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CORRELATION OF SEROLOGY WITH HISTOLOGY BASED DETECTION OF *HELICOBACTER PYLORI* INFECTION IN A TERTIARY CARE TEACHING HOSPITAL, GUJARAT.

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Abstract
The Aim of the study is Correlation of Serology with Histology based Detection of Helicobacter pylori infection. The present study is based on the following objective, first to determine the prevalence of Helicobacter pylori infection from the total number of sample collected from patients presenting with symptoms and secondly, to correlate the non-invasive method (serology) with Invasive Method (Histopathological Examination of Biopsy) for detection of Helicobacter pylori Infection. A total of 60 Serum and Biopsy samples were collected from patients with symptoms of dyspepsia and requiring an Upper Gastrointestinal Endoscopy of both gender in all age group over a period of 1 Year (23rd January 2017 to 31st January 2018) from tertiary care teaching Hospital. The maximum number of patients were in the age group of 41 to 60 years (35%). Of the total 60 patients, 43 (71.67%) were males and 17 (28.33%) were females which indicates male preponderance. The Positivity rate of Modified Giemsa Staining was found to be low (18.3%) as Compared to the Seropositivity Rate (63.3%). 38 (63.3%) out of 60 cases were reported positive and 22 (36.6%) were reported negative by Helicobacter pylori IgG Serological Test. In Modified Giemsa Staining, out of 60 cases, 11 (18.9%) cases were reported as positive and 49 (81.6%) were reported as negative. In our study, Serology by ELFA technique to detect IgG antibodies against Helicobacter pylori did not correlate significantly with presence of Helicobacter pylori on Histology examination of biopsy by modified Giemsa staining.

Key Words: *Helicobacter pylori*, Histopathological Examination, Serological Test, Enzyme Linked Fluorescent Assay (ELFA), Specificity, Sensitivity, IgG Antibody, Prevalence.

Introduction

The discovery of *Helicobacter pylori* in 1982 by Marshall and Warren was the starting point of a revolution concerning the concepts and management of gastrointestinal diseases. *H. pylori* is a Gram negative curved motile rod found in the deeper portion of the gel coating the gastric mucosa. It is extraordinary among bacteria in its ability to colonize and survive in this environment for decades despite host defences and gastric acidity. [(1)] Its relevance to human disease, specifically to peptic ulcer diseases, gastritis and gastric malignancy is indisputable. Over 80% of individuals infected with the bacteria are asymptomatic. [(7)]

Helicobacter pylori is recognized as an important human pathogen by virtue of its association with peptic ulcer diseases, gastric cancer and gastric lymphoma and the high prevalence of infection worldwide [(2)]. In developing nations, infection is acquired early in life with 90% of the population infected with *H. pylori*. In developed countries, the prevalence of infection varies, influenced by the age, race and socioeconomic status of the population studied. [(2,3,4)]

Various diagnostic methods are developed to detect *H. pylori* infection and diagnostic tests with both high sensitivity and specificity, exceeding 90%, are necessary for accurate diagnosis of *H. pylori* infection in clinical practice. Although many diagnostic tests are available now, each method has its own advantages, disadvantages and limitations. The choice of one method or another could be depended on availability and accessibility of diagnostic tests, level of laboratories, clinical conditions of patients and likelihood ratio of positive and negative tests on different clinical circumstances. [(8)]

There are several diagnostic tools, which include invasive and non-invasive methods, for the diagnosis of *H. pylori* infection, are available. But all of the tests have their pitfalls and limitations. Invasive tests, such as culture, histopathology and biopsy urease test require endoscopic biopsy of gastric tissue. There are several non-invasive methods including urea breath test, *H. pylori* Ag detection in stool, *H. pylori* IgG Antibody detection in urine, serum and saliva, are available for the diagnosis of *H. pylori* infection. Each has its own advantage, disadvantage and limitations. [(9)]

In general, the sensitivity and specificity of these diagnostic tests are high, with histology commonly employed as the gold standard. However, some controversy exists as the best test or combination of tests to use in diagnosing *H. pylori* infection, which also depends upon the clinical situation involved [(2,5)].

In contrast to biopsy based methods, non-invasive tests assess the global presence of *H. pylori* in the stomach even when the bacteria are irregularly distributed on the gastric mucosa. Non endoscopic tests, particularly serology, are cheaper and more convenient and thus should be preferred in situations where the additional information yielded by an endoscopy is not needed [(6)].

The present study is therefore planned to determine the prevalence of *H. pylori* infection and to correlate serological method (non-invasive) with histological examination of biopsy (invasive) for the detection of *H. pylori* infection.

Materials and Methods

Source of data: The study was conducted at the Shree Krishna Hospital located in Gokalnagar, Karamsad, and Anand. It is one of the biggest rural based tertiary care and teaching hospital with a capacity of 610 beds.

Ethical Clearance: This is a prospective cross-sectional study. It was conducted after the approval of the Institutional Ethics Committee (IEC) of H. M Patel Centre for Medical Care and Education, Karamsad. The duration of the study is from 23rd January 2017 to 31st January 2018.

Patients with previous gastric surgery, history of gastric resection and complicated peptic ulcer disease were excluded from the study.

Methodology

The study included 60 patients presenting with symptoms of dyspepsia and requiring an upper gastrointestinal endoscopy, attending Shree Krishna Hospital, Karamsad. Details of all the participants such as name, age, sex, brief clinical history and other relevant information were recorded in prescribed case report form .Biopsy samples collected in 10% formalin received from surgery IPD/OPD were processed in Histopathology lab, Central Diagnostic lab. Modified Giemsa staining was performed on the tissue sections in each case. Results of the histological diagnosis were recorded after taking the permission from the concerned Department. For serological testing, blood samples of the patients selected for endoscopy were procured from either Microbiology or Biochemistry lab, collected for other investigations in adequate amount to perform the test. In 4 cases, fresh blood sample (Approx. 3 ml) of the patient was collected after taking the consent.

Serological testing for antibody detection *(H. pylori* IgG) was performed from serum specimens of the patients in an automated instrument mini VIDAS (Biomerieux) based on the principle of Enzyme linked fluorescent assay (ELFA), using the kit VIDAS HPY in Microbiology Lab, Central Diagnostic Laboratory.

Principle of the qualitative detection of *Helicobacter pylori* IgG by ELFA

The assay principle combines a 2 step enzyme Immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR), serves as the solid phase as well as the pipetting device for the

assay. Reagents for the assay are ready to use and are pre-dispensed in the sealed reagent strips. The five reaction steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

After a preliminary washing step, the IgG antibody present in the sample will bind simultaneously to *H. pylori* antigen coating the interior of the SPR. Unbound sample components are washed away. Anti-human IgG antibodies conjugated with ALP are cycled in and out of the SPR and will attach to any human IgG bound to SPR wall. Final wash steps remove's unbound anti human antibody conjugate. During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR (Solid Phase Receptacle). The conjugate enzyme catalysis the hydrolysis of the substrate in to a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is measured by the optical scanner in the mini-VIDAS instrument, a test value is generated and a report is printed for each sample.
100 µl of standard, control and samples were added into the sample well of 'HPY' strips and 'HPY' SPRs were inserted into mini-VIDAS instrument. After the assay was completed, the SPRs and strips were removed from the instrument and discarded in Red Bag

Test Results and Interpretations
Once the assay was completed, results were analysed automatically by the computer.

TV threshold	Interpretation
TV < 0.75	Negative
0.75 ≤ TV < 1.00	Equivocal
TV ≥ 1.00	Positive

- Samples with test values ≥ high threshold were reported as **Positive**.
- Samples with test values < threshold were reported as **Negative.**

Modified Giemsa Staining
The Modified Giemsa staining was used to stain gastric biopsies for demonstration of *H. pylori* organisms.
Sources of error in Modified Giemsa Staining can be Weak Stain/ Deposits, Improper staining and thick tissue section. Results of staining shows *H. pylori* as Dark blue in colour, Background is Pink – pale blue and Nuclei is Blue in colour.

Data Analysis
Modified Giemsa staining was considered as the gold standard method. Sensitivity, Specificity, PPV and NPV value was calculated of the Serological Test. (*H. pylori* IgG antibody detection by ELFA method).

***HELICOBACTER PYLORI* (SEROLOGY)**

FIGURE: 1 Mini VIDAS

FIGURE 2: HPY Strips, HPY SPRs, HPY Standard (S1), HPY Positive Control (C1), HPY Negative Control (C2).

FIGURE 3: Modified Giemsa stained slide of *Helicobacter pylori*

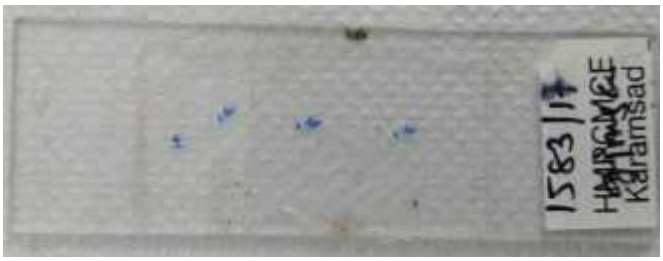


FIGURE 4 (A & B): Photomicrograph of histopathology section of gastric mucosa showing plenty of *Helicobacter pylori*on the surface. Stain: Modified Giemsa & Magnification: x1000

FIGURE 4 A

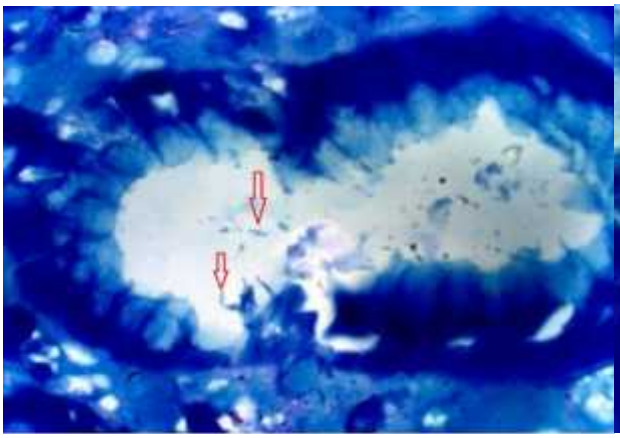
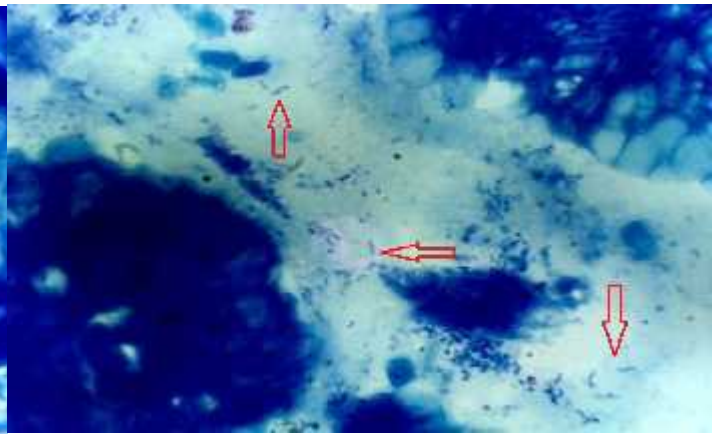


FIGURE 4 B



Observations & Results

This study was conducted in the Department of Microbiology, Pramukhswami Medical College and Shree Krishna Hospital, Karamsad from January 2017 to January 2018.

A total of 60 cases were included in the study. The study population comprised of either gender of any age, presenting with symptoms of dyspepsia and requiring an upper gastrointestinal endoscopy. Details of the history and examination findings were recorded in a predesigned proforma.

Age Wise Distribution of Cases

Table 1 shows the age distribution of the included cases. The maximum number of patients were in the age group of **41 to 60 years (35%).** The mean age of the study population was 48.97 years.

Table 1: Age Wise Distribution of Cases (n=60)

Age Group (years)	Number of patients	Percentage
< 20	02	3.33%
21-40	18	30%
41-60	21	35%
61-80	18	30%
> 80	01	1.67%
Total	60	100%

Gender Wise Distribution of Cases

Table 2 shows the Gender wise distribution of the study cases. Of the total 60 patients, 43 (71.67%) were males and 17 (28.33%) were females.

Table 2: Gender Wise distribution of cases (n=60)

Gender	Number of patients	Percentage
Male	43	71.67%
Female	17	28.33%
Total	60	100%

Correlation of *Helicobacter Pylori* Serology with Modified Giemsa Staining:

Table 3shows that out of 38 cases which were tested positive by serology, only 11 showed visible *Helicobacter pylori* and 27 were negative for *Helicobacter pylori* by Modified Giemsa staining. 22 cases which were Negative by serology were also negative for *Helicobacter pylori* by Modified Giemsa Staining.

The positivity rate of Modified Giemsa staining was found to be low (18.3%) as compared to the seropositivity rate (63.3%)

Table 3: Correlation of *H. pylori* serology with Modified Giemsa Staining (n=60)

SEROLOGY	***H. PYLORI* DETECTION BY MODIFIED GIEMSA STAINING**		**TOTAL (%)**
	POSITIVE	NEGATIVE	
POSITIVE	11	27	**38 (63.3%)**
NEGATIVE	0	22	**22 (36.6%)**
TOTAL	**11 (18.3%)**	**49 (81.6%)**	**60 (100%)**

Figure 1: Modified Giemsa Staining and Serology (by ELFA method) result

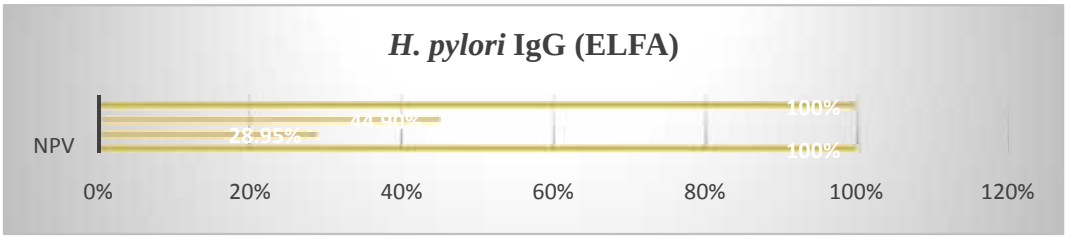
As shown in Figure 1, 38 (63.3%) out of 60 cases were reported Positive and 22 (36.6%) were reported as Negative by *H. pylori* IgG Serological Test. In Modified Giemsa Staining, out of 60 cases, 11(18.3%) cases were reported as Positive and 49 (81.6%) were reported as Negative.
Sensitivity, Specificity, Positive predictive value and negative predictive value of *H. pylori* IgG (ELFA) compared with modified Giemsa staining.

Table 4 Shows the Sensitivity, Specificity, PPV & NPV of *H. pylori* IgG (ELFA) compared with Modified Giemsa Staining.

Table 4: Sensitivity, Specificity, PPV& NPV of *H. pylori* IgG

***H. pylori* IgG (ELFA)**	**SENSITIVITY**	**SPECIFICITY**	**PPV**	**NPV**
	100%	44.90%	28.95%	100%

Figure 2: Sensitivity, Specificity, Positive Predictive Value & Negative Predictive Value of *H. pylori* IgG (ELFA) compared with Modified Giemsa Staining.



Values: True Positive: 11**,** False Negative: 0**,** False Positive: 27**,** True Negative: 22

Discussion

The present study attempted to correlate the serological test *H. pylori* IgG based on the principle of Enzyme linked fluorescent assay with histopathological detection of *H. pylori* by Modified Giemsa staining.A total of 60 patients with upper gastrointestinal symptoms were enrolled in the study. Among them 43 (71.67%) were males and 17 (28.33%) were females. The maximum number of patients in this study were in the age group of 41 to 60 years. (35%).

In the study conducted by Mones M. Abu Shady et al[(10)]out of 100 patients, 57 were males and 43 were females. Similar findings were seen in the study by Ahmed K S et al[(11)] out of 500 patients, 300 were males and 200 were females which is comparable to the present study that showed males were more affected than females.
The prevalence of *H.pylori* infection in the general population varies widely from 40% to 50% in developed countries to as high as 90% in some parts of the developing world. The reported frequency in India has ranged from 31% to 84%.[(12),] In the present study, the prevalence of *H. pylori* infection (using the serological method of diagnosis) was found to be 63.3% and 18.3 % (using the histological method).In one study conducted by Mihai Danciu et al [(13)] showed a prevalence of 58.6 % of *H. pylori* infection (using the serological method of diagnosis) and 51.4 % (using the histological method).

Serological tests are commercially available, easy to preform, and inexpensive and therefore have been recommended for the diagnosis of *Helicobacter pylori* infection. Many serological tests, mainly immunoglobulin G (IgG) based, have been validated against invasive methods. [(14)]The seroprevalence of *H. pylori* infection in the present study was 63.3%, which was similar with the study of Rajan et al, [(15)] that showed seroprevalence of 68.3%. While in other studies conducted by Balan K et al,[(1)] Booth et al [(16)] and Perez-Perez et al [(17)]seroprevalence was low i.e., 35.8%, 30.63% and 56.09% respectively.

In our study, serology by ELFA technique to detect IgG antibodies against *H. pylori* did not correlate significantly with presence of *H. pylori* on histology examination of biopsy by Modified Giemsa staining.Out of 38 cases positive by serology, only 11 (28.9%) showed visible *H. pylori by* Modified Giemsa staining. The positivity rate of histopathological diagnosis of *H. pylori* was low in our study as compared to other studies

conducted by Rajan A et al[15], 30 (73.17%) out of 41 cases , Perez-Perez et al[17], 33 (71.7%) out of 46 cases &Sahar Iqbal et al[18], 25 (83.5%) out of 30 cases.

There can be several explanations for this. It may occur in chronic atrophic gastritis or intestinal metaplasia of gastric mucosa, a common condition in the evolution of *H. pylori* gastritis. In these patients, the gastric cavity becomes hostile to *H. pylori*, the organism disappears from the mucosa but antibody titters persist in the range of diagnostic titters for *H. pylori* infection. [15] In contrast, patients who have had a previous infection with *H. pylori* and where antibody levels were still elevated may have a negative histology and a positive result of serology. [21, 22].

In the present study Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of *H. pylori* IgG serology by ELFA technique against histological examination of biopsy defined as the gold standard was found to be 100%, 44.90%, 28.95% and 100% respectively.

The reported sensitivity and specificity of IgG serology is highly variable, ranging from 30% to 100 %[19, 20, 21]. In a study conducted by Angelo Locatelli[23] and Yoshihisa et al [14] the Sensitivity, Specificity, Positive Predictive value and Negative Predictive value was 64%, 83.7%, 82%, 66.6% and 94.8%, 89%, 91%, 93.6% respectively. In both the studies, ELISA technique for serology was used.When we compared the sensitivity and specificity of serology against histology examination of biopsy defined as the gold standard, failure of the biopsy methods to detect the organisms may decrease the serological true-positives and increase the false positives. False Negative biopsies could occur when the active site of infection was missed because of the patchy distribution of *H. pylori* in the stomach. Multiple biopsy specimens from different areas of the stomach may reduce sampling errors. [14]

Conclusion

The study was based on the "Correlation of Serology with histology based detection of *Helicobacter pylori* infection. The study highlights the following findings: A total of 60 patients with upper gastrointestinal symptoms were enrolled in the study. Among them 43 (71.67%) were males and 17 (28.33%)were females. The maximum number of patients in this study were in the age group of 41 to 60 years (35%). In the present study, the prevalence of *H. pylori* infection (using the serological method of diagnosis) found to be 63.3%and 18.3 % (using the histological method).The seroprevalence of *H. pylori* infection in the present study was 63.3%. In our study, serology by ELFA technique to detect IgG antibodies against *H. pylori* did not correlate significantly with presence of *H. pylori* on histology examination of biopsy by Modified Giemsa staining. Out of 38 cases positive by serology, only 11 (28.9%) showed visible *H. pylori* in biopsy *by* Modified Giemsa staining. The positivity rate of histopathological diagnosis of *H. pylori* was low in our study. In the present study Sensitivity, Specificity, Positive Predictive value and Negative Predictive value of *H. pylori* IgG serology by ELFA technique against histological examination of biopsy defined as the gold standard found to be 100%, 44.90%, 28.95% and 100% respectively. False Negative biopsies could occur when the active site of infection was missed because of the patchy distribution of *H. pylori* in the stomach. Multiple biopsy specimens from different areas of the stomach may reduce sampling errors. Positive IgG serology results are evidence of contact with *H. pylori* but do not necessarily indicate current infection. As this is institutional based limited study further evaluation of the test has to be done with a bigger sample size to arrive at a conclusion for this disparity.

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