

Prevalence of Helicobacter Pylori Infection in Patients with Gastro-Esophageal Reflux Disease in South Bengal.

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Abstract:

Background: *Helicobacter pylori* residing in antral mucosa often found associated with gastroesophageal reflux disease (GERD). **Objective :** Investigating prevalence of *H. Pylori* in GERD patients in South Bengal by serology and histopathology. **Material and methods :** 100 cases selected with 50 controls. Serology done by demonstrating serum IgG . Endoscopic biopsy tissue divided into three portions and subjected to testing for rapid urease test, hematoxylin & eosin stain, Giemsa stain and scanning electron microscopy. **Results :** GERD commonly found in 26-35yrs with male predominance. *H.Pylori* was positive in rapid urease in 38/ 100 cases followed by IgG Elisa positivity (34) and modified Giemsa (27). 23 cases showed positivity by all three methods. 30 males ,9 females with GERD showed *H.Pylori* infection. **Conclusion :**Prevalence of *H pylori* is low in patients with GERD with statistical significant association with age, socio-economic status but since *H. pylori* infection considered a pre neoplastic condition; determination of its presence is mandatory.

Key words: *H. pylori*, GERD, urease, serology, giemsa.

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I. Introduction

Gastro-esophageal reflux disease (GERD) refers to abnormal exposure of esophageal mucosa to gastric contents. The evidence for an association between *Helicobacter pylori* (*H. pylori*) and GERD remains an enigma and largely uncertain. Patients with GERD frequently present with atypical symptoms other than heart burn or acid regurgitation which affects 21-44% of adult population.¹

Accurate diagnosis of GERD can be difficult with currently accepted criteria of heart burn, acid regurgitation, improvement with proton pump inhibitor, 24 hours oesophageal pH assessment and / or histological evidence of oesophagitis. Recent interest has focused on the relation between *H. pylori* infection and GERD.^{1,2}

H. pylori, a gram negative, flagellated micro-aerophilic, spiral, motile rod with distinctive feature of urease production, resides in antral mucosa; transmitted predominantly by faeco-oral route. In 1982, it was first isolated from gastric biopsy material by Warren and Marshall in Australia, later cultured by Marshall successfully in 1983. ^{1,2} In India 70-90 % population carries *H.pylori* as an acquired infection before the age of 10 years compared to 25-30 % in developed countries with increasing prevalence with age. ^{1,2}

Apart from GERD, it is also associated with type B chronic gastritis, duodenal and gastric ulcer, mucosa associated lymphoid tissue lymphoma (MALToma) and gastric adenocarcinoma.^{1,2} The International Agency for Research on cancer of WHO recommends *H. pylori* be classified as group - 1 carcinogen. ³ It colonizes the mucous layer of gastric antrum, fundus characterized by inflammatory reaction without epithelial invasion causing host tissue damage. It damages directly by affecting epithelial cells or indirectly by inducing an intense host inflammatory response. The diagnosis of *H. pylori* infection is currently based upon endoscopic biopsy based tests - rapid urease test (RUT), histopathological examination (HPE) with Haematoxylin and Eosin (H&E), and Giemsa stain, Scanning Electron Microscopy (SEM), culture and non-invasive tests like urea breath test, stool antigen test, ELISA and PCR. Isolation of *H. pylori* by culture is theoretical gold standard for detection of infection but it has been highly variable with success rate ranging from 30% to 73%.^{1,2,3} The simplest non-invasive test is measurement of anti *H. pylori* IgG antibody by ELISA or immunoblot.

Detection of *H. pylori* specific antibodies is consistent with prolonged chronic mucosal infection with IgA and IgG predominating. Since *H. pylori* specific IgG antibody titres decline after bacterial eradication, serological tests can be useful for monitoring success of therapy and as screening test for epidemiological studies. ^{3,4,5,6} An accurate diagnosis of *H. Pylori* infection is of value in targeting antibiotic based eradication

regimens in symptomatic patients.⁶In the present study, GERD patients subjected to anti H. pylori serology, RUT and HPE with SEM for assessment of H. pylori prevalence in South Bengal with control group.^{5,6,7}

II. Material And Methods

The prospective case control study conducted in a tertiary care teaching hospital in West Bengal for one year after taking clearance from the institutional ethical committee and consent of patient. 100 patients who attended and got admitted in the hospital with symptoms of GERD randomly selected with 50 healthy controls after proper history taking, clinical examination, taking consent and subjected to endoscopy, biopsy. Exclusion criteria comprised patient receiving anti H. pylori within 1 month, proton pump inhibitors within 2 weeks or H₂ receptor blockers within 1 week prior to endoscopy; patients with ischemic heart disease, chronic lung disease, diabetes mellitus, thyroid problems, malignancy, pregnant and lactating females and those on long standing NSAIDs.

Patients underwent two procedures of non-invasive and invasive types. The non-invasive one was serological estimation of IgG and invasive one was collection of endoscopic biopsy followed by HPE, RUT and SEM. Data analysis done by chi-square and student t-test. For serology; 5 ml. clotted blood sample aseptically collected and separated serum used for H.pylori IgG antibody (in case of delay, serum stored from 2-8⁰C up to 7 days or frozen if used > 7 days but < 6 months at -20⁰C.) Estimation done by solid phase enzyme linked immunosorbent assay (ELISA), based on sandwich principle (Kit used – IBI, Hamburg H. pylori IgG ELISA; stored at 2-8⁰C). The results interpreted as negative (<8U/ml); equivocal (8-12U/ml) & positive (>12U/ml). Validity of tests required calibrator OD of > 0.250; antibody index for negative control <0.9 and for positive control >1.2.

For invasive category; endoscopic gastric tissue bits collected in three containers. One subjected to 10% urea solution with phenol red as indicator; second one fixed in 10% formalin and post processing stained by H & E, giemsa stains. The last one subjected to SEM for assessing morphology of H. Pylori. For first tissue, adding freshly prepared solution of phenol red with 10% urea solution (1mg:100ml) which acts as indicator (yellow with pH6.8); colour changed to pink in bacterial load exceeding 2 x 10⁶ and change observed up to 4–6 hours. Result interpreted as RUT positive indicating presence of H. pylori in tissue.

For second tissue; sections stained by standard H & E and giemsa staining methods. H. pylori seen near adherent mucus on luminal side of gastric surface and pit epithelial cells on mounted sections. For third tissue; post washing in normal saline; fixed in 4% glutaraldehyde in 0.1% cacodylate buffer followed by dehydration in ascending alcohol; treated with amyl acetate, subjected to critical point drying and gold coating (IB-2ion coater to make gold coating of 200⁰A). SEM examination done by S-5330 Hitachi. The relationship of GERD with socioeconomic status determined by Kuppuswamy's scale. All statistical tests performed using Statistical Package for Social Sciences (SPSS), Version 10 software for windows. Tests of significance done by chi-square test (X²) and p value < 0.05 considered significant.

III. Results

100 patients included in the study with age ranging from 15-65 years (mean age – 40 yrs). GERD symptoms common in 26-35yrs and commoner in males (68%) compared to females (32%) with ratio of 2.12:1. Reflux with heart burn (47%) was the most common presentation in case of GERD followed by irregular and spicy food intake (46%). 66/100 GERD patients were negative for IgG ELISA test; 34 were positive with cut off titre 10 U/ml. Of 50 controls; 40 showed negative results, 3 equivocal and 7 were positive. 62/100 showed negative urease test with positive in 38/100 cases. Modified Giemsa stain showed least positivity (27 cases). Maximum GERD patients (59 / 100 cases) were negative for all three diagnostic tests. In 23 cases, all three tests were positive, rest of patients showed variable test results.

TABLE : 1 : COMPARATIVE EVALUATION OF TESTS

			No. of cases n = 100
IgG Elisa (+) (n=34)	Urease(+)	Giemsa(+)	23
		Giemsa(-)	08
	Urease(-)	Giemsa(+)	01
		Giemsa(-)	02
IgG Elisa (-) (n=66)	Urease(+)	Giemsa(+)	03
		Giemsa(-)	04
	Urease(-)	Giemsa(+)	00
		Giemsa(-)	59

39% patients with GERD show presence of H.Pylori.

TABLE :2: Presence of H.Pylori in GERD

			+ve for H. Pylori	-ve for H. Pylori
UREASE(+)	GIEMSA(+)	26	26	
	GIEMSA(-)	12	12	
UREASE(-)	GIEMSA(+)	01	01	
	GIEMSA(-)	61		61
Total (n=100)			39	61

A case of H. pylori infection defined as patient presenting with positive symptoms and positive for either RUT and or modified Giemsa stain.

Highest numbers of GERD patients (42) were in lower socio-economic group; among them 23 cases (54.76%) were positive and 19 cases (45.24%) negative for H-pylori infection. Lowest number of cases (8) were in upper socio-economic group; among this only one was positive for H.pylori; thus shows significant association of H.pylori infection with the socio-economic status (p = 0.012). 42 cases found in low socio-economic status; 40 in middle and 18 in upper socio-economic status. Highest percentage of H. pylori positivity (60%)seen in 46-55 years age group; followed by 36-45 years (85.8%); demonstrates a significant association (p = 0.011).

Majority of male GERD patients (30/68) were positive for H. pylori; while 9/32 female patients showed H.pylori presenting a higher percentage of males (44%) compared to females (28.1%) which was found to be statistically insignificant (p = 0.126).Most patients (76 cases) were non-vegetarian and H.Pylori found in 42.1 % of them. Positivity for H.Pylori (7 cases;29.1%) noted in 24 vegetarian GERD patients; which reflects insignificant association with dietary habits (p = 0.257). 57% of GERD patients had normal upper gastrointestinal endoscopy; but H.Pylori found in 24 (42.1%). Non-erosive antral gastritis with esophagitis found in 40 cases with positivity for H.Pylori in 14 (35%).

Most GERD cases (54) showed features of chronic gastritis histopathologically ; among these, (44.4%) showed H. pylori. 27 cases showed normal histopathological finding and H.pylori detected in 11 cases (40.7%). 1/7 cases with features of esophagitis on HPE showed H. pylori (14.28%). Both Intestinal metaplasia(4) and atrophic gastritis (5) showed H.pylori (25%, 40%). 2 GERD cases showed well differentiated adenocarcinoma and one was due to Barret's oesophagus, all were negative for infection. IgG ELISA showed sensitivity of 89%, specificity of 86%, positive predictive value of 70.6% and negative predictive value of 90%.

Though , culture of H.pylori is the theoretical 'gold standard' investigation, it is difficult to perform, slow and expensive. Alternatively, histological demonstration considered as 'gold standard' test in many studies.⁷ The present study accesses the sensitivity and specificity of IgG ELISA by taking histological demonstration of the organism by modified Giemsa stain as 'gold standard'. Of various tests applied to diagnose H.pylori, maximum positivity was with RUT, followed by ELISA, Giemsa and H & E. Positivity with H & E stain depends on higher density of the organism .

TABLE : 3: EFCECACY OF VARIOUS TESTS IN DIAGNOSING H.PYLORI

TEST	(+) H.PYLORI	(-) H.PYLORI
RUT	38	62
H & E	18	82
GIEMSA	27	73
ELISA	34	66

IV. Discussion

The study included 100 patients of GERD in age group of 15 to 65 years. In the present study, 55% patients with this complaint fall in age group of 26 to 45 years. Most were male patients (68 / 100) with male to female ratio of 2.1:1. Most studies observed no gender preponderance, but present study showed male preponderance in GERD. Raul V Destura et al. showed susceptibility profile of H. pylori infection commoner in males.⁸

In the present study, reflux with heart burn for more than twice a week for more than three months was the most common symptom, found in 47 / 100 cases (47%) . These findings are consistent with D Me Namara and C O' Morain, Ireland who reported heart burn in 21-44% of adult population.⁹ Anan Raghunath et al reported heart burn or reflux as predominant symptom in GERD.¹⁰

IgG ELISA showed significant titers (>12u/ml) in 34 cases (34%), Coreley et al observed H.pylori antibody status inversely associated with GERD diagnoses and symptoms.¹¹ The reduced ELISA positivity in the present study may be due to diversity of population with different antibody response. Among 50 healthy controls, IgG ELISA showed significant titers (>12U/ml) in 7 (14%), negative titers (<8u/ml) in 40 (80%) and equivocal titers (8-12 u/ml) in 3 persons (6%). Few healthy controls showed positivity for H. pylori ELISA because antibody may persist up to 6 months or more in sera of previously infected, otherwise healthy individuals.

RUT was positive in 38% cases and negative in 62% . Hacklesberget et al found urease positivity in 38% of GERD¹² while Gesbert and Pedro found urease positivity in 57%.¹³ These differences in urease activity addressed to patchy distribution of organism in biopsy sample or anti-ulcer therapy with proton pump inhibitors where H. Pylori moves proximally in stomach.^{12,13} H.Pylori demonstrated in 27 % cases in modified Giemsa stained sections . Pieramico et al observed prevalence of H. Pylori in 44% cases and 38% controls.¹⁴ All these tests were positive in 23 cases; 8 cases were positive for IgG ELISA and urease but negative for Giemsa stain; may be due to patchy distribution of organism. One case was positive for IgG and Giemsa but negative for urease which could be attributed to low density of H. Pylori and low urease activity. IgG ELISA was positive in two cases but urease test and Giemsa stain were negative due to past infection.

Three cases were negative for IgG ELISA, but urease test and Giemsa stain were positive ; may be due to acute infection of H. Pylori with absent antibody. Four cases were negative for IgG ELISA and Giemsa stain, but positive for urease test ; could be due to acute infection of H. Pylori with patchy distribution of organism. 59 cases were negative for all the tests. The case definition adopted in present study similar to studies done in India or abroad. Some authors defined H. Pylori positivity where both the tests were positive ; while some depended only on modified Giemsa stain .

The total number of cases positive for both RUT and modified Giemsa stain was 26; 12 were positive in only RUT and one was positive for only Giemsa. 61 cases were negative for both RUT and modified Giemsa stain. Prevalence of H. Pylori was 39% amongst GERD cases in present study compared to 41% by Goldblum et al;¹⁵ 38% by Hacklesberget et al ;¹² 57% by Gesbert and Pedro .¹³ But,H.Pylori showed an inverse relationship with GERD in study by Corley et al.¹¹ making the present study consistent with previous studies.

The socio-economic status of patients analyzed by Kuppuswamy's socio-economic status scale showed association of H. pylori infection with low socio-economic status. Previous studies observed similar association, attributed to poor sanitation and hygiene, crowded living conditions, poor water supplies.H. pylori infection is slightly higher below 35 years age in this study. Shrivastav UK et al showed maximum H.pylori positivity in age group > 30yrs (80%).¹⁶ But Gill et al showed highest prevalence of H.pylori in 20-29 years age (70%), followed by 30-39 years (69%) and 40-49 years (60%) which may be due to variation in socio-cultural habits and environmental factors.¹⁷ The infection found to be more in above 30 years group in almost all studies.Present study shows H.Pylori positivity in 44.1% of 68 males and 28.1% of 32 females; showing insignificant correlation (p>0.05); similar to Singh V et al,¹⁸ Shrivastava UK etal¹⁶

H.Pylori was positive in 7/24 (29.1%) vegetarian cases, 32/76(42.1%) non-vegetarians, presenting an insignificant correlation. (p>0.05) ; similar to previous studies..Normal upper G.I. endoscopy noted in 57 cases with H.pylori positivity in 24 cases. Non-erosive antral gastritis with esophagitis found in 40 cases with H.Pylori positivity in 14 (35%). Deformed pylorus found in 3 cases with positivity in 1 (33.3%) ; observations consistent with previous studies.

In H & E stained tissue sections, 24 /54(44.4%) cases of chronic superficial gastritis showed H.Pylori. Normal histopathological findings were negative for H.pylori. 1/7 cases of oesophagitis showed H.pylori (14.28%), well differentiated adenocarcinoma diagnosed in 2 cases and all were negative for H.pylori. 2/ 5 cases of atrophic gastritis showed H.pylori and 1/4 cases of intestinal metaplasia was positive. One case of Barret's oesophagus diagnosed by HPE was negative for H.pylori. Culture of H.pylori considered theoretical 'gold standard' for the organism; but the fastidious slow growing organism requires special culture conditions; so culture alone can miss a proportion of cases due to overgrowth and low bacterial load. Many studies considered histological demonstration of the organism as the 'gold standard' .^{4,5,6} In the present study, modified Giemsa stain considered to be the 'gold standard' test and the sensitivity and specificity for IgG ELISA calculated. The results were sensitivity- 89%, specificity – 86%, positive predictive value – 70.6%, negative predictive value – 95% and prevalence – 0.27 ; consistent with previous studies.Harris P et al. recommended local adjustment of cut off in a given population.¹⁹ Moreover, specificity directly depends on antigen preparation used in the kit. Being non-invasive test, IgG ELISA does not require endoscopy; it is inexpensive, rapid and fulfils the criteria of an ideal screening test. Serology used for monitoring therapeutic effect of antimicrobial treatment for eradication of H.pylori as well as screening sera in epidemiological studies, but cannot be used as confirmatory test because of low specificity and positive predictive value. Rao P et al observed similar results and recommended IgG ELISA as a very good screening test.²⁰ Thus, RUT found to be most sensitive (38%) in comparison to other tests in diagnosing H.Pylori infection and H & E found least sensitive (18%) ;probably due to less density of the organism in different tissue bits.⁶ RUT positive samples selected for SEM examination.

V. Conclusion

Prevalence of H pylori infection is low in patients with GERD with statistical significant association with age, socio-economic status . RUT showed highest number of positive cases, followed by IgG ELISA, modified Giemsa and H & E stain. As H.pylori infection is preneoplastic ; early detection with adequate treatment considered mandatory.

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