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DIAGNOSTIC ACCURACY OF IMMUNOHISTOCHEMISTRY FOR DIAGNOSIS OF HELICOBACTER PYLORI INFECTION IN GASTRIC BIOPSIES RECEIVED IN SIR GANGARAM HOSPITAL LAHORE

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ABSTRACT

Background: *The stomach epithelium is colonised by gram-negative spiral-shaped Helicobacter pylori. Chronic gastritis, ulcers, adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma are linked to Pylori infection. Basic H&E stain can detect it. The particular method of immunohistochemistry is reliable for H. pylori diagnosis. Previous research had mixed outcomes. This research was done to gather local demographic data.*

Objective: *To ascertain the diagnostic precision of immunohistochemistry in identifying Helicobacter pylori infection in stomach samples obtained in the histopathology department using H&E as the gold standard.*

Study Design: *Cross Sectional Study*

Study place and duration: *Department of Pathology, Sir Ganga Ram Hospital, Lahore from 21-6-2024 to 21-12-2024.*

Patients and methodology: *260 samples were received from gastroenterology department and were divided in two parts. On one part, immunohistochemistry was applied and findings were recorded. On second part, H&E was applied and findings were recorded. All the data was recorded in proforma while later on analyzed in SPSS 20.0.*

Results: *Patients in this research ranged in age from 16 to 75 years, with a mean age of 41.73 ± 16.64 years. The male-to-female ratio was 1:2.25, with 80 (30.8%) male and 180 (69.2%) female patients. The immunohistochemical test for H. pylori infection has 85.2% sensitivity, 100% specificity, 100% PPV, 93.7% NPV, and 95.4% diagnostic accuracy.*

Conclusion: *Thus, immunohistochemistry is found to be reliable enough to detect H. pylori infection in gastric biopsies.*

Keywords: *immunohistochemistry, helicobacter Pylori infection, gastric biopsies, histopathology, hematoxylin and eosin*

INTRODUCTION

Helicobacter pylori is a gram negative spiral shaped bacterium which colonizes the gastric epithelium.[1] A mucus layer shields motile, microaerophilic bacteria from the acidic gastrointestinal environment.[2, 3] It affects about more than 50% of the population of the world.[4] Immunohistochemistry detected 37.1% of Helicobacter pylori whereas histology detected 32.4%.[5] Contagious food, water, and poverty spread it. In poorer nations like Pakistan, the infectivity is still high owing to low socioeconomic position, while in wealthy countries, improved standards of living have reduced it.[5] Adenocarcinoma, gastric mucosa-associated lymphoid tissue lymphoma, chronic gastritis, and ulcers are linked to pylori infection. Gut inflammation, including ulcerative colitis and autoimmune illnesses, involves it. Aliancillary studies and immunohistochemistry may increase specificity by 90-100% over normal H&E stain.[6] According to the four

authors, blindly evaluating hematoxylin and eosin slides found H pylori with 100%, 95%, and 100% sensitivity/specificity.[7]

Immunohistochemistry is a special technique that uses specific binding ability between an antibody and antigen to detect Pylori bacteria in gastric biopsies. The main steps after fixation of the specimen is deparaffination, antigen retrieval, blocking, primary and secondary antibody incubation and staining.[8] After immunohistochemistry, dark brown thread like bacteria can be identified on light microscope. Staining pattern of Pylori can be diffuse, dot like granular or spiral.[9]

In this research, immunohistochemistry is tested for its ability to detect helicobacter Pylori in stomach samples from histology. Research shows that immunohistochemistry can diagnose H. pylori. However, prior investigations found different findings. Thus, we aim to undertake this research to see whether immunohistochemistry is trustworthy for local populations. It will boost our knowledge and practice.

OBJECTIVE

To ascertain the diagnostic precision of immunohistochemistry in identifying Helicobacter pylori infection in stomach samples obtained in the histopathology department using H&E as the gold standard.

MATERIALS AND METHODS

Study Design: Cross Sectional Study

Study place and duration: Department of Pathology, Sir Ganga Ram Hospital, Lahore from 21-6-2024 to 21-12-2024.

Sample Size: By using sensitivity & specificity calculator, sample size of 260 cases is calculated with 95% confidence level, prevalence of H. pylori i.e. 37.1%,[5] sensitivity of immunohistochemistry i.e. 87.5% with 8.5% margin of error and specificity of immunohistochemistry i.e. 94.44% with 3.5% margin of error. [10]

Sample technique: Non-probability, consecutive sampling

Sample Selection:

Inclusion Criteria: Gastric biopsies of patients of age 16-75 years, both; male and female patients, received for histopathological examination were included in the study.

Exclusion Criteria: Autolyzed specimen or tissue with processing defects, inappropriately stored samples or patients taking proton pump inhibitors were excluded from the study.

Data Collection Procedure: The ethical review board approved 260 gastroenterology samples. Data collected included name, age, gender, BMI, symptom duration, residence, food, water consumption, smoking (>5 pack years), diabetes (BSR> 200 mg/dl), and hypertension (BP≥140/90 mmHg). Samples were split. Part of the study used immunohistochemistry and documented results. Results from immunohistochemistry were positive or negative. EnVision Detection Systems Peroxidase/DAB, Rabbit/Mouse kit (Dako) identified and visualised bound antibodies, indicating positive immunohistochemistry. The second section used hematoxylin and eosin and reported results. HE staining verified positive or negative findings. Positive urease test, spiral-shaped H. pylori, and modified Giemsa staining indicated a positive H&E staining. Proforma records all data.

Statistical Analysis: It was performed using SPSS system for windows SPSS 20.0. Normality was checked by Shapiro-Wilk test. Mean and standard deviation was calculated for quantitative variables, e.g. age, BMI, duration of symptoms. Qualitative variables gender, residence, diet pattern, water use, smoking, diabetes, hypertension, and H. pylori (detected on immunohistochemistry and H&E stain) were expressed as

frequency and percentage. 2x2 table was generated to calculate sensitivity, specificity, PPV, NPV and diagnostic accuracy of immunohistochemistry taking H&E stain as gold standard.

RESULTS:

In this study, the mean age of patients was 41.73 ± 16.64 years with age range from 16 to 75 years. There were 80 (30.8%) male patients while 180 (69.2%) female patients with male-to-female ratio of 1:2.25. The mean BMI was noted as 22.88 ± 1.77 kg/m^2. The mean duration of symptoms was 5.17 ± 2.76 months. Out of 260 patients, 109 (41.9%) were living in rural areas, 51 (19.6%) were living in urban areas, 93 (35.8%) were residing in semi-urban areas while 7 (2.7%) were residing in urban slums. Out of 260 patients, 161 (61.9%) were taking home made food, while 69 (26.5%) were taking mostly street food (about >3 times per week), 24 (9.2%) were consuming fast food (>3 times per week), while 6 (2.3%) had mess. It was also noted that about 189 (72.7%) patients were using plain tap water, and 71 (27.3%) were using filtered water while no one (0%) using boiled water in routine. Out of 260, 37 (14.2%) were smokers, 88 (33.8%) were diabetic and 128 (49.2%) had history of hypertension. Table I

On immunohistochemistry, there were 69 (26.5%) positive while 191 (73.5%) negative cases. On H&E staining, there were 81 (31.2%) positive while 179 (68.8%) negative cases. Fig I

2x2 contingency table was generated and diagnostic accuracy of immunohistochemistry was calculated. The immunohistochemistry showed sensitivity of 85.2%, Specificity of 100%, PPV of 100%, NPV of 93.7% and overall diagnostic accuracy of 95.4% for diagnosis of H. pylori infection. Table II

Table I: Baselines characteristics of patients enrolled in the study (n = 260)

	F (%), Mean ± SD
Age (in years)	41.73 ± 16.64 (16-75)
Gender	
Male	80 (30.8%)
Female	180 (69.2%)
BMI (in kg/m^2)	22.88 ± 1.77
Duration of symptoms (in months)	5.17 ± 2.76
Residence	
Rural	109 (41.9%)
Urban	51 (19.6%)
Semi-urban	93 (35.8%)
Urban slums	7 (2.7%)
Diet pattern	
Home-made	161 (61.9%)
Street food	69 (26.5%)
Fast food	24 (9.2%)
Mess	6 (2.3%)
Water usage	
Tap water	189 (72.7%)
Filtered	71 (27.3%)
Boiled	0 (0%)
History	
Smoking	37 (14.2%)
Diabetes mellitus	88 (33.8%)
Hypertension	128 (49.2%)

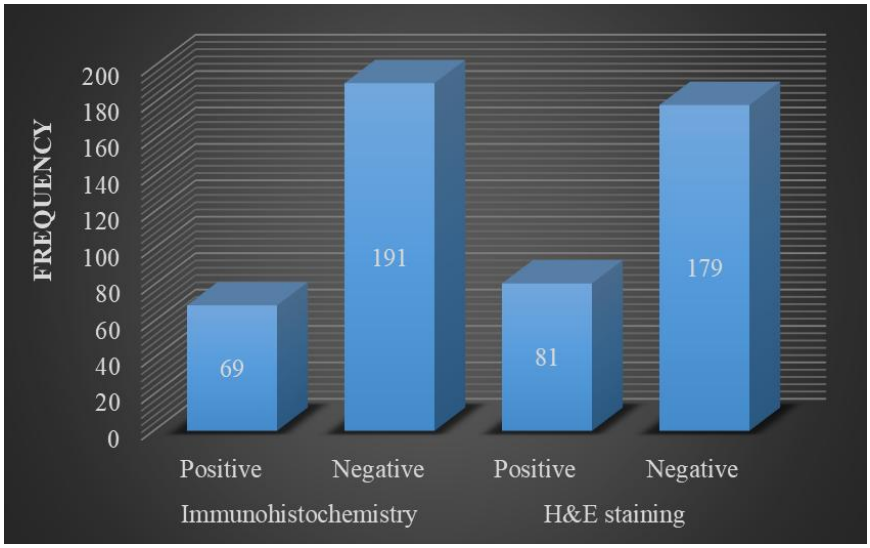

Fig 1: Distribution of positive and negative findings on immunohistochemistry and H&E staining

Table II: 2x2 table showing accuracy of immunohistochemistry for diagnosis H pylori against H&E staining

		H&E staining		Total
		Positive	Negative	
Immunohistochemistry	Positive	69	0	69
	Negative	12	179	191
Total		81	179	260

Sensitivity: 85.2%, Specificity: 100%, PPV: 100%, NPV: 93.7% and diagnostic accuracy: 95.4%.

DISCUSSION:

Accurate H. pylori diagnosis is essential for managing several gastroduodenal disorders.[11] Each invasive and non-invasive H. pylori diagnostic test has its pros and cons in various clinical settings. In clinical practice, there is no gold standard, although different methods provide more trustworthy outcomes.[12-14]

H. pylori infection is initially detected by histology, the gold standard. Staining is important in histology, and routine H&E staining, Giemsa, Warthine-Starry, Hp silver stain, toluidine blue, acridine orange, McMullen, Genta, Dieterle, and immunohistochemical stain may identify H. pylori.[15, 16] In clinical practice, H&E stain is frequently adequate to diagnose H. pylori infection, even though immunohistochemical stain is most sensitive and specific. For biopsy specimens with moderate or severe chronic gastritis but no H. pylori in H&E staining, ancillary stain is advised. If using auxiliary stain to identify H. pylori, immunohistochemical stain is best.[15, 17]

In our research, immunohistochemistry had 85.2% sensitivity, 100% specificity, 100% PPV, 93.7% NPV, and 95.4% diagnostic accuracy for H. pylori infection. Immunohistochemical staining is sensitive, specific, rapid, and has the lowest interobserver variance for H. pylori.[18]

A comparable research by Jahanzeb and Nagi reported that Immunofluorescent has 90% sensitivity and 100% specificity for H. pylori detection.[19] Our investigation almost matched these results. Another research by Akeel et al. found that immunohistochemical staining has 87.50% sensitivity and 94.44% specificity for H. pylori infection identification.[10]

A retrospective study of 200 gastric biopsy specimens by Smith et al. found that H&E can detect H. pylori in 91% of cases and 100% of cases, making it the fastest and cheapest test for gastric biopsies.[20]

Immunohistochemical staining had better sensitivity (87.5%) and specificity (94.44%) than H&E and modified Giemsa staining, concurring with earlier investigations.[21, 22] Immunohistochemical staining cannot assess histopathological changes like H&E. Direct histopathological staining for H. pylori identification is

most reliable with immunohistochemical staining. Immunohistochemical staining is sensitive and selective because it uses particular antibodies, can identify unusual bacterial forms such coccoid forms, and has low false-positive rates. When standard regular procedures fail to identify H. pylori in chronic gastritis caused by limited infection or unusual bacterium distribution in afflicted tissue, immunohistochemistry is indicated. High cost limits immunohistochemical staining for regular diagnostics. Therefore, immunohistochemistry labelling is advised for mild H. pylori infection.[10, 23]

CONCLUSION:

Thus, immunohistochemistry is found to be reliable enough to detect H. pylori infection in gastric biopsies. Now in future, instead of going for H&E staining, we will use immunohistochemistry to detect H. pylori infection in gastric biopsies.

CONFLICT OF INTEREST: None

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