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DIAGNOSTIC CONCORDANCE OF HEMATOXYLIN-EOSIN AND GIEMSA STAINING OF GASTRIC BIOPSIES WITH CHEMILUMINESCENT IMMUNOASSAY FOR STOOL ANTIGEN DETECTION TO DIAGNOSE *Helicobacter pylori* INFECTION

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Abstract: Introduction. Identification of *Helicobacter pylori* is very important for timely treatment. **Objective.** To determine the level of diagnostic agreement between hematoxylin-eosin and Giemsa staining of gastric biopsies with chemiluminescent immunoassay for detection of antigen in stool to diagnose *Helicobacter pylori* infection. **Methodology.** Retrospective observational descriptive study. Statistical analysis was performed using Jamovi 2.3.28 statistical software. Cohen's kappa index was applied to determine the diagnostic agreement between hematoxylin-eosin staining and Giemsa staining with chemiluminescent immunoassay for detection of *Helicobacter pylori* antigen in stool. **Results.** The diagnostic agreement when comparing the hematoxylin-eosin stain with the chemiluminescent immunoassay resulted in an acceptable level of agreement by obtaining a value of 0.40 in Cohen's kappa index; the diagnostic agreement when comparing the Giemsa stain with the chemiluminescent immunoassay resulted in a moderate level of agreement by obtaining a value of 0.45 in Cohen's kappa index. **Conclusion.** Giemsa staining has a better performance; however, since there is no gold standard, it is necessary to evaluate the performance of an additional methodology based on clinical criteria for an accurate diagnosis.

Keywords: *H. pylori, concordance, chemiluminescent immunoassay, Giemsa, hematoxylin-eosin.*

INTRODUCTION

Helicobacter pylori is a Gram-negative bacillus with the ability to colonize the gastric mucosa, there are influential factors such as: inflammation, altered gastric acid production and tissue damage (Murray, 2013). Persistent infection is associated with different pathologies such as: chronic gastritis, gastric ulcers, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma, 20% of people infected by *H. pylori* present a progressive disease course that starts with chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia and finally cancer (Marín et al., 2018); therefore a timely diagnosis is necessary.

In Guatemala, in 2015, Matta conducted a review of 284 files with registration of gastric cancer, finding record of performing endoscopy in 248 (87%) patients, histological tests were performed to diagnose *Helicobacter pylori* infection in 69 of them, indicating a positivity of 31.Histological tests were performed to diagnose *Helicobacter pylori infection in* 69 of them, indicating a positive result of 31.9% (22/69); 47.1% of the cases were classified as intestinal type gastric adenocarcinoma and 29.9% as diffuse type, in the intestinal type adenocarcinoma 45.5% (10/22) were positive for *Helicobacter pylori*.

On the other hand, in 2016, in Peru a study was conducted with 100 gastric biopsy samples that sought to measure the application of Giemsa staining and hematoxylin - eosin staining in the diagnosis of *H. pylori*, a sensitivity of 76% and specificity of 73% was found (Garay, 2016); in 2014, in Ecuador, a study was conducted with 103 gastric biopsies with the objective of applying utility indexes between hematoxylin - eosin and Giemsa staining, a sensitivity of 78%, specificity of 70%, positive predictive value of 66% and negative predictive value of 80% was obtained (Escudero, 2014).

In addition to the identification of *H. pylori* through histochemical staining, the diagnosis can be made with immunological tests, such as the chemiluminescent immunoassay for detection of antigens in stool, as shown in the study conducted by Resina (2021), which included 72 patients who underwent the stool chemiluminescent antigen test and compared it with the breath test as a reference method, a sensitivity of 75% with a confidence interval of 95% and a specificity of 96% was obtained.

For the diagnostic approach of *Helicobacter pylori* infection in gastric biopsies received and processed by the Department of Clinical Laboratories and Pathology of the Roosevelt Hospital, hematoxylin-eosin staining and Giemsa staining are performed; however, no diagnostic concordance studies have been performed between these stains compared with other methods such as chemiluminescence immunoassay available at the Endocrinology and Specialties Laboratory of the Roosevelt Hospital.

The present research seeks to demonstrate the diagnostic concordance of hematoxylin-eosin staining and Giemsa staining in gastric biopsies for the diagnosis of *Helicobacter pylori* compared to chemiluminescent immunoassay for the detection of *Helicobacter pylori* antigens in stool in order to identify the best diagnostic method to contribute to the prevention of pathologies secondary to *Helicobacter pylori* infection and their progression in the Guatemalan population.

METHODOLOGY

TYPE OF STUDY

Retrospective observational descriptive.

POPULATION

Gastric biopsies received and processed with hematoxylin-eosin and Giemsa stain by the Department of Clinical Laboratories and Pathology of Roosevelt Hospital, which have chemiluminescent immunoassay results for *Helicobacter pylori* antigen detection performed by the Endocrinology and Specialties Laboratory of Roosevelt Hospital.

SAMPLE

Gastric biopsies received and processed with hematoxylin-eosin and Giemsa staining and their diagnostic interpretation for *Helicobacter pylori* in the Department of Clinical Laboratories and Pathology of Roosevelt Hospital that meet the inclusion criteria during the period from January to July 2022 and that have a chemiluminescent immunoassay result (LAISON ® *Helicobacter pylori* SA) for detection of *Helicobacter pylori* antigen in stool.

SAMPLING DESIGN

Non-probabilistic, of consecutive cases during the corresponding period.

VARIABLES

Age, sex, hematoxylin-eosin stain, Giemsa stain, chemiluminescent immunoassay, final diagnosis.

DATA PROCESSING AND ANALYSIS

The values of epidemiological variables, results obtained from the diagnostic interpretation of *H. pylori* in gastric biopsies using hematoxylin-eosin staining and Giemsa staining and chemiluminescent immunoassay for detection of antigens in feces were transcribed and standardized in a database elaborated in Microsoft Excel 2010, this database was analyzed with the statistical software Jamovi 2.3.28.

For the measurement of diagnostic agreement between hematoxylin-eosin staining and Giemsa staining with the chemiluminescent immunoassay for detection of *Helicobacter pylori* antigen in stool, Cohen's kappa index was applied. The reference values for the interpretation of the kappa index were: 0.00 poor concordance, 0.01 - 0.20 slight concordance, 0.21 - 0.40 acceptable concordance, 0.41 - 0.60 moderate concordance, 0.61 - 0.80 considerable concordance and 0.81 - 1.00 almost perfect concordance.

For the epidemiological characterization of the sample, a description of age was made through absolute frequencies and percentages, and measures of central tendency and dispersion were used for the age variable.

The frequency of positivity for *Helicobacter pylori* in gastric biopsies was calculated using hematoxylin-eosin staining and Giemnsa staining. The frequency of positivity for *Helicobacter pylori* obtained by chemiluminescent immunoassay for detection of antigens in stool was calculated.

In addition, other diagnostic efficacy indicators were measured, such as: sensitivity, specificity, positive predictive value, negative predictive value and accuracy, with a confidence level of 95%.

ETHICAL ASPECTS

Confidentiality was maintained throughout the research. The Department of Clinical Laboratories and Pathology of the Roosevelt Hospital and the Department of Teaching and Research of the Roosevelt Hospital were authorized to carry out this research. Authorization registered in Act 747, item No. 3 dated May 7, 2024.

RESULTS

A total of 153 gastric biopsies were included in this research, the age of the participants was in the range of 18 to 88 years, with a mean of 51 years and a standard deviation of 14.8 years, 82.50% (121 / 153) of the participants were female.

Positivity in tests to diagnose *Helicobacter pylori* infection was 28.1% (43/153) with hematoxylin eosin staining, 31.4% (48/153) with Giemsa staining and 16.3% (21/153) in the chemiluminescent assay for detection of *H. pylori* antigen in stool; positivity for *H. pylori* was found in 43.1% (66 /153) in at least one of the tests evaluated (Table 1).

	Result	f	%	95% CI	
				Inferior	Superior
HE	Negative	110	71.9%	64.1%	78.9%
	Positive	43	28.1%	21.1%	35.9%
Giemsa	Negative	105	68.6%	60.6%	75.9%
	Positive	48	31.4%	24.1%	39.4%
Chemiluminescent immunoassay	Negative	128	83.7%	76.8%	89.1%
	Positive	25	16.3%	10.9%	23.2%
Diagnosis	Negative	87	56.9%	48.6%	64.8%
	Positive	66	43.1%	35.2%	51.4%

Table 1. Diagnostic test positivity for H. pylori (n= 153)

HE: Hematoxylin-eosin

Source: Own elaboration

In the gastric biopsies evaluated, gastric antrum mucosa was found in 140 (91.5%), of which 62 were positive for *H. pylori* infection. Gastric atrophy was reported in 42 (27.5%) of the gastric biopsies, of which 30 were positive for *H. pylori* infection. Metaplasia was observed in 25 (16.3%) of the biopsies, 18 of these were positive for *H. pylori* infection; inflammatory activity was identified in 60 (39.2%) of the biopsies, 49 of these were positive for *H. pylori* infection (Table 2).

	Diagnosis				Total	
	Negative		Positive			
Mucosa	**f**	**%**	**f**	**%**	**f**	**%**
Antral	78	89.7 %	62	93.9 %	140	91.5 %
Cast body	1	1.1 %	1	1.5 %	2	1.3 %
Fundica / antral	8	9.2 %	3	4.5 %	11	7.2 %
Atrophy						
Negative	75	86.2 %	36	54.5 %	111	72.5 %
Positive	12	13.8 %	30	45.5 %	42	27.5 %
Metaplasia						
Negative	80	92.0 %	48	72.7 %	128	83.7 %
Positive	7	8.0 %	18	27.3 %	25	16.3 %
Activity						
Negative	76	87.4 %	17	25.8 %	93	60.8 %
Positive	11	12.6 %	49	74.2 %	60	39.2 %

Table 2. *Characteristics of gastric biopsies (n= 153)*

Source: Own elaboration

The detection of *Helicobacter pylori* infection by hematoxylin-eosin staining of gastric biopsies had acceptable agreement with the chemiluminescent immunoassay for detection of *Helicobacter pylori* antigen in stool (kappa index of 0.40) and moderate agreement between Giemsa staining and the chemiluminescent immunoassay (kappa index of 0.45) (Table 3).

HE	Chemiluminescent immunoassay					
	Negative		Positive		Total	
	f	%	f	%	f	%
Negative	103	67.3 %	7	4.6 %	110	71.9 %
Positive	25	16.3 %	18	11.8 %	43	28.1 %
Giemsa						
Negative	101	66.0 %	4	2.6 %	105	68.6 %
Positive	27	17.6 %	21	13.7 %	48	31.4 %
Kappa index hematoxylin eosin				0.40		
Kappa Giemsa Index			0.45			

Table 3. *Hematoxylin-eosin and Giemsa concordance, with chemiluminescent immunoassay*

HE: Hematoxylin-eosin

Source: Own elaboration

The specificity and positive predictive value values observed for detection of *Helicobacter pylori* infection were 100% for hematoxylin--eosin, Giemsa and chemiluminescent assay stains. The sensitivity and negative predictive value for hematoxylin-eosin staining were 65.2% and 79.1% respectively; sensitivity and negative predictive value for Giemsa staining were 72.7% and 82.9% respectively; sensitivity and negative predictive value for chemiluminescent immunoassay were 37.9% and 68.0% (Table 4).

DISCUSSION

Identification of *Helicobacter pylori* is based on direct and indirect evidence, and is determinant for timely treatment. In a study by Farouk (2018) in Malaysia, where they evaluated 104 gastric biopsies, the participants were in the range of 10 to 85 years, with a mean of 56.2 years; these data are similar to the present investigation in which the 153 participants were in the range of 18 to 88 years, with a mean of 51 years. Alkhamiss (2020) in Saudi Arabia, in a research that evaluated 49 gastric biopsy, found that 63.2% of their participants were female, in comparison to this research where 82.5% (121 / 153) of the participants were female; in contrast to that reported by Farouk (2018), whose population had predominantly male participants corresponding to 63.5%.

A wide range of diagnostic tests for *Helicobacter pylori* are currently available and can be classified into two broad groups: invasive and non-invasive. The choice of which test to use will depend on the clinical scenario, costs and performance of the test. Among the invasive methods, histological evaluation of the gastric mucosa by biopsy with hematoxylin-eosin staining allows the detection of *Helicobacter pylori* infection (Dore 2021), while allowing the recognition of morphological characteristics of the gastric mucosa of clinical importance such as

Parameter	HE	95% CI	Giemsa	95% CI	CLIA	95% CI
Result						
Positive	28.1%	21.1% - 35.9%	31.4%	24.1% - 39.4%	16.3%	10.9% - 23.2%
Negative	71.9%	64.1% - 78.9%	68.6%	60.6% - 75.9%	83.7%	76.8% - 89.1%
Concordance						
Sensitivity	65.2%	52.4% - 76.5%	72.7%	60.4% -83.0%	37.9%	26.2% - 50.7%
Specificity	100.0%	95.8% - 100%	100.0%	95.8% - 100%	100.0%	95.8% - 100%
VPP	100.0%		100.0%		100.0%	
VPN	79.1%		82.9%		68.0%	

Table 4. *Correlation of tests*
HE: Hematoxylin-eosin
CLIA: Chemiluminescent immunoassay
PPV: Positive predictive value
NPV: Negative predictive value
Source: Own elaboration

inflammatory activity, atrophy and metaplasia; diagnostic accuracy can be increased if auxiliary stains, such as Giemsa staining, are incorporated. Among the non-invasive tests, the detection of antigen in stool allows to know the active presence of *Helicobacter pylori* infection. The test evaluated in this research is based on chemiluminescent immunoassay.

Alkhamiss (2020) in Saudi Arabia found a positivity for *Helicobacter pylori* infection of 26.5% in gastric biopsies evaluated with hematoxylin eosin staining and 28.5% with Giemsa staining; in the present investigation, positivity for *Helicobacter pylori* infection was found to be 28.1% (43 / 153) in gastric biopsies evaluated with hematoxylin eosin staining and 31.4% (48 / 153) with Giemsa staining (Table 1). Ramírez-Lázaro (2016) in Spain performed *Helicobacter pylori* antigen detection tests, to 290 patients with dyspepsia, finding a positivity of 41.7% for *Helicobacter pylori* infection, also Resina in Madrid, Spain performed stool antigen tests for *Helicobacter pylori* detection in 307 patients of which 16.2% were positive; in the present investigation a positivity of 43.1% (66/153) was obtained using any of the three methodologies analyzed (Table 1) and specifically the chemiluminescent immunoassay test for *Helicobacter pylori* antigen detection showed a positivity of 16.3% (25 / 153).

Histological observation of gastric biopsies provides the advantage of direct visualization of *Helicobacter pylori* as well as other morphological features that may be associated with *Helicobacter pylori* infection and gastric cancer precursor lesions such as atrophic gastritis and intestinal metaplasia (Yadav, 2022). In the present investigation it was found that 91.5% (140 / 153) of the gastric biopsies evaluated presented characteristics of gastric antrum mucosa, in comparison Alkhamiss (2020) who found in his population of gastric biopsies evaluated that 42.8% corresponded to gastric antrum, 30.The gastric atrophy in our research was identified in 27.5% (42 / 153) of the gastric biopsies evaluated, 30 of these were positive for *Helicobacter pylori* infection; Castaneda (2020) in 270 gastric biopsies submitted for study found that 53.3% presented atrophy characteristics.

An important characteristic to identify in gastric biopsies due to its clinical importance is intestinal metaplasia, in the present investigation 16.3% (25 / 153) of the evaluated biopsies presented characteristics of intestinal metaplasia, of these 18 were

positive for *Helicobacter pylori* infection, Alkhamiss (2020) reported in his study 6.1% (3 / 49) of positivity for metaplasia, while Castaneda (2020) reported 90.7% (245 / 270) in his studied population. Gastric activity corresponds to the presence of neutrophils migrating through the gastric glands, in the present investigation 39.2% (60 / 153) of the gastric biopsies presented some degree of activity and 49 of these positive for *H. pylori*, Alkhamiss (2020) found a similar percentage of activity in his gastric biopsies evaluated, 30.6% (15 / 49) (Table 2).

The sensitivity, specificity and concordance of histological tests for the detection of *Helicobacter pylori* depend on the technique employed and may be affected by factors such as the experience of the observer in the case of staining. The choice of test for *Helicobacter pylori* stool antigen detection is indicated based on the patient's clinical presentation; the initial diagnostic test may be supplemented by another test, or a different test may be added during monitoring of the infection. The stool antigen test is highly cost-effective in countries with moderate prevalence of *Helicobacter pylori*, in addition to being a non-invasive and automated test using chemiluminescence immunoassay methodology as evaluated in this research, which is contrasted with hematoxylin-eosin and Giemsa histochemical stains.

Table 3 shows the interactions of positivity and negativity of the tests under study to measure the concordance between them using Cohen's kappa index; the diagnostic concordance between hematoxylin-eosin staining and immunoassay was 0.40, interpretable as acceptable concordance, and the diagnostic concordance between Giemsa staining and immunoassay was 0.45, interpretable as moderate. Keskin (2022) in an investigation evaluated histochemical stains of hematoxylin eosin and Giemsa in gastric biopsies compared against the detection test for *Helicobacter pylori* antigen in stool, obtained a kappa index of 0.62 demonstrating a considerable concordance between the tests.

The results presented in Table 4 show the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of hematoxylin-eosin and Giemsa staining in gastric biopsies and immunoassay for detection of *H. pylori* antigen in feces. Of the tests evaluated for diagnosis of *H. pylori* infection, Giemsa staining showed the highest accuracy with sensitivity 72.7%, specificity 100%, PPV 100% and NPV 82.9%, followed by hematoxylin-eosin staining with sensitivity 65.2%, specificity 100%, PPV 100% and NPV 79.1%, in third place the immunoassay test by chemiluminescence methodology with sensitivity 37.9%, specificity 100%, PPV 100% and NPV 68.0%; the values reported in other studies vary according to the inclusion or exclusion criteria applied to the sample selection and other variables applied; Keskin (2022) reported in his research that the histochemical stains hematoxylin eosin and Giemsa in the histological evaluation compared against the detection of antigen in stool provides sensitivity 81.3%, specificity 66.7%, PPV 50% and NPV 89.7%, when comparing stool antigen detection against histochemical stains, sensitivity 47.6%, specificity 82.4%, PPV 62.5% and NPV 71.8%; Alkhamis (2020) comparing hematoxylin-eosin versus Giemsa stains for the detection of *Helicobacter pylori* in gastric biopsies found for hematoxylin-eosin sensitivity of 66.67% and specificity of 91.18%, for Giemsa sensitivity of 93.33% and specificity of 99.9%.

Helicobacter pylori infection is an infection that varies in frequency according to the populations studied, for this reason it is opportune to find the best diagnostic methods that adapt to the needs of our country, histochemical stains performed by manual procedures can decrease their performance due to the human factor, which improves considerably with the use of automated stains, likewise the interpretation of the stains being an obser-

ver-dependent technique is susceptible to error. There are other histochemical stains that allow a more efficient visualization of *Helicobacter pylori* bacillus such as Warthin-Starry staining but represent higher costs (Farouk 2018), as well as stains that have antigen-antibody reaction as a basis such as immunohistochemistry.

Hematoxylin-Eosin and Giemsa stains, as well as chemiluminescent immunoassay are tests currently available at Roosevelt Hospital; all have advantages and have diagnostic limitations, so the choice of one or more tests is made on the basis of clinical findings and the clinical-diagnostic correlation provides guidance for the proper management of *Helicobacter pylori* infection.

Several limitations were present in the research, among them the histochemical staining processes used were performed manually because automated methods were not available in the Department of Clinical Laboratories and Pathology at the time of the research, this limitation can be addressed in the future by comparing the diagnostic performance of manual histochemical stains against automated ones. Not knowing whether the patients included in the investigation had antibiotic or proton pump inhibitor treatment prior to gastric biopsy and chemiluminescent immunoassay is a limiting factor for the performance of the tests evaluated by affecting histologic interpretation and immunoassay results, which could be addressed in a prospective study including only patients who have not been exposed to this class of drugs previously.

CONCLUSION

Diagnostic agreement when comparing hematoxylin-eosin staining with chemiluminescent immunoassay resulted in an acceptable level of agreement by obtaining a value of 0.40 on Cohen's kappa index; diagnostic agreement when comparing Giemsa staining with chemiluminescent immunoassay resulted in a moderate level of agreement by obtaining a value of 0.45 on Cohen's kappa index.

In general, Giemsa staining has a better performance compared to hematoxylin-eosin staining and chemiluminescent immunoassay; since there are no culture and molecular techniques for the diagnosis of *Helicobacter pylory* infection in the Department of Clinical Laboratories and Pathology of the Roosevelt Hospital, it is necessary to evaluate the use of an additional methodology to histochemical techniques based on clinical criteria for an accurate diagnosis.

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