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Prevalence Of *Helicobacter pylori* And Its Serological Detection By Elfa Method.

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ABSTRACT

Helicobacter pylori (*H. pylori*) infection is a significant global health concern, strongly associated with gastric diseases such as gastritis, peptic ulcer, and gastric cancer. This study aims to assess the prevalence of *H. pylori* infection using its serological detection by Enzyme-Linked Fluorescent Assay (ELFA) method and evaluate its diagnostic sensitivity. The research problem of this study typically revolves around understanding the extent of *H. pylori* infection in a specific population and assessing the effectiveness of the Enzyme-Linked Fluorescent Assay (ELFA) in detecting the bacterium. A total of 120 clinical samples were collected during normal endoscopic procedure of patients with abdominal discomfort or duodenal ulcers, comprising 60 male and 60 female patients. Enzyme-Linked Fluorescent Assay (ELFA) using VIDAS family machine is employed to detect the *H. pylori* IgG antibody. The results showed an overall *H. pylori* IgG antibody positivity rate of 52.5% (63/120). Among males, 65% (39/60) tested positive, while 35% (21/60) were negative. In females, 40% (24/60) were positive, and 60% (36/60) were negative. The findings indicate a higher prevalence of *H. pylori* IgG antibodies in males compared to females. The ELFA method demonstrated robust sensitivity in detecting *H. pylori* antibody providing reliable diagnostic insights. These results underscore the importance of gender-specific strategies for the detection and management of *H. pylori* infections. Further studies are recommended to explore the method's performance in diverse populations.

Keywords: *Helicobacter pylori*, IgG Antibody, Prevalence, ELFA.

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INTRODUCTION

Helicobacter pylori(H. pylori), previously known as *Campylobacter pylori*, are Gram-negative, spiral, microaerophilic bacterium that colonizes the human gastric mucus layer in approximately half of the world's population [1]. The bacteria enter the body and lives in the digestive tract. After many years, in some peoples they can cause sores, called ulcers, in the lining of stomach or the upper part of small intestine. Infection with *H. pylori* is common [2]. Up to 85% of people infected with *H. pylori* never experience symptoms or complications. It is transmitted from person to person through close contact and exposure to faecal matter or vomitus more of the world gets access to clean` water and sanitation [3]. For some people, an infection can lead to cancer [4]. For some people, Infection with *H. pylori* trigger a chronic gastric inflammation that can progress to atrophy, peptic ulcer, distal gastric adenocarcinoma and primary gastric mucosa associated lymphoid tissue lymphoma. *Helicobacter pylori* is a group I carcinogen cause Mucosa-associated lymphoid tissue (MALT) lymphoma is a low-grade B-cell marginal zone lymphoma [23].

The prevalence of *Helicobacter pylori* infection shows high geographic variability, markedly from country to country and in a country, region to region. *Helicobacter pylori (H. pylori*) infection is still a big issue in gastroenterology field [5]. Its relationship with gastrointestinal malignancies now is widely known and the extra-gastrointestinal manifestation of this epidemic bring new problems. Although the prevalence is decreasing in developed countries, the resistance rate of some strains to standard therapy needs more attention and new strategies [6].

Testing for *H. pylori* is not routinely recommended, it is recommended if peptic ulcer disease or low-grade gastric MALT lymphoma is present, after endoscopic resection of early gastric cancer, for first-degree relatives with gastric cancer, and in certain cases of dyspepsia. Several methods of testing exist; including invasive and noninvasive testing method [7]. These tests determine the presence of *H. pylori*. Invasive method include Biopsy by endoscopy has traditionally been used to obtain gastric or duodenal tissue specimens for subsequent staining, culture, or direct urease detection. With biopsy false positive results can occur in infected individuals due to non- uniform distribution of *H. pylori* in the sample or by obtaining tissue with non-viable or non-urease producing *H. pylori*. Invasive method also involves patient discomfort, risk, and costly to perform. Noninvasive methods include urea breath test and serological methods. Urea breath test detect *H.pylori* presence via its highly active urease production urea labeled with c^{14} or c^{13} ingested by the patient, and presence of exhaled carbon dioxide is determined via scintillation or mass spectrometry.

Serological methods such as enzyme immune assays are inexpensive, quick, and easy to perform when compared to invasive methods. The major advantage is that serological methods do not relay on the accuracy of sampling. In epidemiological studies, serum tests could offer high sensitivity and specificity [8].

Serum assaying of anti-*H pylori* IgG and IgA antibodies could be used for the determination of prevalence of acute and chronic infections [9]. In general, the serum levels of anti-*H pylori* IgG antibodies were increased in the presence of infection and could be used as a marker.

H. pylori IgG detection using Enzyme Linked Fluorescent Assay (ELFA) is an automated qualitative test for use on the instrument of the VIDAS family, for the detection of anti-*Helicobacter pylori* IgG antibodies in human serum or plasma (EDTA). VIDAS *H. pylori* assay is indented as an aid in diagnosis of *H. pylori* infection in an adult symptomatic population [10, 11]. Through this study the serological detection of *H. pylori* by ELFA technique is employed. The prevalence and detection of *Helicobacter pylori*, is associated with gastrointestinal disorders, have been pivotal areas of research in the field of clinical diagnostics. Enzyme-Linked Fluorescent Assay (ELFA) has emerged as a sophisticated technique contributing to the accurate identification of *H. pylori* infections. ELFA leverages the principles of enzyme-linked immunosorbent assay (ELISA) combined with fluorescence detection [12]. This method involves binding specific antibodies to *H. pylori* antigens, creating a reaction that produces a measurable fluorescent signal. ELFA's sensitivity and specificity make it a reliable tool for detecting *H. pylori* antibodies or antigens in patient samples [13, 14].

As we delve into this exploration, we analyzed the prevalence of *H. pylori* infections across diverse populations and regions. Simultaneously, scrutinized the efficacy of ELFA in providing rapid and precise results, underscoring its potential impact on enhancing diagnostic accuracy and aiding in the timely management of *H. pylori*-related conditions. Through this investigation, we aim to contribute valuable

insights into both the prevalence patterns and the diagnostic landscape of *H. pylori*, shedding light on advancements that hold promise for improved healthcare outcomes.

Importance of the Study

Public Health: Understanding prevalence helps in tailoring prevention and treatment strategies.

Clinical Relevance: Identifying infection early can lead to timely interventions and reduce the risk of complications.

Research Contribution: Adding to the body of knowledge about *H. pylori* and its impact on gastrointestinal health. This study's findings can guide both clinical practice and public health policies regarding *H. pylori* management.

Aim

The aim of the study is the evaluation of the prevalence of *Helicobacter pylori* and it's serological detection by a ELFA technique. a sensitive and specific diagnostic tool for identifying *H. pylori* infections.

Objectives

- Diagnostic Accuracy: Assess the effectiveness of the ELFA method in detecting *H. pylori* antibodies.
- Prevalence Study: Determine the prevalence of H. pylori infections in a specific population. This can help identify at-risk groups and inform public health strategies.

STUDY SETTINGS AND DESIGN

The study was conducted at the department of Microbiology laboratory in KVM Hospital, Cherthala, Kerala, India. The study conducted was a cross-sectional study, during the period of January 2018 to June 2018. Total of 120 patients sample test were done for the study.

Requirements

Specimen

- Sera or plasma

Inclusion Criteria

- Adult patients with a normal endoscopic examination or showing a peptic disease included.

Exclusion Criteria

- Previous Treatment for H. pylori, Recent Gastrointestinal Surgery, pregnancy

Collection

Serum sample was collected when venipuncture was performed for pre-endoscopic blood work.

- 2ml of blood sample is collected by venipuncture method
- Serum is separated by centrifugation.
- 100 microliter serum is required for the test

ELFA Technique

Principle of the Assay

The assay principle combines a two step enzyme immunoassay sandwich method with a final fluorescent detection(ELFA).All of the assay steps as well as the assay temperature are controlled

automatically by the instrument .The solid space receptacle(SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for this assay are ready to use and pre dispensed in the sealed reagent strips. After preliminary wash and sample dilution steps, the sample is cycled in and out of the SPR for a specific length of time. IgG antibodies to *H. pylori* present in the specimen will bind to *the H. pylori* antigen coating in the interior of the SPR. Unbound sample components are washed away.

Anti human IgG antibodies conjugated with alkaline phosphates are cycled in and out of the SPR and will attach to any human IgG bound to the SPR well. A final wash step removes the unbound anti – human antibody conjugate.

During the final detection step, the substrate (4 methyl umbellyferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalysis the hydrolysis of this substrate into a fluorescent product (4 methyl umbelliferon), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is measured by the optical scanner in the instrument . At the end of the assay, results are automatically calculated by the instrument, a test value is generated and a report is printed for each sample.

Procedure

- Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.
- Use one HPY strip and HPY SPR for each sample, control and standard to be tested.
- The test is identified by the HPY code on the instrument.
- Mix the standard, controls and samples.
- For this test, the standard, control and sample test portion is 100 micro liters.
- Insert the HPY SPR's and HPY strips into the instrument.
- Initiate the assay as directed in the operator's manual.
- The assay will be completed within 35 minutes.

Result And Interpretation

- Once the assays completed, results are analyzed automatically by the computer.
- Fluorescence measured twice in Reagent strips reading cuvette for each sample tested.
- The first reading is a background reading of the substrate cuvette before the SPR is introduced in to the substrate.
- The second reading is taken after incubating the substrate with enzyme remaining on the interior of the SPR.
- The RFV [Relative Fluorescence Value] is calculated by subtracting the background reading from the final result.
- This calculation appears on the result sheet.
- The test value obtained by dividing the patient RFV value by the standard RFV value.
- The test value then compared to the threshold stored by the instrument and a final result is interpretated.
- Result with the test values less than the lower threshold indicate that the patient does not have detectable anti-*H. pylori* antibodies.

Threshold And Interpretation Of Result

Test Value Threshold	Interpretation
TV<0.75	NEGATIVE
0.75<TV<1.00	Equivocal
TV>=1.00	Positive

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OBSERVATION AND RESULTS

Total Samples	Number Of Positives				Number Of Negative			
	Male	%POSITIVITY	Female	%POSITIVITY	Male		female	
120	39	32.5%	24	20%	21	17.5%	36	30%

Contingency Table

	Positive	Negative	Total
Male	39	21	60
Female	24	36	60
Total	63	57	120

Statistical Analysis

Proportions

Proportion of Males

$$pm=39/60=0.65$$

Proportion of Females

$$pf=24/60=0.4$$

Overall Proportion of Positive Cases

$$P=39+24/120=63/120=0.525$$

Confidence Intervals

Overall Confidence Interval

Using the formula for the confidence interval for the overall proportion:

$$CI=P\pm Z\times\sqrt{P(1-P)/n}$$

Where:

- $P=0.525$
- $n=120$
- $Z\approx1.96$ for a 95% confidence level.

Standard Error (SE)

$$SE=\sqrt{0.525\times(1-0.525)/120}$$

$=\sqrt{0.525\times0.475/120}$
$\approx\sqrt{0.002073}$
≈0.0455

Confidence Interval

$$CI=0.525\pm1.96\times0.0455\approx0.525\pm0.0893$$

Thus, the overall confidence interval is approximately (0.436,0.614)

Hypothesis Testing

Null Hypothesis (H0)

The proportions of positive cases in males and females are equal.

Alternative Hypothesis (Ha)

The proportions of positive cases in males and females are different.

Test Statistic Calculation

Combined Proportion:

$$P=63/120=0.525$$

Now calculate the standard error for the difference in proportions:

$$SE=\sqrt{P(1-P)}\ (1/nm+1/nf)$$

Where

- nm=60
- nf=60

Calculating

$$SE=\sqrt{0.525\times0.475(1/60+1/60)}$$
$$=\sqrt{0.525\times0.475\times2/60}$$
$$\approx\sqrt{0.0083}\approx0.091$$

Calculating the Z-score

$$Z=pm-pf/SE$$

$$=0.65-0.4/0.091$$
$$\approx0.250.091\approx2.75$$

P-value Calculation

For Z=2.75

The corresponding p-value is approximately 0.003 (two-tailed test).

CONCLUSION

P-value: Approximately 0.003.

Significance: Since the p-value (0.003) is less than 0.05, we reject the null hypothesis.

Summary of Findings

Prevalence of H. pylori antibodies

- Males: 65%
- Females: 40%
- Overall: 52.5%

95% Confidence Interval for Overall Proportion

(43.6%, 61.4%)

Statistical Significance

There is a significant difference in the prevalence of H. pylori antibodies between males and females.

DISCUSSION

The results of our study reveal important insights into the gender distribution and overall prevalence of the condition under investigation. Total of 120 patients sample tests were done for the study , comprising 60 males and 60 females, who were came in the hospital at gastroenterology department with abdominal discomfort or duodenal ulcer and they were undergoing normal endoscopic procedures. The blood samples are collected and tested for IgG antibodies of *H. pylori* by ELFA method using VIDAS family machine. The result data indicates that **65%** of the positive cases were males. This suggests that males might be at a higher risk of the condition under study in this specific sample. The frequency of *H. pylori* infection in patients diagnosed with duodenal ulcers is approximately 80% in all age groups.

The prevalence of *Helicobacter pylori* infection exhibits considerable geographic diversity, influenced by factors like socioeconomic conditions, hygiene practices, and sanitation levels [15]. Higher prevalence is often observed in developing regions and among individuals with lower socioeconomic status. This suggests a potential link between the living environment and *H. pylori* transmission [16].

Moreover, studies have indicated a correlation between age and infection, with a higher prevalence in older individuals. The mode of transmission, primarily through oral-oral or fecal-oral routes, contributes to the widespread dissemination of the bacterium. Cultural practices, dietary habits, and family structure can also impact the likelihood of H. pylori acquisition.

In a study, *H. pylori* infection has been found worldwide, but geographical distribution of prevalence varies widely. It is always higher in developing countries (70 - 90%) than in industrialized countries (20 - 30%), higher prevalence being associated with low socio-economic levels [17]. In Caucasian populations in the United States and other industrialized countries, H. pylori infection is infrequent in childhood but with each year of age the prevalence increases 0.5 - 2%, reaching about 50% in those who are 60 or older. Prevalence rates appear to be higher in blacks and Hispanics than in whites [18, 19]. The frequency of H. pylori infection in patients diagnosed with duodenal ulcers is approximately 80% in all age groups [20].

The study titled *"Comparative evaluation of the diagnostic tests for Helicobacter pylori and dietary influence for its acquisition in dyspeptic patients: A rural hospital-based study in central India"* by Kaore NM, Nagdeo NV, and Thombare VR, aims to evaluate and compare the diagnostic tests for *Helicobacter pylori* (*H. pylori*) infection in a rural hospital setting in central India. Additionally, the study examines the role of dietary factors in the acquisition of H. pylori in dyspeptic patients [21].

Our study correlate with study of Hooi, J. K. Y., Lai, W. Y., Ng, W. K., et al they performed a systematic search of the MEDLINE and EMBASE databases for studies of the prevalence of H pylori infection published from January 1, 1970 through January 1, 2016. They analyzed data based on United Nations geoscheme regions and individual countries. They used a random effects model to calculate pooled prevalence estimates with 95% confidence intervals (CIs), weighted by study size, extrapolated 2015 prevalence estimates to obtain the estimated number of individuals with H pylori infection. In this systematic review and meta-analysis to assess the prevalence of H pylori infection worldwide, observed large amounts of variation among regions-more than half the world's population is infected. These data can be used in development of customized strategies for the global eradication [22].

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CONCLUSION

This study aimed to assess the prevalence of *H. pylori* IgG antibodies in a sample of 120 individuals using the ELFA technique. The findings reveal significant differences in antibody prevalence between genders, which are summarized as follows:

Prevalence Rates: The overall prevalence of *H. pylori* antibodies in the sample was 52.5%, with 39 out of 60 males (65%) testing positive and 24 out of 60 females (40%) testing positive.

Statistical Significance: A statistical analysis indicated a significant difference in the prevalence rates between males and females, with a calculated p-value of approximately 0.003. This p-value is less than the conventional significance level of 0.05, leading us to reject the null hypothesis that there is no difference between the two groups.

Confidence Intervals: The 95% confidence interval for the overall prevalence of positive cases was found to be between 43.6% and 61.4%, indicating that we can be 95% confident that the true prevalence lies within this range.

Implications: The findings suggest that *H. pylori* infection may be more prevalent in males than in females in the studied population. This difference could have implications for targeted screening and treatment strategies, as well as for understanding the epidemiology of *H. pylori* infections.Further studies are recommended to explore the underlying factors contributing to the observed gender differences, including lifestyle, dietary habits, and environmental factors. Additionally, expanding the sample size and including diverse demographics could enhance the generalizability of the findings.

In summary, this study highlights the significance of gender in the prevalence of *H. pylori* antibodies and emphasizes the need for tailored public health strategies to address this infection.

Figure 1: Mini VIDAS

Figure 2: HPY strip before test.

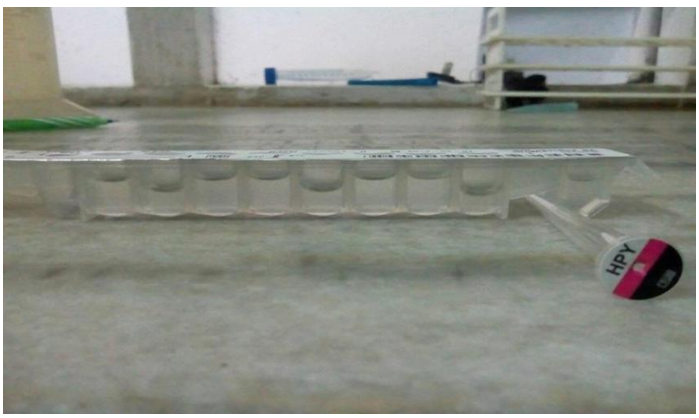

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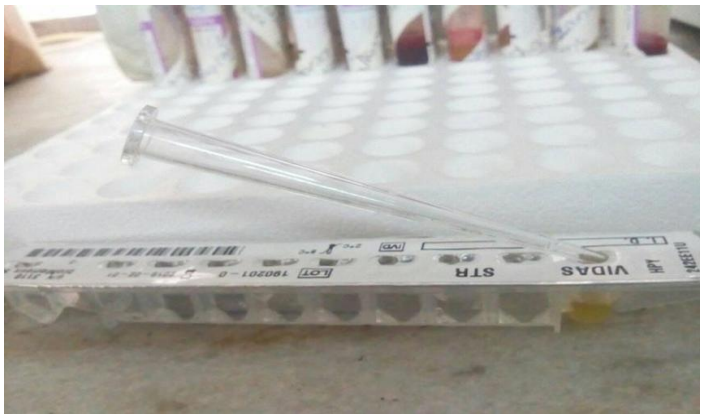
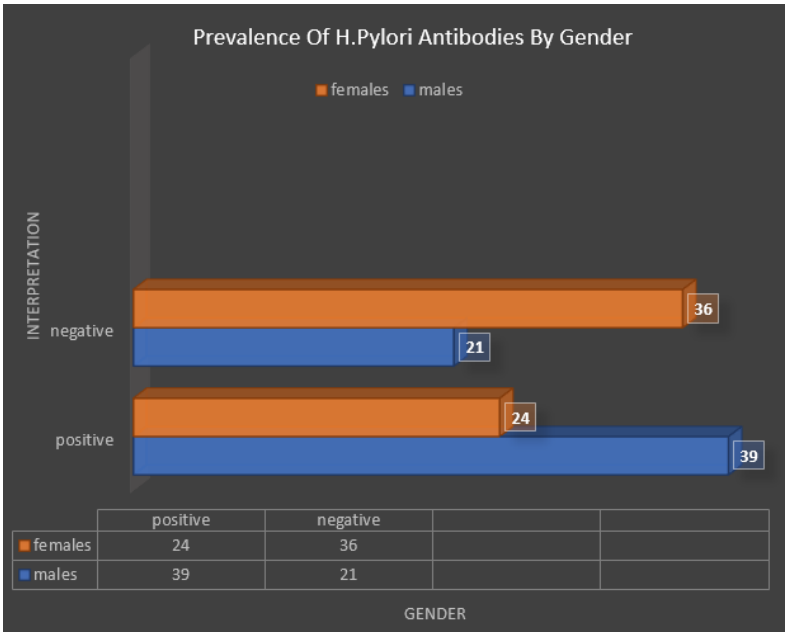
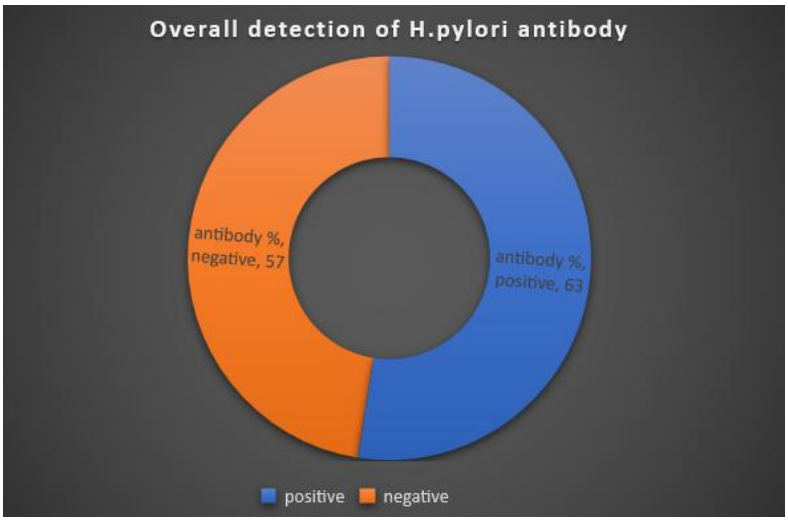
Figure 3: HPY strip after test



Graphical Representation

Comparison of the proportions of positive cases between males and females.





Pie Chart: Shows The Distribution Of Positive And Negative Cases Overall

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