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Andes pediatr. 2022;93(3):400-409
DOI: 10.32641/andespediatr.v93i3.3667

ORIGINAL ARTICLE

Immunotyping of lymphocytes in the gastric mucosa of patients infected by *H. pylori* in two regions with contrast in the risk of developing gastric cancer

Inmunotipificación de linfocitos en mucosa gástrica de pacientes infectados por *H. pylori* en dos regiones geográficas con contraste en el riesgo de desarrollar cáncer gástrico

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Received: February 9, 2021; Approved: January 2, 2022

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

Due to the lack of specificity of the symptoms, it is not possible to evaluate the characterization of the initial alterations caused by H. pylori, which makes the endoscopy an unlikely option to evaluate the mucosa in recently infected individuals.

What does this study contribute to what is already known?

The comparison between the inflammatory response in populations with differences in gastric cancer risks presents a pattern characterized by the bacterial load and the significant damage to the mucosa caused by this bacterium.

Abstract

Helicobacter pylori (*H. pylori*) infection involves multiple factors internal and external to the host. Among the internal factors, the immune response plays a fundamental role in the process of antigen presentation, lymphocytic response and cytokine-mediated regulatory response that are directly associated with disease progression and prognosis. **Objective:** To compare the immune response in gastric mucosa of *H. pylori* infected patients in two regions comparing the risk of developing gastric cancer. **Patients and Method:** 71 participants with symptoms of dyspepsia were included. The samples for biopsies were collected from different regions of the gastric mucosa; the identification of *H. pylori* was carried out by culture and polymerase chain reaction (PCR) of the ureA gene. For the characterization of the histopathological alterations and the immunophenotyping of lymphocytes, anti-human mouse monoclonal antibodies specific for each antigen were used: T lymphocytes: CD3 and CD8; B lymphocytes: CD20; Natural Killer Cells: CD56; Macrophages: CD68. **Results:** The prevalence of *H. pylori* was 83.1%, the predominant types of gastritis were chronic gastritis and multifocal atrophic

Keywords:
Helicobacter pylori;
Children;
Cancer;
Gastric Cancer;
Anatomy;
Histology.

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Edited by:
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How to cite this article: Andes pediatr. 2022;93(3):400-409. DOI: 10.32641/andespediatr.v93i3.3667

gastritis with intestinal metaplasia (63.4% and 22.5%, respectively). The cellular response was characterized mainly by polymorphonuclear lymphocytes and positive anti-CD8 reactivity both in stroma and epithelium. **Conclusions:** Multifocal atrophic gastritis was more prevalent in the high-risk region for gastric cancer (GC) while non-atrophic gastritis and the expression of CD3 and CD8 antigens in the foveolar epithelium was higher in the low-risk region.

Introduction

Helicobacter pylori (*H. pylori*) infection increases the risk for gastric adenocarcinoma. However, the development of the neoplasm is the result of a prolonged, multifactorial process preceded by precursor and presumably sequential histopathologic lesions with cellular alterations associated with gastric microenvironment abnormalities[1,2].

H. pylori is an organism with large genomic diversity and high capacity to simultaneously colonize the same tissue with multiple strains[3]. Although no mutagenic or carcinogenic strain has been identified, immunological and molecular studies divide *H. pylori* into two groups based on the presence of the *cagA* antigen that is usually co-expressed by the vacuolating cytotoxin with greater capacity to damage the gastroduodenal mucosa and even increase the risk for gastric adenocarcinoma[4,5].

In Colombia, some areas are at extremely high risk for the development of intestinal-type gastric adenocarcinoma, with prevalence rates of up to 150/100,000 inhabitants[6]. In populations such as Pasto, which is at high risk for gastric cancer, *H. pylori* infection presents hyperendemic characteristics, with prevalence rates of 52% at 2 years of age, increasing to 80% at 9 years of age, and in adults, a prevalence rate of 93% has been reported[7]. However, in populations such as Tumaco, which is at low risk for cancer, the prevalence of infection is very high but the incidence and mortality rates for gastric cancer are very low. This phenomenon was first observed in Africa and was called the "African enigma"[8]. In Colombia, the Atlantic and Pacific coasts have presented very low rates of gastric cancer despite high rates of *H. pylori* infection, which could be called "the coastal enigma"[9].

In some populations, the causes that result in a carcinogenic effect are unknown, but not in others. It is known that the type of chronic gastritis caused by the infection varies considerably; patients with duodenal ulcer caused by *H. pylori* present gastritis predominantly in the gastric antrum, without loss of glands (atrophy), called non-atrophic gastritis or diffuse antral gastritis, without high rates of gastric cancer[10]. In other patients, gastritis is associated with multifocal atrophy and intestinal metaplasia, and they often present high gastric ulceration and are at higher risk for gastric cancer[11].

Although the influence of genetic factors is known[12], epidemiological evidence in humans does not support a genetic basis as the primary determinant of gastric cancer. On the contrary, environmental factors appear to have a predominant influence and it is now postulated that modulation of the bacterial-induced inflammatory response may be a major factor in the outcome of infection that could lead to the development of disease and cancer[13]. The objective of this research was to compare the immune response in gastric mucosa of *H. pylori*-infected patients in two geographic regions with different risks of developing gastric cancer.

Patients and Method

Design

Descriptive study with 35 and 36 patients from the Municipalities of Pasto and Tumaco, respectively. Patients with symptoms of dyspepsia, with indication for endoscopic evaluation by esophagogastroduodenoscopy as part of their diagnostic procedures, were selected. Seven biopsies were collected from each patient as part of the routine examination (one from the greater curvature at 5 cm from the pylorus, four from the lesser curvature immediately below the angular incisure, and two from the middle part of the gastric body) for the identification of *H. pylori* by microbiological and molecular methods, and for histochemical evaluation and immunophenotyping of lymphocytes in gastric mucosa.

Ethical Aspects

This research was approved by the Institutional Human Ethics Review Committee (CIREH) of the *Universidad del Valle*. Informed consent was obtained from all adult participants and the parents or guardians of the minor, as well as the assent of the children.

Infection Definition

Molecular identification is considered the Gold Standard test for the identification of *H. pylori*, therefore, molecular, microbiological, and immunohistochemical tests were performed, however, molecular

identification was considered the reference test to establish positivity for *H. pylori*.

H. pylori isolation and culture

Gastric mucosal samples were homogenized in 200 µL of sterile saline solution 0.89%. The homogenized sample was seed on Columbia agar plates (Oxoid, Basingstoke, Hampshire, England) with 7% defibrinated sheep blood plus *H. pylori* selective supplement (Dent) and incubated in microaerophilic conditions (6% O_2, 6% CO_2, 88% N_2) using a BBL CampyPak Plus Envelope (Becton Dickinson, Nashville, TN, United States) at 37°C for 4 to 8 days [14]. The presence of translucent, non-hemolytic, 1-2 mm convex colonies growing in a microaerophilic environment was considered as criteria for the identification of *H. pylori*. Colonies compatible with *H. pylori* were transferred to Columbia agar with 10% defibrinated sheep blood for purification and identification by urease, catalase, oxidase, and Gram stain[15].

DNA extraction from *H. pylori* isolates

From a Petri dish, colonies were transferred to 1.0 ml of PBS 1X, pH 7.2, and centrifuged for 2 min at 13000 rpm. The cell button was re-suspended in 300 µL of extraction buffer [proteinase K 100 µg/ml, sodium dodecyl sulfate (SDS) 0.5%, ethylenediaminetetraacetic acid (EDTA) 5 mM, Tris-HCl 10 mM, pH 8.0, and 276 µL of distilled water]; then, the cell button was homogenized and placed in a dry bath (Labnet®) at 56°C for 18 hr, and subsequently, proteinase K was inactivated at 76°C for 10 min and 5M NaCl was added. The supernatant was vortexed for 15 sec and centrifuged for 5 min at 13000 rpm; later, 2 volumes of absolute ethanol were added and centrifuged for 20 min at 13000 rpm at 4°C, and then it was precipitated by centrifugation at 13000 rpm. The decant was precipitated by adding 2 volumes of absolute ethanol 70%, mixed and centrifuged for 5 min at 13000 rpm, and the supernatant was discarded. The DNA button was dried by inversion for 10 min. Precipitated DNA was re-suspended in 100 µl TE buffer (Tris 10 mMol, EDTA 1 mMol) and stored at -20°C.

Molecular identification of *H. pylori*

The molecular identification was performed by PCR amplification of the ureA gene [16]. The following reagents were added to a 0.2 mL tube: 1X PCR buffer (5X Green Buffer, Promega®), 1 µM MgCl2 solution (Promega®), 0.25 mM dNTPs [(dATP, dCTP, dGTP, dTTP); (Promega®)], 50 pmol/µL of each primer (forward 3'-AAGACATCACTATCAACG-5'/reverse 5'-CCCGCTCGCAATGTCTAA-3'), 0.5 U of GoTaq DNA polymerase (Promega®), and 25 ng of *H. pylori* genomic DNA equal to 25 µL. Amplification was performed at 95°C/2 min, followed by 35 cycles (95°C/1 min, 54°C/1 min, and 72°C/1 min) and a final amplification at 72°C/15 min.

Amplicon electrophoresis

All amplicons obtained in the PCR reactions were run on 2% agarose gel (SeaKem, FMC Biolabs) and stained with an ethidium bromide solution (Invitrogen, Carlsbad, CA, United States) at 0.5 µg/mL, in an electrophoresis chamber (Fotodyne Inc., Hartland, WI, United States). This process was performed by an EC-105 compact power supply (Thermo Fisher Scientific Inc., Asheville, NC, United States) at 75V for 40 min.

Histopathology

Each biopsy collected for histological evaluation was fixed, processed, and examined separately, with a dehydration protocol of 2hr, followed by kerosene embedding in the first 24hr. During the embedding process, the biopsies were carefully positioned so that the mucosal plane was perpendicular to the cut surface, ensuring a proper evaluation of the entire mucosal thickness. Histological cuts of 5µm thickness were made with a microtome (Accu-Cut ® SRM) and then three mucosal sections were placed in a slide previously treated with acid alcohol and heat-fixed at 56°C overnight. The following evaluations were performed: gastritis severity, mucus depletion, infiltration by polymorphous nuclear neutrophils, presence of mononuclear cells and intraepithelial lymphocytes, epithelial damage, and abundance of *H. pylori*.

Histochemical evaluation of gastric mucosa

To determine the presence of *H. pylori*, the modified Giemsa stain was used to look for curved and spiral bacilli, and the negative cases were stained with the modified Steiner stain. To evaluate the integrity and amount of mucus of the superficial epithelium, Alcian Blue/PAS staining was used at pH 2.57. Each biopsy was evaluated separately, and the histopathological alterations found in the gastric mucosa were graded according to the visual analog scale described by Dixon[17-19].

Lymphocyte immunophenotyping in gastric mucosa

Leukocyte antigens present in gastric mucosa-associated lymphoid tissue were identified and characterized using mouse anti-human monoclonal antibodies specific for each antigen. The markers evaluated included T-cell markers CD3 (M7193, Dako®, 1:400 dilution) and CD8 (M7103, Dako®, 1:400 dilution) to characterize the T-cell infiltrate and CD20 (M0755, Dako®, 1:200 dilution) to characterize B-cells. CD56 (M7074, Dako®, 1:400 dilution) was used to identify

Natural Killer cells, CD68 (M0814, Dako®, 1:200 dilution) to identify macrophages, and M0748 antibody (Dako®, 1:2000 dilution) to mark human myeloperoxidase.

Immunohistochemistry was performed by indirect immunoperoxidase assay at room temperature (24°C) in three steps. Endogenous peroxidase blocking was performed by incubating in 3% hydrogen peroxide for 5 minutes. The histological sections were then incubated for 1 hour with monoclonal antibodies for human leukocyte antigens (CD3, CD8, CD20, CD56, and Myeloperoxidase). Subsequently, gastric mucosal samples were re-incubated with biotinylated goat anti-mouse secondary antibody (Dako, Carpinteria, CA) for 30 minutes at 1:200 dilution, followed by the addition of the avidin-biotin-peroxidase complex (Dako, Carpinteria, CA) for 30 minutes. Diaminobenzidine hydrochloride (DAB) (Sigma, Saint Louis, MO) was used to detect the color of the end-product. Sections were counterstained with Harris hematoxylin according to conventional methodology.

Quantification of the lymphocyte response in gastric mucosa

Four gastric mucosa biopsy specimens were collected from each subject to be studied (2 from the antrum and 2 from the body) and, from each fragment, three histological sections were placed on each slide for the quantification evaluation of lymphocytes in 12 histological sections. In each of the sections, the number and type of lymphocytes (T, B, Natural Killer, or Macrophage) found in the lamina propria, lymphoid follicles, and intraepithelial region were quantified according to the following: for intraepithelial lymphocytes, the number, and type of lymphocytes found in an area containing 100 contiguous epithelial cells were counted; for lamina propria, the number of T and B lymphocytes found in 11 areas, each one of 0.0025mm^2; and for lymphoid follicles, the number of T and B lymphocytes found in 8 areas were counted. The evaluation was performed with an Olympus CH-2 microscope at 400X magnification. To delimit and quantify the areas, a calibrated eyepiece graticule 5x5mm (12-578 Fisher®) and a stage micrometer calibration slide were used.

Data analysis

This work was planned to describe the histopathological characteristics and type of leukocyte antigen expressed in the lymphoid tissue associated with the gastric mucosa. Mean, range, and proportion were used for descriptive data of the variables studied in the groups. Means were used for normal distributions and medians for nonparametric variables. Group comparisons were made using the chi-square test with statistical methods based on procedures described by Snedecor and Cochan[20]. The null hypothesis was rejected at $p < 0.05$ and was considered borderline at $p = 0.05$-0.10.

Results

Characterization of histopathologic alterations according to the city of origin

Regarding the absence of prevalence of gastric mucosal colonization by *H. pylori*, this was observed in 11.4% and 16.9% of the samples from Pasto and Tumaco, respectively.

Multifocal atrophic gastritis, considered a precursor lesion of gastric carcinoma, was significantly more frequent in Pasto compared with Tumaco ($p = 0.55$) (Table 1). There were no differences in the prevalence of *H. pylori* infection or other parameters evaluated according to the place of origin. Figure 1 shows the mixed inflammatory response of lymphocytes, plasmacytes, and polymorphonuclear cells (PMNs) to hematoxylin-eosin staining.

Characterization of leukocyte antigen expression according to the presence of *H. pylori*

Myeloperoxidase and CD8 antigen expression on lymphocytes was significantly higher in the lamina propria of patients in whom *H. pylori* was not identified during histopathological evaluation ($p = 0.07$ and $p = 0.01$, respectively). The positivity of the remaining markers was similar for leukocytes located in lymphoid follicles, mucous neck cells, and foveolar cells (Table 2). Figure 2 shows the presence of CD20+ lymphocytes and CD56+ Natural Killer cells in the glandular epithelium.

Characterization of leukocyte antigen expression according to the city of origin

The expression of CD3 and CD8 antigens in leukocytes located in the foveolar epithelium was higher in Tumaco than in Pasto ($p = 0.03$ and $p = 0.06$, respectively). When evaluating the lamina propria, it was found that in Pasto, NK lymphocytes ($p = 0.06$) and CD3 lymphocytes were higher. In Tumaco, the most frequent expression of antigens was myeloperoxidase and B lymphocytes, but only macrophages showed a significant difference ($p = 0.007$) (Table 2).

Finally, Figure 3 shows the presence of CD8+ lymphocytes in the glandular epithelium of the antral gastric mucosa and CD3+ lymphocytes in the lymphoid follicles.

Discussion

The type of chronic gastritis caused by *H. pylori*

infection varies considerably; in our study, we reported two geographic regions with a high prevalence of *H. pylori* infection that show differences in the type of gastritis. In Tumaco, non-atrophic gastritis was more frequent, similar to that described in patients with duodenal ulcer caused by *H. pylori* who have predominant gastritis in the gastric antrum without atrophy, called non-atrophic gastritis or diffuse antral gastritis, and other investigations have demonstrated that these patients do not present high rates of gastric cancer [10]. In Pasto, the predominant gastritis was associated with multifocal atrophy and intestinal metaplasia, conditions where gastric ulceration with a high risk of gastric cancer may be frequent[21].

In Colombia, there is great variability in the prevalence of gastric cancer and precursor lesions in different regions, such as the Andean region, which, compared with the Atlantic Coast, has a high frequency of gastric cancer and multifocal atrophic gastritis. Cities located in the mountain regions of Colombia have a significantly higher prevalence of multifocal atrophic gastritis compared with the Atlantic coast (25.9% vs. 9.5%). Similarly, in the Andean region, the prevalence of gastric cancer is higher than in the Atlantic coast (11.1% vs. 4.7%)[9].

It is notable that, when comparing the two regions, their results are similar. The coastal regions of our country, the Atlantic and Pacific coasts, have different types of gastritis compared with the Andean zone. In a delimited geographical region such as Nariño, the risk of gastric cancer and its precursor lesions is generalized and seems to be limited to the Andean mountain region. In Tumaco, as in the Atlantic Coast, the risk of gastric cancer and precursor lesions is lower, with a type of predominantly non-atrophic gastritis similar to that described in North America and European countries. Although in

Table 1. Relative frequencies (N%) of histopathological characteristics of gastric mucosa.

Characteristics	City	Absent	Slight	Moderate	Severe	p Value
Polymorphonuclear neutrophils in lamina propria	Pasto	2.9	8.6	25.7	62.9	0.176
	Tumaco	19.4	8.3	22.2	50.0	
Lymphocytes in lamina propria	Pasto		2.9	68.6	28.6	0.319
	Tumaco	5.6	8.3	66.7	19.4	
Lymphocytes in faveolar epithelium	Pasto	82.9	14.3	2.9		0.366
	Tumaco	69.4	16.7	11.1	2.8	
Mucus depletion	Pasto	5.7	22.9	11.4	60.0	0.055
	Tumaco	22.2	5.6	16.7	55.6	
Chronic multifocal atrophic gastritis	Pasto	45.7	37.1	8.6	8.6	0.009
	Tumaco	80.6	11.1		8.3	
Intestinal metaplasia	Pasto	68.6	14.3	5.7	11.4	0.150
	Tumaco	86.1	11.1	2.8		
H. pylori	Pasto	11.4	22.9	31.4	34.3	0.154
	Tumaco	16.9	14.1	31.0	38.0	

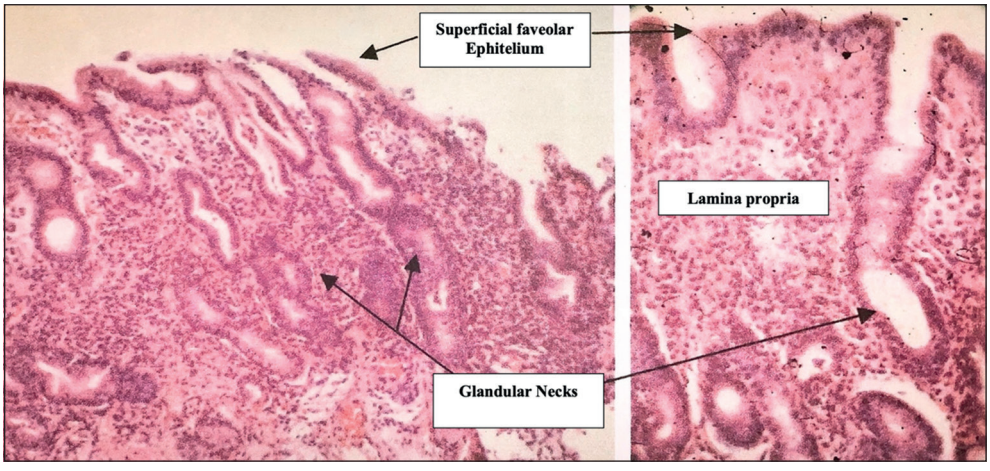

Figure 1. Hematoxylin-Eosin staining of the antral gastric mucosa, lamina propria and ganglion epithelium where the inflammatory response of mixed type with lymphocytes, plasmocytes and polymorphonuclear is evident.

Table 2. Distribution of immunotyping of leukocytes in the gastric mucosa according to the presence of *H. pylori* and geographical location

	Presence *H. pylori*							Geographical location						
Anatomical site	*H. pylori*	B Lympho-cytes	T Lympho-cytes	Natural killer	Ma-crophages	CD8 Lym-phocytes	Myelope-roxidase	City	B Lympho-cytes	T Lympho-cytes	Natural killer	Ma-crophages	CD8 Lym-phocytes	Myelope-roxidase
Faveolar epithelium	Positive	0.0	14.6	1.84	0.75	15.6	2.6	Pasto	0.0	13.4	2.5	0.7	14.7	3.1
	Negative	0.0	22.3	0.75	1.0	26.1	3.0	Tumaco	0.0	18.3	0.9	0.9	19.9	2.4
	p Value	1.0	0.12	0.33	0.67	0.23	0.69	Valor p.	1.0	0.03	0.27	0.29	0.06	0.89
Glandular necks	Positive	0.10	20.72	6.22	0.68	18.3	4.53	Pasto	0.02	20.9	5.1	0.7	16.6	3.6
	Negative	0.0	19.50	5.08	0.50	16.2	3.91	Tumaco	0.13	20.2	6.9	0.6	19.2	5.2
	p Value	0.42	0.67	0.40	0.91	0.19	0.59	Valor p.	0.55	0.28	0.29*	0.96	0.46	0.35
Lamina propria	Positive	75.08	824.0	112.00	361.41	410.6	236.0	Pasto	106.0	933.5	273.7	299.2	514.5	325.0
	Negative	117.44	938.19	228.32	343.96	525.5	381.5	Tumaco	114.5	904.7	145.4	393.3	497.8	386.4
	p Value	0.18	0.24	0.012	0.71	0.01	0.07	Valor p.	0.72*	0.69*	0.06	0.007*	0.64*	0.31*
Lymphoid follicles	Positive	2700.9	2709.3	871.2	811.0	1261.4	846.1	Pasto	2909.3	2742.5	911.1	1103.1	1178.7	641.8
	Negative	3326.5	2814.9	918.5	990.5	1226.4	652.6	Tumaco	3523.6	2850.1	909.9	821.1	1284.5	727.0
	p Value	0.73	0.42	0.83	0.84	0.58	0.58	Valor p.	0.60	0.72	0.64	0.87	0.28	0.43

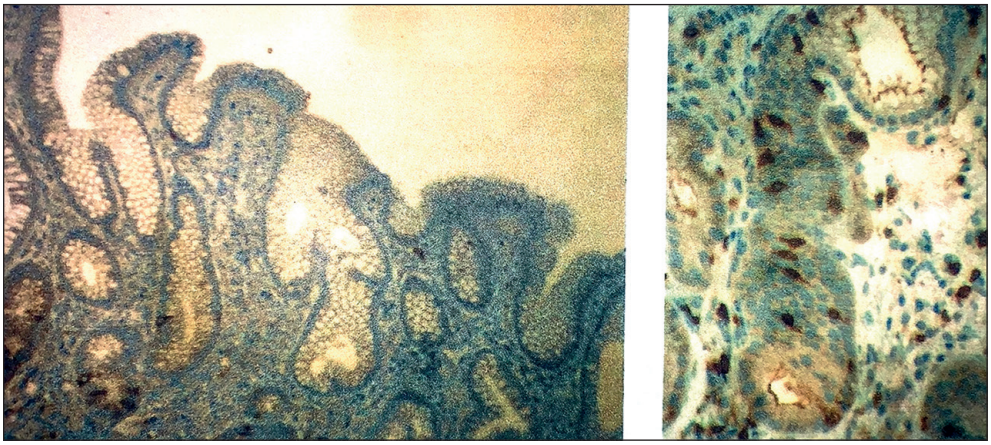

Figure 2. Immunohistochemical staining with targeted monoclonal antibodies to identify CD20+ B lymphocytes and monoclonal antibodies for Natural Killer CD56+ cell identification.

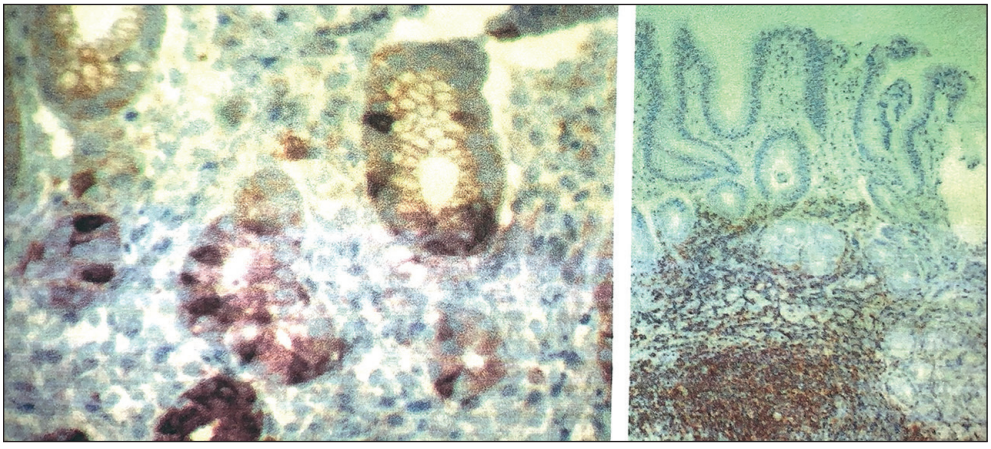

Figure 3. Immunohistochemical staining with monoclonal antibodies to identify CD3+ and CD8+ leukocytes.

the United States the Afro-descendant ethnic group in Louisiana is at higher risk of gastric cancer than the rest of North America[22], we see that ethnicity does not make a difference either, because the white population of Europeans and North Americans shares the low risk of the Afro-descendant population of Colombia.

The presence of *H. pylori* also does not explain these differences in the prevalence of precursor lesions between the mountain and coastal regions of Nariño. In order to answer this question, the distribution of *H. pylori* genotypes associated with virulence was evaluated in a prospective design in these two populations which showed differences in the risk of gastric cancer, but a similar prevalence of *H. pylori* infection [11]. It was found that in patients from Pasto at high risk for the development of gastric cancer, *H. pylori* genotypes associated with virulent *cagA*-positive and *vacA* s1 and m1-positive strains were significantly more frequent than in Tumaco.

The above findings suggest that *H. pylori* genotypes associated with virulent phenotypes may partially explain the differences; other factors such as human genetic polymorphism and diet are suspect and may play a key role in the complex process of gastric carcinogenesis and should be studied in future research.

Unlike the small intestine and colon, in the normal gastric mucosa, there is no lymphoid tissue associated with the mucosa[23], however, when present, it is usually related to the different types of gastritis. Leukocytes could play a fundamental role in the inflammatory process. There are few studies aimed at characterizing the associated leukocyte subpopulations and the inflammatory response in gastric mucosa but, in general, they show controversial results. These differences may be explained by the difficulty in standardizing measurement methods[23].

In the inflamed mucosa of the small intestine and colon, most of the intraepithelial cells express a phenotype of probably cytotoxic T lymphocytes, whereas, in the lamina propria, the helper T lymphocyte phenotype predominates. In line with these findings, the results of this study show in the stomach a lymphocyte distribution similar to that described in the intestine in cases of gastritis associated with *H. pylori* infection[24].

In both gastric body and gastric antrum biopsies, intraepithelial cells expressed almost exclusively the probably cytotoxic T phenotype. In contrast, in the extraepithelial spaces, there was a greater presence of B lymphocytes and the helper T phenotype. These results differ from other studies which describe a predominance of CD4+ lymphocytes and agree with quantifications that show a predominance of CD8+ lymphocyte subpopulation group and that patients with *H. pylori*-associated gastritis present an increased number of NK cells, coinciding with increased levels of interleukins IL-4 and IL-6[24].

In murine models infected with *H. felis*[25], it is observed that the associated gastritis is characterized by the presence of CD4+ and CD8+ lymphocytes and, during the experiments of urease immunization, it was observed that the increase of CD8+ cells may be related to the presence of residual bacteria on the apical surface or between epithelial cells. This observation is relevant since, in our work, we found that in some cases in the absence of *H. pylori*, the inflammatory response in the gastric mucosa was associated with CD8+ and CD56+ lymphocytes. This finding could also indicate that residual bacteria not detected with the histochemical stain used to evaluate *H. pylori* were present or that the immune response was efficient and successfully controlled the colonization of the bacteria and that resolution of the inflammation may take time.

The characterization of the initial alterations caused by *H. pylori* infection is impossible to evaluate in humans due to the lack of specificity of the symptoms associated with acute symptoms and because probably most of the acquisitions of the infection may be asymptomatic, making unlikely the indication of performing an endoscopy that would eventually help us to evaluate the gastric mucosa in individuals who recently were infected. In this work, we found in most of the gastric mucosal specimens an inflammatory response with a predominance of mononuclear cells. In some of them, a predominance of polymorphonuclear neutrophils was evident, but with the presence of lymphocytes, plasmacytes, and macrophages.

The clinical picture of chronicity was already established and the presence of PMNs was the process manifestation of acute activity probably associated with the continuous contact with the noxa that is damaging. In animal models of gastritis induced by *H. pylori* and *H. felis*, it is described that neutrophils predominate in the initial stages of infection and later mononuclear leukocytes are the predominant cells. In humans, it is very difficult to study early gastritis; in general, the processes already installed are evaluated and coincide with what has been observed in murine models[26].

In this work, we found significantly higher NK cell activity (CD56+) in the foveolar epithelium of patients with chronic atrophic gastritis and in the lamina propria of the gastric mucosa of patients from Pasto. These results are important because of the possibility that NK cells participate in the etiopathogenesis of multifocal atrophic gastritis through an autoimmune mechanism. NK cells are functionally relevant in the initial defense against many infectious agents[2]; their activation and associated secretion of IFN-γ (interferon-gamma) play an important role in macrophage activation through an antigen-independent mechanism as they promote the expansion of the T-cell response by an antigen-dependent process to collectively form a Th1-type response profile [27].

Direct contact of lymphocytes and *H. pylori* is considered to induce IFN-γ production and efficiently stimulate NK cell activity [28], probably by the action of *H. pylori* lipid A which also induces the production of tumor necrosis factor-alpha. Paradoxically, this type of lipid has strong immune activity and low endotoxin activity[29]. CD56+ cells seem very important in the gastric mucosal immune response because they were detected in all compartments, foveolar and mucous neck cells, lamina propria, and lymphoid follicles, however, the interleukins associated with their presence could contribute to cell damage and be cofactors of glandular destruction and atrophy.

In conclusion, in our study, we found that the lymphoid tissue associated with the gastric mucosa is predominantly diffusely organized in the lamina propria with frequent formation of lymphoid follicles. Our comparison of the inflammatory/immune response in two populations at risk for gastric cancer describes a pattern characterized by increased bacterial load and increased mucosal damage to the epithelium, probably representing direct bacterial damage. The cellular response included a nonspecific acute response characterized mainly by PMNs, as well as a specific immune response, especially T lymphocytes, both intraepithelial and stromal. Multifocal atrophic gastritis was more frequent in Pasto and non-atrophic gastritis in Tumaco. The immunological responses to *H. pylori* infection are most probably related to this dichotomic result.

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.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

Acknowledgements

The authors appreciate the support of: The Laboratory of Microbiology and Molecular Biology and the Laboratory of Histopathology of the Department of Pathology of the Universidad del Valle, for facilitating their facilities for the development of this study.

References

1. de Brito BB, da Silva FA, Soares AS, et al. Pathogenesis and clinical management of *Helicobacter pylori* gastric infection. World J Gastroenterol. 2019; 25(37):5578-5589. doi: 10.3748/wjg.v25.i37.5578.
2. Correa P. The gastric microenvironment determines *Helicobacter pylori* colonization [editorial; comment]. Am J gastroenterol. 1995;90(9):1379-81.
3. Matta AJ, Pazos AJ, Bustamante-Rengifo JA, Bravo LE. Genomic variability of *Helicobacter pylori* isolates of gastric regions from two Colombian populations. World J Gastroenterol. 2017;23(5):800-9. doi: 10.3748/wjg.v23.i5.800.
4. Van Doorn Lj, Figueiredo C, Megraud F, et al. Geographic distribution of vacA allelic types of *Helicobacter pylori*. Gastroenterology 1999;116(4):823-30.
5. Kim A, Servetas SL, Kang J, et al. *Helicobacter pylori* bab Paralog Distribution and Association with cagA, vacA, and homA/B Genotypes in American and South Korean Clinical Isolates. PLoS One 2015;10(8):e0137078. doi: 10.1371/journal.pone.0137078.
6. Cuello C, Correa P, Haenszel W, et al. Gastric cáncer in Colombia. I. Cancer risk and suspect enviromental agents. J Natl Cancer Inst 1976;57(5):1015-20.
7. Rosero YL, Arévalo-Jaimes BV, Delgado MP, et al. Evaluation of *Helicobacter pylori* Infection and Clarithromycin Resistance in Strains From Symptomatic Colombian Children. J Pediatr Gastroenterol Nutr. 2018;67(5):601-4. doi: 10.1097/MPG.0000000000002016.
8. Bray F, Ferlay J, Soerjomataram I, Siegel RL Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2018;68:394-424.
9. Bravo LE, Muñoz N. Epidemiology of cancer in Colombia. Colomb Med. 2018;49(1):9-12. doi: 10.25100/cm.v49i1.3877.
10. Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 2015;64(9):1353-67. doi: 10.1136/gutjnl-2015-309252. Epub 2015 Jul 17.
11. Caguazango JC, Pazos ÁJ. Microbiota according to gastric topography in patients with low or high risk of gastric cancer in Nariño, Colombia. Biomedica 2019; 39(Supl. 2):157-71. doi: 10.7705/biomedica.v39i4.4520.
12. Guan E, Tian F, Liu Z. A novel risk score model for stomach adenocarcinoma based on the expression levels of 10 genes. Oncol Lett. 2020;19(2):1351-67. doi: 10.3892/ol.2019.11190.
13. Javed S, Skoog EC, Solnick JV. Impact of *Helicobacter pylori* Virulence Factors on the Host Immune Response and Gastric Pathology. Curr Top Microbiol Immunol. 2019;421:21-52. doi: 10.1007/978-3-030-15138-6_2.
14. Salih BA. *Helicobacter pylori* infection in developing countries: the burden for how long? Saudi J Gastroenterol. 2009;15(3):201-7. doi: 10.4103/1319-3767.54743.
15. Dunn BE, Campbell GP, Perez-Perez GI, Blaser MJ. Purification and characterization of urease from *Helicobacter pylori*. J Biol Chem. 1990;265(16):9464-9.
16. Pruszkowski J, Ziolkowski G, Gonciarz Z, Besser P. Isolation of *Helicobacter pylori* from gastric mucosa depending on the growth medium used. Med Dosw Microbiol 1994;46:305-11 [PMID: 7603132].
17. Madan EK, Kemp J, Westblom TU, Subik M, Sexton S, Cook J. Evaluation of staining methods for identifying *Campylobacter pylori*. American J Clin Pathol. 1988;90(4):450-3.
18. Garvey WF, Bigelow F. Modified steiner for the demonstration of spirochetes. J Histotechnol. 1985;8(1):15-7.
19. Dixon MF, Yardle GJ, Correa P. Classification and grading of gastritis. The update sydney system. Am J Surg Pathol. 1996;29(10):1161-81.
20. Snedecor GW, Cochran WG. Statistical methods. 6th. Ed. Ames IA. Iowa State University Press 1967.
21. Archampong TN, Asmah RH, Richards CJ, et al. Gastro-duodenal disease in Africa: Literature review and clinical data from Accra, Ghana. World J Gastroenterol. 2019;25(26):3344-58. doi: 10.3748/wjg.v25.i26.3344.
22. Ulanja MB, Beutler BD, Rishi M, et al. Influence of race and geographic setting on the management of gastric adenocarcinoma. J Surg Oncol. 2019; 120(2):270-9. doi: 10.1002/jso.25503.
23. Tursi A, Gasbarrini G. Acquired gastric mucosa-associated lymphoid tissue (MALT): a review with special emphasis on association with extraigastric diseases and management problems of gastric MALT. J Clin Gastroenterol. 1999;29(2):133-7.
24. Wu YY, Hsieh CT, Tsay GJ, et al. Recruitment of CCR6+ Foxp3+ regulatory gastric infiltrating lymphocytes in *Helicobacter pylori* gastritis. Helicobacter. 2019;24(1):e12550. doi: 10.1111/hel.12550.
25. Wu X, Li X, Dang Z, Jia Y. Berberine demonstrates anti-inflammatory properties in *Helicobacter pylori*-infected mice with chronic gastritis by attenuating the Th17 response triggered by the B cell-activating factor. J Cell Biochem. 2018;119(7):5373-81. doi: 10.1002/jcb.26681.
26. Fox JG, Blanco M, Murphy JC, et al. Local and systemic immune responses in murine Helicobacter felis active chronic gastritis. Infect Immun. 1993;61(6):2309-15.
27. Wan R, Wang ZW, Li H, et al. Human Leukocyte Antigen-G Inhibits the Anti-Tumor Effect of Natural Killer Cells via Immunoglobulin-Like

Transcript 2 in Gastric Cancer. Cell Physiol Biochem. 2017;44(5):1828-41. doi: 10.1159/000485819.
28. Yuceyar H, Saruc M, Kokuludag A, Terzioglu E, Goksel G, Isisag A. The systemic cellular immune response in the *Helicobacter pylori*-associated duodenal ulcer and chronic antral gastritis. Hepatogastroenterology 2002;49(46):1177-9.
29. Ogawa T, Suda Y, Kashihara W, Hayashi T, Shimoyama T, Kusumoto S. Immunobiological activities of chemically defined lipid A from *Helicobacter pylori* LPS in comparison with Porphyromonas gingivalis lipid A and *Escherichia coli*-type synthetic lipid A (compound 506). Vaccine. 1997;15(15):1598-605.