Original Article

Two Easy and Early Methods for the Diagnosis of H. Pylori i.e: Serum Anti H. Pylori Antibodies and Stool Antigen

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ABSTRACT

OBJECT: Two easy methods for the diagnosis of h pylori i.e: Serum Anti H Pylori Antibodies & Stool Antigen.

MATERIALS AND METHODS: Hundred serum and stool samples collected from the department of surgery, and Medicine were registered at Department of Pathology and Diagnostic and Research Lab LUMHS, Jamshoro/ Hyderabad for performing H. pylori diagnosis were included in this Study. Patients of all age groups and both sexes with complain of dyspepsia, or having the clinical suspicion of H. pylori associated gastritis were included. Patients having H/O NSAIDs, or eradication therapy were excluded. A specially designed proforma was used for recording the relevant data. The Period of study was 15-10-2009 to 28-02-2011.

RESULTS: From 100 cases of, H. pylori antibodies were found in 68(68 %). 38(55.9%) were male and 30(44.1%) were females. Sensitivity of this technique was 75.55% and specificity 60%. p value 0.007. From stool samples collected from 100 patients *H. pylori* antigen were positive in 48 (48%) cases detected on immunochromatography technique. Stool antigen positive was in 28 (58.3%) males and 20(41.7%) Female patients. The stool antigen sensitivity was 52.74% and specificity 90% P value (0.011).

CONCLUSION: H. pylori positivity is highly significant in histological examination of endoscopic biopsies on H&E and Giemsa Stains The detection of anti H. pylori antibodies from serum by ELISA method and the H. pylori antigen from stool samples by immunochromatographies method, can be non invasive simplest, least expensive and alternatives in patients unwilling for endoscopy.

KEY WORDS: Helicobacter pylori (H. pylori), endoscopy, serological and stool antigen HpSA, and Anti H. pylori IgG antibodies, MALT lymphoma (mucosa associated lymphoid tissue lymphoma).

INTRODUCTION

Helicobacter pylori are motile, non-sporulating gram negative, microaerophilic organisms. They are S-shaped, flagellated, spiral rods measuring, 2.5-3.5µm long & 0.5-01µm wide(1), and can grow under micro aerophlic condition (5% 02, 10% CO2 85% N2) at 37oC. They produce a number of enzymes namely urease, catalase, oxidase etc(2).

H pylori represent medically the most common & important world wide infectious disease. The bacterium have the etiologic factor for the formation of peptic ulcer. In addition, helicobacter pylori established the malignancy of stomach ie: adenocarcinoma & MALT lymphoma (3)Up to 80% of gastric carcinomas. (4). It has a world-wide distribution with a high prevalence rate in developing countries with increasing age. It is causally related to chronic active antral gastritis and is highly associated with duodenal and gastric ulcers. (5). It usually survive in acidic medium of stomach by means of urease enzyme, which converts urea into ammonia and C02, enabling itself to neutralize acid in its immediate environment.(6). The histological examination of gastric biopsy showed correlation between H pylori and gastric cancer and those without H pylori infection. In population with H pylori infection there is increase six fold risk of gastric cancer as compared those population who have no gastric H pylori (7,8). Helicobacter pylori colonize the stomachs of approximately Half of all humans. In the absence of treatment, H. pylori can persist throughout the life of the host. Although mostly infected individuals are asymptomatic, a significant proportion develops peptic ulcer disease, chronic atrophic gastritis (ChAG) or gastric adeno-carcinoma. The precise mechanisms by which H. pylori causes gastric cancer are unknown.

Histological examination of endoscopic biopsies for detection of H. pylori disease is still considered gold standard method, but the H. pylori can be detected by serological analysis and HpSA test which are proved to be simplest, least expensive, effective one and less time consuming. Although having disadvantage de-

spite it has disadvantage to detect acute and chronic infection and MALT lymphoma, still it is considered as a best tool for screening and early detection for primary care of patients, diagnosis and eradication therapy, and for mass screening purpose in those patients who are unwilling for the endoscopy.

The widely used non invasive methods, i.e: stool antigen test, urea breath test, & serological testing. In epidemiological studies the commonly used test is of serology to see the incidence & prevalence of infection. The study was performed, & compare the specificity & sensitivity between two commonly used tests i.e. HpSA, and Anti H. pylori IgG antibodies, and evaluate their usefulness in our patients in detecting H.pylori and formulate recommendations after consideration the cost effectiveness, time factor sensitivity, specificity. Analysis of the above methods and recommendation that which method is best as a single diagnostic tool. This study will be useful for earlier diagnosis with comprehensive information of the disease status with possible minimum expenses and more confident results in our patients.

MATERIALS AND METHODS

Institution based Prospective comparative study. The study was conducted at Pathology Department in collaboration with Department of Medicine, and Surgery, LUMHS Jamshoro. The duration of study was 15-10-2009 to 28-02-2011. After endoscopic antral biopsies of hundred patients, the same patients were also included for two other techniques for the detection of H P infection. Their serum samples and stool specimens were included in this Study for performing H. pylori diagnosis on (ELISA Test Kit Applied for Anti H pylori IgG: Monobind Inc. (company) Lake Forest, CA 92630, USA) and a rapid immunochromatography for the detection of Helicobacter pylori antigens in stool specimens (biotech USA) techniques respectively. Samples were collected from the department of surgery, and Medicine registered at Department of Pathology, Diagnostic and Research Lab LUMHS, Jamshoro/ Hyderabad. Patients of all age groups and both sexes with complain of dyspepsia, or having the clinical suspicion of H. pylori associated gastritis were included in this study. Patients having H/O NSAIDs, or eradication therapy were excluded from study. A printed proforma specially designed for recording the relevant clinical and other details of each case. Clinical findings and relevant data of patients were collected from concerned Doctors, and directly from the patients.

Statistical Analysis:

The data were entered and analyzed in statistical program SPSS version 16.0. Qualitative data (frequencies and percentages) such as gender, age groups, H pylori stool antigen were presented as n(%) and chi square test was applied to compare the proportions between the groups. P value <0.05 was considered as statistical significant level.

RESULTS

Blood samples were obtained from 100 patients included in the study for separation of serum for analysis on ELISA technique. Out of 100 cases, H. pylori antibodies were found in 68(68 %) cases. Amongst which 38 (55.9%) were male and 30(44.1%) female **(Table I)**. The sensitivity of this technique was seen 75.55% and specificity 60%. P Value 0.007 when compared with gastric biopsies **(Table II)**. Fresh stool samples were collected from same 100 patients in sterilized bottle H. pylori antigen were detected on immunochromatography method and were found positive in 48 (48%) cases. Stool antigen for H pylori positivity was seen in 28(58.3%) male patients and in 20 (41.7%) Female patients (Table I).The values of stool antigen sensitivity was 52.74% and specificity 90%.and P Value was 0.011 when compared with gastric biopsies(**(Table III)** In our study, on analysis of serum of 90 histopathological positive cases for detection of H. pylori antibodies on ELISA technique to compare with gold standard method 66 (73.3%) were seen positive but 24 (26.7%) negative and out of 10 histologically H. pylori negative, 02 were seen positive but 08 negative on serum analysis for H. pylori antibodies. Performed stool examination of 100 cases to see HpSA. 90% were histopathologicaly positive cases for H. pylori, show 47 (52.22%) positivity on immunochromatography method. Amongst 10 histologicaly H. pylori negative cases only 01 was seen positive but 09 were found negative. (Table I)

TABLE I: SERUM ANTI H PYLORI ANTIBODIES AND H PYLORI STOOL ANTIGEN (n =100)

Serum anti H pylori Antibodies	Male	Female	Grand total
age group	11-85	11-85	
Positive	38 (55.9%)	30 (44.1%)	68 (68%)
Negative	18(56.2%)	14(43.7%)	32 (32%)
Total	**56**	**44**	**100**
H pylori stool antigen			
Positive	28 (58.3%)	20 (41.7%)	48 (48%)
Negative	28(53.8%)	24(46.1%)	52 (52%)
Total	**56**	**44**	**100**

TABLE II: SENSITIVITY, SPECIFICITY AND P VALUES OF SERUM ANTI HP ANTIBODIES VERSUS STOOL ANALYSIS FOR HP ANTIGEN (n=100)

	Sensitivity	Specificity	P-values
Gastric biopsies vs serum anti Hp antibodies	73.3%	80%	0.007 (significant)
Gastric biopsies vs HpSA	52.2%	90%	0.011 (significant)

DISCUSSION

Since 52 years endoscopy has been used for the upper gastrointestinal disorders It helps to see the underlying gastrointestinal disorder, i.e: ulcer disease, however the serological HP tests have predictive value for the management of dyspepsia. The handling & storage of sample shows error, that's why the histological methods are considered as gold method to confirm the infection. However on biopsy if level of infection is low and patchy, it will be missed on endoscopy.

In the present study the frequency of serum Anti H. Pylori was seen in 68% patients. This was in comparison of Luthra GK et al. (11) who reported the similar frequency i.e. 63% of serum anti HP antibodies whereas an other study conducted by Satti SA et al. (9) who showed 87.7% frequency of serum Anti HP antibodies. This difference is because of unawareness of Hygiene and old study.

We also found 48% frequency of HPSA in our results which is lower than Chisholm SA et al.(15) and Gisberg JP (10), this was due to more antigens present in stool in above study and they have more advance than us in diagnostic methods.

Our study showed lower number of positive cases which may be due to insufficient amount of antigen in the stools. Difference of HpSA results from other study is due to the difference of climate and may be quality of kit.

CONCLUSIONS

The diagnosis from anti H. pylori antibodies by ELISA method and the H. pylori antigen from stool samples by immune-chromatographies are non invasive simplest, least expensive methods in patients who are not willing for endoscopy. H. pylori positivity is highly significant in histological examination endoscopic biopsies on H&E and Giemsa Stains. It will be more useful if gastric biopsies should be taken from proper site and multiple biopsies by endoscopists as gastritis is a patchy inflammatory disease, in these circumstances cases may be detected by serum or stool analysis as seen in this study.

SUGGESTIONS AND RECOMMENDATIONS

There are various methods to detect H pylori but serological and stool antigen tests should be routinely used to detect H pylori, because they are effective, simple, easy and least expensive. Patients who are unwilling for endoscopy, they should be recommended for these tests and mass screening may also carried out on theses investigations.

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