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Comparative evaluation of stool antigen immunoassay and blood antibody test methods for the screening of *Helicobacter pylori* infection in asymptomatic population at tertiary care hospital

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Abstract
Helicobacter pylori is a bacterial pathogen associated with various gastrointestinal disorders, including gastritis, peptic ulcers, and even gastric cancer. Early detection of *H. pylori* infection in asymptomatic individuals can aid in preventing the progression of these diseases. This research paper aims to compare the diagnostic accuracy and cost-effectiveness of two commonly used screening methods, stool antigen immunoassay and blood antibody tests, for detecting *H. pylori* infection in asymptomatic individuals attending a tertiary care hospital. The study will involve a large sample of asymptomatic participants who will undergo both types of tests, and the results will be compared to a reference standard, such as endoscopy or biopsy. The findings will provide valuable insights into the most efficient and reliable method for *H. pylori* screening in individuals without apparent symptoms, contributing to better disease management and prevention strategies.

Keywords: Blood Antibody, *Helicobacter pylori*

Introduction
Helicobacter pylori are a Gram-negative bacterium that is believed to be a key reason for chronic gastritis, gastric cancer, peptic ulcer illness, and lymphoid tissue lymphoma of the gastric mucosa. All of these conditions are related to inflammation of the stomach lining. It is estimated that some percentage of the whole population is responsible for transporting this bacterium. It has been hypothesized that the prevalence of *H. pylori* infection is greater in non-industrial nations in comparison to created nations. Oro-oral, faeco-oral, gastro-oral, gastro gastric and individual-to-individual transmissions are some of the several modes of transmission that have been postulated by logical literary works. Other modes of transmission include transfer from animal to human. Some of the factors that can increase a person's likelihood of contracting *H. pylori* include their race, socioeconomic standing, where they live (Especially in rural areas), their age, the quality of the sterile environment, the food they eat, the quality of the water they drink, and the level of education they have received. The tests that are performed in order to determine whether or not an individual has been infected with *H. pylori* may be divided into two primary categories: invasive and non-invasive. The invasive methods, which include culture, endoscopy, and biopsy for histology as well as a rapid urease test evaluation, are generally considered to be more accurate than the non-invasive methods [1].

The use of serology, urea breath tests, and stool antigen examinations are all included in the non-harmful procedures. The urea breath test is considered to be the most reliable method out of the three non-invasive techniques available for determining whether or not an individual has *H. pylori* infection. However, despite its precision, the urea breath test has been criticized for being unreliable in very young children, cumbersome, expensive, and, as a result, unavailable in certain countries. In most cases, the serologic tests are carried out in order to identify the presence of specific flowing antibodies against *H. pylori*. They are financially accessible, simple to perform, and reasonable, but they have been answered to be temperamental for the determination of *H. pylori* because they can't separate between active and asymptomatic colonization and past and current *H. pylori* infection [2].

Objectives

1. To compare the prevalence of *H. pylori* in subjects with dyspepsia with age and sex matched controls.
2. To determine the relevance of *H. pylori* screening by the stool antigen test in Indian subjects with dyspepsia.

Research Methodology

The accuracy of the test that is used to determine whether or not an individual has an infection with *H. pylori* is extremely important to the success of any strategy that is chosen. The purpose of this investigation is to compare and contrast two invasive examinations with two painless "tests. In addition, risk variables associated with *H. pylori* infection were investigated and analyzed".

Permissions and Ethical Consideration

"For the sample collection, the appropriate consents were obtained from the pertinent specialists, and authorizations were obtained from the patients for both the meeting and the sample collection. Patients were provided information on the purpose of this research, and prior to any sample collection or meeting, patients were given the opportunity to verbally consent to participate in the study" [3].

Patients

Patients who were willing to be examined by their primary care physician based on clinical reasons were considered to be qualified members who could have *H. pylori* infection. There was no upper age limit for members of the organization.

Gastric biopsies

An examination of gastric biopsies was carried out by a specific group of medical professionals, during which the patient underwent upper gastrointestinal endoscopy and a biopsy was obtained, which was then placed in a sterile phosphate support for ultra-quick urease testing (URUT) and for engrave spreads. Tissue for the biopsy was obtained with the use of conventional endoscopic biopsy forcipes. The gastro scopes (Olympus GIF Type Q40 - 2300903) and the Olympus Video trolley television z CLE-10 were employed in the procedure".[4]

Blood Sample for Serological Evaluation

A plain vial devoid of any anticoagulant was used to collect approximately 5 milliliters of venous blood from each patient. The patient's name, together "with the patient number and the date of collection, was written on the vial". The vial was heated in a water bath at 37^0c for half an hour, or blood had been clotted. After the blood had been clotted, it was centrifuged, and the serum was aspirated into a few (200 µl) Eppendorf tubes. These tubes were then stored at -70 ^{0}C until they were analyzed.

Sample Processing

Blood sample

In order to detect IgG/IgM with an enzyme-linked immunosorbent assay (ELISA), serum was allowed to warm up to room temperature (ELISA kit). Tests were performed on 96 wells and were manufactured in Germany [5].

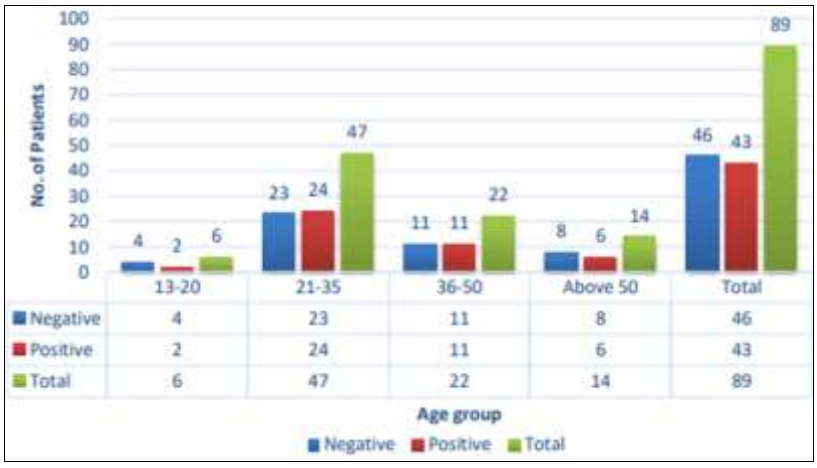
Principle of the ELISA test

The *H. pylori* antigen that has been sterilized is coated on the microwells' outermost layer. Patients' serum that has been diluted is then added to the wells, and any *H. pylori* IgM/IgG-explicit antibody that is present ties itself to the antigen if it is there. Everything that wasn't bound up is lost in the flood. Compound form is introduced, which creates a link between the antibody-antigen complex and the antibody. A wash is performed on the abundance compound form before the addition of an answer of TMB Reagent. At a certain point in time, the catalyst-form synergist reaction comes to an end. The strength of the coloring that is produced "is proportional to the amount of IgM/IgG-explicit antibody that is present in the example. The results are analyzed using an ELISA reader, which is then compared and contrasted with a calibrator and a control".

Results and Discussion

***H. pylori* Infection among the Study Sample**

As was demonstrated previously, the only people who were considered to have a dynamic *H. pylori* infection were those who tested positive for URUT or for possibly positive levels of methylene blue. This is because these individuals were the only ones whose results indicated that they had a dynamic infection. Only
48.3 percent of the cases met this threshold, which indicates that they did not have adequate proof. The following Table No.1 provides a breakdown of the *H. pylori* infection rate among persons of varying ages in India. According to the information contained in the table, persons between the ages of 21 and 35 years old had the most substantial improvement in their situation (51.1%). Graph No.1



Graph 1: Age distribution of *H. pylori* positive subjects"

Table 1: Age distribution of *H. pylori* positive subjects"

Age (Year)	*H. pylori* infection				Total		*P*- Value
	Negative		Positive				
	No	%	No	%	No	%	
13-20	4	66.7	2	33.3	6	100	0.832
21-35	23	48.9	24	51.1	47	100	
36-50	11	50.0	11	50.0	22	100	
Above 50	8	57.1	6	42.9	14	100	
Total	46	51.7	43	48.3	89	100	

Risk factors For *H. pylori* Infection"

For the acquisition of *H. pylori*, the writing had a great number of risk factors that were entangled. In addition, there were a variety of reports, some of which contradicted one another, about virtually all of the contentious factors. The majority of the analyzed written material suggested that these differences might be attributed to differences in either the methods of information collection or the geological characteristics of the area. The purpose of this study was to determine the risk variables associated with the acquisition of *H. pylori* by the study population [6].

Personal variables

The age group 21–35 years old had the highest proportion of participants with good outcomes (51.1%), whereas the age group 13–20 years old had the highest proportion of patients with negative outcomes. There were 89 subjects who completed all of the material (66.7%). The most significant favorable outcome was observed in females, where it was found to be 53.1%, in contrast to male, where it was observed to be "45.6%. There were no significant factual results associated with age or sex that might be taken into consideration as a risk factor". The weight group that ranged from 98 to 118 kilograms had the fewest positive results, whereas the weight group that ranged from 35 to 55 kilograms had the highest percentage of positive results (87.5%). There were no significant differences seen in terms of marital status. Subjects who were married and those who were not had nearly the same level of influence Table No.2

Life style variables

The way of life differs from one region to another and is influenced by a wide range of elements, including economic variations, geological dispersion, and religious practices. These types are employed in the process of justifying contradictory results obtained by distinct professionals. According to the data presented "in Table No.3, there is not a significant difference in the prevalence of *H. pylori* infection between smokers and nonsmokers. The percentage of people who had favorable results after quitting smoking was 49.1% whereas the percentage of people who had positive outcomes after smoking was 46.9%. In point of fact, even the number of cigarettes smoked does not appear to impact or increase the likelihood of becoming infected. There are statistically significant differences between the persons who drink tea and those who don't drink tea, and the P-value for this comparison is 0.045" [7].

Subjects who consume coffee on a regular basis have a 41.0% lower risk of becoming infected with the *H. pylori* bacteria, in comparison to those who don't consume coffee on a regular basis (54.0%). In any event, the thing that really matters is not something that can be measured in terms of its size. People who drank more than five cups of coffee per day had a much-reduced risk of becoming infected with *H. pylori* than those who drank less than five cups per day.

Based on a quantitative analysis of the available data, the kind of water consumed when the individual was a child has the potential to be regarded as a risk factor with a P-value of 0.018. As can be seen in Table No.3, the proportion of subjects who had a positive outcome was significantly higher in those who had consumed water from a region or well when they were younger (53.2%), in contrast to those who had consumed separated water when they were adolescents (16.7%), who only had positive outcomes. In any event, the kind of water consumed as an adult had no effect on the outcome of the *H. pylori* infection. In order to determine the individuals' levels of oral hygiene, we asked them whether or not they suffered from dental grumbles. The prevalence of *H. pylori* infection was considerable in participants who suffered from oral gripes (64.3%), but the significance of this result could not be determined [8].

It was determined that coming into contact with different species posed a possible threat. The persons who do not handle creatures or those who do handle creatures were identified as the subjects of the study. It was discovered that 50.0% of the people who had contact with animals had a favorable response, whereas only 47.8% of the people who don't have contact with animals did. The patients who travelled had a positive outcome of 41.2%, while patients who didn't travel had a positive outcome of 50.0%, and there was no factually significant difference noticed between the two groups' positive outcomes. The subjects were questioned as to whether or not they had travelled outside the country.

Drugs history

The historical context of prescriptions for headache medicine, various calming pharmaceuticals, and a gastrointestinal treatment known as a proton pump inhibitor (PPI) was analyzed, and the results revealed that there were no significant differences between the groups. This information is presented in Table No.4 [9].

Antibiotics intake during the last month

A look at the patient's use of antibiotics over the course of the previous month is summarized in Table No.5, which reveals that the investigation revealed no statistically significant findings [10].

Table 2: Personal factors

Variable		*H. pylori* infection				P- value
		Negative		Positive		
		No.	%	No.	%	
Age	13-20 y	4	66.7	2	33.3	0.832
	21-35y	23	48.9	24	51.1	
	36-50y	11	50.0	11	50.0	
	>51y	8	57.1	6	42.9	
Sex	Male	31	54.4	26	45.6	0.323
	Female	15	46.9	17	53.1	
Weight	35-55	1	12.5	7	87.5	0.125
	56-76	23	57.5	17	42.5	
	77-97	20	52.6	18	47.4	
	98-118	2	66.7	1	33.3	
Marital Status	Married	38	50.7	37	49.3	0.440
	Single	8	57.1	6	42.9	

Table 3: Life style variables

Variable		*H. pylori* infection				P-Value
		Negative		Positive		
		No	%	No	%	
Smoking	No	29	50.9	28	49.1	0.507
	Yes	17	53.1	15	46.9	
If smoking No. of cigarettes/day	1-20	13	48.1	14	51.9	0.208
	>20	4	80.0	1	20.0	
Drink Tea	No	1	14.3	6	85.7	0.045
	Yes	45	54.9	37	45.1	
If Drink tea No. of cup/day	1-5	36	54.5	30	45.5	0.564
	>5	9	56.3	7	43.8	
Drink Coffee	No	23	46.0	27	54.0	0.158
	Yes	23	59.0	16	41.0	
If Drink Coffee No. of cup/day	1-5	20	55.6	16	44.4	0.194
	>5	3	100	-	-	
Type of drinking water During childhood	Municipality or well water	36	46.8	41	53.2	0.018
	Filtered water	10	83.3	2	16.7	
Type of drinking water During adulthood	Municipality or well water	8	50.0	8	50.0	0.548
	Filtered water	37	50.7	34	46.5	
Dental complains	Yes	5	35.7	9	64.3	0.156
	No	41	54.7	34	45.3	
Consumed Drug	No	28	49.1	29	50.9	0.336
	Yes	18	56.3	14	43.8	
Consumed antibiotics in the last month	No	23	46.9	26	53.1	0.218
	Yes	23	57.5	17	42.5	
Contact with animals	No	36	52.2	33	47.8	0.532
	Yes	10	50.0	10	50.0	
Travelling abroad	No	36	50.0	36	50.0	0.351
	Yes	10	58.8	7	41.2	

Table 4: Drug intake relation to *H. pylori* infection"

Do you take drug	*H. pylori* infection				Total		*P*- Value
	Negative		Positive				
	No	%	No	%	No	%	
No	28	49.1	29	50.9	57	100.0	0.336
Yes	18	56.3	14	43.8	32	100.0	
Total	46	51.7	43	48.3	89	100.0	

Table 5: Antibiotics intake relation to *H. pylori* infection"

Do you take antibiotics	*H. pylori* infection				Total		*P*-Value
	Negative		Positive				
	No	%	No	%	No	%	
No	23	46.9	26	53.7	49	100.0	0.218
Yes	23	57.5	17	42.5	40	100.0	
Total	46	51.7	43	48.3	89	100.0	

Socioeconomic status

The degree of education, monthly income, kind of convenience, number of rooms, number of people living in each room, type of water supply, and the sewage system were all documented and related to *H. pylori* infection for each and every one of the 89 participants who had participated in this study". As can be "shown in Table No.6, the level of education had no significant effect whatsoever on the prevalence of *H. pylori* infection". Those who had completed middle school had the highest significant increase in their likelihood of a favorable result (66.7%), while those who had completed college had the lowest likelihood (36.8%) [11].

Subjects' month-to-month salary was ordered to be distributed "to those with 20,000 Rs., 20,000-30,000 Rs., 30,000-40,000 Rs., and more than 40,000 Rs respectively". Subjects who had more than 40,000 incidents each month showed the greatest decrease in favorable result (22.20%). In spite of this, the significance of this conclusion was not very high [12]. No major differences were seen in terms of the kind of convenience, the "number of persons who lived in the convenience, the type of water, or the type of the sewage system" [13].

The study included both 46 individuals (20 males and 26 females) and 46 controls (20 males and 26 females). In all, there were 46 participants [14]. The average age of the patients was found to be 40.87±13.31 years, whereas the average age of the controls was found to be 40.83±13.20 years. There was nothing of quantitative significance to be found in any of the two meetings' mean times Table No.6. The ages of the two test participants and the controls ranged from 18 to 70 years, with 18 years serving as the base age [15].

Table 6: Demographic characteristics of subjects and control

Variable		Subjects (n=46)	Control (n=46)	□2	P-Value
Sex	Male	20(43.50%)	20(43.50%)	0.00	1.0
	Female	26(56.50%)	26(56.50%)		
Age (Years)	<30	10(21.70%)	10(21.70%)	t=0.016	0.987
	30-39	13(28.30%)	14(30.40%)		
	40-49	13(28.30%)	12(26.10%)		
	≥50	10(21.70%)	10(21.70%)		
	Mean Age (SD)	40.87(13.31)	40.83(13.20)		
Occupation	Students	5(10.90%)	6(13.0%)	7.18	0.127
	Traders	15(32.60%)	13(28.30%)		
	Civil Servants	18(39.10%)	26(56.50%)		
	Artisans	8(17.40%)	1(2.20%)		

Conclusion

"This study is the first of its kind to be conducted in the India to identify *H. pylori* infection and to attempt to determine potential risk factors associated with the microorganism's acquisition. As a direct result of the study that was done, the following conclusions might be drawn": The prevalence of *H. pylori* infection was found to be 48.3% among 89 individuals who had upper endoscopy. "The Ultra Rapid Urease test turned proven to be reliable, easy to prepare for and carry out, not expensive, and capable of providing the patient with the results quickly while they were still in the doctor's office. In any event, the selective use of the rapid urease test for the examination of *H. pylori* cannot be proposed in individuals who have a history of drug use". The use of drugs and anti-toxins appears to hamper tests that rely on either the presence of *H. pylori* or its mobility. The evaluation found that not one of the tests that were used to conduct the study could be relied upon to accurately diagnose an active *H. pylori* infection. The HpSAg test is a non-invasive, straightforward, and accurate method for determining whether *H. pylori* is present in a stool sample; nonetheless, its utilization for determination has to be investigated.

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