Arginase-1 and Treg Profile Appear to Modulate Inflammatory Process in Patients with Chronic Gastritis: IL-33 May Be the Alarm Cytokine in H. pylori-Positive Patients

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Helicobacter pylori (H. pylori) is a highly prevalent bacterium in our environment, directly involved in various upper digestive tract diseases, such as gastritis, peptic ulcer, and gastric cancer. Several molecules activating the immune system have been reported to be involved in containing H. pylori infection. This study is aimed at analyzing the mRNA expression of the cytokines IFN-γ, IL-17, IL-10, TGF-β, IL-6, IL-22, IL-23, and IL-33; transcription factors T-bet, RORC, and FOXP3; enzymes ARG1, ARG2, and NOS2; and neuropeptides VIP and TAC and their respective receptors VIPR1 and TACR1 in the stomach lining of patients with severe digestive disorders. One hundred and twenty six patients have been evaluated, presenting with symptoms in the upper digestive tract, with the clinical indication for an Upper Digestive Endoscopy exam. Two fragments of the mucosa of the gastric body and antrum have been collected for anatomopathological examination and to analyze the expression of enzymes, cytokines, and transcription factors using qPCR. Expression of the ARG1 gene was seen as significantly higher in the group of patients with chronic inactive gastritis than in the control group. Expression of the TGF-β gene and its FOXP3 transcription factor was significantly higher in the group of chronic inactive gastritis patients than in the control. Expression of IFN-γ, IL-17, IL-10, and TGF-β and the transcription factors, T-bet and RORC, in the presence or absence of H. pylori showed no significant difference. However, the expression of FOXP3 was significantly lower in H. pylori-positive patients than that in H. pylori-negative patients. ARG1 and Treg profile appeared to be modulating the inflammatory process, protecting patients from the tissue lesions with chronic inactive gastritis. Furthermore, we suggest that IL-33 may be a crucial mediator of the immune response against an infection, after gastric mucosal damage.

1. Introduction

Helicobacter pylori (H. pylori) is a gram-negative spiral bacterium that colonizes the gastroduodenal mucosa of humans [1]. H. pylori infection represents a key factor in the etiology of various gastrointestinal diseases, from chronic active gastritis without clinical symptoms, to peptic ulceration, gastric adenocarcinoma, and the lymphoma of lymphoid tissue associated with gastric mucosa [2]. Although half of the world’s population is infected with this bacterium, 80% of such individuals do not manifest the disease symptoms [3]. Studies have showed that the interaction between H. pylori subtype and the host immune response profile contributes to the chronicity of the disease and progression to more severe gastric disorders, thus explaining the different clinical manifestations of the infection caused by this microorganism
In the stomach, *H. pylori* enters the gastric mucus layer, not invading the gastric tissue; thus, the contact between *H. pylori* and phagocytic cells probably occurs infrequently, unless there exist ruptures in the epithelial gastric barrier [6]. It is believed that the microenvironment can result in a spectrum of phenotypes and macrophage functions *in vivo*; furthermore, classical M1 and an alternative M2-activated macrophage phenotypes are at the extremes of that spectrum [7]. Depending on the type of pathogens (pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)) and an environment rich in IFN-γ, the infection or tissue injury promotes the classic activation of highly pro-inflammatory type I M1 macrophages, expressing high levels of nitric oxide synthase (NOS2), interleukin- (IL-) 1β, and tumor necrosis factor (TNF), aimed at the destruction of the pathogens [8]. Nitric oxide (NO) can kill the bacteria *in vitro*; yet, the survival of *H. pylori* appears to be dependent on the amount of NO produced, which further depends on the availability of L-arginine as its substrate [9]. Alternately, the activation of type 2 macrophage (M2), which produce arginase, favors the healing and repair of lesions, with the secretion of high levels of IL-10 and TGF-β [10, 11]. The balance of the expression of NOS2 and arginase defines iNOS-dependent microbialicidal capacity in an inflammatory environment; the deletion of arginase 2 (ARG2) in an experimental model results in increased gastritis and decreased bacterial load during *H. pylori* infection [12].

The interaction between *H. pylori* strains and the host immune response profile contributes to the chronicity of the disease and/or the progression to the more severe gastric disorders; this may explain the different clinical manifestations of the infection caused by this microorganism [4]. Although it is known that *H. pylori* infection triggers a predominantly Th1-like response [3, 4], the role of Th1 cells in *H. pylori* infection is not fully understood [13]. In addition to the Th1 profile, the Th17 profile may be associated with the regulation of stomach colonization by *H. pylori* [14]. Since IL-23 is an important cytokine in the Th17 profile maintenance, the expression of IL-23 after *H. pylori* infection appears to increase the recruitment of neutrophils at the site of infection [15]. The Treg profile associated with TGF-β production is described in the pathogenesis of the inflammatory process of the gastric mucosa [16]. This cytokine increases the production of Treg cells through the positive regulation of the FOXP3 transcription factor, inhibiting the activation and proliferation of antigen-specific T cells and exerting an anti-inflammatory effect [16]. In the normal gastric mucosa, TGF-β plays an important role in the mucosal homeostasis maintenance under physiological conditions [17]. Nevertheless, the increase in the TGF-β expression in the gastric mucosa is important to control the inflammation induced by the bacterium. However, it is believed that *H. pylori* may secrete some soluble proteins that induce TGF-β production in the gastric epithelial cells and monocytes, favoring the colonization of host cells, thus contributing to the pathogenesis of the infection [18]. Considerably suppressed levels of TGF-β1 have been observed in individuals exposed to *H. pylori*; this decrease was associated with *H. pylori* infection in patients with peptic ulcers [19]. Generally, TGF-β is a multifunctional cytokine that plays important roles in gastric inflammation through various regulatory mechanisms. Furthermore, gastric mucosa presents capsaicin-sensitive sensory nerve (CSSN)—its activation can be involved in symptoms and in the modulation of the inflammatory process. Neuropeptides are involved in the signaling of CSSN and may play an important role in the modulation of the immune response. Among these, vasoactive intestinal polypeptide (VIP) and substance P (SP) have anti- and proinflammatory effects, respectively [20].

Another cytokine studied in response to *H. pylori* infection is IL-33; however, its mechanism of action is not yet fully understood [21]. It has been indicated as an alarming cytokine against *H. pylori* infection. The expression of IL-33 mRNA was shown to be significantly greater in biopsies of patients with *H. pylori* infection, as compared to the uninfected patients. Additionally, IL-33 mRNA expression levels were significantly lower in patients with chronic gastritis, as compared to those with active gastritis [22].

In the present study, the expression of the cytokine genes IFN-γ, IL-17, IL-10, TGF-β, IL-6, IL-22, IL-23, and IL-33; transcription factors T-bet, RORC, and FOXP3; enzymes ARG1, ARG2, and NOS2; and neuropeptides VIP and TAC and their respective receptors VIPR1 and TACR1 in the stomach fragments of patients with upper digestive tract symptoms was compared in the presence or absence of *H. pylori*.

## 2. Materials and Methods

### 2.1. Sample Characterization

126 patients with symptoms of the upper digestive tract disorder and with the clinical indication of the Upper Digestive Endoscopy (UDE) examination were selected. Patients were randomized; those using Proton Pump Inhibitors (PPIs), histamine blockers (H2 blockers), antibiotics, or corticosteroids were instructed to discontinue the medication at least two weeks prior to the UDE exam. The UDE exams were performed at the Federal University of the Triângulo Mineiro (UFTM). Biopsies of the esophageal distal mucosa, gastric body, and antrum were collected from all patients during examination, according to present standardization in the literature and procedures established by the Brazilian Society of Digestive Endoscopy (SOBED). Fragments from the biopsies of the distal third of the esophagus, gastric body, and antrum were fixed in 4% buffered formalin, wherein 5 μm thick histological sections were cut; the slides were stained with hematoxylin-eosin (HE) for routine histopathological study or with the silver-based Warthin-Starry technique for *H. pylori* detection. The histological findings of the gastric mucosa were interpreted according to the Sidney classification, with the modifications/graduation proposed by the Houston meeting [23]. An additional fragment of the gastric body mucosa was collected and placed in RNAlater® (Life Technologies Corporation®, USA) for RNA extraction.

The patients were grouped according to the anatomopathological study (APS) of the gastric body regions; 44 patients had active chronic gastritis, 20 had chronic inactive
gastri
tis, and 32 were without gastritis but with some inflam-
matory process in the antrum. The control group of 30 did
not present any type of inflammatory process in any analyzed
region of the stomach (body and antrum). This project was
approved by the Research Ethics Committee (REC) of the
University of Uberaba (UNIUBE), report number 350.874.

2.2. mRNA Extraction and Reverse Transcription. The gastric
body fragments were thawed, transferred to sterile Eppen-
dorf vials, and crushed manually with sterile metal pistils to
obtain mRNA using the total RNA Isolation System SV kit
(Promega, USA), according to the manufacturer’s guidelines.
Each sample was macerated, resuspended in 175 μL Lo Lysis
Buffer, and homogenized in the vortex. After the addition
of 350 μL Dilution Buffer, the samples were placed in a water
bath at 70°C for 3 min, and then centrifuged at 13000 xg for 3
min. Further steps consisted of the addition of different solu-
tions, followed by centrifugations at 13000 xg for 1 minute, in
the following order: addition of 200 μL of 95% ethanol, cen-
trifugation; 600 μL RNA wash solution, centrifugation; 50 μL
of DNase Incubation Mix (consisting of 40 μL Yellow Core
Buffer, 5 μL MnCl₂, and 5 μL DNase I per sample), incuba-
tion at room temperature for 15 min; addition of 200 μL of
DNase Stop Solution, centrifugation; 600 μL RNA wash
solution, centrifugation; and 250 μL RNA wash solution,
centrifugation. After the last phase, the basket containing
the RNA was transferred to an eluate tube and 30 μL of
nuclease-free water was added; thereafter, centrifugation
was followed at 13000 xg for 1 minute to obtain the mRNA.
In the end, the mRNA was quantified in a NanoDrop® 2000
spectrophotometer (Thermo Scientific) and stored in a
freezer at -80°C until reverse transcription. For the prepara-
tion of complementary DNA (cDNA), the high-capacity
cDNA reverse transcription kit (Applied Biosystems, USA)
was used, according to the manufacturer’s instructions. First,
the RT Master Mix was prepared for each sample—its com-
ponents are as follows: 2 μL RT buffer, 0.8 μL dNTP Mix, 2
μL random primers, 1 μL MultiScribe reverse transcriptase,
and 4.2 μL nuclease-free water, totaling 10.0 μL per reaction.
Then, 10 μL of the RT Master Mix and 10 μL of the mRNA
were mixed and placed in a thermocycler in the following
conditions: 25°C for 10 min, 37°C for 120 min, and 85°C
for 5 min. In the end, the cDNA was quantified using a
NanoDrop® ND1000 spectrophotometer and stored at
-20°C until further use.

2.3. qPCR. The quantitative expression of the ARG1, ARG2,
NOS2, VIP, VIPR1, TAG, TACR1, IFN-γ, IL-17, IL-10,
TGF-β, IL-6, IL-22, IL-23, T-bet, RORC, and FOXP3
was analyzed by qPCR in the StepOnePlus™ Real-Time
PCR Systems (Applied Biosystems, Foster, CA, USA) using
the threshold cycle comparison method.

All experiments were mounted on 96-well plates of
MicroAmp® Fast 96-Well Reaction Plate (Applied Biosys-
tems, Foster, USA), and probes from the TaqMan® sys-
tem (Applied Biosystems, USA) were used, following the
manufacturer’s guidelines. The reaction used the following
components: 5.0 μL of TaqMan® Universal PCR Master
Mix (reference 4304437, Applied Biosystems, USA), 0.5 μL
of TaqMan® Gene Expression Assay (probes), and 4.5 μL
dNA, per sample, totaling 10 μL in each well. After assembling
the plates, they were sealed with an adhesive
(MicroAmp™ Optical Adhesive Film—Thermo Scientific),
centrifuged, and placed in the previously programmed
thermal cycler. The amplification reaction comprised 2
min at 50°C, 10 min at 95°C for polymerase activation,
and 40 cycles of 95°C for 15 s and of 60°C for 1 min, ideal
temperatures for denaturation and annealing, respectively,
of cDNA strands. For each cDNA sample, an amplification
curve of each elected target and an internal gene calibrator
(β-actin) was obtained. The results were analyzed based
on the value of the CT (cycle threshold)—cycle threshold
comparative; this is the point corresponding to the number
of cycles where the amplification reaches a given threshold.
The arithmetic form to obtain the relative quantification of
dNA was QR = 2−ΔΔCT.

2.4. Statistical Analysis. Data was analyzed using the
StatView® software (Abacus concept, USA) and IBM SPSS
Statistics® (IBM Corporation®) (Version 23). The continu-
ous variables were submitted to normality and variance
(Kolmogorov-Smirnov) tests. Nonparametric tests were
applied to analyze relative levels of mRNA. The variables pre-
sent a nonnormal distribution. They were analyzed by the
nonparametric Mann-Whitney tests for comparison between
two groups or Kruskal-Wallis followed by the Dunn posttest
for comparison between three or more groups. Data was
expressed as median, with minimum and maximum values
and percentiles. Results were considered statistically signif-
ant when p < 0.05.

3. Results

3.1. Patient Assessment. We evaluated 126 biopsy samples of
the gastric body of patients with upper digestive tract
complaints. The mean age was 44.90 (±15.5) years, with 84
females age 44.9 (±15.9) years and 42 males age 44.8
(±14.9) years.

H. pylori was present in the gastric antrum of 42 (33.3%) and
in the gastric body of 43 (34.1%) of the 126 patients
studied, as determined by APS.

Among the 126 participants of the clinical questionnaire
(Table 1), no significant difference was found in relation to
the clinical data with the presence of H. pylori in the gastric
antrum diagnosed by APS.

Based on anamnesis alone and in clinical situations such
as epigastric pain, heartburn, smoking, alcoholism, obesity,
and diabetes among others, a previous diagnosis of the
H. pylori infection was not obtained. Epigastric pain was
the most frequent symptom, affecting 105 (83.3%) patients,
followed by heartburn, manifested in 86 (68.3%) patients
(p > 0.05) (Table 1).

On comparing male and female patients harboring
H. pylori in the gastric antrum, a higher prevalence of the
bacterium in females was observed, although not statistically
significant. Of the 84 (66.7%) female patients, 28 (22.2%) were
positive for the presence of bacteria in the gastric

Table 1: Comparison of clinical data with the presence of *H. pylori* studied in the gastric antrum through the PAD (*N* = 126).

| Clinical data                | Number | *H. pylori* + | *H. pylori* - |
|------------------------------|--------|---------------|---------------|
| Male                         | 42 (33.3%) | 14 (11.1%) | 28 (22.2%)   |
| Women                        | 84 (66.7%) | 28 (22.2%) | 56 (44.4%)   |
| Epigastric pain              | 105 (83.3%) | 35 (27.8%) | 70 (55.6%)   |
| Nausea                       | 56 (44.4%) | 21 (16.7%) | 35 (27.8%)   |
| Pirose                       | 86 (68.3%) | 27 (21.4%) | 59 (46.8%)   |
| Dysphagia                    | 30 (23.8%) | 15 (11.9%) | 15 (11.9%)   |
| Regurgitation                | 42 (33.3%) | 17 (13.5%) | 25 (19.9%)   |
| Smoking                      | 22 (17.5%) | 6 (4.8%)   | 16 (12.7%)   |
| Ethnicism                    | 50 (39.7%) | 20 (15.9%) | 30 (23.8%)   |
| Obesity                      | 27 (21.4%) | 13 (10.3%) | 14 (11.1%)   |
| Diabetes                     | 12 (9.5%)  | 5 (4.0%)   | 7 (5.6%)     |
| Previous treatment GERD      | 48 (38.1%) | 16 (12.7%) | 32 (25.4%)   |
| Previous treatment of gastritis | 37 (29.4%) | 8 (6.3%)   | 29 (23.0%)   |
| Previous treatment *H. pylori* | 19 (15.1%) | 3 (2.4%)    | 16 (12.7%)   |

*p* < 0.05.

antrum, while of the 42 (33.3%) males, 14 (11.1%) were positive to the gastric antrum (Table 1).

There existed no statistically significant difference when we compared the patients who had undergone previous *gastroesophageal reflux disease* (GERD) treatment. Of the 126 participants, 48 (38.1%) had been treated for GERD; among them, 16 (12.7%) had the bacteria present in the gastric antrum, while 32 (25.4%) were not positive for *H. pylori*.

For analysis of gene expression, samples were grouped according to the inflammatory process in the control, mean age was 43.50 (±15.7) years (19 females and 11 males); no gastritis, mean age was 44.70 (±15.1) years (21 females and 11 males); active gastritis, mean age was 44.0 (±15.0) years (29 females and 15 males); and chronic inactive gastritis, mean age was 49.0 (±17.3) years (15 females and 5 males), groups.

3.2. Analysis of Relative Gene Expression. The analysis of the gene expression of ARG1, ARG2, and NOS2 was performed in the patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group. It was observed that the ARG1 gene expression was significantly upregulated in the group of patients with chronic active gastritis, as compared to the control group (Kruskal-Wallis; *p* = 0.02) (Figure 1(a)). The other groups showed no significant difference (Kruskal-Wallis; *p* > 0.05) (Figures 1(c) and 1(e)). Additionally, these three genes were analyzed in the patient groups and classified by APS as *H. pylori*-positive or *H. pylori*-negative. No significant difference was observed on comparing the expression of ARG1, ARG2, or NOS2 genes in the presence or absence of *H. pylori* (Mann-Whitney; *p* > 0.05) (Figures 1(b), 1(d), and 1(e)).

Furthermore, the expression of VIP, VIPR1, TAC, and TACR1 was analyzed in patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group; no significant difference was observed among the groups (Kruskal-Wallis; *p* > 0.05) (Figures 2(a) and 2(c)). No significant difference was observed on comparing the expression of VIP, VIPR1, TAC, and TACR1 genes in the presence or absence of *H. pylori* (Mann-Whitney; *p* > 0.05) (Figures 2(b) and 2(d)).

Similarly, while analyzing the expression of the IFN-γ, IL-17, and IL-10 genes and the T-bet and RORC transcription factors, in patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group, no significant difference was observed (Kruskal-Wallis; *p* > 0.05). Nevertheless, the expression of the TGF-β gene and its FOXP3 transcription factor was significantly higher in patients with chronic inactive gastritis, as compared to the control group (Kruskal-Wallis; *p* = 0.02 and *p* = 0.03, respectively) (Figure 3(g)). On comparing the expression of the IFN-γ, IL-17, IL-10, and TGF-β and the T-bet and RORC transcription factors in the presence or absence of *H. pylori*, no significant difference was observed (Mann-Whitney; *p* > 0.05) (Figures 3(b), 3(d), and 3(f)). However, the FOXP3 expression was significantly lower in the *H. pylori*-positive patients, as compared to the *H. pylori*-negative patients (Mann-Whitney; *p* = 0.01) (Figure 3(h)).

On analyzing the gene expression of IL-6, IL-22, IL-23, and IL-33 in patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group, no significant difference was observed (Kruskal-Wallis; *p* > 0.05). However, the IL-33 gene expression was significantly upregulated in the *H. pylori*-positive patients, as compared to the *H. pylori*-negative patients (Mann-Whitney; *p* = 0.03) (Figure 4(f)).

4. Discussion

The present study analyzed the mRNA expression of a set of mediators involved in inflammatory response modulation. The expression of genes encoding cytokines, chemokines, neuropeptides, and enzymes involved in the effector mechanisms and the regulators of inflammation was analyzed. The study evaluated gastric body biopsies obtained during the digestive endoscopy examination, performed in patients with...
Figure 1: (a) Number of relative mRNA copies of ARG1 present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; *p* = 0.02); (b) number of relative mRNA copies of ARG1 present in biopsies of patients with or without *H. pylori* (Mann-Whitney; *p* > 0.05); (c) number of relative mRNA copies of ARG2 present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; *p* > 0.05). (d) Number of relative mRNA copies of ARG2 present in biopsies of patients with or without *H. pylori* (Mann-Whitney; *p* > 0.05); (e) number of relative mRNA copies of iNOS present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; *p* > 0.05); (f) number of relative mRNA copies of iNOS present in biopsies of patients with or without *H. pylori* (Mann-Whitney; *p* > 0.05). The horizontal line represents the median, the bar percentile of 25%–75%, and the vertical line represents the percentile of 10%–90%. * indicates significant *p* value.
dyspepsia. We observed a significant upregulation in the expression of the ARG1 gene in the group of patients with chronic inactive gastritis, as compared to the control group. The ARG1 enzyme, which catalyzes the hydrolysis of arginine to ornithine and urea, is produced in the liver under the stimulation of IL-4, IL-10, and TGF-β, as well as by immune response cells, such as activated neutrophils at infection sites. It can modulate the immune response and functions in the process of tissue regeneration [24]. In our study, association of ARG1 with a type of chronic inactive gastritis suggested that it may be involved in the regulation of inflammation and the repair processes arising in acute inflammatory lesions. In our study, no significant difference was found in the expression of the genes ARG2 and NOS2, which also have L-arginine as the substrate. ARG2 hydrolyzes L-arginine in the same way as ARG1, while NOS2 produces nitric oxide, a potent microbicidal agent associated with the growth inhibition of microorganisms, causing tissue damage in the process. Thus, the association of ARG1 with chronic inflammation may indicate an ongoing repair process [25]. Conversely, some authors associate the presence of arginases, especially ARG2, with the escape mechanisms of H. pylori, by causing a low availability of L-arginine and thus inhibiting the NOS2-dependent microbicidal activity. Moreover, the expression of arginase by H. pylori itself was associated with its carcinogenic activity [26]. We believe that the patients with chronic

Figure 2: (a) Number of relative mRNA copies of VIP and VIPR1 present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; p > 0.05); (b) number of relative mRNA copies of VIP and VIPAC present in biopsies of patients with or without H. pylori (Mann-Whitney; p > 0.05); (c) number of relative mRNA copies of TAC and TACR1 present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; p > 0.05). (d) Number of relative mRNA copies of TAC and TACR1 present in biopsies of patients with or without H. pylori (Mann-Whitney; p > 0.05). The horizontal line represents the median, the bar percentile of 25%–75%, and the vertical line represents the percentile of 10%–90%. * indicates significant p value.
Figure 3: Continued.
in active gastritis established a mechanism of protection against infection, and with an increase in \textit{ARG1}, established a modulation process for regeneration.

The TGF-\(\beta\) and FOXP3 transcription factor expression was significantly higher in the group of chronic inactive gastritis patients, as compared to the control group. In an \textit{H. pylori} infection, when the Th1 profile response was predominant, there was an establishment of the effector mechanisms that reduced the number of bacteria; however, a greater epithelial damage could occur with the subsequent progression of gastritis to gastric atrophy. \textit{H. pylori} has an important interface with TGF-\(\beta\), since it can induce TGF-\(\beta\) production in a VacA-dependent manner [27]; furthermore, it has a potential modulating effect on its signaling, through the interaction between CagA and Smad3 [28]. The results presented here, in which a high mRNA expression of TGF-\(\beta\) is associated with chronic inactive gastritis, suggest that the cytokine is associated with mechanisms of inflammation control and regeneration processes.

Corroborating this hypothesis, we found a low mRNA expression of FOXP3 in \textit{H. pylori}-positive samples. FOXP3 encodes a signaling molecule associated with Treg cells, the process of regulation of the immune response; generally, its expression is less in the presence of the bacterium, suggesting it can repress its production, reducing the pressure of immunoregulation and the establishment of effector mechanisms with the potential ability to control bacterial growth. It is important to consider that these same mechanisms have a potential effect of damaging the mucosa.

We found no significant difference in the gene expression of IFN-\(\gamma\), IL-17, and IL-10 and the T-bet and RORC transcription factors in the groups of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group; the same was noted when we compared to the group of \textit{H. pylori}-positive or \textit{H. pylori} -negative patients. Alternatively, some studies observed an upregulation of IL-17 and downmodulation of Treg cells in peptic ulcer associated with \textit{H. pylori} infection [29, 30]. The differences in the observations between these two studies may be due to the characteristics of the sample pooling process. Moreover, previous studies have showed an association with peptic ulcer, a complication not observed in our study. Hence, we believe that the Treg profile seems to be modulating the inflammatory process of patients with chronic inactive gastritis, protecting them from tissue lesions and worsening of the gastritis.

Furthermore, in the present work, the IL-33 gene expression was significantly upregulated in \textit{H. pylori}-positive patients, as compared to the \textit{H. pylori}-negative patients.

We also analyzed the gene expression of IL-6, IL-22, IL-23, and IL-33 in patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group and found no significant difference. The IL-33 has been
Figure 4: (a) Number of relative mRNA copies of IL-6 present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; \( p > 0.05 \)); (b) number of relative mRNA copies of IL-6 present in biopsies of patients with or without H. pylori (Mann-Whitney; \( p > 0.05 \)); (c) number of relative mRNA copies of IL-22 and IL-23 present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; \( p > 0.05 \)); (d) number of relative mRNA copies of IL-22 and IL-23 present in biopsies of patients with or without H. pylori (Mann-Whitney; \( p > 0.05 \)); (e) number of relative mRNA copies of IL-33 present in biopsies of patients with chronic active gastritis, chronic inactive gastritis, no gastritis, or the control group (Kruskal-Wallis; \( p > 0.05 \)); (f) number of relative mRNA copies of IL-33 present in biopsies of patients with or without H. pylori (Mann-Whitney; \( p = 0.03 \)). The horizontal line represents the median, the bar percentile of 25%–75%, and the vertical line represents the percentile of 10%–90%. * indicates significant \( p \) value.
described as an alarming indicator of tissue damage. It is highly expressed in the gastric mucosa and appears to potently activate Th2 immunity and the regulatory cells of the immune system [31]. Studies suggest that it may play a key role during *H. pylori* infection. Other studies have observed an increase in the expression of IL-33 in biopsies of patients infected with *H. pylori*, as well as in cases of active gastritis [22]. IL-33 production during an infection can modulate Th1- and Th17-exacerbated inflammation, contributing to lesser tissue damage. Its ability to activate Th2 response and regulation also induces differentiation of the M2 macrophages. These participate in tissue repair processes and express arginase, associated with chronic inactive gastritis in this study. The data suggest that IL-33, in addition to acting as an alarming cytokine against *H. pylori* infection, is associated with the repair mechanisms, production of the anti-inflammatory mediators, and the possible differentiation of M2 macrophages, modulating the inflammatory processes and stimulating repair in the injured tissues. Thus, IL-33 may be a crucial mediator of the immune response in infection, after damage to the mucosal epithelial tissues.

5. Conclusion

Thus, ARG1 and Treg profile appear to be modulating the inflammatory process, protecting chronic inactive gastritis patients from tissue lesions. Furthermore, we suggest IL-33 may be a crucial mediator of the immune response in infection, after gastric mucosal damage.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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