

Immune response Th1/Th2 to *Helicobacter pylori* and Helminths in co-infected patients

Respuesta inmune Th1/Th2 en pacientes co-infectados con *Helicobacter pylori* y helmintos

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What do we know about the subject matter of this study?

The inflammation associated with *Helicobacter pylori* infection is related to gastric lesions. Such inflammation is caused by the Th1-type CD4⁺ immune response in the gastric mucosa on the inflammatory cells of the host.

What does this study contribute to what is already known?

Helminth infections cause the polarization of the Th2-type CD4⁺ immune response and can influence the *H. pylori* pro-inflammatory response from a Th1-type CD4⁺ profile to a less harmful Th2-type CD4⁺ response.

Abstract

Introduction: Inflammation associated with *Helicobacter pylori* (*H. pylori*) infection is linked to the development of a gastric precancerous lesion. Helminth infections could influence the pro-inflammatory response to such infection from LTCD4⁺ Th1 to a less harmful LTCD4⁺ Th2 response. **Objective:** To characterize the polarization of the LTCD4⁺ Th2 immune response in co-infected patients with *H. pylori* and helminths from low-risk areas for developing gastric cancer. **Patients and Method:** We analyzed 63 patients infected by *H. pylori* (40 adults and 23 children). Through the Multiplex Analysis technology (xMAP), we determined the serum profiles of the interleukins associated with the polarization of the immune response of LTCD4⁺ Th1 (IL-1 β , INF- γ , TNF- α) as well as the LTCD4⁺ Th2 (IL-4, IL-10, and IL-13). The ratio between helminths co-infection status in *H. pylori*-infected patients and the polarization of the immune response mediated by LTCD4⁺ Th1 and LTCD4⁺ Th2 was assessed using a Mixed Effects Logistic Regression Model. **Results:** The frequency of helminths was similar between adults (15%) and children (17%). The polarization of the immune response was more prevalent in LTCD4⁺ Th1. Serum values of interleukins associated with the immune response polarization of LTCD4⁺ Th1 (IL-1 β , INF- γ , and TNF- α) and LTCD4⁺ Th2 (IL-4, IL-10, and IL-13) were independent of helminths infection status. **Conclusion:** The prevalence of intestinal parasitic infection was high and the immune response polarization was mainly LTCD4 + Th1.

Keywords:

Infection;
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Introduction

Since its discovery in 1983¹, *Helicobacter pylori* (*H. pylori*) has been recognized as an etiological agent of acute and chronic gastritis as well as an important risk factor in the predisposition to diseases such as peptic ulcer, the appearance of MALT (Mucous Associated Lymphoid Tissue) type lymphoma, and is one of the main determinants of gastric cancer², the third cause of cancer mortality in Colombia³.

It is estimated that more than 50% of the world's population is infected with *H. pylori*, however, a few of them develop a more serious gastrointestinal disease⁴. In most cases, the infection is acquired in childhood and persists throughout life, causing chronic inflammation and development of lesions in the gastric mucosa, since the host's immune response is not capable of removing this bacterium^{5,6}.

The immune response to pathogens varies according to the pattern of interleukin secretion. In general, the polarization of that response towards Th1-type CD4⁺ profile is related to intracellular microorganisms including bacteria, protozoa and fungi, production of interleukin-1 beta (IL-1 β), interferon-gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α); while helminths induce polarization of the immune response towards Th2-type CD4⁺ with production of interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-10 (IL-10), and interleukin-13 (IL-13), and induce activation of B-lymphocytes and their differentiation. The degree of polarization may vary depending on the nature of the infection and its persistence in the host^{7,8}.

The inflammation associated with *H. pylori* infection is caused by Th1-type CD4⁺ in the gastric mucosa through the release of IFN- γ by host inflammatory cells^{9,10}.

Since concurrent helminth infections induce polarization of the Th2-type CD4⁺ immune response¹¹ and are endemic in developing countries with high prevalence of *H. pylori* infection, helminth infections may modulate the pro-inflammatory response to *H. pylori* Th1-type CD4⁺ infection to a less harmful Th2-type CD4⁺ response.

The objective of this study is to determine the immune response profile in people with *H. pylori* and helminthiasis coinfection in Tumaco, Nariño, Colombia, where the prevalence of infection is > 80%¹². This new knowledge will allow defining more frequent interleukin profiles in populations with high prevalence of *H. pylori* and at low risk of developing gastric cancer, which may show great differences regionally.

Patients and Method

Type of study and population

A descriptive study was conducted in the Tumaco region including 63 people, 40 adults with a histopathological diagnosis of chronic *H. pylori*-associated gastritis and 23 children in the Growth and Development program at Tumaco health centers who tested positive for *H. pylori* through the [¹³C]-Urea breath test, to compare the Th1- and Th2-type CD4⁺ immune response.

A blood sample (15 ml) for immune profile evaluation and stool samples for intestinal parasites detection were collected from each participant during three different cycles. The cycle is defined as the time at which the antiparasitic therapy was administered and the respective blood and stool samples were collected, each with three months apart. In each cycle, a control coprological examination was performed. Patients with a positive diagnosis of intestinal parasite infection received antiparasitic treatment with Albendazole (two doses of 400 mg each 15 days apart) and Tinidazole (2 g per day for two days) within 15 days after obtaining the coprological results.

The research was approved by the Institutional Committee for the Review of Human Ethics (CIREH) of the *Universidad del Valle*. All adult participants and the child's parents or legal guardians gave informed consent, as did the children's assent.

Histopathology procedures

Three gastric mucosa fragments were obtained from each participant, one from the body mucosa of the anterior middle wall and two from the antral region, one in the lesser curvature adjacent to the angular incisure and the other one in the anterior face 5 cm from the pylorus. Each fragment obtained was fixed, processed, and examined separately, with a 2-hour dehydration protocol, followed by paraffin embedding in the first 24 hours. Histological sections were cut into microtomes (Accu-Cut® SRM) and stained with hematoxylin-eosin (H & E). To determine the presence of *H. pylori*, modified Giemsa staining was used to detect curved and spiral bacilli¹³.

[¹³C]-Urea Breath Test (UBT)

The first sample of exhaled air was collected in a 10-ml Exetainer® vial. A glucose solution containing 100 mg of [¹³C]-Urea (Cambridge Isotope Laboratories, USA) was immediately administered for ingestion. Another breath sample was collected 30 min after the ingestion of the solution. The concentration of stable [¹³C]-Urea radioisotope gas was determined at baseline and 30 min post-ingestion through the Iris®-Doc Infra-Red Isotope analyzer (Wagner Analyzen Tech-

nik, Bremen, Germany)¹⁴. The $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio measurement was compared with a reference gas of known isotope ratio.

Diagnosis of intestinal parasite infection

Two aliquots of each stool sample were emulsified in 10% neutral formalin for direct examination by flotation in zinc sulfate solution¹⁵. A third aliquot was immersed in Sheather's sugar¹⁶, for trichrome staining as a control and comparison gold standard¹⁵. The parasite identification was carried out by morphology following the Guidelines of the American Society of Parasitology. Patients diagnosed with intestinal parasite infection were treated with Albendazole (two doses of 400 mg each 15 days apart) and Tinidazole (2 g per day for two days) within 15 days after obtaining the results.

Characterization of the Th1- and Th2-type CD4⁺ immune response

Serum profiles determination of interleukins associated with Th1-type CD4⁺ (IL-1 β , INF- γ , and TNF- α) and Th2-type CD4⁺ (IL-4, IL-10, and IL-13) immune response polarization was established using xMAP® Technology according to the manufacturer's instructions.

Statistical analysis

The normality assumption for the value distribution of each interleukin was addressed by analyzing the residuals in ANOVA of repeated measurements through the Shapiro-Wilk test. We used the Friedman test to determine if there were significant differences in each interleukin in the study's cycles. A mixed-effects logistic regression model was developed to study the relationship between helminthiasis coinfection status in *H. pylori*-infected patients and the polarization of the Th1-type CD4⁺ and Th2-type CD4⁺-mediated immune response. The response variable was the presence or absence of helminths and the predictor variables, age, sex, and interleukin measurements that characterize the Th1-type CD4⁺ and Th2-type CD4⁺ immune response.

Results

The average age (average \pm standard deviation) in the adult participants was 49.6 \pm 5.0 years and were mostly women (80%). The average age of the children was 11.0 \pm 2.4 years and also most of them were female patients (57%). The adults and children patients were colonized by *H. pylori* according to the results of the histopathology and Urea (UBT) tests.

Intestinal parasite infection

The frequency of intestinal parasite infection was higher in adults than in children (67% and 48% res-

pectively) with a $p = 0.001$ value and helminth infestation occurred regardless of age.

Protozoa were more frequent in adults, $p = 0.058$ (table 1). Trichocephalus (*T. trichiura*) and Ascaris (*A. lumbricoides*) were the most common helminth species in adults and children. In children, there were no *Enterobius*, *Strongyloides*, and *Ancylostoma*; and in adults, no *Giardia* (Figure 1).

Adult and child cytokine profile

Adults presented high serum levels of IFN- γ and TNF- α , however, these interleukins related to the Th1-type CD4⁺ response profile showed no variation in the different cycles. In contrast, serum values of IL-1 β , IFN- γ , and TNF- α in children were significantly higher in cycle III (Table 2). When studying the interleukins related to the Th2-type CD4⁺ response profile, an important increase in the serum levels of IL-4-, IL-10, and IL-13 was observed in children through the different cycles. In adults, the behavior was similar only in IL-4 values. The other interleukins (IL-10 and IL-13) showed a significant decrease in serum concentrations in cycle III, compared with the values of the cycle I (Figure 2).

Th1- and Th2-type CD4⁺ immune response in Helicobacter pylori and helminthiasis coinfecting

In children and adults, IFN- γ and TNF- α interleukins have the highest serum values, which suggests a predominance of the Th1-type CD4⁺ immune response profile, however, serum determinations of the different interleukins related to the Th1- Th2-type CD4⁺ immune response do not depend on the intestinal helminth infestation status (table 2).

Mixed-effects logistic regression model

All variables were included (cycles, IL-1 β , IFN- γ , TNF- α , IL-4, IL-10, and IL-13) adding the randomized intercept to assess the influence of each patient throughout the study. The model was adjusted individually

Table 1. Prevalence of protozoa and helminths

Parasites	Adults	Pediatric	p-value
	n (%)	n (%)	
Without parasite infection	13 (32)	12 (52)	0.001
With parasite infection	27 (67)	11 (48)	
Sole Protozoa	21 (52)	7 (30)	
Sole Helminthes	3 (7)	1 (4)	
Co-infection	3 (7)	3 (13)	0.058
All protozoa	24 (60)	10 (43)	
All helminthes	6 (15)	4 (17)	

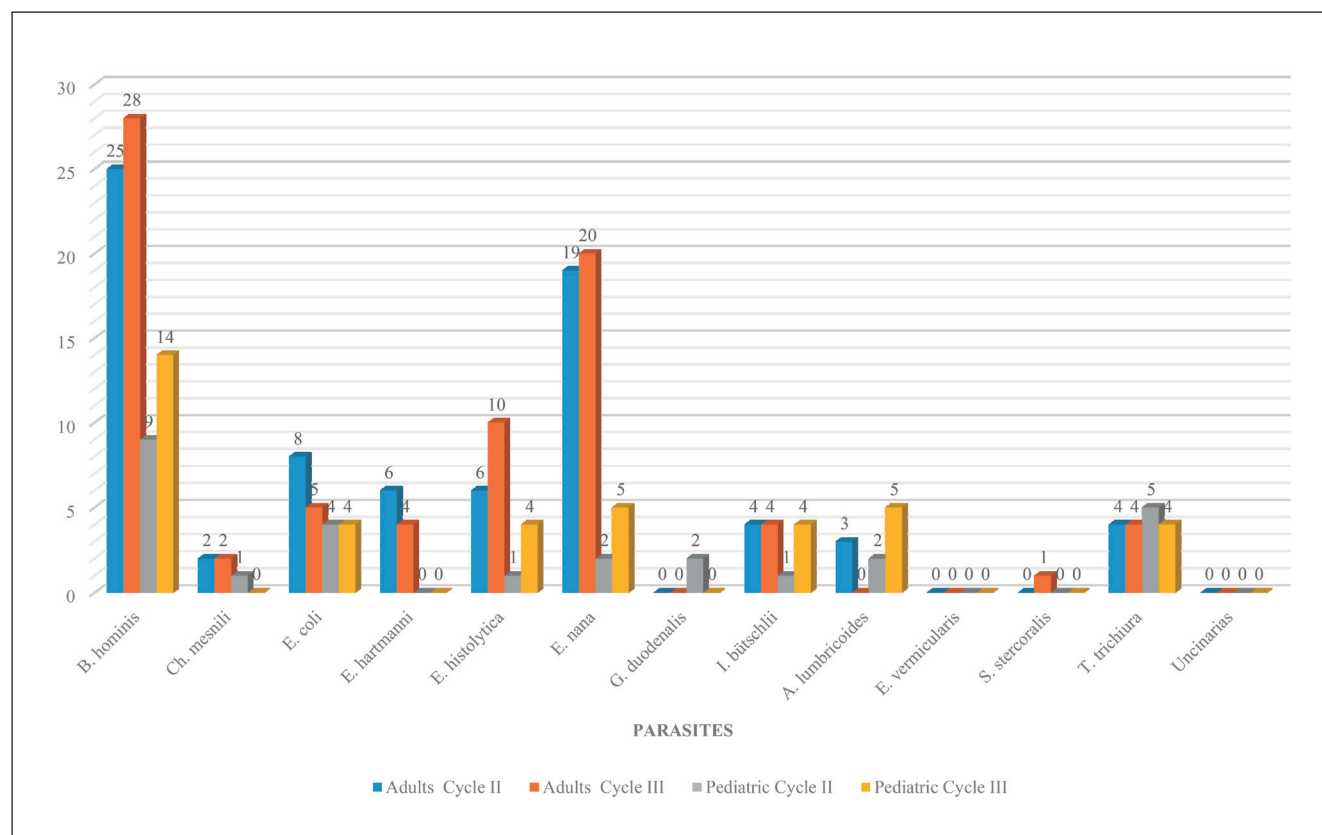


Figure 1. Parasite species detected in stool samples according to the study cycle.

Table 2. Descriptive Analysis for helminths per cycle

Immune Response LTCD4 ⁺	Adults			Pediatric	
	Cycle	Positive	Negative	Positive	Negative
		n (%)	n (%)	n (%)	n (%)
Th1 – Cytokines					
IL-1β	II	1.41 (1.4)	1.8 (2.2)	3.4 (2.7)	3.6 (2.6)
	III	1.4 (0.3)	1.6 (1.8)	4.2 (1.5)	3.5 (1.4)
IFN-γ	II	46.9 (26.7)	73.9 (84.6)	97.1 (109.5)	123.1 (64.1)
	III	73.2 (38.6)	78.3 (55.8)	94.0 (37.5)	102.0 (44.7)
TNF-α	II	16.6 (10.1)	32.5 (42.7)	70.7 (99.2)	62.3 (29.8)
	III	21.2 (11.0)	29.6 (28.7)	41.7 (16.3)	49.4 (20.0)
Th2 – Cytokines					
IL-4	II	2.2 (1.3)	2.4 (1.3)	2.5 (2.3)	3.1 (1.3)
	III	2.6 (0.8)	3.0 (1.3)	3.9 (1.8)	3.2 (1.5)
IL-10	II	6.2 (5.4)	4.4 (5.9)	3.2 (5.2)	6.3 (8.8)
	III	4.3 (5.2)	4.5 (6.5)	6.3 (5.1)	7.2 (11.7)
IL-13	II	5.2 (2.5)	5.4 (5.7)	6.2 (2.9)	5.8 (1.7)
	III	4.8 (3.0)	4.0 (1.8)	7.1 (1.8)	7.0 (3.0)

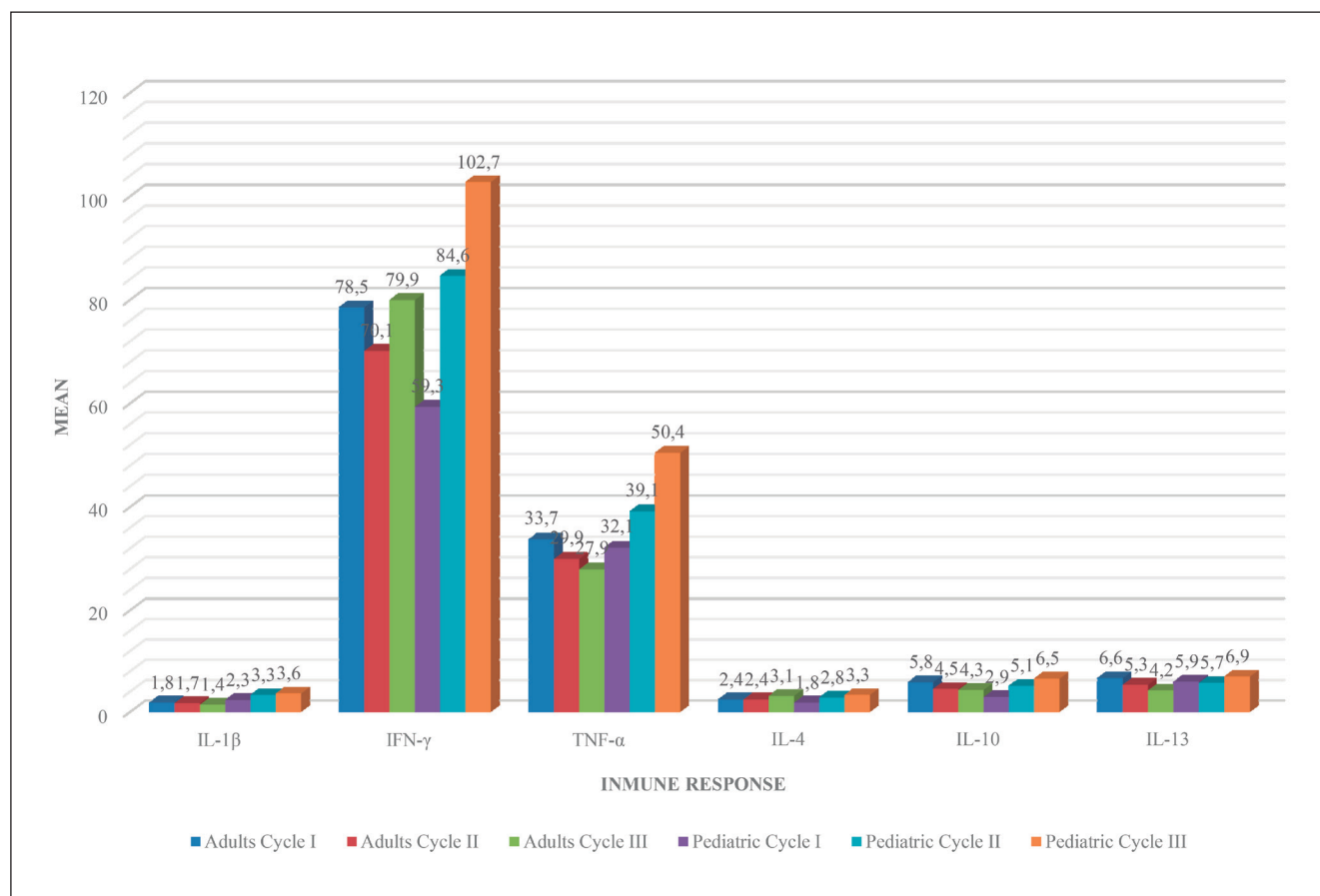


Figure 2. LT CD4⁺ Th1 cytokine profile (pg/ml) in adults and children, by cycle.

Table 3. Ratio of the interleukins profile associated with polarization of immune response type LTCD4⁺ Th1/Th2 and the state of co-infection by *Helicobacter pylori* and helminths

Parameters	Adults			Children		
	Estimation	IC95%	p- value	Estimation	IC95%	p- value
Intercept	-0.142	(-2.704; 2.419)	0.913	-1.721	(-4.4758; 1.0320)	0.220
Cycles	-0.178	(-1.534; 1.178)	0.797	0.316	(-1.1980; 1.8307)	0.682
IL-1 β .	0.250	(-0.368; 0.869)	0.428	-0.096	(-0.7893; 0.5956)	0.784
IFN- γ	0.0156	(-0.153; 0.024)	0.157	0.062	(-0.0410; 0.1656)	0.237
TNF- α	-0.064	(-0.015; 0.047)	0.329	-0.012	(-0.1169; 0.0913)	0.809
IL-4	-0.718	(-1.803; 0.367)	0.195	-0.116	(-2.5107; 2.2783)	0.924
IL-10	0.078	(-0.035; 0.192)	0.176	-0.080	(-0.2809; 0.1191)	0.428
IL-13	0.02762	(-0.133; 0.188)	0.737	-0.043	(-0.4178; 0.3305)	0.819
$\sigma^2_{u_{i0}}$	0.06783			0		
Deviance	61.5			49.4		

Reference Category: Patients infected by *Helicobacter pylori* without helminthiasis.

in both adults and children. There was no relationship between *H. pylori* and helminthiasis coinfection status and Th1- and Th2-type CD4⁺ immune response profiles (table 3).

Discussion

During childhood, *H. pylori* infection is acquired and its prevalence increases dramatically during the

first four years of life^{11,17}. Some studies suggest that during the first years of life, it is at home that the infection is contracted, although there are still large gaps in knowledge regarding the predictors of initial acquisition^{18,19}.

This study was conducted with participants with positive infection by *H. pylori*, both adults and child population, from Tumaco, Colombia, a place located at sea level, where sanitary and environmental conditions (heat and humidity) favor the life cycle and transmission of geo-parasites such as helminths²⁰. This study did not evaluate the presence of common comorbidities in tropical areas, such as malaria or leishmaniasis, as other authors have done²¹.

The protozoa (*Blastocystis hominis* and *Endolimax nana*) were the most prevalent intestinal parasite infection in the adult and child population, both considered non-pathogenic^{17,20} and the most frequent helminths in the pediatric population were *Ascaris lumbricoides* and *Trichuris trichiura*.

An outstanding point was the techniques used to collect, process, store, and transport the coprological samples since, despite the distance between the collection site (Tumaco) and the processing one (Cali), cystic forms, trophozoites, and adult parasitic forms were identified which was also favored by the use of analysis techniques (direct, trichrome, and Sheather's sugar).

In searching for models of the host immune response to *H. pylori* infection, it has been suggested that the polarization of the immune response towards a Th1-type CD4⁺ profile contributes to gastric mucosal injury^{2,5}. However, other authors find no association between *H. pylori* infection and circulating pro-inflammatory interleukins, which has been specifically identified in children and associated with presumed *H. pylori* inactivity during childhood when there is no ulcerated tissue²¹.

In our study, we found that adult and pediatric participants infected with *H. pylori*, explain the polarization of the immune response towards Th1-type CD4⁺ in both populations. In adults, there was a higher concentration of IFN- γ and TNF- α in all cycles, but no statistically significant difference. This may be related to the chronic nature of *H. pylori* infection, triggering an immune response that mainly produces an inflammatory reaction⁶. A similar finding was observed in the pediatric population, where higher concentrations of pro-inflammatory interleukins were associated with the polarization of the immune response towards a Th1-type CD4⁺ profile, with higher IFN- γ and TNF- α levels, in contrast to what has been published²¹. We must be careful in interpreting the results because these immune response models (Th1- and Th2-type CD4⁺) are theoretical in humans and most of the evidence for these models come from murine ones.

Helminths are the best example of a stimulus to induce a reaction in Th2-type CD4⁺ cells in humans and experimental models, as eosinophilia, mastocytosis, and high IgE production have been observed in affected tissues^{22,23}.

On the one hand, in our case, we found in adult patients high concentrations of IL-10 and IL-13 at the beginning of the study, but there was a slight increase of IL-4 at the end when they had already received two doses of antiparasitic medication. This may reflect the easy reinfection of the population living in risk areas²². On the other hand, we found in the pediatric population higher levels of IL-13 at the beginning of the study; however, in the final stage, the most pro-inflammatory interleukins were higher than the anti-inflammatory ones, therefore, we found that there was a partial relationship between co-infection status (*H. pylori*-helminths) and the polarization of the immune response towards a Th2-type CD4⁺ profile, especially in children.

In other investigations, in adult and pediatric patients with gastritis or ulcer, the expression of the Th1 response against Th2 has been compared with normal patients, showing that patients infected with *H. pylori* have higher concentrations of Th1 against Th2, however, in non-infected patients, a higher expression of Th2 against Th1 was observed²⁴, suggesting that the immune response of children and adults against an *H. pylori* infection shows mainly Th1 concentrations.

This increase in Th1 is directly proportional to the degree of gastritis and, therefore, to the inflammation of the gastric mucosa. This difference in secretion against patients with positive *H. pylori* infection compared with the negative ones may indicate a key role for this cytokine against gastritis caused by *H. pylori*²⁴.

This study contrasts with previous publications on the modulation of host immune response in *H. pylori* and helminthiasis coinfection. Although there are reports in the literature that in murine models the polarization of the immune response is evident for a Th2-type CD4⁺ profile²⁵ and the immune basis confirms a cellular response mediated by Th2-type CD4⁺ in the presence of intestinal parasites associated with high levels of IgE and IgG1²⁶ also evident in certain populations, our study found no such association or at least it was only partially so during the bivariate analysis as explained above.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human

experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

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