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ORIGINAL ARTICLE

Impairment of Esophageal Barrier Integrity: New Insights into Esophageal Symptoms in Post-COVID-19

Dayllanna Stefanny Lopes Lima Feitosa[1] · Larissa Gurgel Mota Saraiva[1] · Maria Klayre Araújo de Sousa[1] · Lara Mara Gomes da Silva[1] · Isabela Caldas Borges[1] · Thiago Andrade Ribeiro[1] · Quésia Reis Lederhos[1] · Rôney Reis de Castro Silva[1] · Suliana Mesquita Paula[1] · Marco Antônio de Freitas Clementino[2] · Alexandre Havt[2] · Marcellus Henrique Loiola Ponte Souza[1] · Armênio Aguiar dos Santos[2] · Miguel Angelo Nobre Souza[1,3]

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Abstract

Background The COVID-19 pandemic, caused by SARS-CoV-2, has unveiled a range of symptoms beyond the respiratory system, including significant gastrointestinal manifestations.

Aims This study explores the prevalence and intensity of gastroesophageal symptoms in post-COVID-19 patients and the integrity of the esophageal epithelial barrier.

Methods We conducted a prospective longitudinal cohort study with 55 patients hospitalized due to COVID-19 at a University Hospital. Patients were evaluated during hospitalization and between 3 and 6 months post-discharge, using validated questionnaires for gastrointestinal and gastroesophageal reflux symptoms. Additionally, 25 of these patients underwent upper digestive endoscopy, with esophageal mucosal biopsies analyzed for transepithelial electrical resistance (TER), permeability, and expression of inflammatory cytokines and cell junction proteins. Data expressed as mean EPM, inference by two-way ANOVA.

Results Results were considered statistically significant at $p < 0.05$. There were significant increases in heartburn and acid reflux symptoms in post-COVID-19 patients, as measured by the GSRS questionnaire. Biopsies from post-COVID patients revealed increased esophageal permeability when compared to non-COVID patients in acidic media (pH 2: non-COVID-19: 717.8 ± 168.2 vs. post-COVID-19: 1377.6 ± 316.4), suggesting compromised mucosal barrier. Furthermore, IL-8 levels and expression of Claudin-2 were elevated in these patients.

✉ Miguel Angelo Nobre Souza
mans@ufc.br

Dayllanna Stefanny Lopes Lima Feitosa
daylana@alu.ufc.br

Larissa Gurgel Mota Saraiva
larissagurgel@alu.ufc.br

Maria Klayre Araújo de Sousa
klayresousa@alu.ufc.br

Lara Mara Gomes da Silva
laramara@alu.ufc.br

Isabela Caldas Borges
isabelacaldas@alu.ufc.br

Thiago Andrade Ribeiro
thiagoandrader@alu.ufc.br

Quésia Reis Lederhos
quesialederhos1986@gmail.com

Rôney Reis de Castro Silva
roney@alu.ufc.br

Suliana Mesquita Paula
suliana@alumni.usp.br

Marco Antônio de Freitas Clementino
mclementino@ufc.br

Alexandre Havt
ahavt@ufc.br

Marcellus Henrique Loiola Ponte Souza
souzamar@ufc.br

Armênio Aguiar dos Santos
meno@ufc.br

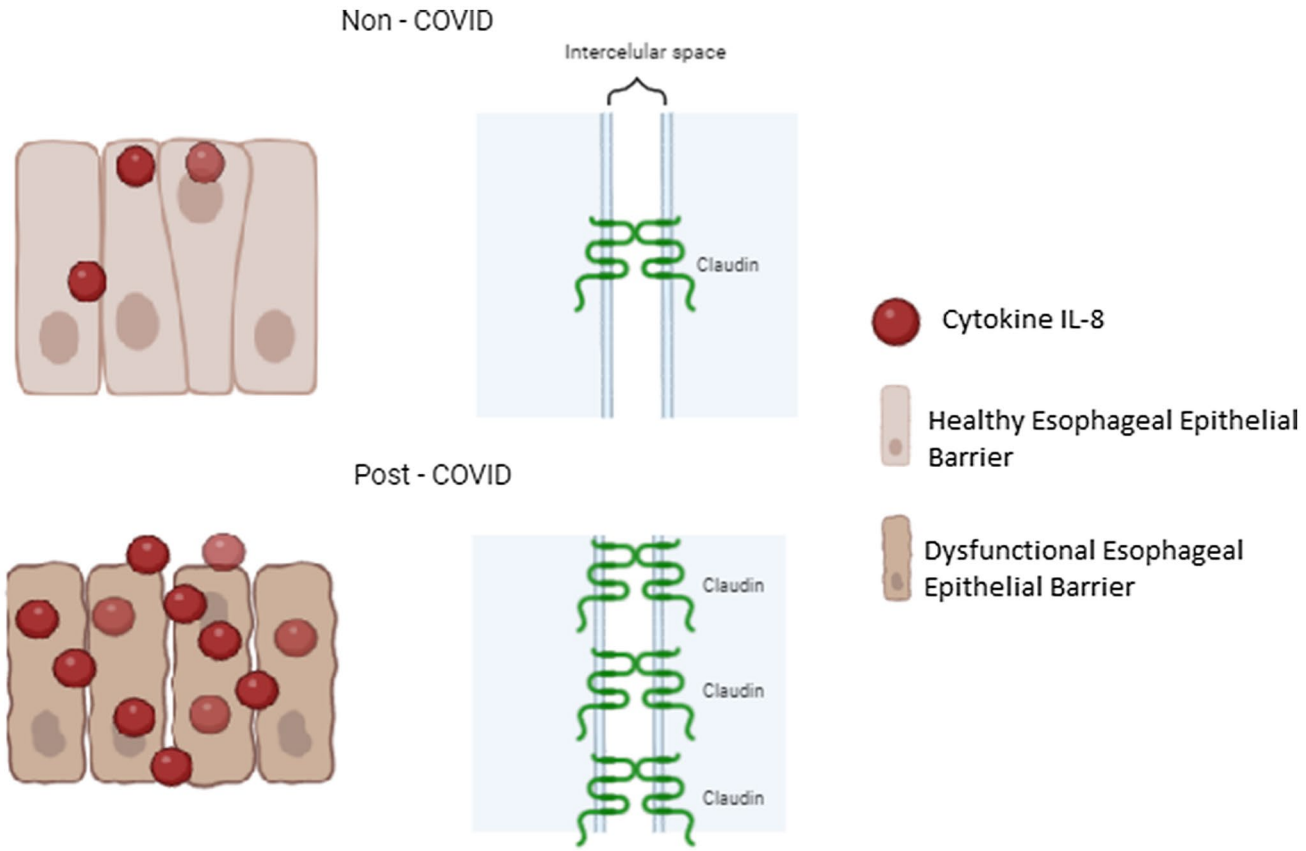
[1] Department of Medicine, School of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil

[2] Department of Physiology and Pharmacology, Federal University of Ceará, Fortaleza, Ceará, Brazil

[3] Institute of Biomedicine for Brazilian Semi-Arid (IBISAB), Coronel Nunes de Melo Street, 1315, Rodolfo Teófilo, Fortaleza, CE 60.430-270, Brazil

Conclusions The data suggested that COVID-19 infection may cause lasting damage to the esophageal epithelial barrier, increasing its permeability and provoking an exacerbated inflammatory response. These changes may explain the prevalence of post-infection gastroesophageal symptoms. Our findings underscored the importance of continuous monitoring and the development of therapeutic strategies to mitigate gastroesophageal effects in patients recovering from COVID-19.

Graphical Abstract



Keywords COVID-19 · SARS-CoV-2 · Esophageal barrier · Gastroesophageal symptoms · Esophageal permeability · Inflammatory cytokines · Claudin-2

Introduction

The COVID-19 pandemic has profoundly impacted society, presenting immense challenges in the prevention, treatment, and recovery of affected individuals (Youssef [1]). In addition to respiratory symptoms, both clinical and experimental studies have reported a range of gastrointestinal manifestations in COVID-19 patients. The virus has been detected in the esophagus, stomach, duodenum, and rectum [2–5].

A study by Gu et al. [6] highlighted the presence of gastrointestinal symptoms in COVID-19 patients, which may occur independently or in combination with the classic respiratory features of the disease. Moreover, there is growing evidence that these symptoms can persist for months following hospital discharge [7, 8]. Such long-lasting effects are referred to as "long COVID," a condition characterized by persistent symptoms despite complete microbiological recovery [9]. Long COVID is believed to result from a systemic cytokine-mediated inflammatory response [10] and tissue hypoxia [11].

Esophageal inflammation associated with reflux may result from the caustic effects of refluxed gastric contents on the mucosa [12, 13], as well as from lymphocytic infiltration originating in the submucosa and affecting the epithelial surface [14]. Both mechanisms lead to increased levels of

inflammatory cytokines, such as IL-8 [15, 16], and enhanced expression of junctional proteins like Claudin-2 [17–19]. As a result, the permeability of the esophageal mucosa increases [20], further exacerbating inflammation and tissue injury [21].

Several studies have explored the interaction between SARS-CoV-2 infection, increased gastrointestinal mucosal permeability, and the production of inflammatory cytokines [22–24]. IL-8 has been implicated as a key player in the pathogenesis of gastroesophageal reflux disease (GERD), while claudins have been identified as critical regulators of junctional barrier integrity in the esophageal epithelium [2, 25–29]. In addition, tissue hypoxia—common to both COVID-19 and reflux disease—may aggravate mucosal injury by compromising barrier function, thereby increasing permeability to inflammatory agents [11, 30].

Accordingly, the present study aims to investigate changes in the resistance and permeability of the esophageal epithelial barrier, explore their potential association with inflammatory mechanisms triggered by COVID-19, and assess their relationship with post-COVID-19 esophageal symptoms.

Methods

Study Design and Ethics

This prospective longitudinal cohort study enrolled patients infected with COVID-19 at a university hospital. Hospitalized individuals were recruited by invitation and interviewed as part of the research protocol. The study was conducted in full compliance with the ethical principles outlined in the Declaration of Helsinki. Approval was obtained from the local ethics committee (protocol number: 5.342.989), and written informed consent was obtained from all participants prior to their inclusion in the study.

Study Population

A total of 55 patients (both male and female) hospitalized during the COVID-19 pandemic were included. All participants had laboratory-confirmed COVID-19 via polymerase chain reaction (PCR) testing performed during their hospital stay. Demographic and clinical data were extracted from electronic medical records. Participants were contacted again 3 to 6 months after hospital discharge for follow-up.

Symptom Questionnaire

Gastrointestinal symptoms were assessed using the validated Gastrointestinal Symptom Rating Scale (GSRS). The GSRS includes two questions specific to esophageal dysfunction: one on heartburn, defined as "an unpleasant stinging or burning sensation in the chest," and another on acid reflux, defined as "a sensation of regurgitating small quantities of acid or flow of sour or bitter fluid from the stomach up to the throat." Symptom severity was scored on a 7-point Likert scale (1–7), and participants were considered symptomatic if they reported moderate to severe discomfort (score ≥ 4). Individual symptom scores were analyzed by comparing responses during hospitalization versus 3 to 6 months post-discharge, as shown in Fig. 1A and B.

The GSRS was selected due to its well-documented reliability and validity, with established reference values in the general population [31]. Additionally, 6 months post-discharge, 25 COVID-19 patients and 8 non-COVID-19 controls without gastroduodenal symptoms underwent upper gastrointestinal endoscopy. Four biopsies of the distal esophageal mucosa were collected from each individual for further analysis.

Using Chamber Experiments

Esophageal biopsy samples were first inspected under magnification, then mounted in Ussing chambers (Mussler

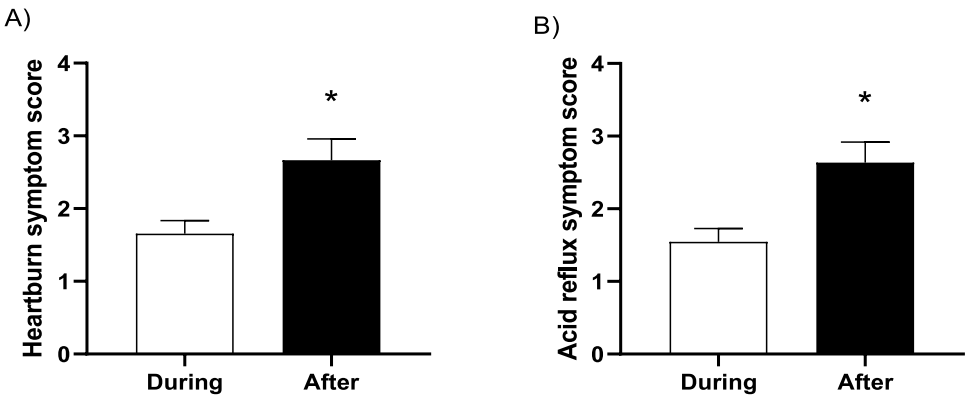


Fig. 1 Evolution of the intensity and frequency of heartburn and acid reflux symptoms during and after infection with the SARS-CoV-2 virus. Data were obtained from scores on the Gastrointestinal Symptom Rating Scale (GSRS), assessing symptoms of heartburn (**A**) and acid reflux (**B**). $N = 55$ patients per group. Student's T-test. $^{*}p < 0.05$ vs. During

Scientific Instruments, Aachen, Germany). Samples were maintained in Krebs–Henseleit buffer (pH 7.4) for evaluation of basal transepithelial electrical resistance (TEER). One sample was exposed to Krebs–Henseleit solution at pH 2 to evaluate potential epithelial injury [32]. TEER was calculated according to Ohm's Law using voltage deflections from bipolar current pulses (50 µA, 200 ms duration) applied every 6 s through platinum electrodes. TEER changes were expressed as a percentage of baseline resistance and corrected for tissue area ($\Omega \cdot cm^2$) [33].

After TEER measurements, epithelial permeability was assessed. Biopsies remained in the chambers while the luminal solution was replaced with a pH 7.4 Krebs solution containing fluorescein (375 kDa; 1 mg/mL) as a paracellular permeability marker. Fluorescein concentrations were measured at 30-min intervals for 90 min (T30, T60, T90) using a fluorescence reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany). Fluorescein concentrations were calculated in $\mu mol/cm^2$ based on a standard calibration curve [32].

Inflammatory Cytokine Assay

Esophageal cytokine levels were quantified using a MILLIPLEX® Cytokine/Chemokine 4-plex assay kit (Millipore Corporation, Billerica, MA, USA). The panel included IL-1β, IL-6, IL-8, and TNFα. Assays were performed on biopsy extracts using the Luminex® MAGPIX® instrument and xPONENT® software (Luminex Corporation, Austin, TX, USA).

Western Blot Analysis

Claudin-2 expression in esophageal biopsies was assessed by Western blot. Tissues were homogenized in chilled RIPA lysis buffer (Merck Millipore, Billerica, MA, USA) containing protease inhibitor cocktail (1:100; Sigma-Aldrich, St. Louis, MO, USA), PMSF (1:800; Amresco, Solon, OH, USA), and Pepstatin (1:800; Roche, Basel, Switzerland). Homogenates were centrifuged (3000 rpm, 15 min, 4 °C), and supernatants were collected. Protein samples (30 µg) were separated by 10% SDS-PAGE and transferred to PVDF membranes (Amersham-GE Healthcare, UK) overnight at 4 °C using a Mini Trans-Blot system (Bio-Rad, Hercules, CA, USA) and transfer buffer (25 mmol/L Tris, 192 mmol/L glycine, 5.5% methanol, 0.5% SDS; Amresco).

Membranes were blocked with 5% bovine serum albumin (Inlab, São Paulo, Brazil) and incubated overnight at 4 °C with primary antibodies: mouse anti-Claudin-2 (1:1000; Abcam, Cambridge, UK) and mouse anti-β-actin (1:1000; Cell Signaling Technology, Danvers, MA, USA). After washing, membranes were incubated with HRP-conjugated secondary antibodies (anti-mouse IgG, 1:1000; Cell Signaling Technology). Bands were visualized using ECL™ Prime chemiluminescent reagent (Bio-Rad) and imaged on a ChemiDoc™ MP System (Bio-Rad). Densitometric analysis was performed using Image Lab™ 5.1 software, and Claudin-2 expression was normalized to β-actin levels within the same gel/membrane.

Statistical Analysis

Symptom questionnaire data were analyzed using paired Student's t-tests and Chi-square or Fisher's exact tests. Ussing chamber data were evaluated using unpaired Student's t-tests and two-way ANOVA. Western blot and cytokine assay data were analyzed with unpaired Student's t-tests. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

Results

Study Population

A total of 55 patients were enrolled in the study, with a predominance of males (61.8%) and a mean age of 46.6 years (Table 1). Over 70% of the cohort had mild comorbidities, with hypertension (36.6%), diabetes mellitus (25.4%), and obesity (25.4%) being the most frequent. No participant presented with cardiovascular, pulmonary, hepatic, or renal insufficiency, nor with other severe comorbidities beyond COVID-19. The most commonly

Table 1 Clinical and demographic characteristics of patients hospitalized for COVID-19

Potential covariants		Total (%) n=55
Gender	Male	34 (61.82)
	Female	21 (38.18)
Age (average)		46.6
Previous comorbidities		39 (70.91)
Nausea/vomiting		15 (27.27)
Diarrhea		19 (34.54)
Dyspnea		33 (60)
Oxygen therapy		41 (74.54)
Corticosteroid		47 (85.45)
Antibiotic		49 (89.27)
Anticoagulant		32 (58.18)
Hospitalization time >8 days		38 (69.10)

Table 2 Prevalence of patients with reflux symptoms according to the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire

GSRS		During n = 55 (%)	After *n* = 55 (%)	*p* value
Heartburn	yes	5 (9.09)	18 (32.73)	0.002
Acid reflux	yes	6 (10.91)	17 (30.91)	0.009

Responses with an intensity and frequency score greater than or equal to 4 were considered symptomatic

$P < 0.05$

Chi-square (and Fisher's exact) test

reported symptoms during hospitalization were dyspnea (60.0%), diarrhea (34.5%), and nausea or vomiting (27.3%). The majority of patients (69.1%) were hospitalized for fewer than 8 days. During the hospitalization period, 41 patients (74.5%) required oxygen therapy, 49 (89.3%) received antibiotics, 47 (85.5%) were treated with corticosteroids, and 32 (58.2%) were prescribed anticoagulants. Only two patients used proton pump inhibitors.

Gastroesophageal Symptoms Following COVID-19

A significant increase in gastroesophageal symptoms was observed during post-COVID-19 follow-up. Heartburn scores significantly increased after hospitalization (1.65 ± 0.18 vs. 2.63 ± 0.29; $p = 0.003$), as did acid reflux scores (1.54 ± 0.18 vs. 2.63 ± 0.28; $p = 0.0001$) (Fig. 1). The prevalence of symptomatic individuals also increased (Table 2). Heartburn was reported by 9% ($n = 5$) during hospitalization, increasing to 32.7% ($n = 18$) after recovery ($p = 0.002$). Similarly, acid reflux was reported by 10.9% ($n = 6$) during hospitalization, rising to 30.9% ($n = 17$) after discharge ($p = 0.009$). There was no statistically significant difference when comparing symptom prevalence before versus after COVID-19 infection (heartburn: $p = 0.284$; acid reflux: $p = 0.274$). No significant associations were found between the presence of post-COVID-19 heartburn or acid reflux and comorbidities such as hypertension, diabetes, or obesity (Chi-square and Fisher's exact tests).

Esophageal TEER and Permeability

Baseline transepithelial electrical resistance (TEER) of esophageal biopsies did not differ significantly between post-COVID-19 patients ($n = 21$) and non-COVID-19 controls ($n = 9$) (162.55 ± 11.38 vs. 145.06 ± 19.84 $\Omega \cdot cm^2$) (Fig. 2A). In both groups, TEER significantly decreased following exposure to acidic Krebs solution (pH 2) compared to neutral conditions (pH 7.4), indicating epithelial barrier disruption in response to acid. TEER reduction was comparable between COVID-19 and non-COVID-19 controls [non-COVID: pH 7.4 = 98 ± 2.87 vs. pH 2 = 80 ± 6.56; post-COVID: pH 7.4 = 100.7 ± 0.93 vs. pH 2 = 86.3 ± 4.54] (Fig. 2B–C).

Esophageal permeability, assessed via fluorescein flux, was significantly elevated in the post-COVID-19 group exposed to acidic Krebs solution (pH 2) compared to the same group under neutral pH conditions (1377.57 ± 316.37 vs. 398.01 ± 96.78 $\mu mol/cm^2$; $p < 0.05$). Moreover, post-COVID-19 patients showed significantly greater permeability than non-COVID-19 controls when both were exposed to pH 2 Krebs solution (1377.57 ± 316.37 vs. 717.80 ± 168.29 $\mu mol/cm^2$; $p < 0.05$) (Fig. 2D).

Inflammatory Cytokine Expression

Among the inflammatory markers assessed in esophageal mucosal samples, only interleukin-8 (IL-8) levels were significantly elevated in post-COVID-19 patients compared to non-COVID-19 controls (17.88 ± 2.85 vs. 8.33 ± 0.78 pg/mg protein; $p < 0.05$) (Fig. 3B). No significant differences were observed for IL-1β, IL-6, or TNFα.

Claudin-2 Expression

Western blot analysis revealed a significant increase in Claudin-2 expression in esophageal tissues of post-COVID-19 patients relative to controls (0.588 ± 0.116 vs. 0.241 ± 0.082 arbitrary units; $p < 0.05$) (Fig. 3E), suggesting disruption of epithelial tight junction integrity.

Discussion

A significant increase in heartburn and acid reflux symptoms has been observed in patients after COVID-19 infection. This phenomenon is associated with increased esophageal permeability to acidic pH, indicating a compromised mucosal barrier. Esophageal biopsies from post-COVID-19 patients revealed elevated expression of the inflammatory cytokine IL-8. Additionally, increased expression of the junctional protein Claudin-2 was detected. These findings suggest that both inflammation and alterations in cell junctions persist after recovery from COVID-19.

Most patients recruited during hospitalization had previously diagnosed mild comorbidities. Prior research has demonstrated that individuals with preexisting medical are more vulnerable to COVID-19 [34, 35]. However, common comorbidities—such as hypertension, diabetes, or obesity—showed no association with heartburn or acid reflux, suggesting they did not modulate reflux symptoms in our cohort.

The increase in esophageal symptoms reported by COVID-19 patients indicates that the virus can affect not

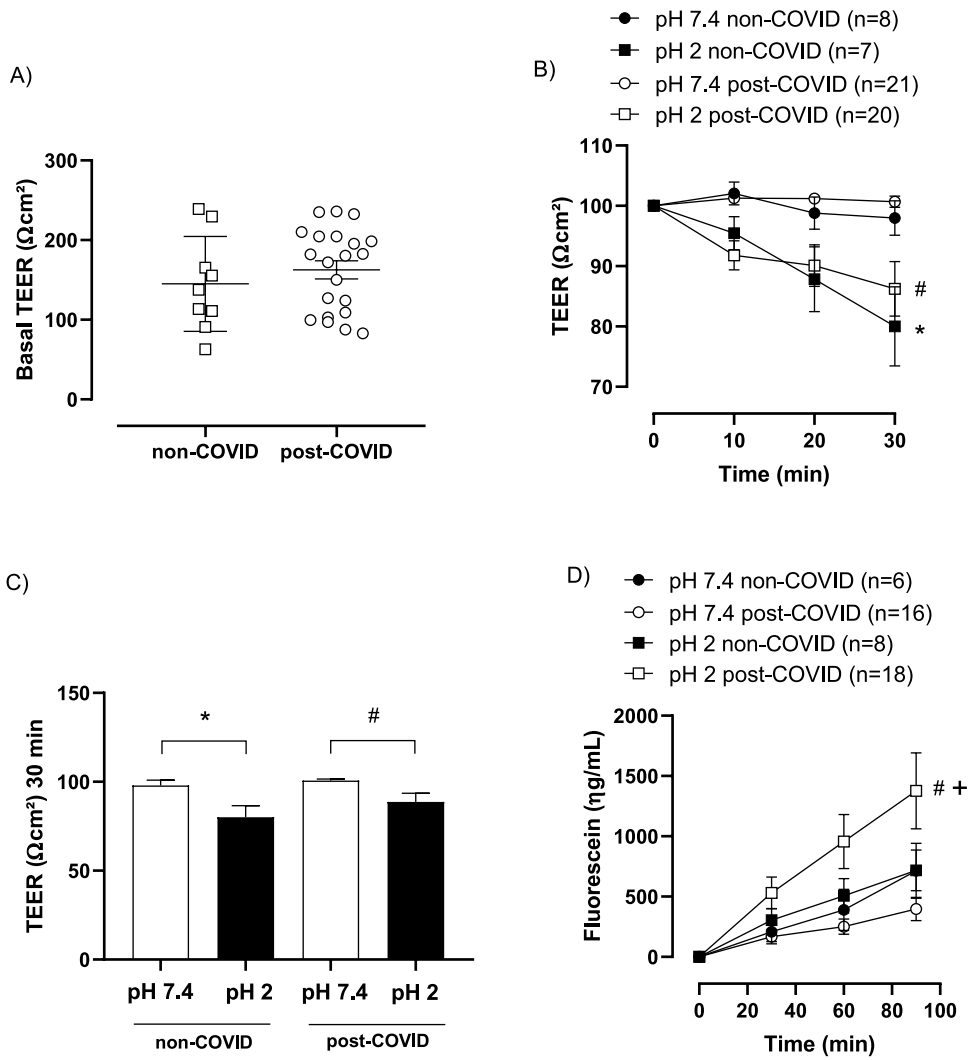


Fig. 2 Transepithelial electrical resistance and Permeability of esophageal tissue from healthy subjects (non-COVID) and post-COVID patients. Basal transepithelial electrical resistance (TEER) (**A**) recording of esophageal tissue from non-COVID healthy subjects ($n = 9$) and post-COVID-19 patients ($n = 21$). Transepithelial electrical resistance of non-COVID and post-COVID patients over 30 min (**B**) and at 30 min (**C**) in the presence of neutral pH 7.4 and acid pH 2.0. Permeability of non-COVID and post-COVID patients over 90 min (**D**) in the presence of neutral pH 7.4 and acid pH 2.0. The number of esophageal biopsies analyzed are in parentheses. Results expressed as mean ± SEM and analyzed by two-way ANOVA test and Student's T-test when appropriate, $^{*}p < 0.05$ *vs.* pH 7.4 non-COVID, $^{\#}p < 0.05$ *vs.* pH 7.4 post-COVID, $^{+}p < 0.05$ *vs.* pH 2 non-COVID

only the respiratory system but also the gastrointestinal tract [6, 36]. Following infection, participants reported increased symptoms of heartburn and acid reflux, which may be initially attributed to dietary changes during hospitalization, emotional stress [37, 38], and the use of medications such as anti-inflammatories [39]. However, increased mucosal permeability may represent an independent cause of these symptoms, as seen in patients with visceral hypersensitivity [40]. The observed increase in permeability may therefore help explain the post-COVID-19 symptoms. Although symptoms appeared similar to those experienced prior to hospitalization, this study did not directly measure pre-hospitalization symptoms,instead, baseline data were based on patient recall. As with any subjective data, esophageal symptom reports are subject to recall bias and multiple influencing factors and should be interpreted cautiously. Nevertheless, the authors argue that symptoms may have worsened due to mucosal damage, as evidenced by increased Claudin-2 expression—an objective finding of this study.

Transepithelial electrical resistance (TEER) measurements showed no significant differences between non-COVID-19 and post-COVID-19 patients. This may be because TEER reflects both transcellular and paracellular conductance. In contrast, the fluorescein permeability assay evaluates only paracellular permeability [41–43]. Thus, the observed increase in permeability is likely due to altered tight junction integrity.

This study is the first to demonstrate that, under acidic exposure (pH 2.0), fluorescein permeability in esophageal biopsies increased progressively, reaching a maximum effect at 90 min. Notably, this increase was significantly greater in post-COVID-19 patients compared to non-COVID controls. This could be due to an exacerbated inflammatory response that impairs normal tissue function and compromises the gastrointestinal barrier [44]. Damage to the epithelial barrier can permit the translocation of pathogens and inflammatory molecules into systemic circulation, potentially causing further tissue injury, as observed in other viral and bacterial infections [45–47]. Impairment of the esophageal epithelial

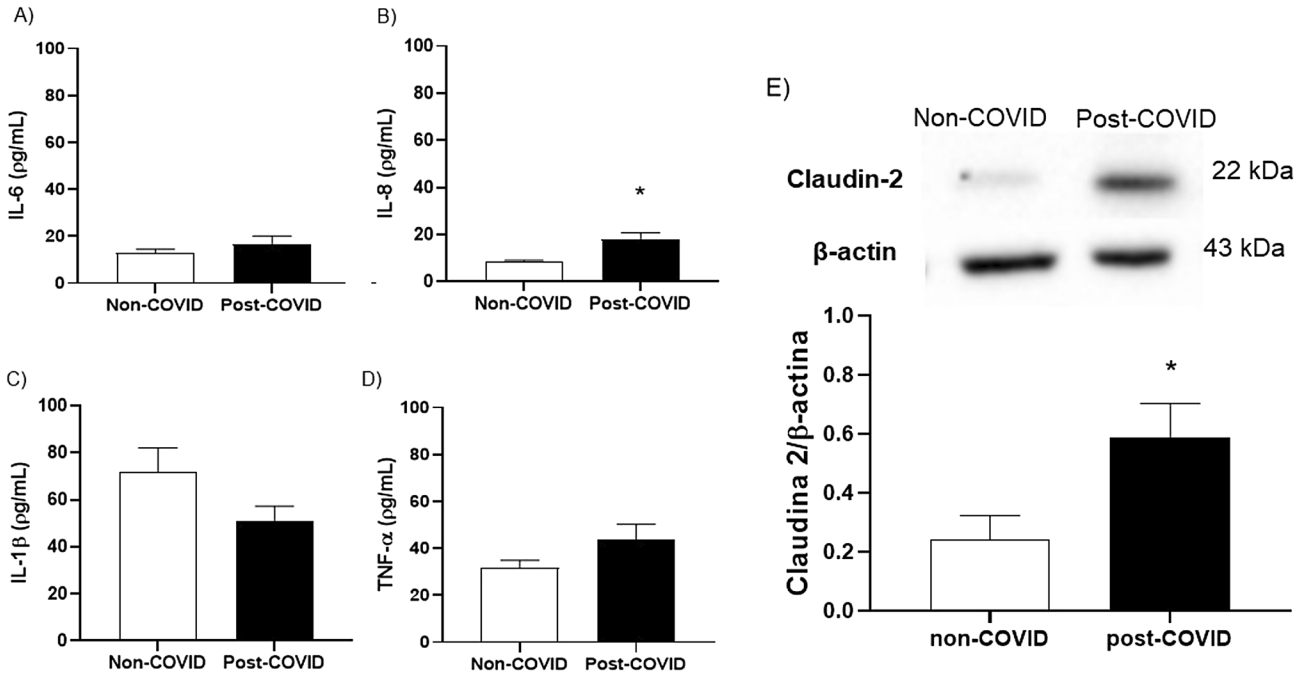


Fig. 3 Inflammatory biomarkers and Claudin-2 protein expression in biopsies of the distal portion of the esophagus from patients after COVID-19. Inflammatory cytokines IL-6 (**A**), IL-8 (**B**), IL-1β (**C**), and TNF-α (**D**) of non-COVID and post-COVID patients were analyzed by Multiplex assay. Expression of claudin-2 protein (E) by Western Blot in biopsies from patients with esophagitis. $N=$ 6–8 (non-COVID) and $N=$ 21–26 (post-COVID) per group. Results expressed as mean ± SEM and analyzed by Student's T-test, $^{*}p<0.05$ vs. non-COVID

barrier has also been documented in both healthy controls and patients with GERD [48–51]. Therefore, it is plausible that COVID-19 directly impacts the integrity of the esophageal epithelial barrier and its response to acid exposure.

The increased inflammation and permeability may be further aggravated by tissue hypoxia, a known consequence of COVID-19 [52, 53]. The interrelationship between hypoxia, inflammation, and intestinal permeability is well-established, with each factor influencing the others and potentially worsening clinical outcomes [49–51]. These mechanisms may underlie the increased esophageal symptoms, as hypoxia can intensify inflammation. Supporting this, 74.5% of hospitalized patients received oxygen therapy—a standard treatment for COVID-19-related hypoxemia.

Elevated IL-8 levels in post-COVID-19 patients have also been reported in other studies, where intense pro-inflammatory stimuli triggered cytokine release leading to esophageal epithelial barrier damage [54, 55]. A hallmark of COVID-19 is the acute elevation of inflammatory markers—a "cytokine storm"—in affected tissues [56]. The increased IL-8 observed post-COVID-19 suggests ongoing immune activation, potentially contributing to the persistent inflammation. Indeed, elevated IL-8, alongside anti-SARS-CoV-2 antibodies, has been associated with more severe disease, including severe inflammation, lung injury, and the need for ventilatory support [57].

Other diseases, such as inflammatory lung conditions [58], influenza [59], gastrointestinal disorders such as dyspepsia [60], worsening of intestinal symptoms [61], and GERD [14, 15, 62], have also been associated with elevated IL-8. This pro-inflammatory cytokine plays a central role in the development and maintenance of esophageal symptoms [44, 63, 64]. Thus, IL-8 may contribute both to the persistent inflammation and the increased permeability observed in esophageal biopsies from post-COVID-19 patients.

The increase in esophageal symptoms in this cohort may also be explained by elevated expression of Claudin-2 in the esophageal epithelium. Claudins are integral cell–cell adhesion proteins that form tight junctions and regulate barrier integrity. Unlike most Claudins, Claudin-2 forms paracellular pores, and its overexpression increases epithelial permeability [65]. It is also involved in forming cation-selective channels and regulating water transport [66]. In various pathologies, IL-8 has been linked to Claudin regulation, including in Helicobacter pylori infection [67], Barrett's esophagus [68, 69], and non-erosive reflux disease, where Claudin-2 expression increases fivefold, accompanied by expansion of intercellular spaces and greater paracellular permeability [28]. Claudin-2 upregulation has also been implicated in diarrhea [70]. Additionally, inflammatory

cytokines affect various tight junction components across tissues, with increased expression linked to a wide range of symptoms, including alveolar fibrosis, pulmonary edema, conjunctivitis, male infertility, and gastrointestinal dysfunction [71].

In summary, increased levels of inflammatory cytokines such as IL-8 and elevated Claudin-2 expression may disrupt the esophageal epithelial barrier, leading to increased permeability in post-COVID-19 patients. These changes may contribute to persistent symptoms such as acid reflux and heartburn, leaving a lasting impact on the gastrointestinal system. This study aims to enhance clinical and therapeutic understanding of post-COVID-19 conditions and may encourage further research into strategies for modulating inflammation and improving outcomes in affected individuals. The findings underscore the importance of long-term follow-up for patients recovering from COVID-19, especially those with ongoing gastrointestinal symptoms.

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Data Availability The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but can be requested from the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study protocol was reviewed and approved by Research Ethics Committee of the Hospital Complex of the Federal University of Ceará (CEP/HUWC-UFC), approval number 5.342.989.

Consent to participate All participants and/or their legal guardian gave their consent by completing the Free and Informed Consent Form (TCLE), approved by the same CEP/HUWC-UFC committee.

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