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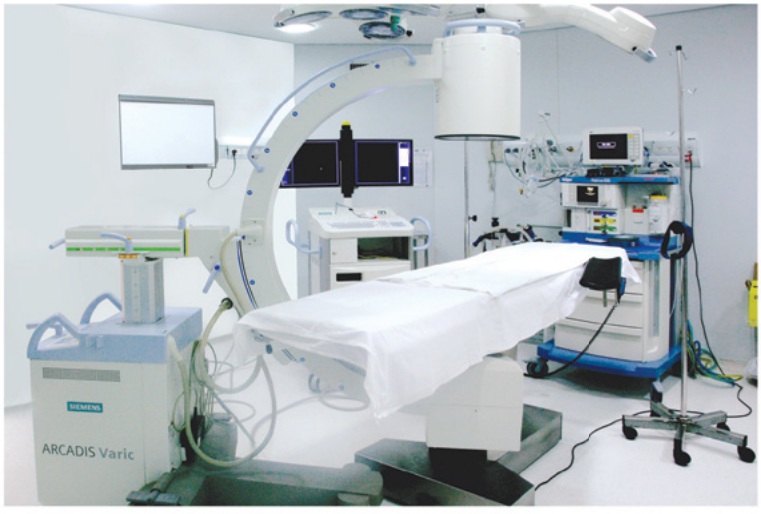

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Advances in therapeutic endoscopy

Galvão Neto M, Grecco E, Souza T. Advances in therapeutic endoscopy. Arq Gastroenterol. 2018;55(3):201.

The therapeutic endoscopy has evolved in an impressive way in recent years, even in areas before under surgical domain. Endoscopic treatment of early cancer, for example, through the development of mucosectomy techniques (EMR – Endoscopic Mucosal Resection) and the most elaborate and effective submucosal dissection (ESD – Endoscopic submucosal resection) that can resect larger areas, are techniques used in the esophagus, stomach, duodenum and colon.

Still in oncology, the palliation of advanced tumors by means of prostheses is a reality of the day to day of endoscopy and the addition of the therapeutic endosonography allowed advances until previously difficult to imagine, such as deriving the bile ducts in a transluminal way through the stomach and duodenum.

In the benign diseases of the biliary tract, the colangiopancreatic endoscopy (ERCP) has long already become indispensable and is gold standard but it does not cease to evolve. The addition of therapeutic cholangioscopy with laser lithotripsy and access by endosonography is already able to drain and exploit decisively the gallbladder.

Into the management of gastroesophageal reflux disease (GERD) after frustrated attempts in the decade of 80, the endoluminal treatment is back with radiofrequency procedures, submucosal resection at the level of gastric esophageal transition or with the manufacture of anti-reflux valves. Also, in GERD, it impresses the possibility of performing the ablation of Barrett's esophagus, even with dysplasia, by radiofrequency, mucosectomy, submucosal dissection or cryoablation.

The treatment of obesity was restricted to behavioral changes, diets, physical exercise and medications for overweight patients in mild obesity. For severe obesity the option was restricted to the bariatric surgery. Currently, bariatric endoscopy emerges as an option for patients with overweight and obesity grade II with interventions on the stomach through equipment (devices) that occupy space such as the intragastric balloons and interventions that alter the anatomy, reducing the stomach volume through endosuture. In addition to these actions on the stomach, bariatric endoscopy advances with procedures in the small intestine intending to treat metabolic alterations such as type 2 Diabetes and NASH, with the possibility of proximal intestinal deviations, through an intestinal sleeve anchored in the duodenum or with distal intestinal deviations by means of endoscopic anastomosis through magnetic rings. There is also the possibility of remodeling the duodenal mucosa by means of endoscopic ablation. Also, in bariatric endoscopy, the endoluminal approach is practically the first option in the treatment of complications of bariatric surgeries such as stenosis and fistulas.

Thus we outline a brief summary of how the therapeutic endoscopy has advanced and how this progress is being made in solid bases of scientific evidence, thus demonstrated, as an example, in two articles published in this edition of the **Archives of Gastroenterology**.

Yamazaki et al.[1] evaluate through an experimental model the learning of endoscopic dissection (ESD), one of the most complex endoscopic procedures, with clear objectives and methodology demonstrating results based on depth of resection with microscopy and defining the relationship with complications and learning. Coronel et al.[2] demonstrate through a well-structured meta-analysis of randomized prospective articles the effectiveness of endoscopic treatment of gastroesophageal reflux disease. These two articles of great scientific quality that match the standards of the **Archives of Gastroenterology** come from the excellent school of Endoscopy of the University of São Paulo that is renewed every day.

Manoel **GALVÃO NETO**[1,2,3]
Eduardo **GRECCO**[1,2]
Thiago **SOUZA**[1,2,4]

Galvão Neto M, Grecco E, Souza T. Avanços de Endoscopia terapêutica. Arq Gastroenterol. 2018;55(3):201.

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ORIGINAL ARTICLE

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Inflammatory bowel disease: impact on scores of quality of life, depression and anxiety in patients attending a tertiary care center in Brazil

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ABSTRACT – **Background** – Inflammatory bowel disease frequently affects patients at working age, compromising their quality of life in several levels: physical, psychological, familial and social. Few studies have evaluated the impact of Inflammatory bowel disease on quality of life, anxiety and depression in Brazilian patients. **Objective** – Evaluate quality of life and its correlation with psychological aspects of patients with inflammatory bowel disease through the Inflammatory Bowel Disease Questionnaire and Hospital Anxiety and Depression Scale. **Methods** – Cross-sectional study; Inflammatory Bowel Disease Questionnaire, Short Form-36 and Hospital Anxiety and Depression Scale were applied to consecutive outpatients in a tertiary referral center for inflammatory bowel disease. Harvey-Bradshaw Index and Truelove scores were used to evaluate Crohn's disease and ulcerative colitis activity. Sample calculation: 113 patients for a significance level of 5%, power of 90% and a correlation coefficient of at least 0.3 between scales. Statistical analysis: Student-t test, Pearson and Spearman correlations. **Results** – One hundred twenty patients participated in the study; mean age: 41.7 years; female: 58.3%; Crohn's Disease: 69 patients. No low scores for quality of life were found across the four Inflammatory Bowel Disease Questionnaire domains; the Short Form-36 showed low scores in physical limitations (47.2±42.4) and emotional aspects (49.8±43.4); Hospital Anxiety and Depression Scale score presented a mean of 9.5±2.7 for anxiety and 8.7±2.0 for depression. Quality of life was decreased and Hospital Anxiety and Depression Scale did show increased indices of anxiety and depression, in both diseases only when clinically active. **Conclusion** – Inflammatory Bowel Disease Questionnaire and Hospital Anxiety and Depression Scale showed that outpatients of a tertiary care center for inflammatory bowel disease in Brazil presented good quality of life. The worst quality of life was associated with the intensity of the disease activity.

HEADINGS – Inflammatory bowel disease. Crohn's disease. Ulcerative colitis. Quality of life. Anxiety. Depression.

INTRODUCTION

Inflammatory bowel disease (IBD), in its both forms – Crohn's disease (CD) and ulcerative colitis (UC) – are chronic illnesses with unpredictable clinical course. Frequently affect young people of a working age, compromising their quality of life (QoL), physical, psychological, familial and social dimensions of life[1-6]. A biopsychosocial understanding of illness describes clinical outcome and disease exacerbation as influencing and strongly influenced by both biological and psychosocial factors[7,8].

Studies conducted in other countries have primarily aimed at evaluating the validity, reliability and sensitivity of the quality of life disease-specific instrument, the Inflammatory Bowel Disease Questionnaire (IBDQ), though not being designed to measure the impact of the diseases themselves on the subjects. The influence of disease activity on the association between mood disorders and IBD is unclear[9]. Few studies have used the Hospital Anxiety and Depression Scale (HADS) instrument to estimate the influence of these diseases on the psychological aspects of patients[7,9-12].

There are rare studies that assess the impact of IBD on QoL using a Brazilian population sample[10,13]. The knowledge about anxiety, depression and quality of life is an important issue to promote a good care for this patients. This study aimed to assess the impact of IBD on quality of life and the psychological effects of these diseases on patients attending an outpatient tertiary care clinic in southern Brazil and the correlation with disease's activity.

METHODS

A cross-sectional study was conducted in a population of patients with inflammatory bowel disease, through application of the quality of life assessment tool IBDQ[16] and the Short Form 36 Health Survey (SF-36)[11,12,14-16]. Anxiety and depression were evaluated using the Hospital Anxiety and Depression Scale (HADS)[17,18]. All versions have been validated for the Brazilian-Portuguese language.

Patient selection

A total of 128 consecutive patients with inflammatory bowel disease were included in the study, all of whom attended an outpatient care clinic at a referral center for the treatment of IBD. All were approached either before or after their routine visit to the clinic and invited to participate in the study.

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Assessement

Three questionnaires were applied. The IBDQ, having 32 closed questions with a 7 point response scale, where 1 signifies worst quality of life and 7 equates to the best. The scores were expressed as follows: ≥200 = excellent; 151-199 = good; 101-150 = regular and ≤100 = bad. The SF-36, having eleven questions; the scores were coded, summed up and converted onto a scale from 0 to 100, denoting worst possible to best possible health status, respectively. The HADS questionnaire, having 14 questions divided into two sub-scales to measure anxiety and depression, with classification of scores from 0 (no distress) to 21 (maximum distress).

The questionnaires are considered to be easily understood, self-reported instruments and were completed by patients without any external help.

The clinical indices of UC severity (Truelove) and CD activity (Harvey Bradshaw) were calculated at the clinic on the day of consultation, when the questionnaires were also completed.

Sample size

The sample size calculation was based on the study of Pontes et al. (2004)[19]. For a significance level of 5%, a statistical power of 90% and a correlation coefficient of at least 0.3 between the scales was used, obtaining a minimum total of 113 patients.

Statistical analysis

Quantitative variables were described through average and standard deviation, and qualitative variables through absolute and relative frequencies.

The Student t-test was applied to assess the differences between the inflammatory bowel diseases for QoL scores (IBDQ and SF-36) and anxiety and depression scores (HADS).

The relationships between the scales were evaluated using Pearson's correlation coefficient (symmetric distribution) or Spearman's correlation coefficient (asymmetric distribution).

The level of significance adopted was 5% ($P \leq 0.05$) and the analyses were performed using SPSS (Statistical Package for the Social Sciences) version 13.0.

Ethical considerations

The study protocol was approved by the local Research Ethics Committee, and all patients signed a Consent Form.

RESULTS

Eight patients (6.7%) from a sample of 128 were excluded due to incorrect completion of questionnaires. The IBDQ was correctly completed by 113 patients, 115 for the SF-36, and 123 for the HADS. From the 120 patients evaluated, 70 (58.3%) were female; the mean ±SD age was 41.2±13 years; 69 (57.5%) presented with Crohn's disease and 51 (42.5%) with Ulcerative Colitis; 85.7% of CD and 64.6% of UC patients were in remission.

Patient IBDQ scores indicated a good quality of life for all fields of the questionnaire in 62 (58.4%) patients. The best scores were observed in the Functional Capacity and Social Aspects domains, averaging over 65 points. There was no difference observed between the two diseases in any of the domains. (TABLE 1). Lower scores for quality of life were shown in only two of the eight SF-36 domains: the mean (±SD) of the Physical Limitations and Emotional Aspects were 47.2±42.4 and 49.8±43.4, respectively, with both results being below 50, which is half of the variation from 0 to 100 points. Stratifying patients according to the presence or absence of activity of the disease the QoL is decreased in both diseases when in activity. (TABLE 2).

TABLE 1. Comparison of diseases through the Student t-test for independent samples.

Variables	CD Average ± DP	UC Average ± DP	*P*
IBDQ	n = 68	n = 45	
Intestinal symptoms	53.7 ± 11.1	49.4 ± 14.6	0.097
Systemic symptoms	23.8 ± 6.9	22.6 ± 7.3	0.371
Social aspects	26.5 ± 8.1	25.1 ± 9.1	0.391
Emotional aspects	56.9 ± 17.4	55.5 ± 19.4	0.696
Total	160.9 ± 39.3	152.7 ± 46.9	0.312
SF- 36	n = 61	n = 46	
Functional capacity	66.7 ± 28.5	64.4 ± 27.6	0.688
Physical limitations	50.8 ± 42.6	42.4 ± 42.1	0.311
Pain	59.4 ± 27.1	56.0 ± 24.8	0.514
General health	57.1 ± 10	55.6 ± 11.8	0.488
Vitality	56.3 ± 16.3	52.5 ± 14.9	0.219
Social aspects	69.8 ± 26.5	64.4 ± 32	0.365
Emotional aspects	53.3 ± 43.9	45.2 ± 42.7	0.343
Mental health	62.6 ± 25	57.9 ± 25.5	0.346
HADS	n = 67	n = 48	
Anxiety	9.3 ± 2.4	9.8 ± 3.0	0.296
Depression	8.4 ± 1.8	9.1 ± 2.2	0.094

IBDQ: Inflammatory Bowel Disease Questionnaire. HADS: Hospital Anxiety and Depression Scale.

TABLE 2. Associations between quality of life, anxiety and depression with disease activity.

Variables	Remission	Active	*P*
	n (%)	n (%)	
IBDQ	n=83	n=23	<0.001
Bad	5 (6.0)	10 (43.5)*	
Regular	20 (24.1)	9 (39.1)	
Good	37 (44.6)*	3 (13.0)	
Excelent	21 (25.3)*	1 (4.3)	
Anxiety	n=80	n=25	0.040
Presence	40 (50.0)	19 (76.0)*	
Absence	40 (50.0)*	6 (24.0)	
Depression	n=80	n=25	0.005
Presence	36 (45.0)	20 (80.0)*	
Absence	44 (55.0)*	5 (20.0)	

IBDQ: Inflammatory Bowel Disease Questionnaire. * Test for a statistically significant association waste adjusted to 5% significance.

The HADS questionnaire scores ranged from 0 to 21, and patients presented a mean score of 9.5 for the anxiety and 8.7 for the depression sub-scales, with the median being 9; the minimum points for anxiety and depression were 5 and 2 with the maximum being 16 and 14, respectively. The evaluation in UC population did not detect statistically significant manifestations of anxiety and depression. Indeed, we observed higher levels of depression only in patients with active Crohn's disease. TABLE 3.

TABLE 3. Associations between quality of life, anxiety and depression with the Harvey-Bradshaw Activity Index for Crohn's disease.

Variables	Remission	Activity	*P*
	n (%)	n (%)	
IBDQ	n=54	N=9	0.003
Bad	3 (5.6)	4 (44.4)*	
Regular	14 (25.9)	3 (33.3)	
Good	23 (42.6)	2 (22.2)	
Excelent	14 (25.9)	0 (0.0)	
Anxiety	n=52	n=9	0.276
Presence	27 (51.9)	7 (77.8)	
Absence	25 (48.1)	2 (22.2)	
Depression	n=52	n=9	0.001
Presence	20 (38.5)	9 (100)*	
Absence	32 (61.5)*	0 (0.0)	

* Test for a statistically significant association waste adjusted to 5% significance.

When correlated with the Harvey-Bradshaw index for Crohn's disease, an association was found between a greater degree of disease activity and lower quality of life and higher anxiety scores of patients.

There was a statistically significant direct association between the scores for almost all domains of the SF-36 (except general health) with the IBDQ scores, showing a good correlation between both questionnaires. Patients with higher anxiety scores presented lower scores for quality of life on the IBDQ. There was no association between scores of the IBDQ with depression.

There was a statistically significant, inverse association between the Truelove activity index and the SF-36 scores for pain, vitality and mental health, and for all areas of the IBDQ. The same was not observed with the scores from the HADS questionnaire. TABLE 4.

Our date demonstrated that there was no statistically significant association between the physical limitations and general health domains of the SF-36 with the HBI, although all other domains presented with an inverse association. (data not shown).

TABLE 4. Associations between quality of life, anxiety and depression with the severity of Ulcerative Colitis Index (Truelove).

Variables	Remission	Activity	*P*
	n (%)	n (%)	
IBDQ	n=29	n=14	0.003
Bad	2 (6.9)	6 (42.9)*	
Regular	6 (20.7)	6 (42.9)	
Good	14 (48.3)*	1 (7.1)	
Excelent	7 (24.1)	1 (7.1)	
Anxiety	n=28	n=16	0.127
Presence	13 (46.4)	12 (75.0)	
Absence	15 (53.6)	4 (25.0)	
Depression	n=28	n=16	0.661
Presence	16 (57.1)	11 (68.8)	
Absence	12 (42.9)	5 (31.3)	

IBDQ: Inflammatory Bowel Disease Questionnaire.

DISCUSSION

Our study showed, rather surprisingly, a low disease impact on quality of life scores for patients with IBD attending the outpatient clinic of a tertiary care referral center in Brazil. Additionally, a low disease impact was also detected on anxiety and depression scores in the same population of patients when their disease is in remission.

This study demonstrated that QoL of CD and UC patients in remission had a better QoL when compared with patients in activity by clinical indices usually applied in clinical settings.

These findings can probably be explained by the high disease remission rates observed in our study, 85.7% for CD and 64.6% for UC. The reason for this is probably related to the fact that our study sample consisted of outpatients, who were interviewed during previously scheduled consultations, whereas patients showing more intense disease activity were more likely to not wait for appointments and to be seen on an off-schedule basis, thus not participating in our study.

Our main objective was to evaluate the impact of inflammatory bowel disease on patient quality of life, using a disease-specific Health-related Quality of Life questionnaire (IBDQ), as well as through generic questionnaires. Our population demonstrated a good quality of life according to the IBDQ, and a lower quality of life in only two areas of the SF-36, physical limitations and emotional aspects. The IBDQ showed that patients presented acceptable scores, with a total mean ±SD for CD and UC of 160.9±39.3

and 152.7±46.9, respectively, a result similar who obtained mean scores (±SD) of 177.6±39.6 for CD and 178.5±35.3 for UC, but in contrast to IGLESIAS et al.[20] in which patients with CD had lower scores in all domains of the SF-36 and low scores in the Systemic Symptoms section of the IBDQ.

No difference in quality of life was observed between patients with Crohn's disease and those with ulcerative colitis, results also found in other studies using the IBDQ, such as Cohen et.al.[2] who studied 50 patients in southern Brazil, and BLANCO et. al.[21] and Casellas et. al.[22] who assessed the quality of life for 120 and 289 Spanish patients, respectively. In contrast, comparing our study and the above, the finding of Taleban et al.[23], obtained a good correlation of QoL and activity in the disease only to UC using another method of verification of disease activity the "Mayo endoscopic score".

When comparing the IBDQ with the generic SF-36, it was seen that patients showed lower quality of life scores in two domains (Physical Limitations and Emotional Aspects), as also observed by Pontes et. al. (2004)[19]. Our study showed an association between all domains of the SF-36 (except general health) with the IBDQ, whereas Pontes found no correlation between the Intestinal Symptoms component of the IBDQ with the SF-36.

Few studies have utilized HADS in IBD patients[24]. Our results are similar to the findings of Knowles et al. (2011)[25] who found that when evaluating patients with CD, 55% presented with moderate anxiety and 41% with moderate depression. Both studies support the idea that keeping patients in remission has a positive impact on their lives, reducing depression levels although our results suggest that there no impact on anxiety.

In the study of Lonnfors et al.[26] Quality of Life was evaluated in 4670 patients in 25 countries starting an online questionnaire developed by EFCCA, European Federation of Crohn's and ulcerative colitis Associations, who demonstrated that patients with IBD had limitations in various situations of life which was also demonstrated in the study by Morandkihani[27].

Whilst comparing the IBDQ and HADS instruments, no association was demonstrated between the IBDQ domains with the depression sub-scale, Zhang et al.[28] used IBDQ and other tools for analysis of depression and observed a prevalence within this study differently from our study. It is worth noting the consistency of the results: deterioration in QoL and anxiety scores were associated with greater activity of the IBD. The total scores for the HADS correlated with all domains of the IBDQ, particularly so in the area of mental health.

The choice of Truelove and Harvey-Bradshaw indices was determined by the simplicity and ease of data collection, with values only being calculated on the consultation day. The HBI shows good correlation with the Crohn's Disease Activity Index (CDAI) and can replace it without interfering with the quality of the study[6,29-33].

The HBI showed a direct correlation with scores of anxiety and decreased quality of life, as also demonstrated by Pontes et al.[18,19].

Three points can be considered as limitations to our study. A- Questionnaires: the large number of questions to be answered could potentially tire patients; the layout of the SF-36 questionnaire with the use of long tables and many rows and columns could lead to patient confusion and incorrect completion. B- Population sample: patients were evaluated in an elective medical consultation setting, whereas those with greater disease activity were more likely to be seen in the Emergency Room or directly admitted to hospital, therefore not being subject to the questionnaires[34-36]. However, this comprised only a small number of cases, with care for almost all patients with IBD attending this institution coming under the control of the authors. Furthermore, whenever necessary, patients with more urgent conditions were promptly scheduled for a regular outpatient visit. As a consequence and with the recruitment period being almost 1 year, these patients were seen by our team in a time span close to the flare-up of their diseases, meaning that any major repercussion on their quality of life would probably not have been missed by our research. C- We did not verify the correlation of quality of life, anxiety and depression with the phenotype of the diseases.

Our study presented significant strengths: a low probability that a sampling bias was responsible for our results as the sample consisted of consecutive patients attending a specialist clinic dedicated to the care of IBD patients at a reference university hospital, part of the state health system; the number of patients evaluated met the study sample size calculation; individuals were evaluated sequentially; the same investigator evaluated all patients; it is the first study to evaluate in a systematic manner anxiety and depression in a population with IBD in Brazil through use of the HADS questionnaire, validated for the Brazilian-Portuguese language; the study showed that all three questionnaires, SF-36, IBDQ and HADS, were sensitive to the health status of patients with IBD, having a good correlation with disease activity indices; the concept that the IBDQ and HADS questionnaires can be considered reliable, easily understood, self-applicable and cost effective, is also supported.

We believe that the incorporation of these questionnaires into our clinical practice will contribute to the assessment of IBD impact on the quality of life of our patients. This broader assessment can, therefore, contribute to a more appropriate and individualized care plan for these patients.

However, it is necessary to conduct further studies in order to evaluate whether IBD in low prevalence countries such as Brazil[37,38], express the same phenotype and severity patterns as in high prevalence countries. If a low incidence of patients with severe disease is found in these countries, it could possibly explain our findings of the low impact on QoL in our IBD patients.

In conclusion, the IBDQ and HADS questionnaires showed that outpatients attending a tertiary care center for IBD in Brazil presented with good quality of life, with no differences being observed between patients with CD and UC. Quality of life for these patients is significantly reduced with increased IBD activity.

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Authors' contribution

Calixto RP: scientific, intellectual, conception and design of the study; acquisition, analysis and interpretation of data; statistics analysis; manuscript preparation; critical revision. Flores C: conception and design of the study, critical revision. Francesconi CF: conception and design of the study, critical revision.

Calixto RP, Flores C, Francesconi CF. Doença inflamatória do intestino: impacto no score da qualidade de vida, depressão e ansiedade em pacientes atendidos em um centro de atendimento terciário no Brasil. Arq Gastroenterol. 2018,55(3):202-7.

RESUMO – **Contexto** – A doença inflamatória do intestino afeta frequentemente os pacientes em idade de trabalho, comprometendo a qualidade de vida em vários níveis: físico, psicológico, familiar e social. Poucos estudos avaliaram o impacto da doença inflamatória do intestino na qualidade de vida, ansiedade e depressão em pacientes brasileiros. **Objetivo** – Avaliar a qualidade de vida e sua correlação com os aspectos psicológicos dos pacientes com doença inflamatória intestinal através do Questionário da Doença Inflamatória do Intestino e da Escala de Ansiedade e Depressão Hospitalar. **Métodos** – Foi realizado um estudo transversal, com uma amostra de pacientes consecutivos, nos quais foram aplicados os questionários de perguntas fechadas sobre Qualidade de Vida através dos questionários: Inflammatory Bowel Disease Questionnaire, Short Form Health Survey 36; e ansiedade e depressão: Hospital Anxiety and Depression em suas versões validadas para a língua portuguesa praticada no Brasil. Foram aplicados em pacientes ambulatoriais consecutivos em um centro de referência terciária para doença inflamatória intestinal. Os índices Harvey-Bradshaw Index e Truelove foram utilizados para avaliar a doença de Crohn e a atividade da colite ulcerativa. Cálculo da amostra: 113 pacientes para um nível de significância de 5%, força de 90% e um coeficiente de correlação de pelo menos 0,3 entre as escalas. Análise estatística: teste Student-t, correlações Pearson e Spearman. **Resultados** – Cento e vinte pacientes participaram do estudo; idade média: 41,7 anos; feminino: 58,3%; doença de Crohn: 69 pacientes. Não foram encontrados escores baixos para a qualidade de vida nos quatro domínios do questionário da Inflammatory Bowel Disease; O Short-Form-36 mostrou baixa pontuação em limitações físicas (47,2±42,4) e aspectos emocionais (49,8±43,4); O índice da escala Hospital Anxiety and Depression apresentou uma média de 9,5±2,7 para ansiedade e 8,7±2,0 para depressão. A qualidade de vida foi diminuída e a Hospital Anxiety and Depression mostrou índices aumentados de ansiedade e depressão, em ambas as doenças somente quando clinicamente ativo. **Conclusão** – O questionário da Inflammatory Bowel Disease e a Escala de Hospital Anxiety and Depression mostraram que os pacientes ambulatoriais de um centro de cuidados terciários para doença inflamatória do intestino no Brasil apresentaram boa qualidade de vida. A pior qualidade de vida foi associada à intensidade da atividade da doença.

DESCRITORES – Doenças inflamatórias intestinais. Doença de Crohn. Colite ulcerativa. Qualidade de vida. Ansiedade. Depressão.

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ORIGINAL ARTICLE

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US-guided percutaneous core liver biopsy: analysis of 171 cases from a single oncology service

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ABSTRACT – **Background** – Though strongly suggestive of metastasis, focal lesions on liver scans of oncological patients require histological confirmation for the prescription of adequate treatment. **Objective** – To evaluate the safety and efficacy of US-guided percutaneous core liver biopsy. **Methods** – Descriptive, cross-sectional study based on secondary data from 171 patients submitted to US-guided percutaneous core liver biopsy at the diagnostic radiology service of the Ceará Cancer Institute (ICC, Brazil) between February 2010 and March 2015. Quantitative data were expressed in absolute numbers or percentages, with emphasis on the rate of complications observed within six hours after the procedure. **Results** – The overall accuracy was 96.4%. The overall rate of complications was 2.3%, three quarters of which was due to hemorrhage. Age over 50 years was positively associated with accuracy. No deaths occurred within the period of observation. **Conclusion** – Our findings support the claim that the use of thick biopsy needles improves diagnostic accuracy. The few complications observed were non-lethal and predominantly hemorrhagic.

HEADINGS – Neoplasm metastasis. Liver. Image-guided biopsy, adverse effects. Ultrasonography, methods.

INTRODUCTION

In oncology, biopsies are often used to confirm suspicion of malignancy. Despite the existence of a known primary tumor, the concurrent emergence of focal lesions on liver scans does not necessarily imply metastasis, but requires diagnostic confirmation by biopsy for the prescription of adequate treatment.

US-guided percutaneous core biopsies are currently considered an effective means of diagnosing lesions found almost anywhere in the body. In fact, in many scenarios it is the primary diagnostic method. Acceptance of this method is evidenced by its rapid dissemination over the past years, in step with the progressive drop in the use of diagnostic laparotomy[1].

The liver may be biopsied with several techniques and approaches, including percutaneous blind biopsy, US-guided biopsy, CT-guided biopsy, transjugular biopsy and laparoscopic biopsy. In this study, only US-guided percutaneous core biopsy was used due to the possibility of real-time monitoring, which offers improved safety and accuracy, especially when biopsying abdominal and pelvic structures. Despite being operator-dependent, US has a range of advantages, including low cost and absence of ionizing radiation[2,3].

There are, however, also contraindications for percutaneous core biopsy. According to some authors[4,5], these include: A) Coagulation disturbance (confirmed by the presence of one or more of the following: prothrombin time <60% of control, partial thromboplastin time 5 sec longer than control, and platelet count <60,000/mm^3); B) Massive ascites (increased abdominal wall tension, with echograhy-confirmed ascites refractory to treatment with diuretics); C) Accentuated chronic anemia associated with chronic kidney failure, hematocrit count ≤29%, and use of anticoagulants (heparin); D) Morbid obesity (20% above the ideal body weight).

Many types of biopsy needles are available on the market, with varying gauges, shapes and sample extraction mechanisms. In general, needles may be classified as thin (gauges 20-23) or thick (gauges 16-19). Thin needles are appropriate for collecting samples for cytological and, in some cases, histological studies. They can transgress the bowels at minimal risk and are associated with low rates of hemorrhage when used to sample vascularized lesions. Five or more fragments can be aspired with this type of needle[3].

Thick needles provide more satisfactory samples for both cytological and histological studies (only 2-3 fragments are necessary). Moreover, certain types of benign and malignant lesions require larger samples for diagnosis. Though still considered low, the risk of hemorrhage is significantly greater for thick needles than for thin needles. It should therefore be verified on contrast CT or US Doppler before the procedure whether the lesion is highly vascularized or not[1,6].

Liver biopsies are relatively safe, with an overall complication rate of 1% and a mortality rate of 0.1% or less when performed percutaneously. Most complications (60%) occur within the first two hours, and 80% (with hemorrhage as the most common) occur within ten hours of the procedure. Hemorrhagic complications are more frequent in patients with malignancy and/or acute liver failure, chronic active hepatitis and cirrhosis[7]. At our service, patients are therefore observed for 24 hours after biopsies.

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The primary objective of this study was to evaluate the diagnostic accuracy and safety of US-guided percutaneous core liver biopsy in patients attending a diagnostic imaging and radiology service in Northeastern Brazil. The secondary objectives were:

- Describe the US-guided percutaneous core biopsy technique adopted at our service, including post-biopsy care and management of complications.
- Identify the main causes of inconclusive results.

METHODS

In this descriptive, cross-sectional study, we analyzed secondary data from an intentional sample of 171 patients submitted to US-guided percutaneous core liver biopsy at the diagnostic imaging and radiology service of *Haroldo Juaçaba* Hospital (Ceará Cancer Institute / ICC), covering the period February 2010 to March 2015. The patients were identified by a search in the digital histopathology archives of the ICC pathology service (*PathoControl 8*), using the descriptors "hepatic" and "liver".

Thus, the sample consisted of patients with hepatic lesions detected by imaging, with or without a known primary tumor, referred to our service by their physicians (most often oncologists) for the determination of etiology.

Demographic, laboratory and clinical data were retrieved from the patients' records, including platelet and hematocrit count, prothrombin and thromboplastin time, histopathological and immunohistochemical findings, and information on postoperative complications.

The results were expressed as absolute (n) and relative frequency (%), mean, median, maximum and minimum values and standard deviation. All analyses were performed with IBM SPSS Statistics, v. 21.0. The study protocol was approved by the ICC research ethics committee and filed under entry #44484915.0.0000.5528.

The standard procedure for US-guided percutaneous core liver biopsy used at our service is based on Khatie and coworkers[2]:

1. Review clinical indications for the procedure. Analyze previous imaging test results if available to make sure the use of US guidance is safe for the patient.
2. Check whether the patient's coagulation status allows to carry on the biopsy with safety. If not, ask the patient's physician (or a hematologist) to prescribe treatment to restore normal values.
3. Obtain the patient's informed written consent.
4. Perform biopsy under local anesthesia with 2% lidocaine.
5. Provide postoperative care.
6. Check the quality control of the pathological report to determine the percentage and causes of unsuccessful biopsies.

In the absence of contraindications (steps 1 and 2), written consent was requested. Before signing the form, patients were given relevant information about the procedure, including the reason for the biopsy and the possible occurrence of complications (e.g., bleeding and infection) and postoperative pain (the latter is rarely important and easily controlled with proper analgesics). Likewise, patients were informed about the need to remain in postoperative observation to monitor for and manage complications, if any.

Prior to the biopsy, 12 hours of fasting was required. Following the recommendations of Shankar and coworkers[1], the vast majority of the biopsies were performed with the patient in a comfortable supine position, although in some cases other positions (e.g., oblique, and lateral or ventral decubitus) were preferred in order to reduce the distance to the target, favoring diagnostic accuracy. Only local anesthesia was used. Sedative or analgesic medication was not administered routinely. A peripheral venous access was provided for hydration.

The patients in this study were biopsied with thick needles (18 gauge) in order to collect at least two fragments of each lesion. Stored in labelled vials with formaldehyde, the samples were sent to the laboratory for pathological evaluation.

After the procedure, common dressing was applied to the wound. The patients were then placed in lateral decubitus on top of the biopsy site for a 2-hour rest with monitoring of vital signs (pulse and arterial pressure) and possible complaints at 30-min intervals. This was followed by a 4-hour rest in dorsal decubitus (totaling 6 hours of observation at our service). Then the patients were submitted to abdominal echography for the detection of liquid in the abdominal cavity (an indirect sign of hemorrhagic complications). If negative, the patients were allowed to eat and transferred to a ward for observation until completing 24 hours.

RESULTS

In our sample of 171 patients, the male sex was slightly predominant (89 vs 82) (TABLE 1). At the time of biopsy, the average age was 61.5 years (range: 26-89; median 63; mode 72).

As shown in TABLE 1, most procedures were performed in patients over 50 years of age, regardless of sex, due to the indicating a positive association between cancer and age related to cellular aging and progressive loss of cell recovery capabilities.

TABLE 1. Demographic and diagnostic data of 171 patients submitted to US-guided percutaneous core liver biopsy at the Ceará Cancer Institute, Brazil. February 2010 to March 2015.

	n	%
Sex		
Female	82	48.0
Male	89	52.0
Age		
≤ 50	31	18.6
> 50	136	81.4
Number of fragments		
1	52	30.6
> 1	118	69.4
Size of fragment		
≤ 1.2 cm	90	52.9
> 1.2 cm	80	47.1
Overall accuracy	165	96.4

All patients had preoperative laboratory findings in the safe range for the procedure, except one case of coagulation disturbance promptly treated and corrected by the patient's physician. The lowest platelet count observed was 80,000/mm³.

Most patients (69.4%) had more than one fragment extracted. Nearly half the fragments (47.1%) were larger than 1.2 cm. Results were satisfactory for 165 patients, corresponding to an overall accuracy of 96.4%. The remainder (n=6; 3.6%) were inconclusive.

In the present study, age over 50 years was the only factor influencing overal accuracy (*P*=0.044) (TABLE 2).

TABLE 2. Influence of demographic and diagnostic factors on the accuracy of US-guided percutaneous core liver biopsy in a sample of 171 patients biopsied at the Ceará Cancer Institute, Brazil. February 2010 to March 2015.

	Accuracy	*P*-value
Sex		
Female	97.6% (80/82)	0.466
Male	94.3% (84/89)	
Age		
≤ 50	90.3% (28/31)	0.044
> 50	97.8% (133/136)	
Number of fragments		
1	94.2% (49/52)	0.293
> 1	97.5% (115/118)	
Size of fragment		
≤ 1.2 cm	95.6% (86/90)	0.493
> 1.2 cm	97.5% (78/80)	

In the group of inconclusive results, the average number of extracted fragments was 1.5, and the average fragment size was 1.2 cm. Only two patients with inconclusive results had repeat biopsies. One of these had three fragments extracted, the largest of which measuring 1.4 cm, but once more the presence of extensive fibrosis/necrosis prevented reaching a conclusion. The other had two fragments extracted, the largest of which measuring 1.7 cm, yielding a satisfactory result, despite the presence of fibrosis/necrosis.

The histopathological and immunohistochemical analyses identified primary liver tumors in 15 patients, secondary tumors in 117, indifferentiated neoplasia in 19, and non-neoplastic lesions in 3. In 11 patients, no lesion was found.

In the group of 117 secondary tumors, the most frequent type was biliodigestive, followed by neuroendocrine, coloretal, squamous cell and breast (FIGURE 1).

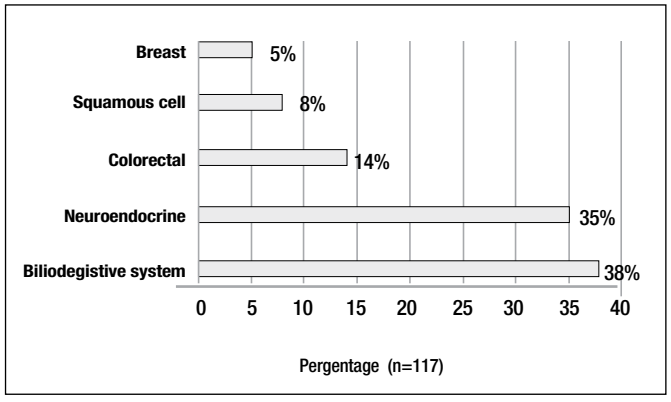

FIGURE 1. Distribution of relative frequency of secondary liver tumors in 117 out of a sample of patients submitted to US-guided percutaneous core liver biopsy at the Ceará Cancer Institute, Brazil. February 2010 to March 2015.

The four complications observed in the sample of 171 patients were predominantly hemorrhagic and correspond to an overall rate of ~2.3%. Half were early occurrences (<6 hours) and half were late occurrences (6-24 hours) (TABLE 3). No deaths were observed during the 24-hour observation period adopted at our institution.

TABLE 3. Complications of US-guided percutaneous core liver biopsy according to type and time of occurrence in a sample of 171 patients biopsied at the Ceará Cancer Institute, Brazil. February 2010 to March 2015.

Complications	Early (<6h)	Late (6-24h)	Total
Transient hypotension (vasovagal reaction)	-	1	1
Intraperitoneal hemorrhage	2	-	2
Intrahepatic or subcapsular hematoma	-	1	1

DISCUSSION

Although it is a common situation in oncology, synchronic or metachronous liver lesions, even using US, CT-scan and MRI, may still raise doubts as to its neoplastic nature, as well as whether it is a new primary or metastatic lesion. Under these circumstances the US-guided needle biopsy appears as a low-cost, safe and accurate option[8,9].

At our service, thick needles (18 gauge) are preferred as they provide samples more easily interpreted by pathologists, minimizing the need for repeat biopsies due to inconclusive results. On the other hand, hemorrhagic complications are more frequent with thick needles than with thin needles[6]. According to Shankar and colleagues,[1] the thicker the needle, the more accurate the diagnosis and the higher the rate of complications. This may be true in some settings, but in this study the rate of complications was low, no urgency surgical intervention was necessary, and the few complications observed were non-lethal. While core biopsy with thin needles is associated with high diagnostic accuracy and a low rate of complications, it requires the availability of an experienced cytopathologist. Core biopsy with thick needles (<19 gauge) does not[3].

The observed complications were predominantly (75%) hemorrhagic (intraperitoneal hemorrhage and intrahepatic or subcapsular hematoma). Hemorrhage is a common complication and the main cause of mortality in the follow-up of biopsy patients. It usually results from the inadvertent perforation of a dilated portal vein or aberrant arteries and may cease spontaneously or, if severe, require blood transfusion and surgical intervention[10].

Other much less common complications have been described in the literature, including peritonitis, septicemia, pneumothorax and hemothorax. One of our patients presented transient vasovagal hypotension, a minor complication easily corrected with hydration. Pain was not classified as a complication in this study since it is inherent to invasive procedures, short-lived, easily managed and non-life-threatening.

In one study, overall positivity was found to be independent of needle gauge, but 21 gauges was associated with the greatest levels of safety[6]. In our sample, conclusive and inconclusive results did not differ significantly with regard to fragment size and number. However, most inconclusive results were associated with extensive fibrosis/necrosis. In other words, conclusiveness was not dependent on fragment size and number, but poor fragment quality (i.e., extensive fibrosis/necrosis) compromised diagnostic accuracy and was the main reason for repeat biopsies.

CONCLUSION

The preference for thick needles in US-guided percutaneous core liver biopsies is justified by the high accuracy achieved and the small percentage of inconclusive results. In this study, age over 50 years had a significant impact on accuracy, reflecting the well-documented association between cancer and old age. Few complications were observed, most of which were hemorrhagic, but none resulted in death. The presence of extensive fibrosis/necrosis in the samples compromised diagnostic accuracy and was the main reason for repeat biopsies.

Authors' contribution

Parente FVC: made substantial contributions to conception and design, acquisition of data; and in drafting the article. Moura EA: made contributions to acquisition of data; and analysis of data. Santos JAM: participated in doing the biopsies, drafting the article and revising it critically. Lima MVA: made substantial contributions to conception and design, wrote part of the article, gave final approval of the version to be submitted and revised version.

Parente FVC, Moura EA, Santos JAM, Lima MVA. Biópsia percutâneas de lesões hepática guiada por ultrassonografia: análise de 171 casos de um único centro oncológico. Arq Gastroenterol. 2018,55(3):208-11.

RESUMO – **Contexto** – Lesões focais nos exames de imagem do fígado em pacientes oncológicos, embora sejam achados fortemente sugestivos de envolvimento metastático, permanece a necessidade de confirmação histológica, a fim de que se institua uma terapia apropriada. **Objetivo** – Verificar a segurança e a eficácia do procedimento de biópsia hepática percutânea guiada por ultrassom, realizado pelo serviço de Radiologia e Diagnóstico por Imagem do Instituto do Câncer do Ceará (ICC). **Métodos** – Estudo transversal, descritivo, baseado em dados secundários de 171 pacientes, submetidos a biópsias hepáticas percutâneas, guiadas por ultrassonografia, realizadas no ICC, de fevereiro de 2010 a março de 2015. Os dados quantitativos obtidos foram apresentados em forma de números absolutos ou percentuais, com ênfase nas taxas de complicações, ocorridas nas primeiras seis horas de observação hospitalar. **Resultados** – A acurácia geral foi de 96,4%. Encontramos uma taxa global de complicações de 2,3%, sendo que 75% delas foram de natureza hemorrágica. Não verificamos a ocorrência de óbitos dentro do período de observação pós-biópsia. **Conclusão** – A utilização de agulhas calibrosas, parece, de fato, estar relacionada **à** melhoria na acurácia diagnóstica, com baixas taxas de complicações, sobretudo as hemorrágicas, contudo não letais. No presente trabalho, a idade mostrou-se um fator modificador da acurácia.

DESCRITORES – Metástase neoplásica. Fígado. Biópsia guiada por imagem, efeitos adversos. Ultrassonografia, métodos.

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ORIGINAL ARTICLE

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Comparison between the endoscopic findings and the histological diagnosis of antral gastrites

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ABSTRACT – **Background** – Gastritis is a very common disorder that is widely distributed worldwide, representing one of the most prevalent pathological entities in Gastroenterology and Digestive Endoscopy. **Objective** – This study aims to analyze the correlation between the endoscopic findings and the histological diagnosis of antral gastritis. **Methods** – In this study, 92 reports of upper digestive endoscopy were performed between November 2014 and January 2015, including biopsy of the antral gastric mucosa, comparing the endoscopic and histological findings, which were classified according to the Sidney System. The 92 exams included 35 men and 57 women, ranging in age from 15 to 84 years. The most frequent indication was epigastric pain. **Results** – Of the 92 examinations analyzed, the histological diagnosis of antral gastritis appeared in 75 exams, 59 endoscopic reports contained the diagnosis of antral gastritis, and 33 endoscopic findings were normal. The kappa coefficient was 0.212 (P<0.05), indicating that there was no significant agreement between the endoscopic findings and the histological diagnosis of antral gastritis. **Conclusion** – We conclude that histology represents the gold standard method for the diagnosis of antral gastritis and that in daily clinical practice, biopsies should always be performed, regardless of the endoscopic findings.

HEADINGS – Gastritis, diagnosis. Endoscopy. Gastric mucosa. Biopsy.

INTRODUCTION

The term gastritis was first used in 1728 by Stahl. There is still controversy about the term gastritis, mainly due to the lack of correlation between the clinical, endoscopic and histological manifestations. Gastritis is a very common condition, with a wide distribution worldwide, and its prevalence increases with age. After the age of 60, the prevalence of gastritis varies from 50% to 100% and appears to be higher in low socioeconomic populations. Its main etiological factor is *Helicobacter pylori*, which has high incidence (approximately 50% in the world population) and is marked by the presence of mucosal inflammation, representing the stomach's response to an injury[1]. Histologically, gastritis exhibits cellular lesion, regenerative process, inflammatory infiltration of the mucosa and the presence of lymphoid follicles [2].

In the 1960s, the endoscopic era began with the introduction of flexible endoscopy; later, with the introduction of the biopsy channel in the appliances, directed collection of gastric mucosa became possible. In 1990, a multidisciplinary committee developed the Sydney Classification System with the aim to standardize the different terminologies used, trying to define the endoscopic, histological and etiological aspects whenever possible. In 1994, a new consensus was held in Houston (i.e., the modified Sidney Classification). The Sidney System for the classification of gastritis establishes two major divisions that interact: histological and endoscopic[1,2].

Histology includes the following findings: inflammation, inflammatory activity, glandular atrophy, intestinal metaplasia, dysplasia, *H. pylori* detection, evolutionary characteristics (acute or chronic) and gradation (mild, moderate, intense). Hematoxylin-eosin is the stain used in microscopy. One of the practical consequences of this system is the inclusion of endoscopic biopsies for investigation of gastroduodenal disease[1,2].

The diagnosis of gastritis can only be established by gastric biopsy. At least five biopsy specimens are recommended: the large and small curvatures of the distal antrum; the angular incisura; and the anterior and posterior walls of the proximal body. Unfortunately, the correlation between endoscopic and histological appearances is weak. Endoscopy is usually used for the diagnosis of possible causes of dyspepsia. In general practice, different aspects can be found during endoscopy; however, there is no consensus on the association of endoscopic gastric findings and histopathological conditions[3,4].

Although poor, correlations between endoscopic findings and histological changes have been detected in many studies[5-8]. Good correlations were reported only in the severe types of gastritis or normal endoscopy[4,7,9]. Given the divergence between the studies in the literature, the objective of this study was to evaluate the degree of agreement between the endoscopic and histological reports regarding the diagnosis of antral gastritis in upper digestive endoscopy examinations.

METHODS

To accomplish this study, 250 histological reports conducted between November of 2014 and January of 2015 were initially collected, including products of biopsies of upper digestive en-

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doscopies and colonoscopies. From this initial group, reports that presented biopsy material fragments of antral mucosa were selected for convenience, for a total of 100 reports (initial sample). In this selection, the reports referring to colonoscopies were excluded, as were those that contained biopsies than those of antral gastric mucosa. After the selection of the histological reports, we performed an active search for the corresponding endoscopic reports, which were stored on the report room computers. In this search, 08 endoscopic reports were not found, requiring their exclusion and resulting in a final sample of 92 reports for the study (n=92).

The endoscopic and histological reports were elaborated according to the Sydney Classification. The Sidney System for the classification of gastritis presents two major divisions that interact: histological and endoscopic.

Endoscopic classifications included 1. Topography (pan gastritis, body gastritis, antrum gastritis); 2. Category (enanthematous/exudative, erosive flat and elevated, atrophic, hemorrhagic, reflux, hyperplastic); and 3. Intensity (mild, moderate, marked)[10].

Histology included the following findings: inflammation, inflammatory activity, glandular atrophy, intestinal metaplasia, dysplasia, *Helicobacter pylori* detection, evolutionary characteristics (acute or chronic) and gradation (mild, moderate, intense). Hematoxylin-eosin was the stain used in microscopy. The following data were analyzed and computed: age, sex, indication of the exam, presence or absence of antral, endoscopic and histological gastritis and presence or absence of *H. pylori*[10].

Endoscopy

To perform the upper digestive endoscopy examinations, the patients remained in absolute fast for a minimum period of 8 hours. After directed anamnesis and signing of the consent form, the patients were referred to the examination room, where pulse oximetry, noninvasive blood pressure (BP) and heart rate were monitored. Patients were given simethicone (40 drops via oral) and lidocaine spray (10 jets in the oropharynx). The exams were performed with patients in left lateral decubitus and under superficial venous sedation, administering midazolam 5 mg and fentanyl 50 mcg, reserving the use of propofol for selected cases. In the exams, four fragments of gastric mucosa, two fragments of antrum (small and large curvatures) and two fragments of gastric body (small and large curvatures) were collected for biopsy material. The collected fragments were placed in separate flasks containing 10% formalin solution and then sent to the pathology laboratory[11-14].

Data analysis

To determine whether the endoscopic findings corresponded to the histological findings for antral gastritis, the kappa concordance index test was performed, with a significance level of $P<0.05$. For the data analysis, MEDCALC software was used.

RESULTS

Of the 92 patients included in the study, 57 were males and 35 females, ranging in age from 15 to 84 years. The most prevalent indications were epigastric pain, pyrosis, dyspepsia and *H. pylori* eradication control.

Regarding the endoscopic reports analyzed, 59 presented antral gastritis, while 33 were normal. Upon analyzing the histopathological reports, 75 presented antral gastritis, while 17 were normal (TABLE 1).

TABLE 1. Relationship between endoscopy and histology for the diagnosis of antral gastritis.

Endoscopic and histological gastritis	49
Normal endoscopy and histology	7
Endoscopic gastritis with normal histology	10
Histological gastritis with normal endoscopy	26

Histological investigation of the presence of *H. pylori* was performed in 90 patients: 42 had *H. pylori* infection, while 48 did not present *H. pylori* (TABLE 2).

In this study, the kappa coefficient was applied to evaluate the relationship of antral gastritis diagnosis between the endoscopic and histological methods, with a value of 0.212 (confidence interval [0.08-0.34] and $P<0.05$).

TABLE 2. Relationship between *H. pylori* infection and the presence of endoscopic and histological gastritis.

	Histopathology		Endoscopy	
H. pylori	+	–	+	–
Present	42	0	26	16
Absent	31	17	32	16

DISCUSSION

Some studies have shown that there is a poor correlation between the endoscopic findings and the histological diagnosis of gastritis. The aim of this study was to evaluate the correlation between endoscopic and histological diagnosis of antral gastritis.

Data from 92 upper digestive endoscopies were examined in an original study addressing the issue of concordance between endoscopic and histological diagnoses of antral gastritis. The main finding found in this study showed that there was a low correlation between the endoscopic findings and the histological diagnosis, which was demonstrated by the kappa index of 0.212. Because agreement is generally considered substantial when associated with a kappa index greater than 0.6, the value of 0.212 reflects a poor correlation in this context[15-17].

Some studies have shown that there is a poor correlation between endoscopic findings and histological diagnosis of gastritis[5,6,18,19]. Kaur and Raj[20], in a study to assess the correlation between histological gastritis and endoscopic findings, showed that there was a poor correlation between them. They concluded that endoscopic findings are an unreliable predictor of histological gastritis. A study by Fung et al.[6] in dyspeptic patients showed that the endoscopic diagnosis was relatively imprecise in specific types of gastritis. They showed that among 33 dyspeptic patients diagnosed with endoscopic gastritis, histological confirmation was detected in 3/9, 10/14 and 0/6 cases of chronic atrophic gastritis, chronic (superficial) gastritis and acute gastritis, respectively. A study by Redéen et al.[21] in 488 adult individuals selected from a general population showed that, except for the absence of visible vessels and folds in the gastric body, endoscopic findings had very limited value in the evaluation of histological gastritis. Calabrese et al.[19],

in a prospective study evaluating the correlation of endoscopic findings with histological changes and *H. pylori* infection, showed that the correlation between endoscopic findings and histological diagnoses of gastritis was poor and concluded that biopsies were mandatory in all patients. A study by Jonsson et al.[22] in 210 dyspeptic patients showed that the endoscopic diagnosis correlated significantly with histological changes in the duodenal bulb, but not in the stomach. A study by Elta et al.[23] concluded that the histological and endoscopic findings in the stomach of patients with symptomatic erosive gastroduodenitis correlated poorly, while there was good correlation in the duodenum.

One limitation of our study was that different medical professionals performed the endoscopies and the histopathological exams in the referenced laboratory.

The main contribution of our study is that in clinical practice, the endoscopic diagnosis of antral gastritis should always be confirmed by histology. Biopsies should always be performed regardless of the endoscopic findings because they do not have such a high cost, are easy to perform with a low complication rate and are indispensable for diagnosis.

CONCLUSION

Our study showed that gastritis cannot be safely diagnosed by endoscopy, assuming histology to be the gold standard method. This conclusion is consistent with most studies in this field, and we agree with other authors who have concluded that histology is mandatory for accurate diagnosis. If the diagnosis of gastric inflammation is of clinical relevance, biopsies should always be performed, regardless of the endoscopic findings.

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Authors' contribution

Bertges LC: final approval of the article. Dibai F: data collection. Bezerra G: data collection. Oliveira ES: data collection. Aarestrup FM: statistical analysis. Bertges KR: review of the manuscript and study supervision.

Bertges LC, Dibai FN, Bezerra G, Oliveira ES, Aarestrup FM, Bertges KR. Comparação entre os achados endoscópicos e o diagnóstico histológico de gastrite antral. Arq Gastroenterol. 2018,55(3):212-5.

RESUMO – **Contexto** – Gastrite é uma afecção muito comum, de larga distribuição mundial, representando uma das entidades patológicas mais prevalentes em Gastroenterologia e Endoscopia Digestiva. **Objetivo** – Este estudo tem por objetivo analisar a correlação entre os achados endoscópicos e o diagnóstico histológico de gastrite antral. **Métodos** – Nesse estudo, foram analisados 92 laudos de endoscopia digestiva alta, realizados entre novembro de 2014 e janeiro de 2015, que continham biópsia de mucosa gástrica antral, comparando-se os achados endoscópicos e histológicos, que foram classificados segundo o Sistema Sidney. Os 92 exames analisados englobaram 35 homens e 57 mulheres, com idade variando entre 15 e 84 anos. A indicação mais frequente foi epigastralgia. **Resultados** – Dentre os 92 exames analisados, o diagnóstico histológico de gastrite antral apareceu em 75 exames, sendo que 59 laudos endoscópicos continham o diagnóstico de gastrite antral e 33 laudos endoscópicos foram normais. O coeficiente kappa foi 0,212 com $P<0,05$, mostrando que não há concordância significativa entre os achados endoscópicos e o diagnóstico histológico de gastrite antral. **Conclusão** – Concluímos que a histologia representa o método padrão-ouro para o diagnóstico de gastrite antral, e que na prática clínica diária, biópsias devem ser sempre realizadas, independente dos achados endoscópicos.

DESCRITORES – Gastrite, diagnóstico. Endoscopia. Mucosa gástrica. Biópsia.

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ORIGINAL ARTICLE

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Vitamin D deficiency among inflammatory bowel disease patients in Argentina: a cross-sectional study

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ABSTRACT – Background – An association has been established between low serum values of vitamin D and inflammatory bowel disease. There is a lack of evidence on whether this association is still observed in regions where sun exposure throughout the year is higher. **Objective** – To compare the prevalence of vitamin D deficiency between inflammatory bowel disease patients and healthy controls. **Methods** – Inflammatory bowel disease patients were consecutively enrolled as cases. Age and gender-matched healthy subjects who agreed to undertake a determination of serum vitamin D were enrolled as controls. Demographic features, medical treatment, need for hospital admission at diagnosis, steroid treatment, smoking, need for surgical treatment were evaluated as factors associated with vitamin D deficiency. **Results** – Overall, 59 patients with a diagnosis of either Crohn's disease or ulcerative colitis were enrolled, as well as 56 controls. Median age was 41 years (19-79) and 56% were male. Vitamin D deficiency was observed in 66.1% of inflammatory bowel disease patients versus 21.42% of healthy controls (OR 7.15 (3.1-16.48), *P*=0.001). Among inflammatory bowel disease patients, male gender, disease duration, moderate-to-severe disease and hospital admission at the moment of diagnosis were found to be associated with vitamin D deficiency. On multivariate analysis, only longer disease duration [(OR 1.01 (1-1.06)] and hospital admission at diagnosis [(OR 5.63 (1.01-31.61)] were found to be significantly associated with the latter. **Conclusion** – Vitamin D deficiency was more frequent among inflammatory bowel disease patients. Longer disease duration and need for hospital admission at diagnosis were associated to vitamin D deficiency among these patients.

HEADINGS – Ulcerative colitis. Crohn's disease. Vitamin D.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of immune-related disorders that may affect different anatomical sites throughout the digestive tract: as a matter of fact, two well-defined clinical entities constitute the vast majority of IBD cases: Crohn's disease (CD) which can affect virtually any site of the digestive tract and can also involve different layers of the gastrointestinal wall and ulcerative colitis (UC), which affects only the mucosa and is distributed solely throughout the colon[1].

The etiology of IBD is not well understood. It is believed that they derive from an aberrant chronic immune response towards an unknown luminal antigen, in genetically-predisposed subjects[2]. As a consequence, there is a myriad of both pro-inflammatory and anti-inflammatory biologic agents which are inappropriately secreted or inactivated among IBD subjects[3].

Vitamin D3 – or cholecalciferol – is a liposoluble vitamin which has been classically related to phosphocalcic metabolism[4]. However, in recent years a considerable amount of evidence has suggested that vitamin D3 has several important immunological functions: it can promote lymphocyte differentiation, can induce interleukin-10 production as well as decrease the production of pro-inflammatory cytokines such as Interferon γ[5].

Several studies of different methodological nature have suggested that vitamin D3 deficiency may have a significant role in terms of the magnitude of the inflammatory response observed among IBD patients. For instance, there seems to be a correlation between vitamin D3 values in serum and the severity of the inflammatory response among CD patients[6]. This correlation has also been observed among UC patients.

As it is known, vitamin D3 activity is related to solar exposure[7]. Most of the evidence regarding vitamin D3 and IBD comes from Northern Hemisphere countries, in which solar exposure and thus vitamin D deficiency can be a prevalent condition. Coincidentally, IBD incidence is slightly higher in many countries from the Northern Hemisphere than from the Southern Hemisphere.

There is also a lack of local evidence regarding the prevalence of vitamin D deficiency among IBD patients and possible risk factors that could be potentially associated with vitamin D deficiency. Hence, we sought to compare vitamin D3 serum values between a cohort of IBD patients versus healthy volunteers and to determine which clinical features among IBD patients were linked to a higher odds of low vitamin D values.

METHODS

Study design and population

A cross-sectional study was undertaken. The study protocol was properly reviewed and approved by our Institution's Internal Review Board. Adult patients with a diagnosis of either UC or

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CD were consecutively enrolled from June 2014 to January 2016. The vast majority of these patients were initially diagnosed and followed up on a regular basis at our Institution – only 5% (n=3) of these patients were referred for treatment from another Institution. Also, age and gender-matched asymptomatic individuals were enrolled for vitamin D determination; these were regarded as controls. We excluded patients with conditions that can be associated with vitamin D deficiency: cirrhosis, chronic kidney disease, hypoparathyroidism.

Outcome measures

After signing informed consent, both cases and controls were subject to a blood extraction to determine serum vitamin D3 levels. A cutoff value of 30 ng/mL was considered for the definition of vitamin D deficiency. Vitamin D3 levels were compared between cases and controls. The proportion of IBD patients with vitamin D deficiency was estimated. The following variables were registered and compared between IBD patients with and without vitamin D deficiency: age, gender, disease distribution, disease duration, need for hospital admission at diagnosis, smoking, need for biological therapy, steroid treatment, clinical severity at the moment of vitamin D determination. For the latter, either Crohn's Disease Activity Index (CDAI) or Mayo score were used: moderate-to-severe disease were defined as a CDAI >220 and Mayo score >6.

Statistical analysis

Stata software was used for this purpose (v11.1, Statacorp, College Station, Texas, USA). Categorical variables were described as percentages; numerical variables were described as mean with their standard deviation or – in cases of non-parametrical variables, as median with their range. For the comparison of categorical variables, Fisher exact test was used. In the case of numerical variables, either Student t test or Mann-Whitney test were used. Odds Ratios (OR) with their corresponding 95% Confidence Intervals (95%CI) were estimated. A univariate analysis was performed to determine the variables significantly associated with vitamin D deficiency among IBD patients, followed by a multivariate analysis including all variables with a p value of less than 0.1 on univariate analysis. Multivariate analysis was performed following a logistic regression model.

RESULTS

From June 2014 to January 2016, 59 patients with a diagnosis of either CD or UC were consecutively enrolled as cases, as well as 56 healthy controls. All patients undertook serum vitamin D determination. Clinical characteristics of IBD patients are shown in TABLE 1. Median age was 41 years (19-79) and 56% (n=33) were male. Overall, 76.27% (n=45) had a diagnosis of UC and 23.73% (n=14) of CD; 34% (n=20) were receiving immunomodulatory treatment with either azathioprine or 6-mercaptopurine, whereas 23.73% (n=14) were under concomitant treatment with biologics.

Vitamin D deficiency was observed in 66.1% (n=39) of IBD patients versus 21.42% (n=12) of healthy controls (OR 7.15 (3.1-16.48), p 0.001), as shown in FIGURE 1. Mean vitamin D values were 23.5±9.3 UI/mL and 38.5±7.6 UI/mL, respectively (P<0.05). FIGURE 2 shows the comparison of vitamin D deficiency between UC and CD patients, showing no significant differences in terms of vitamin D deficiency (P=0.2).

TABLE 1. Main characteristics of patients with inflammatory bowel disease.

	% (n/N)
Age	41 (19-79)
Gender (%M)	56 (33/59)
Diagnosis	
Ulcerative colitis	76.27 (45/59)
Crohn's disease	23.73 (14/59)
Age at diagnosis	31 (16-64)
Disease extension	
Crohn's disease	
Ileal	14.28 (2/14)
Ileocolonic	28.57 (4/14)
Colonic	57.15 (8/14)
Ulcerative colitis	
Rectal	15.55 (7/45)
Left-sided colitis	20 (9/45)
Extensive colitis	64.45 (29/45)
Smoking	13.56 (8/59)
Treatment with 5-ASA	
Crohn's disease	78.57 (11/14)
Ulcerative colitis	97.78 (44/45)
Need for steroid treatment	
Crohn's disease	85.71 (12/14)
Ulcerative colitis	66.67 (30/45)
Treatment with immunomodulator	
Crohn's disease	42.85 (6/14)
Ulcerative colitis	31.11 (14/45)
Treatment with biologics	
Crohn's disease	50 (7/14)
Ulcerative colitis	15.55 (7/45)
Need for admission at diagnosis	27.11 (16/59)
Need for surgical treatment	
Crohn's disease	21.42 (3/14)
Ulcerative colitis	6.66 (3/45)

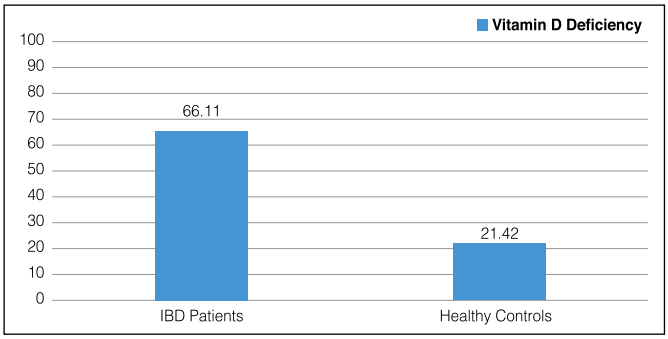

FIGURE 1. Comparison of vitamin D deficiency between inflammatory bowel disease patients and healthy controls.

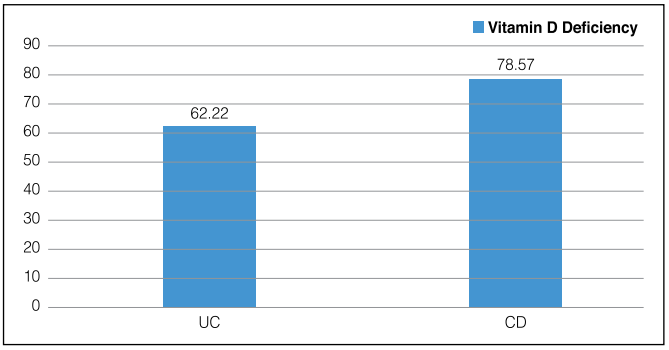

FIGURE 2. Comparison of vitamin D deficiency between ulcerative colitis and Crohn's disease patients.

When analyzing the features among IBD patients significantly related to vitamin D deficiency (TABLE 2), male gender (53.85% vs 25%, *P*=0.04), longer disease duration [12 (4-22) vs 8 (2-16) years, *P*=0.05], need for steroid treatment (79.49% vs 55%, *P*=0.05), moderate-to-severe disease activity at the moment of vitamin D serum determination (69.23% vs 35%, p 0.01) and hospital admission at the moment of diagnosis (35.91% vs 10%, *P*=0.03) were found to be associated. On multivariate analysis (TABLE 3), only longer disease duration [(OR 1.01 (1-1.06)] and hospital admission at diagnosis [(OR 5.63 (1.01-31.61)] were found to be independently associated with vitamin D deficiency.

TABLE 2. Comparison of Clinical Features between IBD patients with and without Vitamin D deficiency.

	Vitamin D deficieny (%, n/N)	Normal Vitamin D values (%, n/N)	OR (95%CI)	*P*
Age	42 (20-62)	45 (18-69)	N/A	0.27
Gender (%M)	53.85 (21/39)	25 (5/20)	3.5 (1.06-11.52)	0.04
Hospital admission on diagnosis	35.91 (14/39)	10 (2/20)	5 (1.2-27.02)	0.03
Need for steroid treatment	79.49 (31/39)	55 (11/20)	3.17 (1-10.81)	0.05
Disease duration	12 (4-22)	8 (2-16)	N/A	0.05
Need for biological treatment	28.21 (11/39)	15 (3/20)	2.22 (0.52-9.39)	0.25
Smoking	12.82 (5/39)	15 (3/20)	0.83 (0.17-3.96)	0.8
Moderate-to-severe disease activity*	69.23 (27/39)	35 (7/20)	4.17 (1.33-13.11)	0.01
Need for surgical treatment	12.82 (5/39)	10 (2/20)	1.32 (0.23-7.63)	0.7

*At the moment of vitamin D determination.

TABLE 3. Multivariate analysis results.

Variable	OR (CI 95%)
Gender	3.3 (0.56-19.58)
Hospital admission on diagnosis*	5.63 (1.01-31.61)
Need for steroid treatment	2.71 (0.68-10.83)
Disease duration*	1.01 (1-1.06)
Moderate-to-severe disease activity at the moment of vitamin D determination	2.37 (0.7-8.26)

*Significant values from a statistical point of view.

DISCUSSION

Our study confirms the increased prevalence of vitamin D deficiency among IBD patients as well as its association with certain features showing disease activity and severity. These findings are consistent with what other authors have suggested in previously published papers[8-10].

It is well known that vitamin D exerts a significant function regulating the phosphocalcic metabolism; its deficiency is a key component of osteopenia and osteoporosis – a common finding among IBD patients[11]. However, growing evidence point towards other key functions vitamin D has, such as anti-inflammatory, anti-proliferative as well as anti-apoptotic functions[12]. Vitamin D can modulate both adaptive and innate immune response by means of its influence on T and B lymphocytes as well as dendritic cell and macrophage function: these cells can express vitamin D receptors on its surface, which can bind cholecalciferol and 25 (OH)-cholecalciferol and turn them into 1,25 (OH)-cholecalciferol, an active metabolite with autocrine and paracrine actions. Vitamin D3 has been shown to suppress Th1 lymphocytic response, leading to a decrease in the excretion of pro-inflammatory cytokines such as interferon γ, interleukin-2 and tumor necrosis factor α[13].

Additionally, vitamin D inhibits dendritic cell differentiation[14]; furthermore, dendritic cells can induce the conversion of 25-OH cholecalciferol into 1,25 OH-cholecalciferol, which in turn helps promote monocyte differentiation as well as CD4+ inhibition. The evidence derived from experimental models have proved a crucial association between chronic inflammation and vitamin D. However, these models do not necessarily prove a causal association between vitamin D deficiency and IBD, nor it answers the therapeutic benefit of vitamin D administration among IBD patients.

Our results show a significant difference in terms of mean serum vitamin D concentration between IBD patients and otherwise healthy controls. This finding has been previously observed in several observational studies[8-10]. However, the vast majority of such studies were undertaken in geographical locations where both vitamin D deficiency and IBD incidence are relatively high; this could lead to a potential association bias, a bias that can be avoided with data from geographical places where sun exposure – and consequently, vitamin D levels – are higher. There is a relative lack of evidence from mild-temperature places such as South American countries.

We have found some relevant clinical features that IBD patients with vitamin D deficiency show. It is interesting that, on univariate analysis, key factors related to a more severe disease – in both CD as well as UC – such as need for steroid treatment during disease

evolution, disease duration, moderate-to-severe disease activity at the moment of vitamin D determination and need for hospital admission at the moment of diagnosis were significantly related to vitamin D deficiency. On multivariate analysis, the need for hospital admission at the moment of IBD diagnosis as well as the disease duration were independently associated with the odds of vitamin D deficiency. These findings could have two potentially feasible explanations. First of all, the probable relationship between the degree of vitamin D deficiency and IBD severity; for instance, among CD patients, those with need for surgical resection, or gastrointestinal stenosis, or need for steroid treatment at the moment of diagnosis were significantly related to a more profound vitamin D deficiency[15,16]. Additionally, those IBD patients on remission fail to show a similar prevalence of vitamin D deficiency than patients with active disease. These observations support the idea that the absence of vitamin D anti-inflammatory properties enhance IBD inflammatory activity and thus become a risk factor of a more severe disease course.

On the other hand, the association between vitamin D deficiency and disease severity may be due to the intermittent or continuous exposure to high doses of steroids that these patients may have. Most patients who require admission, especially at the time of diagnosis, may receive intravenous steroids, which are then switched to orally administered steroids; it could also be argued that those patients with longer duration of disease may experience a higher amount of relapses and thus, may receive a non-neglectable amount of steroids. As it has been demonstrated by Skversky et al.[17], chronic exposure to steroids constitutes an independent predictor of severe vitamin D deficiency. The question, hence, is whether vitamin D deficiency is a cause of a more severe disease or instead, vitamin D deficiency is a consequence of the inevitable exposure to medications that patients with a more severe evolution suffer – a question that observational studies like ours do not fully address.

Limitations should be mentioned. Mainly, sample size was relatively small, with a rather low proportion of patients with severe disease, as witnessed by the relatively low proportion of patients who required biological treatment and/or surgical interventions. This could underestimate the true weight that the latter may have as predictors of vitamin D deficiency. On the other hand, this is one of the few studies on the subject performed in a South American setting, apart from a recently published study by Kotze et al.[18].

In conclusion, we found a higher prevalence of vitamin D deficiency among IBD subjects when compared to healthy controls. Moreover, both hospital admission at the moment of diagnosis as well as longer disease duration were found to be significantly associated with the odds of showing vitamin D deficiency among IBD patients, regardless of their disease activity and severity.

Authors' contribution

Torella MC: patient enrollment, bibliographic search, draft design. Lasa J: design of the study, statistical analysis. Rausch A: patient enrollment, draft design. Zubiaurre I: bibliographic search, critical review of manuscript draft.

Torella MC, Rausch A, Lasa J, Zubiaurre I. Deficiência de vitamina D entre pacientes com doença inflamatória intestinal na Argentina: um estudo transversal. Arq Gastroenterol. 2018,55(3):216-20.

RESUMO – **Contexto** – Uma associação foi estabelecida entre os baixos valores séricos de vitamina D e doença inflamatória intestinal. Falta evidência se esta associação ainda é observada em regiões onde a exposição ao sol durante todo o ano é maior. **Objetivo** – Comparar a prevalência de deficiência de vitamina D entre pacientes com doença inflamatória intestinal e indivíduos controles saudáveis. **Métodos** – Pacientes com doença inflamatória intestinal foram consecutivamente selecionados. Indivíduos saudáveis combinados da mesma idade e gênero que concordaram em fornecer uma determinação da vitamina D do soro foram considerados como controles. Características demográficas, tratamento médico, necessidade de admissão hospitalar no diagnóstico, tratamento de esteroides, tabagismo, necessidade de tratamento cirúrgico foram avaliados como fatores associados à deficiência de vitamina D. **Resultados** – No geral, 59 pacientes com diagnóstico de doença de Crohn ou colite ulcerosa foram observados, bem como 56 controles. A idade mediana era de 41 anos (19-79) e 56% eram do sexo masculino. A deficiência de vitamina D foi observada em 66,1% dos pacientes com doença inflamatória intestinal versus 21,42% dos controles saudáveis (OR 7,15 (3.1-16.48), *P*=0,001). Entre os pacientes com doença inflamatória intestinal, sexo masculino, duração da doença, doença de moderada a severa e admissão hospitalar no momento do diagnóstico foram associados com a deficiência de vitamina D. Na análise multivariada, apenas a duração da doença [(OR 1; 1 (1-1,06)] e a admissão hospitalar no diagnóstico [(OR 5,63 (1,01-31,61)] foram encontradas significativamente associadas ao último. **Conclusão** – A deficiência de vitamina D foi mais frequente entre os pacientes com doença inflamatória intestinal. Maior duração da doença e necessidade de admissão hospitalar no diagnóstico foram associadas à deficiência de vitamina D entre esses pacientes.

DESCRITORES – Colite ulcerativa. Doença de Crohn. Vitamina D.

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ORIGINAL ARTICLE

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Usefulness of gastric submucosal dissection depth to evaluate skill acquirement in short term training courses in ESD: an experimental study

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ABSTRACT – Background – Endoscopic submucosal dissection (ESD) is a complex endoscopic procedure, with high rates of adverse events and technical difficulties. To overcome that problem, many training centers published the importance of animal models for skill acquirement in ESD. However, no study has used the submucosal dissection depth (DSUB) as a parameter to evaluate the learning curve in ESD, which might be a relevant factor since an optimal resection plane is important to achieve a curative resection and avoid intraoperative complications. **Objective** – This study aimed to assess ESD skill acquirement after short-term training sessions by evaluating the submucosal dissection depth (DSUB) and the association with adverse events. **Methods** – This experimental study included 25 experienced endoscopists in therapeuthic procedures (>5years) and 75 specimens resected by ESD (three resections / endoscopist). Learning parameters (resection time, size, en bloc resection rate, bleeding, perforation and submucosal dissection depth) were prospectively evaluated. The percentages of DSUB of all specimens resected were calculated. **Results** – All specimens were resected from the gastric body (n=75). The mean size of the resected specimens was 23.97±7.2 mm. The number of adverse events, including bleeding, perforation, and death, were 17 (22.67%), 3 (4%), and 0 cases, respectively. The average mean time by the third dissection decreased from 28.44±9.73 to 18.72±8.81 min (P<0.001). The proportion of DSUB in the bleeding and non-bleeding group were respectively 37.97%±21.13% and 68.66%±23.99%, indicating a significant association between DSUB and bleeding incidence (P<0.001). The ROC curve analysis indicated a cut-off point of 61% (sensitivity, 64%; specificity, 94%) of submucosal dissection depth associated with bleeding. Therefore, when ESD was performed at a depth of >61% of the submucosal layer, the risk for bleeding during the procedure decreased (PPV, 0.97; 95% CI, 0.85–0.99). **Conclusion** – Improvement in the learning curve in ESD and a better cognitive ability were seen by the third dissection in these short term training courses. And a significant association between DSUB and the risk of bleeding.

HEADINGS – Endoscopic mucosal resection, education. Gastric mucosa. Gastroscopy. Treatment outcome.

INTRODUCTION

Endoscopic submucosal dissection (ESD) has been gaining acceptance in several countries[1-3]; however, the difficulty level combined with adverse events, such as bleeding and perforation, limit the use of ESD by endoscopists. The risk of adverse events depends on the experience and expertise of the operator, patient comorbidities (e.g., hypertension, diabetes, bleeding disorders, anticoagulant use), and factors intrinsic to lesions such as the location, size, and degree of submucosal invasion[4].

In Japan, it is believed that beginners of ESD can achieve acceptable expertise levels after performing 20 to 30 resections[5-12]. This teaching method is difficult to replicate in western countries because of the low incidence of gastric cancer, which limits the learning curve in ESD.

Considering the importance of learning ESD, most western countries proposed ESD training in experimental animals before performing it in humans[5].

In several studies on ESD training that used *in vivo* and *ex vivo* animal models, the assessment of skill acquisition is based on the analysis of variables such as resection time, *en bloc* resection, complete lateral margin resection, bleeding and perforation[5-11].

The analysis of these parameters is essential but does not include the resection depth of the gastric submucosa. This is an important factor because endoscopic treatment of EGC is only acceptable when the lesion extends below 500 μm into the submucosa. [i.e., to the first submucosal (SM1) layer]. Therefore, the resection depth should reach at least the SM1 layer[12].

This concept should be considered to achieve an optimal resection plane and consequently, curative resection.

In addition, there may be an association between the submucosal dissection depth (DSUB) and the risk for bleeding[13,14]. This raises the concern about whether DSUB is an important factor to be analyzed during ESD training in live porcine models.

The primary objective is to evaluate the knowledge gain on short-term training courses in ESD, by using DSUB and other

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known parameters such as resection time, volume of submucosal injection, en bloc resection rate, specimen size, and adverse events (bleeding and perforation).

The secondary objective is to evaluate the association between DSUB and the risk of adverse events (bleeding and perforation).

METHODS

This experimental study was conducted between July 2011 and July 2013 at the Gastrointestinal Endoscopy Unit (Gastroenterology Department), Experimental Surgery Division and Department of Pathology (LIM05) of the University of São Paulo Medical School.

Selection of participants

A questionnaire was given to all physician participants to assess the years of medical practice; specialty; years of endoscopy experience; number of endoscopies, colonoscopies and endoscopic retrograde cholangiopancreatography (ERCPs) examinations performed/ year and previous experience of ESD. Voluntary informed consent was signed by all participants.

Inclusion criteria

- Medical professional with a specialization in endoscopy.
- Minimum experience of 5 years in endoscopy.
- Experience in advanced therapeutic endoscopic techniques.
- No previous experience in ESD.

Exclusion criteria

- Execution of ESD in different parts of the gastric body.
- Use of a technique different from the standard technique
- Failure to fill out the questionnaire
- Specimens that were missing, unlabeled or defective

Endoscopic submucosal dissection training technique in live porcine models

Gastric lesions were simulated in a live porcine model by aspirating the gastric mucosa using a distal attachment cap (Olympus D-201-10704), which was inserted into the tip of the endoscope. For a uniform analysis, all lesions were created at the gastric body.

The technique, standardized by Hosokawa et al.[15] begins with circumferential marking of the lesion (FIGURE 1A) using a needle knife (Olympus, KD-620LR) at a distance of approximately 2 to 3 mm outside the lesion. This circumferential marking was performed using electrocautery in coagulation mode (soft coagulation, 30 W).

A solution containing 20% mannitol and indigo carmine was injected into the submucosa using a 23-gauge sclerosis catheter (Olympus NM-200L-0423) to form a submucosal cushion and enable appropriate elevation of the lesion (FIGURE 1B).

Using a needle knife and electrocautery (Endocut, 40 W), a pre-incision was made approximately 1 to 2 mm outside the marked area. An IT-Knife (KD-611L) was inserted into the pre-incision so that the submucosal dissection outside the demarcated area would encircle the entire lesion (FIGURES 1C, 1D, 1E). Submucosal dissection was performed until the lesion was completely resected (FIGURES 1F,1G,1H).

The resected specimen was removed using a foreign body forceps (Olympus GF-47L-1), stretched and fixed with pins on a Styrofoam plate, and subsequently transferred to a container with 10% formalin solution. The specimens were labeled regarding the gastric region resected, operator name, and the number of the operating table. Specimens that were missing, unlabeled, or defective were not eligible for the study. Pathologists were blinded to any information regarding the resected specimens or the outcomes during ESD training.

In this study, intraprocedural bleeding is defined as any visible bleeding episode that develops during the submucosal dissection. In the event of intraprocedural bleeding, the methods of choice used for hemostasis were endoscopic clips (Olympus HX-610-090 and 135), electrocautery, or injection methods. The injection method of choice was adrenaline combined with saline solution of 1:10,000 or 1:20,000. Postprocedural bleeding was not assessed since the porcine models would be euthanized soon after the procedure. In cases of perforation, the treatment involved the use of metal clips (Olympus HX-135 and 610-090).

Laparotomy for gastric wall samples

After endoscopic resections were done, laparotomy was conducted to obtain a transmural sample of the gastric wall (greater curvature of the body) of each live pig used in the study as a reference (control group) to estimate the depth of submucosal resection by ESD.

Histological examination and measurement of the gastric layer

Histopathological examination of the resected specimens was performed using hematoxylin and eosin staining.

The thickness of each gastric layer was measured using stereological techniques. Stereology is a set of methods used to quantify morphological structures enabling the interpretation of solid structures using two-dimensional images (e.g., tissue sections, radiological images, and ultrasound images).

Stereology has some advantages over traditional measurement methods. Conventional methods measure two-dimensional images, such as the direct measurement of a histological section in micrometers, using a microscope. In contrast, stereological techniques enable the estimation of volume, density, area, number of cells, and other data in a three-dimensional format using two-dimensional information of the target object. Therefore, several statistical and geometrical analyses may be performed (e.g., sample size, uniform randomization of the sectioned areas, and isotropy) while maintaining statistical rigor. The advantage of stereological

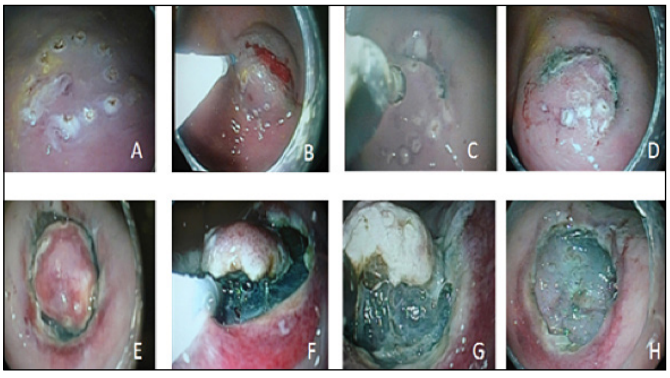

FIGURE 1. Schematic representation of the endoscopic submucosal dissection (ESD) technique. **A)** Marking of the lesion; **B)** Submucosal injection; **C, D, E)** Pre-cut and circunferencial incision; **F, G)** Submucosal dissection; **H)** Complete resection. [Source: Gastrointestinal Endoscopy Unit University of São Paulo Medical School (photos) and Hosokawa et al.[15]].

studies is the production of numerical (objective) results, with good reproducibility and rapid assessment, in addition to the low cost of the equipment used[16].

Nyengaard et al.[17] described the applicability of these methods in estimating the number of cells, total volume, and average length of stomach in rats using histological images of the gastric wall.

Stereological techniques are often used in experimental neuroscience studies, particularly those of brain areas that are inaccessible by other methods, for the accurate quantification of volume, area, and spatial distribution of neural cells[18], making this method practical and feasible for measuring the area of gastric layers.

In this study, the measurement of the gastric layer area was performed by evaluating the area percentage (Av_{comp}) of each compartment using a point counting method[19]. The tissue sections with the deepest dissection depth of each specimen were chosen for this analysis (ΣP_{comp}) and compared with gastric transmural samples (ΣP_{Tot}) obtained on the same area where ESD was performed. For this purpose, photomicrographs of tissue section were created, with a magnification of 4×, and a point test system was superimposed on the images. The incident points on each of the compartments of interest were differentially counted, and Av_{comp}[19,20] was calculated using the following formula[1]:

$Av_{comp} = \Sigma P_{comp}/\Sigma P_{Tot}$	(1)
Where, ΣP_{comp} is the sum of the number of incident points in one compartment. ΣP_{Tot} is the sum of the number of incident points in all compartments of the gastric wall.	

The software program Image J was used for all counts and measurements. Each counter type is shown in the left column and corresponds to one of the layers evaluated (FIGURE 2).

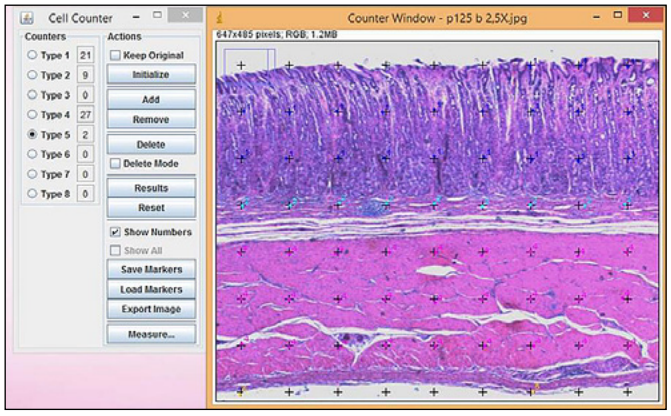

FIGURE 2. Representation of counting the incident points using Image J [Source: Experimental Air Pollution Laboratory, Department of Pathology (LIM 05) FMUSP].

Initial sample

Twenty five endoscopists were eligible for the study and they were divided into eight groups (3-4 participants/ group). Each group had two to three live porcine models available for a 1-day training session. Each endoscopist performed at least three resections. Seventeen live porcine models were used for the study. A total of 92 specimens (17 operated transmural sample and 75 resected by ESD) eligible for the study (FIGURE 3).

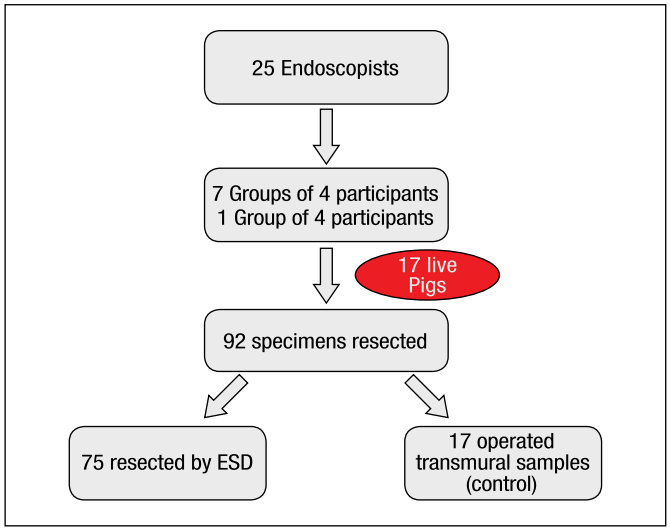

FIGURE 3. Schematic representation of number of participants, resected specimens and live porcine models.

Study flowchart

The study was divided into two phases, corresponding to different assessment periods. The objective of phase 1 was to evaluate training in dissections performed by each participant during ESD. Phase 2 included the histological analysis of the resected specimens (FIGURE 4).

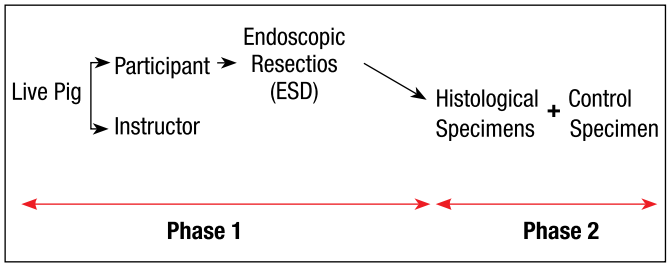

FIGURE 4. Schematic representation of the study phases.

ESD training evaluation (phase 1)

The day before the endoscopic dissections, all participants received a 4-hour lecture on the key points of ESD. The lecture was given by an experienced endoscopist (>300 cases of gastric ESD). Videos were also presented to demonstrate ESD cases previously performed by the lecturer.

The lecturer gave a practical demonstration of how to execute endoscopic dissection using a live porcine model. Subsequently, each subject performed a minimum of three endoscopic resections. All procedures were performed at the gastric body and under the supervision of experts.

Each trainee performed all resections on the same day. After all participants of each group finished the first resection, they would start the second resection, and so on. None of them were instructed to perform deeper resections.

The variables of interest collected after each resection are as follow: resection time (ΔTR: min) starting from the marking of the lesion to the end of submucosal dissection; volume of submucosal injection (mL); size of the resected specimen (mm); location of the lesion; and incidence of bleeding, perforation and death.

These variables were defined according to current medical literature as criteria of technical proficiency[5,6,11,20-27]. The variables were collected by an independent observer after each dissection: first (D1), second (D2), and third (D3). Thus, the outcome of phase 1 was to evaluate ESD training in a live porcine model using the aforementioned technical criteria.

Submucosal dissection depth (phase 2)

The thickness of the gastric wall varies among live pigs. Therefore, to enable the assessment of endoscopic resection depth, gastric transmural samples were collected (laparotomy) in the same area where endoscopic resections were performed. The submucosal compartments of these samples (control specimen) were used as a parameter to evaluate how deep the endoscopic resection has been taken i.e, submucosal dissection depth (FIGURE 5). After the resection, the specimen were fixed and forwarded to the Pathology Department with no further information about the endoscopic dissection outcomes on phase I and II or any other information about the resected specimens on D1, D2 and D3. The pathologists were instructed to analyse and measure the submucosal dissection depth only.

The primary outcome assessed in this phase was to evaluate the association between DSUB and variables collected in phase 1.

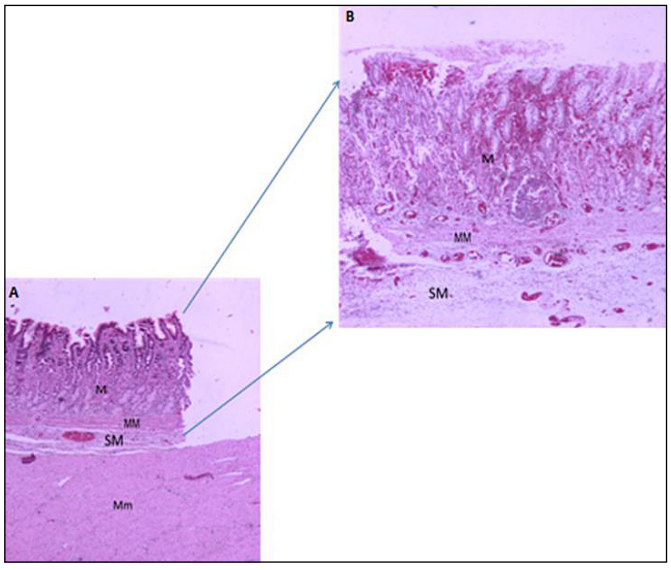

FIGURE 5. Photomicrographs of cross-sections of the gastric wall of pigs after resection. **A)** Transmural specimen resected by surgery (control specimen). **B)** Specimen endoscopically resected using ESD in the same pig. Identification of gastric compartments (layers). M: mucosa; MM: muscularis mucosa; SM: submucosa; Mm: muscle [Source: Experimental Air Pollution Laboratory, Department of Pathology (LIM 05) FMUSP].

Ethical considerations, anesthesia, and euthanasia

The study was approved by the Ethics Committee on Animal Use and the Research Ethics Committee of the University of Sao Paulo Medical School. Male and female domestic pigs of a hybrid breed (Landrace × Pietran × Duroc), with an average weight of 20±2 kg, were used in this study and previously subjected to a veterinary evaluation to rule out any obvious clinical infectious diseases. The procedure was performed under general anesthesia. During the procedure, temperature, oxygen saturation, and heart rate were monitored[28,29].

After the endoscopic procedure, the animals were euthanized by a method recommended by the Federation of Laboratory Animal Science Associations and Guidelines on Euthanasia[29,30].

Statistical analysis

Quantitative variables were presented as mean ± standard deviation; and all qualitative variables were presented as frequencies (%) with 95% confidence interval (CI) calculated. Median values were given for outliers.

Non-parametric analysis of variance for repeated measures was used to evaluate the effect of repeating dissections over time[31]. Measurements assessed at baseline (D1) and in the final dissection (D3) were compared using the non-parametric Dunnett test[32].

The association among the factors involved in complications was evaluated using the Mann–Whitney test for quantitative variables and Fisher's exact test for qualitative variables.

Finally, based on the submucosal dissection depth receiver operation characteristic (ROC) curve analysis was performed to determine the optimal depth necessary to reduce the risk of bleeding, calculating the area under the ROC curve (AUC). A cut-off point was estimated using the Youden- Index to maximize the sensitivity and specificity desirable to create the test (maximum= sensitivity + specificity-1), so that an "optimal cut-off" point with a maximal sensitivity and specificity could be determined on the ROC curve. Subsequently, 95% CIs were calculated for all diagnostic measures: AUC, sensitivity, specificity, and positive and negative predictive values.

P values of <0.05 were considered statistically significant. All statistical analyses were performed using the software program R version 3.1.2[33].

RESULTS

A total of 92 specimens (75 ESDs and 17 control specimens) were resected and included in the study. The mean size of the resected specimens was 23.97±7.2 mm.

Specimens were resected from the greater curvature and anterior wall of the gastric body. The resection mean time was 23.97±11.74 minutes.

In addition, 94.67% en bloc resections were performed, and specimen fragmentation occurred in four (5.33%) cases.

The adverse events were divided into two types: intraprocedural bleeding [17 (22.67%) cases] and perforation [3 (4%) cases]. There were no cases of death.

Assessment of ESD learning

• Trend analysis

The trend analysis of variables enabled the observation of the effect of repeating the dissections (from D1 to D3). Interestingly, deepening of the submucosal resection was seen during the study but resection time and bleeding rate were the most significant trend. (TABLE 1).

Variables of interest versus adverse events

• Bleeding

There were 17 cases (22.67%) of bleeding among the 75 resections. The association between the variables of interest and the presence or absence of bleeding was analyzed (TABLE 2).

TABLE 1. Values of the variables analyzed in D1, D2, and D3.

	D1 (n=25)	D2 (n=25)	D3 (n=25)	*P* value
Resection time (min)*	28.44 ± 9.73	24.76 ± 14.24	18.72 ± 8.81	<0.001
Injection volume (mL)*	25.24 ± 13.17	21.16 ± 13.36	23.72 ± 15.34	0.241
Size (mm)*	22.68 ± 6.89	22.6 ± 7.33	26.64 ± 6.9	0.17
Bleeding (%)**	8 (32%) (17.18–51.78)	5 (20%) (8.56–39.71)	4 (16%) (5.95–35.43)	0.047
Perforation (%)**	0 (0%)	0 (0%)	3 (12%) (3.49–30.99)	0.07
Fragmented specimens (%)**	1 (4%) (0–21.43)	1 (4%) (0–21.43)	2 (8%) (1.24–26.34)	0.77
Submucosal dissection depth (%)*	53.5 ± 23.76	61.8 ± 26.47	69.82 ± 27.86	0.073

*Values are expressed as mean ± standard deviation. **Values with 95% confidence interval.

TABLE 2. Association between the variables of interest and bleeding.

	Bleeding (n=17)	No Bleeding (n=58)	*P*
Resection time (min)*	25.71 ± 10.82	23.47 ± 12.04	0.339
Submucosal injection (mL)*	22.29 ± 13.28	23.69 ± 14.18	0.704
Fragmented specimens (%)	0	6.9	1.0
Em bloc resected specimens (%)	100	93.1	1.0

*Values are expressed as mean ± standard deviation (SD).

Factors, such as resection time (*P*=0.339), volume of submucosal injection to lift the lesion (*P*=0.704), and specimen fragmentation during resection (*P*=1) did not influence the risk of intraoperative bleeding (TABLE 2).

• Perforation

There were three (4%) cases of perforation among the 75 resections. These cases occurred in D3. In addition, there was no significant association among resection time (*P*=0.344), volume of the submucosal injection (*P*=0.223), specimen fragmentation (*P*=0.154), and perforation risk during resection (TABLE 3).

TABLE 3. Association between the variables of interest and perforation.

	Perforation (n=3)	Non perforation (n=72)	*P*
Resection time (min)*	17.33 ± 2.89	24.25 ± 11.9	0.344
Submucosal injection (mL)*	15 ± 2.65	23.72 ± 14.08	0.223
Fragmented specimens (%)	33.33	4.17	0.154
En bloc resected specimens (%)	66.67	95.83	0.154

*Values are expressed as mean ± standard deviation (SD).

• Submucosal dissection depth

Histological analysis of the submucosa enabled the measurement of D_{SUB} and the association between D_{SUB} and adverse events. No significant results in the comparative analysis between perforation and the depth of submucosal dissection were seen (*P*=0.324) due to few cases of perforation (n=3).

Other variables such as resection time and submucosal injection volume were compared with $D_{SUB,}$ showing a non-associable trend between these factors.

On the other hand, significant association between D_{SUB} and bleeding risk during the procedure was seen (*P*<0.001). The D_{SUB} mean value in the bleeding group was 37.97%±21.13 % and the non-bleeding group was 68.66%±23.99%.

Few outliers were seen, so calculating the median value was preferable where submucosal dissection depth were significantly different between the bleeding and non-bleeding groups, corresponding to 27.95% and 73.11%, respectively (FIGURE 6).

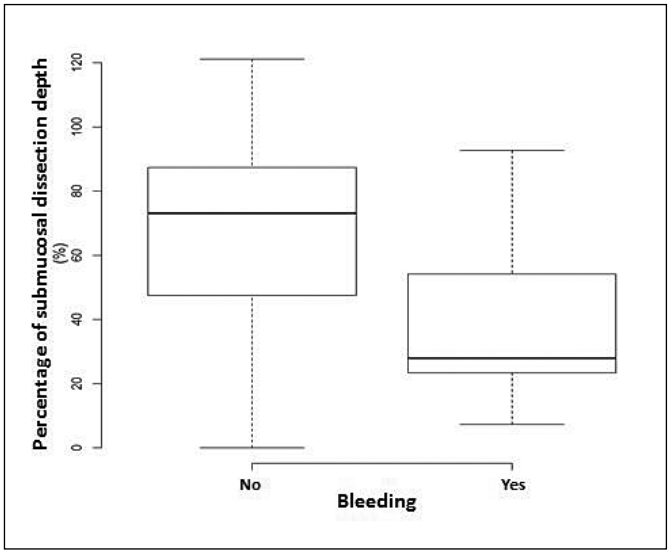

FIGURE 6. Sample distribution between bleeding and non-bleeding group.

• ROC curve analysis

Based on the submucosal dissection depth, ROC curve analysis was performed to determine the optimal depth necessary to reduce the risk of intraprocedural bleeding and the AUC was calculated. According to this analysis, the point with the largest AUC was defined as the point having the greatest association with bleeding. Optimal cutoff points were determined on the basis of maximum values of the Youden Index, which indicates the minimum distance from the upper left corner to the point of the ROC curve (FIGURE 7).

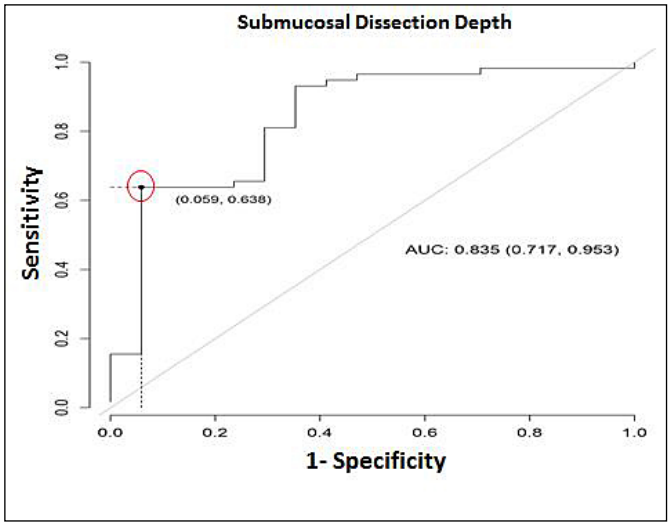

FIGURE 7. ROC curve analysis of bleeding development after endoscopic submucosal dissection. The resulting cutoff point of the submucosal dissection depth for bleeding was 61% (specificity, 94%; sensitivity, 64%). Sensitivity is shown in the Y-axis, and specificity is shown in the X- axis.

The results of estimating the cutoff point (TABLE 4) with strong association between D_{SUB} and bleeding was 61% (sensitivity, 64%; specificity, 94%). Therefore, when D_{SUB} was larger than 61% of the submucosal area, there was a strong probability of the absence of bleeding during the procedure (PPV, 0.97; 95% CI, 0.85–0.99). Consequently, the risk for bleeding was high when the submucosal dissection depth was less than 61% (NPV, 0.43; 95% CI, 0.30–0.97).

TABLE 4. Results in estimating the cutoff point based on the Youden--Index.

	Submuc. dissect. depth (DSUB)		
	ESTIMATE	95%CI	
CUTOFF	61.03	NA	NA
Se	0.64	0.5	0.76
Sp	0.94	0.71	1
PPV	0.97	0.85	0.99
NPV	0.43	0.3	0.97
DLR positive	10.84	1.6	73.33
DLR negative	0.38	0.27	0.55
FP	1	NA	NA
FN	21	NA	NA

Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; DLR positive: positive diagnostic likelihood ratio; DLR negative: negative diagnostic likelihood ratio; FP: false positive; FN: false negative.

DISCUSSION

ESD was developed in Japan in the 1990s[16]. This technique quickly revolutionized the treatment of early cancer by enabling the resection of lesions larger than 2 cm, with excellent oncological control.

However, a minimum learning curve is needed to achieve a safe resection and remain within the recommended oncological standards[6,23]. It is expected that most elaborate learning programs are from Japan, where the training algorithm is divided into two primary stages: theoretical preparation and practical training[23,25].

In Japan, practical training does not usually involve the use of simulators. The procedure is performed in humans, by endoscopists under direct supervision of an expert[24].

Unfortunately, the extensive experience of ESD in Japan cannot be replicated in western countries, which limits the opportunities for learning the technique. Simulators are valuable tools because they enable the development of the necessary cognitive ability and diminish the period to achieve an optimal learning curve[8,21,23,34].

In complex endoscopic procedures such as ESD, it is necessary to select a simulator capable of reproducing a reliable tactile sensation that is similar to a real procedure and providing an opportunity to treat potential complications such as perforation and bleeding[23].

In this scenario, live pigs better simulates the human stomach because of anatomical similarities, particularly with regard to the gastric wall layers, vascularization, gastric secretions, peristaltic movements, and the possibility of bleeding[35-38].

The main disadvantages of using live animals are the logistics for animal care, high cost, and ethical considerations[39].

At present, in countries with limited experience in ESD, simulators are the only option as a starting point for ESD training[5,40,41].

Therefore, different algorithms have been proposed for training, which seeks to integrate the accumulation of theoretical knowledge (diagnosis, indications, complications, and accessories), training under expert supervision, and visits to specialized centers in Japan[22].

In many reports the proficiency indicators for ESD training are resection time, en bloc resection rate, and safety (low incidence of adverse events)[5,13,21-24]. In this study we used a short-term training course in ESD so the number of procedures per student were fewer than other published papers[8,10], however it was possible to observe a progressive improvement in the resection time (P<0.001) and reduction in the bleeding rate (P=0.047), which do not differ from those in the literature[5-13,21-24].

In addition, histological analysis of the specimens indicated an increase in the submucosal dissection depth (P=0.073), demonstrating a trend toward deepening of the dissection plane.

What really differs from other studies is that all participants were experienced endoscopists who already had expertise in other therapeutic procedures which was a determining factor for a good performance, even after only three resections.

However, there were few cases of perforation (n=3), all of them on the third dissection which might lead to the assumption that participants were less cautious during ESD due to the self-confidence gained during the training.

Berr et al.[42] conducted a case series study in which they demonstrated that an endoscopist with previous experience on interventional procedures, after a theoretical learning period in Japan, succeeded in conducting ESD in his home country without the guidance of an expert, with a perforation rate of 14% and a bleeding rate of 4%[42], which are not unacceptably high, considering that

complication rates in Japan varies between, 3% to 7% for bleeding and 1% to 12% for perforation[43,44]. Thus, perhaps, the endoscopists experience in therapeutic procedures might have more relevance than the number of resections to achieve an ideal learning curve.

Moreover, resection depth has never been determined to be an important variable to be analyzed during ESD training in animal models. This factor is important because of two principles. a) The Japanese Gastric Cancer Association[27] reported that one of the criteria of curability is when the tumor does not penetrate the first submucosa layer or reaches a depth of up to 500 μm from the SM1, which means that when endoscopic resection is indicated, the endoscopist should go deep enough to include this small portion of the submucosa. b) The gastric vasculature responsible for bleeding during ESD is mostly from the ramified vascular network located in the superficial submucosal layer[45], which suggests that the best strategy to prevent intraprocedural bleeding is to maintain the appropriate dissection depth to reach the avascular stratum located just above the muscle layer where fewer but larger vessels arise, enabling the easy identification to perform cauterization[13].

Toyonaga et al.[45] observed that the ideal dissection plane was closest to the muscle layer because of the presence of fewer and larger caliber vessels, making their identification easier and reducing the risk for bleeding (FIGURE 8).

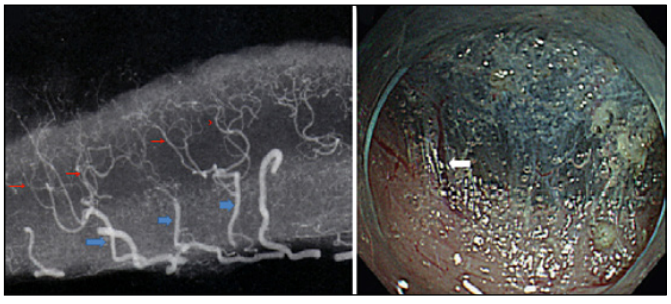

FIGURE 8. Vasculature of the gastric submucosal layer. **A)** Contrast photomicrograph of the submucosal vessels of the deepest submucosal layer (blue arrow) and its branches located in the superficial submucosal layer (red arrow). **B)** Image obtained during ESD, showing larger caliber vessel (white arrow) near the muscle layer [Source: Takashi Toyonaga, Department of Endoscopy, Kobe University Hospital].

However, there are no clinical studies to support this theory. Association studies between risk of intraprocedural bleeding and the depth of submucosal dissection would be difficult to conduct, primarily because of the clinical variability among patients, other risk factors (hypertension, coagulopathy, cirrhosis, diabetes, anticoagulant use), and factors intrinsic to the lesion (size, location, submucosal invasion, and recurrence)[4,46,47]. These confounding factors are eliminated using experimental animals.

The D_{SUB}s of the specimens resected by ESD were calculated using stereological methods based on point counting and estimated in percentage of the total gastric wall (transmural gastric sample).

As a result, we could observe that the number of cases of intraprocedural bleeding significantly decreased when endoscopic resections were performed at deeper levels ($P<0.001$).

This result corroborates the hypothesis held by some authors, concerning the importance of finding the ideal submucosal dissection plane for better visualization of vessels and consequently, reducing the risk for bleeding[13,45].

Based on the Youden-Index a cut-off point was estimated to reduce the risk of bleeding.

The resulting cut-off point was 61% (sensitivity, 64%; specificity, 94%), given that sensitivity was defined as the probability that the submucosal resection rate was above the cut-off point in cases in which bleeding did not occur and specificity was defined as the probability that the submucosal resection rate was below the cut-off point in cases in which bleeding occurred. The fact that bleeding could harm the patient, favors the use of a cut-off point with greater specificity to avoid bleeding (true negatives).

Therefore, when the submucosal resection rate was higher than 61%, there was a strong likelihood that bleeding would not occur during the procedure (PPV, 0.97; 95% CI, 0.85–0.99), and the risk for bleeding increased when resection was below 61% (NPV, 0.43; 95% CI, 0.30–0.97).

This statistical inference indicates that the evaluation of endoscopic resection depth in conjunction with other variables (resection time, en bloc resection rate, size and adverse events) might be important in the teaching process during ESD training in live porcine models. Other factors contribute to the bleeding risk in ESD, but we were able to show the importance of measuring the submucosal dissection depth during ESD training on animal models. We used the D_{SUB}, not as the only but one of the variables to achieve an ideal learning curve in ESD.

CONCLUSION

The short-term training model allowed cognitive improvement from D3, with shorter resection time, reduced bleeding rate and deeper submucosal resection rates.

There was a significant association between submucosal dissection depth and the bleeding incidence during ESD training.

Authors' contribution

Yamazaki K, Mestieri LH, Veras MM, Sakai P and Moura EGH: protocol/project development, data collection and management. All authors contributed significantly to the analysis, literature review and writing of the final submitted version, and share responsibility for the contents of this paper.

Yamazaki K, Moura EGH, Veras MM, Mestieri LH, Sakai P. A aplicação da profundidade de dissecção da submucosa gástrica na avaliação do aprendizado em ESD: um estudo experimental. Arq Gastroenterol. 2018,55(3):221-9.

RESUMO – **Contexto** – A técnica de ESD (Endoscopic Submucosal Dissection) é um procedimento endoscópico de grande complexidade, com alto índice de complicações e dificuldades técnicas. Para superar este problema, muitos centros de treinamento em endoscopia vêm publicando a aplicabilidade dos modelos animais para a aquisição de competência em ESD. Entretanto, a profundidade de ressecção nunca foi utilizada como parâmetro de aprendizagem, o que pode ser um fator relevante a ser ensinado, dado que atingir o plano de dissecção ideal é de suma importância para uma ressecção curativa e na prevenção de complicações intraoperatórias. **Objetivo** – Analisar o aprendizado em ESD em treinamentos de curta duração através da avaliação da profundidade de submucosa ressecada; e sua associação com complicações. **Métodos** – Estudo experimental; incluídos 25 endoscopistas com experiência em procedimentos terapêuticos (> 5anos) e 75 peças ressecadas por ESD sendo uma média de três resseções por endoscopista. Os parâmetros de aprendizagem (tempo de ressecção, tamanho, taxa de ressecção em bloco, sangramento, perfuração e análise histológica da camada submucosa) foram prospectivamente avaliados. A percentagem de profundidade de submucosa ressecada foi calculada. **Resultados** – Todas as ressecções foram realizadas no corpo gástrico (n=75). O tamanho médio das peças ressecadas foi de 23,97±7,2 mm. O número de complicações como sangramento, perfuração e morte foram respectivamente, 17 (22,67%), 3 (4%) e 0 casos. Na terceira dissecção, tempo médio do procedimento diminuiu de 28,44±9,73 para 18,72±8,81 minutos (P<0,001). O grupo que teve sangramento durante o procedimento ressecou 37,97%±21,13% da camada submucosa e o grupo sem sangramento ressecou 68,66%±23,99%, demonstrando uma associação significante entre a profundidade de dissecção submucosa e a incidência de sangramento (P<0,001). De acordo com a análise de curva ROC, o valor de corte da profundidade de submucosa ressecada para a ocorrência de sangramento é de 61% (64% sensibilidade, 94% especificidade), logo quando o ESD é realizado em uma profundidade maior do que 61% da camada submucosa o risco de sangramento durante o procedimento diminui (VPP=0,97; IC95%:0,85-0,99). **Conclusão** – O modelo de treinamento de curta duração possibilitou um aprendizado da técnica de ESD mostrando uma melhora cognitiva dos alunos já na terceira dissecção. Existe uma associação significativa entre a profundidade de ressecção da submucosa com o risco de sangramento.

DESCRITORES – Ressecção endoscópica de mucosa, educação. Mucosa gástrica. Gastroscopia. Resultado do tratamento.

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Pancreatic cancer in Brazil: mortality trends and projections until 2029

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ABSTRACT – **Background** – Pancreatic cancer is one of the main cancer-related causes of death in developed countries, and one of the most lethal malignant neoplasms. This type of cancer is classified as the ninth most frequent in the world. **Objective** – Analyze temporal trends for pancreatic cancer in Brazil in the period 2000-2014 and calculate mortality projections for the period 2015-2029. **Methods** – Ecological study, with temporal series, based on information provided by the Brazilian Mortality Information System. Analysis included deaths due to pancreatic malignant neoplasms in Brazil in the period 2000-2014, and analyzed according to sex, age group and Brazilian geographic regions. Projections were made until 2029 in five-year periods, calculated in Nordpred (within the R software). Mortality trends were analyzed by Joinpoint regression. **Results** – Between 2000 and 2014, there were 112,533 deaths due to pancreatic cancer in Brazil. Age-standardised rates was 5.1 deaths/100,000 men and 3.81 deaths/100,000 women. The highest rates were registered for the Midwest region, for both genders. Projections indicated that for the five-year period 2025-2029 there will be increased mortality rates for men in the Northeast and Midwest regions. Joinpoint analysis for Brazil did not reveal significant increases for women (APC=0.4%; 95% CI: -0.2; 1.0), however, there was a significant increasing mortality trend for men (APC= 3.7%; 95% CI: 0.6-7.0) in the period 2000-2004, followed by a stable period, an then another period of significant increases after 2010. These figures are mostly explained by variations in the Brazilian demographic structure. **Conclusion** – Pancreatic cancer mortality is unequally distributed across Brazilian regions and genders, and during the next two decades the differences will be accentuated.

HEADINGS – Pancreatic neoplasms. Mortality. Forecasting. Demography.

INTRODUCTION

Pancreatic cancer is one of the main cancer-related causes of death in developed countries, and one of the most lethal malignant neoplasms in the world[1]. Considering the year 2012, 337 thousand people were diagnosed with pancreatic cancer in the world, while 330 thousand people died because of this disease. This type of cancer is classified as the ninth most frequent in the world, despite its rarity. Due to its high mortality rates, pancreatic cancer is the seventh cancer-related cause of death in the world[2].

The symptoms of pancreatic cancer are initially insidious and gradually progress over time, including epigastric pain with or without posterior irradiation, weight loss, discomfort, nausea and fatigue. Jaundice is a characteristic sign of cancers in the head of the pancreas, due to the compression of the common bile duct. Sometimes a tumor can extend to the duodenum or stomach, leading to the obstruction of the gastric outlet[3].

The two main types of pancreatic cancer are adenocarcinoma (responsible for 85% of cases) and pancreatic endocrine tumors (which represent less than 5% of cases)[1]. Only 10%-10% of patients are diagnosed in initial stages, when surgical resection can be an option. More than 90% of individuals are diagnosed in advanced stages. Due to the bad prognosis and late treatment, the survival rates during the first year of diagnosis is very low (10%-20%) and decreases to 5% in the fifth year[4].

Currently there are no adequate, safe, effective and sensible monitoring strategies in terms of costs, to be implemented for the general population, even for those with significant risk factors, such as exposure to tobacco and advanced age[5]. Moreover, environmental and genetic factors contribute to the etiology of pancreatic cancer. Individuals with family history of pancreatic cancer have a higher risk of developing the disease and this risk increases with the number of first-degree relatives affected. Family history is related to germinal mutations in genes CDKN2A, BRCA2, PALB2, STK11 and PRSS1, which have been demonstrated to increase the risk of developing pancreatic cancer[6].

The consumption of tobacco is the most consistently risk factor established for pancreatic cancer, contributing to 25% of cases. Other suspected risk factors include the excessive consumption of alcohol, chronic pancreatitis, and dietary-endocrine factors[7]. Emerging molecular studies suggest that the carcinogenic effect of hyperglycemia, the mitogenic effect of hyperinsulinemia associated with obesity and chronic inflammation in diabetes can be considered as risk factors involved in the proliferation and metastasis of pancreatic cancer[8].

Pancreatic cancer occurs at all ages, but the incidence peak occurs between 60 and 80 years of age. Less than 10% of cases occur in individuals under the age of 55, and the average onset age is 71 years[3]. Pancreatic cancer rates are considerably higher in the Afro-American population than in any other racial group. Men present higher incidence rates than women[9].

As a disease with remarkable mortality, comprehension of the geographic distribution of pancreatic cancer and the behavior of rates throughout time is important, as the analysis of the epidemiological situation is necessary to support the planning of public

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health measures for vulnerable groups. To this date, few studies have examined the epidemiology of pancreatic cancer in South America, especially in Brazil. Therefore, the objective of the study presented herein is to analyze the temporal trends of pancreatic cancer in Brazil and its geographic regions in the period 2000-2014 and calculate mortality projections for the period 2015-2029.

METHODS

A temporal series, ecological study was carried out, based on secondary data registered by the Brazilian Mortality Information (SIM), made available by the Informatics Department of the Unified Health System (Brazil's publicly funded healthcare system). Analysis included deaths due to pancreatic malignant neoplasms (C25) categorized from the International Statistical Classification of Diseases and Related Health Problems – 10th revision (ICD-10) occurred in Brazil in the period 2000-2014, and analyzed according to sex, age group and Brazilian geographic regions.

Although within recent years it is recognized that SIM experimented a significant improvement in quality, the utilization of secondary data on mortality is subject to under-registry. For the correction of under-registered deaths by pancreatic cancer, information was obtained from the Redistribution of the Deaths per Chapters, corrected by Active Search Investigation, an initiative of the Ministry of Health, with data provided by the Informatics Department of the Unified Health System.

The correction factor was calculated for each age group, period, region and sex, from the percentage difference between the amount of deaths reported to SIM and redistributed deaths, based on Chapter II (Neoplasms) of ICD-10. The difference (D) was expressed in decimal values with 1 corresponding to a 100% change, with the possibility of obtaining values higher than 1 as some locations presented redistributed values above those reported to SIM (Equation 1). When the redistributed value was less than what was reported to SIM, a negative difference was obtained.

$$D = \frac{NR - NS}{NS}$$

In Equation 1, NR is the number of redistributed deaths by neoplasms, and NS is the number of neoplasm-related deaths reported to SIM.

The difference obtained (D) was added to the value 1 to establish a correction value (F, in agreement with Chapter II - neoplasms), as the number 1 represents a neutral factor in a multiplication, according to Equation 2:

$$F = 1 + D$$

Factor F was then multiplied by the number of deaths by suicide. It was assumed that the correction factor for Chapter II was applicable to pancreatic cancer, as shown by Equation 3:

$$OC = F \times NOS$$

In equation 3, OC is the corrected number of deaths due to pancreatic cancer, and NOS is the number of deaths due to pancreatic cancer reported to SIM.

With information on the readjusted number of deaths, standardized mortality rates were calculated, adjusted to the world population, per 100,000 inhabitants[10]. Population data by region, sex and age were obtained from demographic censuses and inter-census projections, available at the website of the Brazilian Institute of Geography and Statistics.

The temporal trends of pancreatic cancer mortality in Brazil and its geographic regions were analyzed, and projections were made until 2029 in five-year periods (2015-2019, 2020-2024 and 2025-2029).

Analysis of mortality trends utilized Joinpoint regression, with software Joinpoint Regression Program (National Cancer Institute, Bethesda, Maryland, USA), version 4.4.0. The objective of the analysis is to identify the occurrence of possible joinpoints, where significant changes in trends occurred.

The applied method identified joinpoints based on the model with up to three change points. The final model selected was the most adjusted method, with *Annual Percentage Change* (APC) based on the trend of each segment, estimating whether these values were statistically significant to a 0.05 level. The significance tests utilized were based on the Monte Carlo permutation method and on the calculation of the annual percentage variation of the ratio, utilizing the logarithm of the ratio.

In the description of trends, the terms "significant increase" or "significant decrease" mean that the slope of the trend is statistically significant ($P<0.05$).

Predictions were made for each period utilizing the age-period-cohort model of the Nordpred program (Cancer Registry of Norway, Oslo, Norway), within statistical program R. Data were compiled in five-year blocks. The results of the predictions are presented for the total of observed and expected deaths for each period in Brazil and its five geographic regions. For each period, adjusted mortality rates were calculated based on the age-standardised rates (ASR)[10].

The annual changes in the number of deaths were calculated for the last projected period (2025-2029) in comparison with the last observed period (2010-2014), where the proportion of this change could be due to risk of die due to pancreatic cancer or due to demographic changes (size or structure of population). These two components could be different from zero and present a positive or negative direction. Calculation follows Equation 4[11]:

$$\Delta_{tot} = \Delta_{risk} + \Delta_{pop} = (N_{fff} - N_{off}) + (N_{off} - N_{ooo})$$

Δtot is the total change, Δrisk is the change in function of risk, Δpop is the change in function of population, *Nooo* is the number of observed cases, *Nfff* is the number of projected cases and Noff is the number of expected cases when mortality rates increase during the observed period.

RESULTS

Between 2000 and 2014, there were 112,533 deaths due to pancreatic malignant neoplasms in Brazil, with 50.2% of deaths affecting male individuals and 49.8% affecting females. The standardized mortality rates to the world population for Brazilian men varied between 4.2 deaths/100,000 inhabitants in 2000, to 5.1 deaths/100,000 inhabitants in 2014. For women, rates varied between 3.6 deaths/100,000 inhabitants in 2000 to 3.81 deaths/100,000 inhabitants in 2014. The highest mortality rates were registered for the Midwest region, for both sexes. High mortality rates in men of the Southeast region should also be highlighted (FIGURE 1).

Joinpoint analysis for Brazil did not reveal significant increases for mortality in the female sex (APC=0.4%; 95% CI: -0.2; 1.0), however, there was a significant increasing mortality trend for men

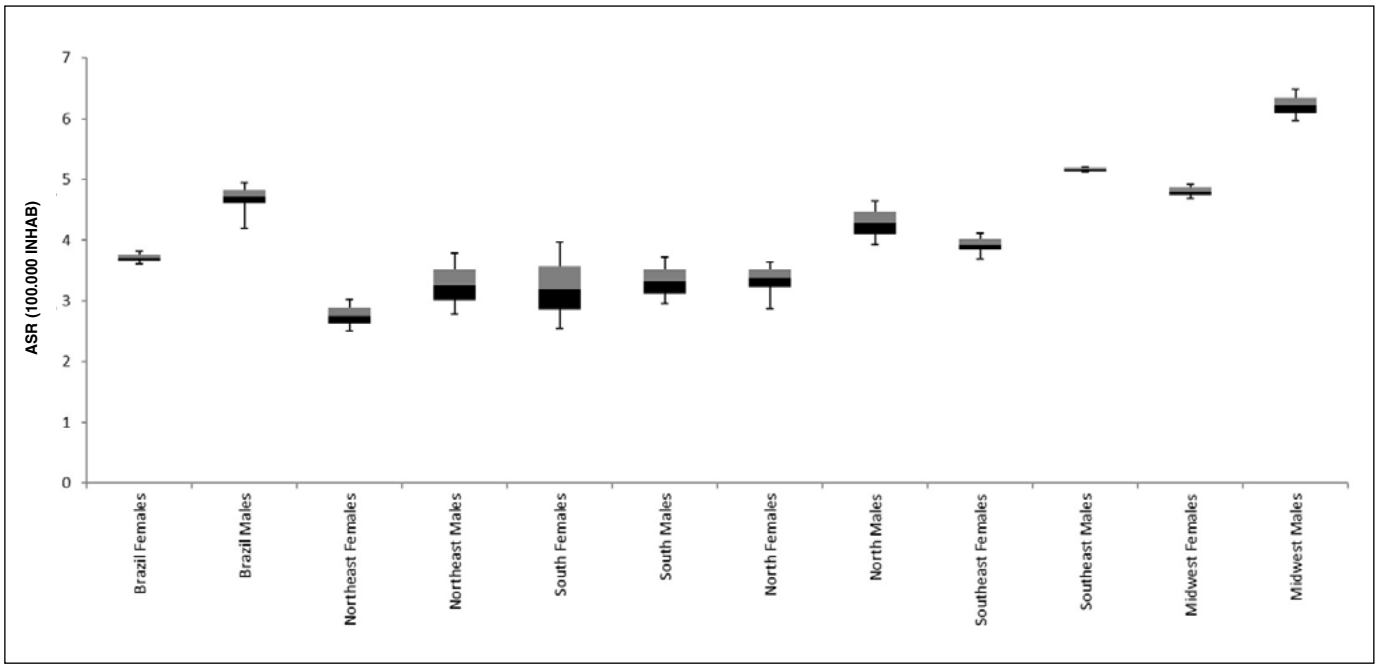

FIGURE 1. Standardized pancreatic cancer mortality rates for Brazil and its geographic regions, according to sex, for the period 2000–2014. Brazil, 2017.

(APC= 3.7%; 95% CI: 0.6-7.0) in the period 2000-2004, followed by a stable period, an then another period of significant increases after 2010. The Northeast region registered a significant increase in female (APC= 1.4%; 95% CI: 0.2-2.6) and male (APC= 2.3%; 95% CI: 1.4-3.3) mortalities. For the Midwest region, the female sex presented two joinpoints: a significant increasing period until 2004 (APC= 6.2%), followed by a stable period (APC= -2.5%), and then in 2010 a period of significant increase (APC=4.2%) occurred. The North and South regions did not present significant trends for either sexes (TABLE 1).

TABLE 2 and TABLE 3 presents the number of deaths and standardized mortality rates for the observed and projected periods, for women and men, respectively. Data analysis for Brazil for the five-year period 2025-2029 resulted in the expected occurrence of 38,551 and 41,952 deaths in females and males, respectively, due to pancreatic cancer. Mortality rates for females will not present considerable increases in the future; Brazilian, Midwest and North regional rates will present decreasing rates throughout the period. For the male sex, the same behavior will be observed, but the Northeast and Midwest rates will present increasing trends until 2029.

TABLE 1. Temporal trends for pancreatic cancer mortality in Brazil.

	Number of Deaths	APC1 (95% CI)	Joinpoint	APC2 (95% CI)	Joinpoint	APC3 (95% CI)
Females						
Brazil	56,066	0.4 (-0.2; 1.0)				
Northeast	10,894	1.4*(0.2-2.6)				
North	1960	-3.1 (-8.0-1.8)				
Midwest	2764	6.2* (0.8-12.0)	2004	-2.5 (-5.4-0.5)	2010	4.2* (0.5-8.1)
Southeast	28,307	3.0* (0.7-5.3)	2005	-4 (-12.0-5.3)	2008	1.1 (-0.3-2.5)
South	11,600	0.4 (-1.0-1.3)				
Males						
Brazil	56,517	3.7* (0.6-7.0)	2004	-1 (-2.9-0.9)	2010	2.7* (0.2-5.2)
Northeast	10,411	2.3*(1.4-3.3)				
North	2378	1.7(-1.0-3.9)				
Midwest	3295	1.3 (-0.3-2.9)				
Southeast	28,173	0.1 (-0.4-0.6)				
South	11,844	0.6 (-0.1-1.3)				

*Statistically significant P<0.05. APC: annual percentage change; CI: confidence intervals.

TABLE 2. Pancreatic cancer mortality in females, for Brazil and its geographic regions.

	Observed			Projected		
	2000-2004	2005-2009	2010-2014	2015-2019	2010-2024	2025-2029
Brazil						
Age (yr)						
0-54	2263	2895	3307	4018	4013	4265
55-74	6986	8361	10265	12909	15782	18386
≥ 75	5627	7165	9202	10356	12663	15900
ASR	2.58	3.06	2.72	2.36	2.09	1.89
Northeast						
Age (yr)						
0-54	494	591	728	925	1037	1097
55-74	1349	1565	1969	2384	2886	3419
≥ 75	1043	1351	1804	2021	2514	3083
ASR	2.62	2.75	2.90	3.02	3.08	3.10
North						
Age (yr)						
0-54	122	170	162	147	138	142
55-74	211	318	354	400	441	476
≥ 75	148	205	263	295	357	445
ASR	2.58	3.06	2.72	2.36	2.09	1.89
Midwest						
Age (yr)						
0-54	128	169	214	264	296	328
55-74	334	429	585	773	984	1203
≥ 75	215	289	403	498	682	928
ASR	3.31	3.32	3.43	3.43	3.45	3.45
Southeast						
Age (yr)						
0-54	1099	1386	1567	1753	1795	1895
55-74	3579	4152	5115	6519	8027	9336
≥ 75	2925	3775	4718	5335	6400	8021
ASR	3.93	3.94	3.95	3.95	3.98	4.01
South						
Age (yr)						
0-54	399	553	616	699	713	731
55-74	1411	1828	2173	1945	3374	3920
≥ 75	1170	1484	1957	2289	2853	3655
ASR	4.58	4.90	4.86	4.85	4.85	4.87

ASR: age-standardised rates.

TABLE 3. Pancreatic cancer mortality in males, for Brazil and its geographic regions.

	Observed			Projected		
	2000-2004	2005-2009	2010-2014	2015-2019	2010-2024	2025-2029
Brazil						
Age (yr)						
0-54	3301	3966	4429	7912	4626	5139
55-74	7595	9394	11739	11533	15112	21030
≥ 75	4152	5249	6692	7347	7541	15783
ASR	4.52	4.73	4.84	4.89	4.89	4.82
Northeast						
Age (yr)						
0-54	577	749	876	1027	1103	1210
55-74	1255	1662	2103	2596	3134	3581
≥ 75	858	1033	1297	1319	1663	2093
ASR	2.92	3.36	3.54	3.70	3.77	3.75
North						
Age (yr)						
0-54	184	224	229	260	264	284
55-74	291	367	502	656	811	918
≥ 75	149	179	254	257	345	479
ASR	3.19	3.26	3.45	3.52	3.50	3.39
Midwest						
Age (yr)						
0-54	206	257	296	359	377	402
55-74	427	511	750	1055	1397	1686
≥ 75	187	264	396	485	651	883
ASR	4.08	4.10	4.56	4.92	5.13	5.14
Southeast						
Age (yr)						
0-54	1707	1972	2138	2284	2266	2325
55-74	3883	4742	5767	7428	8937	10085
≥ 75	2048	2596	3322	3738	4691	6146
ASR	5.09	5.18	5.19	5.16	5.08	4.98
South						
Age (yr)						
0-54	600	751	872	997	1002	985
55-74	1659	2058	2567	3344	4113	4729
≥ 75	836	1127	1376	1609	2056	2682
ASR	5.99	6.29	6.36	6.43	6.48	6.51

ASR: age-standardised rates.

FIGURE 2 presents mortality rates due to pancreatic cancer, for the observed and projected periods, according to the influence of risks and population structure of Brazil and its regions. This calculation showed that there will be an increase in mortality rates, and these numbers are mainly explained by variations in the Brazilian demographic structure for all Brazilian geographic regions and for both sexes. For females, the reduction of risks must be highlighted along with the positive influence of demographic changes to explain the increase in the number of deaths in the future.

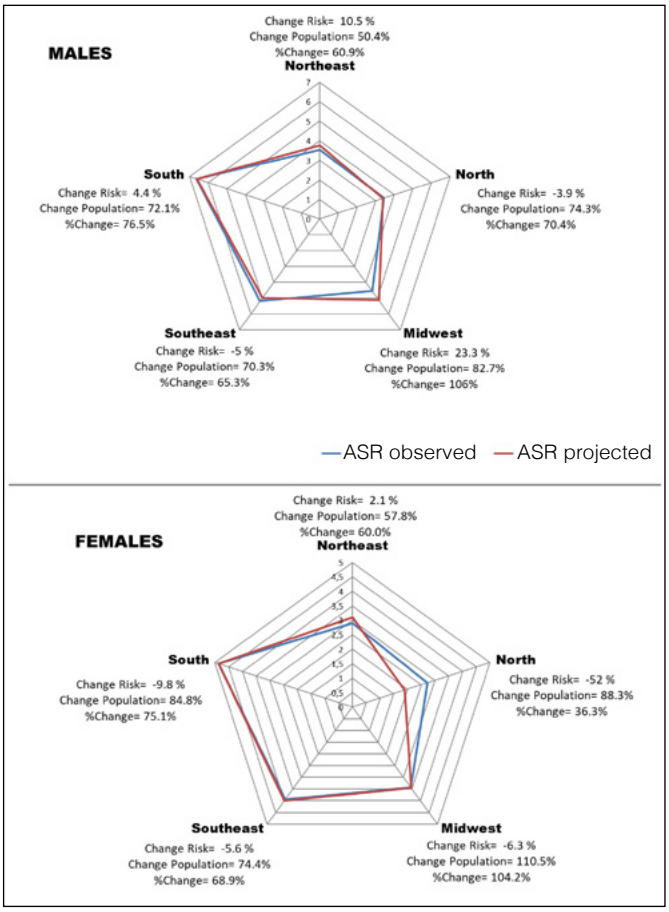

FIGURE 2. Age-standardised rates (ASR) total change (change), relative change due to risk (risk) and changed population (pop), between 2010-2014 (observed) and 2025-2029 (predicted) for pancreatic cancer mortality in Brazil.

DISCUSSION

Increasing trends were verified for pancreatic cancer mortality in Brazil throughout the period comprehended between 2000 and 2014, with important regional variations and higher rates for the male sex. When compared with the highest and lowest rates in the world, the Brazilian mortality rates for pancreatic cancer were intermediate: North America and Eastern Europe present 6.9 and 6.8 deaths/100,000 inhabitants, respectively, and South Africa and Central Asia present under 1.0 death/100,000 inhabitants[1,12].

Pancreatic cancer is more frequent in men. The ratio between sexes, in general, is 6:1 in developed countries and 1:1 in developing countries. Globally, approximately 173,000 deaths occur due to pancreatic cancer in men, with 156,000 deaths in women. In developed countries, mortality rates are 7-9/100,000 in men and 4,5-6/100,000 in women[2,13]. The results presented herein showed that Brazilian mortality rates follow the behavior observed in developed countries.

As verified herein for Brazil, in several parts of the world increasing trends were also observed for pancreatic cancer mortality. Serbia, one of the countries with the highest pancreatic cancer mortality rates in the world, presented increasing trends for both sexes in the period 1991-2010, with 1.6% annual growth (95% CI: 1.1-2.0) for men and 2.2% (95% CI: 1.7-2.7) for women[14]. In Korea, pancreatic cancer increased considerably between 1983 and 1994, with APC=9.82% in men and 12.57% in women[15]. In Canada, analysis of mortality trends between 1992 and 2005 revealed a 7% decrease in standardized mortality, per age group, in men (from 11.18/100,000 in 1992 to 10.38/100,000 in 2004) and a 3% increase in women (from 7.98/100,000 in 1992 to 8.23/100,000 in 2004)[16]. In Italy, increasing trends were observed for the female sex only (APC=0.7% from 1989 to 2010)[17].

Regarding pancreatic cancer, long term survival rates remain very low, despite improvements in recent decades. One-year survival for patients improved between 1981 and 2010, from 17.0% to 19.9%, and then to 28.2%, with a 42% increase when comparing the second and third decades. This indicates a general progress in the epidemiology of this cancer, either due to early diagnosis or improvements in treatment and care. However, five-year survival increased from 3.1% to 4.4%, and then to only 6.9% throughout the last thirty years; i.e., only few patients affected by pancreatic cancer survive for more than five years after resection[18].

The variation in incidence and mortality rates for pancreatic cancer around the world can be an indicator of differences in the prevalence of several risk factors, between sexes and between races, which in turn are a result of different levels of development across countries[9]. The hypotheses drawn to explain this include the fact that female steroid hormones could play a protective role. There are also several risk factors with established prevalence (e.g., consumption of tobacco, diabetes and heredity) and other still not elucidated (e.g., excessive ingestion of alcohol, high body mass index), which are under consideration to explain racial differences in pancreatic cancer incidence. However, further research is still required to determine the extension of genetic contribution and other factors towards the differences detected in pancreatic cancer incidence in men and women, and in different races[18].

In Brazil, the prevalence of the main risk factors related to pancreatic cancer were estimated by the National Health Survey, carried out in 2013, which revealed that the prevalence of diabetes and consumption of tobacco in the population were, respectively, 6.2% (19) and 15%[20]. The Telephone Survey Vigilance System for Risk Factors and Protection regarding chronic diseases (Vigitel) verified that the prevalence of obesity and overweight were, respectively, 15.8% and 48.5% in the Brazilian population[21]. For all these studied factors, it was also observed that the Brazilian population presents different exposure patterns, where prevalence vary across regions and according to sociodemographic characteristics, which could explain the pancreatic cancer mortality patterns observed herein.

It is also important to consider that international differences in incidence and mortality rates could reflect the capacity of diagnosis and changes in the utilization of several types of diagnosis techniques. In 2012, Europe represented a third of global incidence,

probably reflecting a more precise diagnosis of pancreatic cancer. It must be highlighted that a share of incidence and mortality differences across countries could also be attributed to the quality of registries, with variable coverage, integrity and precision[1].

Early detection of pancreatic cancer through radiographic images and high resolution endoscopic ultrasound has increased sensitivity regarding the detection of lesions, although conventional tomographies also provide detailed views of pancreatic tumors in relation to the superior mesenteric artery, celiac axis, superior mesenteric vein, and portal vein. This image technique is still the preferred choice for initial evaluation of the majority of patients with suspected pancreatic cancer[22]. Besides the currently utilized serologic markers, new markers, including those obtained from tumor blood or tissue, are being evaluated for early or more precise detection and prediction[23].

In general, screening programs involve multidisciplinary specialist teams and a combination of image techniques, usually based on endoscopic ultrasound. However, data have not generated a widely accepted screening protocol. Consensus practice recommendations, based mostly on the opinion of specialists, suggest a risk threshold increased 10 times to select the individuals that could benefit from screening, but this threshold excludes patients with significant risk (increases between 5 and 10 times). Results and cost efficiency analyses were also not currently possible, due to the lack of uniform screening practices. Although counseling and genetic tests were recommended as a part of the treatment of selected patients, there is little published information on the impact of genetic tests on these patients[5]. There are no current screening recommendations for pancreatic cancer, and therefore primary prevention of risk factors is paramount.

Although Brazil presents intermediate mortality rates, the structure and organization of health services could be predictors of the mortality rates observed in the country. In Latin America (e.g., Brazil, Mexico), large urban centers concentrate the majority of oncology services, as a consequence of extreme internal migration and development of these areas due to regional and local socioeconomic demands. The populations of rural areas must travel to large urban centers to receive treatment, consult with specialists and be assisted by diagnosis centers. These difficulties generate delays in diagnosis and treatment of patients, which results in worse prognosis in comparison with patients that already reside in urban centers[24].

This particular situation can explain the inequalities observed in the mortality rates across Brazilian geographic regions, where increasing trends were verified for the poorest regions of the country, such as the Northeast. In Brazil, the distribution of hierarchic levels for patient care is unequal, with high disparities: areas with better urban structures (Southeast and South regions), count with well-equipped and distributed health systems, while the North and Northeast regions suffer with the absence of intermediate hierarchic levels. Besides the traditional concentration of equipment and services, with scarce intermediate-level healthcare centers (as occurs in the Northeast), the North region is also affected by a scarce occupation of the territory, which is negatively reflected on the organization and distribution of the offer of health services[25,26]. The unequal allocation of resources, concentration of health professionals in urban centers, and the lack of investments in resources and infrastructure lead to the reproduction of socioeconomic inequalities in the assistance of individuals with cancer[24].

Our prediction model for Brazil estimated that, in 2025-2029, pancreatic cancer will be responsible for 38,551 deaths in women and 41,952 deaths in men. Besides Brazil, other countries have also demonstrated the impact of this type of cancer in future mortality. Projections carried out for Switzerland estimate that in 2025-2029, pancreatic cancer could be the main cause of deaths related to gastrointestinal cancers[27]. In Canada, the result is similar: it is expected that, in 2031, the number of cases of pancreatic cancer more than doubles, as a result of an exponential increase in the population over the age of 65[16]. Cancer mortality projections for the U.S.A. show that, in 2030, pancreatic cancer will be one of the three main cancer-related causes of death, for men and women, with a predicted occurrence of 63,000 deaths in 2030[28].

Any attempt to minimize the impact of pancreatic cancer mortality in Brazil must, in the future, also include adequate planning of services, besides the identification of reference centers, regionalization of treatment, reduction of distances travelled by patients and organization of treatment demands per region[29]. Although Brazil counts with an universal health system, the challenge for the next years will be the development of a system that is capable of responding to changes in health risks and in assistance necessities, due to the demographic changes that the country has been experiencing.

Addressing pancreatic cancer is a great challenge for the future. There is a clear necessity of investing in basic, translational and clinical research nowadays, as a preparation for the expected dramatic increase in mortality due to this type of cancer in the next 10-20 years. Changes in pancreatic cancer mortality rates require high investments in the comprehension and identification of early detection strategies and therapeutic targets that can be tested in clinical trials[29]. Also, new and more effective preventive measures oriented towards the main risk factors, such as obesity and tobacco consumption, could result in lower incidence and mortality risks (lower than those predicted).

One of the limitations of this study regards the national registry of deaths by cancer in Brazil. It must be mentioned that, in the past, there have been issues with data reliability, especially in the North and Northeast regions, but improvements have been implemented since the 2000's[30].

CONCLUSION

In conclusion, data analysis showed an unfavorable epidemiological situation related to pancreatic cancer in Brazil, considering regional differences in the spatial distribution of cancer and also the increasing temporal trends observed in the last 15 years (which will continue throughout the next decades). The results of the projections clearly showed the inequalities embedded in pancreatic cancer mortality per geographic region, which must be considered in the development of public policies and planning of health services.

Authors' contribution

Barbosa IR, Santos CA and Souza DLB contributed to study conception and design; Barbosa IR contributed to data acquisition, data analysis and interpretation, and writing of article; Santos CA and Souza DLB contributed to editing, reviewing and final approval of article.

Barbosa IR, Santos CA, Souza DLB. Câncer pancreático no Brasil: tendências e projeções da mortalidade até 2029. Arq Gastroenterol. 2018,55(3):230-6.

RESUMO – **Contexto** – O câncer de pâncreas é uma das principais causas de morte relacionadas ao câncer em países desenvolvidos, e uma das neoplasias malignas mais letais. Este tipo de câncer é classificado como o nono mais frequente do mundo. **Objetivo** – Analisar as tendências temporais do câncer de pâncreas no Brasil no período de 2000-2014, e calcular as projeções de mortalidade para o período de 2015-2029. **Métodos** – Estudo ecológico, de séries temporais, baseado em informações advindas do Sistema de Informações sobre Mortalidade brasileiro. A análise incluiu os óbitos por neoplasias malignas pancreáticas no Brasil, no período de 2000 a 2014, avaliados segundo sexo, faixa etária e regiões geográficas brasileiras. As projeções foram feitas até 2029, em períodos de cinco anos, calculados no Nordpred (no software R). E as tendências de mortalidade foram analisadas por regressão *Joinpoint*. **Resultados** – Entre 2000 e 2014, ocorreram 112.533 mortes por câncer de pâncreas no Brasil. A taxa padronizada foi de 5,1 mortes /100.000 homens e 3,81 mortes /100.000 mulheres. As maiores taxas foram registradas para a região Centro-Oeste, para os dois gêneros. As projeções indicaram que, para o quinquênio 2025-2029, haverá aumento nas taxas de mortalidade de homens nas regiões Nordeste e Centro-Oeste. A análise do joinpoint para o Brasil não revelou aumento significativo para as mulheres (APC=0,4%; IC95%: -0,2; 1,0), entretanto, houve um aumento significativo da tendência de mortalidade para homens (APC=3,7%; IC95%: 0,6-7,0) no período 2000-2004, seguido de um período de estabilidade e, em seguida, aumento significativo após 2010. Esses resultados são explicados principalmente por variações na estrutura demográfica brasileira. **Conclusão** – A mortalidade por câncer de pâncreas está distribuída de forma desigual nas regiões e gêneros brasileiros e, nas próximas duas décadas, as diferenças serão acentuadas.

DESCRITORES – Neoplasias pancreáticas. Mortalidade. Previsões. Demografia.

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ORIGINAL ARTICLE

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The changing trend of mortality caused by gastrointestinal cancers in Iran during the years 2006-2010

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ABSTRACT – **Background** – Cancers are one of the most important causes of death in the world. According to their high incidence and mortality, gastrointestinal cancers have particular importance among other cancers. **Objective** – Therefore, this study was conducted to investigate the mortality change trends of gastrointestinal cancers in Iran. **Methods** – This study was performed by analyzing the reported mortality data in 29 provinces of Iran in 2006-2010. Mortality trend of gastrointestinal cancers was drawn for both sexes in the study years and disaggregated by age groups and their frequency distribution. The WinPepi software was used for analysis. **Results** – In the years 2006-2010, the mortality rate of, gastric, colorectal, liver and pancreatic cancers, has significantly increased. Totally, gastrointestinal mortality is higher in men than women. Also, the results showed that by increasing age, death from these cancers also increased. **Conclusion** – The most important causes of death from gastrointestinal cancers were gastric, liver and colorectal cancers in Iran and because of their increasing trend in the country, performing preventive interventions for the cancers' risk factors is necessary.

HEADINGS – Gastrointestinal Neoplasms, mortality. Age factors. Sex factors. Middle East.

INTRODUCTION

Cancers are one of the most important causes of death in the world[1]. More than 70% of these deaths occur in developing and undeveloped countries[2]. Gastrointestinal cancers include gastric, esophagus, colorectal, small intestine, lips, mouth and pharynx, liver, biliary system and pancreas cancers[3], which are especially important due to their high incidence and mortality among other cancers[4,5]. Gastric cancer is the 2nd, liver cancer is the 3rd and esophagus cancer is the 6th cause of death among cancers in the world[6]. Cancer is the 3rd cause of death after cardiovascular disease and accidents in Iran[7]. The most important causes of death caused by gastrointestinal cancers in Iran, have been gastric, liver, esophagus and colorectal cancers[8].

There are different reports about each one of gastrointestinal cancers' mortality in the world. The number of deaths caused by gastric cancer in the western countries and even in the countries with high incidence of gastric cancers like Japan, Korea, Colombia, Ecuador and Russia has been decreased[9]. However, the mortality caused by liver cancer has had an increasing rate in Mexico and USA in 2000 to 2006[10]. The deaths from colorectal cancer have been decreased in both sexes in some countries[11]. Also during the years 1992 to 2002, an increasing trend of death caused by pancreatic cancer has been observed in Romania, Albania, Spain, Croatia and Korea[12], while the death trend was decreased in men in Ireland, England, Switzerland, Austria and Poland countries[13]. The trend of deaths from gastric cancer has been decreasing in the years 1970 to 2000 in some of the Latin American countries like Colombia, Venezuela, Ecuador, Costa Rica and increasing in some others like Mexico, Canada and the United States[14]. On the basis of WHO report in the years 2008 and 2012, generally, a decreasing trend of death from gastrointestinal cancers has been shown except for colorectal and pancreatic cancers which have had a small increasing trend all over the world[6,15]. Improvement of lifestyle like decreased salt intake, keeping food in the refrigerator and also decreased prevalence of *H. pylori*, is somewhat related to the decreasing trend of gastric cancer[16]. Reduction in death trend caused by colorectal cancer in some countries has been due to its screening and therapy developments[11]. Also, it has been observed that alcohol usage and hepatitis infection correlate with increasing deaths from liver cancer[17]. Changes in diagnostic methods[13] and smoking[18] are correlated with the changed trend of deaths from pancreatic cancer in different parts of the world.

For planning in the health care system, providing sufficient fund for necessary instruments, and training expert people, knowledge about mortality is necessary. In Iran, gastrointestinal cancers are the cause of repeated visits and re-hospitalization and lead to high costs imposed on the patient[8]. Until now, we have had no study determining the process of mortality caused by gastrointestinal cancers (separately in different parts of the gastrointestinal tract) in recent years in the country, so considering the fact that epidemiological information is the prerequisite of successful planning for decreasing death, the health centers need to know this information to find the right solution for cancer prevention. Therefore, this study was carried out aiming at studying mortality change trend caused by gastrointestinal cancer in Iran.

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METHODS

This cross-sectional study was performed by analyzing data of the published country report of mortality in 29 provinces in 2006-2010. The data were collected from information and technology management group center and applied research of the Ministry of Health and Medical Education from different sources including NOCR (Network for Oncology Communication and Research), cemeteries, hospitals and health centres. Their homogenization was done and for grouping them in 17 groups on the basis of mortality causes, we used ICD10 of World Health Organization[3]. In this study, the data of death caused by gastrointestinal cancers (ICD code: C15-C26) including gastric, esophagus, colorectal, small intestine, lips, mouth and pharynx, liver, pancreatic and biliary system were extracted from data for all causes of deaths.

The extracted information was checked on the basis of frequency, percentage of all deaths and also the number of deaths per one hundred thousand people divided by sex and age groups for existence of deaths from gastrointestinal cancers.

Statistical analysis

After checking the data and eliminating the missing data, we used Excel 2010 for analysis and WinPepi 2-1 for drawing figures and tables in order to determine the significance of the death trend change from gastrointestinal cancers during a 5-year period. Crude mortality rates for cancer diseases were calculated as the ratio of deaths due to cancers and the population of the provinces of Iran; standardized rates were calculated by the direct method as the ratio between total expected deaths in template of WinPepi analysis software.

RESULTS

Investigating the mortality caused by gastrointestinal cancer among per hundred thousand people during 2006-2010 in all ages and gender groups showed that the death from gastrointestinal cancer slightly increased from 23.86 in 2006 to 25.16 in 2010 (TABLE 1). Moreover, gastrointestinal cancer mortality rate was higher for male (FIGURE 1 and TABLE 1). The Gastrointestinal cancers mortality rate was changed from 29.72 in 2006 to 30.99 in 2010 in men and it was increased from 17.81 in 2006 to 19.18 in 2010 in women (TABLE 1), the trend of death from lips and mouth cancer did not have a significant increase between 2006 and 2010 (P=0.95). Moreover, Esophagus cancer mortality rate did not have a significant increase in this time (P=0.15) (TABLE 1 and 2).

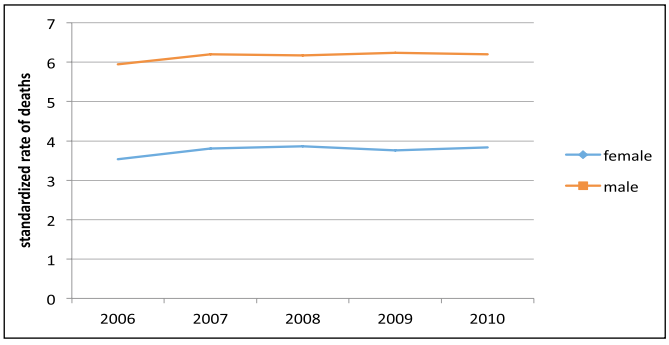

FIGURE 1. The trend of gastrointestinal cancers divided by gender in 29 provinces of Iran from 2006 to 2010.

TABLE 1. Standardized death per 100,000 people (all ages) due to gastrointestinal cancers during the 2006 to 2010 by sex, separately in different parts of the gastrointestinal tract (Statistical significance was attributed to P value <.05.)

Cause of death	ICD code	2006			2007			2008			2009			2010			P-value
		Female	Male	Total	Female	Male	Total	Female	Male	Total	Female	Male	Total	Female	Male	Total	
Gastrointestinal cancers	C15-C26	17.81	29.72	23.86	19.02	30.10	25.09	19.30	30.85	25.15	18.90	31.29	25.17	19.18	30.99	25.16	<0.001
Lips, mouth and pharynx cancer	C00-C14	0.03	0.26	0.283	0.28	0.30	0.29	0.22	0.27	0.24	0.29	0.35	0.32	0.27	0.26	0.26	0.95
Esophagus cancer	C15	2.56	3.76	3.17	2.67	3.84	3.26	2.80	3.48	3.14	2.57	3.60	3.09	2.58	3.61	3.10	0.15
Gastric cancer	C16	7.64	14.71	11.23	7.93	15.47	11.75	7.44	15.09	11.31	7.49	14.78	11.18	7.53	14.51	11.06	0.02
Small intestine cancer	C17	1.19	1.74	1.47	1.21	1.63	1.42	1.24	1.87	1.56	1.27	1.92	1.60	1.25	1.96	1.61	<0.001
Colon cancer	C18	1.40	2.01	1.71	1.75	2.35	2.06	1.91	2.51	2.21	1.74	2.58	2.16	1.79	2.49	2.15	<0.001
Rectal cancer	C19-21	0.21	0.25	0.23	0.20	0.32	0.26	0.25	0.36	0.31	0.25	0.36	0.31	0.30	0.50	0.40	<0.001
Liver and bile system cancer	C22-C24	3.81	5.73	4.78	4.22	5.85	5.04	4.41	5.84	5.14	4.09	6.06	5.09	4.61	6.11	5.37	<0.001
Pancreas cancer	C25	0.70	1.25	0.98	0.77	1.22	0.10	1.02	1.42	1.22	0.96	1.41	1.19	0.83	1.55	1.19	<0.001
Others Gastrointestinal cancers	C26	0	0	0	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	<0.001	<0.001	<0.001	0.023

TABLE 2. Standardized death of gastric cancers per 100,000 people per year studied separately by age groups and sex

Years	<5			5-14			15-49			50-69			>70		
	Female	Male	Total	Female	Male	Total	Female	Male	Total	Female	Male	Total	Female	Male	Total
2006	1.25	2.27	1.78	0.08	0.27	0.18	4.03	5.89	4.97	61.43	100.88	80.98	267.32	430.68	353.63
2007	1.54	2.17	1.87	0.11	0.19	0.15	4.02	6.39	5.22	64.39	99.39	81.64	295.71	470.19	388.52
2008	0	0	0	0.18	0.22	0.20	4.01	5.78	4.90	64.27	99.91	81.81	302.03	479.57	395.77
2009	1.68	1.43	1.55	0.11	0.17	0.14	3.80	6.03	4.93	60.66	101.98	80.99	302.03	484.63	397.69
2010	1.65	2.10	1.88	0.13	0.15	0.14	3.98	5.85	4.92	59.83	97.34	78.28	307.65	497.08	406.08
P-value	0.15	0.14	0.86	0.49	0.18	0.40	0.49	0.40	0.27	0.09	0.39	0.05	<0.001	<0.001	<0.001

However, other gastrointestinal cancers, including gastric, small intestine, colorectal, liver and pancreas, had a significant increase in this period (TABLE 1 and 2). The results showed that among gastrointestinal cancers, gastric cancer had the highest mortality rates (TABLE 2 and FIGURE 2). The lowest mortality of gastrointestinal cancers belongs to lips and mouth cancers (TABLE 2).

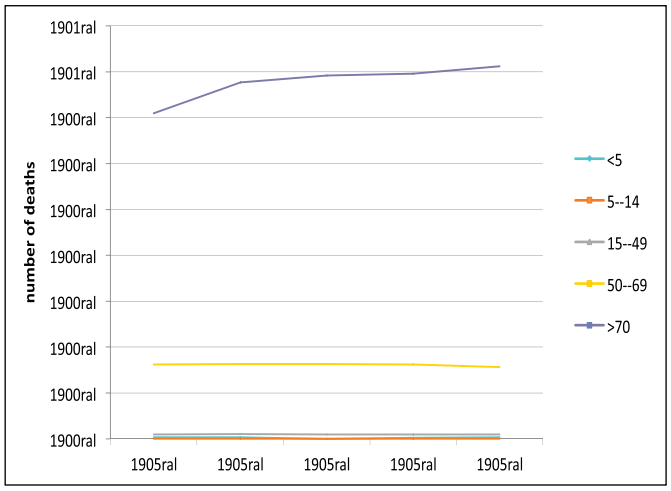

FIGURE 2. The trend of gastrointestinal cancers divided by age groups in 29 provinces of Iran from 2006 to 2010, Separated in different age groups.

The calculation of mortality from gastrointestinal cancers in all the study year in age and gender groups showed that deaths from these cancers increased with aging. The mortality due to gastrointestinal cancers in the age groups under 5 and 5-14 years is the lowest rate while this trend increased with aging, so that the highest mortality observed was in more than 70 years old age groups. Also, its trend has had a significant increase since the year 2006 from 353.63 to 406.08 (TABLE 2 and FIGURE 3).

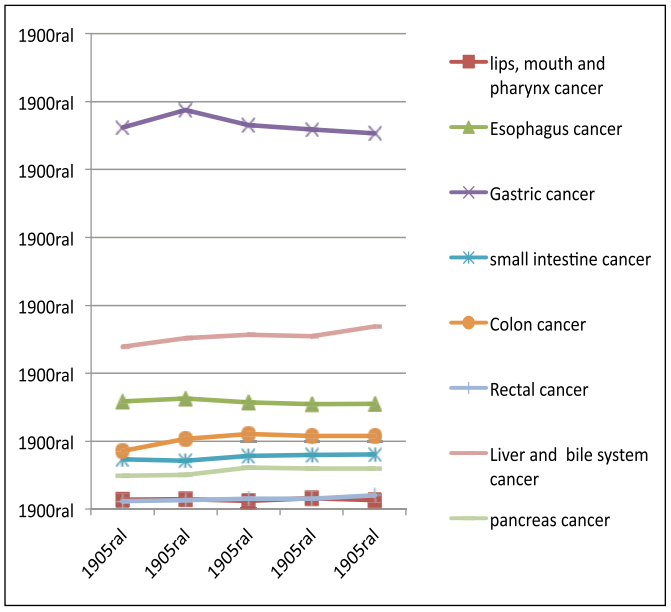

FIGURE 3. Comparison of gastrointestinal cancers incidence trend divided by age groups in 29 provinces of Iran from 2006 to 2010.

DISCUSSION

The results of this study revealed that the mortality trend of gastric, colorectal, liver and pancreatic cancers had been developing between 2006 and 2010 in Iran. Ganji et al.'s[8] Study in Tehran showed that gastric, hepatobiliary, liver cirrhosis, esophageal and colon cancers were the most important causes of death from gastrointestinal disorders between 2001 and 2005. Different studies show an increasing and decreasing trend of deaths caused by gastric cancer in the world. Bertuccio et al.[9] showed that the trend of deaths caused by gastric cancer has decreased from 1980 to 2005 in western countries, United States and even in high incidence gastric cancer countries like Japan, China, Korea, Colombia, Ecuador and Russia. The result of this decrease is correlated with better food preservation, declined smoking levels, controlling H-pylori infection, especially during childhood and adolescents[9] and improvement of diagnosis and treatment methods[19].

In Center et al.'s study in years 1998 to 2002, the decreasing trend of mortality due to colorectal cancer has been observed in many developed countries all around the world like the United States, Australia, New Zealand, Austria, France, Germany, Ireland and also some of Asian and Eastern European countries like Japan, Czech Republic, Slovakia, Latvia and South Africa by the side of the economy. This decline may be related to early diagnosis by screening[11], better treatment of colorectal cancer and more survival[20]. Unlike the decreasing trend of death from gastric and colorectal cancers mentioned in several studies, Pour Hosseingholi et al.[12] showed an increasing trend of gastric and colorectal cancers in Iran during the years 1995 to 2003. Access to colorectal cancers screening in Iran is very poor because of costs and the problems of the health care system[21]. This may be effective in the ascending trend of colorectal cancer mortality[12]. Some studies have shown that gastric cancer is the main cause of deaths from cancers among Latin American men, Asian countries and Iran[22,23]. The results of the present study also showed that among gastrointestinal cancers, the gastric cancer had the highest rate of mortality in Iran during the study years. Most of the gastric cancer sufferers in Iran are in the development stages of the disease and are non-curable[24-26] and their surveillance is rather low[27]. Lack of early diagnosis plans for diagnosing gastric cancer patients in the first stages of illness has been effective in increasing mortality trends[12]. In their study, they showed that the amount of mortality from liver cancer had been increased from 1999 to 2004 in Iran[28], which correlates with the results of the present study. The death trend from liver cancer has been heterogeneous in the European countries in 1970 to 1996. The increasing trend of deaths caused by this cancer in some of European countries, like Italy, is due to infection with hepatitis C and better diagnosis of liver cancer in cirrhosis patients[29]. The highest rate of liver cancer in East Asian countries is due to the high prevalence of hepatitis B virus[6,30]. 80% of patients with liver cancer in Iran have been infected with hepatitis B[31,32]. Also, Hajiani et al.'s[33] study in the south of Iran showed that the main cause of liver cancer is hepatitis B and C. Pancreatic cancer is the deadliest cancer among gastrointestinal cancers and only 4% of sufferers live more than 5 years[34]. Hariharan et al.[13] showed an increasing trend of mortality caused by pancreatic cancer in both sexes in Romania, Albania, Spain, Croatia and Korea and decreasing trend of death in Ireland, England, Switzerland, Austria and Poland during 1992 to 2002. In many studies, similar to our study, the correlation between smoking and mortality of pancreatic cancer has been observed[19-21].

As reported by WHO in 2012, death caused by gastric, colorectal, liver, pancreas and esophagus cancers has been higher in every hundred thousand people in men than women and also the results of this report showed that in Iran, the death due to gastrointestinal cancers was higher among Iranian men than women[15]. Also, studies have shown that the trend of mortality from colorectal, gastric, liver and pancreatic cancers is higher in men than women and the number of deaths also increases with ageing[12].

Among the limitations of this study, we can mention low calculation of mortality from cancers in Iran because of the weakness of mortality registration system[22]. Also, the results of this study were just related to age and gender data and did not check the mortality trend in different cities of Iran in order to determine the risk full areas by the side of deaths from gastrointestinal cancers. It is suggested that some studies determine the dispersion of deaths caused by these cancers in all parts of Iran in order to be able to design the needed plans in health and treatment field more efficiently. The epidemiological studies such as studying the time trend of disease outbreak and mortality and disability from them in different countries of the world cause the emergence of differences and similarities due to the comparison of their results. Consequently, these varieties are a probe for finding the results. For instance, by considering the increasing trend of deaths caused by gastric and colorectal cancers in Iran and comparing it with a decreasing trend of deaths from these cancers in developed countries, we should improve the existing diagnosis, treatment and preventive methods in Iran in comparison with different areas. Correction of lifestyle, educational methods for prevention and screening has definitely a significant effect on achieving the expected goal.

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Authors' contribution

Salehi F: idea of study. Ahmadi Soodejani S S: data collection. Ahmadi A: statistical analysis. Shahini Shams Abadi M: writing of text.

Salehi F, Ahmadi A, Ahmadi Soodejani S S, Shahini Shams Abadi M. A mudança da tendência de mortalidade causada por câncer gastrointestinal no Irã durante os anos 2006-2010. Arq Gastroenterol. 2018,55(3):237-41.

RESUMO – **Contexto** – O câncer é uma das causas mais importantes de morte no mundo. De acordo com a sua alta incidência e mortalidade, o câncer gastrointestinal tem particular importância entre outros tipos de neoplasias. **Objetivo** – Este estudo foi conduzido para investigar as tendências de mudança de mortalidade de câncer gastrointestinal no Irã. **Métodos** – Foi realizada pesquisa analisando-se os dados de mortalidade relatados em 29 províncias do Irã entre 2006-2010. A tendência de mortalidade de câncer gastrointestinal foi delineada para ambos os sexos nos anos de estudo e desagregadas por grupos etários em sua distribuição de frequência. O software WinPepi foi usado para análise. **Resultados** – Nos anos 2006-2010, a taxa de mortalidade por câncer gástrico, colorretal, de fígado e pancreático aumentou significativamente. A mortalidade por câncer gastrointestinal é maior nos homens do que nas mulheres e, além disso, os resultados mostraram que com o aumentar a idade, a morte causada por estes cânceres também aumentou. **Conclusão** – As causas mais importantes da morte entre cânceres de origem gastrointestinal no Irã foram o gástrico, o hepático e o colorretal e devido a sua tendência crescente no país, a realização de intervenções preventivas para os fatores de risco é necessária.

DESCRITORES – Neoplasias gastrointestinais, mortalidade. Fatores etários. Fatores sexuais. Oriente Médio.

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ORIGINAL ARTICLE

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Impact of small bowel capsule endoscopy in iron deficiency anemia: influence of patient's age on diagnostic yield

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ABSTRACT – **Background** – Iron deficiency anemia remains one of the main indications to perform small bowel capsule endoscopy. Literature suggests that diagnostic yield is influenced by patient's age but with conflicting results regarding age cutoff. **Objective** – We aimed to clarify the differences in diagnostic yield and incidence of specific findings according to age. **Methods** – Retrospective single-center study including 118 patients performing small bowel capsule endoscopy in the study of iron deficiency anemia. Videos were reviewed and small bowel findings that may account for anemia were reported. Incomplete examinations were excluded. Findings were compared between patients ≤60 and >60 years. **Results** – Patients had a mean age of 58 years old (SD ±17.9) with 69.5% females (n=82). The overall diagnostic yield was 49% (58/118), being higher among patients >60 years (36/60, diagnostic yield 60%) than those ≤60 years (20/58, diagnostic yield 34%), (P<0.01). Angioectasias were more frequent in patients >60 years (45% vs 9%, P<0.01). Patients ≤60 years presented more frequently significant inflammation (Lewis score >135 in 10.3% vs 1.7%, P<0.05) and other non-vascular lesions (24% vs 10%, P=0.04). **Conclusion** – In our cohort small bowel capsule endoscopy diagnosed clinically relevant findings in the setting of iron deficiency anemia in almost half the patients. Diagnostic yield was higher in patients older than 60 years (60%), with vascular lesions being more frequent in this age group. Despite the lower diagnostic yield in patients ≤60 years, significant pathology was also found in this age group, mainly of inflammatory type.

HEADINGS – Anemia. Capsule endoscopy. Small intestine.

INTRODUCTION

Iron deficiency anemia (IDA), the most frequent cause of anemia[1], has a prevalence of 2%-7% in the general population and is particularly frequent in the elderly, with a prevalence of 10-12[2]. Since chronic occult gastrointestinal bleeding is the main cause for IDA, upper and lower gastrointestinal endoscopies are the first line diagnostic procedures[3]. However, about 30% of patients will have normal bidirectional endoscopy[4], making small bowel endoscopy the next step in the diagnosis.

Capsule endoscopy has become an important aid in clinical practice, providing the diagnosis in many situations that were previously hard to identify. Occult gastrointestinal bleeding (OGIB) remains one of the main indications to perform small bowel capsule endoscopy (SBCE)[5]. In this setting, when compared with other small-bowel imaging techniques, SBCE has significantly higher diagnostic yield (DY) than small bowel follow-through, push enteroscopy, CT enteroclysis, CT angiography and MRI, and is comparable to double balloon enteroscopy[6-8]. In face of such evidence, current guidelines recommend capsule endoscopy as the first-line investigation of OGIB[9].

In the setting of IDA, SBCE has a pooled diagnostic yield of 66.6%[10] influencing subsequent clinical management in 72%-75% of patients with positive SBCE[11]. The most frequent findings are angioectasias but other vascular abnormalities, inflammatory lesions or small bowel tumors can also arise[10].

Current literature suggests that diagnostic yield is influenced by patients' age but with conflicting results regarding the age cutoff[11-14]. Also, current evidence suggests that diagnostic findings on SBCE performed in the setting of IDA are influenced by patients' age[11-13]. We aimed to assess the diagnostic yield of SBCE in patients with IDA after negative upper endoscopy and colonoscopy, and to clarify the differences in DY and incidence of specific findings according to patients' age.

METHODS

Retrospective study in a University affiliated Hospital Gastroenterology Department, highly experienced in SBCE. All videos of consecutive SBCE performed in the study of IDA between September 2012 and August 2015 were reviewed, and small-bowel findings that could account for IDA were considered relevant. Those findings included visible hemorrhage or hematic residues, angioectasias, erosions, ulcers, varices, polyp/tumor and significant villous atrophy in proximal small bowel. Patients with inflammatory lesions had their Lewis Score (LS) calculated and were categorized according to the defined and validated cut-offs in three groups – non-significant inflammation if LS<135, mild inflammation if LS 135-<790 and moderate to severe inflammation if LS ≥790[15].

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Anemia was defined as hemoglobin below 12g/dL for women and 13g/dL for men and iron deficiency was defined as ferritin <15ug/L for patients with negative inflammatory markers, and ferritin <50ug/L for those with elevated inflammatory markers[3].

All patients had undergone upper and lower gastrointestinal endoscopy before SBCE. SBCE was performed with PillCam® SB2 or SB3 (Medtronic, Minneapolis, MN, USA) using the standard protocol for our unit – clear liquid diet the day before the procedure and a 12h night-fast; 30 minutes before capsule ingestion patients were given 100 mg of Simethicone (Aero-OM®, OM Pharma) and 1h after ingestion they returned to our unit for real time visualization; at this point, if the capsule remained in the stomach, the patient was given Domperidone (Motilium®, Janssen). If after prokinetic administration the capsule remained in the stomach, it was passed into the duodenum by upper gastrointestinal endoscopy[16,17].

The complete video obtained in each SBCE was reviewed by two gastroenterologists with vast experience in capsule endoscopy using Rapid® Software, at 8-12 frames per second rate, using when needed FICE technology[18,19].

We collected data on age, gender, capsule type (PillCam® SB2 or SB3), quality of bowel preparation, gastric and small bowel transit time and presence of relevant findings in segments other than small bowel. Incomplete SBCE and repeat examinations for the same patient were excluded. SBCE findings were compared between patients ≤60 and >60 years.

Statistical analysis was performed using SPSS v.21.0 and a two-tailed *P* value <0.05 was defined as indicating statistical significance. Categorical variables were presented as frequencies and percentages, and compared with the use of Fisher's exact test or chi-square test, as appropriate. Continuous variables were presented as means and standard deviations and compared with the use of Student's *t*-test.

This study was performed in compliance with ethical standards and all patients signed an informed consent form and consensual contraindications for SBCE procedure were respected as have been described elsewhere[20].

RESULTS

From September 2012 to August 2015 a total of 357 SBCE were performed, 127 in the study of obscure OGIB. Out of these 127 patients, only 118 were considered for the purpose of this study – five patients had positive stool blood test without anemia and other four capsules were incomplete.

Patients had a mean age of 58±17.9 years old (minimum 20, maximum 86 years old) with 69.5% females (n=82). At the time of SBCE mean hemoglobin was 9.9±1.4 g/dL. SBCE was performed with PillCam® SB3 in 64 (54.2%) patients. Preparation quality was considered good in the vast majority of patients (66.9%). Information regarding patients' usual medications and comorbidities are depicted in TABLE 1.

The overall DY was 49.0% (58/118). No differences were found for DY between PillCam® SB2 and SB3 (50% vs 45.3%, *P*>0.05) nor between patients with good small bowel preparation when compared with moderate or bad preparations (41.8% vs 58%, *P*>0.05).

The most frequently reported findings were angioectasias in 27.1% of SBCE. The frequencies of endoscopic findings are depicted in FIGURE 1.

First, second and third tercile findings were reported in 51%, 25% and 30% of SBCE, respectively, and mean small-bowel transit time was 274±105 minutes. No differences were found between SB2 and SB3 for first (42.6% vs 57.8%, *P*>0.05), second (25.9% vs 23.4%, *P*>0.05) and third tercile findings (33.3% vs 26.6%, *P*>0.05) or relevant findings detection (50% vs 45.3%, *P*>0.05). SB3 had a significantly higher detection of villous atrophy (0% vs 9.3%, *P*=0.03) but both capsules had comparable performances regarding other findings detection.

TABLE 1. Patients usual medication and comorbidities.

Usual medication	Percentage (%)
Acetylsalicylic acid	23.7
Clopidogrel	0.8
Warfarin	12.7
Low wheight molecular heparine	0.0
Comorbidities	
Chronic renal failure	15.3
Hypertension	64.4
Dyslipidemia	56.8
Diabetes	23.7
Chronic liver disease	5.1

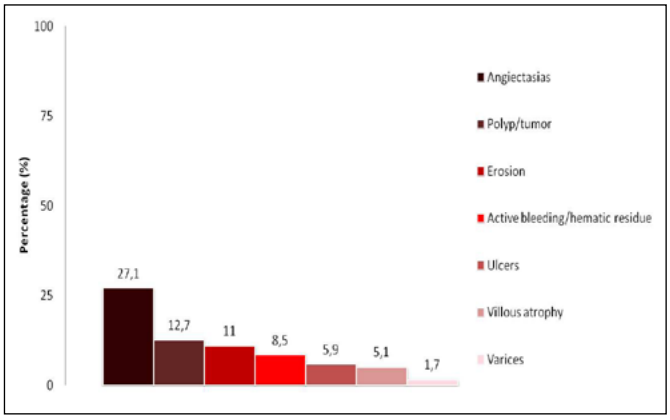

FIGURE 1. Frequency of relevant findings.

For patients with small-bowel inflammatory lesions, a LS>135 was reported in 5.9% of SBCE (n=6).

Relevant findings in segments other than the small bowel were reported in 20.3% of examinations, mostly in the stomach (17.8%) being erosions the most frequently reported finding in these segments (10.2%). Half of patients with extra-small bowel findings also had relevant small bowel findings.

DY was significantly higher for males (63.9% vs 40.2%, *P*<0.02). Also, patients with diagnostic SBCE were significantly older (62.4 vs 54.9 years, *P*=0.02) and had longer small bowel transit time (303.6 min vs 247.1 min, *P*<0.01). Even for PillCam®SB3, longer small bowel transit time is significantly associated with significant finding detection on SBCE (254 vs 312 min, *P*=0.026). DY was not influenced by antiplatelet/anticoagulant drugs or patients' co-morbidities (hypertension, dyslipidemia, diabetes mellitus, and chronic renal or hepatic disease).

The mean age of patients with angioectasias was 67±12.5 years, while the mean age of patients with non-diagnostic SBCE was 55±18.4 years. Patients with inflammatory lesions had a mean age of 45±15.7 years (TABLE 2).

TABLE 2. Mean age according to findings.

Finding	Patients mean age ±SD (years)
Angioectasia	67 ± 12.5
Polyp/tumor	60.9 ± 18.0
Erosion	56.1 ± 20.6
Active bleeding/hematic residue	60.6 ± 19.8
Ulcers	44.9 ± 16.0
Villous atrophy	66.3 ± 18.0
Varices	82.0 ± 5.7
No findings	55 ± 18.4
Lewis score >135	45 ± 15.7

Statistically significant differences between DY were found between patients ≤60 years (20/58, DY 34%) and those >60 years (36/60, DY 60%), (P<0.01). Angioectasias were more frequently found in patients >60 years (45% vs 9%, P<0.01). Also, dyslipidemia and hypertension were significantly more frequent in patients with angiectasia (51.2% vs 71.9%, P=0.04 and 58.1% vs 81.3%, P=0.02, respectively). Patients ≤60 years presented more frequently significant inflammation (Lewis score >135 in 10.3% vs 1.7%, P<0.05) and non-vascular lesions (erosions, ulcers, villous atrophy and polyp/tumor) (24% vs 10%, P=0.04) (FIGURE 2). No differences were found in the detection of visible hemorrhage/hematic residues, ulcers, erosions, varices, polyp/tumor and villous atrophy between the two groups.

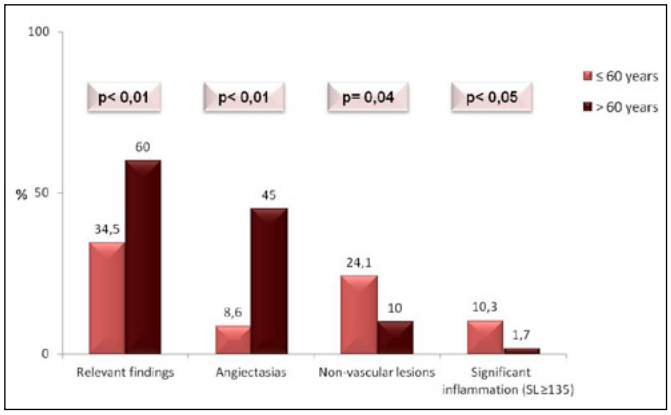

FIGURE 2. SBCE findings in IDA according to age.

DISCUSSION

SBCE is currently widely accepted and recommended as first-line investigation tool in OGIB. Our study confirms that SBCE has a significant role in the study of IDA providing valuable diagnostic information in nearly half of patients. Other investigators have reported DY ranging from 27% to 77%[7,21] and Koulaouzidis et al. reported, in a recent systematic review, a pooled diagnostic yield of 66.6%[10]. We were also able to confirm that SBCE has greater diagnostic performance in older patients, with 60% DY for patients over 60 years. The latter finding strengthens previous evidence that older patients have higher DY, however different authors have presented different age cut-offs and, until now, no clear age delimitation was consistently established[11-14]. The higher DY in patients over 60 years is usually attributed to their higher comorbidity and medication burden since previous studies reported that chronic liver disease, hematologic disease, chronic renal failure and anticoagulants intake influence diagnostic yield in older patients[11]. In our cohort such co-morbidities and therapies were not significantly associated with higher diagnostic yield which may indicate that other factors influence DY. Despite higher DY in older people, younger patients have significant pathology detected by SBCE in our cohort (34% DY) and other authors reported DY of 28%-50% in younger age groups[11,13,14].

In our cohort, we found significantly higher DY in male gender. Some authors have reported that SBCE has low DY in pre-menopausal women with iron-deficiency anemia (13.7%)[22]. Since nearly a third of the women in our sample have ≤45 years, and we did not consider pre-menopausal status as an exclusion criterion for our study, this may account for the differences found between genders. What is more, the inclusion of young women irrespective of their pre-menopausal status may contribute to the lower diagnostic yield reported in younger patients.

We also found that patients with longer small bowel transit time (SBTT) have higher DY. Faster small bowel transit can eventually translate into a higher risk of missed lesions, particularly in the first tercile and several authors reported higher diagnostic yield with longer SBTT, including for SBCE performed in OGIB study[23]. PillCam® SB3 aims to overcome this limitation, by providing adaptable frame rate technology. However, in our cohort, even for PillCam®SB3, longer small bowel transit time is significantly associated with significant finding detection on SBCE.

In our cohort the most frequent finding were angiectasias in 27.1% of all patients, particularly in patients >60 years, who had angiectasia in 45% of cases, and in patients with hypertension and dyslipidemia. The former finding is in concordance with the literature that consistently associates higher angioectasia detection with older patients[11-14,24]. The pathogenesis of GI angioectasias is not fully understood, but some authors advocate that angioectasias should be regarded as degenerative lesions of aging, caused by chronic intermittent low-grade obstruction of veins, capillaries, and arterioles that supply the mucosa[25]. Also, previous authors have described hypercholesterolemia and hypertension as predictors of angioectasias, comorbidities increasingly more frequent with age and that may contribute to the higher prevelance of this finding in older patients[24].

We found significant inflammation, assessed by a Lewis Score>135, more frequently in younger patients, with a mean age of 45±15.7 years. For the majority of these patients Crohn's disease (CD) diagnosis was established during follow up, which is in agreement with the previously described high diagnostic accuracy and sensitivity of LS>135 for CD diagnosis[26]. This finding is also consistent with the widespread knowledge that inflammatory enteritis are more prevalent in younger populations and stresses the need to consider this diagnosis in patients presenting solely with IDA.

Even though all patients in our cohort had undergone upper and lower gastrointestinal endoscopy before SBCE, relevant findings in segments other than small bowel were reported in 20.3% of examinations, mostly in the stomach (17.8%). Other authors have reported extra-small bowel findings in 7% to 23%[27,28] of SBCE performed in the study of OGIB. Despite significant extra-small bowel findings detection rates, both Gilbert et al.[29] and Selby et al.[30] proved that a second look endoscopy before SBCE has low diagnostic yield, is not cost-effective, and is not recommended in

current guidelines[9]. Furthermore, in our sample, half of patients with extra-small bowel findings also had relevant small bowel findings reinforcing the need to assess this gastrointestinal segment in patients with previous unremarkable upper and lower gastrointestinal endoscopy.

Limitations of our study include its retrospective nature, making our sample widely heterogeneous in terms of therapeutical approach.

CONCLUSION

In our cohort SBCE diagnosed relevant findings in the setting of IDA in almost half the patients. The DY was higher in patients older than 60 years (60%), with vascular lesions being more frequent in this age group. Despite the lower DY in patients ≤60 years, significant pathology is also found in this age group, mainly of inflammatory type.

Authors' contribution

Xavier S performed the literature search, collected and analyzed clinical data, designed the text structure and wrote the text. Magalhães J and Moreira MJ contributed to analysis and interpretation of data and made several critical corrections and revisions. Rosa B and Cotter J suggested the theme to be reviewed, and made the several critical corrections and revisions, including English editing, until the submitted version was achieved. All authors approved the final version of the article.

Xavier S, Magalhães J, Rosa B, Moreira MJ, Cotter J. Impacto da enteroscopia por cápsula na anemia ferropénica: influência da idade do doente no rendimento diagnóstico. Arq Gastroenterol. 2018,55(3):242-6.

RESUMO – **Contexto** – A anemia ferropénica constitui uma das principais indicações para realização de enteroscopia por cápsula. A literatura sugere que o rendimento diagnóstico é influenciado pela idade do doente, contudo, não é consensual o grupo etário para o qual o rendimento diagnóstico é maior. **Objetivo** – Clarificar as diferenças de rendimento diagnóstico e incidência de achados específicos de acordo com a idade. **Métodos** – Estudo retrospetivo unicêntrico. Incluídos 118 doentes que realizaram sistematicamente enteroscopia por cápsula no estudo de anemia ferropénica. Todos os vídeos foram revistos e foram reportados os achados no intestino delgado que pudessem ser a causa da anemia ferropénica. Excluídas enteroscopia por cápsula incompletas. Comparados os achados entre doentes com ≤60 e >60 anos. **Resultados** – Doentes com idade média de 58 anos (SD ±17,9), 69,5% do género feminino (n=82). O rendimento diagnóstico global foi de 49% (58/118), sendo superior em doentes >60 anos (36/60, rendimento diagnóstico 60%) do que em doentes ≤60 anos (20/58, 34%). As angiectasias foram mais frequentemente reportadas em doentes >60 anos (45% vs 9%, P<0,01). Nos doentes com ≤60 anos foi mais frequentemente reportada inflamação significativa (Score de Lewis >135 em 10,3% vs 1,7%, P<0,05) e lesões não vasculares (24% vs 10%, P=0,04). **Conclusão** – Na nossa amostra, a enteroscopia por cápsula revelou-se importante no estudo da anemia ferropénica detectando achados relevantes em cerca de metade dos doentes. O rendimento diagnóstico foi maior em doentes com mais de 60 anos (60%), sendo as lesões vasculares mais frequentes neste grupo. Apesar do menor rendimento diagnóstico em indivíduos até aos 60 anos, foi detectada patologia relevante neste grupo, em especial do tipo inflamatório.

DESCRITORES – Anemia. Endoscopia por cápsula. Intestino delgado.

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ORIGINAL ARTICLE

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Correlation between nonalcoholic fatty liver disease features and levels of adipokines and inflammatory cytokines among morbidly obese individuals

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ABSTRACT – **Background** – Nonalcoholic fatty liver disease (NAFLD) is the commonest hepatopathy worldwide. **Objective** – To investigate the correlations between NAFLD histopathological features and the levels of adipokines (adiponectin, leptin, and resistin) and circulating inflammatory markers (interleukin-6 [IL-6], interleukin-8 [IL-8], tumor necrosis factor alpha [TNF-α], and C-reactive protein [CRP]). **Methods** – This is an exploratory cross-sectional study, which enrolled 19 women with obesity who underwent bariatric surgery. Biochemical characteristics evaluated included the levels of adiponectin, leptin, resistin, IL-6, IL-8, TNF-α, and CRP. NAFLD was assessed through histological examination of liver biopsies carried out during the surgical procedures. **Results** – The mean age of the study group was 37.3±8.2 years old; mean BMI was 36.2±2.5 kg/m^2. Among individuals with liver fibrosis, the levels of IL-8 were significantly higher (24.4 ± 9.7 versus 12.7 ± 6.6; *P*=0.016726). The intensity of fibrosis presented a significant negative correlation with the levels of adiponectin (R= -0.49379; *P*=0.03166); i.e. the higher the levels of adiponectin, the lower the intensity of fibrosis. The intensity of steatohepatitis presented a significant negative correlation with the levels of adiponectin (R= -0.562321; *P*=0.01221); this means that the higher the levels of adiponectin, the lower the intensity of steatohepatitis. **Conclusion** – Adiponectin levels were inversely correlated with the severity of fibrosis and steatohepatitis, whereas IL-8 levels were higher in individuals with liver fibrosis among individuals with obesity and NAFLD undergoing bariatric surgery. The use of these markers to assess NAFLD may bring significant information within similar populations.

HEADINGS – Obesity. Fatty Liver. Adipokines. Cytokines. Interleukins.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the commonest liver disease around the world, with a worldwide prevalence of 25%, according to a recent systematic review conducted by Younossi et al. Its prevalence has presented a significant rise over recent years, as a direct consequence of the obesity and diabetes epidemics, turning into a source of public health concern[1,2]. Since it may present more aggressive phenotypes, which lead to severe forms as nonalcoholic steatohepatitis, cirrhosis, and even liver cancer, there are estimates that NAFLD will be the major indication for liver transplantation by 2030 in the United States (US)[3,4].

The pathophysiology of NAFLD is multifactorial and involves several interconnected mechanisms, such as insulin resistance, lipotoxicity, imbalance of inflammatory mediators, endotoxinemia, among others[5]. The role of the metabolically active compounds secreted by the adipose tissue and collectively known as adipokines, as well as of many circulating inflammatory mediators and markers, appears to be significant on the pathogenesis of NAFLD, albeit poorly understood to date[6-8].

Adiponectin, leptin, and resistin are the most studies adipokines. Adiponectin is a hormone released by the adipose tissue, with anti-inflammatory properties such as inhibiting the effects of pro-inflammatory cytokines, mainly tumor necrosis factor alpha and interleukin-6; its circulating levels are usually lower in individuals with obesity[9]. Leptin is involved in the regulation of the circadian cycle and satiety in the central nervous system. Its levels are usually higher in individuals with obesity, although its effects are suppressed in this situation due to a phenomenon called leptin resistance; weight loss often leads to a decrease in its levels associated with an increase of its effects[10]. Resistin is a pro-inflammatory adipokine whose main properties are inducing inflammation and insulin resistance, angiogenesis, and proliferation of smooth muscle cells[11].

There are several substances produced by immune cells with active metabolic and immunomodulating functions; they are collectively named cytokines and are usually regarded as mediators and markers of the inflammatory process. Interleukin-6 [IL-6] is a glycoprotein released by a number of cells, mainly monocytes, macrophages, lymphocytes, fibroblasts, keratinocytes, endothelial

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cells, and some tumor cells; it is a regulator of the differentiation of CD4+ T-cells and its levels markedly increase in acute inflammation and mildly to moderately in chronic inflammatory conditions[12]. Interleukin-8 (IL-8) is a pro-inflammatory cytokine released by macrophages and epithelial cells whose main function is promoting chemotaxis of neutrophils and T-cells[13]. The tumor necrosis factor alpha (TNF-α) is a cytokine produced by macrophages in response to endotoxinemia, inflammation and cancer; higher levels are usually associated with the acute inflammatory response, but are also linked to obesity[14]. The C-reactive protein (CRP) is an acute-phase protein secreted by the liver that increases following IL-6 secretion by macrophages and T-cells; its levels are also mildly elevated in chronic inflammation[15].

Since adipokines, cytokines, and inflammatory markers may present clinical relevance to be used targets for the research of novel therapies, diagnosis methods and assessment of severity of NAFLD, the evaluation of their correlations with the histological abnormalities of this disease presents a significant importance.

This study aims to investigate the correlations between NAFLD histopathological features and the levels of adipokines (adiponectin, leptin, and resistin) and circulating inflammatory markers (IL-6, IL-8, TNF-α, and CRP).

METHODS

This is an exploratory cross-sectional study, which enrolled 19 women with obesity who underwent bariatric surgery at a university hospital from January through December 2015. This study underwent evaluation and was approved by the institutional Ethics Review Board under the reference UNICAMP/289.425. Bariatric surgery was indicated according to the National Institutes of Health Consensus Statement criteria[16]. The surgical technique used in all of the individuals was the Roux-en-Y gastric bypass (RYGB). Surgery was indicated for individuals who presented obesity for at least five years, with at least two unsuccessful attempts to conservative treatment, with a body mass index (BMI) equal or above 40 kg/m^2, or equal or above 35 kg/m^2 associated with obesity-related comorbidities. The inclusion criteria were: women aged from 18 to 65 years old, which underwent RYGB. The exclusion criteria were: subjects who belonged to vulnerable groups (mentally ill, institutionalized or aged below 18 years old); recent or previous abuse of alcohol; antecedents of viral acute or chronic hepatitis; serologic abnormalities regarding hepatitis B or C virus; and previous bile duct obstruction.

All subjects who undergo bariatric surgery at this institution take part in a preoperative weight loss program which lasts 4 to 12 weeks and is comprehended by weekly consultations carried out by a multidisciplinary team. Individuals undergo surgery once a minimal 10% preoperative weight loss is achieved or since the minimal body mass index (BMI) of 35 kg/m^2 for subjects with obesity-related morbidities or 40 kg/m^2 for those free of comorbidities is reached[17]. No liquid or very low-calorie diet was prescribed in the immediate preoperative period to specifically reduce liver fat volume. All the lab examinations were collected on the day immediately prior to the surgical procedure.

Main characteristics regarding demographics and anthropometric parameters were assessed. Biochemical characteristics evaluated included the levels of adiponectin, leptin, resistin, IL-6, IL-8, TNF-A, and CRP, which were determined in the plasma by means of Western-blot analysis (SpectraMax i3, Molecular Devices, CA, EUA) at a 540-nm-wavelength. These laboratory assays were collected the day prior to surgery in a fasting state.

NAFLD was assessed through histological examination of liver biopsies carried out during the surgical procedures. All the histological examinations were performed by the same pathologist. Liver abnormalities were classified into three categories: 1) steatosis; 2) fibrosis; 3) steatohepatitis. Each category was divided accordingly as absent or present. The severity of each abnormality was stratified into four categories: absent (0), mild (1), moderate (2), or severe (3).

Statistical analysis

Data were expressed as means ± standard deviation. For comparison of proportions, chi-square and Fisher's exact tests were carried out. To compare continuous measures between independent or correlated groups, the Mann-Whitney test was used. Spearman's correlation coefficients (values of R) were calculated to assess the association between variables and the outcomes analyzed. The values of R vary from -1 to 1; values next to the extremities signal negative or positive correlations, respectively. The significance level adopted was 5% (P-value <0.05). For the execution of analysis, it was used Statistic Analysis System (SAS) software for Windows version 9.2.

RESULTS

The mean age of the study group was 37.3±8.2 years old; mean BMI was 36.2±2.5 kg/m^2. All of the studied individuals presented mild steatosis at the liver biopsy examination. In relation to fibrosis, 15.8% presented no fibrosis, 63.1% mild fibrosis, and 21.1% moderate fibrosis. Regarding liver inflammatorty activity, 36.8% presented no steatohepatitis, 52.6% mild steatohepatitis, and 10.6% moderate steatohepatitis. The main demographic, anthropometric and the distribution of NAFLD features are presented in TABLE 1.

TABLE 1. Characteristics of the study group.

Age (years)	37.3 ± 8.2
Gender	
Female	19 (100%)
Weight (kg)	94.9 ± 9.2
BMI (kg/m^2)	36.2 ± 2.5
NAFLD features	
Steatosis	
mild	100%
Fibrosis	
absent	15.8%
mild	63.1%
moderate	21.1%
Steatohepatitis	
absent	36.8%
mild	52.6%
moderate	10.6%

NAFDL: nonalcoholic fatty liver disease; BMI: body mass index.

Among individuals with liver fibrosis, the levels of IL-8 were significantly higher (24.4±9.7 versus 12.7±6.6; *P*=0.016726); all other variables evaluated did not differ between the individuals with or without fibrosis. TABLE 2 shows the complete comparisons. None of the variables evaluated differed between the individuals with or without steatohepatitis. TABLE 3 details the complete comparisons.

TABLE 2. Comparison between individuals with and without liver fibrosis.

	Non-fibrosis	Fibrosis	Value of *P*
Age (years)	35 ± 5.3	37.8 ± 8.7	0.610032
BMI (kg/m^2)	35.8 ± 1.4	36.3 ± 2.7	0.767814
Adiponectin	207.3 ± 21.5	186.4 ± 58.3	0.556420
IL-6 (pg/dL)	39.7 ± 24	28.5 ± 17.1	0.336081
IL-8 (pg/dL)	12.7 ± 6.6	24.4 ± 9.7	0.016726
Resistin (pg/dL)	46.6 ± 76	51.7 ± 33.3	0.843854
Leptin (pg/dL)	102.8 ± 31.4	101.1 ± 29.5	0.92151
TNF-α (pg/dL)	315.4 ± 427.4	105.3 ± 229.9	0.217523
CRP (pg/dL)	63.4 ± 23.6	71.9 ± 26.5	0.610032

BMI: body mass index; IL-6: interleukin-6; IL-8: interleukin-8; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein; pg/dL: picograms per deciliter.

TABLE 3. Comparison between the individuals with and without steatohepatitis.

	Non-steatohepatitis	Steatohepatitis	Value of *P*
Age (years)	33.7 ± 4.9	39.4 ± 9.1	0.147749
BMI (kg/m^2)	35.8 ± 2.4	36.5 ± 2.7	0.573183
Adiponectin	217.1 ± 28	173.7 ± 60.3	0.093356
IL-6 (pg/dL)	31.8 ± 22.8	29.4 ± 15.8	0.794518
IL-8 (pg/dL)	12.6 ± 5.7	15.6 ± 9.3	0.445529
Resistin (pg/dL)	45.2 ± 37.7	54.2 ± 42	0.644999
Leptin (pg/dL)	104.1 ± 32.5	99.8 ± 28	0.767814
TNF-α (pg/dL)	206.2 ± 326.7	99 ± 229.4	0.411142
CRP (pg/dL)	72.9 ± 18.2	69.2 ± 29.8	0.767814

BMI: body mass index; IL-6: interleukin-6; IL-8: interleukin-8; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein; pg/dL: picograms per deciliter.

The intensity of fibrosis presented a significant negative correlation with the levels of adiponectin (R= -0.49379; *P*=0.03166); this means that the higher the levels of adiponectin, the lower the intensity of fibrosis. FIGURE 1 presents graphical representations of the correlation analysis of each variable evaluated with the severity of fibrosis and the respective correlation coefficients and values of P. Similarly, the intensity of steatohepatitis presented a significant negative correlation with the levels of adiponectin (R= -0.562321; *P*=0.01221); this means that the higher the levels of adiponectin, the lower the intensity of steatohepatitis. None of the other evaluated variables presented significant correlations with the intensities of both fibrosis and steatohepatitis. FIGURE 2 presents graphical representations of the correlation analysis of each variable evaluated with the severity of steatohepatitis and the respective correlation coefficients and values of *P*.

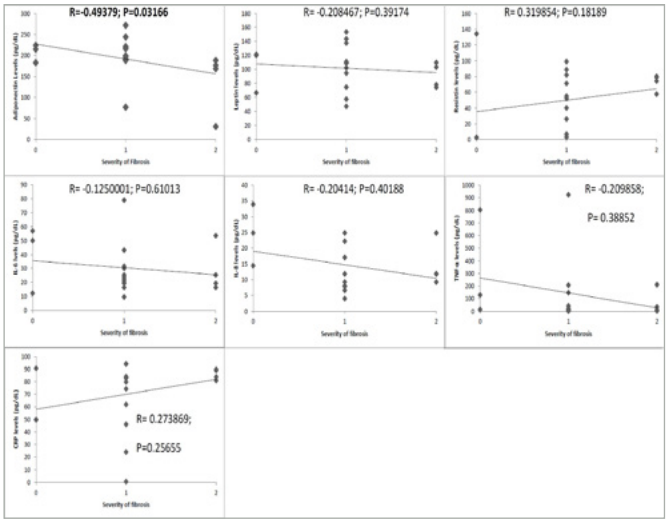

FIGURE 1. Correlations between adipokine/cytokine profiles and severity of fibrosis. IL-6: interleukin-6; IL=8: interleukin-8; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein; R: coefficient of correlation; *P*: value of *P*.

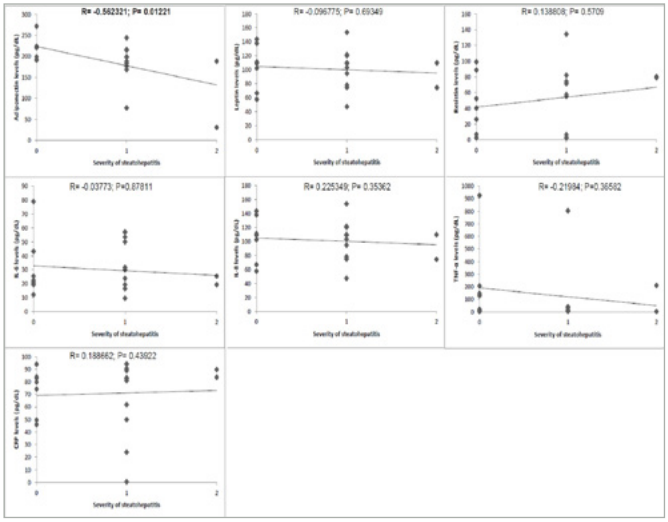

FIGURE 2. Correlations between adipokine/cytokine profiles and severity of steatohepatitis. IL-6: interleukin-6; IL=8: interleukin-8; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein; R: coefficient of correlation; *P*: value of *P*.

DISCUSSION

The interplay between the liver metabolism and circulating levels of active substances with pro or anti-inflammatory properties is a complex pathophysiological process which is not completely understood. However, due to obesity and fatty liver epidemics, the analysis of the role of these compounds presents a great significance as a way to gain insight into the mechanisms of chronic liver injury and plan therapies targeting these molecules or, at least, their use as markers of severity of disease.

The current study showed significant negative correlations between the levels of adiponectin and the severity of both liver fibrosis and steatohepatitis, signaling a protective effect of adiponectin in

regard to the progression of NAFLD to more aggressive forms. This role may be potentially linked to the anti-inflammatory properties of adiponectin, as well as its insulin sensitizing effect. Both effects are likely to act conjointly to, at least, stabilize the liver injury in the context of NAFLD. The relationship between adiponectin and hepatoprotection has been previously observed in the literature[18-21]. Since bariatric surgery leads to increases in the levels of adiponectin[22-25], it is reasonable to suppose that this mechanism is involved to some degree in the significant improvement of NAFLD reported after surgery[26-29].

There is evidence that leptin levels are associated with liver disease[30-32]; it is possible that the present study did not show significant correlations because all of the studied individuals presented obesity and thus tended to present high levels. The role of resistin in the development and progression of NAFLD is not so well established and the previous available evidence showed mixed results, with some authors reporting a significant association with NAFLD[33-35], and others demonstrating no differences[36-38]. D'Incao et al. have observed that leptin levels were inversely correlated with the degree of steatosis, and also that resistin levels were inversely correlated with fibrosis stages[18].

Higher levels of IL-8 among individuals with liver fibrosis were also observed in the present study. Previous studies have demonstrated that IL-8 is strongly activated in chronic liver disease, thus likely contributing to liver inflammation; Zimmerman et al.[39] suggested a possible role of IL-8 for recruitment and activation of hepatic macrophages. The increased levels of IL-8 among individuals with NAFLD compared to healthy controls have also been demonstrated by Jarrar et al.[40] and Hasanaliyeva et al.[41]. The latter study pointed out the IL-8 levels were also independently associated with fibrosis among individuals with NAFLD. There is scarce evidence on the influence of bariatric surgery on IL-8 levels; Klein et al.[42] observed a significant decrease in its levels, thus this may also be involved in the improvement of NAFLD after surgery.

Although CRP, TNF-α, and IL-6 did not differ statistically among the studied individuals, there are previous evidence of their significant influence on the NAFLD pathophysiology[43-46]. The lack of significance in regards to these markers in the present study may be caused by the small number of individuals analyzed, or by the selection bias due to the high frequency of NAFLD within this population.

This study has some limitations that should be taken into account. Firstly, it was performed in a small patient population; this occurs primarily due to the high costs of the assays utilized in this study. Furthermore, there was not a control group with healthy individuals and all the studied individuals presented some degree of NAFLD, facts that limit a complete explanation of the findings and further extrapolation. The major caveat of this approach is that it is not possible to fully determine which features should certainly be ascribed to which of the three modalities of liver disease considered, as they overlap in the analysis. Finally, the complete inflammatory panel involves a number of mediators which were not analyzed in this study; since there is a complex interplay among these compounds, it may also avoid ultimate conclusions. Despite these caveats, the results of the present study permit significant insights in regards to the progression of liver disease among individuals with obesity and reinforce the possibility of using some of these molecules as markers for diagnosis and following, or even targets for potential therapies.

CONCLUSION

Adiponectin levels were inversely correlated with the severity of fibrosis and steatohepatitis, whereas IL-8 levels were higher in individuals with liver fibrosis among individuals with obesity and NAFLD undergoing bariatric surgery.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Statement of human and animal rights

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Baltieri L, Chaim EA, Chaim FDM, Utrini MP, Gestic MA, Cazzo E. Correlações entre características da doença hepática gordurosa não-alcoólica e os níveis de adipocinas e citocinas inflamatórias em indivíduos submetidos à cirurgia bariátrica. Arq Gastroenterol. 2018,55(3):247-51.

RESUMO – **Contexto** – A doença hepática gordurosa não-alcoólica (DHGNA) é a hepatopatia mais comum no mundo. **Objetivo** – Investigar correlações entre as apresentações histopatológicas da DHGNA e os níveis de adipocinas (adiponectina, leptina e resistina) e marcadores sistêmicos de inflamação (interleucina-6 [IL-6], interleucina-8 [IL-8], fator de necrose tumoral alfa [TNF-α] e proteína C reativa [PCR]). **Métodos** – Estudo transversal exploratório envolvendo 18 mulheres com obesidade submetidas à cirurgia bariátrica. As características bioquímicas avaliadas incluíram os níveis de adiponectina, leptina, resistina, IL-6, IL-8, TNF-α e PCR. A DHGNA foi avaliada através de exams histológicos de biópsias hepáticas realizadas durantes as cirurgias. **Resultados** – A idade média foi 37,3±8,2 anos; o índice de massa corporal (IMC) médio foi 36,2±2,5 kg/m^2. Entre os indivíduos com fibrose hepática, os níveis de IL-8 foram significativamente mais altos (24,4±9,7 versus 12,7±6,6; *P*=0,016726). A intensidade da fibrose apresentou uma correlação negativa significativa com os níveis de adiponectina (R= -0,49379; *P*=0,03166), demonstranso que, quanto maiores os níveis de adiponectina, menor a intensidade da fibrose. A intensidade da esteato-hepatite apresentou uma correlação negativa significativa com os níveis de adiponectina (R= -0,562321; *P*=0,01221), demonstrando que quanto mais altos os níveis de adiponectina, menor a intensidade da esteato-hepatite. **Conclusão** – Os níveis de adiponectina correlacionaram-se negativamente com a severidade da fibrose e da esteato-hepatite, enquanto os níveis de IL-8 foram maiores entre os indivíduos com fibrose hepática. O uso destes marcadores pode trazer informações significativas sobre a DHGNA em populações com obesidade.

DESCRITORES – Obesidade. Fígado gorduroso. Adipocinas. Citocinas. Interleucinas.

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ORIGINAL ARTICLE

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Analysis of risk factors and postoperative complications in patients with Crohn's disease

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ABSTRACT – **Background** – The post-operative complications rate is greater in patients with Crohn's disease than in other abdominal surgeries due to other benign conditions. Prevention and management of such complications are important factors in the care of these patients. **Objective** – The objectives of this research are to analyze the rate of postoperative complications and the major risk factors in patients with Crohn's disease. **Methods** – A descriptive and retrospective study based on analysis of medical records of patients with Crohn's disease undergoing ileal and/or colonic resection, which analyzed the main surgical complications and their major risk factors. **Results** – Forty-four surgical procedures and thirty-seven patients were analyzed. Most were female (56.7%). Postoperative complications were observed in 18 (40.9%) surgeries. The disease duration (P=0.04), the penetrating behavior (P=0.013), the time between diagnosis and the first surgery (P=0.04), malnutrition with low body mass index (BMI), duration of surgery (P=0.016), and the size of the removed specimen (P=0.014) were associated with higher rates of complications. The use of drugs blocking tumor necrosis factor up to eight weeks before surgery was not significantly associated with higher complications rates or increased need for reoperation. **Conclusion** – The complication rate observed in this study is similar to published data. The duration of the disease, the penetrating behavior, the size of the removed specimen, the duration of the surgery, and BMI are important risk factors for perioperative complications in Crohn's disease.

HEADINGS – Secondary prevention. Risk factors. Digestive system surgical procedures. Postoperative complications. Inflammatory bowel disease.

INTRODUCTION

Crohn's disease (CD) can cause transmural inflammation and can affect any segment of the digestive tract (especially terminal ileum and perianal region). It is characterized by a discontinuous involvement of the gastrointestinal tract, with affected areas interspersed with areas of normal mucosa. Unlike ulcerative colitis, CD is commonly associated with complications such as abscesses, fistulas and stenosis[1,2].

The worldwide incidence of CD in the adult population ranges from 0.1 to 20.2 per 100,000 people per year, and its prevalence in North America is 26 to 199 cases per 100,000 inhabitants per year[3-5]. The incidence of CD in the Midwest of São Paulo state during the years of 2001 to 2005 was 3,5 cases per 100.000 people, and its prevalence was 5,65 cases per 100.000 people[6]. Although South America is considered an area with a low frequency of CD, its incidence is rising in the countryside of both the São Paulo and Minas Gerais states in the past few decades[7,8].

Despite the reduction in the need for surgery due to recent advances in the clinical management of CD, it is estimated that between 70% and 90% of patients with CD will require surgical intervention at some point during the progression of the disease[9].

Several authors[10-12] have already proven that patients with inflammatory bowel diseases (IBD) are more prone to perioperative complications, due to pre-existing clinical condition, nutritional status and drug use, especially corticoids and immunomodulators. However, the rates of perioperative complications vary widely (5% to 60%)[10-13] according to the group. Some factors widely influence the rate of perioperative complications: presence of multidisciplinary team, experience of the surgeon, center of reference to treat IBD, medications in use and duration.

Regarding the medications, there is a growing concern about the safety of perioperative use of biologic anti-TNF (tumor necrosis factor) agents, as Infliximab (IFX) and Adalimumab (ADA). In a recent meta-analysis article[14], with extensive review of the literature, the postoperative complications associated with the use of anti-TNF were evaluated. One of the few multicenter studies performed in Brazil, analyzing 76 patients with CD did not show an increased risk of complications with the use of biological therapy[15].

Thus, the objective of this study is to analyze the rate of perioperative complications and to identify their risk factors in patients with intestinal CD undergoing surgical treatment.

METHODS

Ethical aspects of the survey

This survey was approved by the Department of Surgery and Orthopedics, Botucatu Medical School, São Paulo State University – UNESP. Grant #2014/01091-2, São Paulo Research Foundation (FAPESP). Ethics Committee approval number: 3547/2010.

A retrospective observational study was conducted through the collection of data from medical records of patients with CD submitted to abdominal surgical intervention.

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All patients diagnosed with CD and monitored at the IBD outpatient clinic of Botucatu Medical School who underwent abdominal surgery related to CD itself in the last 10 years were included in this study. The only exclusion criteria was insufficient data on the medical records.

The patients were classified according to Montreal Classification for CD[16], which establishes three parameters of classification: age of onset, behaviour of the disease, and location.

The following complications were analyzed: fistulae, abscesses, anastomotic dehiscence, and abdominal sepsis. Socio-demographic data (sex, ethnicity, age, schooling), clinical features (BMI, age at diagnosis, disease duration, family history, history of smoking, use of corticosteroids, immunomodulators or anti-TNF drugs within 8 weeks prior to surgery, Montreal classification of CD), and surgical characteristics (age at first surgery, time between diagnosis and first surgery, need for more than one surgery, number of surgeries, surgical indication, surgical time, technique and position of the anastomosis, presence of complications and their classification, and size of the removed specimen) were also analyzed.

Complications were classified according to the method proposed by Clavien-Dindo[17] (grade I to V), that stratifies them according to the therapy that was necessary for their treatment, which makes this model ideal for retrospective studies, such as the present.

The variables were analyzed in a descriptive way, including their relative and absolute frequencies (for the categorical variables) and the calculation of the mean or median and the standard deviation (for the quantitative variables). The variables were then separated into clinical and surgical, and analyzed associatively using chi-square or Fisher's exact test (for categorical variables), and Student's t-tests or the Mann-Whitney (for the statistical variables), assuming a value of statistical significance of P=0.05. The program used for such analyzes was STATA 12.0[18].

RESULTS

Descriptive analysis

A total of 44 surgical procedures were analyzed in 37 patients, 21 (56.7%) female, 32 (86.5%) white, and 18 (49%) with complete secondary education. The mean age at diagnosis is 32.5 years (standard deviation [SD] = 11.4); the mean age of the patients at the first surgery is 35.7 years (SD = 10.9), and the median time between diagnosis and the first surgery is 1 year.

Only 8.1% of the patients had a family history of CD; 21.6% of the patients have a history of current or previous smoking; and 12 (32.4%) required more than one surgery.

The highest incidence of CD (67.6%) occurred between 12 and 40 years of age (A2 from Montreal); the most common location (75.7%) of the disease was ileocolonic (L3 of Montreal); and stricturing (B2) and penetrating (B3 Montreal) behaviors showed the same frequency (48.65%). Complications occurred in 16 patients (46.2%), and the most common was the presence of fistulas (35.1%).

Of the total number of surgeries, 70.5% were elective; 6 surgeries (13.7%) required blood transfusion; and the approach of all surgeries performed was through laparotomy. The mean duration of the surgeries was 181.1 minutes (SD=43.9), and the median length of the removed specimen was 25 cm, with a range of 8.5 to 99 cm.

The rate of postoperative complications was 40.9% (18 complicated surgeries, out of a total of 44). Half of these complications (nine) occurred within 30 days of the surgical procedure. There were 15 (34.1%) surgeries complicated by fistulas; 10 (22.7%) surgeries complicated by abscesses; 5 (11.3%) surgeries complicated by anastomotic dehiscence; and 2 (4.5%) surgeries complicated by abdominal sepsis. Of the 18 complications occurred, 5 (11.3%) were classified as IIIa; 11 (25%) as IIIb; and 2 (4.5%) as IVb, according to Clavien-Dindo[14] as shown in TABLE 1.

TABLE 1. Incidence of complications.

Variables	N	%
Complications		
Any complication	18	40.9
Fistula	15	34.1
Abscess	10	22.7
Anastomotic dehiscence	05	11.3
Abdominal sepsis	02	4.5
Clavien-Dindo[13] classification		
IIIa	05	11.3
IIIb	11	25.0
IVb	02	4.5

Associative analysis

Of the 37 studied patients, 12 (32.4%) required more than one surgery. There was no significant association between the need for additional surgery and gender, ethnicity, schooling or smoking.

There was no significant difference between the need for additional surgery in the future and elective or emergency indication for the surgery; the use of corticosteroids, immunomodulators or biological up to 8 weeks before surgery; anastomotic features or extension of the specimen removed in the first operation. However, family history for CD (P=0.028) and duration of disease in years (P=0.04) were significantly associated with the need for additional surgery, as shown in TABLE 2.

There was a significant difference (P=0.013) in the clinical behavior of disease in the groups with and without general complications after surgery (including fistulas, abscesses, wound dehiscence and sepsis). The penetrating behavior (B3 of Montreal) was associated with a higher frequency of complications.

In addition, there was also a significant difference (P=0.02) in the extension of the removed specimen in the surgeries with postoperative complications when compared to the extension of the specimen in the uncomplicated surgeries. Removal of specimens greater than 25 cm was associated with a higher rate of complications such as fistulae, abscesses, anastomotic dehiscence, and abdominal sepsis (P=0.019, relative risk of 3.69), as shown in TABLE 3. None of the drugs (corticoids, immunomodulators and biologics) was associated with a higher occurrence of complications.

According to the classification of Clavien-Dindo[17], the severity of the studied complications was not significantly influenced by the type of surgical indication, the use of drugs (corticoids, immunomodulators or biologics) up to 8 weeks prior to surgery, anastomosis technique or size of the removed specimen, as shown in TABLE 4.

TABLE 2. Clinical characteristics of the patients according to the need for additional surgery after the first surgery.

Clinical characteristics	One surgery (n=25)		More than one surgery (n=12)		*P* (Fisher)	Relative Risk (CI)
Categorical variables	N	%	N	%		
Family history					0.028	
No	25	73.5	09	26.5		1
Yes	00	0	03	100		3.77 (2.15–6.61)
Schooling					0.52	
Secondary education or less	20	69.0	09	31.0		1
Graduate	05	62.5	03	37.5		1.2 (0.42–3.44)
Smoking					0.48	
No	19	65.5	10	34.5		1
Yes	06	75.0	02	25.0		0.75 (0.19–2.66)
Quantitative variables	Average (DP)		Average (DP)		*P* value	
Age at diagnosis (years)	33.5 (12.3)		30.5 (9.2)		0.53	
Disease duration (years)	10.7 (6.9)		17.7 (9.7)		0.04	

TABLE 3. Surgical characteristics according to the presence of postoperative complications.

Surgical characteristics	Absence of complications (n=26)		With complications (n=18)		*P* (Fisher)	Relative Risk (CI)
Categorical variables	N	%	N	%		
Surgical indication					0.44	
Elective	19	61.3	12	38.7	0.64 (X^2)	1
Emergency	07	53.8	06	46.2		1.19 (0.57–2.48)
Biological drugs up to 8 weeks prior to surgery					0.21	
Yes	06	46.1	07	53.9	0.25 (X^2)	1
No	20	64.5	11	35.5		0.65 (0.32–1.31)
Extension of the removed specimen (cm)					0.019	
≤ 25	16	84.2	03	15.8		1
> 25	05	41.7	07	58.3		3.69 (1.17–11.58)
Information unavailable	05	–	08	–	–	–
Quantitative variables	Average (DP)		Average (DP)		*P* value	
Surgical time (minutes)	192 (34.57)		167.5 (52.64)		0.27	
Extension of the removed specimen (cm)	29.40 (23.75)		41.55 (18.21)		0.02	

TABLE 4. Relation between surgical characteristics and postoperative complications, according to the therapy necessary to treat them[17].

Surgical characteristics	Classification according to the model proposed by Clavien-Dindo[14]						*P* (Fisher)
	IIIa (n=05)		IIIb (n=11)		IVb (n=02)		
Categorical variables	N	%	N	%	N	%	
Surgical indication							1.00
Elective	03	60.0	08	72.7	01	50.0	
Emergency	02	40.0	03	27.3	01	50.0	
Biological drugs up to 8 weeks prior to surgery							0.29
Yes	01	20.0	06	54.5	00	00.0	
No	04	80.0	05	45.5	02	100.0	
Anastomosis (technique)							
Mechanical	02	40.0	06	54.5	01	50.0	1.00
Manual	02	40.0	03	27.3	00	00.0	
Information unavailable	01	20.0	02	18.2	01	50.0	
Extension of the removed specimen (cm)							0.13
≤ 25	00	0.0	01	09.0	02	100.0	0.04 (X^2)
> 25	02	40.0	05	45.5	00	00.0	
Information unavailable	03	60.0	05	45.5	00	00.0	

There was a significant difference between the group of patients with and without fistulas in the postoperative period regarding to the clinical behavior of the disease (P=0.003). The penetrating behavior (B3 of Montreal) was associated with a higher frequency of fistulas. Thus, there is a tendency to maintain the penetrating behavior after surgery.

The time between diagnosis and the first surgery was also different between these two groups (P=0.04), with the longest periods associated with the group with fistulas. There was no significant difference between gender, ethnicity, family history or smoking among these groups. There was no significant association between the use of corticosteroids, immunomodulators and biological agents up to 8 weeks prior to surgery and the presence of fistulas, nor between the characteristics of the anastomosis and the presence of fistulas.

The only significant association related to the presence of abscesses after surgery was the Body Mass Index (BMI) of the patients, with the highest BMIs being associated with the abscess group (P=0.02). There was no significant association between the presence of abscesses and gender, ethnicity, family history, smoking, use of medications (corticoids, immunomodulators and biologics), or anastomotic features.

There were no significant associations between the variables studied and the presence of anastomotic dehiscence or abdominal sepsis.

DISCUSSION

The present study analyzed 44 surgical procedures in 37 high-complexity patients in a reference hospital of the Midwest of São Paulo state. Its results show that treatment of CD remains challenging, especially when the disease has a penetrating behavior. The number of patients and the retrospective design of the study are limitations. However, despite this limitation, the study is important to analyze CD in the Midwestern Sao Paulo state, and its results corroborate with many other studies in the literature.

The gender distribution of the patients in this study is similar the one reported in the literature[19]. The ileocolonic location is the most common among patients undergoing conventional (open) surgery for CD in several studies found in the literature[13,20]. The highest incidence of CD between 12 and 40 years of age (A2 of Montreal) is also reported in the literature as the most common among patients requiring surgery[13].

The literature reports[13] a lower rate of postoperative complications in laparoscopic surgeries, especially in cases of mild to moderate non-fistulizing CD. However, it was not possible to study the difference between the surgical approaches, since all the surgeries performed in the present study had a conventional approach. The need for reoperation is also lower in laparoscopic approach surgeries, according to the literature[13]. Although it is available in our group, the hospital cost policy difficult the use of laparoscopy, especially at the time when the study was conducted. Recently in the hospital, an inflammatory bowel disease surgical team was assembled, and this team began to use the laparoscopic approach to CD patients, with only a few recent cases operated. New studies will be conducted to evaluate the results of this new approach in our group.

The rate of postoperative complications was 40.9% (18 complicated surgeries, out of 44), which is consistent with the rates reported in the literature[13,21,22]. The fact that the hospital in which the study was conducted is the only tertiary referal hospital for the entire midwest of the state of São Paulo, which comprehend more than 2 million people, may explain the relatively high value of complications. In this scenario, the patients that are referred to us usually have a more aggressive and severe disease, and they usually have been using medications for a long time, increasing the risk of postoperative complications.

Regarding the high rate of fistula, the authors believe that it occurred because the patients that are referred to our group usually have been treating CD for a long time, have a severe clinical state, many of them have chronic obstructive symptoms and malnutrition, and many of them were operated in an urgent scenario. Besides, at the time of this study, every gastrointestinal surgeon in our hospital operated on CD patients – including surgical residents; nowadays we have implanted an inflammatory bowel disease surgical team that comprises two expert surgeons. With this measure, the results seem to be subjectively improved over the last year. Another possible explanation is that the low number of patients included in this study had a negative influence on this analysis.

In a recent retrospective study[23], published in 2015, both the ileocolonic location and the penetrating behavior of CD were associated with a greater surgical recurrence of the disease, resembling what was found in the present study, in which the latter were associated with a higher incidence of postoperative complications. The complexity of the treatment of penetrating-behavior CD was again highlighted in a recent systematic review[24] and in a recent retrospective muticentric study[25]. All of that indicates that the penetrating behavior is an indication of severity and poor prognosis on CD.

The use of corticosteroids, despite showing a clear benefit in the treatment of the acute phase of inflammatory bowel diseases, is associated with negative repercussions in the postoperative period by most authors, such as increasing the rate of postoperative complications[26-28] and increasing postoperative mortality[29]. In the present study, however, no relation was observed between the presence of complications and corticosteroid therapy up to 8 weeks prior to surgery, probably because of the small sample. The authors believe that an increase in the patient number would confirm this relation.

There are controversies in the literature regarding the influence of biological therapy on the rate of postoperative complications. There are meta-analyzes showing that these drugs exert a protective effect against postoperative complications[30,31], and there are others showing an increase in the rate of postoperative complications[21,32-35] related to the use of these drugs. In the present study, there was no evidence that the biological drugs influence the rate of postoperative complications, resembling what was found in recent studies[22,25].

The removal of a specimen greater than 25 cm was associated with both higher rates of general complications and higher incidence of postoperative fistulas. Thus, it seems that conservative resections regarding specimen extension are a way to reduce the rate of postoperative complications.

Another important alternative regarding the reduction of the rate of complications and the need for reoperation is the laparoscopic approach, which is not routinely practiced in our group in patients with CD.

There are only a few papers in the international literature discussing this matter, especially in Latin America, and the results presented in this paper contribute to the current discussion regarding the perioperative complications of IBD, especially CD.

CONCLUSION

Treatment of CD, despite numerous advances, remains challenging, with high rates of postoperative complications. Family history, duration of disease, extension of the removed specimen, penetrating behavior and degree of nutrition are important risk factors for the presence of complications in the postoperative period.

Conservative resections of the segments affected by CD may be an important measure aimed at reducing the rate of postoperative complications.

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Authors' contribution

Saad Hossne R: idealizer and coordinator of the project and editor of the article. Sassaki LY: coordinator of the project and editor of the article. Baima JP: reviewer of the article. Meira Júnior JD, Campos LM: data collectors and text writers.

Saad Hossne R, Sassaki LY, Baima JP, Meira Júnior JD, Campos LM. Análise dos fatores de risco e complicações pós-operatórias em pacientes portadores de doença de Crohn. Arq Gastroenterol. 2018,55(3):252-7.

RESUMO – **Contexto** – O índice de complicações pós-operatórias é maior em pacientes com doença de Crohn do que em outras cirurgias abdominais decorrentes de outras afecções benignas. A prevenção e o manejo de tais complicações constituem importante fator no tratamento desses pacientes. **Objetivo** – Os objetivos deste trabalho são analisar a taxa de complicações pós-operatórias e os principais fatores de risco em pacientes portadores de doença de Crohn. **Métodos** – Estudo descritivo e retrospectivo, baseado na análise dos prontuários dos pacientes com doença de Crohn submetidos a ressecções ileais e/ou colônicas, analisando as principais complicações cirúrgicas e os principais fatores de risco relacionados as mesmas. **Resultados** – Foram analisados 44 procedimentos cirúrgicos em 37 pacientes. A maioria dos pacientes era do sexo feminino (56,7%). Complicações pós-operatórias foram observadas em 18 (40,9%) cirurgias. A duração da doença (P=0,04), o comportamento penetrante (P=0,013), o tempo entre o diagnóstico e a primeira cirurgia (P=0,04), a desnutrição com baixo índice de massa corpórea (IMC), o tempo de duração da cirurgia (P=0,016), e o tamanho do espécime retirado (P=0,014) estiveram associados a maiores taxas de complicações. O uso de fármacos bloqueadores do fator de necrose tumoral até oito semanas antes da cirurgia não foi significativamente associado a maiores taxas de complicações ou a maior necessidade de reoperação. **Conclusão** – A taxa de complicações observadas neste trabalho é semelhante aos dados da literatura. A duração da doença, o comportamento penetrante, o tamanho do espécime retirado, o tempo de duração da cirurgia e o IMC são importantes fatores de risco para complicações peri-operatórias em doença de Crohn.

DESCRITORES – Prevenção secundária. Fatores de risco. Procedimentos cirúrgicos do sistema digestório. Complicações pós-operatórias. Doença inflamatórias intestinais.

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ORIGINAL ARTICLE

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Metachronous colorectal liver metastases has better prognosis – is it true?

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ABSTRACT – **Background** – Liver metastases from colorectal cancer are an important public health problem due to the increasing incidence of colorectal cancer worldwide. Synchronous colorectal liver metastasis has been associated with worse survival, but this prognosis is controversial. **Objective** – The objective of this study was to evaluate the recurrence-free survival and overall survival between groups of patients with metachronous and synchronous colorectal hepatic metastasis. **Methods** – This was a retrospective analysis of medical records of patients with colorectal liver metastases seen from 2013 to 2016, divided into a metachronous and a synchronous group. The Cox regression model and the Kaplan-Meier method with log-rank test were used to compare survival between groups. **Results** – The mean recurrence-free survival was 9.75 months and 50% at 1 year in the metachronous group and 19.73 months and 63.3% at 1 year in the synchronous group. The mean overall survival was 20.00 months and 6.2% at 3 years in the metachronous group and 30.39 months and 31.6% at 3 years in the synchronous group. Patients with metachronous hepatic metastasis presented worse overall survival in multivariate analysis. The use of biological drugs combined with chemotherapy was related to the best overall survival prognosis. **Conclusion** – Metachronous colorectal hepatic metastasis was associated with a worse prognosis for overall survival. There was no difference in recurrence-free survival between metachronous and synchronous metastases.

HEADINGS – Colorectal neoplasms. Neoplastic metastasis. Liver. Prognosis. Survival.

INTRODUCTION

Hepatic metastases from colorectal cancer are an important public health problem due to the increasing incidence of colorectal cancer worldwide[1]. Hepatic metastases from colorectal cancer may be present in 15% to 20% of cases at diagnosis and in up to 60% of cases during the life of these patients[2]. The liver is the only site of metastasis in 20% to 50% of cases[3]. Hepatic metastases may be present from the diagnosis of primary colorectal neoplasia or even be diagnosed before it. In this case, they are called synchronous metastases when they occur in the first 6 months after diagnosis of primary colorectal neoplasia[4]. Metastases diagnosed after 6 months are called metachronous[4]. However, this definition is heterogeneous, since other time intervals are adopted between the diagnosis of the primary tumour and of the liver metastasis, ranging from 0 to 12 months[5].

Numerous therapeutic innovations have been developed in the last decade with the intention of improving the prognosis and increasing the survival of patients with hepatic metastasis[6-8]. Such advances in therapy were able to contribute to this goal, with new surgical techniques such as associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), staged resections, selective ligation of the portal vein branch, use of intraoperative ultrasound, and modern chemotherapy regimens associated with biological agents[9-12].

Synchronous colorectal liver metastasis is classically associated with worse prognosis, being a component of several prognostic scores[13-17]. However, there are discordant studies showing that the survival of synchronous liver metastasis does not differ from metachronous metastasis[18-20]. In view of the divergent results, there is a need for a new approach on the subject, given the rapid evolution of systemic and surgical treatments for colorectal liver metastases.

The objective of this study was to evaluate the recurrence-free survival and overall survival between groups of patients with metachronous and synchronous colorectal hepatic metastasis.

METHODS

A retrospective analysis of the data of patients with colorectal liver metastases treated at the Clinical Hospital of the Federal University of Goias from 2013 to 2016 was performed. Patients were divided into two groups: metachronous group (time between diagnosis of primary tumour and liver metastasis greater than 6 months) and synchronous group (time less than or equal to 6 months). Patients with multiple primary neoplasms or unavailable data were excluded from the study.

Clinical, anatomical, and surgical characteristics and their distributions between the two groups were evaluated. The variables studied were age, gender, location of primary tumour, extrahepatic disease, carcinoembryonic antigen (CEA), *KRAS*/*NRAS* gene ex-

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pression, degree of cell differentiation, number of liver metastases, number of hepatic segments affected, distribution of metastases, surgical treatment performed, chemotherapy scheme, biological drugs, use of intraoperative ultrasound, transfusion of blood products, surgical complications, size of the surgical margins of resected liver metastasis, and recurrence site.

Recurrence-free survival and overall survival were compared between the metachronous and synchronous groups. The follow-up period was at least 12 months in both groups, with periodic computer tomography (CT) and seric carcinoembryonic antigen (CEA) protocol. PET-SCAN was also used at the multidisciplinary team's discretion. The effect of all variables studied on survival in the total study population in univariate and multivariate analyses was also studied.

The chi-squared test was used to compare the clinical, anatomical, and surgical variables between the synchronous and metachronous groups. Survival curves were obtained using the Kaplan-Meier method, and the log-rank test was used to compare the groups. The Cox regression model was used in multivariate analysis to identify the variables associated with recurrence-free survival and overall survival; it included variables that presented *P*-values <0.1 in the univariate analysis. The risk prediction was exposed through the *P*-value, hazard ratio (HR), and 95% confidence interval (95% CI). The differences were considered statistically significant when $P<0.05$. Statistical analysis was performed using SPSS® for Windows®, version 16.0.

This study was approved by the Research Ethics Committee of the Clinical Hospital of Federal University of Goias (protocol number 1,595,740).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

RESULTS

A total of 59 records were analysed, of which 54 were included in the study. One patient had no diagnosis of liver metastasis confirmed on follow-up imaging and was excluded; three patients were excluded due to the absence of histological evidence of colorectal primary neoplasia; and one patient was excluded because of two primary neoplasms of different origins. Thus, the metachronous group consisted of 16 patients, and the synchronous group contained 38 patients.

Clinical aspects

The mean age of the patients was 58.6 years in the metachronous group and 57.1 years in the synchronous group. There was a predominance of males in both groups.

The median CEA value was 34.53 ng/mL in the metachronous group and 12.45 ng/mL in the synchronous group. The genetic study of the *KRAS*/*NRAS* family showed the prevalence of the wild type in both groups.

A moderate degree of cell differentiation predominated in both groups.

The use of biological therapy associated with the chemotherapy regimen (5-fluorouracil and/or capecitabine associated with oxaliplatin and/or irinotecan) was more frequent in the synchronous group, but without statistical significance. The biological agents used in the study were bevacizumab, cetuximab, and panitumumab.

Among the patients operated on for hepatic metastasis, the patients in the synchronous group underwent more preoperative chemotherapy treatments than the metachronous group, but without statistical significance. The comparison of clinical features between the groups is displayed in TABLE 1.

TABLE 1. Clinical features of patients in synchronous and metachronous groups.

	Synchronous		Metachronous		*P*-value
	n	%	n	%	
Age					
≤ 60 years	19	50.0	9	56.3	0.675
> 60 years	19	50.0	7	43.7	
Gender					
Male	20	52.6	10	62.5	0.505
Female	18	47.4	6	37.5	
CEA					
<50 ng/mL	26	68.4	10	66.7	0.902
≥50 ng/mL	12	31.6	5	33.3	
KRas/NRas					
Mutated	12	46.2	3	33.3	0.503
Wild-type	14	53.8	6	66.7	
Differentiation grade					
Well	3	9.7	1	6.7	
Moderate	23	74.2	13	86.7	0.606
Poor	5	16.1	1	6.7	
Biological drugs					
No	16	44.4	11	68.8	0.105
Yes	20	55.6	5	31.3	
Preoperative chemotherapy					
Yes	10	55.6	2	40.0	0.538
No	8	44.4	3	60.0	

Chi-squared test. CEA: carcinoembryonic antigen.

Anatomical aspects

Primary colorectal neoplasia occurred more frequently in the left colon in both groups. Metastatic disease occurred exclusively in the liver at higher frequency in both groups.

The metachronous group had up to three hepatic lesions in most of the cases, unlike the synchronous group, which presented more lesions, but without statistical significance. Both groups showed a predominance of up to four hepatic segments affected by metastases.

Hepatic metastatic disease was predominantly unilobar in the metachronous group, unlike the synchronous group, in which there was a predominance of bilobar metastasis, but without statistical significance. The comparison of the anatomical characteristics between the groups is displayed in TABLE 2.

TABLE 2. Anatomical characteristics of patients with synchronous and metachronous colorectal liver metastases.

	Synchronous		Metachronous		*P*-value
	n	%	n	%	
Location of primary tumour					
Rectum	12	31.6	5	31.3	0.981
Colon	26	68.4	11	68.8	
Colon affected					
Right	6	23.1	3	27.3	0.786
Left	20	76.9	8	72.7	
Extrahepatic disease					
No	22	57.9	9	56.3	0.911
Yes	16	42.1	7	43.8	
Number of liver metastases					
≤3	18	47.4	9	56.3	0.551
>3	20	52.6	7	43.8	
Number of hepatic segments affected					
≤4	21	55.3	10	66.7	0.448
>4	17	44.7	5	33.3	
Distribution of metastases					
Unilobar	14	36.8	9	56.3	0.188
Bilobar	24	63.2	7	43.8	
Hepatic lobe affected					
Right	10	71.4	4	50.0	0.315
Left	4	28.6	4	50.0	

Chi-squared test.

Surgical aspects

Approximately one-third of the patients in the metachronous group and almost half of the patients in the synchronous group underwent surgical treatment of hepatic metastasis. The most performed surgeries were non-anatomical surgeries in both groups.

Intraoperative hepatic ultrasound was used as an auxiliary diagnostic resource in the majority of cases, and in most cases, there was no need for intraoperative blood transfusion in both groups.

Postoperative surgical complications were more frequent in the metachronous group, but without statistical significance.

Among the liver resections, excluding laparotomies with findings of unresectable disease, R0 surgeries were performed in most surgeries in both groups. Surgical margins were disease free and ≥1 cm in both groups.

In the metachronous group, extrahepatic pulmonary recurrence was predominant, whereas in the synchronous group, hepatic recurrence was more frequent, but without statistical significance. The comparison of surgical features between the groups is displayed in TABLE 3.

TABLE 3. Surgical features of patients in synchronous and metachronous groups.

	Synchronous		Metachronous		*P*-value
	n	%	n	%	
Surgical treatment					
No	20	52.6	11	68.8	0.274
Yes	18	47.4	5	31.2	
Hepatic surgery					
Nodulectomy	7	38.9	2	40.0	0.552
Segmentectomy	6	33.3	2	40.0	
Right hepatectomy	1	5.6	1	20.0	
Laparotomy	4	22.2	0	0.0	
Colorectal neoplasms surgery					
Elective	21	61.8	9	60.0	0.907
Emergency	13	38.2	6	40.0	
Intraoperative ultrasound					
No	5	29.4	0	0.0	0.168
Yes	12	70.6	5	100.0	
Blood transfusion					
No	12	70.6	4	80.0	0.678
Yes	5	29.4	1	20.0	
Surgical complications					
No	14	77.8	3	60.0	0.423
Yes	4	22.2	2	40.0	
Hepatic resection					
R0	11	78.6	4	80.0	0.805
R1	2	14.3	1	20.0	
R2	1	7.1	0	0.0	
Surgical margins					
≥1cm	6	54.5	3	60.0	0.953
<1cm	2	18.2	1	20.0	
Positive	3	27.3	1	20.0	
Recurrence					
Yes	9	81.8	3	75.0	0.770
No	2	18.2	1	25.0	
Recurrence site					
Liver	5	55.6	1	33.3	0.528
Lung	2	22.2	2	66.7	
Liver and lung	1	11.1	0	0.0	
Lung and brain	1	11.1	0	0.0	

Chi-squared test.

Recurrence-free survival

The mean recurrence-free survival in the metachronous group was 9.75 months and 50% at 1 year, while in the synchronous group, the mean was 19.73 months and 63.3% at 1 year, as shown by the recurrence-free survival curves for the metachronous and synchronous groups (FIGURE 1).

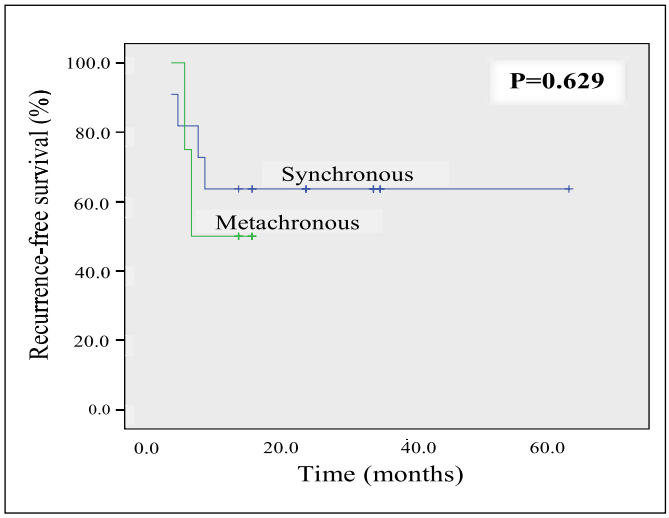

FIGURE 1. Recurrence-free survival curves in metachronous and synchronous groups.

Univariate analysis was performed using the Cox model of all patients undergoing potentially curative liver surgery. The variable surgical margins showed an association with recurrence-free survival in the univariate analysis, but it did not have statistical significance in the multivariate analysis.

Overall survival

The mean overall survival in the metachronous group was 20.00 months and 6.2% at 3 years, while in the synchronous group, the mean was 30.39 months and 31.6% at 3 years, as shown by the overall survival curve for the metachronous and synchronous groups (FIGURE 2).

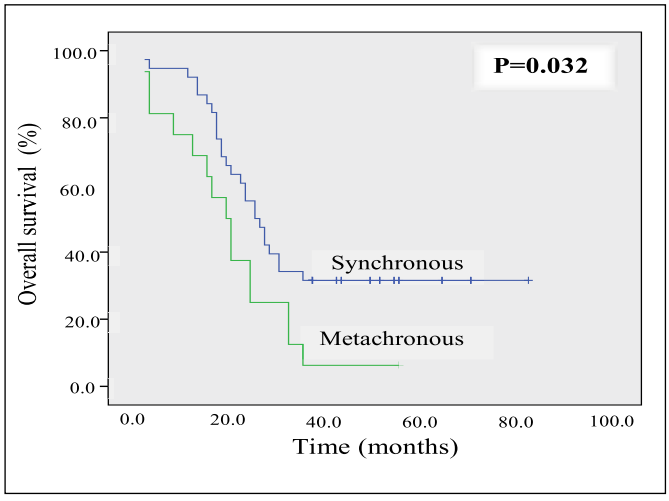

FIGURE 2. Overall survival curves in metachronous and synchronous groups.

A univariate analysis of the total study population was performed using the Cox model. For the multivariate analysis, we chose the variables age, metachronicity, extrahepatic disease, number of liver metastases, number of hepatic segments involved, distribution of liver metastases, use of biological drugs, and surgical treatment. The Cox multivariate analysis revealed metachronous hepatic metastasis as an independent variable related to worse overall survival (P=0.036) and chemotherapy treatment combined with biological drugs as an independent variable related to better overall survival (P=0.001). The multivariate analysis of overall survival is shown in TABLE 4.

TABLE 4. Multivariate analysis of prognostic factors of overall survival.

	Hazard Ratio	95% CI		*P*-value
Age ≤60 years	1.73	0.78	3.85	0.180
Metachronicity	2.66	1.06	6.63	0.036*
Extrahepatic disease	1.24	0.41	3.76	0.705
Liver metastasis >3	3.12	0.69	14.04	0.138
Hepatic segments involved >4	1.64	0.37	7.26	0.515
Bilobar metastasis	0.76	0.25	2.26	0.616
Biological drugs	0.21	0.08	0.50	0.001*
Surgical treatment	0.38	0.12	1.20	0.099

Cox regression model. * P<0.05.

DISCUSSION

Metachronous colorectal liver metastasis is associated with better survival, especially in studies of prognostic scores[13-17]. However, its actual influence on prognosis is controversial[18-20]. The present study evaluated the survival of patients with colorectal liver metastasis in the metachronous and synchronous groups and the clinical, anatomical, and surgical characteristics of each group. Metachronous colorectal liver metastasis was associated with worse overall survival, an association that contravenes evidence of a worse prognosis for synchronous hepatic metastasis[13-17,23]. Neo et al. conducted a database study and found a 3-year overall survival of 33% in metachronous metastases and 28% in synchronous metastases, with no significant difference[18]. Mekenkamp et al. also found no difference between the survival of patients with metachronous and synchronous metastasis[19]. Knopke et al. found worse survival in patients with synchronous colorectal liver metastasis, with a mean overall survival of 53 months for metachronous metastasis and 39 months for synchronous metastasis[16]. Kuo et al. also found an association between synchronous hepatic metastasis and worse prognosis, with lower overall survival (26.9% vs 48.1%) and recurrence-free survival (13.8% vs. 38.9%) rates[23].

In the present study, some factors could explain the worse prognosis of metachronous metastasis. First is the oligosymptomatic nature of colorectal liver metastasis, which can sometimes delay the performance of follow-up examinations and consequently delay the diagnosis of metachronous metastasis[24]. This does not usually occur in patients with synchronous metastasis, since staging examinations are performed routinely at the time of diagnosis of the primary tumour. Another important factor is the influence of the neoplastic tumour microenvironment, in which the association of

different molecular markers in metachronous and synchronous metastases is studied, which could be related to different carcinogenesis mechanisms, levels of tumour aggressiveness, and prognoses[25,26].

In this study, another variable was also related to prognosis: the use of biological drugs was associated with better overall survival. This result may be related to the inversion of the predicted prognosis for metachronous metastasis. In our service, patients with synchronous colorectal liver metastases are treated with high-dose chemotherapy regimens based on the association of 5-fluoracil with oxaliplatin or irinotecan, and nearly half of these patients also received biological agents such as bevacizumab, cetuximab, and panitumumab. The same type of chemotherapeutic treatment was performed in patients who developed metachronous metastases, but 70% of these patients were exposed to these drugs before the appearance of liver metastasis, which theoretically could generate some degree of resistance to the chemotherapeutic and biological agents in the microbiological environment of the metastatic tumour[3,19].

The metachronous and synchronous groups of this study were homogeneous in the characteristics analysed. Some studies have reported the association of unfavourable characteristics with synchronous hepatic metastases. For example, Tsai et al. found bilobular distribution, greater tumour number, and greater tumour diameter in synchronous metastases[21], and Mekenkamp et al. found greater tumour invasion of the primary neoplasia in the synchronous group[19]. Ng et al. had a more heterogeneous group, with a higher prevalence of women and a higher mean age in the metachronous group, as well as fewer curative surgeries and more complications in the synchronous group[22]. The present study found no significant difference between the groups in any of the analysed variables. The homogeneity of the metachronous and synchronous groups in this sample strengthens our results because it reduces the biases related to the unfavourable clinical characteristics that could unbalance the groups statistically.

The study has some limitations, mainly related to the retrospective model of data collection from medical records and to the short period of follow-up.

The association of the synchronicity of liver metastasis with prognosis is not an exhausted topic of discussion and remains controversial, with divergent results. Therefore, there is a need for prospective studies with longer follow-up to evaluate the prognosis of metachronous and synchronous liver metastases in the context of modern multimodal therapeutic strategies.

CONCLUSION

Metachronous colorectal hepatic metastasis was associated with a worse prognosis for overall survival. There was no difference in recurrence-free survival between groups with metachronous and synchronous metastases.

Authors' contributions

Quireze Junior C: study design, interpretation of data, preparation of manuscript, critical revision of manuscript. Brasil AMS: Study literature review, data collect, preparation of manuscript. Morais LK: Study material contribution, literature review, critical revision of manuscript. Campion ER: study material contribution, interpretation of data, critical revision of manuscript. Taveira EJF: study material contribution, critical revision of manuscript. Rassi MC: study material contribution, critical revision of manuscript. All authors read and approved the final manuscript.

Quireze Junior C, Brasil AMS, Morais LK, Campion ER, Taveira EJF, Rassi MC. Metástase hepática colorretal metacrônica tem melhor prognóstico – é verdade? Arq Gastroenterol. 2018,55(3):258-63.

RESUMO – **Contexto** – As metástases hepáticas de câncer colorretal representam um importante problema de saúde pública devido à incidência crescente de câncer colorretal pelo mundo. A metástase hepática colorretal sincrônica está associada a pior sobrevida, no entanto, o pior prognóstico é assunto controverso. **Objetivo** – O objetivo do estudo foi avaliar a sobrevida livre de recorrência e a sobrevida global entre os grupos de pacientes com metástase hepática colorretal metacrônica e sincrônica. **Método** – Análise retrospectiva através de revisão de prontuários de pacientes com metástase hepática colorretal atendidos no período de 2013 a 2016, divididos em grupos metacrônico e sincrônico. Foram utilizados o modelo de regressão de Cox e o método de Kaplan-Meier com teste de Log-rank para comparação de sobrevida entre os grupos. **Resultados** – A média de sobrevida livre de recorrência no grupo metacrônico foi de 9,75 meses e 50% em 1 ano, e no grupo sincrônico 19,73 meses e 63,3% em 1 ano. A média de sobrevida global no grupo metacrônico foi de 20,00 meses e 6,2% em 3 anos, e no grupo sincrônico 30,39 meses e 31,6% em 3 anos. Os pacientes com metástase hepática metacrônica apresentaram pior sobrevida global em análise multivariada. O uso de drogas biológicas associadas ao tratamento quimioterápico foi relacionado ao melhor prognóstico em sobrevida global. **Conclusão** – A metástase hepática colorretal metacrônica foi associada a pior prognóstico na sobrevida global. Não houve diferença na sobrevida livre de recorrência entre as metástases metacrônica e sincrônica.

DESCRITORES – Neoplasias colorretais. Metástase neoplásica. Fígado. Prognóstico. Sobrevida.

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Acute gastroenteritis associated with norovirus GII.4 variants

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ABSTRACT – **Background** – Norovirus (NoV) is an important etiologic agent of acute gastroenteritis and infects individuals of all ages, especially children in Brazil and worldwide. NoV GII.4 was the most prevalent genotype worldwide because of your extensive genetic diversity. In Brazil, especially in the Northeast, few studies have been developed for identify and molecularly characterize NoV. **Objective** – The present study aimed to detect and describe the molecular epidemiology of NoV associated with acute gastroenteritis. **Methods** – The viral RNA extracted from stool samples were subjected to Nested RT-PCR and the genotypes were determined by nucleotide sequences analysis. In total, 278 stool samples assisted at Aliança Hospital in the city of Salvador, with acute gastroenteritis were examined, between March 2009 and July 2012. **Results** – A high NoV rate (54.2%) was identified in children under 5 years of age. We detected the circulation of different NoV GII.4 variants in Salvador, during the study period as Den Haag 2006b, New Orleans 2009 and Sydney 2012. **Conclusion** – These findings reinforce the need to study the molecular epidemiology of NoV infections in acute gastroenteritis.

HEADINGS – Norovirus. Gastroenteritis. Phylogeny.

INTRODUCTION

Norovirus (NoV) belonging to *Caliciviridae* family is recognized as a leading agents of acute nonbacterial gastroenteritis[1]. NoV infects individuals of all ages, especially children and the elderly, and rarely occurs with other viruses that cause gastroenteritis. NoV is a non-enveloped virus with icosahedral capsid morphology whose viral genome is a positive-stranded RNA of approximately 7.5 kb in length, organized into three open reading frames (ORF1-3). The ORF1 encodes a non-structural polyprotein, including RNA-dependent RNA polymerase, a highly conserved region used for the diagnosis and identification of NoV. ORF2 and ORF3 encode the major capsid protein (VP1) and the minor structural protein (VP2), respectively[2].

The NoV can be classified into five genogroups (GI-GV). Three of these genogroups (GI, GII and GIV) occur in human infections; however, most strains belong to GI and GII. The genogroups can be further subclassified into genotypes, with 8 genotypes found in GI (GI.1-GI.8) and 21 in GII (GII.1-GII.21). Among them, NoV GII.4 was the most prevalent genotype worldwide because of your extensive genetic diversity[3].

In Brazil, NoV was first reported in 1993 and since then, the presence of GI and GII has been reported in several regions of the country, in the North, Midwest, Southeast and Northeast[4,5,6,7,8,9]. NoV is an etiologic agent frequently reported in gastrointestinal disorders in Brazil and, especially in the Northeast, few studies have been developed for this purpose. Therefore, the aim of this study was to detect and molecularly characterize NoV in stools samples of patients from the city of Salvador, Bahia, Brazil.

METHODS

We examined 1,281 stool samples from patients with acute gastroenteritis assisted at Aliança Hospital in the city of Salvador, Bahia, between March 2009 and July 2012. Of these, 321 samples were positive by serology. From the serologically positive samples, the molecular tests were performed, 278 samples being positive by RT-PCR. The definition of acute gastroenteritis used in this study was the occurrence of diarrhea and other symptoms such as vomiting, fever and abdominal pain. The Ethics Committee for Human Research of Salvador University approved this study (protocol number 04.11.12).

Viral RNA was extracted from 140 mL of 10% stool supernatant using the QIAmp viral RNA kit (QIAGEN, Germany), according to the manufacturer's instructions and then subjected to a nested multiplex reverse transcription polymerase chain reaction (RT-PCR) assay. Nested RT-PCR was used for the detection of the NoV RNA polymerase gene. A first-round RT-PCR was performed using outer primers CAL-32 (5'-ATGAATATGAATGAGGATGG-3') and MO3-N (5'-TCAGATGGGTCTTCATGATTGG-3'). These primers span nucleotide positions 4490-5127 of the reference sequence (M87661: Hu/NoV/GI.1/Norwalk virus/1968/US) and amplify a 638-bp outer product. The second-round of PCR was done using inner primers JV-12 (5'-ATACCACTATGATGCAGATTA-3') and ACAL-36 (5'-GACAAAACAGAAGGACCAAT-3')[10]. These inner primers generate a 428-bp final nested fragment. Amplification was performed in a GeneAmp PCR System 2400 thermal cycler (Perkin Elmer). The first-round RT-PCR cycling conditions were as follows: 48°C/45 min (RT-step), 94°C/2min, followed by 35 cycles

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of 94°C/30s, 37°C/30s and 68°C/50s and one extension step of 68°C/7min. For the second-round PCR cycling, conditions were: 94°C/3min followed by 30 cycles of 93°C/30s, 37°C/15s, 72°C/30s plus a final extension step of 72°C/5min[10]. The PCR products were analyzed by electrophoresis in a 2% agarose gel and ethidium bromide staining (1mg/mL). PCR products were purified with QIAquick® PCR purification Kit (QIAGEN, Germany) according to the manufacturer's instructions and nucleotide sequencing of the PCR products was performed by ACTgene, Biotech Center of the Universidade Federal do Rio Grande do Sul (UFRGS), using the inner primers with Big Dye Terminator® and ABI-Prism 3500 sequencer (Applied Biosystems, USA). Nucleotide sequences were aligned and edited with Clustal W software available in the BioEdit program (http://mbio.ncsu.edu/bioedit.html) Sequence Editor 7.0, and then compared with the NoV sequences published in GenBank, using the Basic Local Alignment Search Tool (BLAST) application. Phylogenetic analysis was performed by the Maximum Likelihood method based on the Kimura two-parameter model with MEGA version 6 software[11]. Statistical support for the tree was evaluated by bootstrapping based on 1000 repetitions. Nucleotide sequences obtained from this study were deposited in GenBank under accession numbers KF307752.1, KF307753.1, KF307754.1, KF307755.1, KF307756.1, KF307757.1, KF265495.1, KF265496.1 and KF265497.1.

RESULTS AND DISCUSSION

Previously we reported the molecular detection of NoV in young adults[6] and in the present study we extended the identification of NoV in all age groups (TABLE 1). In total, 151 of the 278 stool samples were detected in children under 5 years old, identifying a high rate of infection in this age group (54.32%) and suggesting that this is a group more susceptible to NoV infection. The high incidence of NoV infection in children seems to be related to the low preexisting NoV-specific antibodies and the decline in the cases of rotavirus infection[12]. In Brazil, after the introduction of rotavirus vaccines, has been observed this decline and the NoV has been considered as the main etiological agent of viral gastroenteritis in children[13].

TABLE 1. Distribution of stool samples analyzed by age groups

Age group (years)	n	%
0-5	151	54.32
6-15	34	12.23
16-21	16	5.75
22-59	52	18.70
Above 60	25	9.00
Total	278	100

Several states in Brazil reported the presence of NoV based upon sequence data of NoV strains registered in GenBank. The genetic relationship among of these strains with others characterized in the present study was established by phylogenetic analysis. The phylogenetic tree was based on the K2 model (Kimura 2-parameter) of nucleotide substitution and similarity value - InL= -787.546 (FIGURE 1).

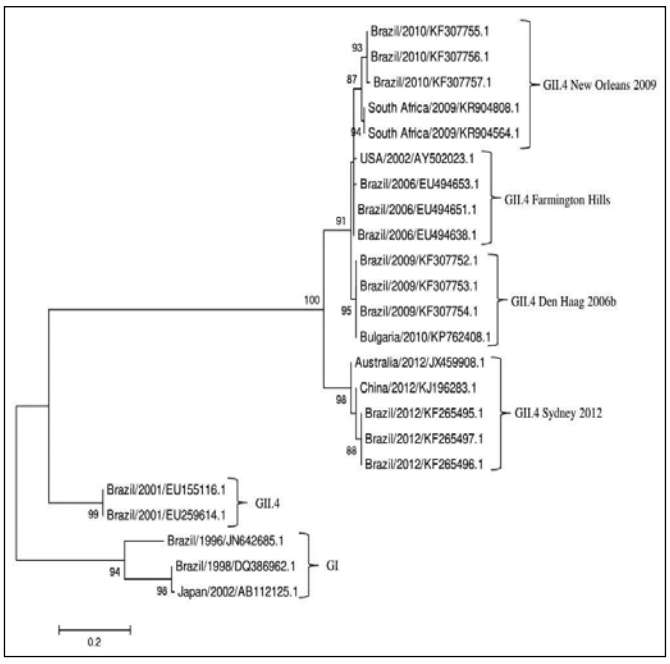

FIGURE 1. Phylogenetic tree based on a partial fragment of the RNA-dependent RNA polymerase gene on human Norovirus GI and GII. Strains denomination: state of origin/year of collection/GenBank access number.

Based on this model, the phylogenetic tree revealed three distinct phylogenetic groups. The GI group was used as an out group and the strains of the GII.4 group displayed divergences in sequence alignment which led to the separation into two distinct groups. Previously, we reported the genetic diversity of NoV and suggested that GII.4 is a predominant circulating genotype in NoV outbreaks in Brazil[6]. The predominance of GII.4 genotype was also reported in other study in Bahia with children up to 3 years of age with acute gastroenteritis[7].

The strains identified in the present study (Brazil/2009/KF307752.1, Brazil/2009/KF307753.1, Brazil/2009/KF307754.1, Brazil/2010/KF307755.1, Brazil/2010/KF307756.1, Brazil/2010/KF307757.1, Brazil/2012/KF265495.1, Brazil/2012/KF265496.1, Brazil/2012/KF265497.1) and previous identified by our group in 2006 (Brazil/2006/EU494638.1, Brazil/2006/EU494651.1, Brazil/2006/EU494653.1) formed a distinct, well-supported clade (bootstrap value D 100). Interestingly, the phylogenetic position of these strains obtained in Salvador demonstrated that these strains have similarity with GII.4 variants circulating around the world. Since 1995, NoV GII.4 was the most prevalent genotype worldwide because of your extensive genetic diversity and every 2 to 3 years new GII.4 variants have emerged, such as US95/96 strain in 1995, Farmington Hills in 2002, Hunter in 2004, GII.4 Den Haag2006b in 2006, New Orleans 2009 in 2009 and Sydney 2012 in 2012[14,15]. In Brazil, different variants were detected circulating in Northeast, South and Southeast regions during the period 2004-2012. The first variant detected was Asia 2003 in 2004 (described as a recombinant strain), followed by Hunter 2004, Farmington Hills, Yerseke 2006a, Den Haag 2006b, New Orleans 2009 and Sydney 2012. However, it was observed that the variants circulating in Brazil spread and were replaced by different dynamics[16].

In this study, the phylogenetic tree topology shows that the strains previously identified in Bahia in 2006 (Brazil/2006/

EU494638.1, Brazil/2006/EU494651.1, Brazil/2006/EU494653.1) form a cluster with the strain USA/2002/AY502023.1, defined in GenBank as GII.4 Farmington Hills. The strains circulating in 2009(Brazil/2009/KF307752.1, Brazil/2009/KF307753.1, Brazil/2009/KF307754.1) form a cluster with the strain Bulgaria/2010/KP762408.1 (bootstrap value D 95), suggesting that these strains are Den Haag 2006b. This variant was detected circulating for the first time in Brazil in 2006, was the predominant strain during the period of 2006-2008 and was observed co-circulating with Yerseke 2006a during 2008-2009[16]. The strains circulating in 2010 (Brazil/2010/KF307755.1, Brazil/2010/KF307756.1, Brazil/2010/KF307757.1) form a cluster with a New Orleans 2009 variant (South Africa/2009/KR904808.1 and South Africa/2009/KR904564.1). In Brazil this variant was first detected in 2009 and was observed circulating until 2011, being subsequently replaced by the Sydney 2012 variant in 2012[16], as observed in this study, where strains circulating in 2012 (Brazil/2012/KF265495.1, Brazil/2012/KF265496.1, Brazil/2012/KF265497.1) form a cluster with China/2012/KJ196283.1 and Australia/2012/JX459908.1 (bootstrap value D 98).

CONCLUSION

This is the first report of NoV GII.4 variants in Bahia. The identification of different NoV strains is a significant public concern, considering that the new strains are often associated with new outbreaks of gastroenteritis. In addition, they are often more virulent than the previous one and may lead more severe clinical manifestations, mainly in children, elderly and immunocompromised patients. These findings reinforce the need to study the molecular epidemiology of NoV infections in acute gastroenteritis. Further studies are necessary in order to identification the NoV in other parts of the country and identificate the genetic variability of this virus. Finally, the development of a national database of Brazilian strains of NoV could provide greater insight into the virus and its epidemiological impact on the population, as seen in the European countries that have the NoroNet, a database who share surveillance and research data on Norovirus.

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Authors' contribution

Sardi SI and Campos GS: conception and design of research; Paula FL: performed experiments, analyzed data, interpreted results of experiments, drafted manuscript; Tigre, DM and Fernandes FMC: analyzed data, interpreted results of experiments; Paula FL, Sardi SI and Campos GS: edited, revised and approved final version of manuscript.

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RESUMO – **Contexto** – Norovírus (NoV) é o agente etiológico mais importante nas gastroenterites agudas e infecta indivíduos de todas as idades, especialmente crianças no Brasil e no mundo. O NoV GII.4 é o genótipo mais prevalente em todo o mundo devido a sua elevada diversidade genética. No Brasil, principalmente no Nordeste, poucos estudos têm sido desenvolvidos a fim de identificar e caracterizar molecularmente o NoV. **Objetivo** – O presente estudo teve como objetivo detectar e descrever a epidemiologia molecular do NoV associado com gastroenterite aguda. **Métodos** – RNA viral extraído a de amostras de fezes foi submetido a amplificação por Nested-RT-PCR e o genótipo determinado por analise da sequência de nucleotídeos. Um total de 278 amostras de pacientes atendidos no Hospital Aliança, na cidade de Salvador, com gastroenterite aguda foram examinados, entre março de 2009 a julho de 2012. **Resultados** – Uma alta taxa de NoV (54,2%) foi identificado em crianças de até 5 anos de idade. Detectou-se a circulação de diferentes variantes de NoV GII.4 em Salvador, durante o período do estudo, tais como Den Haag 2006b, New Orleans 2009 e Sydney 2012. **Conclusão** – Estes achados reforçam a necessidade de maiores estudos para esclarecer a epidemiologia molecular das infecções por NoV em casos de gastroenterite aguda.

DESCRITORES – Norovirus. Gastroenterite. Filogenia.

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Hepatitis B and C in pregnant women attended by a prenatal program in an universitary hospital in Rio de Janeiro, Brazil: retrospective study of seroprevalence screening

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ABSTRACT – **Background** – Hepatitis B and C are diseases with high morbimortality and constitute a global public health problem. In Brazil, the prevalence is not homogeneous, oscillating among different regions, but it is estimated that currently about 1% of the population present chronic disease related to the B virus and that there are 1.5 million infected with the C virus. Despite the development of hepatitis B vaccine, improvement in diagnostic methods and therapeutic advances in the field of viral hepatitis, there is still a large number of people who continues to be infected by these viruses, especially in populations at risk and also due to several factors, including vaccination and migration policies. Vertical and perinatal transmissions are of great importance in the epidemiology of viral hepatitis and the blood tests performed during prenatal care constitute a great opportunity for screening and identifying these viruses. **Objective** – To evaluate the seroprevalence of markers for B and C viruses in women who underwent prenatal care at the *Hospital Universitário Antônio Pedro (Antonio Pedro University Hospital)* from 2006 to 2013 and to compare the results found with regional data and those described in the specific literature. **Methods** – A descriptive, cross-sectional, quantitative study with retrospective data collected from 635 records of pregnant women attended at the Prenatal Service of the *Hospital Universitário Antônio Pedro*, Niterói, state of Rio de Janeiro, from March 2006 until December 2013. The database was built in the Microsoft Office Access program and was later exported to Microsoft Office Excel. For the processing and analysis of the data, it was used the SPSS (Statistical Package for Social Science, IBM) version 22.0, for Windows. **Results** – Twelve cases with positive HBsAg (1.9%), 189 cases with positive anti-HBs (35.9%) and seven positive anti-HCV patients (1.3%) were observed. There was no significant association between age and positivity for HBsAg, anti-HBs and anti-HCV (P =0.205, 0.872 and 0.676, respectively). There was a direct relationship between the anti-HBs positivity and the last four years of the study (P<0.0001). **Conclusion** – A high prevalence of HBsAg was observed, higher than the expected for the evaluated region; there was a prevalence of anti-HCV, consistent with the current Brazilian reality; and a likely low rate of hepatitis B immunization, with a relatively high rate of susceptibility to this infection and no case of co-infection between B and C viruses and HIV. It is emphasized not only the need to trace hepatitis B and C, without exceptions, during prenatal care, since even though the current advances in therapy may not cure, at least they may allow a better quality of life for patients with chronic disease and the mandatory completion of immunoprophylaxis in all newborns. Special attention should be given to those patients susceptible to HBV, with prompt diagnosis and referral for specific vaccination.

HEADINGS – Seroepidemiologic studies. Hepatitis B. Hepatitis C. Pregnancy. Pregnant women.

INTRODUCTION

Hepatitis B virus (HBV) and C virus (HCV) are diseases with high morbimortality. HBV infection continues to be a global public health problem, with changes in its epidemiology due to several factors, including vaccination and migration policies. Approximately two billion people have been infected by HBV, and 20 million are infected annually, existing about 240 million chronic carriers of this virus. The variable geographic prevalence is highest in Africa and Asia[1,2].

In Brazil, despite the introduction of the vaccine in the Western Amazon in 1989 and progressive efforts of immunization and prevention by the *Sistema Único de Saúde* (SUS), hepatitis B transmission is still a reality. The country was previously considered to be of intermediate endemicity, with great heterogeneity among regions and areas of high prevalence, especially in the Amazon Basin. Between 2004 and 2009, a populational study reported lower prevalence in all regions of Brazil. The most recent results indicated a reduced prevalence of hepatitis B throughout the country, classifying Brazil as being of low endemicity. Most studies showed a prevalence of HBV inferior to 1%[3-7].

Although there is a HCV pandemic, its distribution is variable in different regions of the world. The World Health Organization (WHO) estimates that 3% of the world's population is chronically infected with this virus and that 3–4 million people are infected each year, with 130–170 million chronic carriers at high risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. Approximately 350,000 deaths occur each year due to HCV-related

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liver disease. Although HCV infection incidence rate is apparently slowing in the developed world, deaths from liver diseases secondary to HCV will continue to increase over the next 20 years[8-10].

Brazil, with about 1.5 million chronically infected people, is considered by the WHO as a country with intermediate density. The country has a vast territory with unequal population density and HCV incidence rates are variable, with the majority of cases concentrated in the most populated areas. Population-based and blood donor studies revealed lower prevalences than estimated, placing Brazil as low endemicity[4,11]. From 1999 to 2016, 319,751 cases of hepatitis C were detected in the country with anti-HCV or HCV-RNA reagents and 155,032 cases considering those that displayed both markers positive. Most of these cases were observed in the Southeast (24.5%) and South (5.5%) regions. This detection rate with anti-HCV and HCV-RNA reagents displayed a growth trend between 2003 and 2016 in all Brazilian regions. From 2015 on, cases were confirmed using only HCV-RNA, offering a more precise definition of an infection case, and consequently increasing the detection rate in all regions of the country. The number of deaths related to this etiology has increased over the years in all regions of Brazil[4].

The prevalence of HBV and HCV in a population is related to risk factors associated with the transmission, such as blood transfusion and blood products, dental and surgical procedures, use of tattoos, accidents with biological material, use of injectable substances, besides the sexual and vertical routes[12,13].

Maternal-fetal transmission is of great importance in the epidemiology of viral hepatitis. In children of HBV infected mothers, vertical transmission carries a risk of progression to chronicity in 70%–90% of the cases[14,15].

The present study had as objectives: to determine the seroprevalence of hepatitis B and C in the prenatal patients of the *Hospital Hospital Universitário Antônio Pedro*, Niterói, Rio de Janeiro; to compare the data found with the regional data and those described in the specific literature and to provide demographic, clinical and epidemiological data to the literature that may be used for the formulation of policies in the area.

There are few studies in national and international literature addressing the prevalence of hepatitis in pregnant women. As this is a still worldwide worrying and the early detection in prenatal care can avoid a series of deleterious outcomes for the pregnant woman and its child, the concern of deepening the study in this area arose with a more accurate evaluation of the epidemiological profile of pregnant women from the region. Thus, prenatal exams provide a great opportunity for screening and identifying these viruses in this population that could serve as a natural reservoir for future generations.

METHODS

A cross-sectional, descriptive study of the quantitative type with retrospective data collection was carried out, with medical records of pregnant women who attended the Prenatal Service of the *Hospital Universitário Antônio Pedro* (HUAP) from March 2006 to December 2013. The hospital, located in Niterói, attends patients from the Metropolitan Region II of Rio de Janeiro, which also includes the municipalities of São Gonçalo, Maricá, Itaboraí, Tanguá, Silva Jardim and Rio Bonito. Appointments were made with the MV2000 – Hospital information system, used at HUAP since 2006. The study period ran from March 2006 to December 2013. The data were collected from prenatal records, via previously validated protocols.

All the records of pregnant women registered in the system were eligible for inclusion in the study, reaching a total of 13,771 attendances (first and subsequent prenatal appointments). Finally, 635 randomly generated records were selected by querying the Oracle database of the MV2000 system. General variables such as age, municipality of origin, year of follow-up, gestational age, prenatal risk, number of visits performed during prenatal care, previous obstetric and clinical history, intercurrences during pregnancy or delivery, beginning of sexual life, number of partners, gestational outcome, and specific variables such as history of vaccination for hepatitis B and the results of viral markers for hepatitis B and C. As for age, patients were categorized into three groups: under 20, 20 to 35 and over 35 years of age. For the purpose of this study, the frequency of prenatal appointments was categorized into three groups and considered "insufficient" (less than six visits), "adequate" (six to ten visits) and "optimal" (more than ten visits). Regarding the number of pregnancies, the women were divided into three groups: primigravidae (single gestation), multiparous (between two and three gestations) and large multiparous (above three gestations). The gestational outcomes considered were vaginal delivery, cesarean section, abortion and ignored (delivered elsewhere than in HUAP and not reported in the medical record). In "reported vaccination" there were three possible answers: yes, no, unknown (not mentioned in the medical records). The viral markers were defined as positive, negative and absent (not requested or the result was not documented in the medical record). The database was built in the Microsoft Office Access program and was later exported to Microsoft Office Excel. For the processing and analysis of the data, it was used the SPSS (Statistical Package for Social Science, IBM) version 22.0, for Windows. For the age, the average, standard deviation (SD), median and confidence interval (CI) were calculated. This variable was also explored by verifying the distribution of normality, to test the established hypotheses. From the Kolmogorov-Smirnov test it was defined that the Mann-Whitney test would be used to verify the existence of significant differences between the ranked averages of the ages in the groups of viral markers, positive and negative. The Kruskal-Wallis tests with Tukey's post-Hoc test were used to verify if there was a difference between the years of research (eight categories) and the age of the pregnant women. Categorical variables were analyzed via relative and absolute frequency tables. To verify the possible association between these variables, the Chi-square test was conducted, employing the Fisher's exact test. In addition to the verification of the *P*-value, in the binary tabulations, the odds ratio was established observing the absence of the number 1 in the CI 95%. All tests presented $P<0.05$. The research complied with Resolution 196/1996, which deals with research with human beings and was approved by the Research Ethics Committee of the *Universidade Federal Fluminense* (UFF), under opinion 977.674/2015 and CAAE 41681014.6.0000.5243.

RESULTS

The age of pregnant women varied from 12 to 46 years, with median and average of 27 years (26.4–27.5), with standard deviation ±6.92 (SD). In relation to the age groups, there were 98 women under the age of 20, 465 women between 20 and 35 years and 72 of them over the age of 35 years. There was no significant difference between the age of the patients during the years of research ($P=0.384$). The

number of appointments ranged from 1 to 16, with an average of 7. An adequate number of appointments predominated in women over 35 years of age. The number of pregnancies ranged from 1 to 8 (average of 2.3). From 469 completed deliveries, 164 (34.9%) were vaginally and 305 (65.1%) were delivered via cesarean section. The main indications of cesarean section were: iterativity, cephalopelvic disproportion and acute fetal distress. The clinical-demographic characteristics of the study population are summarized in TABLE 1.

TABLE 1. Clinical-demographic characteristics of pregnant women attended by age group in HUAP during the 2006-2013 period.

Variables	Age Group			Total
	< 20 years (N=98)	20-35 years (N=465)	>35 years (N=72)	
Research year	n (%)	n (%)	n (%)	n_t (%)
2006	17 (17.3)	71 (15.2)	9 (12.5)	97 (15.3)
2007	21 (21.5)	84 (18.2)	14 (19.5)	119 (18.7)
2008	10 (10.2)	62 (13.3)	11 (15.3)	83 (13.1)
2009	10 (10.2)	36 (7.7)	5 (6.9)	51 (8.1)
2010	13 (13.3)	47 (10.2)	5 (6.9)	65 (10.2)
2011	6 (6.1)	58 (12.5)	12 (16.7)	76 (12.0)
2012	11 (11.2)	57 (12.2)	11 (15.3)	79 (12.4)
2013	10 (10.2)	50 (10.7)	5 (6.9)	65 (10.2)
Municipality of origin	n (%)	n (%)	n (%)	n_t (%)
Niterói	42 (42.8)	208 (44.7)	35 (48.6)	285 (44.9)
São Gonçalo	38 (38.8)	176 (37.8)	26 (36.1)	240 (37.8)
Others	18 (18.4)	81 (17.5)	11 (15.3)	110 (17.3)
At risk	n (%)	n (%)	n (%)	n_t (%)
Yes	66 (67.3)	322 (69.2)	65 (90.3)	453 (71.3)
No	32 (32.7)	143 (30.8)	7 (9.7)	182 (28.7)
Gestations	n (%)	n (%)	n (%)	n_t (%)
1	72 (73.4)	124 (26.7)	7 (9.7)	203 (32.0)
2-3	23 (23.5)	244 (52.5)	39 (54.2)	306 (48.2)
>3	3 (3.1)	97 (20.8)	26 (36.1)	126 (19.8)
Appointments	n (%)	n (%)	n (%)	n_t (n_t%)
<6	44 (44.9)	150 (32.3)	18 (25.0)	212 (33.4)
6-10	40 (40.8)	229 (49.2)	40 (55.5)	309 (48.6)
>10	14 (14.3)	86 (18.5)	14 (19.5)	114 (18.0)
Outcome	n (%)	n (%)	n (%)	n_t (%)
Vaginal delivery	32 (32.6)	114 (24.5)	18 (25.0)	164 (25.8)
Cesarean section	35 (35.7)	231 (49.7)	39 (54.1)	305 (48.0)
Abortions	3 (3.1)	7 (1.5)	3 (4.2)	13 (2.1)
Ignored	28 (28.6)	113 (24.3)	12 (16.7)	153 (24.1)
Intercurrences	n (%)	n (%)	n (%)	n_t (%)
Yes	38 (38.8)	154 (33.1)	25 (34.7)	217 (34.2)
Not reported	60 (61.2)	311 (66.9)	47 (65.3)	418 (65.8)

N: number of patients evaluated; n: total per variable; n_t(%) total per variable/percentage of the total number of patients evaluated (N=635).

As for the viral markers, 12 (12/631) returned positive HBsAg (1.9%), 189 (189/526) positive anti-HBs (35.9%) and seven (7/560) positive HCV (1.3%). The response to HBV vaccination was positive in 23 (3.6%) patients and negative in 35 (5.5%). This information was not included in 577 (90.9%) medical records. The results of the hepatitis B and C markers and the vaccination history for HBV and markers per year of this survey can be seen in TABLE 2 and FIGURE 1 respectively.

TABLE 2. Serological markers of hepatitis B and C and vaccination history against hepatitis B virus by age group evaluated. HUAP/2006-2013.

Variáveis	Age Group			Total
	< 20 years (N=98)	20-35 years (N=465)	>35 years (N=72)	
HBsAg	n (%)	n (%)	n (%)	n_t (%)
Positive	1 (1.0)	11 (2.4)	-	12 (1.9)
Negative	97 (99.0)	451 (97.0)	71 (98.6)	619 (97.5)
Not listed	-	3 (0.6)	1 (1.4)	4 (0.7)
Anti-HBs	n (%)	n (%)	n (%)	n_t (%)
Positive	38 (38.8)	139 (29.9)	12 (16.7)	189 (29.8)
Negative	44 (44.9)	246 (52.9)	47 (65.3)	337 (53.1)
Not listed	16 (16.3)	80 (17.2)	13 (18.0)	109 (17.2)
Anti-HCV	n (%)	n (%)	n (%)	n_t (%)
Positive	1 (1.0)	5 (1.1)	1 (1.4)	7 (1.1)
Negative	83 (84.7)	408 (87.7)	62 (86.1)	553 (87.1)
Not listed	14 (14.3)	52 (11.2)	9 (12.5)	75 (11.8)
Vacinação contra HBV	n (%)	n (%)	n (%)	n_t (%)
Yes	5 (5.1)	17 (3.7)	1 (1.4)	23 (3.6)
No	7 (7.1)	26 (5.6)	2 (2.8)	35 (5.5)
Unknown	86 (87.8)	422 (90.7)	69 (95.8)	577 (90.9)

N: number of patients evaluated; n: total per variable; n_t(%): number of cases found in the total number of cases evaluated for each variable.

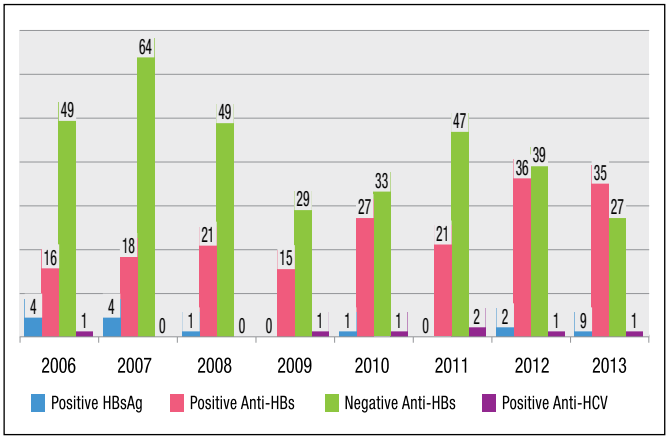

FIGURE 1. Result of the main viral markers per year of research. HUAP/2006–2013.

The positive HBsAg cases predominated in the 20–35-year-old age group (90%), with an average of 26 years, but according to the Mann-Whitney test, the difference between the age groups was not significant (P=0.205). There was a prevalence of positive HBsAg cases in the years 2006, 2007 and 2012 (over 80%) – FIGURE 1. According to the number of pregnancies, 41% were primigravidae and 58% were multiparous. Regarding the history of previous vaccination, six women denied having been vaccinated for hepatitis B and in six cases this fact was unknown. Other possible risk factors for HBV infection were identified in only three cases: habitual use of injectable medication due to chronic disease, drug-using sexual partner and mother with hepatitis B, the latter suggesting vertical HBV transmission. The diagnosis in 67% of the cases was carried out in prenatal care. It was possible to identify, according to reports in the medical records, four cases of inactive patients with HBsAg, one of acute hepatitis already in convalescence and two of patients with chronic hepatitis, one positive HBeAg and the other negative HBeAg. There was no case of co-infection with HCV.

Anti-HBs were positive in 189 cases (34.2%), with predominance in the 20–35 years age group (73.5%), with the average age in this range being 26.1 years. These positive results were more observed from the year 2010 onwards, especially in the 20–35 years age group and 47% of cases from 2006 to 2013. Only in 2013 the number of cases with positive anti-HBs was higher than that of negative anti-HBs (FIGURES 1 AND 2). There was a direct relationship between positive anti-HBs and the last four years (2010–2013) of the study (P<0.001; OR=2.22; 95%CI: -1.543–3.205), but this relationship was not confirmed for the variable "age", when compared with the years of research (P=0.872). There is a direct relationship between the anti-HBs positivity and the positive history for previous vaccination against hepatitis B (P=0.001; OR=8,125; CI 95%= 2.208–29.901). FIGURE 3 displays the vaccination history for HBV and the relationship with the result of anti-HBs over the years of research.

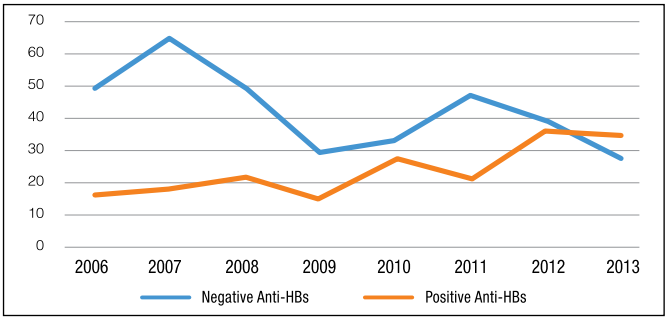

FIGURE 2. Outcome of anti-HBs over the years of research. HUAP/2006–2013.

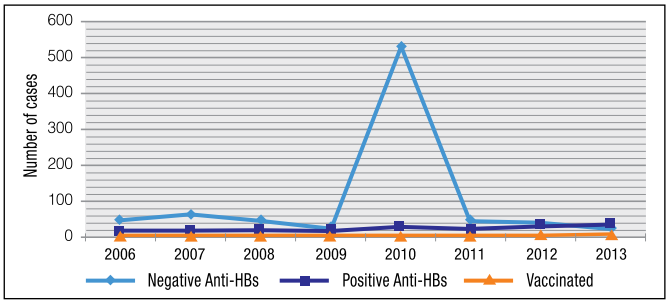

FIGURE 3. Relationship between positive history for vaccination and positive and negative Anti-HBs. HUAP/2006–2013.

In the first three years of the study period (2006–2009) anti-HBs were negative in 162 cases, representing 48% of the negative cases in these years. From 2009 onwards, negative anti-HBs were proportionally less frequent, although peaks were observed in 2011 and 2012 (FIGURE 3). There was no significant difference between the average age of patients that returned positive and negative for anti-HBs (P=0.907).

Seven patients with HCV-positive serologies (1.3%) were identified, and five cases (71.4%) were diagnosed in the prenatal period. The age ranged from 18 to 46 years, with a mean of 28.8 years and blood transfusion was considered a risk factor for three patients, and it was not possible to identify the presence of risk factors for the remaining cases.

DISCUSSION

In the pregnant women group, average maternal age was 27 years, outlining the profile of pregnant women as a young population that, from a reproductive point of view, is considered optimal because it represents a lower perinatal risk[16]. There was a significant percentage of pregnant adolescents (younger than 20 years) who reached 15.4% of the total number of women evaluated. Despite falling rates, the incidence of pregnant adolescents in Brazil is considered high, and it is estimated that 20%–25% of all pregnant women in Brazil are adolescents[17,18]. Manfré and collaborators[19] observed in a study on Latin America that among 25% of the poorest population, one in three births originates from an adolescent mother, increasing this proportion even more in rural areas. Pregnancy in this population group has been considered a public health problem in some countries, since it can lead to obstetric complications, with repercussions for the mother and the newborn, as well as psychosocial and economic problems[20].

Late pregnancies (older than 35 years) have been increasing in Brazil and in the world, demonstrating a trend that is taking place mainly in industrialized countries and is considered a pre-existing gestational risk factor by the Ministry of Health, requiring special attention in prenatal care. Late pregnancy was observed in 11.3% of the women studied, lower than the 13.0% found in the literature[21,22].

The fertility rate, which is an estimate of the number of children a woman has throughout her life, has been declining in Brazil since the end of the 1960s, from 2.4 to 1.8 in the period from 2000 to 2015, but remained higher in the rural area, with about 2.7 children per woman. The Southeast Region has the lowest national average, equal to 1.75. Factors such as the expansion of urbanization, sexual education and family planning, with more widespread use of contraceptive methods, greater participation of women in the labor market, and higher costs of raising and educating children appear to be contributing to this decline. In the present study there was a predominance of multiparous, with the average number of pregnancies found in the evaluated period equal to 2.3, higher than that found for the Southeast region according to the demographic census of 2010[23].

The definition of an ideal number of appointments is important for the elaboration of health care protocols and resource planning. The Prenatal and Birth Humanization Program makes it possible to classify as appropriate every prenatal follow-up initiated up to the fourth month of gestation with six or more appointments, one in the first trimester, two in the second and three in the third trimester[24]. The last National Demography and Health Survey, conducted in 2006, revealed a 80.9% coverage for prenatal care with

six or more visits[25]. The average score was equal to seven visits, with 66.7% of the cases with more than six queries defined in this study as an optimal number, although this proportion is lower than the one observed in Brazil[26]. Inferior proportional adherence to the appointments was observed in the age of less than 20 years, and this fact was also demonstrated in studies conducted in other Brazilian regions, which showed an association between pregnancy in adolescence and a smaller number of prenatal appointments[27]. The highest percentage of appointments with a number greater than six (75.0%) were found in the group above 35 years of age, probably because the pregnancies of elderly women have been considered as high risk, mainly due to increased incidence of hypertensive syndromes, premature rupture of membranes, presence of diabetes, and a greater chance of complications for the newborn[28].

There was a predominance of cesarean delivery in this study, 65% of cases, even though WHO recommends that this percentage should not exceed 15%. The prevalence of cesarean section over vaginal delivery occurred in all age groups evaluated. The high rate of cesarean deliveries in the Brazilian context stands out as alarming, especially when compared to data of countries with low rates (14.0%–18.0%) such as the Netherlands, Czech Republic, Slovakia, Norway and Sweden and even those with rates considered high (30.0%–33.0%) such as the United States, Portugal, Australia, Korea, Italy and Mexico[29]. It is important to note that the HUAP prenatal outpatient clinic is a reference for pregnancy with any type of risk, obstetric or not, and that, of the cases studied here, more than 71% were pregnant women considered to be at risk.

The prenatal blood tests represent a valuable opportunity for the diagnosis of hepatitis B and its manifestations, contributing to the decrease of the prevalence of this disease and its complications. Machado Filho et al. reported a variation of the prevalence of HBsAg from 0.3% to 1.7% in Brazil, except in some regions of the Amazon. Similar data are found in other countries, except in regions of Africa and Asia where prevalence can reach up to 15%.[30] In an epidemiological study conducted in Brazil, the overall prevalence of the HBsAg marker for the Brazilian capitals was 0.37%[6].

In pregnant women, the prevalence of hepatitis B varies according to the endemicity of the infection in the geographic region and population studied. However, there are few studies on the screening of the disease in the gestational period in Brazil, especially in the state of Rio de Janeiro. Souza et al. reported a prevalence of chronic HBV among Brazilian pregnant women ranging from 0.5% to 8.7%, depending on the region studied[5].

Twelve women positive for HBsAg were detected in this study, with an estimated prevalence of 1.9%. This prevalence varied according to the years of research when the cases were observed (4.1% in 2006, 3.4% in 2007, 1.2% in 2008, 1.5% in 2010 and 2.5% in 2012). Contrary to the literature[31], in this study there was no growth in its prevalence with the increase in age.

In the Rio de Janeiro state, the detection rate of Hepatitis B showed significant growth until 2009, displaying a decrease in 2010, with a new increase in 2011, exhibiting a new downward trend as of 2012[32].

The prevalence of HBV presented here has important implications for both health managers and for the effectors of the health service, especially physicians involved in its care, and emphasize the importance of carrying out similar surveys in this region.

Studies on the vertical transmission of hepatitis B are rare in Brazil, since the HBsAg research began to compose the prenatal routine only after 2005[33]. The Ministry of Health, through the Epidemiological Bulletin of Viral Hepatitis, presented the vertical transmission of hepatitis B as the third most frequent category of infection, only below the sexual and domiciliary contact, and the reported transmission rate was 5.9%[4].

In the presence of chronic HBV, a greater incidence of maternal and fetal complications has been reported if compared to the rest of the population, but these data are still conflicting[34]. In five cases of positive HBsAg patients, complications related specifically to gestation (retrocorionic hematoma, oligohydramnios and premature amniorrexis) were found. In relation to newborns, prematurity was reported in three cases. There were insufficient data in the records to assess possible cases of HBV via vertical transmission.

In the absence of maternal antiviral treatment or immunoprophylaxis when indicated, the chronification percentage of HBV-infected newborn by vertical transmission may reach 90%, with an increased risk of developing disease-related complications at an early stage in life[7,15,35]. In the 1990s, WHO recommended that hepatitis B vaccination must be incorporated into national vaccination programs. Also in this same decade the vaccine was offered by the SUS. Since 1998, the National Immunization Program of the Ministry of Health has begun to recommend universal vaccination of children from birth[36].

Anti-HBs were positive in 189 pregnant women. This positivity was more observed as of 2010, especially in the 20–35 age group, and 2013 was the only year in which the number of cases with positive anti-HBs was higher than those with negative anti-HBs. This direct relationship between the anti-HBs positivity and the last years of the research was statistically significant ($P<0.0001$) and probably related to the improvement of vaccination coverage in the country in recent years.

The low relative immunization rate observed in this population of pregnant women requires better investigation and should be clarified if it was due to an inadequate medical recommendation for vaccination or the noncompliance of the patients to this recommendation, or if it was the result of a possible defective immune response to the vaccination.

Regarding HCV in the evaluated group, seven patients with positive anti-HCV were identified, with a global prevalence estimated at 1.3%. The prevalence found in this research was higher than those reported by Nunes and collaborators (0.1%)[37], Rodrigues Neto et al. (0.3%)[38] and Carvalho et al. (0.9%)[39], but similar to that found in a 2010 study led by researchers from University of Pernambuco[40], in which the prevalence of all Brazilian capitals was 1.38%. Licata and collaborators[41] reported a prevalence among pregnant women between 1% and 2% in the United States and Europe but could reach up to 8% in developing countries.

Studies in Brazil did not detect differences between the prevalence of hepatitis C in pregnant women and in the general population[42]. However, these prevalences may be underestimated since the practice of screening was adopted only in pregnant women belonging to groups that are more likely to be infected and may ignore a large number of HCV cases[43]. According to WHO, Brazil is considered a country of intermediate endemicity. However, population-based and blood donor studies revealed lower prevalences than those estimated, placing Brazil as low endemicity[4,5,7]. This divergence is probably explained by the use of seroprevalence studies in specific groups that do not necessarily represent the general population[39].

The age of positive anti-HCV patients in the present study ranged from 18 to 46 years, with an average of 28.8 years (SD

±9.04). Pereira and collaborators[44] reported an increase in exposure to HCV with age. Oliveira et al.[45] also observed an association between positive anti-HCV and age over 40 years. In their study, Wang and colleagues[46] suggested that hepatitis C affects people of all ages, but there is a peak incidence between 20 and 39 years of age and a higher prevalence rate in the 30–49 years range. In the casuistry of the present study, six patients were older than 25 years and all were born before 1989 and no statistically significant difference was observed in age and anti-HCV positivity (P=0.676).

As in the case of hepatitis B, hepatitis C transmission is relatively stable in Brazil in terms of number of new cases diagnosed each year during the period between 2004 and 2014, except for 2013. The majority of cases reported in Brazil are concentrated in the Southeast and South regions[4]. In any case, the HCV in pregnant women addresses the need for a routine investigation of this infectious disease during prenatal care. Vertical HCV transmission is less frequent than that of HBV and might encompass between 3% and 10% of the cases[47,48]. The patients in this study had no complications during pregnancy or childbirth attributable to HCV. Intercurrences with newborns were also not reported.

FINAL CONSIDERATIONS

This is the first study that evaluates the seroprevalence of hepatitis B and C in pregnant women attended in the city of Niterói (RJ) and, although it is limited to the population of pregnant women attended at HUAP and does not represent the population as a whole, it can serve as a base for the adoption of public policies in the municipal and state scenario, contributing to changes. A high prevalence of HBV, above that expected for the region, was observed; a prevalence for HCV consistent with the current Brazilian reality; a low rate of hepatitis B immunization, with a high index of susceptibility to this infection; and no case of co-infection.

The availability of the vaccine against HBV and the appearance of new drugs that can control the chronic disease, besides the knowledge of measures that prevent transmission, including vertical transmission, has a real chance of controlling this disease.

To prevent the prevalence of HCV from continuing to increase worldwide, a global effort is needed to strengthen care for primary prevention, including the development of vaccines and new approaches to secondary and tertiary prevention, reducing cases of chronic liver disease and improving the survival of the carriers.

There is a need to encourage research on the prevalence and incidence of these diseases in pregnant women, in order to plan strategies for prevention, prophylaxis and treatment of these infections, avoiding the damaging effects on the mother and her child and consequently raising costs to the health system.

It is important to carry out more research in pregnant women in this area as well as the prenatal screening of hepatitis B and C, with no exceptions, since even though the current advances in therapy may not cure, at least they may allow a better quality of life for patients with chronic disease. It is also emphasized the need for mandatory immunoprophylaxis for all newborns and special attention should be given to those patients susceptible to HBV, with prompt diagnosis and referral for specific vaccination.

Authors' contribution

Barros MMO performed the research and wrote the paper; Ronchini KROM and Soares RLS designed the research and analyzed the data.

Barros MMO, Ronchini KROM, Soares RLS. Hepatite B e C em gestantes atendidas em um programa de pré-natal em um hospital universitário no Rio de Janeiro, Brasil: estudo retrospectivo de rastreamento da soroprevalência. Arq Gastroenterol. 2018,55(3):267-73.

RESUMO – **Contexto** – As hepatites pelo vírus B e C são doenças com elevada morbimortalidade e um problema de saúde pública global. No Brasil a prevalência não é homogênea, variando entre as diferentes regiões, mas estima-se que atualmente cerca de 1% da população apresente doença crônica relacionada ao vírus B e que haja 1,5 milhões de infectados pelo vírus C. Apesar do desenvolvimento da vacina contra a hepatite B, da melhoria nos métodos diagnósticos e dos avanços terapêuticos no campo das hepatites virais, ainda é grande o número de pessoas que continuam sendo infectadas por esses vírus, principalmente nas populações sob algum tipo de risco e devido a vários fatores incluindo políticas de vacinação e migração. A transmissão vertical e também a perinatal têm grande importância na epidemiologia das hepatites virais e os exames realizados durante o pré-natal constituem uma oportunidade única de rastreio e identificação destes vírus. **Objetivo** – Avaliar a soroprevalência de marcadores para os vírus B e C em mulheres que realizaram a assistência pré-natal no Hospital Universitário Antônio Pedro no período de 2006 a 2013 e comparar os resultados encontrados com os dados regionais e os descritos na literatura específica. **Métodos** – Estudo transversal, descritivo, do tipo quantitativo, com coleta retrospectiva de dados em 635 prontuários de gestantes atendidas no Serviço de pré-natal do Hospital Universitário Antônio Pedro, Niterói, estado do Rio de Janeiro no período de março de 2006 a dezembro de 2013. O banco de dados foi construído no programa Microsoft Office Access, sendo posteriormente exportado para Microsoft Office Excel. Para o processamento e análise dos dados, foi utilizado o pacote estatístico SPSS (*Statistical Package for Social Science, IBM)* versão 22.0, para Windows. **Resultados** – Foram observados 12 casos com HBsAg positivo (1,9%), 189 casos com anti-HBs positivo (35,9%) e sete pacientes positivas para o anti-HCV (1,3%). Não foi observada associação significativa entre a faixa etária e a positividade do HBsAg, anti-HBs e anti-HCV (P=0, 205, 0,872 e 0,676 respectivamente). Houve relação direta entre a positividade do anti-HBs e os últimos quatro anos da pesquisa (P<0,0001). **Conclusão** – Foi observada uma prevalência alta do HBsAg, acima daquela esperada para a região avaliada; uma prevalência para o anti-HCV concordante com a realidade brasileira atual; um índice provavelmente baixo de imunização contra a hepatite B, com índice relativamente alto de susceptibilidade para esta infecção e nenhum caso de coinfecção entre o vírus B, C e o HIV. Enfatiza-se não só a necessidade da triagem das hepatites B e C, sem exceções, durante o pré-natal, já que os avanços atuais na terapêutica poderão se não curar, pelo menos possibilitar uma melhor qualidade de vida para as pacientes com doença crônica e da realização mandatória da imunoprofilaxia em todos os recém-natos. Atenção especial deverá ser dada àquelas pacientes susceptíveis ao HBV, com pronto diagnóstico e encaminhamento para a realização da vacinação específica.

DESCRITORES – Estudos soroepidemiológicos. Hepatite B. Hepatite C. Gravidez. Gestantes.

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ORIGINAL ARTICLE

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Insulin resistance reduction after sustained virological response with direct acting antiviral: not every population improves

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ABSTRACT – **Background** – Hepatitis C virus (HCV) infection is a serious public health problem, that affects approximately 170 million people worldwide. Chronic HCV infection is associated with hepatic insulin resistance and an increased risk of diabetes HCV-infected patients has been well documented. **Objective** – To assess the homeostasis model assessment of insulin resistance (HOMA-IR) index in patients treated with direct acting antiviral (DAAs) medication in the sustained virological response (SVR), categorized by the presence or absence of cirrhosis. **Methods** – A prospective study was conducted. Data were collected at the beginning of treatment (t-base) and in the twelfth week after the completion of treatment (t-SVR12). The inclusion criteria were presence of: HCV infection (RNA-HCV positive), age ≥18 years, completion of DAAs' therapy, and presence of diabetes with use of oral hypoglycemic agents. All samples were collected during the study period. The exclusion criteria were: presence of HBV/HIV co-infection, hepatocellular carcinoma at baseline, diabetic patients taking insulin and transplanted patients (liver/kidney). Fibrosis was assessed by hepatic elastography or biopsy (METAVIR). Cirrhosis was determined by clinical results or imaging. HOMA-IR was calculated as fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5) The patients were divided into two groups: the general study population (all patients, including the diabetic patients) and the special population (patients with normal values of HOMA-IR, which is >2.5, and without diabetes). The delta HOMA-IR value was calculated as: HOMA-IR at t-base – HOMA-IR at t-SVR12. For the descriptive statistical analysis, the paired t-test and generalized linear model assuming the log binding function were performed. A *P* value of < 0.05 was considered significant. **Results** – We included 150 patients, and 75 were cirrhotic. The mean age was 55.3±9.97 and body mass index was 27.4±5.18. Twenty-two (14.67%) were diabetic patients using oral hypoglycemic agents, and 17 (11%) were cirrhotic. In the general study population, the mean glucose and HOMA-IR values increased at t-SVR12, but insulin decreased. Delta HOMA-IR was negative at t-SVR12, but there was no significant difference. Excluding diabetic patients and those with normal HOMA-IR values (<2.5), mean glucose, insulin and HOMA-IR decreased at t-SVR12. Delta HOMA-IR decreased significantly at t-SVR12 (*P*: 0.02). **Conclusion** – In the general population, glucose and HOMA-IR values increased at t-SVR12, but insulin decreased. In the special population, glucose, insulin, HOMA-IR and Delta HOMA-IR decreased at t-SVR12.

HEADINGS – Hepatitis C. Insulin resistance. Antiviral agents.

INTRODUCTION

Hepatitis C virus (HCV) infection is a serious public health problem, that affects approximately 170 million people worldwide[1]. It continues to be the leading cause of death from liver disease and, despite recent medical advances in HCV therapy, it is the principal indication for liver transplantation in the United States[2,3]. Eighty-five percent of the infected people fail to achieve clearance of the virus and become chronically infected[4]. Approximately 20% may develop hepatic cirrhosis within 20 years, characterized by progressive replacement of the functional hepatic architecture by non-functional fibrotic tissue. Each year, 6% of cirrhotic patients are expected develop terminal hepatic disease, and 4% to develop hepatocellular carcinoma (HCC)[5,6].

Chronic HCV infection is associated with hepatic insulin resistance (IR) and an increased risk of diabetes in HCV-infected patients has been well described[7]. This large prevalence of type 2 diabetes (T2DM) in HCV remains even when cirrhotic patients are excluded from the analysis[8-10], indicating that cirrhosis could be diabetogenic by itself[11]. Although insulin resistance (IR) has also been detected also in subjects with minimal or no fibrosis, an increase of homeostatic model assessment of insulin resistance (HOMA-IR) has been associated with the presence of more advanced fibrosis[12]. Other studies confirmed these findings and showed a correlation between the degree of fibrosis and IR[13,14]. T2DM and IR were observed to be independent predictors of liver-related mortality[15,16], moreover IR was associated with fibrosis progression and the stage of fibrosis[12].

In patients receiving interferon (IFN)-based therapy, Romero-Gomez et al.[17] showed that IR was associated with lower sustained virological response (SVR) rates, independent of a viral genotype. Baseline IR did not affect the outcomes of direct acting antiviral (DAA) -based therapy and HOMA-IR scores had no effect on virological response to interferon free therapy[18,19].

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IFN based therapy improved the IR in SVR[20], however, it is unclear if this effect occurs during direct acting antiviral therapy. Thus, our aim was to assess HOMA -IR behavior in patients treated with DAAs that reached the SVR, categorized by the presence or absence of cirrhosis.

METHODS

In this prospective study, we assessed patients treated with interferon free therapy from 2015 to 2017. The study was conducted at the Viral Hepatitis Outpatient Clinic of Botucatu Medical School and was approved by the Committee on Ethics in Research of the Botucatu Medical School. The data were collected at the beginning of treatment (baseline: t-base) and in the twelfth week after treatment was completed (t-SVR12).

The inclusion criteria were: presence of chronic hepatitis C infection (presence of RNA-HCV); age ≥18 years; completion of DAAs' therapy; presence of diabetes with intake of oral hypoglycemic agents, and samples collected during the study period. The exclusion criteria were as follows: HBV and/or HIV co-infection; hepatocellular carcinoma at baseline; diabetic patients taking insulin and transplanted patients (liver and kidney).

Fibrosis was assessed by hepatic elastography or biopsy (METAVIR classification)[21]. Cirrhosis was determined clinically or by imaging examination. HOMA-IR was calculated as fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5)[22]. To determine the delta HOMA-IR value, the difference was calculated as the HOMA-IR at t-base – HOMA-IR at t-SVR12.

Descriptive statistical analyses were performed to establish a comparison of the delta HOMA-IR between the groups: the general study population (all patients, including diabetics patients) and the special population (We excluded patients with normal values of HOMA-IR, which is <2.5, and with diabetes). For delta HOMA-IR, delta insulin and delta glucose, the paired t test was performed. A generalized linear model assuming log-binding function was performed for delta HOMA-IR evaluated by fibrosis. A *P*-value of <0.05 was considered significant.

RESULTS

Of the 274 consecutive patients, 150 were included, and 75 were cirrhotic. Six patients died during the treatment period,15 were excluded for taking insulin, 10 did not achieve SVR, 3 had a hepatic nodule at baseline, 7 underwent kidney or liver transplantation and 83 did not have data for HOMA-IR in all the periods indicated.

The mean age was 55.3±9.97 and body mass index (BMI) was 27.4±5.18. Twenty-two (14.67%) were diabetic patients who required oral hypoglycemic agents, and 17 (11%) were cirrhotic (*P*=0.01).

In TABLE 1 the baseline results of the general study population are provided. Mean glucose and HOMA-IR increased at t-SVR12 (102.36±24.52 and 3.72±3.26 respectively), but insulin decreased (14.03±10.32).

In TABLE 2 the changes in of delta glucose, delta insulin and delta HOMA-IR values are provided.

Altough the delta HOMA-IR was negative at t-SVR12, there was no statistical difference. When we evaluated the delta HOMA-IR based on the presence of advanced fibrosis or not, no statistical difference was found either. (*P*=0.58).

TABLE 1. Baseline results in the general population.

	t-base (n=150)
Age	55.3 ± 9.97
BMI	27.4 ± 5.18
F≤2 / F3 / F4(%)	54(36%) / 21(14%) / 75(50%)
Diabetics taking oral hypoglycemic agents	14.67% / F≤3 (3%) / F4 (11%)
Glucose	100.65 ± 19.7
Insulin	14.46 ± 10.26
HOMA-IR	3.69 ± 2.99

t-base: baseline; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance.

TABLE 2. Values of delta glucose, delta insulin and delta HOMA-IR in the general population.

	Mean delta (n=150)	*P*-value
Delta glucose	-1.71 ± 15.61	0.18
Delta insulin	0.48 ± 8.82	0.5
Delta HOMA-IR	-0.009 ± 2.66	0.96

Delta: t-base–t-SVR; HOMA-IR: homeostasis model assessment of insulin resistance; t-base: baseline; t-SVR12: sustained virological response.

Analyses of special population – when we excluded patients with normal values of HOMA-IR, which is <2.5 and with diabetes.

Excluding diabetic patients and those who had normal HOMA-IR values (HOMA-IR <2.5) there were 75 patients in total, 21 (28%) were F≤2, 12 (16%) were F3 and 42 (56%) were F4 (TABLE3).

In this population, the mean glucose, insulin and HOMA-IR values decreased at t-SVR12 (98.25±14.58, 16.56±7.64, 4.09±2.13), respectively.

TABLE 3. Baseline results in the population without diabetic patients and normal values of HOMA-IR TW0.

	t-base (n=75)
Age	55.98 ± 9.47
BMI	28.66 ± 5.18
F≤2 / F3 / F4(%)	21(28%) / 12(16%) / 42(56%)
Glucose	100.85 ± 15.09
Insulin	19.46 ± 8.92
HOMA-IR	4.86 ± 2.44

t-base: baseline; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance.

In TABLE 4 delta glucose, delta insulin and delta HOMA-IR are provided after excluding diabetic patients and those who had normal values of HOMA-IR (<2.5). The Delta HOMA-IR decreased significantly at t-SVR12. When categorized by fibrosis, there was no statistical difference (*P*=0.98).

TABLE 4. Values of delta glucose, delta insulin and delta HOMA-IR in patients with values of HOMA-IR >2.5 and non-diabetics.

	Mean delta (n=75)	*P*-value
Delta glucose	2.6 ± 12.49	0.07
Delta insulin	2.9 ± 9.88	0.01
Delta HOMA-IR	0.76 ± 2.81	0.02

Delta: t-base–t-SVR; HOMA-IR: homeostasis model assessment of insulin resistance; t-base: baseline; t-SVR: sustained virological response.

DISCUSSION

Numerous studies suggest that HCV can harm insulin signaling and increase the onset of T2DM[23,24]. In HCV-infected patients the reported prevalence of IR has increased from 30% to 70%, irrespective of the severity of hepatic disease, and is genotype-specific[2,13]. In our study, the incidence of T2DM was 14.6%, and 11% of patients were cirrhotic and 3.33% were non cirrhotic (P<0.01).

Diabetes mellitus caused by cirrhosis is called hepatogenous diabetes. This type of diabetes is most likely caused by IR with a lower hepatic extraction of insulin or by some dysfunction of pancreatic beta cells; however, this mechanism is not completely understood[25]. Classification of patients according to hepatogenous diabetes or hereditary T2DM to better determine the effect of HCV eradication in these conditions, would be beneficial[26]. However, this is not always possible because there is no standard definition of hepatogenous diabetes, even in international guidelines.

The impact of IR on the treatment efficacy of DAAs has greatly reduced because of the recent development of high potency antivirals[16].However, post-treatment outcomes with respect to glucose abnormalities have rarely been investigated. Thus, the correlation and/or changes of IR with treatment efficacy of DAAs in a real-world scenario needs more assessment.

Our data showed that the delta HOMA-IR increased at SVR12, but the result was not statistically significant, when the general study population, including diabetic patients, were studied. Additionally, there was no association between HOMA-IR and low and high levels of fibrosis. In this population, there was a slight increase of glucose and HOMA-IR, while the insulin levels decreased. A study by Jeef-Fu Huang et al.[19], that included 65 native Tawainese patients with chronic hepatitis C treated with DAAs assessing HOMA-IR, it was observed that, the levels of HOMA-IR decreased at SVR12, compared to baseline, even though there was no statistical difference. In addition, the mean glucose levels decreased in diabetic patients at SVR12. The study did not show the level of fibrosis in the patients. Another study that consisted of 110 patients with chronic hepatitis C, advanced fibrosis and T2DM that analyzed the impact of SVR on fasting glucose and glycated hemoglobin, also showed a significant reduction in fasting glucose, as SVR12 was the only factor associated with this improvement[27]. However, they did not evaluate HOMA-IR.

There is no consensus regarding the cut-off value for diagnosing IR[28,29]. Some studies use a HOMA-IR value between 1.5 and 3.0 to determine insulin resistance[17,30-32]. In our study, we used HOMA-IR >2.5 for the diagnosis of insulin resistance. In Brazil, there was a populational study of non-diabetic patients without HCV infection from the Brazilian Study of Metabolic Syndrome[32].In this study the optimal diagnostic level of HOMA-IR was between 2.3 and 2.7, which is close to the level used in our study.

In our study, on excluding diabetic patients and those with a HOMA-IR <2.5 (special population), a decrease in glucose, insulin and HOMA-IR values at t-SVR12 was observed. A study of 133 HCV genotype 1 patients with advanced liver fibrosis without T2DM[33] that evaluated the impact of HCV clearance by anti-viral treatments on IR and glycemic control, showed similar results to ours. Glucose, insulin and HOMA-IR values decreased at the end of treatment, resulting in values that remained constant after SVR12. The HOMA-IR values were associated with advanced liver fibrosis, however in our study, we did not find a significant association between the delta HOMA-IR and fibrosis stage. This might be attributable to the small study population of 75 patients after excluding diabetic patients and those with HOMA-IR <2.5.

CONCLUSION

Glucose and HOMA-IR increased at t-SVR12, but insulin decreased in the general population. The delta HOMA-IR increased, but there was no association with the stage of fibrosis. Excluding diabetic patients and those who had normal values of HOMA-IR, glucose, insulin, HOMA-IR and delta HOMA-IR decreased at t-SVR12. In addition, no association with fibrosis was found.

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Authors' contribution

Designed the study and wrote the paper: Andrade VG and Silva GF. Performed the reasearch: Yamashiro FS, Oliveira CV, Moreira A and Winckler FC. Revised the manuscript for final submission: Silva GF.

Andrade VG, Yamashiro FS, Oliveira CV, Moreira A, Winckler FC, Silva GF. Redução da resistência à insulina após resposta virológica sustentada com agentes antivirais diretos: nem toda população melhora. Arq Gastroenterol. 2018,55(3):274-8.

RESUMO – **Contexto** – A infecção pelo vírus da hepatite C (VHC) é um grave problema de saúde pública, que afeta aproximadamente 170 milhões de pessoas no mundo. A infecção crônica pelo VHC está associada à resistência à insulina hepática e a um risco aumentado de diabetes. Os doentes infetados pelo VHC foram bem documentados. **Objetivo** – Avaliar o modelo de avaliação da homeostase do índice de resistência à insulina (HOMA-IR) em pacientes tratados com medicação antiviral de ação direta na resposta virológica sustentada (RVS), categorizada pela presença ou ausência de cirrose. **Métodos** – Foi realizado um estudo prospectivo. Os dados foram coletados no início do tratamento (t-base) e na décima segunda semana após o término do tratamento (t-RVS12). Os critérios de inclusão foram presença de: infecção pelo VHC (RNA-VHC positivo), idade ≥18 anos, conclusão da terapia de antivirais de ação direta e presença de diabetes com uso de hipoglicemiantes orais. Todas as amostras foram coletadas durante o período do estudo. Os critérios de exclusão foram: presença de coinfecção VHB/HIV, carcinoma hepatocelular no início do estudo, pacientes diabéticos em uso de insulina e pacientes transplantados (fígado/rim). A fibrose foi avaliada por elastografia hepática ou biópsia (METAVIR). A cirrose foi determinada por resultados clínicos ou exames de imagem. O HOMA-IR foi calculado como insulinemia de jejum (μU/mL) x glicemia de jejum (mmol/L) /22,5). Os pacientes foram divididos em dois grupos: a população geral do estudo (todos os pacientes, incluindo os diabéticos) e a população especial (pacientes com valores normais de HOMA-IR, que é <2,5 e sem diabetes). O valor do delta HOMA-IR foi calculado como: HOMA-IR no t-base – HOMA-IR no t-RVS12. Para a análise estatística descritiva, foram utilizados o teste t pareado e o modelo linear generalizado, assumindo a função de ligação logarítmica. Um valor de P<0,05 foi considerado significativo. **Resultados** – Foram incluídos 150 pacientes e 75 eram cirróticos. A idade média foi de 55,3±9,97 e o índice de massa corpórea foi de 27,4±5,18. Vinte e dois (14,67%) eram pacientes diabéticos em uso de hipoglicemiantes orais e 17 (11%) eram cirróticos. Na população geral do estudo, os valores médios de glicose e HOMA-IR aumentaram na t-SVR12, mas a insulina diminuiu. O delta HOMA-IR foi negativo em t-SVR12, mas não houve diferença significativa. Excluindo pacientes diabéticos e aqueles com valores normais de HOMA-IR (<2,5), a média de glicose, insulina e HOMA-IR diminuiu no t-RVS12. O delta HOMA-IR diminuiu significativamente em t-RVS12 (P: 0,02). **Conclusão** – Na população geral, os valores de glicose e HOMA-IR aumentaram no t-RVS12, mas a insulina diminuiu. Na população especial, glicose, insulina, HOMA-IR e delta HOMA-IR diminuíram no t-RVS12.

DESCRITORES – Hepatite C. Resistência à insulina. antivirais.

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ORIGINAL ARTICLE

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Honey-derived *Lactobacillus rhamnosus* alleviates *Helicobacter pylori*-induced gastro-intestinal infection and gastric inflammation in C57BL/6 mice: an immuno-histologic study

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ABSTRACT – Background – *Helicobacter pylori* (*H. pylori*) has been introduced by since 1983 by Marshal and Warren to play the main role in the pathophysiology of gastritis and gastric ulcers. Almost half of the world population1 is infected by *H. pylori*. Current therapeutic regimen against *H. pylori* includes the use of a proton pump inhibitor plus two or more antibiotics. However, the efficacy of this regimen is decreasing mainly due to antibiotic resistance and side effects of medications. This fact has resulted in public interest in other therapeutic options and the role of probiotics merits special attention in this regard. **Objective** – This study aims to evaluate the efficacy of honey-derived *Lactobacillus rhamnosus* on *H. pylori*-induced gastric inflammation and gastro-intestinal infection in C57BL/6 Mice. **Methods** – The 24 C57BL/6 Mice were randomly divided into three groups of eight mice each. All the mice were fed with 1cc suspension containing $5*10^{10}$ CFU/ mL of ATCC43504 strains of *H. pylori* for 3 consecutive days, twice daily via polyethylene gavage tubes. At the end of 4th week, infection with *H. pylori* was confirmed with stool Ag (ELISA) and following sacrifice of one mouse from each group, histopathologic study confirmed gastritis. The groups were subjected to different therapies as stated, 1: without Bismuth (Bi), Omeprazole (Om) and *L. rhamnosus* prescription, 2: Bi, Om and Clarithromycin (Cl) and 3: Bi, Om plus 1cc of suspension of 10^9 CFU/mL of *L. rhamnosus*. After 2 weeks, the stool was analyzed for Ag and the mice were sacrificed for evaluation of histopathologic changes. **Results** – Treatment with *L. rhamnosus* group provided Zero titer of stool Ag and was associated with improved gastric inflammation in all subjects, similar to the clarithromycin group. **Conclusion** – Honey-derived *L. rhamnosus* probiotics provides similar results as clarithromycin in terms of improvement of *H. pylori* infection and gastritis in C57BL/6 Mice model, without its cons of antibiotic resistance.

HEADINGS – *Helicobacter pylori*. *Lactobacillus rhamnosus*. Stomach ulcer. Inbred C57BL mice. Honey.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram negative microaerophilic bacillus which is motile and pathogenic due to possessing flagella. *H. pylori* infection is pandemic and its prevalence ranges from 41.35% to 72.3% in different countries, worldwide[1,2]. In many developing countries, the infection rate rises to 80% to 90% of the adult population. Prevalence of infection is variable between the countries and between the races and ethnicities in a certain country and is dependent on socioeconomic status of population residing in that environment.

Barry Warren and Robin Marshall were the first to isolate *H. pylori* in 1983 which resulted in their achievement of Nobel Prize in physiology for their pioneering work on *H. pylori* and its role in gastritis and peptic ulcer disease. In 1994 the International Agency for Research on Cancer (IARC), a subordinate organization of the World Health Organization (WHO), identified *H. pylori* as a "group 1 (definite carcinogen)"[3]. *H. pylori* infection is identified as the main cause of chronic gastritis, peptic ulcer disease, MALToma[4-6] and gastric adenocarcinoma[7,8].

Many therapeutic regimens have been introduced since the establishment of their pathogenic hypothesis containing bismuth, proton pump inhibitors and one or two antibiotics[9] (mainly tetracyclin, metronidazole, amoxicillin and clarithromycin)[10].

However, considering the evolution of resistant strains of *H. pylori*[11], therapeutic modalities other than antibiotics are necessary and lactobacillus probiotics[12,13] plays a crucial role in this regard[14,15].

Probiotics have the potential to inhibit *H. pylori* due to production of organic acids, peroxides and bactericides[16] as well as competitive inhibition of attachment sites on gastric mucosa. Taking into account that triple or quadruple therapeutic regimens result in 85% to 90% eradication of *H. pylori* makes other therapeutic approaches interesting and necessary in this field.

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Here in, considering the potential role of probiotics in inhibition of *H. pylori* infection and *H. pylori*-induced gastritis, we evaluated the application of honey-derived[17] *Lactobacillus rhamnosus* (*L. rhamnosus*) as a probiotic in the C57BL/6 mice. In this animal study, we tend to compare the results of *H. pylori* infection and *H. pylori*-induced gastritis via stool Ag and Histopathologic exam, respectively between the treatment groups.

METHODS

Animal population

Twenty-four male C57BL/6 mice aged 8-10 weeks were randomly divided into three groups of 8 mice each. Mice were maintained at the animal house under standard conditions (food and water ad libitum, 12:12 hours light/dark cycle, 21±3°C). All experiments involving mice were approved by the Animal Care Committee of Alborz University of Medical Sciences, Karaj, Iran.

H. pylori culture

Helicobacter pylori (Marshall et al.) Goodwin et al. (ATCC® 43504D-5™) was cultured on Brucella agar media containing sheep blood and Trypticase soy agar, in a microaerophilic environment and incubated for 5 to 7 days in 37 degrees of centigrade. After this incubation period, transparent spherical colonies of *H. pylori* appeared which were identified using gram stain and urease, catalase and oxidase tests. The DNA of strain of ATCC® 43504D-5™ was also confirmed with QIAamp DNA Micro Kit (Quiagen).

Animal preparation and induction of *H. pylori* infection

All the mice were fed with 1cc suspension containing 10^8-10^{10} CFU/mL of ATCC43504 strains of *H. pylori* for 3 consecutive days, twice daily via polyethylene gavage tubes. (FIGURE 1) ATCC43504 strains of *H. pylori*.

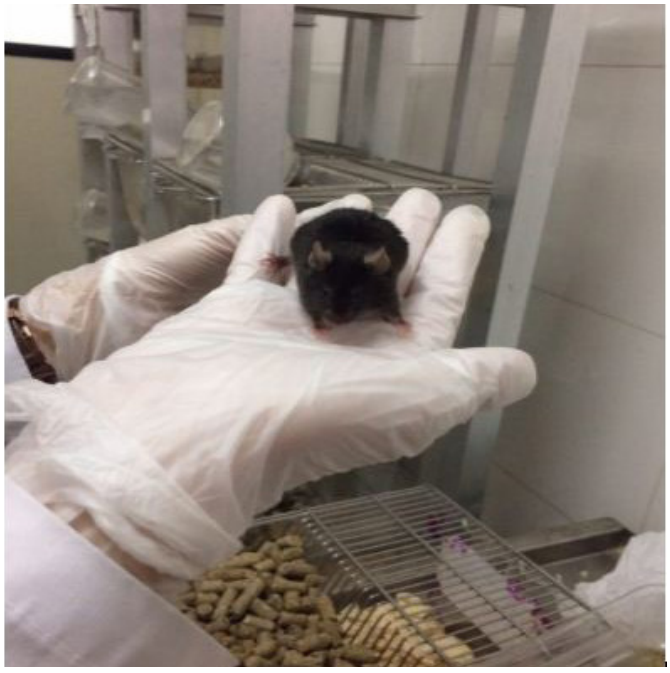

FIGURE 1. Feeding of suspension containing 10^8 CFU/mL of *H. pylori* to the mice via gavage polyethylene tubes.

Confirmation of *H. pylori* infection and gastritis

Infection with *H. pylori* was confirmed with stool Ag (RIA test kits); At the end of 4th week, 30 mg fresh stool was obtained and 1cc of extraction solution was added to the stool tubes. Fifty microliters of stool suspension were added to wells pre-coated with *H. pylori* antibody and incubated for 60 minutes at room temperature. Results were read at 540 nm wavelength and concentrations above 0.505 µg/mL were considered positive for *H. pylori* stool Ag. All mice tested positive at the end of 4th week of ingestion of *H. pylori*.

After treatment all mice killed under anesthetic condition by euthanasia protocole. Mucosal tissue was obtained through a longitudinal incision in the stomach and prepared with hematoxylin-eosin(H&E) and Giemsa staining. Histopathologic study confirmed gastritis in all three.

Preparation of *L. rhamnosus*

L. rhamnosus was derived from honey gathered from mountains of Mazandaran province in northern Iran and cultured in MRS agar media under microaerophilic condition for 48 hours in 37 degrees of centigrade. The colonies were solved in Phosphate-buffered saline (PBS) solution with a dosage of 10^9 CFU/ mL.

Treatment protocols

The mice were subjected to different therapies as stated below;

1: without Bi, Om and *L. rhamnosus* prescription.

2: Daily Bi, Om for the first week and Clarithromycin (Cl) with dosage of 20 mg/kg QD through the second week.

And 3: Daily Bi, Om for the first week plus 1cc of suspension of 10^9 CFU/mL of *L. rhamnosus* QD through the second week.

Evaluation of gastritis and status of *H. pylori* infection after treatment

At the end of the second week of treatment, the stool was tested for Ag and all the remaining seven mice in each group were sacrificed for evaluation of histopathologic changes in the mucosa of stomach. (Stated above in Confirmation of *H. pylori* infection and gastritis section).

RESULTS

The results for stool Ag titer for the seven mice in each treatment group is summarized in TABLE 1.

TABLE 1. *H. pylori* stool Ag titer following treatment.

	#1	#2	#3	#4	#5	#6	#7
Group 1	0.225	0.230	0.525	0.852	1.101	1.011	1.012
Group 2	0.052	0.065	0.045	0.032	0.022	0.00	0.00
Group 3	0.050	0.055	0.065	0.045	0.012	0.00	0.00

Considering 0.505µg/ml as the threshold for a positive stool Ag test, infection persisted in 71.4% of the subjects in the first group following treatment; however, all seven mice were successfully treated in both the clarithromycin and *L. rhamnosus* groups.

Histopathology also revealed the resolution of inflammation in gastric mucosa of all mice following treatment with clarithromycin as well as probiotic *L. rhamnosus*. (FIGURE 2).

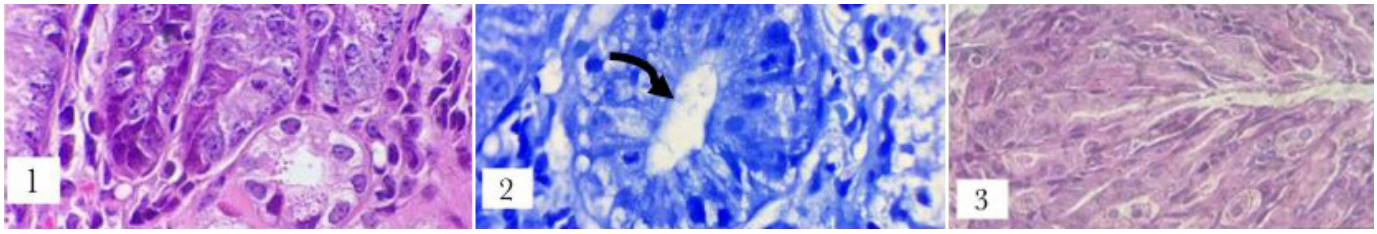

FIGURE 2. Histopathologic slides of gastric mucosa of C57BL/6 mice under * 100 magnification (1: H&E staining of normal gastric mucosa prior to infection, 2: Giemsa staining of infected mouse with black arrow pointing to the *H. pylori* attached to the gastric mucosa via its flagella, 3: H&E staining, confirming the resolution of inflammation following treatment with *L. rhamnosus*.

DISCUSSION

Helicobacter pylori has been the cornerstone of studies addressing gastritis and gastric cancer after its introduction by Marshall and Warren in 1983. Studies on the epidemiology, genetics, pathophysiology and treatment of *H. pylori* has been overwhelming within the last three decades. *H. pylori* is equipped with several virulence factors[18] such as flagella, colonization factors, urease and peroxidase enzymes and vacuolating cytokine A (Vac A)[19] which has made it a very potent agent evading human immune system and resistant to antimicrobials.

Antibiotics has been presented as a potent treatment against *H. pylori* infection causing significant reduction in the rate of gastritis and cancers associated with this infection. Amoxicillin, tetracyclin, metronidazole, clarithromycin and levofloxacin[20] are the main antibiotics utilized in eradication of *H. pylori* infection. However, concerns has arised regarding the use of antibiotics as the resistant strains appeared in the last two decades. Reports of antibiotic resistance vary in different countries. For example, pharmacoepidemiologic studies in Iran, reported resistance to metronidazole, amoxicillin and clarithromycin in 60%, 40%-50% and 30% of the population, respectively.

Beside evolution of resistance, antibiotics sustain several side effects including GI irritation, appearance of infections due to changes in normal flora, influence on the pharmacokinetics of other medications, etc, which has resulted in seeking for alternative therapeutic options.

Probiotics has emerged as novel treatment option in treating several infectious conditions such as bacterial vaginosis, gastrointestinal ulcer and burns. Probiotics may play a role as adjunctive treatment in *H. pylori* infections and possibly in prophylaxis. A wide range of probiotic strains (*L. acidophilus*, *L. johnsonii*, *L. gasseri*, *Bifidobacterium longum* and bioyoghurts) have been studied in *H. pylori* infection and the results are variable[21]. In this study we investigated the effect of sequencial treatment (following adminstration of bismuth and omeprazole) with *L. rhamnosus*, derived from honey, on *H. pylori* infection in C57BL/6 mice.

Previous studies have revealed that *L. rhamnosus* is effective against *H. pylori* infection following antibiotic therapy (as a prophylactic measure to reduce reinfection). This study shows that it is useful as a therapeutic option, and is as effective as antibiotic therapy as the sole treatment to eradicate *H. pylori*.

We hypothesize that the efficacy of *L. rhamnosus* as a therapeutic to eradicate *H. pylori* may rely on bismuth's effect on depolarization of *H. pylori* membrane[22,23] prior to adminstration of *L. rhamnosus* which makes it vulnerable to the bacteriocines released from this probiotic.

Another interesting issue to be mentioned is the derivation of *L. rhamnosus* colonies from honey, which might justify the several studies that present honey as a remedy for *H. pylori* infection[24-29].

A limitation of other studies evaluating the effect of honey on *H. pylori* infection could be the extraction of different bacterial species from different honey types and sources. In the current study, we identified *L. rhamnosus* with genetic sequencing which should be examined with other honey types as well. This animal study warrants a human clinical trial with honey-derived *L. rhamnosus* as a treatment of *H. pylori* infection.

CONCLUSION

Honey-derived *Lactobacillus rhamnosus* is as effective as clarithromycin in eradication of *H. pylori* infection and cure of gastritis, in the C57BL/6 mice.

Authors' contribution

Asgari B: executing tests and acquisition of data. Kermanian F: performing specialised tests and interpretation of data. Derakhshan N: writting and professional editting of manuscript critically. Asna-Ashari M: search literature and data collection. Sadat ZRN: data collection. Yaslianifard S: conception and design writting of text critical revision of the article for important intellectual content. At the end all authors approved the final revision of the manuscript.

Asgari B, Kermanian F, Derakhshan N, Asna-Ashari M, Sadat ZRN, Yaslianifard S. O mel-derivado de *Lactobacillus rhamnosus* alivia a infecção gastro-intestinal induzida pelo Helicobacter pylori-e inflamação gástrica em C57Bl/6 ratos: um estudo imuno-histológico. Arq Gastroenterol. 2018,55(3):279-82.

RESUMO – **Contexto** – O *Helicobacter pylori* (*H. pylori*) foi reconhecido em 1983 por Marechal e Warren como protagonista principal na fisiopatologia de gastrite e úlceras gástricas. Quase metade da população mundial está infectada por *H. pylori*. O regime terapêutico atual contra *H. pylori* inclui o uso de um inibidor da bomba de prótons associada a dois ou mais antibióticos. No entanto, a eficácia deste regime está diminuindo principalmente devido à resistência aos antibióticos e efeitos colaterais de medicamentos. Este fato resultou no interesse público em outras opções terapêuticas e o papel dos probióticos merece atenção especial a este respeito. **Objetivo** – Este estudo visa avaliar a eficácia do mel-derivado do *Lactobacillus rhamnosus* na inflamação gástrica e infecção gastrointestinal *H. pylori*-induzida em camundongos C57Bl/6. **Métodos** – Vinte e quatro camundongos C57Bl/6 foram divididos aleatoriamente em três grupos de oito camundongos cada. Todos os ratos foram alimentados com suspensão de 1cc contendo 5*1010 UFC/mL de cepas ATCC43504 de *H. pylori* por 3 dias consecutivos, duas vezes por dia através de gavagem por tubos de polietileno. No final da 4ª semana, a infecção com *H. pylori* foi confirmada pelo antígeno fecal (ELISA) e após o sacrifício de um rato de cada grupo, o estudo histopatológico confirmou gastrite. Os grupos foram submetidos a diferentes terapias, como indicado, 1: sem prescrição de bismuto (BI), Omeprazol (Om) e *L. rhamnosus*, 2: Bi, Om e claritromicina (CL) e 3: Bi, Om mais 1cc de suspensão de 109 UFC/mL de *L. rhamnosus*. Após 2 semanas, as fezes foram analisadas para o antígeno e os ratos foram sacrificados para a avaliação das alterações histopatológicas. **Resultados** – O tratamento com o grupo *L. rhamnosus* forneceu o título zero de antígeno e foi associado com a inflamação gástrica melhorada em todos os camundongos, similar ao grupo claritromicina. **Conclusão** – O probiótico mel-derivado *L. rhamnosu*s fornece resultados semelhantes ao da claritromicina em termos de melhoria da infecção *H. pylori* e gastrite em C57Bl/6 camundongos modelos, sem os inconvenientes de resistência aos antibióticos.

DESCRITORES – *Helicobacter pylori*. *Lactobacillus rhamnosus*. Úlcera gástrica. Camundongos Endogâmicos C57BL. Mel.

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ORIGINAL ARTICLE

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Target, prescription and infusion of enteral nutritional therapy of critical patients in intensive care unit

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ABSTRACT – Background – Enteral nutritional therapy (ENT) is the best route for the nutrition of critically ill patients with improved impact on the clinical treatment of such patients. **Objective** – To investigate the energy and protein supply of ENT in critically ill in-patients of an Intensive Care Unit (ICU). **Methods** – Prospective longitudinal study conducted with 82 critically ill in-patients of an ICU, receiving ENT. Anthropometric variables, laboratory tests (albumin, CRP, CRP/albumin ratio), NUTRIC-score and Nutritional Risk Screening (NRS-2002), energy and protein goals, and the inadequacies and complications of ENT were assessed. Statistical analysis was performed using the Chi-square or Fischer tests and the Wilcoxon test. **Results** – A total of 48.78% patients were at high nutritional risk based on NUTRIC score. In the CRP/albumin ratio, 85.37% patients presented with a high risk of complications. There was a statistically significant difference (P<0.0001) for all comparisons made between the target, prescription and ENT infusion, and 72% of the quantities prescribed for both calories and proteins was infused. It was observed that the difference between the prescription and the infusion was 14.63% (±10.81) for calories and 14.21% (±10.5) for proteins, with statistically significant difference (P<0.0001). In the relationship between prescription and infusion of calories and proteins, the only significant association was that of patients at high risk of CRP/albumin ratio, of which almost 94% received less than 80% of the energy and protein volume prescribed (P=0.0111). **Conclusion** – The administration of ENT in severely ill patients does not meet their actual energy and protein needs. The high occurrence of infusion inadequacies, compared to prescription and to the goals set can generate a negative nutritional balance.

HEADINGS – Enteral nutrition. Energy intake. Dietary proteins. Critical care. Intensive care units.

INTRODUCTION

Severely ill patients admitted to the intensive care unit (ICU) are in a hypercatabolic state, attempting to survive the acute phase of the disease and stress[1,2]. The patient responds with intense protein catabolism and, consequently, rapidly loses stocks of muscle mass for the production of new proteins for healing, for the immune system and replacement of muscle and hepatic glycogen[1,3,4]. One of the consequences of the response to hypercatabolism during severe disease is malnutrition, with a negative impact on clinical outcomes such as increased mortality risk, time and cost of hospitalization, greater clinical and infectious complications, longer healing time of pressure ulcers and surgical wounds and more fragile quality of life[3-5].

Enteral nutritional therapy (ENT) is the best route for the nutrition of critically ill patients with improved impact on the clinical treatment of such patients, according to the American Society of Parenteral and Enteral Nutrition (ASPEN) and The European Society for Clinical Nutrition and Metabolism (ESPEN)[6]. Proper indication and monitoring can prevent and treat malnutrition and improve immune response, thus preventing clinical and infectious complications in the critically ill[6-8].

A recent study by Bendavid et al.[9], on nutritional practices in ICUs, found that the preferred feeding route is the enteral route and that ENT is initiated in the first days of hospitalization and that nutritional goals are seldom reached; this shows that achieving energy and protein goals in critically ill patients is one of the challenges in ICUs. Another study, conducted by Tsai et al.[4], found an average delivery of 49 to 70% of the study energy and protein goals for the critically ill patient. Two other studies[10,11] have shown that critically ill patients receive 60% and 56% of their energy and protein needs, respectively, and that a protein supply below 80% of the set goals is associated with higher mortality rates[9,10].

Monitoring and identifying complications associated with ENT supply, identifying the most frequent inadequacies, may facilitate management and a better selection of nutritional therapy[10,11]. Complications and intolerances associated with ENT administration may occur frequently[12,13], and some associated factors such as a high residual gastric volume, vomiting and diarrhea may prevent an adequate supply of ENT[12,14], as well as routine ICU interventions such as extubation, imaging and surgical procedures[15].

Considering the relevance of the therapy in critically ill patients, the objective of this study was to investigate the energy and protein supply of ENT in critically ill in-patients in an ICU of a university hospital.

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METHODS

Study design, ethical approval and inclusion and exclusion criteria

A longitudinal prospective study was carried out in a university hospital ICU, after approval by the Research Ethics Committee of the institution (opinion No. 1,754,082) and the signing of the Free and Informed Consent Form (FICF), in the state of São Paulo, Brazil, from 2016 to 2017.

The inclusion criteria adopted were: admission to the ICU, over 18 years of age and receiving exclusively enteral nutritional therapy (EENT). Patients with another route of nutritional therapy (NT) (oral or parenteral) were excluded; patients whose ENT was discontinued and patients in whom another NT form was introduced, even if concomitant with ENT, or in less than 3 days in EENT were also excluded as well as those with incomplete records of nutritional status, lacking exams or other information essential for the survey in medical records.

Initially, 142 patients were recruited to participate in the study. After the review of the inclusion and exclusion criteria, 82 patients remained. Out of the 60 excluded patients, 20 (24.4%) stayed less than 3 days in the ICU, 16 (19.5%) had undergone less than 3 days EENT, 18 (21.9%) submitted incomplete data entered in their medical records and 6 did not sign the FICF. Thus, the study was developed with 82 (N=82) adults and older patients of both genders who received EENT, through oroenteral tube (OET), nasoenteral tube (NET), jejunostomy (J) or gastrostomy (G), under clinical or surgical treatment. The indication of oroenteral tube (OET) is part of a protocol indicated for patients in mechanical ventilation, such as prevention of hospital pneumonia, standardized in the hospital where the research was carried out.

Data collection

Data such as gender, age, length of stay and diagnosis were collected. All patients were evaluated for nutritional status at the beginning and at the end of the ENT, as well as every 5 days during ENT stay, taking into account the following indicators: anthropometry, laboratory tests, Nutritional Risk Screening-2002 (NRS) and NUTRIC-score. The energy and protein requirements and the inadequacies or complications during enteral diet administration were also evaluated. All patients were evaluated in the first 24 hours of hospitalization and monitored until the time of hospital discharge or death.

A) Anthropometry

To determine the patient's nutritional conditions by anthropometry, data on body weight and height were collected to compute the body mass index (BMI). In the BMI calculation, the criteria established by the WHO, 1995[16] for adults up to 60 years of age and by Lipschitz (1994)[17] for the elderly (>60 years) were considered. When it was not possible to assess the weight in bedridden patients, the body weight was estimated using the formula of Chumlea et al.[18].

B) Laboratory tests

- Albumin

Serum albumin dosage was classified following Blackburn et al.[19], who defined the following cutoff points:- >3.5mg/dL (reference values); between 2.8-3.5 mg/dL (mild depletion); between 2.1-2.7 mg/dL (moderate depletion) and <2.1 mg/dL (severe depletion).

- C Reactive protein (CRP)

For CRP analysis, the dosages performed every 5 days, the same day or the next day (maximum 2 days before or after) of the albumin dosage were used. The CRP dosing result was used to calculate the CRP/Albumin inflammatory-nutritional index, and it was not used alone to evaluate the patient. The patient was fasted for 8 hours before collection and the cut-off point for the inflammatory assay was <0.5 mg/dL[20].

- CRP/ALB relationship

The inflammatory / nutritional index PCR/Albumin was used to assess the risk of severely ill patients according to the following risk factors for complications: CRP/albumin ratio <0.4 (without risk); 0.4-1.2 (low risk); 1.2-2.0 (medium risk) and >2.0 (high risk)[20,21].

C) Nutritional risk screening (NRS-2002)

The NRS-2002 is a tool for the assessment of the nutritional risk of in-patients and was applied on admission or within 24 hours afterwards. Results were interpreted by a numerical score, where the score ≥3 indicates that the patient is at nutritional risk, and score <3, at no nutritional risk[22].

D) Nutrition risk in the critically ill score (NUTRIC-score)

The NUTRIC-score is a tool for assessing the nutritional risk of critically ill patients, and its control variables include: Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), age, number of comorbidities and the total number of days of hospitalization before admission to the ICU. The NUTRIC-score was applied to determine the nutritional risk in the first 48 hours of ICU patient admission, and the values ≥5 were considered indicative of a higher nutritional risk[23].

E) Energy and protein targets determination

The daily patients' energy and protein requirements were estimated based on the recommendations of the new ASPEN nutritional therapy guidelines[24] (TABLE 1).

TABLE 1. Energy and protein targets determination.

	BMI (Kg/m²)		
Energy Target (Kcal/Kg of current weight/day)	<25	30–50	>50
	25–30	11–14	22–25
	BMI (Kg/m²)		
Protein Target (g/Kg of current weight/day)	<30	30–40	>40
	1.2–2	2	2.5

F) ENT monitoring

- Comparison between the prescribed energy and protein value and the infused energy and protein value

The infusion of the enteral diet and the inadequacy between the prescription and delivery of ENT were reviewed daily. The analyses of the energy and protein value prescribed, actually delivered within 24 hours after the prescription, as well as the causes of non-infusion of the diet, were carried out by comparing the diet volume prescribed and the volume actually administered, by checking the entries in the medical records. We also analyzed the actual infusion of the prescribed diet, in relation to the goals defined by the calculation of the patients' energy and protein needs.

- Review of ENT inadequacies

The complications associated with ENT and that directly impacted the infusion of the prescribed diet were classified as follows: gastrointestinal (diarrhea, constipation, abdominal distension); mechanical (loss, obstruction or displacement of the probe); (surgery, exams, procedures such as tracheostomy, extubation); others (death, discharge from the ICU, fasting and unexplained delays). The frequency of the occurrence of these complications was evaluated based on the entries in the medical records.

Statistical analysis

Statistical analysis was performed with the aid of the SAS[25] program. For the characterization of the sample, a descriptive analysis was performed using frequency tables for the categorical variables and position and dispersion measurements for the continuous variables. Subsequently, the Wilcoxon test for related samples was used to compare descriptive and infused measurements. To verify association or to compare proportions, the Chi-square test or Fisher's exact test was used when necessary. The significance level adopted for the statistical tests was 5%.

RESULTS

Characteristics of the study population

The mean age of the studied population was 60.23 (±18.51) years, with 60.98% (n=50) males and 39.02% (n=32) females. The patients' mean body weight was 70.74 kg (±15.75) and the mean height was 165.49 cm (±10.3). Patients remained on average 15.88 (±7.78) days in the ICU, of which; 12.84 (±8.24) days on EENT. Among the NT routes, the following routes were most frequently used: oroenteral in 84.15% (n=69), nasoenteral in 13.41% (n=11) and ostomy in 2.44% (n=2) of the patients. Regarding the origin of the patients in the hospital, before being transferred to the ICU, 70.73% came from the emergency room (hospital emergency care services), 26.83% came from inpatient wards, 1.22% from the surgical center and 1.22% came from another hospital. Among the patients studied, 50% had a clinical diagnosis and 50% had a surgical diagnosis.

Nutritional status, nutritional risk and risk of complications

At the beginning of NT 59.76% (n=49) of the patients were eutrophic and 8.54% (n=7) malnourished according to the BMI. All patients (100%) were at high nutritional risk by the NRS-2002. In the assessment with the NUTRIC score, 51.22% of the patients (n=42) presented low risk (NUTRIC <5) and 48.78% (n=40) presented high nutritional risk. For the evaluation of the risk of complications of the CRP/albumin ratio, 85.37% (n=70) of the patients presented, at some point in the evaluation, a high risk.

Outcome of NT

Regarding the final outcome of EENT, 57.32% (n=47) of the patients had a favorable outcome (31 patients were discharged from the ICU, one patient initiated parenteral nutrition concomitant to ENT and 15 patients initiated concomitant oral diet with ENT or had the ENT discontinued and were switched to oral diet). In the present study, 42.68% (n=35) of the patients had a poor outcome and died (n=31) or had their ENT discontinued due to palliative treatment (n=4).

Target, prescription and energy and protein infusion

Regarding the targets, prescription and energy and protein infusion of patients receiving EENT, it was verified that the patients' daily energy goal was 2,132.91±337.88 kcal per day. The mean energy requirement was 1,432.69±407,00 kcal per day and the mean infusion was 1,114.50±437.37 kcal per day. The mean protein target was 113.96±26.35 g per day. The mean protein prescription was 62.35±18.43 g of daily protein and the mean protein infusion was 47.58±19.01 g per day. For the protein, the mean infusion was 0.67 g/kg body weight, and the mean energy infusion was 15.76 Kcal/kg body weight, well below the recommendations of 1.2 to 2.5 g protein per Kg weight and 25-35 Kcal per kg weight[24].

Comparison between target, prescription and calorie and protein infusion

A comparison of the goals, prescription and infusion of calories and proteins of patients receiving EENT are reported in TABLE 2. A statistically significant difference (P<0.0001) was observed for all comparisons made between the target, prescription and infusion of EENT. For calories, prescription was on average 68.07% of the value of the calculated goal, and 53.44% of the energy goal was infused. For protein, the prescription was on average 57.92% of the value of the target, and an average 43.72% of the quantities of the protein goal was infused. Seventy two percent of what was prescribed for both calories and proteins was infused (TABLE 2).

TABLE 2. Comparison between target, prescription and infusion of calories and proteins of patients under EENT (N=82).

Variable	Diff ± dp average	Median	%	Value-*P*
Calories (kcal)				
M x P	700.22 ± 473.01	676.15	68.07	<0.0001*
M x I	1018.42 ± 498.47	1004.59	53.44	<0.0001*
P x I	318.19 ± 185.29	305.79	72.26	<0.0001*
Protein (g)				
M x P	51.61 ± 32.64	53.48	57.92	<0.0001*
M x I	66.39 ± 31.14	66.86	43.72	<0.0001*
P x I	14.77 ± 8.07	13.59	72.26	<0.0001*

M x P: goal versus prescription; M x I: goal versus infused; P x I: prescribed versus infused; Mean Diff: Mean of difference in values between target, prescribed and infused. Values expressed as mean ± standard deviation; significance level P<0.05. *Wilcoxon test for related samples (null hypothesis: median equal to zero).

TABLE 3 shows the percentage of prescription and infusion in relation to the energy and protein target and the difference between what was prescribed and infused. It was observed that the difference between the prescription and the infusion was 14.63% (±10.81) for calories and 14.21% (±10.50) for proteins. In both cases, the difference was statistically significant (P<0.0001) (TABLE 3).

Comparison between goals and energy and protein infusion and variables of nutritional status and risk and outcome of the nutritional therapy

Considering that 80% or more infusion of the defined goals for calories and proteins would be optimal, FIGURES 1 and 2 show the results of energy and protein infusions in relation to the defined goals and the prescription. The results show that 89.09% (n=73) of the patients did not receive an average of 80% or more of the calories infusion and that 97.56% (n=80) of the patients did not receive an infusion average of 80% or more of protein, when compared to the defined goal (FIGURE 2). If we compare the calorie and protein infusion with the prescription, 59.76% (n=49) of the patients received on average below 80% of the prescribed dosage (FIGURES 1 and 2).

TABLE 4 shows the association between those patients who received an infusion greater than or equal to or less than 80% in relation to the energy and protein goals, in the different variables of state and nutritional risk and NT outcome. There was no statistically significant difference between the variables reviewed and the patients who received above or equal to, or below 80% of the energy goal. For the protein target, it was not possible to make the same comparison because, in only two cases, the infusion was equal to or greater than 80% of the defined goal.

Comparison between prescription and energy and protein infusion and the variables of the state and nutritional risk and NT outcome

TABLE 5 shows the association between energy and protein prescriptions and what was infused in the different variables reviewed. The only significant association was that of patients at high risk for the CRP/Albumin ratio. Among these, almost 94% received less than 80% of the energy and protein volume prescribed (P=0.0111).

TABLE 3. Percentage comparison between target, prescription and energy and protein infusion and differences between prescribed and infused (N=82).

Variable	Average (%)	DP (%)	Minimum	Median	Maximum	*P*-value
Calories (%)						
M x P	68.07	19.29	29.40	67.39	109.69	
M x I	53.44	20.79	9.11	50.42	99.19	<0.0001*
Difkcal	14.63	10.81	35.56	13.27	53.89	
Proteins (%)						
M x P	57.92	22.66	21.24	54.83	119.64	
M x I	43.72	19.47	6.59	39.86	97.44	<0.0001*
Difp	14.21	10.50	0.19	11.63	64.99	

M x P: goal versus prescription; M x I: goal versus infused; Difcal: difference between prescribed and infused calories; Difp: difference between prescribed and infused protein. Values expressed as mean ± standard deviation; significance level P <0.05. * Wilcoxon test for related samples (null hypothesis: median equal to zero).

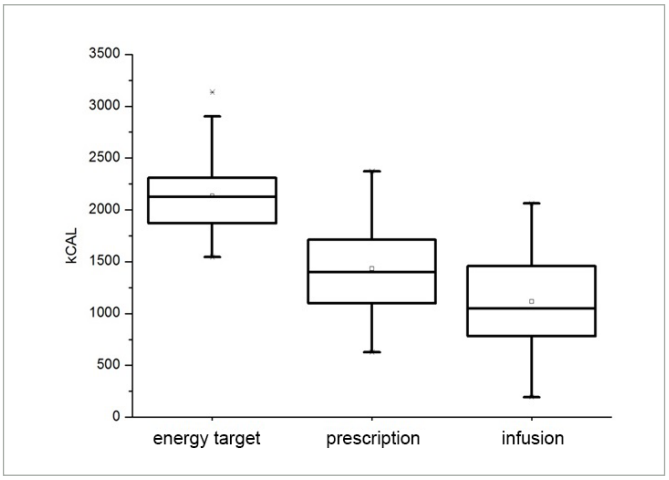

FIGURE 1. Relationship between target, prescription and energy infusion.

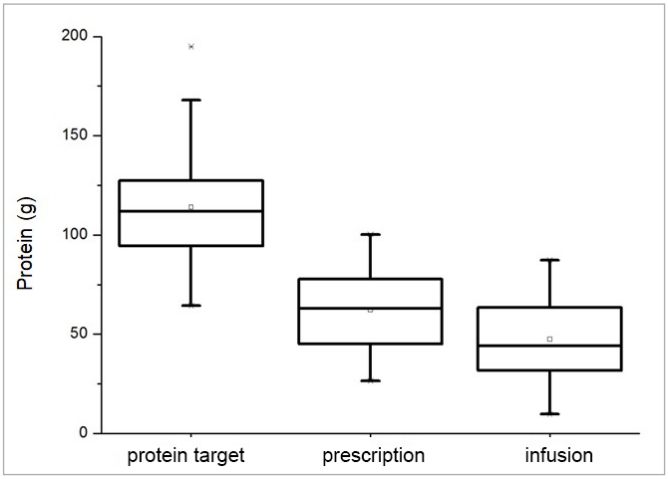

FIGURE 2. Relationship between target, prescription and protein infusion.

TABLE 4. Association between goals and energy and protein infusion, and the variables of the state and nutritional risk and NT outcome (N=82).

Variable		Target X Energy Infusion			*P*-value
		≥ 80% (%)	< 80% (%)	Total N (%)	
IMC	Overweight	4 (44.44)	22 (30.14)	26 (31.71)	0.6489*
	Eutrophic	5 (55.56)	44 (60.27)	49 (59.76)	
	Low weight	-	7 (9.59)	7 (8.54)	
NUTRIC score	No risk	4 (44.44)	38 (52.05)	42 (51.22)	0.7347*
	At risk	5 (55.56)	35 (47.95)	40 (48.78)	
Outcome	Good	5 (55.56)	42 (57.53)	47(57.32)	1.0*
	Bad	4 (44.44)	31 (42.47)	35 (42.68)	
CRP/Alb Relationship	High risk	7 (77.78)	63 (86.30)	70 (85.37)	0.6134*
	Low, medium and no risk	2 (22.22)	10 (13.70)	12 (14.63)	
		Target X Protein Infusion			
IMC	Overweight	2 (100)	24 (30)	26 (31.71)	
	Eutrophic	-	49 (61.25)	49 (59.76)	
	Low weight	-	7 (8.75)	7 (8.54)	
NUTRIC score	No risk	1 (50)	41 (51.25)	42 (51.22)	
	At risk	1 (50)	39 (48.75)	40 (48.78)	
Outcome	Good	1 (50)	46 (57.50)	47 (57.32)	
	Bad	1 (50)	34 (42.50)	35 (42.68)	
CRP/Alb Relationship	High risk	2 (100)	68 (85)	70 (85.37)	
	Low, medium and no risk	-	12 (15)	12 (14.63)	

BMI: body mass index; CRP/Alb ratio: relationship between C-reactive protein and albumin. The values expressed in% compare values of the same goal (<80% or ≥80%). P-value was not calculated for the protein target, since only two cases were ≥80%. * Fisher's exact test.

TABLE 5. Association between prescription and energy and protein infusion and the variables of the state and nutritional risk and NT outcome (N=82).

Variable		Prescription X Energy and Protein Infusion			*P*-value
		≥ 80% (%)	< 80% (%)	Total N (%)	
IMC	Overweight	13 (39.39)	13 (26.53)	26 (31.71)	0.0507*
	Eutrophic	20 (60.61)	29 59.18)	49 (59.76)	
	Low weight	-	7 (14.29)	7 (8.54)	
NUTRIC score	No risk	18 (54.55)	24 (48.98)	42 (51.22)	0.6210**
	At risk	15 (45.45)	25 (51.02)	40 (48.78)	
Outcome	Good	21 (63.64)	26 (53.06)	47(57.32)	0.3424**
	Bad	12 (36.36)	23 (46.94)	35 (42.68)	
CRP/Alb Relationship	High risk	24 (72.73)	46 (93.88)	70 (85.37)	0.0111*
	Low, medium and no risk	9 (27.27)	3 (6.12)	12 (14.63)	

BMI: body mass index; CRP/Alb ratio: relationship between C-reactive protein and albumin. The values expressed in% compare values of the same goal (<80% or ≥80%). * Fisher exact test. ** Qui-square test.

DISCUSSION

The hypothesis that the nutritional goals of severely ill patients receiving EENT are seldom met, was verified in this study, that confirmed that complications and inadequacies during the ENT supply negatively affect the energy and protein balance of critically ill patients. Differences between goals and energy and protein infusion were significant in this study. Only 53.44% of the energy value (15.76 Kcal/kg of weight) was infused; when compared to the target, and 43.72% of the protein (0.67 g/kg body weight) was infused; with respect to the goal, amounts extremely lower than those recommended by McClave et al.[24], in the ASPEN guidelines were infused. Teixeira et al.[26], observed that TNEE patients received 74.4% of the energy target and 74.1% of the protein target. In another study, Campanella et al.[27], found infusion of 72.2% of the energy goal and 71.4% of the protein goal.

Insufficient infusion of energy and protein was also reported by Heyland et al.[28], who observed that ICU patients received 61.2% of the energy targets and 57.6% of the protein targets, and 74% of them received less than 80% of the targets set.

In this study, 72.26% difference between energy and protein prescription and infusion was similar to the results found in the literature, such as in the secondary analysis of an international database of 2270 patients, where the infusion was 61% and 57% of energy and protein prescriptions, respectively[9]. The study by Santana et al.[12] found that patients admitted to the ICU on EENT received 76% of the energy prescription and 69% of the protein prescription. Another study on the shortage of the nutritional supply of critically ill patients[14] showed that they received 63% of the total energy and protein prescribed.

In this study we observed that, on average, 68.07% of the energy requirements and 57.92% of the protein requirements had been prescribed. Similar results were found by McClave et al.[29], reporting that only 65% of the patients received adequate prescription compared to the calculated goals, and that only 51% was actually infused, and Weijs et al.[30], who found 75% energy goals and 72% protein targets infusion in severely ill patients.

Discrepancies with regard to energy and protein goals and infusions may be justified if the dietary volume changes after the onset of ENT, in severely ill and clinically unstable patients[14]. NT related complications and intolerances, such as diarrhea, vomiting, high gastric residue, among others, that were observed in 30.5% of ICU patients, hinder delivery of the programmed diet, generating an energy and protein deficit[31]. The use of enteral formulas with a caloric and protein content lower than the recommendations, contributes to the difficulty of reaching the needs defined[13]. The challenge is to attain the goals set using standard enteral formulas, which would be sufficient for only 25% of the patients[30].

In this study, the difficulty of severely ill patients to receive the nutritional goals was made clear; 89.09% did not reach a minimum of 80% of their energy target and only 2% reached a minimum of 80% of their protein target, but there was no difference between the low energy and protein supply and the severity condition of the patients, measured by the nutritional risk scores and CRP/Alb ratio. No significant difference was observed between the outcome of the patient and the infusion of the nutritional goals, evidencing that the difficulty of NT infusion in critically ill patients occurs independently of the severity of the patient's condition. Choi et al.[32] also found no significant difference in mortality nor in length of stay in the ICU among groups of patients receiving energy input lower than or equal to the defined goals.

However, several studies on the successful outcome of severely ill NT patients correlated low energy and protein supply with the worse clinical outcomes, evidencing an increase in infectious complications, days of mechanical ventilation, longer ICU stay and a higher frequency of pressure injuries[14,31,33].

According to Weijs et al.[30], defining and achieving individual energy and protein goals, reduces 50% mortality of critically ill patients; however, ensuring only the energy supply is not sufficient to obtain better clinical and nutritional outcomes, highlighting the importance of protein supply in patients receiving ENT for several days.

CONCLUSION

The administration of ENT in critically ill patients does not fulfill their actual energy and protein needs, and the high occurrence of infusion inadequacies, regarding prescription and the delivery goals established, can generate a negative nutritional balance.

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Study limitations

Some difficulties in obtaining information in the medical records and from the patients' own family members posed difficulties in carrying out this study. Because it is a prospective study, the daily loss of patients and the difficulty in obtaining information from the other teams involved in patient care made it difficult to evaluate some of the outcomes.

Authors' contribution

IBJ conceived and designed the study, collected and analyzed data, and wrote the manuscript. VALM helped to conceive the study, supervised the research, helped to write the manuscript and reviewed the manuscript. JLBA contributed to the interpretation of the data. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

José IB, Leandro Merhi VA, Aquino JLB. Meta, prescrição e infusão da terapia nutricional enteral de pacientes críticos em unidade de terapia intensiva. Arq Gastroenterol. 2018,55(3):283-9.

RESUMO – Contexto – A terapia nutricional enteral (TNE) é a melhor via para a nutrição de pacientes críticos e com melhores impactos no tratamento clínico desses pacientes. **Objetivo** – Investigar a oferta energética e proteica da TNE em pacientes críticos, internados em uma unidade de terapia intensiva (UTI) de um hospital universitário. **Métodos** – Um estudo prospectivo longitudinal foi conduzido com 82 pacientes críticos internados em uma UTI, recebendo TNE. Foram estudadas variáveis antropométricas, exames laboratoriais (albumina, PCR, relação PCR/albumina), NUTRIC-score e o Nutritional Risk Screening (NRS-2002), metas energéticas e proteicas e as inadequações e complicações da TNE. A análise estatística foi realizada utilizando-se os testes Qui-quadrado ou Fischer e o teste de Wilcoxon, com nível de significancia de $P<0,05$. **Resultados** – Na avaliação pelo NUTRIC score, 48,78% apresentaram alto risco nutricional. Na relação PCR/albumina, 85,37% apresentaram alto risco de complicações. Verificou-se diferença estatisticamente significante ($P<0,0001$) para todas as comparações efetuadas entre a meta, prescrição e infusão da TNE, sendo infundido 72% do que foi prescrito tanto para caloria como para proteína. Observou-se que a diferença entre a prescrição e a infusão foi de 14,63% (±10,81) para caloria e de 14,21% (±10,5) para proteína, com diferença estatisticamente significante ($P<0,0001$). Na relação entre prescrição e infusão de calorias e proteínas, a única associação significativa foi a dos pacientes com alto risco para a relação PCR/albumina, destes; quase 94% receberam menos que 80% do volume energético e proteico prescrito ($P=0,0111$). **Conclusão** – A administração da TNE em pacientes graves, não supre suas reais necessidades energéticas e proteicas. A alta ocorrência de inadequações da infusão, comparadas à prescrição e às metas definidas podem gerar balanço nutricional negativo.

DESCRITORES – Nutrição enteral. Ingestão de energia. Proteínas na dieta. Cuidados críticos. Unidades de terapia intensiva.

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ORIGINAL ARTICLE

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The onset of clinical manifestations in inflammatory bowel disease patients

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ABSTRACT – **Background** – The diagnosis of inflammatory bowel disease is often delayed because of the lack of an ability to recognize its major clinical manifestations. **Objective** – Our study aimed to describe the onset of clinical manifestations in inflammatory bowel disease patients. **Methods** – A cross-sectional study. Investigators obtained data from interviews and the medical records of inflammatory bowel disease patients from a reference centre located in Brazil. **Results** – A total of 306 patients were included. The mean time between onset of symptoms and diagnosis was 28 months for Crohn's disease and 19 months for ulcerative colitis. The main clinical manifestations in Crohn's disease patients were weight loss, abdominal pain, diarrhoea and asthenia. The most relevant symptoms in ulcerative colitis patients were blood in the stool, faecal urgency, diarrhoea, mucus in the stool, weight loss, abdominal pain and asthenia. It was observed that weight loss, abdominal pain and distension, asthenia, appetite loss, anaemia, insomnia, fever, nausea, perianal disease, extraintestinal manifestation, oral thrush, vomiting and abdominal mass were more frequent in Crohn's patients than in ulcerative colitis patients. The frequencies of urgency, faecal incontinence, faeces with mucus and blood, tenesmus and constipation were higher in ulcerative colitis patients than in Crohn's disease patients. The mean time from the onset of clinical symptoms to the diagnosis of Crohn's disease was 37 months for patients with ileocolonic location, 26 months for patients with ileum location and 18 months for patients with colon location. In ulcerative colitis patients, the mean time from the onset of symptoms to diagnosis was 52 months for proctitis, 12 months for left-sided colitis and 12 months for extensive colitis**. Conclusion** – Ulcerative colitis presented a high frequency of intestinal symptoms, and Crohn's disease showed a high frequency of systemic manifestations at the onset of manifestation. There was a long delay in diagnosis, but individuals with more extensive disease and more obvious symptoms showed a shorter delay.

HEADINGS – Inflammatory bowel diseases. Crohn disease. Ulcerative colitis.

INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract that may present pleomorphic clinical manifestations. The most common forms are ulcerative colitis (UC) and Crohn's disease (CD)[1,2].

CD may affect the entire digestive tract from the mouth to the perianal region; its inflammatory process is characterized by discontinuous and segmental injuries that affect all layers of the intestine (transmural inflammation)[3]. The location is closely related to the clinical manifestation[4,5]. The symptoms of CD are heterogeneous but generally include abdominal pain, weight loss and chronic diarrhoea[1,4,6].

UC is characterized by diffuse inflammation of the colon restricted to the mucosa that usually affects the rectum and presents a proximal extension in a symmetrical and continuous pattern[1,3,7]. Symptoms depend on the extent and severity of the disease and more commonly include bloody diarrhoea, rectal bleeding and/or rectal urgency, but these patients also describe faecal incontinence, tenesmus and abdominal pain. Systemic symptoms of malaise, anorexia, fever or even nausea and vomiting may be present mainly during a severe attack[6-9].

IBD can lead to disability and can have a significant impact on quality of life, with significant mental health problems, including depression. Moreover, patients develop professional and social constraints that interfere with work and recreational activities and result in decreased sexual satisfaction compared to the general population[6,10-12].

The incidence of IBD is highest in the United States, the United Kingdom and Scandinavia[13]. Moreover, studies from IBD centres in Brazil report a clear increase in the prevalence of IBD in recent years[14,15]. Despite this rise, IBD is still a relatively unknown disease, and its diagnosis is often delayed[13]. Our study aimed to describe the onset of clinical manifestations presented by these patients.

METHODS

This was a cross-sectional and observational study. The data were collected in the reference centre for inflammatory bowel disease at Hospital Geral Roberto Santos, Bahia, Brazil. The study was conducted between April 2015 and July 2016. The sample included patients from the outpatient unit. This study included consecutive IBD patients (≥18 year of age). Patients were interviewed about clinical manifestations. Information about the Montreal classification was

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collected from medical records. The following variables were included in the study: sex, age at diagnosis, age at onset of the symptoms, time from symptoms to diagnosis, the Montreal classification, nausea, vomiting, abdominal pain, diarrhoea, incontinence, faecal urgency, tenesmus, abdominal distension, the presence of blood and mucus in the stool, weight loss, appetite loss, constipation, malnutrition, anaemia, fever, asthenia, insomnia, abdominal mass, mouth ulcers, perianal disease and extraintestinal diseases. The study was approved by the institutional ethics committee. Informed written consent was obtained from each patient. Data were analysed with the Statistical Package for Social Sciences (version 21.0, SPSS, Chicago, IL).

RESULTS

This study included 306 patients. Epidemiological data are presented in TABLE 1. Occasionally, patients received a diagnosis before clinical manifestation.

TABLE 1. Epidemiological and clinical characteristics of inflammatory bowel disease patients (n=306).

Characteristics	Crohn's disease (n=141)	Ulcerative colitis (n=165)
Sex n (%)		
Female	89 (63.1%)	102 (61.1%)
Male	52 (36.9%)	65 (38.9%)
Age at diagnosis (in years)		
Mean	36 (±14)	38 (±12)
Range	13–78	16–78
Age at onset of symptoms (in years)		
Mean	33 (±14)	36 (±12)
Range	9–78	3–77
Time to diagnosis (in months)	28 (±48)	19 (±46)

The patients were classified according to the Montreal classification, but 21 (6.7%) IBD patients could not be classified (TABLE 2).

The mean time from the onset of clinical symptoms to the diagnosis of CD was 37 months for patients with ileocolonic location, 26 months for patients with ileum location and 18 months for patients with colon location. In UC patients, the mean time from the onset of symptoms to diagnosis was 52 months for proctitis, 12 months for left-sided colitis and 12 months for extensive colitis.

TABLE 3 shows the frequency of each one of the clinical manifestations for all patients.

TABLE 4 presents the onset of clinical manifestations according to the location for CD patients.

TABLE 5 presents the onset of clinical manifestations according to the age at diagnosis for CD patients.

TABLE 6 presents the onset of clinical manifestations for UC patients according to the extent of disease.

DISCUSSION

IBD remains a poorly understood disease. This lack of knowledge has caused delays in IBD diagnosis. Furthermore, there are few studies regarding the onset of clinical manifestations of IBD. Our study contributes as a pioneering evaluation of this subject in Brazil.

TABLE 2. Montreal classification of inflammatory bowel disease patients (n=285).

Crohn's disease	n (%)
Age at diagnosis (A)	
A1 <16 years	11 (7.9%)
A2 17-40 years	83 (59.7%)
A3 > 40 years	44 (31.7%)
Location (L)	
Ileum (L1)	25 (18.7%)
Colon (L2)	55 (41.0%)
Ileocolon (L3)	49 (36.6%)
Isolated upper digestive (L4)	–
L4 + L1	1 (0.7%)
L4 + L2	1 (0.7%)
L4 + L3	3 (2.2%)
Behaviour (B)	
No stricturing/no penetrating (B1)	54 (39.7%)
Stricturing (B2)	17 (12.5%)
Penetrating (B3)	11 (8.1%)
Perianal disease (p)	
B1 + p	24 (17.6%)
B2 + p	12 (8.8%)
B3 + p	18 (13.2%)
Ulcerative colitis	
Extension	
Proctitis (E1)	27 (17.9%)
Left-sided colitis (E2)	67 (44.4%)
Extensive colitis (E3)	57 (37.7%)

TABLE 3. The onset of clinical manifestations in inflammatory bowel disease patients (n=306).

Clinical manifestations	CD (n=141) n (%)	UC (n=165) n (%)	IBD (n=306) n (%)
Weight loss	127 (90%)	128 (76.6%)	255 (82.8%)
Abdominal pain	119 (84.4%)	121 (72.5%)	240 (78%)
Diarrhoea	113 (80%)	133 (79.6%)	246 (80%)
Asthenia	113 (80%)	118 (70.7%)	231 (75%)
Faecal urgency	104 (74%)	139 (83.2%)	243 (79%)
Appetite loss	97 (68.8%)	86 (51.5%)	183 (59.4%)
Malnutrition	94 (66.7%)	51 (30.5%)	145 (47%)
Anaemia	88 (62.4%)	74 (44.3%)	162 (52.6%)
Mucus in the stool	85 (60.3%)	129 (77%)	214 (70%)
Blood in the stool	78 (55.3%)	141 (84.4%)	219 (71%)
Tenesmus	78 (55.3%)	104 (62.3%)	182 (59%)
Abdominal distension	73 (54%)	78 (46.7%)	154 (50%)
Insomnia	73 (51.2%)	69 (41.3%)	142 (46%)
Faecal incontinence	70 (49.6%)	104 (62.3%)	174 (56.5%)
Fever	66 (46.8%)	52 (31%)	118 (38.3%)
Nausea	64 (45.4%)	52 (31%)	116 (37.7%)
Perianal disease	62 (44%)	7 (4.2%)	69 (22.4%)
Extraintestinal diseases	60 (42.6%)	68 (40.7%)	128 (41.6%)
Mouth ulcers	55 (39%)	28 (16.3%)	83 (27%)
Vomiting	47 (33.3%)	31 (18.6%)	78 (25.3%)
Abdominal mass	23 (16.3%)	11 (6.6%)	34 (11%)
Constipation	13 (9.2%)	20 (12%)	33 (10.7%)

TABLE 4. The onset of clinical manifestations according to the Montreal classification for location in Crohn's disease patients (n=134).

Clinical manifestations	Ileum (n=26) n (%)	Colon (n=56) n (%)	Ileocolon (n=52) n (%)
Weight loss	22 (84.6%)	53 (94.6%)	46 (88.5%)
Abdominal pain	22 (84.6%)	49 (87.5%)	42 (80.8%)
Diarrhoea	22 (84.6%)	52 (96.3%)	38 (74.5%)
Asthenia	23 (88.5%)	48 (85.7%)	35 (67.3%)
Faecal urgency	15 (57.7%)	46 (82.1%)	37 (71.2%)
Appetite loss	18 (69.2%)	44 (64.3%)	29 (55.8%)
Malnutrition	15 (57.7%)	39 (69.6%)	34 (65.4%)
Anaemia	17 (65.4%)	38 (67.9%)	27 (51.9%)
Mucus in the stool	16 (61.5%)	36 (64.3%)	28 (53.8%)
Blood in the stool	08 (30.8%)	38 (67.9%)	29 (55.8%)
Tenesmus	114 (2.3%)	36 (64.3%)	26 (50%)
Abdominal distension	16 (61.5%)	23 (41.1%)	30 (57.7%)
Insomnia	12 (46.2%)	30 (53.6%)	28 (53.8%)
Faecal incontinence	07 (26.9%)	34 (60.7%)	27 (51.9%)
Fever	11 (42.3%)	27 (48.2%)	22 (42.3%)
Nausea	15 (57.7%)	26 (46.4%)	19 (36.5%)
Perianal disease	10 (38.5%)	25 (44.6%)	21 (40.4%)
Extraintestinal diseases	14 (53.8%)	22 (39.3%)	19 (36.5%)
Mouth ulcers	11 (42.3%)	24 (42.9%)	17 (32.7%)
Vomiting	10 (38.5%)	21 (37.5%)	12 (23%)
Abdominal mass	03 (11.5%)	06 (10.7%)	12 (23.1%)
Constipation	02 (7.7%)	03 (5.4%)	03 (5.8%)

TABLE 5. The onset of clinical manifestations according to the Montreal classification for age at diagnosis of Crohn's disease patients (n=134).

Clinical manifestations	< 16 yr (n=11) n (%)	17 to 40 yr (n=80) n (%)	> 40 yr (n=43) n (%)
Weight loss	11 (100%)	78 (94%)	35 (80%)
Abdominal pain	10 (91%)	69 (83%)	37 (84%)
Diarrhoea	09 (81.8%)	69 (83%)	34 (77.3%)
Asthenia	09 (81.8%)	70 (84.3%)	31 (70.5%)
Faecal urgency	09 (81.8%)	60 (73.3%)	32 (72.7%)
Appetite loss	07 (63.6%)	59 (71%)	29 (66%)
Malnutrition	09 (81.8%)	56 (67.5%)	27 (61.4%)
Anaemia	10 (91%)	54 (65%)	21 (47.7%)
Mucus in the stool	06 (54.5%)	54 (65%)	23 (52.3%)
Blood in the stool	09 (81.8%)	47 (56.6%)	20 (45.5%)
Tenesmus	08 (72.7%)	47 (56.6%)	21 (47.7%)
Abdominal distension	04 (36.4%)	44 (53%)	25 (56.8%)
Insomnia	04 (36.4%)	40 (48.2%)	28 (63.6%)
Faecal incontinence	08 (72.7%)	39 (47%)	23 (53.3%)
Fever	05 (45.5%)	38 (45.8%)	20 (45.5%)
Nausea	07 (63.6%)	41 (50%)	14 (31.8%)
Perianal disease	05 (45.5%)	41 (50%)	14 (31.8%)
Extraintestinal diseases	06 (54.4%)	33 (40%)	19 (43%)
Mouth ulcers	07 (63.6%)	35 (42,2%)	11 (25%)
Vomiting	05 (45.5%)	28 (33.7%)	12 (27.3%)
Abdominal mass	02 (18.2%)	14 (17%)	06 (13.6%)
Constipation	02 (18.2%)	06 (7.2%)	03 (6.8%)

TABLE 6. The onset of clinical manifestations according to the Montreal classification of ulcerative colitis patients (n=151).

Clinical manifestations	Proctitis (n=27) n (%)	Left-sided colitis (n=67) n (%)	Extensive colitis (n=57) n (%)
Blood in the stool	22 (81.5%)	59 (88%)	48 (84.2%)
Faecal urgency	18 (66.7%)	59 (88%)	50 (87.7%)
Diarrhoea	19 (70.4%)	62 (92.5%)	52 (91.2%)
Mucus in the stool	19 (70.4%)	55 (82%)	42 (73.3%)
Weight loss	17 (63%)	53(79%)	47 (82.5%)
Abdominal pain	19 (70.4%)	50 (74.6%)	42 (73.7%)
Asthenia	14 (52%)	47 (70%)	48 (84.2%)
Tenesmus	17 (63%)	47 (70%)	32 (56%)
Faecal incontinence	15 (55.6%)	47 (70%)	34 (59.6%)
Appetite loss	16 (59.3%)	33 (49.3%)	29 (51%)
Abdominal distension	12 (44.4%)	36 (53.7%)	24 (42%)
Anaemia	06 (22.2%)	33 (49.3%)	29 (51%)
Insomnia	10 (37%)	28 (42%)	24 (42%)
Fever	09 (33.3%)	18 (27%)	22 (38.6%)
Nausea	08 (27%)	23 (34.3%)	19 (33.3%)
Malnutrition	06 (22.2%)	17 (25.4%)	23 (40.4%)
Extraintestinal diseases	08 (29.6%)	26 (38.8%)	28 (49%)
Vomiting	05 (18,5%)	13 (19.4%)	12 (21%)
Mouth ulcers	03 (11%)	12 (18%)	11 (19.3%)
Constipation	04 (14.8%)	02 (3%)	03 (5.3%)
Abdominal mass	02 (7.4%)	06 (9%)	03 (5.3%)
Perianal disease	0%	03 (4.5%)	01 (1.8%)

The majority of patients in our study had UC (54%), following the trend of other Brazilian studies[14-17] and studies from other countries in Latin America[18-20], the USA[21,22], Asia (83.2% UC)[23], France (64% UC)[24] and Denmark (54% UC)[25]. One study from North America demonstrated that UC is more common in the Hispanic population[26]. Several studies in the world have shown that even though UC is still the most prevalent IBD in some places, CD has been the most incident IBD[27,28]. In places with a higher incidence of IBD, the number of CD patients already surpasses that of UC patients[27,29]. In our study, we noticed a small difference in the numbers of CD and UC patients.

The two diseases were more prevalent among females, following the trend in other countries[19,24,26,29,30]. The mean age at diagnosis in our study was 37 years. This finding follows results from Rio de Janeiro for both IBDs (36.7 years)[15], as well as results from São Paulo (35-36 years)[17] and other countries such as Colombia (37.3 years), the Netherlands (34 years)[18] and Asia (36.3 years)[24]. Our data confirmed a trend showing a younger mean age at diagnosis for CD than for UC. This finding is probably explained by the tendency of CD to affect patients at a younger age[23,31].

The mean time between the onset of symptoms and diagnosis was 23 months. This time was longer in CD patients (28 months) than in UC patients, which aligned with findings from previous studies[2,14,32,33]. This timing reflects a noticeably delayed diagnosis compared to countries such as Peru (14.6 months)[18], Iran (15.8 months)[30] and other international centres, in which the time between the onset of symptoms and diagnosis is 1 to 9 months[3,25,31,34,35]. The greater delay in the diagnosis of CD in relation to UC may be due to the lower frequency of alarming symptoms and a higher frequency of unspecific and insidious systemic symptoms, which, in some cases, may be confused with other aetiologies such as irritable bowel syndrome (IBS). Researchers

have suggested that characteristics that are similar between irritable bowel syndrome and IBD have been associated with a delay in the diagnosis of Crohn's disease[36,37].

The main clinical manifestation was weight loss, followed by diarrhoea, abdominal pain, faecal urgency, asthenia and blood in the stool. These findings are similar to results from the USA[34]. The description of fever during the onset of symptoms occurred in 38.3% of IBD patients. The extraintestinal manifestations at the beginning of the symptoms corresponded to reports from 41.6% of patients, slightly higher than findings presented in a study from Iran, in which 32.4% of IBD patients presented at least one extraintestinal manifestation at diagnosis[30,38]. The CD patients presented more weight loss, abdominal pain, asthenia, appetite loss, malnutrition, anaemia, insomnia, fever, nausea and vomiting than UC patients. The UC patients reported more blood and mucus in the stool, urgency, faecal incontinence and tenesmus. More recently, a study suggested that dyssynergic defecation is highly prevalent in IBD patients[39].

The most common symptom described in CD patients was chronic diarrhoea[40]. Abdominal pain and weight loss were seen prior to diagnosis in 80 and 60% of the Caucasian patients, respectively[32,41]. A Chinese study showed that the main manifestations of CD were non-specific symptoms such as abdominal pain (83.3%), diarrhoea (46.2%), weight loss (45.4%) and fever (37.1%)[42]. These results were similar to the Caucasian population and our sample, except for diarrhoea that presented a less frequency in the Chinese population. The most common symptom in our sample was weight loss, affecting 90% of the patients. This finding could be explained by the delay in the diagnosis. The extraintestinal involvement was quite relevant in CD patients, being far higher than that reported by the ECCO (European Crohn's and Colitis Organization) consensus[38]. It was noteworthy that systemic manifestations such as weight loss and asthenia showed a higher frequency than some other digestive symptoms associated with IBD.

The main clinical manifestations in UC patients observed in our study were stool blood, diarrhoea, faeces mucus, weight loss, abdominal pain and asthenia. Data from developed countries, Asian Centers and from our study are similar about the main symptoms at the onset of the clinical manifestation[42]. As reported by the second ECCO guideline for ulcerative colitis, the main symptom of UC is visible blood in the stool[7]. Our finding is in agreement with this statement, showing that blood in the stool was the main symptom. There is no doubt that blood in the stool is a crucial symptom for the physician to suspect ulcerative colitis even if the clinician is not specialized in IBD.

CD patients presenting ileal disease frequently have systemic manifestations, and these results were confirmed in our study. Furthermore, CD patients with small intestine involvement usually present diarrhoea without urgency. In our study, urgency and faecal incontinence were frequent manifestations in colonic CD patients and in UC patients since urgency and possible faecal incontinence are symptoms related to cases of colonic and rectal involvement due to the loss of rectal distensibility[39]. We noticed that colonic Crohn's disease may present symptoms similar to those of UC. A new and important finding about location in the present study was that patients with isolated colonic involvement compared to other locations presented a higher frequency of alarming symptoms such as mucus and bloody diarrhoea, urgency, faecal incontinence, tenesmus, abdominal pain, weight loss, malnutrition, anaemia, and fever. This high frequency of alarming clinical manifestations may be the reason for a shorter time to diagnosis of colonic CD than found with other disease locations.

The left-sided colitis patients reported more diarrhoea with blood and mucus in the stool, faecal urgency and abdominal pain than proctitis patients, which may explain the earlier diagnosis of left-sided colitis patients. On the other hand, patients with proctitis presented a lower frequency of manifestations, which may contribute to a delay in the diagnosis of these patients compared to patients with other UC locations.

Patients diagnosed before the age of 16 years presented the highest frequency of most symptoms. Otherwise, patients over 40 years of age generally had a lower frequency of symptoms, similar to the findings of the studies of Charpentier and Conley[29,32]. Clinical manifestations were mild in older patients compared with those in younger patients as described by Butter[43]. This difference is possibly due to a less active immune system. On the other hand, the high frequency of insomnia may be a typical symptom of patients with advancing age.

In the onset of the clinical manifestations of UC, the presence of intestinal symptoms was described more frequently by UC patients than by CD patients. Otherwise, CD patients usually presented with more pronounced systemic symptoms. This characteristic of CD contributes to a delayed diagnosis, especially when the patient seeks care with a team not familiar with IBD.

Finally, our sample included patients who typically had an established diagnosis of IBD at follow-up. Memory biases may have occurred. In the future, prospective studies involving incidental cases may lead to clearer characterization of the onset of clinical manifestations of IBD in patients.

CONCLUSION

Ulcerative colitis presented a high frequency of intestinal symptoms, and Crohn's disease showed a high frequency of systemic manifestations. There was a long delay in diagnosis, but individuals with disease in more extensive locations and more pronounced symptoms showed a shorter delay in diagnosis. The characterization of the onset of symptoms is critical so that health professionals can suspect IBD from the onset of the disease, contributing to an earlier diagnosis.

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Authors' contributions

Nóbrega VG: data collection, survey execution, statistical analysis and wrote the paper. Silva INN, Brito BS, Silva J, and Silva MCM: data collection and prepare the database. Santana GO: wrote the project, wrote and reviewed the paper.

Nóbrega VG, Silva INN, Brito BS, Silva J, Silva MCM, Santana GO. Manifestações clínicas iniciais em pacientes com doença inflamatória intestinal. Arq Gastroenterol. 2018,55(3):290-5.

RESUMO – **Contexto** – O diagnóstico da doença inflamatória intestinal é frequentemente retardado pela falta de capacidade para reconhecer as suas principais manifestações clínicas. **Objetivo** – Nosso estudo teve como objetivo descrever as manifestações clínicas iniciais em pacientes com doença inflamatória intestinal. **Métodos** – Estudo transversal. Os pesquisadores obtiveram dados por entrevistas e registros médicos de pacientes com doença inflamatória intestinal em um centro de referência localizado na Bahia. **Resultados** – Foram incluídos 306 pacientes. O tempo entre o início dos sintomas e o diagnóstico foi de 28 (±48) meses para doença de Crohn e 19 (±46) meses para colite ulcerativa. O tempo médio desde o início dos sintomas clínicos até o diagnóstico de doença de Crohn foi de 37 meses para pacientes com localização do ileocolon, 26 meses para a localização do íleo e 18 meses para a localização do cólon. Nos doentes com colite ulcerativa, o tempo médio desde o início dos sintomas até ao diagnóstico foi de 52 meses para proctite, 12 meses para colite no lado esquerdo e 12 meses para colite extensa. As principais manifestações clínicas em pacientes com doença de Crohn foram perda de peso, dor abdominal, diarreia e astenia. Os sintomas mais relevantes da colite ulcerativa foram sangue nas fezes, urgência fecal, diarreia, muco nas fezes, perda de peso, dor abdominal e astenia. Observou-se que a perda de peso, dor abdominal e distensão, astenia, perda de apetite, anemia, insônia, febre, náusea, doença perianal, manifestação extraintestinal, afta oral, vômitos e massa abdominal foram mais frequentes na doença de Crohn. A frequência de urgência e incontinência fecal, fezes com muco e sangue, tenesmo e constipação foram maiores na colite ulcerativa. **Conclusão** – A colite ulcerativa apresentou alta frequência de sintomas intestinais e a doença de Crohn mostrou alta frequência de manifestações sistêmicas. Houve um grande atraso no diagnóstico, mas indivíduos com doença mais extensa e sintomas mais exuberantes mostraram um atraso menor.

DESCRITORES – Doenças inflamatórias intestinais. Doença de Crohn. Colite ulcerativa.

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REVIEW

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The efficacy of the different endoscopic treatments versus sham, pharmacologic or surgical methods for chronic gastroesophageal reflux disease: a systematic review and meta-analysis

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ABSTRACT – **Background** – Endoscopic antireflux treatments for gastroesophageal reflux disease (GERD) are still evolving, and most of the published studies address symptom relief in the short-term. **Objective** – We aimed to perform a systematic review and meta-analysis focused on evaluating the efficacy of the different endoscopic procedures. **Methods** – Search was restricted to randomized controlled trials (RCTs) on MedLine, Cochrane, SciELO, and EMBASE for patients with chronic GERD (>6 months), over 18 years old and available follow up of at least 3 months. The main outcome was to evaluate the efficacy of the different endoscopic treatments compared to sham, pharmacological or surgical treatment. Efficacy was measured by different subjective and objective outcomes. **Results** – We analyzed data from 16 RCT, totaling 1085 patients. The efficacy of endoscopic treatments compared to sham and proton pump inhibitors (PPIs) treatment showed a significant difference up to 6 months in favor of endoscopy with no heterogeneity (P<0.00001) (I^2: 0%). The subgroup analysis showed a statistically significant difference up to 6 months in favor of endoscopy: endoscopy vs PPI (P<0.00001) (I^2: 39%). Endoscopy vs sham (P<0.00001) (I^2: 0%). Most subjective and objective outcomes were statistically significant in favor of endoscopy up to 6 and 12 months follow up. **Conclusion** – This systematic review and meta-analysis shows a good short-term efficacy in favor of endoscopic procedures when comparing them to a sham and pharmacological or surgical treatment. Data on long-term follow up is lacking and this should be explored in future studies.

HEADINGS – Gastroesophageal reflux, therapy. Gastrointestinal endoscopy. Follow-Up Studies. Review.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a disease defined as a chronic condition resulting from the reflux of gastroduodenal contents into the esophagus and adjacent organs. It is characterized by symptoms of retrosternal burning (heartburn) and acid regurgitation. Occurring in 6.3% of the US adult population at a frequency of at least twice a week[1,2] There has been an increasing prevalence of GERD (10%-20%) in adults in Western populations in recent decades. It is estimated that up to 28% of adults have weekly symptoms of retrosternal burning and acid regurgitation[3]. In Brazil, about 12% of the population is affected by the disease[4].

The symptoms of persistent mild reflux affect the physical, psychological well-being and quality of life of patients. Uncontrolled GERD can result in complications, including erosive esophagitis, with consequent peptic stenosis, and extraesophageal manifestations that require additional therapy. GERD also increases the risk of developing Barrett's esophagus and subsequent esophageal adenocarcinoma. Recent reports demonstrate a worldwide increase in the annual incidence of esophageal cancer in parallel with the increasing prevalence of GERD[5,6].

The use of proton pump inhibitors (PPIs) in conjunction with lifestyle modifications continues to be the primary therapy. However, the effectiveness of this intervention is often hampered by adherence, costs and risks associated with the long-term use of PPIs. Anti-reflux surgery is an option for patients with refractory symptoms or in those in whom medical therapy is contraindicated or undesirable. Surgery is based on the reconstruction of the antireflux barrier, usually associated with the posterior closure of the diaphragmatic hiatus. These operations can be performed in an open fashion and more recently laparoscopically[7,8].

Surgical treatment, although effective in the short term, may be associated with non-negligible morbidity and there is a growing concern about late recurrence[9]. Although conventional surgery has an acceptable safety profile, there has been increasing interest in alternative minimally invasive endoscopic treatments that may offer similar results with an increased safety profile and faster recovery times.

Endoscopic therapies have emerged as a possible treatment options for individuals with GERD, particularly when refractory to the use of PPIs. These techniques can be categorized into three groups: 1) Endoluminal suture or plication of the gastro esophageal

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junction (GEJ) (EndoCinch®, NDO Surgical®, MUSE® Esophyx®); 2) Radiofrequency (RF) thermal therapy of the lower esophageal sphincter (LES) (Stretta®); and 3) Injection or implantation of biopolymers in GEJ (Enteryx®, Gatekeeper®, among others)[10]. Due to the continuous evolution of these therapies, multiple prospective, randomized clinical trials evaluating the benefits of these interventions have been performed.

Two meta-analysis recently described on the literature, one showing an overall increased benefit of transoral incisionless fundoplication (TIF) performed with the EsophyX® device when compared to patients who did not undergo TIF[11]. The second study analyzing the Stretta® procedure, showed that there were no significant changes in physiologic parameters (time spent at a pH less than 4 and lower esophageal sphincter pressure (LESP), ability to stop PPIs, or health related quality of life score (HRQL) when compared with sham therapy[12]. The aim of our study is to perform a systematic review and meta-analysis on the efficacy of all randomized controlled trials evaluating the efficacy of all available endoscopic treatments when compared to a sham procedure or therapy with pharmacologic agents like PPIs or laparoscopic anti reflux surgery (LARS).

METHODS

Protocol and registration

The present systematic review and meta-analysis is performed according with the PRISMA statement[13]. This study was registered at www.crd.york.ac.uk/PROSPERO. Registration number is: CRD42017064534. This study was exempt from ethical approval because analysis involved only de-identify data.

Search strategy

We searched in MedLine (Pubmed), EMBASE, Cochrane Central and SciELO (1980 to March 22, 2018), for the studies assessing the efficacy of all endoscopic treatment for GERD.

Terms used to search Medline

"Gastric Acid Reflux," or 'Esophageal Acid reflux "Gastroesophageal Reflux Disease," or "GERD" AND "Endoscopic treatment," or "Gastrointestinal Endoscopy," or "Surgical Procedures," or "Gastrointestinal Surgeries".

Terms used to search in EMBASE, Cochrane Central, SciELO

(Gastroesophageal reflux disease) AND ("Endoscopic treatment" or "Gastrointestinal Surgical treatment").

The search was restricted to human studies with no language or date of publication restriction in peer- reviewed journals. Two authors (M.C. and B.W.) independently screened each of the potential titles, abstracts in the primary search to exclude studies that did not address the research question of interest, based on pre-specified inclusion and exclusion criteria (detailed below). The full text of the remaining articles was examined to determine whether it contained relevant information. Areas of disagreement or uncertainty in article selection were resolved by consensus, and in discussion with a coauthor (D.T.M). Conference proceedings, which did not undergo peer review, were excluded from our analysis. We attempted to contact the corresponding authors to provide additional information on trials if required.

Study selection

Selection of prospective, randomized clinical trials evaluating the efficacy of the different endoscopic treatments versus any other interventions (sham, PPI, surgery) for chronic GERD was performed. Studies that met the following criteria were included: patients over 18 years of age, undergoing endoscopic procedures for chronic GERD (defined as symptoms equal or over 6 months in duration), more than 3 months follow up period. Types of intervention and controls: Available endoscopic therapies: transoral incisionless fundoplication (TIF2) by the EsophyX® device, surgical plication by NDO surgical® device, radiofrequency therapy by the Stretta® device; endoscopic suturing system by EndoCinch® device, injectable esophageal prostheses by Gatekeeper® device, biocompatible non-resorbable copolymer by the Enteryx® device. Controls were performed via a sham procedure, pharmacological treatments (PPIs) or surgery (LARS). Exclusion criteria: retrospective, prospective non-randomized, studies without full text, studies that were requested to the authors without being answered and studies that compare two endoscopic procedures head to head.

Data extraction and quality assessment

Data on study characteristic, such as, author name, reference, year of publication; sample size and population, type of endoscopic intervention, type of control group (sham, PPI or LARS), subjective or objective outcomes, follow-up period and type of analysis (per protocol or intention to treat) were abstracted onto a standardized data form by at least two authors independently (M.C, B.W, D.T.M). Details of data abstraction are reported in FIGURE 1. The quality of each study was classified according to the risk for bias, considering: the question to be investigated, a correct randomization protocol, an adequate subject allocation,

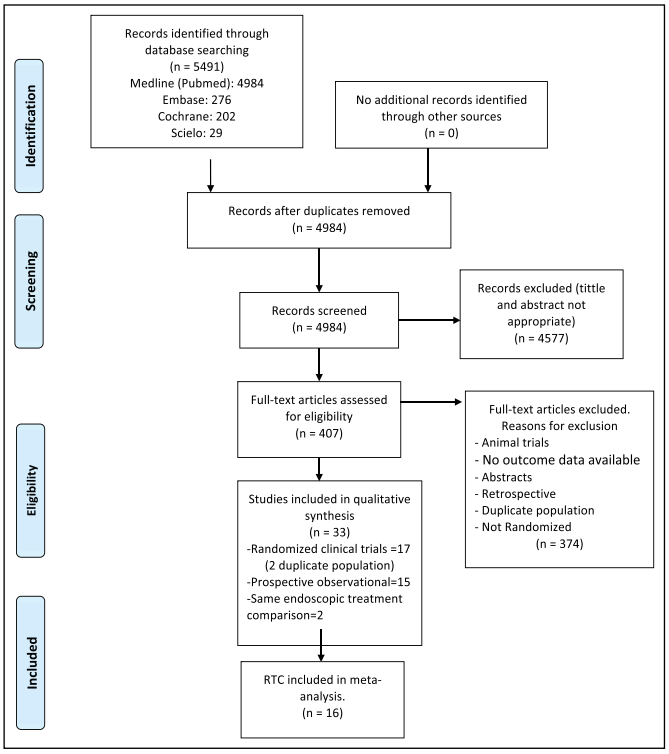

FIGURE 1. Flow diagram of the data extraction methodology.

importance of the blinding, patient losses in each study, each prognostic factor, outcome reporting and analysis by intention to treat or by protocol. In addition, the JADAD scale or score was used to independently assess the methodological quality of each clinical trial[14]. (FIGURE 2.A and FIGURE 2.B).

Outcomes assessed

The main or primary outcome of the study is to measure the overall efficacy of endoscopic treatments versus other interventions (PPI or LARS) or sham procedure for the treatment of chronic GERD. Subgroup analysis was assessed individually

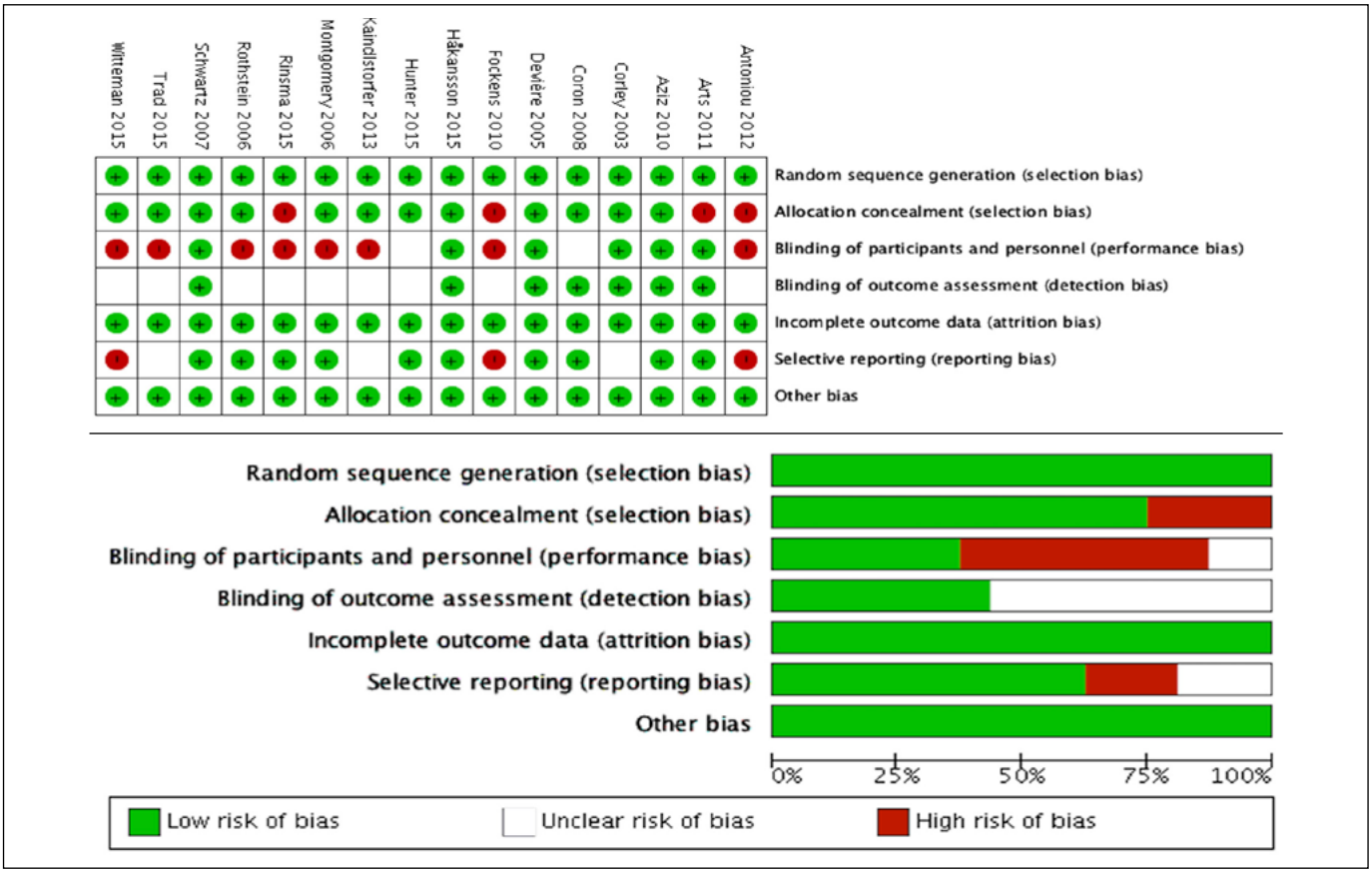

FIGURE 2.A. Summary of risk of bias of included RCT's.

Study	Question	Randomization	Allocation	Double Blinding	Losses	Prognosis	Outcomes	ITT	Jadad Scale
Antoniou, 2012	Yes	Yes	No	No	Yes	Yes	Yes	No	3
Arts, 2011	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	3
Aziz, 2010	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	5
Corley, 2003	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	5
Coron, 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	5
Devière, 2005	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	4
Fockens, 2010	Yes	Yes	No	No	Yes	Yes	Yes	No	3
Håkansson, 2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	5
Hunter, 2015	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	4
Kaindlstorfer, 2013	Yes	Yes	Yes	No	No	Yes	Yes	No	3
Montgomery, 2006	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	4
Risma, 2015	Yes	Yes	No	No	Yes	Yes	Yes	Yes	3
Rothstein, 2006	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	5
Schwartz, 2007	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	5
Trad, 2015	Yes	Yes	Yes	No	Yes	Yes	Yes	No	4
Witteman, 2015	Yes	Yes	Yes	No	Yes	Yes	Yes	No	4

FIGURE 2.B. Summary of risk of bias of included RCT's.

for 3, 6 and 12 months follow up for the different outcomes. The outcomes were categorized as objective: 1) normalization of esophageal acid pH (total proportion of time with a pH <4 in 24-h period)[15]; 2) mean percent of total time of esophageal pH <4 in 24-hours period[15]; 3) healing esophagitis; 4) worsening esophagitis; 5) mean number of reflux episodes; 6) lower esophageal sphincter resting pressure (LESRP); and subjective: 1) time in remission (more than 6 months without the use of PPI); 2) number of patients with GERD health related quality of life (HRQL) score >50 % improvement[16,17]; 3) mean GERD HRQL score[16,17]; 4) elimination of troublesome regurgitation as defined as per the Montreal consensus[16]; 5) heartburn score[18]; 6) DeMeester score[18]. All extracted data were placed according to the intention-to-treat analysis and protocol information.

For this meta-analysis, if there was treatment crossover to other interventions, no data analysis was performed.

Statistical analysis

This meta-analysis, follows the methodology as previously suggested by DerSimonian and Laird[19]. We used a fixed-effect model to determine the efficacy of all endoscopic treatments vs any other type of interventions. Heterogeneity was assessed using the I^2 statistic to estimate what proportion of total variances across studies was due to heterogeneity or chance. As previously reported, I^2 values of 25%, 50%, and 75% represent low, moderate, and high levels of heterogeneity, respectively. Once heterogeneity was noted, to identify potential sources of heterogeneity, subgroup analysis was performed by excluding potential outliers. Visual inspection of publication bias was performed by using a funnel plot and calculated by the Egger test[20]. The absolute risk difference with a 95% confidence intervals (CIs) were calculated for all point estimates, and a P value <0.05 was considered statistically significant. The difference between the main outcome as well as the subgroup analysis was calculated using the risk difference with dichotomous variables and the mean difference with continuous variables. The Mantel Hantzel test for the analysis of categorical variables and inverse variance for continuous variables. The number need to treat (NNT) was also calculated. Statistical analysis was performed using the RevMan 5.3 software (Copenhagen, The Nordic Cochrane Center, The Cochrane Collaboration, 2014) and VassarStats software: Website for Statistical Computation (Richard Lowry 2001-2017 All rights reserved).

RESULTS

A total of 5491 citations were identified by using our search strategy (PubMed, SciELO, EMBASE, and Cochrane databases, provided 4984, 29, 276, and 202 articles respectively) we excluded 5,084 abstracts after initial screening, and assessed 407 full-text articles for eligibility. Of these, 391 studies did not meet inclusion criteria (animal trials, no outcome data available, abstracts, retrospective, duplicate population not randomized, comparison between two endoscopic procedures, comparison of a different technique of the same endoscopic treatment and not relevant). Thus, 16 prospective randomized clinical trials were selected for the final analysis. The schematic diagram of the study selection is illustrated in FIGURE 1. The characteristics of the included studies are summarized in TABLE 1.

A total of 1085 patients were included in the analysis of the endoscopic treatment efficacy in comparison with sham procedure, PPI or LARS. A total of 221 patients underwent TIF2, 145 surgical plications, 81 radiofrequency therapy; 42 endoscopic suturing, 32 injectable esophageal prostheses and 75 biocompatible non-resorbable copolymer. As for the control group a total of 294 patients underwent a sham procedure, 120 received PPIs,

TABLE 1. Descriptive table of RTC's characteristics.

Study/ Publication Year	Population	Intervention Group	Control Group	Outcome (Efficacy)	Follow up (Months)	Final Analysis
Håkansson, 2015[28]	Chronic GERD: 44	TIF2: 22	sham: 22	Time in remission	6	ITT
Hunter, 2015[29]	Chronic GERD: 129	TIF2/placebo: 87	sham + PPI: 42	ETSR	6	ITT
Rinsma, 2015[30]	Chronic GERD: 47	TIF2: 32	PPI: 15	GERD HRQL score	6	ITT
Witteman, 2015[31]	Chronic GERD: 60	TIF2: 40	PPI: 20	>50% GERD HRQL	6	PP
Trad, 2015[32]	Chronic GERD: 63	TIF2: 40	PPI: 23	ETSR	6	PP
Kaindlstorfer, 2013[33]	Chronic GERD: 70	NDO surgical: 37	LARS: 33	DeMeester score	3	ITT
Corley, 2003[34]	Chronic GERD: 64	Stretta: 35	sham 29	>50% GERD HRQL	6	PP
Arts, 2011[35]	Chronic GERD: 22	Stretta: 11	sham: 11	GERD HRQL score	3	ITT
Antoniou, 2012[36]	Chronic GERD: 60	NDO surgical: 30	LARS: 30	GERD HRQL score	3-12	PP
Schwartz, 2007[37]	Chronic GERD: 60	Endocinch: 20	sham: 20 Observation: 20	ETSR	3	ITT
Fockens, 2010[38]	Chronic GERD: 118	Gate Keeper: 75	sham: 43	GERD HRQL score	6	PP
Devière, 2005[39]	Chronic GERD: 64	Enteryx: 32	sham: 32	>50% GERD HRQL	3	ITT
Rothstein, 2006[40]	Chronic GERD: 159	NDO surgical: 78	sham: 81	>50% GERD HRQL	3	ITT
Coron, 2008[41]	Chronic GERD: 43	Stretta: 23	PPI: 20	Time in remission	6-12	ITT
Montgomery, 2006[42]	Chronic GERD: 46	Endocinch: 22	sham: 24	Time in remission	3	ITT
Aziz, 2010[43]	Chronic GERD: 36	Stretta: 12	sham: 12 Observation:12	Time in remission	12	ITT

GERD: gastroesophageal reflux disease, HRQL: health related quality of life, PPI: proton pump inhibitors, LARS: laparoscopic antireflux surgery, TIF: transoral incisionless fundoplication, ITT: intention to treat, ETSR: elimination of troublesome regurgitation, PP: per protocol.

and 63 underwent LARS. Studies consistently scored well on description of study aims, description of main findings, clarity in reporting of unplanned retrospective analyses, appropriate use of statistical tests, and use of accurate main outcome measures, and consistently scored poorly on blinding of subjects and assessors and patent allocation.

According to the risk of bias assessment of each individual study, we observed that a proper outcome description, a question to be investigated, randomization, patient losses and subject group prognosis were properly reported. Adequate allocation was done by 75% (12/16), double blinding was properly described by 40% (7/16) of trials and analysis by intention to treat was done by the 63% (10/16) of the studies. All studies had a JADAD scale over >3 with an overall average of 4.1. (FIGURE 2.A and FIGURE 2.B).

Study outcomes

• Efficacy of endoscopic treatments versus sham and PPI

A total of 707 patients, divided into 3, 6 and 12 follow up periods, from 10 trials were analyzed to evaluate the overall efficacy of the different endoscopic treatment devices versus any other intervention. Endoscopic treatments were performed in 395: Stretta®, Enteryx®, TIF2, NDO surgical®, Endocinch®, and 312 patients from the control group received: sham, PPI, sham + PPI together.

The overall risk-difference analysis (RD) showed a statistically significance difference (P<0.00001) in evaluating the treatment efficacy between the two groups (RD -0.35, 95% CI -0.42, -0.28), in favor of endoscopic treatment and demonstrating no heterogeneity between the trials (I^2: 0%). The Number needed to treat (NNT) was: 2.85. Endoscopic treatments were effective in treating chronic GERD in 62% of the patients in comparison to the 25% of patients from the control group.

For the 3 months follow up subgroup analysis, a total of 263 patients from three trials were included. The RD showed a statistically significance difference (P<0.00001) for the treatment efficacy between the two groups (RD -0.38, 95% CI -0.49, -0.28), in favor of endoscopic treatment and demonstrating no heterogeneity between the trials (I^2: 0%). The NNT was 2.63. For the 6 months follow up subgroup analysis, a total of 377 patients from 6 trials were included. The RD analysis showed a statistically significance difference (P<0.00001) for the treatment efficacy between the two groups (RD -0.36, 95% CI -0.45, -0.26) in favor of endoscopic treatments and demonstrating low heterogeneity between the trials (I^2: 7%). The NNT was 2.77. For the 12 months follow up subgroup analysis, a total of 67 patients from two trials were included. The RD showed no statistically significance difference (P<0.06) for the treatment efficacy between the two groups (RD -0.20, 95% CI -0.41, -0.01) with no heterogeneity between trials (I^2: 0%). (FIGURE 3).

• Subgroup analysis

We decided to make a subgroup analysis for the efficacy of the different endoscopic procedures to any type of intervention and to only sham procedure.

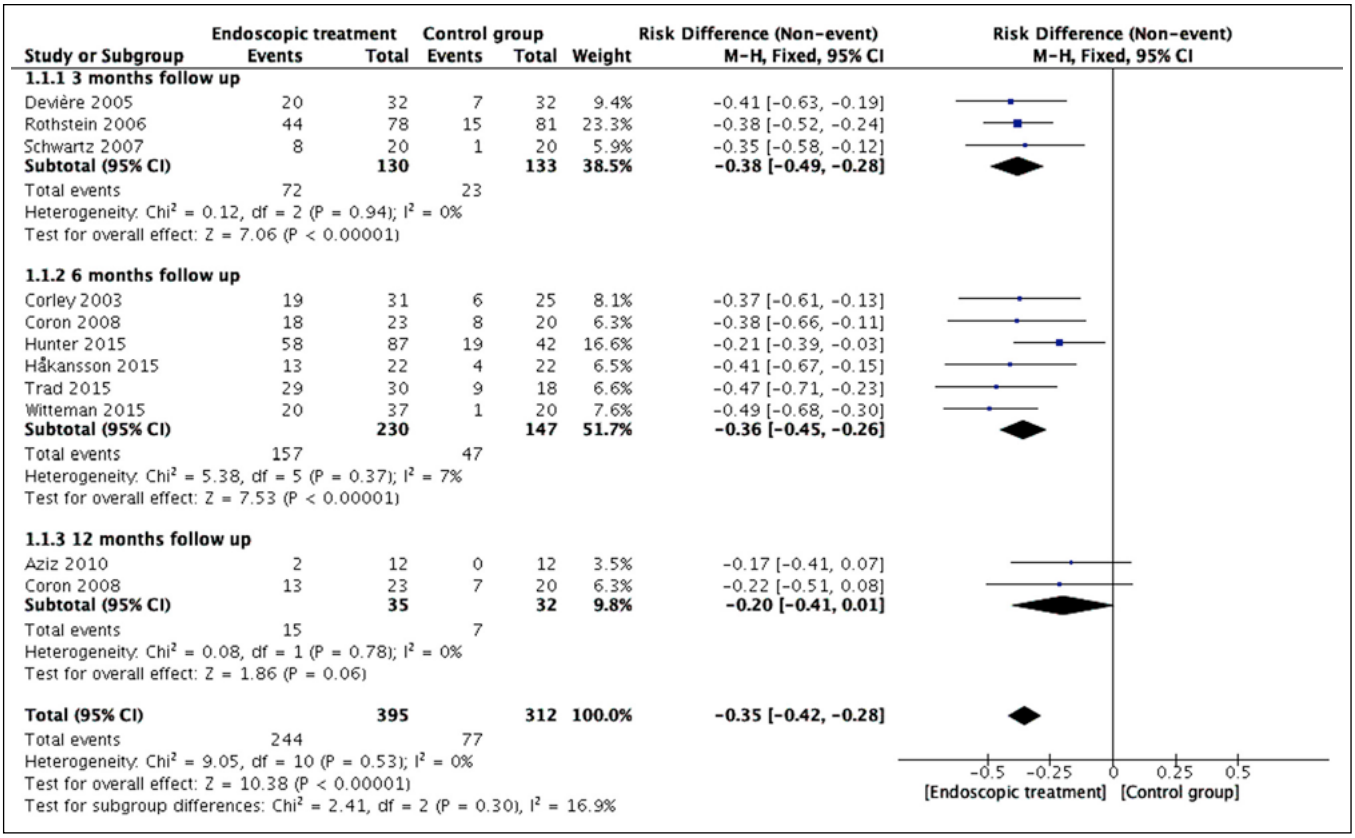

FIGURE 3. Efficacy of endoscopic treatments versus sham and PPI.

• Efficacy of endoscopic treatments versus pharmacological (PPI) treatment

A total of 320 patients from four trials were analyzed. Follow up was divided in 6 and 12 periods. Endoscopic interventions were performed in 200 patients: Stretta®, TIF2, and 120 patients from the control group received: PPI and sham + PPI. The overall RD analysis showed a statistically significance difference (P<0.00001) in treatment efficacy between the two groups (RD -0.33, 95% CI -0.43, -0.22), favoring the endoscopic treatments and demonstrating a low heterogeneity between the trials (I^2: 39%). The NNT was 3.03. The different endoscopic treatments were effective in treating chronic GERD in 69% of the patients in compared to the 37% of patients treated with PPIs or sham + PPI.

For the 6 months follow up subgroup analysis, a total of 277 patients from four trials were included. The RD showed a statistically significance difference (P<0.00001) for the treatment efficacy between the two groups (RD -0.34, 95% CI -0.45, -0.24) favoring endoscopic treatments and demonstrating a moderate heterogeneity between the trials (I^2: 45%). The NNT was 2.94.

For the 12 months follow up subgroup analysis, a total of 43 patients from one trial were included. The RD showed no statistically significance difference (P: 0.15) between the two groups (RD -0.22, 95% CI -0.51, 0.08). (FIGURE 4).

• Efficacy of endoscopic treatments vs sham procedure

A total of 387 patients, from 6 trials were included. Follow up was divided in 3, 6 and 12-month periods. Endoscopic interventions were performed in 195 patients: Stretta®, Enteryx®, TIF2, NDO surgical®, Endocinch®, and 192 patients underwent sham procedure for control.

The overall RD analysis showed a statistically significance difference (P<0.00001) in treatment efficacy between the two groups (RD -0.37, 95% CI -0.46, -0.28), favoring the endoscopic treatments and demonstrating no heterogeneity between trials (I^2: 0%). The NNT was 2.70. The different endoscopic treatments were effective in treating chronic GERD in 54% of the patients in comparison to the 17 % of patients treated with sham.

For the 3 months follow up subgroup analysis, a total of 263 patients from three trials were included. The RD showed a statistically significance difference (P<0.00001) in evaluating the treatment efficacy between the two groups (RD -0.38, 95% CI -0.49, -0.28) favoring endoscopic treatments and demonstrating no heterogeneity between trials (I^2: 0). The NNT was: 2.63.

For the 6 months follow up subgroup analysis, a total of 100 patients from two randomized trials were included. The RD showed a statistically significance difference (P<0.00001) in evaluating the treatment efficacy between the 2 groups (RD -0.39, 95% CI -0.57, -0.21) favoring endoscopic treatments and demonstrating no heterogeneity between the trials (I^2: 0%). The NNT was: 2.56.

For the 12 months follow up subgroup analysis. Total of 24 patients from 1 randomized trial were included. The RD showed no statistically significance difference (P<0.17) between the two groups (RD -0.17, 95% CI -0.41, 0.07). (FIGURE 5).

• Summary of objective outcomes analyzed

Endoscopic therapies had consistent results showing a statistically significant (P<0.0001) (RD -0.42, 95% CI -0.62, -0.21) (I^2: 0%) improvement in healing of esophagitis with no heterogeneity between trials in up to 12 months of follow up. The outcomes of normalization of esophageal acid pH (P<0.03) (RD -0.13, 95% CI -0.26, -0.01) (I^2: 76%), LESRP (P<0.00001) (MD -1.15, 95% CI -1.47, -0.83) (I^2: 94%), mean percent of total time of esophageal pH<4 (P<0.00001) (MD -1.19, 95% CI -1.53, -0.84) (I^2: 78%) and mean number of reflux episodes (P<0.00001) (MD -12.80, 95% CI

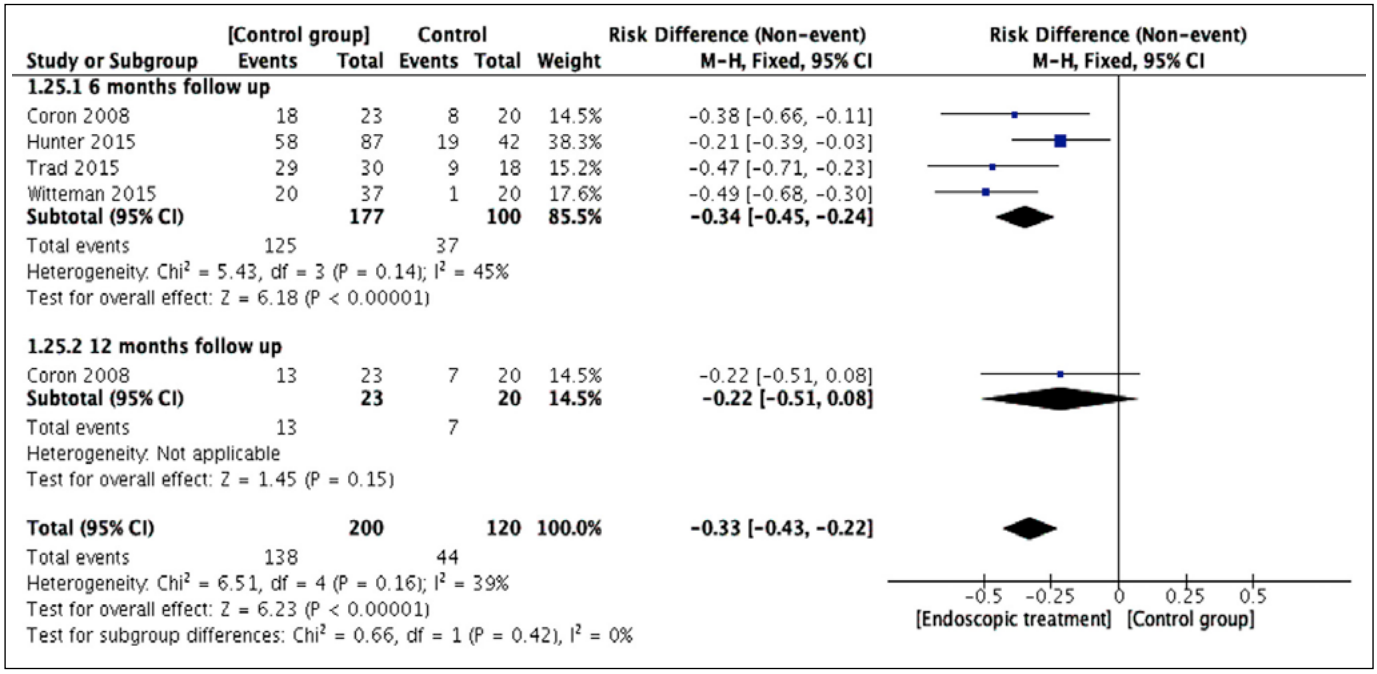

FIGURE 4. Efficacy of endoscopic treatments versus pharmacological (PPI) treatment.

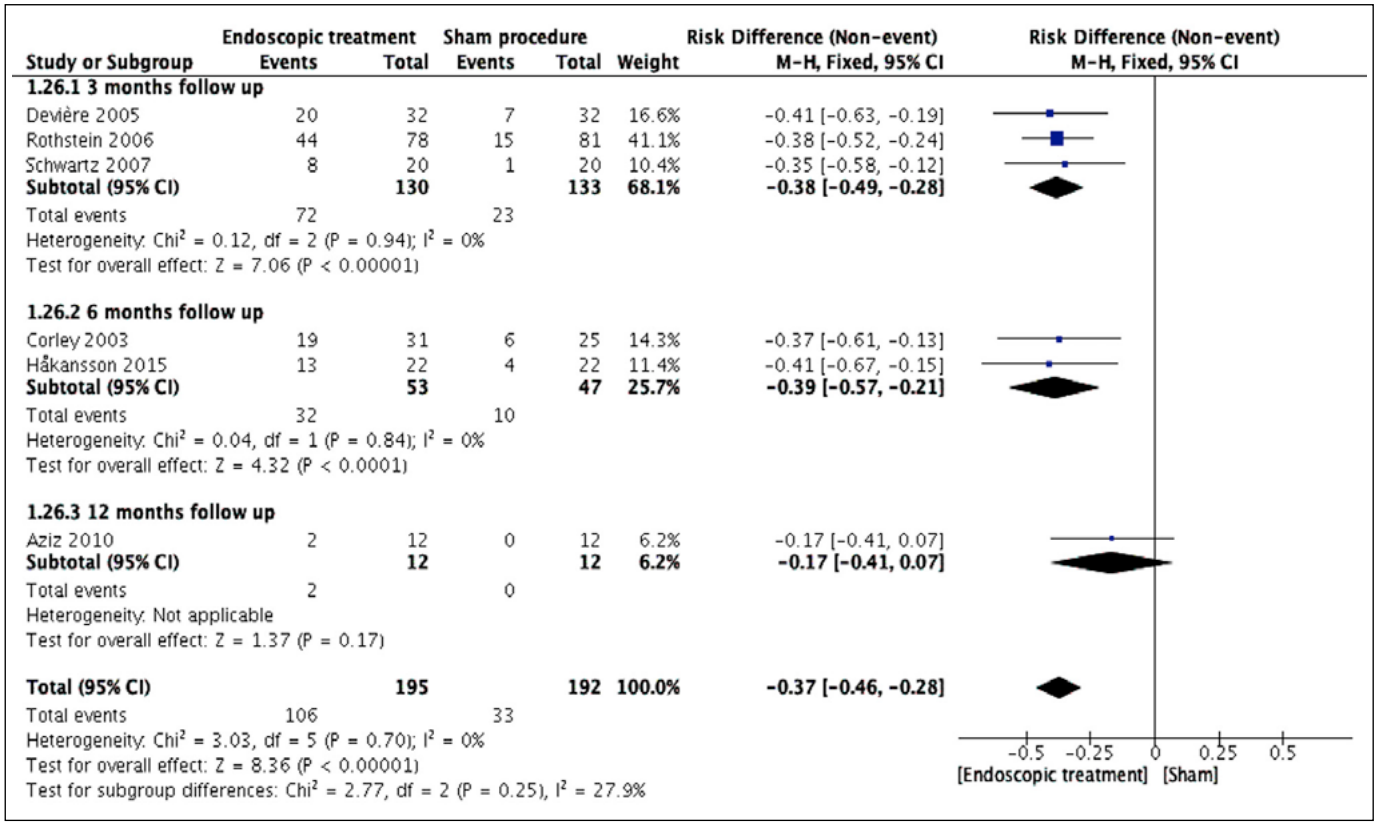

FIGURE 5. Efficacy of endoscopic treatment vs sham procedure.

-15.04, -10.56) (I^2: 98%) were statistically significant in favor to the endoscopic procedures but with a high heterogeneity between trials in up to 12 months of follow up, with the exemption of the mean number of reflux episodes which was statistically significant up to 3 months follow up. When comparing endoscopic therapies only to sham, the results are similar, except for healing of esophagitis since there is no available data to show this comparison.

• Summary of subjective outcomes analyzed

When endoscopic treatments were compared to any other intervention (PPIs or LARS) or sham, the time in remission (P<0.00001) (RD -0.29, 95% CI -0.38, -0.20)(I^2: 0%), number of patients with GERD HRLQ score >50 % improvement (P<0.00001)(RD -0.38, 95% CI -0.47, -0.30) (I^2: 11%), elimination of troublesome regurgitation (P<0.00001) (RD -0.32, 95% CI -0.43, -0.20) (I^2: 10%), were statistically significant in favor to the endoscopic procedures with very low heterogeneity between the trials up to 6 and 12 months follow up. Interestingly, the mean GERD HRQL score (P<0.00001) (MD -0.92, 95% CI -1.24, -0.60) (I^2: 98%), the heartburn score (P<0.00001) (MD -0.53, 95% CI -0.60, -0.46) (I^2: 80%) and De-Meester score (P<0.00001) (MD -5.14, 95% CI -6.43, -3.48) (I^2: 96%), showed statistically significance in favor to endoscopy up to 6 and 12 months but with a high heterogeneity, and the SF-36 score (P<0.00001) (MD 4.75, 95% CI 3.76, 5.74) (I^2: 77%), showed improvement in favor to other therapies compared to endoscopy up to 12 months follow up, but with high heterogeneity between studies. When comparing endoscopic therapies only to sham, the results were similar.

DISCUSSION

A wide-range of GERD patients with poorly controlled symptoms following daily PPIs dependence is actively looking for an effective anti-reflux procedure. Long term PPIs usage is expensive and has several well-known side effects. Laparoscopic Nissen fundoplication is the surgical "gold standard", however, minimally invasive endoscopic treatments may offer the advantages of being safer with faster recovery times.

As stated above, endoscopic treatments for GERD can be categorized into three groups: 1. Endoluminal suture or plication of the gastroesophageal junction; 2. Radiofrequency (RF) thermal therapy of the lower esophageal sphincter (LES); and 3. Injection/implantation of biopolymers in GEJ. This meta-analysis compares the efficacy of different endoscopic procedures to any other intervention (sham procedure, PPIs or LARS). The efficacy of these treatments was measured by different objective and subjective outcomes.

As for the main outcome, which evaluates the general efficacy of the different endoscopic procedures versus sham and PPIs, we observed an overall statistically significant difference favoring the endoscopic procedures with no heterogeneity between the randomized clinical trials. Endoscopic treatment was more effective in treating chronic GERD in 62% of the patients, in comparison to 25 % of patients treated by any of these interventions. In the individual subgroup analysis, we observed a statistically significant difference in favor of the endoscopic procedures in the 3 and 6 months follow up groups, but this difference was not present in studies that

had 12 months of follow up. To date, there are no randomized studies evaluating the efficacy of endoscopic procedures with over 12 months of follow up. In addition, there is treatment crossover since a group of patients who received endoscopic interventions required additional therapies, which limits the follow up analysis.

There are other prospective cohort studies, which reported a prolonged follow up period with good results. For example, for the TIF2 device, Testoni et al.[21] prospectively followed 50 patients, 32 patients completed 3 years and 14 patients completed 6 years of follow-up. Another two prospective studies evaluated the long-term efficacy of the Stretta device, the Dughera et al. study[22] included 86 patients, where 26 patients completed 8 years of follow-up and the Noar et al.[23] evaluated 217 patients and reported a 10-year follow-up. Interestingly, many objective measurements return to baseline but a significant proportion of patients show continued improvement in subjective outcomes and remain off PPIs.

For the Muse system two different trials have been reported, one for 6 months and other for 4 years follow-up, both showing promising objective and subjective outcomes improvement[24,25]. This device was not included in our study because no randomized clinical trials are available to date.

In this meta-analysis, we included RCTs that described the effectiveness of the NDO surgical®, Endocinch®, Enteryx® and Gatekeeper® devices as individually, they show improvement in outcomes. It is important to note that these devices are now off the market in the USA due to safety concerns[26].

Subgroup analysis was performed comparing the efficacy of the different endoscopic procedures versus PPIs and sham procedure separately. For the first subgroup, comparing the efficacy of endoscopic treatments to PPIs, we were able to perform a meta-analysis from four randomized clinical trials and we observed a statistically significant difference favoring the endoscopic treatments with no significant heterogeneity between trials. Endoscopic treatments were more effective in treating chronic GERD in 69% of the patients, in compared to the 37% of patients treated by PPI intervention. In the individual subgroup analysis, we observed a statistically significant difference in favor of the endoscopic procedures in the 6 months follow up group, but this difference was not present in studies that had 12 months of follow up. This difference was lost, probably due to insufficient data since there was only one study evaluating outcomes at this time period[41].

In the analysis of the second subgroup, comparing the efficacy of endoscopic treatments to sham, we observed a statistically significant difference favoring endoscopic treatments with no heterogeneity between trials. Endoscopic treatments were more effective in treating GERD in 54% of the patients compared to the 12% of patients that underwent a sham procedure. As in the previous subgroup, we observed a statistically significant difference in favor of the endoscopic procedures in the 3 and 6 months follow up groups, but this difference was not present in studies that had 12 months of follow up. likely due to insufficient data since there was only one study evaluating outcomes at this time period[43].

As for limitations of this meta-analysis, there is a high degree of heterogeneity when analyzing different outcomes since the reporting of multiple subjective and objective outcomes is not uniform. In addition, very few studies provide reliable information beyond 6 months. Many patients were offered alternative interventions at this time period and the actual benefit of the endoscopic intervention is compromised. Importantly, there was insufficient data to perform a subgroup analysis for LARS separately.

There are several studies comparing laparoscopic surgery to PPIs. A randomized study described by Galmiche et al.[8] comparing LARS vs PPI with a 5-year follow up, showed that both groups presented higher rates of "time in remission" (for LARS, defined as need for acid suppressive therapy and for PPIs, as inadequate symptom control after dose adjustment). A similar, large randomized trial by Hatlebakk et al.[27] comparing LARS vs PPIs at 6-months and 5-years follow up, showed that both therapies were effective in controlling esophageal acid exposure. In our study, the time in remission and esophageal acid exposure rates were statistically significant in up to 6 months with no heterogeneity and 12 months with high heterogeneity, respectively, in favor of endoscopic treatments.

In this review, most of the studies report clinically significant moderate to severe postprocedure related adverse events such as epigastric pain, musculoskeletal pain, dysphagia, sore throat, chest pain, nausea and vomit, bloating and flatulence among others, that were treated clinically, with complete resolution and no major sequelae. Reporting a total of 312 events of 1073 procedures. The event rate of 38% for the endoscopic treatments, 24 % for the sham procedure, 4% for the PPI group and 2% for the LARS group.

However, there are studies that have reported more serious procedure related adverse events. A trial that evaluated the Gatekeeper® device[38] showed two procedure related esophageal wall perforations, one managed surgically and other clinically with no long-term sequelae. A trial evaluating the NDO device, described a procedure related pneumomediastinum and pneumoperitoneum[40], managed clinically and without any further intervention. A trial assessing the NDO vs LARS[33], reported one post- procedure gastric bleeding related to the NDO device, managed clinically without sequelae. From all trials, one death at 11 months after the intervention (TIF2) was reported[31], this mortality case was considered probably unrelated to the intervention.

This meta-analysis provides outcomes of all the endoscopic procedures available to date to treat patients with chronic GERD. Since this patient population is similar across these studies and physiologically, all endoscopic therapies attempt to increase the pressure of the LES by different mechanisms, pooling the results of these RCTs in a systematic fashion, provides a detailed analysis of objective and subjective outcomes that may aid in bridging the gap between medical therapy and conventional surgery in patients who suffer from chronic GERD.

CONCLUSION

The development of alternative treatment options for chronic GERD is of interest. Patients are destined to lifelong PPIs or antireflux surgery, although current conventional surgical approaches have been well studied and are relatively safe, recent advances in minimally invasive endoluminal techniques have introduced the possibility of incisionless procedures.

This systematic review and meta-analysis shows a good short-term efficacy in favor of endoscopic procedures when comparing them to a sham and pharmacological or surgical treatment. Current data on endoluminal therapies for the management of GERD are promising; however, the role of endoscopy within the GERD treatment algorithm remains unclear. More studies, especially in endoscopic plication devices, are necessary because the current available data are limited by conflicting results, lack of long-term efficacy and non-homogeneous outcome reporting. In conclusion, larger prospective, multicenter, randomized studies are necessary,

to identify the role of endoscopic therapies before they can be advocated as an effective GERD solution.

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Authors' contributions

Study concept and design by Coronel MA, Bernardo WM and Moura EGH; acquisition of data, analysis and interpretation of data, statistical analysis by Coronel MA, Bernardo WM, Moura DTH, Moura ETH, Ribeiro IB; drafting of the manuscript and critical revision of the manuscript for important intellectual content by Coronel MA, Bernardo WM, Moura EGH; Study supervision: Bernardo WM, Moura DTH and Moura EGH.

Coronel MA, Bernardo WM, Moura DTH, Moura ETH, Ribeiro IB, Moura EGH. A eficácia dos diferentes tratamentos endoscópicos versus métodos sham, farmacológicos ou cirúrgicos para o refluxo gastroesofágico crônico: uma revisão sistemática e meta-análise. Arq Gastroenterol. 2018,55(3):296-305.

RESUMO – **Contexto** – Os tratamentos endoscópicos para a doença do refluxo gastroesofágico (DRGE) ainda estão em evolução e a maioria dos estudos publicados abordam o alívio dos sintomas em curto prazo. **Objetivo** – Pretendemos realizar uma revisão sistemática e meta-análise focada na avaliação da eficácia dos diferentes procedimentos endoscópicos. **Métodos** – A pesquisa foi restrita a ensaios clínicos randomizados em MedLine, Cochrane, SciELO e EMBASE para pacientes com DRGE crônica (>6 meses), com mais de 18 anos e acompanhamento disponível por pelo menos 3 meses. O principal desfecho foi avaliar a eficácia dos diferentes tratamentos endoscópicos em comparação com o tratamento sham, farmacológico ou cirúrgico. A eficácia foi medida por diferentes resultados subjetivos e objetivos. **Resultados** – Analisamos dados de 16 ensaios clínicos randomizados, totalizando 1085 pacientes. A eficácia dos tratamentos endoscópicos em comparação com o tratamento com sham e inibidores da bomba de prótons mostrou uma diferença significativa até 6 meses a favor da endoscopia sem heterogeneidade (P<0,00001) (I^2: 0%). A análise do subgrupo mostrou diferença estatisticamente significativa até 6 meses a favor da endoscopia: endoscopia vs inibidores da bomba de prótons (P<0,00001) (I^2: 39%). Endoscopia vs sham (P<0,00001) (I^2: 0%). A maioria dos resultados subjetivos e objetivos foram estatisticamente significativos em favor da endoscopia até 6 e 12 meses de acompanhamento. **Conclusão** – Esta revisão sistemática e meta-análise mostrou uma boa eficácia a curto prazo em favor dos procedimentos endoscópicos ao compará-los a tratamento sham, farmacológico ou cirúrgico. Não existem dados sobre o acompanhamento a longo prazo e isso deve ser explorado em estudos futuros.

DESCRITORES – Refluxo gastroesofágico, terapia. Endoscopia gastrointestinal. Seguimentos. Revisão.

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REVIEW

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Association of promoter region polymorphisms of interleukin-10 gene with susceptibility to colorectal cancer: a systematic review and meta-analysis

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ABSTRACT – **Background** – Several epidemiological studies have investigated the association of promoter region polymorphisms of Interleukin-10 (IL-10) gene with colorectal cancer (CRC), while the conclusion is still conflicting and inconclusive. **Objective** – We conducted this meta-analysis to evaluate the association of promoter region polymorphisms of IL-10 with CRC. **Methods** – Eligible articles were identified by a search of several bibliographic databases for the period up to March 15, 2018. The strength of the association was measured by odd ratios with 95% confidence intervals. **Results** – A total of 28 case-control studies with 5,647 CRC cases and 6,908 controls were selected, including 14 studies for IL-10 -1082A>G (rs1800896) polymorphism (2,702 cases and 3,649 controls), eleven studies for -592C>A (rs1800872) polymorphism (3,259 cases and 4,992 controls), and three studies for -819T>C (rs1800871) polymorphism (477 cases and 544 controls). By pooling all eligible studies, we found that the IL-10 -1082A>G and -592C>A polymorphisms were not associated with increased CRC risk in overall population. However, there was significant associations between the IL-10 -819T>C polymorphism and CRC susceptibility under the allele model (A vs G: OR=1.278, 95% CI 1.043-1.566, *P*=0.018) and the recessive model (AA vs AG+GG: OR=1.709, 95% CI 1.026-2.845, *P*=0.039). **Conclusion** – In this meta-analysis we found that IL-10 -819T>C polymorphism was associated with significantly increased risk of CRC; while the IL-10 -1082A>G and -592C>A polymorphisms were not associated with CRC risk. The IL-10 -819T>C polymorphism may be important as suspected predictive factor of CRC occurrence.

HEADINGS – Interleukin-10. Colorectal neoplasms. Genetic polymorphism. Meta-analysis.

INTRODUCTION

Colorectal cancer (CRC) ranks among the three most common cancers in terms of both cancer incidence and cancer-related deaths worldwide[1-4]. CRC is the cancer of the colon and the rectum and approximately two thirds are located in the colon. Differences in the CRC death rates relate to differences in socioeconomic factors, diet, population life span, genetic factors, and to the quality of medical care available[5,6]. The most common risk factors for to CRC include age, the presence of polyps, inflammatory bowel disease, lifestyle, genetic background and family medical history[7]. Life style relating factors such as obesity, physical inactivity, poor diet, cigarette smoking and heavy alcohol consumption account for approximately 80% of all colorectal cancer cases[8]. The sequence of genetic alterations in CRC development is well documented[9]. Genetic conditions such as familial adenomatous polyposis (FAP), Lynch syndrome (HNPCC; hereditary nonpolyposis colorectal cancer) and Gardner's syndrome (considered a subtype of FAP) are genetic risk factors[10], which accounts for 10% of all colorectal cancer cases[11].

Several cytokines that modulate the immunologic response have been implicated in the development of cancer[12]. Interleukin-10 (IL-10) is a multifunctional cytokine involved in both innate and adaptive immune response, and a wealth of evidence supports its regulatory role in carcinogenesis and tumor growth[13,14]. In addition, increased circulating IL10 has been shown in patients diagnosed with different malignancies, such as hepatocellular carcinoma, autoimmune cancers, and leukemia. The IL10 gene (Gene ID: 3586) has been mapped to human chromosome 1q31–q32, spans 5.2 kb and contains five exons[14]. Many SNPs in the promoter and coding region of IL-10 gene were shown to be associated with cancer risk, and also, studies have showed that the genetic variants played important roles in the transcription and protein expression[15].

It is well known that IL-10 gene production can be influenced by the SNPs located within the promoter regions of the gene[15,16]. The IL-10 promoter is highly polymorphic and three single nucleotide polymorphisms (SNPs) have been confirmed in the promoter region of IL-10 including IL-10 -1082A>G (rs1800896), -592C>A (rs1800872) and -819T>C (rs1800871). In recent decade, accumulating evidence has supported the hypothesis that the promoter region of IL-10 polymorphisms correlates with genetic susceptibility of CRC[16,17]. However, the results from the studies were often inconsistent and inconclusive. This inconsistency may derive from a number of issues, including limited sample size of single

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study, different characteristics among studies (such as ethnicity, pathological types, and sources of controls), false-positive errors, lack of power, and minor impacts of IL-10 gene polymorphisms on CRC susceptibility. Therefore, we performed a meta-analysis to comprehensively assess the association of promoter region polymorphisms of IL-10 with CRC risk.

METHODS

Identification of eligible studies

The present meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines. We carried out a search in the internet covering well-known biomedical databases such as PubMed, Excerpta Medica Database (EMBASE), Elsevier Science Direct, Cochrane Library, and Chinese Biomedical Literature Database (CBM) regarding the association of IL-10 polymorphisms with CRC risk up to March 15, 2018. The following keywords were used for searching: (''Interleukin- 10'' OR ''IL-10'') AND (''colorectal cancer'' OR ''CRC'' OR 'colon cancer'') AND (''promoter region'' OR ''promoter'') AND (''polymorphism'' OR ''mutation'' OR ''genotype'' OR ''allele'' OR ''variation'' OR ''variant''). All searched studies were retrieved and the bibliographies were checked for other relevant publications. Review articles and bibliographies of other relevant studies identified were hand searched to find additional eligible studies. We also conducted a manual search of references of original or reviewed articles on this subject to identify additional studies. No language restrictions were applied. Abstracts, case reports and editorials were excluded.

Inclusion criteria

The following criteria were used for the literature selection: (1) evaluating the association between promoter region polymorphisms of IL-10 and CRC risk; (2) case–control or cohort studies comparing CRC cases with healthy or non-cancer controls; (3) the numbers of CRC case and healthy subjects for each genotype were reported or the relevant data was available, and adequate data was provided for calculating the pooled odds ratios (OR) with 95% confidence intervals (CI). Studies was excluded if they were the following: (1) case-only studies; (2) animal studies, abstracts, seminar posters, case reports, letters, or reviews; (3) incomplete data or no usable data were reported; (4) duplicated or studies containing overlapping data; (5) family-based design studies. After deliberate searching, we reviewed all papers in accordance with the criteria defined above for further analysis.

Data extraction

Information was carefully extracted from all eligible papers by two of the authors independently according to the inclusion criteria mentioned above. Data included the following: first author, publication year, country, cancer type, source of control, each genotype frequency of the case and control groups, genotype methods, and the Hardy–Weinberg equilibrium (HWE) value in the control group. Disagreement was resolved by discussion until consensus was reached. If these two authors could not reach a consensus, then a third author was consulted to resolve the dispute.

Statistical analysis

The strength of the association between the IL-10 polymorphisms and the risk of CRC was measured by odd ratios (ORs) with 95% confidence intervals (CI). Z-test was carried out to evaluate the statistical significance of pooled ORs. The pooled ORs were performed for the allele model (B vs A), homozygote model (BB vs AA), heterozygote model (BA vs AA), dominant model (BB + BA vs AA) and recessive model (BB vs BA + AA), respectively. The heterogeneity between studies was assessed with the chi-squared based Q-test. A significant P value (<0.10) was used to indicate heterogeneity among studies. Moreover, the I^2 statistic to quantify the proportion of the total variation due to heterogeneity were used, according to the criteria from the Cochrane Handbook, which categorized it into unimportant (0%–40%), moderate (30%–60%), substantial (50%–90%) and considerable (75%–100%). When the effects were assumed to be homogenous, the fixed-effects model was used (Mantel-Haenszel method)[18]. If obvious heterogeneity was present, the random effects model was used (DerSimonian-Laird method)[19]. Stratification and meta-regression analyses were used to detect the potential heterogeneity among studies. In case–control studies, Hardy–Weinberg equilibrium (HWE) was tested using an online program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl) to evaluate the study quality of genotype data, and P<0.05 was considered statistically significant. A high-quality study was said that its control group was in HWE. Sensitivity analysis by sequentially omitting the single studies and recounting the pooled ORs and 95% confidence intervals (CIs) was performed to estimate the effect of individual studies on overall risk of CRC. The funnel plot was utilized to test the publication bias and Egger's test (linear regression analysis) was used to check the symmetry of funnel plots[20,21]. The statistical analysis for the current meta-analysis study was performed by using the comprehensive meta-analysis (CMA) version 2.0 software (Biostat, USA). All P-values in the meta-analysis were two-sided, and statistical significance was considered when the P-value was less than 0.05.

RESULTS

The initial search of online databases yielded 253 relevant articles, and an additional 4 articles were identified through manually search. A total of 157 articles were excluded after reading the title or abstract because of obvious irrelevance to our criteria, lack of data for calculation and duplication. Finally, a total of 28 case-control studies in 17 publications[22-38] with 5,647 CRC cases and 6,908 controls were selected for the current meta-analysis, including 14 studies[22-35] for IL-10 -1082A>G (rs1800896) polymorphism (2,702 cases and 3,649 controls), eleven case-control studies[22,23,25,26,28,29,32,34,36–38] for -592C>A (rs1800872) polymorphism (3,259 cases and 4,992 controls), and three case-control studies[24,25,28] for -819T>C (rs1800871) polymorphism (477 cases and 544 controls). All of these 28 case-control studies provided sufficient data to calculate the possible relationship between the three polymorphisms of the IL-10 gene and CRC risk. The baseline characteristics of the case-control studies are shown in TABLE 1. Two ethnicities were addressed: 24 studies focused on Caucasian populations and four on Asian populations. The countries of these studies included Scotland, Italy, USA, Spain, Romania, Brazil, Canada, China, India, Korea, Egypt, and Turkey. Three genotyping methods were applied in the present case-control studies such as TaqMan, ARMS-PCR, Sequencing, AS-PCR, MassARRAY, PCR-RFLP, and KASP assay. The distribution of genotypes in the controls was consistent with the HWE for all selected studies, except for three case-control studies for IL-10 -1082A>G[24,28,33].

TABLE 1. Characteristics of studies included in the meta-analysis.

First Author/Year	Country (Ethnicity)	SOC	Genotyping Technique	Case/ Control	Cases					Controls					MAFs	HWE
					Genotypes			Allele		Genotypes			Allele			
IL10 -1082A>G (rs1800896)					**AA**	**AG**	**GG**	**A**	**G**	**AA**	**AG**	**GG**	**A**	**G**		
Macarthur 2005[22]	Scotland (Caucasian)	PB	TaqMan	257/403	61	125	71	247	267	86	202	115	374	432	0.536	0.877
Crivello 2006[23]	Italy (Caucasian)	PB	ARMS-PCR	62/124	16	34	12	66	58	38	60	26	136	112	0.451	0.796
Guntar 2006[24]	USA (Caucasian)	HB	TaqMan	222/207	61	114	47	236	208	55	123	29	233	181	0.437	0.002
Cozar 2007 [25]	Spain (Caucasian)	PB	TaqMan	126/175	42	62	22	146	106	58	87	30	203	147	0.420	0.787
Talseth 2007[26]	Mixed (Caucasian)*	HB	Sequencing	118/100	36	61	21	133	103	33	50	17	116	84	0.420	0.792
Wilkening 2008[27]	Sweden (Caucasian)	PB	TaqMan	304/579	83	146	75	312	296	164	283	132	611	547	0.472	0.639
Cacev 2008[28]	Croatia (Caucasian)	PB	TaqMan	160/160	54	86	20	194	126	43	92	25	178	142	0.443	0.037
Tsilidis 2009 [29]	USA (Caucasian)	PB	TaqMan	205/372	69	101	35	239	171	98	187	87	383	361	0.485	0.903
Miteva 2014[30]	Bulgaria (Caucasian)	PB	ARMS-PCR	119/154	40	57	22	137	101	54	79	21	187	121	0.392	0.349
Burada 2013[31]	Romania (Caucasian)	HB	TaqMan	144/233	65	60	19	190	98	80	118	35	278	188	0.403	0.426
Basavaraju 2015[32]	Scotland (Caucasian)	PB	TaqMan	388/496	58	210	92	351	394	103	261	130	471	521	0.527	0.183
Li 2015[33]	China (Asian)	HB	AS-PCR	102/105	26	66	10	118	86	20	77	8	117	93	0.442	≤0.001
Cai 2016[34]	China (Asian)	HB	MassARRAY	375/382	323	50	2	696	54	343	39	0	725	39	0.051	0.293
Gulubova 2018[35]	Bulgaria (Caucasian)	NS	ARMS-PCR	120/159	47	57	16	151	89	59	78	22	196	122	0.383	0.638
IL-10 -592C>A (rs1800872)					**CC**	**AC**	**AA**	**C**	**A**	**CC**	**AC**	**AA**	**C**	**A**		
Macarthur 2005[22]	Scotland (Caucasian)	PB	TaqMan	258/403	151	99	8	401	115	248	133	22	629	177	0.219	0.455
Crivello 2006[23]	Italy (Caucasian)	PB	ARMS-PCR	62/124	31	28	3	90	34	69	48	7	186	62	0.250	0.719
Talseth 2007[26]	Mixed (Caucasian)	HB	Sequencing	117/99	62	51	4	175	59	50	45	4	145	53	0.267	0.112
Vogel 2007[36]	Danish (Caucasian)	PB	TaqMan	355/753	224	109	22	557	153	455	256	42	1166	340	0.225	0.450
Cozar 2007[25]	Spain (Caucasian)	PB	TaqMan	95/175	52	41	2	145	45	98	63	14	259	91	0.260	0.393
Cacev 2008[28]	Croatia (Caucasian)	PB	PCR-RFLP	160/160	83	64	13	230	90	104	52	4	260	60	0.187	0.399
Tsilidis 2009[29]	USA (Caucasian)	PB	TaqMan	203/361	123	71	9	317	89	213	131	17	557	165	0.228	0.579
Andersen 2012[36]	Danish (Caucasian)	PB	KASP assay	949/1748	596	297	56	1489	409	1072	580	96	2724	772	0.226	0.134
Yu 2014[38]	China (Asian)	PB	PCR-RFLP	298/291	153	114	31	420	176	118	135	38	371	211	0.362	0.949
Basavaraju 2015[32]	Scotland (Caucasian)	HB	TaqMan	387/496	241	134	12	616	158	311	168	17	790	202	0.203	0.323
Cai 2016[34]	China (Asian)	HB	MassARRAY	375/382	221	128	26	570	180	184	158	40	526	238	0.311	0.484
IL-10 -819T>C (rs1800871)					**TT**	**TC**	**CC**	**T**	**C**	**TT**	**TC**	**CC**	**T**	**C**		
Guntar 2006[24]	USA (Caucasian)	HB	TaqMan	222/209	125	76	21	326	118	117	79	13	313	105	0.251	0.944
Cozar 2007 [25]	Spain (Caucasian)	PB	TaqMan	95/175	81	37	9	199	55	98	63	14	259	91	0.260	0.393
Cacev 2008 [28]	Croatia (Caucasian)	PB	PCR-RFLP	160/160	83	64	13	230	90	106	51	3	263	57	0.178	0.262

SOC: source of control, PB: Population based; HB: hospital based; NA: not available; PCR: Polymerase chain reaction; ASPCR: Allele-specific PCR; RFLP: restriction fragment length polymorphism; MAF: minor allele frequency; HWE: Hardy–Weinberg equilibrium. * Australia and Poland.

Quantitative synthesis

The evaluation of the associations of IL-10 -1082A>G, -592C>A and -819T>C polymorphisms with CRC risk are presented in TABLE 2. The results showed that no association of IL-10 polymorphism was observed with the risk of CRC in five genetic models, i.e., allele (A vs G: OR=0.9623, 95% CI 0.893-1.038, *P*=0.318, FIGURE 1.A), homozygote (AA vs GG: OR=0.983, 95% CI 0.836-1.155, *P*=0.835), heterozygote (AC vs GG: OR=0.953, 95% CI 0.843-1.078, *P*=0.444), dominant (AA+AG vs GG: OR=0.921, 95% CI 0.821-1.034, *P*=0.165) and recessive model (AA vs AG+GG: OR=0.986, 95% CI 0.861-1.129, *P*=0.839).

TABLE 2 listed the main results of the meta-analysis of IL-10 polymorphism and CRC risk. When all the eligible studies were pooled into the meta-analysis of IL-10 polymorphism, significantly increased risk of CRC was observed in all the five genetic comparison models, i.e., allele (A vs G: OR=1.497, 95% CI 0.838-1.064, *P*=0.349), homozygote (AA vs GG: OR=2.153, 95% CI 0.705-1.047, *P*=0.132), heterozygote (AC vs GG: OR=1.494, 95% CI 0.826-1.094, *P*=0.481), dominant (AA+AG vs GG: OR=1.584, 95% CI 0.693-1.145, *P*=0.368, FIGURE 1.B) and recessive (AA vs AG+GG: OR=1.753, 95% CI 0.747-1.099, *P*=0.319).

TABLE 2. Results of the association of IL-10 promoter region polymorphisms with CRC risk.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
IL-10 -1082A>G (rs1800896)										
	G vs A	Fixed	10.58	0.337	0.962	0.893-1.038	-0.999	0.318	0.228	0.422
	GG vs AA	Fixed	3.88	0.408	0.983	0.836-1.155	-0.208	0.835	0.826	0.664
	GA vs AA	Fixed	16.60	0.272	0.953	0.843-1.078	-0.766	0.444	0.826	0.688
	GG+GA vs AA	Fixed	0.00	0.458	0.921	0.821-1.034	-1.387	0.165	0.324	0.826
	GG vs GA+AA	Fixed	0.00	0.561	0.986	0.861-1.129	-0.203	0.839	0.228	0.260
IL-10 -592C>A (rs1800872)										
	A vs C	Random	53.69	0.017	0.945	0.838-1.064	-0.937	0.349	0.533	0.544
	AA vs CC	Fixed	37.90	0.097	0.859	0.705-1.047	-1.507	0.132	1.000	0.863
	AC vs CC	Random	45.87	0.047	0.951	0.826-1.094	-0.704	0.481	0.275	0.328
	AA+AC vs CC	Random	84.90	≤0.001	0.891	0.693-1.145	-0.900	0.368	0.350	0.005
	AA vs AC+CC	Fixed	24.83	0.207	0.906	0.747-1.099	-0.997	0.319	0.436	0.625
IL-10 -819T>C (rs1800871)										
	C vs T	Fixed	57.07	0.097	1.278	1.043-1.566	2.368	0.018	1.000	0.636
	CC vs TT	Random	66.94	0.049	1.682	0.651-4.348	1.074	0.283	1.000	0.533
	CT vs TT	Fixed	66.43	0.051	1.011	0.779-1.312	0.083	0.934	1.000	0.988
	CC+CT vs TT	Fixed	52.19	0.123	1.253	0.974-1.611	1.757	0.079	1.000	0.653
	CC vs CT+TT	Fixed	33.49	0.222	1.709	1.026-2.845	2.060	0.039	1.000	0.411

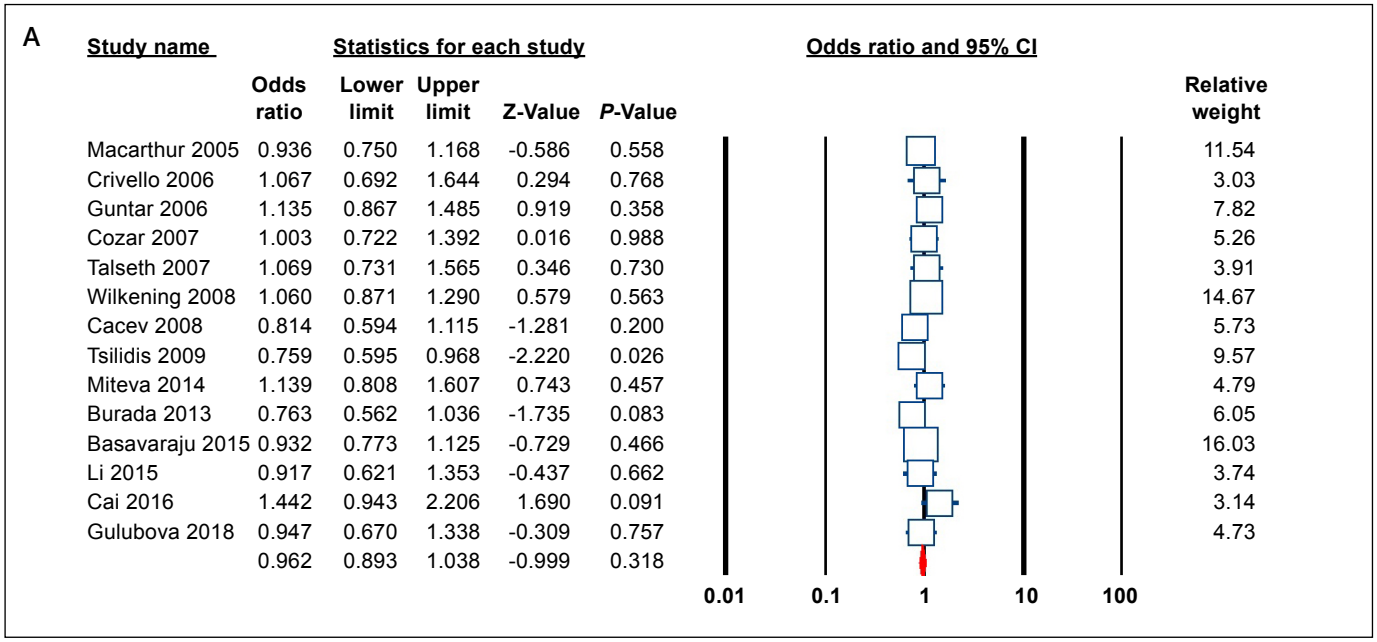

FIGURE 1.A. Forest plots for association of IL-10 -1082A>G and -592C>A polymorphisms with CRC risk. (A) -1082A>G under allele model (G vs. A).

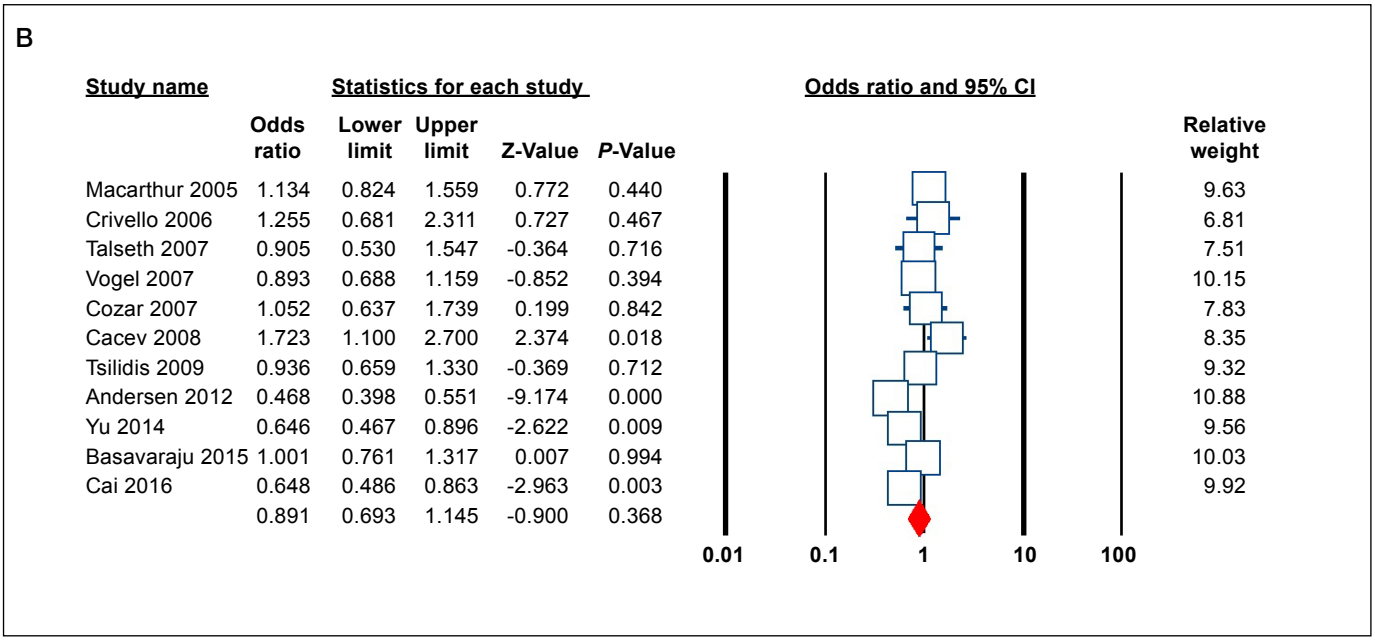

FIGURE 1.B. -592C>A under dominant model (AA+AC vs. CC).

TABLE 2 listed the main results of the meta-analysis of IL-10 -819T>C (rs1800871) polymorphism and CRC risk. When all the eligible studies were pooled into the meta-analysis of IL-10 -819T>C (rs1800871) polymorphism, significantly increased risk of CRC was observed in the two genetic models, i.e., allele (A vs G: OR=1.278, 95% CI 1.043-1.566, *P*=0.018) and recessive (AA vs AG+GG: OR=1.709, 95% CI 1.026-2.845, *P*=0.039).

The studies were further stratified on the basis of ethnicity, source of control, genotyping technique and HWE status. Subgroup analysis did not showed the IL-10 -1082A>G and -592C>A polymorphisms significantly increased risk of CRC (data not showed).

Heterogeneity and sensitivity analyses

TABLE 2 summarizes the results of this meta-analysis for heterogeneity test. For IL-10 -1082A>G polymorphism, there was no a significant heterogeneity in all five genetic models. Our meta-analysis showed little evidence of genetic heterogeneity in the homozygote model of IL-10 -819T>C. We detected significant between-study heterogeneity in the three genetic models, i.e., allele, heterozygote and dominant for -592C>A. One-way sensitivity analyses were performed by iteratively removing one study at a time to assess the stability of the meta-analysis results. The result showed that there was still no significant association of IL-10 -1082A>G and -819T>C polymorphisms with CRC. Significant between-study heterogeneity was still significant after removing one study under the genetic models (data not shown).

Publication bias

We performed Begg's test and Egger's test to assess the publication bias. As shown in the TABLE 2, no obvious publication bias was found according to the obtained *P* values for all the genetic models for IL-10 -1082A>G and -819T>C polymorphisms. However, the results of Egger's regression test and relative asymmetry of funnel plot showed evidence of publication bias for IL-10 -592C>A under the dominant model ($P_{Begg's}$=0.350, P_{Eggers}=0.005, FIGURE 2), suggesting that there was obvious publication bias in the genetic model. Therefore, we have performed the Duval and Tweedie nonparametric "trim and fill" method to adjust for publication bias. The "trim and fill" method did not change conclusion, indicating that our results were statistically robust.

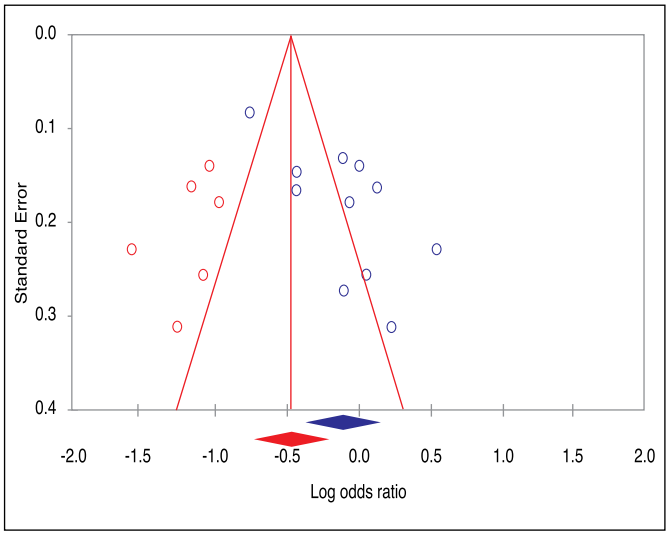

FIGURE 2. Begg's funnel plot of publication bias test before (blue) and after (red) trim-and-fill method for IL10 -592C>A polymorphism with CRC risk under dominant model (AA+AC vs. CC).

Minor Allele Frequencies (MAFs)

The minor allele frequencies (MAFs) of the IL-10 polymorphisms are presented in TABLE 1. The IL10 -1082G, -592A and -819C allele frequencies in the overall populations were 29.35% (5.10%-53.60%), 27.45% (18.70%-36.20%), and 21.9% (17.80%-26.0%), respectively.

DISCUSSION

To date, numerous molecular epidemiological studies have been conducted to evaluate the association between polymorphisms of IL-10 promoter polymorphisms and the risk of CRC, but results have remained conflicting. In this meta-analysis, we identified 28 eligible studies, including 5,647 CRC cases and 6,908 controls, and analyzed the association of IL-10 -1082A>G, -592C>A and -819T>C polymorphisms with susceptibility to CRC. We found that the IL-10 -1082A>G and -592C>A polymorphisms were not associated with CRC risk in overall population. We suggested that the IL-10 -1082A>G and -592C>A polymorphisms could play a protective role in the development of CRC due to a higher incidence found in controls than in cases from the included studies in the meta-analysis.

Previously, association of the IL-10 polymorphisms with CRC risk has been investigated by two meta-analyses. In 2013, Yu et al. performed a comprehensive meta-analysis about IL-10 -819C>T polymorphism and cancer susceptibility. They have found that the IL-10 -819C>T polymorphism was not significantly associated with CRC, breast cancer, lung cancer, hepatocellular carcinoma, prostate cancer, lymphoma, or melanoma[16]. Compared with Yu et al., we only focused on the association of IL-10 polymorphisms with CRC cancer, while Yu et al. analyzed a variety of human malignancies. In 2012, Zhang et al. performed a meta-analysis about IL-10 -1082 A>G and -592 C>A polymorphisms and CRC risk[17]. Compared with Yu et al. and Zhang et al. studies, we have focused on the association of three promoter region polymorphisms with CRC. On the other hand, we also analyzed the association of IL-10 -819T>C polymorphism with CRC risk. Additionally, we identified more eligible studies undertaken to assess the association IL-10 gene promoter polymorphisms and CRC risk.

To the best knowledge, this is the first comprehensive meta-analysis to assess the association of IL-10 -819T>C polymorphism with CRC risk. The current meta-analysis, which included a total of three case–control studies with 477 cases and 544 controls, investigated the association of IL-10 -819T>C polymorphism with CRC risk. We found that the IL-10 -819T>C polymorphism significantly increased risk of CRC in the two genetic models, i.e., allele (A vs G: OR=1.278, 95% CI 1.043-1.566, P=0.018) and recessive (AA vs AG+GG: OR=1.709, 95% CI 1.026-2.845, P=0.039). However, a small number of eligible studies were enrolled for IL-10 -819T>C polymorphism, which may fail to provide enough statistical power to detect a possible or effect of IL-10 -819T>C polymorphism on CRC. Therefore, to achieve more precise correlation, future studies should take ethnic difference in to consideration. However, we suggests that the IL-10 -819T>C polymorphism may be important as suspected predictive factor for CRC occurrence.

Between-study heterogeneity is a potential problem that might affect the interpretation of the results. thus, one of the most important goals of the meta-analysis is to identify the source of heterogeneity[39-42]. Through conducting meta-regression, we found that the heterogeneity could not be explained by ethnicity, source of control, genotyping methods and HWE status in the current meta-analysis. Therefore, we suggested that the heterogeneity may have resulted due to something more than these factors. It is known that the existence of publication bias can influence the conclusions of a meta-analysis. Therefore, the "trim and fill" methods have been developed to deal with publication bias. In the current meta-analysis, we have found possible publication bias between IL-10 -592C>A polymorphism and CRC risk under the dominant model ($P_{Begg's}$=0.350, P_{Eggers}=0.005), adjusting for possible publication bias using the Duval and Tweedie nonparametric "trim and fill" method showed that the results did not adjust, indicating that the overall pooled results should be unbiased.

In interpreting the results, some cautions should be applied. First, there was limited number of eligible studies in the meta-analysis of the association between IL-10 -819T>C polymorphism and risk of CRC. The limited sample size in the meta-analysis may fail to provide enough statistical power to detect a possible or weak effect of IL-10 -819T>C polymorphism on CRC. Therefore, more studies with large sample are needed to give a more precise estimation of the association between IL-10 -819T>C polymorphism and risk of CRC. Second, the included studies involved in the meta-analysis were mainly Caucasian, so it is uncertain whether these results are generalizable to other populations. Moreover, the stratification by ethnicity had little or no information for other ethnicities, which may limited the strength of our results. Thus, strengthening the statistical power will require more data from different ethnicities. Third, although no significant publication bias was detected, we have included only published studies in this meta-analysis, and it is possible that some relevant published and unpublished studies in other languages might be missed, which also publication bias may occur. Fourth, our results were based on unadjusted estimates without adjustment for other risk factors such as age, gender, drinking consumption, environmental factors and other variables, while a more precise analysis should be conducted according to potentially confounding factors. Finally, lack of the original data of the included studies, the interaction of different susceptibility genes, gene-to-environment, and even different polymorphic loci of the same gene interactions due to the limited information of included studies.

In conclusion, this meta-analysis indicated that IL-10 -1082A>G and -592C>A polymorphisms could play a protective role in the development of CRC. However, we found that there was a significant association between IL-10 -819T>C polymorphism and CRC risk. The IL-10 -819T>C polymorphism may be important as suspected predictive factor of CRC occurrence. More extensive studies with large sample sizes, gene-gene and gene-environment interactions are necessary to provide a more reliable estimation of these associations in overall and by ethnicity.

Authors' contributions

Mirjalili SA, Moghimi M and Neamatzadeh H conceived and research design. Abolbaghaei SM and Mazaheri M selected the articles and extracted the data. Aghili K, Jafari M and Zare-Shehneh M performed data analysis. The manuscript was drafted by Mirjalili SA and Neamatzadeh H. Moghimi M and Abolbaghaei SM critically reviewed the manuscript and discussed with the other co-authors. All the authors read and approved the final manuscript.

Mirjalili SA, Moghimi M, Aghili K, Jafari M, Abolbaghaei SM, Neamatzadeh H, Mazaheri M, Zare-Shehneh M. Associação de polimorfismos da região do promotor do gene interleucina-10 com susceptibilidade ao câncer colorretal: uma revisão sistemática e meta-análise. Arq Gastroenterol. 2018,55(3):306-13.

RESUMO – **Contexto** – Vários estudos epidemiológicos têm investigado a associação de polimorfismo da região promotora do gene interleucina-10 (IL-10) com câncer colorretal (CRC), mas por enquanto a conclusão ainda é conflitante e inconclusiva. **Objetivo** – Foi realizada esta meta-análise para avaliar a associação de polimorfismo da região promotora do Il-10 com o câncer colorretal. **Métodos** – Os artigos elegíveis foram identificados por uma pesquisa de várias bases de dados bibliográficas para o período até 15 de março de 2018. A força da associação foi medida por odds ratio (OR) com intervalos de 95% de confiança (IC). **Resultados** – Um total de 28 estudos de casos-controles com 5.647 casos de câncer colorretal e 6.908 controles foram selecionados, incluindo 14 estudos para o polimorfismo de IL-10-1082A>G (rs1800896) (2.702 casos e 3.649 controles), 11 estudos para-592C>A (rs1800872) polimorfismo (3.259 casos e 4.992 controles), e três estudos para-819T>C (rs1800871) polimorfismo (477 casos e 544 controles). Ao reunir todos os estudos elegíveis, verificou-se que o Il-10-1082A>G e-592C>A polimorfismo não foram associados com o aumento do risco de câncer colorretal na população global. No entanto, houve associações significativas entre o polimorfismo IL-10-819T>C e a susceptibilidade de câncer colorretal o modelo alelo (A vs G: OR=1,278; 95% CI 1,043-1,566; *P*=0,018) e o modelo recessivo (AA vs AG + GG: ou =1,709; 95% CI 1,026-2,845; *P*=0,039). **Conclusão** – Nesta meta-análise revelou-se que o polimorfismo IL-10-819T>C foi associado a um risco significativamente maior de câncer colorretal; enquanto o Il-10-1082A>G e-592C>A polimorfismos não foram associados com o risco de câncer colorretal. O polimorfismo IL-10-819T>C pode ser importante como fator preditivo suspeito da ocorrência de câncer colorretal.

DESCRITORES – Interleucina-10. Neoplasias colorretais. Polimorfismo genético. Metanálise.

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Recommendations of the Brazilian Society of Hepatology for the management of acute kidney injury in patients with cirrhosis

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ABSTRACT – Acute kidney injury is a common complication of cirrhosis, occurring in up to 20% of patients hospitalized with cirrhosis. This field is rapidly changing, with significant advances in classification, biomarkers and therapy over the last few years. On the behalf of the Brazilian Society of Hepatology, a panel of experts in Hepatology and Nephrology reviewed published evidence to integrate findings and develop the recommendations presented in this manuscript.

HEADINGS – Acute kidney injury. Disease management. Liver cirrhosis, complications. Risk assessment.

INTRODUCTION

Acute kidney injury (AKI) is a common complication of cirrhosis, occurring in up to 20% of patients hospitalized with cirrhosis[1]. There are many reasons for the development of AKI in cirrhotic patients, such as: i) infections; ii) hypovolemia (loss of fluids associated with bleeding, use of diuretics or gastrointestinal losses); iii) parenchymal nephropathy, iv) nephrotoxicity (drug-induced or contrast-induced nephropathy); v) hepatorenal syndrome – (HRS)[2-4]. A large study of 463 hospitalized cirrhotic patients with AKI evaluated the frequency and the prognosis of the different etiologies of AKI. This study demonstrated that the most frequent cause of AKI among cirrhotic patients was bacterial infection (46%), followed by volume depletion (32%), HRS (13%) and parenchymal nephropathy (9%). Among infections, spontaneous bacterial peritonitis (SBP) and spontaneous bacteremia were the most common, although almost any type of infection could lead to AKI because of the aggravation of circulatory dysfunction commonly found in cirrhosis. The 90-day mortality for the whole series was high (60%), but it was particularly high among patients with AKI associated with infections or HRS[2]. In a multivariate analysis adjusted for potentially confounding variables, the cause of AKI was independently associated with prognosis. The same findings were also reported in another study from Brazil[4]. Not only AKI but also the etiology of AKI seems to play an important role in the prognosis of cirrhotic patients who develop AKI. In this context, the worst outcomes for patients with AKI are associated with HRS or infection.

DIAGNOSTIC CRITERIA

Serum creatinine (SCr), an endogenous biomarker, is traditionally used to evaluate renal function[5]. Nevertheless, SCr level may be affected by a number of factors. In cirrhosis, SCr may still be in the normal range despite significant reductions in renal function because of protein-calorie malnutrition and cirrhosis-related sarcopenia. Sudden changes in renal function may not be accurately evaluated by SCr, which takes time to rise. Another limiting factor for the interpretation of SCr as a biomarker is the laboratory method employed for its determination[6,7]. It is also noteworthy that high levels of bilirubin in the blood may lead to a reduction in SCr levels[8].

The combination of lower production of creatine, higher tubular secretion of creatinine and presence of malnutrition may contribute to a misleadingly lower than expected levels of SCr in cirrhotic patients. Therefore, methods of evaluation of renal function based on SCr must be carefully interpreted in this context.

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There is a body of evidence suggesting that the traditional definition of AKI, based on a fixed cut-off point of SCr above 1.5 mg/dL, poorly reflects the dynamic changes in renal function of cirrhotic patients in different clinical situations[9-13]. In this context, a study demonstrated that the maximum value of SCr associated with normal renal function in cirrhotic patients would be 1mg/dL. Values of SCr between 1.0 mg/dL and 1.5 mg/dL were suggestive of renal failure and were associated with lower survival[13], while values above 1.5 mg/dL were associated with severe AKI with a high specificity. There are studies that suggest that mild elevations of SCr (≥0.3 mg/dL) are associated with significant changes in GFR of cirrhotic patients, and are independently associated with higher incidence of organ dysfunction (OD), longer hospitalization and higher mortality, both in the general population and in cirrhotic patients[9,12-15].

In 2004, the Risk Injury Failure Loss of Kidney Function and End-Stage Kidney Disease (RIFLE) criteria standardized a new definition of AKI based on the variation of SCr, GFR and urine output[16]. RIFLE was innovative because it allowed the staging of patients according to their prognosis. Until then, there were many different definitions of AKI, which complicated comparison of data in distinct studies. In 2007, the Acute Kidney Injury Network (AKIN), a network of nephrologists and critical care physicians dedicated to studying AKI, recognized that mild variations of SCr (0.3 mg/dL in 48 hours) were related to worse prognosis and should be taken into consideration[17]. Following this, the Kidney Disease Improving Global Outcomes (KDIGO) classification combined concepts from both previous classifications[18]. Recently, the International Club of Ascites (ICA) used this classification to recommend new diagnostic criteria for AKI in cirrhotic patients[19]. In this new classification, the ICA-AKI criteria, absolute cut-off points of SCr were abolished, criteria based on urine output were eliminated (urine output is considered an imprecise parameter in cirrhotic patients), and three stages of AKI were defined based on the magnitude of the dynamic changes of SCr compared to baseline (FIGURE 1).

Definition of AKI	
Increase in SCr ≥0.3 mg/dL within 48 hours compared to baseline*; or	
Increase in SCr ≥50% (known or presumed to have occurred during the previous 7 days)	
Staging of AKI	
Stage 1	Increase in SCr ≥0.3mg/dL; or
	Increase ≥1.5x - 2.0x from baseline.
Stage 2	Increase in SCr >2.0x -3.0x from baseline.
Stage 3	Increase in SCr >3.0x from baseline; or
	SCr ≥4.0mg/dL with an acute increase ≥0.3mg/dL; or
	AKI with indication of renal replacement therapy.

FIGURE 1. Definitions of acute kidney injury in cirrhotic patients according to the recommendations of the International Club of Ascites[20]. AKI: acute kidney injury. SCr: serum creatinine. *Serum creatinine value closest to hospital admission, within 3 months. In the absence of a previous creatinine value, serum creatinine at admission should be considered baseline.

After the recommendation of the new diagnostic criteria for AKI in cirrhosis[9-12], some studies suggested that patients with mild AKI (stage 1) and maximum SCr <1.5 mg/dL have more favorable outcomes, similar to those of patients who do not have AKI[10,11,20]. Nevertheless, it should be noticed that these studies evaluated only short term prognosis, and did not consider the impact of mild AKI on longer term mortality. Besides, the etiology of AKI, which is associated with different prognoses[2], was not considered in these studies. Important outcomes, such as occurrence of other complications of cirrhosis, recurrence of AKI and rate of hospital readmission, also were not evaluated. Therefore, additional studies are needed in order to define the possible prognostic contribution of a mixed model of glomerular function evaluation in cirrhotic patients, considering the traditional cut-off point of SCr of 1.5 mg/dL as well as the dynamic changes in SCr proposed by the ICA-AKI classification.

Another application of longitudinal monitoring of SCr involves the evaluation of response to therapeutic measures. Three patterns of response were proposed by ICA[19]: 1) No response: no reduction of SCr; 2) Partial response: regression of at least one stage of AKI, but minimum level of SCr persists at least 0.3 mg/dL above the baseline; 3) Full response: reduction of SCr to levels close to baseline (< 0.3 mg/dL above the baseline).

The new ICA-AKI definition of AKI in cirrhosis corrects, even if not completely, some of the problems related to using SCr as a biomarker of renal function. It considers baseline SCr, and enables earlier diagnosis of AKI from mild changes in SCr levels. Nevertheless, it does not consider the etiology of AKI, which crucially limits its impact on therapeutic decision-making, since treatment of AKI varies according to its cause. Therefore, there is still a need for studying other biomarkers capable of considering the etiology of AKI and aiding early treatment decisions.

Biomarkers

Among the biomarkers studied in the context of AKI, the most extensively evaluated in cirrhotic patients at present is Neutrophil Gelatinase-Associated Lipocalin (NGAL). NGAL is an inflammatory biomarker produced by damaged renal tubular cells (as well as from leukocytes and hepatocytes). Its concentration in urine varies according to the etiology of AKI in cirrhosis[21]. Among patients with AKI, those with pre-renal AKI present the lowest values of urinary NGAL (median 30 µg/g), while patients with acute tubular necrosis (ATN) have much higher levels of urinary NGAL (median 417 µg/g). Patients with HRS present intermediate levels of urinary NGAL (median 76 µg/g). Moreover, urinary NGAL predicts significant outcomes in cirrhotic patients.

A Brazilian pilot study demonstrated that, among nine patients with cirrhosis and bacterial infection, urinary NGAL predicted the development of AKI before the traditional criteria used in cirrhosis[22]. It is noteworthy that despite the diagnosis of AKI according to SCr being made after a mean of 5.4 days after admission, urinary levels of NGAL in those patients increased as early as 6 hours from hospital admission.

Other studies verified an association between urinary NGAL and the clinical course of AKI or 90-day mortality[23,24]. Therefore, measurement of urinary NGAL seems promising for clinical practice, since it could predict relevant clinical outcomes in cirrhotic patients and possibly assist therapeutic decision-making as it could identify the cause of AKI at an early stage.

Diagnosis of hepatorenal syndrome

HRS is a severe complication occurring in patients with cirrhosis and ascites, with an annual incidence around 18%[25]. It is characterized by renal vasoconstriction, reduction of renal perfusion and diminished GFR, which causes a significant decrease in the kidney's capacity to excrete sodium and free water, in the absence of major renal histological damage[26]. The absence of significant histological damage and the recovery of renal function after liver transplantation define the functional characteristics of the syndrome. HRS is the complication of cirrhosis which presents the worst prognosis, having a median survival of around two weeks if left untreated[27]. Therefore, it was long considered a terminal event in the course of cirrhosis. The introduction of treatments that are effective in reversing HRS led to improvement of short-term survival, allowing a significant number of these patients to reach liver transplantation, which is considered the definitive treatment for patients with end-stage liver disease.

Because of the lack of specific tests for HRS, its diagnosis requires exclusion of other causes of AKI in patients with cirrhosis and ascites. The exclusion of acute tubular necrosis (ATN) as a cause of AKI is especially important, since HRS and ATN have different management strategies and prognoses. ATN usually occurs in patients with septic or hypovolemic shock, being characterized by the presence of epithelial cells and granular casts in the urinary sediment analysis, as well as by high urinary excretion of sodium (urinary sodium >40 mEq/L and sodium excretion fraction >2%) and low urinary osmolarity (<350 mOsm/kg). Conversely, patients with HRS do not present alterations in urinary sediment and maintain high capacity of tubular reabsorption of sodium and free water, with values of urinary sodium and sodium excretion fraction under 20 mEq/L and 1% respectively, as well as high urinary osmolarity (>500 mOsm/kg)[28]. The diagnosis of HRS currently must be performed according to the criteria presented in FIGURE 2.

Cirrhosis and ascites
Acute kidney injury according to the new International Club of Ascites criteria (see FIGURE 1).
Absence of structural renal injury, suggested by proteinuria (>500 mg/24 hours), hematuria (>50 red blood cells/high power field) and/or renal alterations on ultrasonography.
Absence of renal function recovery (return of serum creatinine to a final value up to 0.3 mg/dL above the baseline) after two consecutive days of diuretic withdrawal and plasma volume expansion with albumin (1 g/Kg/day, up to a maximum of 100 g/day).
Absence of shock
Absence of current or recent use of nephrotoxic drugs (aminoglycosides, non-steroidal anti-inflammatory drugs, among others).

FIGURE 2. Diagnostic criteria for hepatorenal syndrome-acute kidney injury[19].

The most important change introduced by the new criteria is allowing earlier treatment of HRS, since, according to the previous criteria, the diagnosis could only be done and the treatment initiated if there was a 2-fold increase in SCr to a value ≥2.5 mg/dL within two weeks. The probability of treatment response in HRS is inversely proportional to the value of SCr when treatment begins. This suggests that prognosis is associated with the magnitude of AKI and the promptness of diagnosis, more sensitive criteria were in order[29].

In 1996, ICA classified HRS in two types[30]. HRS type 1 (HRS-1) was characterized by severe and rapidly progressive renal failure, defined by a two-fold increase in SCr to a level ≥2.5 mg/dL within two weeks. Even though HRS-1 could develop spontaneously, it frequently follows a precipitating factor, such as bacterial infection, gastrointestinal bleeding, major surgical intervention or acute hepatitis occurring in a cirrhotic patient. HRS type 2 (HRS-2) is characterized by moderate and steady or slowly progressive decrease in renal function, accompanied by signs of liver failure and arterial hypotension to a lesser degree than in patients with HRS-1. The dominant clinical characteristic of patients with HRS-2 is tense ascites with poor response to diuretic therapy; refractory ascites. It is noteworthy that patients with HRS-2 are particularly susceptible to developing HRS-1. Median survival of patients with HRS-2 (6 months) is significantly lower than that of cirrhotic patients with ascites and preserved renal function[30]. After the adoption of the ICA-AKI criteria for defining HRS (FIGURE 2), the nomenclature of hepatorenal syndrome-acute kidney injury (HRS-AKI) is preferred to HRS-1 and HRS-2.

Recently, new concepts of HRS-2 are being proposed[31], raising the possibility that it is a functional chronic kidney disease, in which case it would be considered for patients with a GFR under 60 mL/minute/ 1.73 m^2 for over three months. It should be distinguished from organic chronic kidney disease. This distinction is of particular importance with the increasing prevalence of non-alcoholic fatty liver disease related to metabolic syndrome as a major worldwide cause of chronic liver disease. It is frequently associated with diabetes and systemic arterial hypertension, both well-documented causes of renal damage.

Recommendations

A. ICA-AKI criteria should be used for the diagnosis and staging of AKI in patients with cirrhosis.
B. Following diagnosis of AKI, the identification of its underlying cause should be pursued in order to manage the condition properly, with favorable impact on prognosis.
C. Using biomarkers for the diagnosis of AKI, although promising, is not currently supported by enough scientific evidence and still cannot be recommended for routine clinical practice.
D. Diagnosis of HRS should be based on HRS-AKI criteria, which allows early indication of therapeutic measures.

TREATMENT OF AKI IN CIRRHOSIS

General measures

Following the diagnosis of AKI, etiology should be determined in order to define the appropriate treatment. In this context, the recently revised recommendations of the ICA are the following[19]:

- Rule out parenchymal renal disease (investigate hematuria, proteinuria or microalbuminuria, and perform a urinary system ultrasound);

- Rule out drug-induced AKI (aminoglycoside antibiotics, non-steroidal anti-inflammatory drugs, vasodilatory drugs, beta-blockers or iodinated contrast media);
- Withdraw or reduce the dosage of diuretics and perform volume expansion in order to rule out prerenal azotemia;
- Consider ATN in the presence of shock;
- Actively investigate infections, especially SBP, and begin early empirical antibiotic treatment if infection is suspected.

Renal function frequently normalizes after the correction of the precipitating factor, especially in initial stages. When kidney injury progresses despite the initial treatment or when AKI is diagnosed at more advanced stages (ICA-AKI stages 2 or 3), volume expansion with albumin 1g/Kg/day for 48 hours is recommended. This must be done carefully, since oliguria is frequent in cirrhosis, and may lead to pulmonary edema. When renal function fails to improve even after volume expansion with albumin and if other causes of kidney injury have been excluded, specific treatment for HRS should be considered.

Albumin

There is robust evidence demonstrating the importance of albumin in the context of decompensated cirrhosis. Initially, the lack of response to volume expansion with albumin should be remembered as a prerequisite for the diagnosis of HRS in cirrhotic patients with AKI[32]. Albumin is capable of preventing loss of renal function in patients submitted to large volume paracentesis, a benefit not seen with other plasma expanders[33]. There is also evidence that albumin improves renal autoregulation curves both in cirrhotic patients with AKI and in those with ascites and normal renal function[34]. In patients with HRS, response to terlipressin is significantly better when it is co-administered with albumin[35]. In patients with SBP, albumin use in conjunction with antibiotic treatment significantly reduces the incidence of HRS and in-hospital mortality, effects which were not identified using hydroxyethyl starch[36,37].

Some studies evaluated the role of albumin infusion in both prevention and treatment of AKI in infected cirrhotic patients[36,38,39]. A randomized controlled trial[36] demonstrated that use of albumin with the antibiotic treatment of SBP led to a lower incidence of AKI when compared to using antibiotics alone. Similarly, in-hospital and 3-month mortalities were significantly lower in patients who received albumin. In that study, albumin was administered at two distinct times: on the day of diagnosis of SBP (D1) patients received 1.5g/Kg of albumin and a second infusion of 1g/Kg on the third day after diagnosis[36].

Recently, a randomized controlled trial analyzed the effects of albumin on 110 cirrhotic patients with infections other than SBP[38]. Albumin, used in similar doses recommended for SBP, led to an improvement of circulatory and renal functions and had a positive impact on survival when the analysis was controlled for other variables which were independently associated with prognosis. Another study[39] showed that albumin infusion was able to postpone the occurrence of AKI, but unable to increase survival. Even though this still is an open question, it is possible that there are benefits of albumin for infections severe enough to negatively affect circulatory function.

Vasoconstrictors

Use of a vasoconstrictor in combination with albumin for the treatment of HRS has been suggested since the 1990s[40]. The most common drugs are terlipressin (an analogue of vasopressin) and noradrenaline (an adrenergic agonist). A combination of midodrine and octreotide was recently shown to be less effective than terlipressin in the treatment of HRS[41].

Terlipressin is administered as an intravenous bolus infusion, initially in doses of 0.5-1.0 mg each 4-6 hours. Treatment response should be evaluated in intervals of 48 hours. In the absence of at least 25% decrease in SCr after the first 48 hours, terlipressin doses could be gradually increased every two days up to the maximum dosage of 12 mg/day. Treatment is maintained for up to 14 days, but the drug can be discontinued earlier in cases of poor response (less than 50% decrease in SCr after seven days of terlipressin at its highest dose or no reduction in SCr after the first three days of therapy[3,19]. Terlipressin was associated with HRS reversal in 34%-44% of patients treated in two international randomized controlled trials[42,43]. In addition a meta-analysis demonstrated its association with HRS reversal, renal function improvement and decreased mortality[44]. When used in continuous infusion, lower doses of terlipressin may be administered, with fewer side effects and without significant loss of effectiveness, compared to bolus infusion[45].

Continuous noradrenaline infusion, in doses of 0.5-3.0 mg/hour, was also associated with HRS reversal, in a similar way to that seen with terlipressin. Even though evidence is scarcer than for terlipressin, noradrenaline is considered to be a valid alternative for the treatment of HRS because of its similar effectiveness, wide availability[46]. Although the drug itself is cheaper, the treatment strategy using noradrenaline for HRS is actually more expensive than a strategy using terlipressin once direct medical costs are included. This is mostly because noradrenaline use requires admission to an intensive care unit, and terlipressin use does not, as shown by a recent Brazilian study[47,48].

Treatment of HRS with a vasoconstrictor and albumin may lead to adverse events in approximately 10% of patients. These effects are usually ischemic (mostly affecting the heart, gut and lower limbs) or related to pulmonary edema. They are managed with drug dose reduction; complete withdrawal of treatment is seldom needed.

Treatment indication for HRS must take into account the stage of AKI according to ICA-AKI criteria. Patients with HRS and ICA-AKI stages 2 or 3 should be immediately managed with a vasoconstrictor and albumin. Treatment for patients at stage 1 should be tailored. Because of the high short-term mortality, treatment should be considered carefully for patients at stage 1b. For those at stage 1a, renal function should be closely monitored, potentially aggravating factors should be avoided, and treatment should be reserved for patients whose AKI progresses to higher stages.

Treatment of HRS-2

Based on the fact that HRS-2 occurs in a context of advanced cirrhosis, the gold standard treatment is liver transplantation. Studies evaluating the role of vasoconstrictors for HRS-2[49,50] gathered small samples of patients and frequently combined HRS-1 and -2. As a general rule, these studies suggested that HRS-2 reversal is more common when a vasoconstrictor is used than in control groups. Nevertheless, the quality of evidence is too low to recommend treatment. A study evaluating treatment of HRS-2 in patients on the liver transplant waiting list[51] was unable to demonstrate that HRS reversal with terlipressin and albumin ultimately influenced the prognoses of the patients. Recurrence rate after treatment interruption is high, around 40%[52]. Therefore, treatment of HRS-2 with a vasoconstrictor still requires more randomized controlled trials clearly showing its benefits in order for it to be formally recommended. At this moment, it is considered a treatment to be used in exceptional circumstances,

possibly recommended for patients who are expected to receive a liver transplant a short time after.

Even though transjugular intrahepatic portosystemic shunt (TIPS) seems to improve renal function[53,54], its role in HRS-2 treatment remains speculative. Therefore, it might be indicated only in very well selected cases.

The best therapeutic option for these patients is liver transplantation. Despite patients with HRS usually needing more blood components, spending more time in the intensive care unit and having longer hospitalizations, 1-year survival is similar to that of patients with normal renal function[55].

Recommendations

A. The diagnosis of HRS requires ruling out other causes of AKI in cirrhosis, especially ATN.
B. The administration of intravenous albumin is recommended for patients with SBP for HRS prophylaxis, and the recommended dose is 1.5 g/Kg at the first day and 1.0 g/Kg at the third day of treatment. More studies are needed in order to assess its role in infections other than SBP.
C. The association of a vasoconstrictor (preferably terlipressin) and albumin is the treatment of choice for HRS.
D. The treatment for HRS is recommended for patients with HRS-AKI stages 2 or 3, independently of the values of SCr at the time of diagnosis. Ttreating patients with HRS-AKI stage 1b should be considered if SCr increases at least 50% over the baseline.
E. Liver transplantation is the treatment of choice for patients with HRS-2. The use of a vasoconstrictor and albumin should be tailored, taking into account the probability of, and time to transplantation.

Renal replacement therapy

Renal replacement therapy (RRT) should be considered for severe AKI, particularly those patients on the waiting list for liver transplantation or where recovery of liver function is anticipated.

The indication for RRT follows standard guidelines and it is not specific for patients with cirrhosis and AKI. Conventional indications include volume overload, severe hyperkalemia, uremia (encephalopathy, pericarditis), severe metabolic acidosis and exogenous intoxication. In patients with HRS, RRT should be indicated in the absence of response or adverse reaction to vasoconstrictors. The assessment of prognosis, eligibility for liver transplantation, advanced stages of *acute on chronic liver failure* (ACLF), should be considered before RRT to avoid futile treatments. Ideal timing to begin RRT is still controversial. Patients that do not fit in conventional indications should be judged on a case-by-case basis.

The choice of the dialytic method is critical for in decompensated cirrhosis or ACLF patients. Worsening of circulatory dysfunction (i.e. severe arterial hypotension) during RRT is a major concern as it may cause organ failure. RRT is particularly poorly tolerated in patients with HRS, due to the profound hemodynamic disturbance that are characteristic of this syndrome.

Acceptable RRT methods are intermittent both conventional hemodialysis or peritoneal dialysis; continuous hemofiltration or continuous hemodiafiltration; prolonged hemodialysis (SLED - Sustained Low Efficiency Dialysis). SLED has the advantage of providing cardiovascular stability and effective clearance of continuous therapies, with the reduced costs of intermittent therapies[56].

The most important limiting factor of intermittent therapies is hemodynamic instability. Hypotension during RRT is associated with dialysis technique (volume and ultrafiltration rate, reduction of plasmatic osmolality) and patient characteristics (hypovolemia, vasodilation, liver failure)[57,58]. Hypotension decreases the effectiveness of RRT and aggravates ischemic injury, delaying the recovery of kidney function[59]. When compared to intermittent therapies, continuous methods offer greater hemodynamic stability, and are often preferred for patients with arterial hypotension[60].

Thrombocytopenia and coagulopathy would limit the use of heparin or other anticoagulants during RRT. However, these coagulation disorders found in patients with cirrhosis do not protect patients against thrombosis during RRT. Due to the reduced blood-flow continuous therapies often require the use of anticoagulation in the dialysis circuit.

Peritoneal dialysis should not be routinely used due to increased risk of infections. Use of dialysis solutions with bicarbonate for patients with hyperlactatemia and regional anticoagulation (only in dialysis circuit) for severe coagulopathy are recommended.

Combined liver-kidney transplantation (CLKT)

Liver-kidney transplantation would be the procedure of choice for patients with end-stage liver and kidney disease. Because of the negative impact on prognosis that renal failure has on liver transplantation recipients, CKLT is usually proposed for these patients. Besides, the postoperative worsening of kidney function is associated with higher morbidity and mortality[61,62].

It is still difficult to identify with certainty which patients may benefit from CKLT. Even though HRS may be entirely reversible with isolated liver transplantation, some patients do not recover. It is likely that these patients have progressed to ATN, and will require long-term RRT and eventual CKLT.

However, it is believed that duration of RRT greater than 6 weeks is a reasonable criterion for eligibility for combined liver and kidney transplantation and this is the basis of the current guidelines.

According to Consensus guidelines published in 2008[63], indications for CKLT in patients with AKI and/or HRS are SCr >2.0 mg/d and RRT for more than 8 weeks without evidence of renal function recovery.

The OPTN (*Organ Procurement and Transplantation Network*) proposed slightly different minimum criteria for CKLT in candidates for LT. This group propose CKLT for patients with prolonged AKI, defined by 1) RRT for over 6 weeks (two dialysis sessions per week for 6 weeks); 2) GFR ≤25 mL/min/ 1,73 m^2 for over 6 weeks. Or 3) a combination of both criteria (GFR ≤25 mL/min/ 1,73 m^2 for 3 weeks followed by 3 weeks in RRT). GRF may be calculated either by MDRD formula or by creatinine clearance[64].

Recommendations

A. Indications of RRT in patients with cirrhosis are the same as those for other patients with renal dysfunction: volume overload, uremia, and severe acid-base and/or electrolyte disorders.
B. Method of RRT should be selected on the basis of the clinical condition of the patient, especially hemodynamic stability. Continuous or hybrid therapies should be the method of choice in patients with hemodynamic instability.
C. Patients with cirrhosis and AKI who require RRT for over 6-8 weeks should be considered for CKLT.

Authors' contribution

Terra C, Mattos AZ, Bittencourt PL: wrote the manuscript. Farias AQ: critically reviewed the manuscript. All authors participated in the panel and approved the manuscript.

Terra C, Mattos ÂZ, Pereira G, Farias AQ, Kondo M, Mattos AA, Medeiros Filho JEM, Strauss E, Dutra FRD, Mazza M, Lopes EP, Sevá Pereira T, Schiavon LL, Carvalho Filho RJ, Fagundes C, Bittencourt PL. Recomendações da Sociedade Brasileira de Hepatologia para manejo da lesão renal aguda em pacientes com cirrose. Arq Gastroenterol. 2018,55(3):314-20.

RESUMO – A lesão renal aguda é uma complicação comum da cirrose, acometendo até 20% dos pacientes hospitalizados. Este tema está em rápida evolução devido aos importantes avanços em novas classificações, biomarcadores e tratamentos que ocorreram nos últimos anos. Em nome da Sociedade Brasileira de Hepatologia, um painel de especialistas em Hepatologia e Nefrologia revisou as evidência publicadas na literatura, integrando os diferentes resultados de estudos, para desenvolver a presente recomendação.

DESCRITORES – Lesão renal aguda. Gerenciamento clínico. Cirrose hepática, complicações. Medição de risco.

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