Detection of Helicobacter pylori antigen in stool by Enzyme Linked Immunosorbent Assay and comparison with conventional methods.

Author: Dr. Krithikaa Sekar, MD

Institute: Sri Lalithambigai Medical College and Hospital, Unit of Dr MGR Educational and Research Institute, Maduravoyal, Chennai-600095

Abstract:

Background: The *H. pylori* have been associated with gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid type (MALT) B-cell lymphomas. In the presence of upper gastrointestinal bleeding, the diagnosis of *H pylori* infection may be compromised. Therefore, it is suggested that noninvasive methods, such as serology, be used to identify *H pylori* infection in these patients. The diagnostic role of Helicobacter pylori stool antigen enzyme immunoassay (HpSA) and simple H pylori antigen cassette test in patients with upper gastrointestinal bleeding remains unclear. Hence we proposed to conduct this study to assess the efficacy of ELISA with conventional methods in detecting H. pylori infection.
Materials and Methods: The details of complete history, clinical feature of the patients subjected to endoscopy were obtained. Preinvasive procedure preparation for oesophago gastro duodenoscopy was performed as per norms. Stool sample is obtained from the patient on the day of endoscopy. Three biopsy sample were collected, two from the gastric antrum and one from the corpus. One biopsy sample from the antrum was used for performing Rapid urease test and the other two samples were placed in 10% formalin and sent for histopathological examination.
Results: 57% of gastritis patients and 56% of duodenitis patients are positive for HpSAg by Enzyme Linked Immunosorbent Assay. Out of the total 120 samples, 30 were positive by both ELISA and gold standard tests. 4 were positive by gold standard but negative by ELISA. 16 were positive by ELSA but negative by gold standard tests. 70 were negative by both ELISA and gold standard tests. Comparing ELISA with the gold standard, the sensitivity was 100%, specificity was 77%, Positive predictive value was 52% and Negative Predictive value was 100%.
Conclusion: Detection of *H. pylori* antigens using HpSA shows a high sensitivity and specificity and might be useful for non-invasive diagnosis of *H. pylori* infection in children and adult patients, hence recommended for the diagnosis of H.pylori infection.

Introduction:

Helicobacter pylori (*H. pylori*) bacteria are a 'slow' bacterial pathogens and the name comes from Latin meaning 'spiral rod of the lower part of the stomach'. It was first isolated in 1983 in Australia by Warren and Marshall and was found to be present in patients suffering from type B gastritis[1]. The *H. pylori* have now been associated with gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid type (MALT) B-cell lymphomas[2]. A variety of host factors and bacterial factors contribute to the pathogenesis of gastrointestinal diseases resulting from *H. pylori* infection. It is intensely antigenic and secretes various factors like urease, catalase, mucinase, lipase, haemolysin and alkaline phosphatase that decrease viscosity of mucus. The production of catalase protects the bacteria against the toxic effects of reactive oxygen metabolites formed in neutrophilic medium from hydrogen peroxide. The multiple polar flagella permit them to penetrate the mucus layer. Adherence of *H. pylori* to gastric epithelial cells and vacuolating cytotoxin are the virulence factors as they are associated with degenerative changes in the epithelial cells[3]. In the presence of upper gastrointestinal bleeding, the diagnosis of *H pylori* infection may be compromised. The Urea Breath Test (UBT) is responsible for a high number of false negative results when it is used to diagnose *H pylori* in patients with upper gastrointestinal bleeding. Coagulation disorders or anticoagulation may prevent a biopsy being taken. The UBT may not be feasible in patients on artificial respiration, or in the presence of impaired consciousness or acute abdominal disease. Therefore, it is suggested that noninvasive methods, such as serology, be used to identify *H pylori* infection in these patients. In some cases, the indication for *H pylori* eradication therapy is based only on a serological test. Serology alone, however, is a rather inaccurate diagnosis method[4,5,6]. An ideal noninvasive test for *H pylori* infection should be safe and acceptable to patients, inexpensive and easy to perform, and with a high degree of sensitivity and specificity[7]. The diagnostic role of Helicobacter pylori stool antigen enzyme immunoassay (HpSA) and simple H pylori antigen cassette test in patients with upper gastrointestinal bleeding remains unclear. Only a few reports have discussed the results of the HpSA test and simple H pylori antigen cassette test in these patients[4,5,6,8]. Pathogen-specific stool antigen tests are a valid alternative to the UBT for noninvasive detection of H. pylori. Much experience has been gained with Premium Platinum HpSA (Meridian Diagnostics, Cincinnati, Ohio), the first enzyme immunoassay (EIA) available for the identification of H. pylori antigens in fecal samples[9]. This test uses polyclonal anti-H. pylori antibodies and has revealed good overall performance in diagnosing H. pylori infection or evaluating the success of eradication therapy. However, some limitations and discrepancies with respect to intertest variations, cut-off-values, and lower accuracy compared to the results seen with UBT after eradication therapy have been reported[9,10,11]. Hence we proposed to conduct this study to assess the efficacy of ELISA with conventional methods in detecting H. pylori infection.

Aim :

Detection of Helicobacter pylori antigen in stool by Enzyme Linked Immunosorbent Assay and comparison with conventional methods.

Objectives:

- Detection of Helicobacter pylori by stool ELISA.
- Detection of Helicobacter pylori by performing Rapid Urease Test
- Detection of Helicobacter pylori by performing Histopathological examination.
- Comparing the results of ELISA and conventional methods.

Materials and Methods:

This was a prospective cross-sectional study conducted on 120 patients attending the OPD of Sri Lalithambigai Medical College and Hospital over a period of 1 year. Patients undergoing upper gastrointestinal endoscopy for complaints suggestive of upper gastro intestinal diseases like gastric ulcer, duodenal ulcer, antral gastritis and gastric carcinoma and those who were not on antibiotics, proton pump inhibitor or Helicobacter eradication therapy within 1 month prior to inclusion in this study were enrolled after consenting process. Patients with severe gastric bleeding and gastric surgery were excluded from this study. The details of complete history, clinical feature of the patients subjected to endoscopy were obtained. Preinvasive procedure preparation for oesophago gastro duodenoscopy was performed as per norms. Stool sample is obtained from the patient on the day of endoscopy. Three biopsy sample were collected, two from the gastric antrum and one from the corpus. One biopsy sample from the antrum was used for performing Rapid urease test and the other two samples were placed in 10% formalin and sent for histopathological examination.

Invasive and non-invasive procedures involved:

	Diagnostic Test	**Method of Organism Identification**
Invasive	Rapid Urease Test	Urease production
	Histology	Morphologic features and location
Noninvasive	Antibody detection	Immunologic Response
	Urea Breath Test	Urease production
	Stool Antigen Test	Antigen detection

Biopsy Sample: Patients fasted overnight before endoscopy. Endoscopy was done using fiber optic endoscope. The endoscope and the biopsy forceps were rinsed thoroughly with water and soaked in 2% gluteraldehyde for 20 minutes and were thoroughly rinsed with sterile normal saline just before the collection of biopsy sample.

Stool Sample: Stool sample was collected on a sterile container on the day of endoscopy and stored at - 20° C.

Rapid Urease Test: The test was done using PYLODRY urease kit. The kit opened and an antral biopsy sample placed on the urea strip and one drop of distilled water added followed by closing of the kit. Colour change from yellow to pink at room temperature within two hours, were taken as positive.

Histopathology: One specimen from the gastric antrum and one from the corpus were fixed in 10% formalin, paraffin sections were made and stained with Haematoxylin and Eosin and examined for Helicobacter pylori.

Enzyme Linked Immunosorbent Assay: COPROELISA kit was used for the detection of Helicobacter pylori antigen in the stool samples. The test was performed according to the kit manufacturer's instructions.

Results:

DEMOGRAPHIC PROFILE OF STUDY POPULATION

AGE	MALE	FEMALE	TOTAL	PERCENTAGE
10 – 19	0	2	2	1.66
20 – 29	12	0	12	10
30 – 39	10	10	20	16.67
40 – 49	10	10	20	16.67
50 – 59	20	18	38	31.67
60 – 69	12	6	18	15
70 – 79	8	0	8	6.67
80 - 89	2	0	2	1.66
TOTAL	**74**	**46**	**120**	**100**

Out of a total of 120 cases 74 (61.67%) were males and 46 (38.33%) were females.

CHART 1

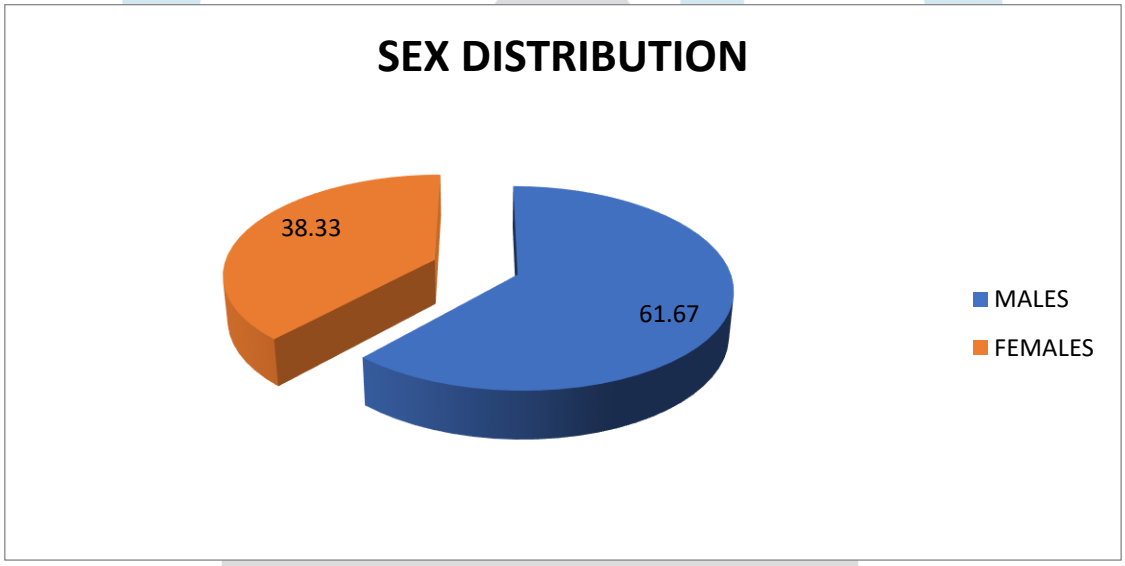


CHART 2

AGE DISTRIBUTION

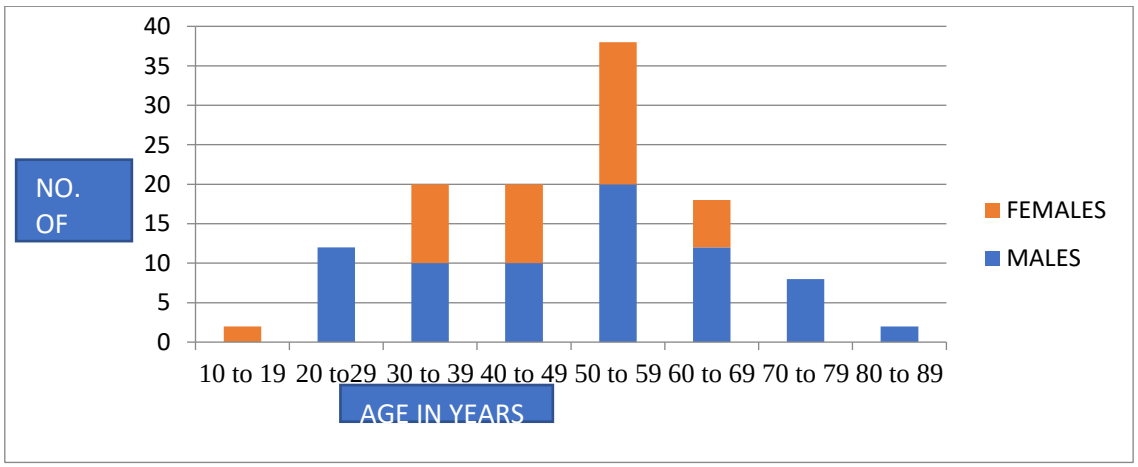


TABLE 2
SYMPTOMS AND SEX DISTRIBUTION IN RELATION TO CLINICAL DIAGNOSIS

SYMPTOMS	MALE	FEMALE	PERCENTAGE
Epigastric pain	56	40	80
Dyspepsia	50	30	67
Vomiting	35	23	48
Loss of appetite	13	9	18
Weight loss	16	18	28
Haematemesis	9	3	10
Malena	4	1	4

Epigastric pain was the predominant symptom followed by dyspepsia in both male and female patients.

TABLE 3

CATEGORIZATION OF THE STUDY POPULATION BASED ON ENDOSCOPIC DIAGNOSIS

ENDOSCOPIC DIAGNOSIS	TOTAL	PERCENTAGE
Gastritis	56	47
Oesophagitis	38	32
Lax LES	22	18
Duodenitis & Duodenal ulcer	18	15
Gastric ulcer	10	8
Normal study	20	17

CHART 3

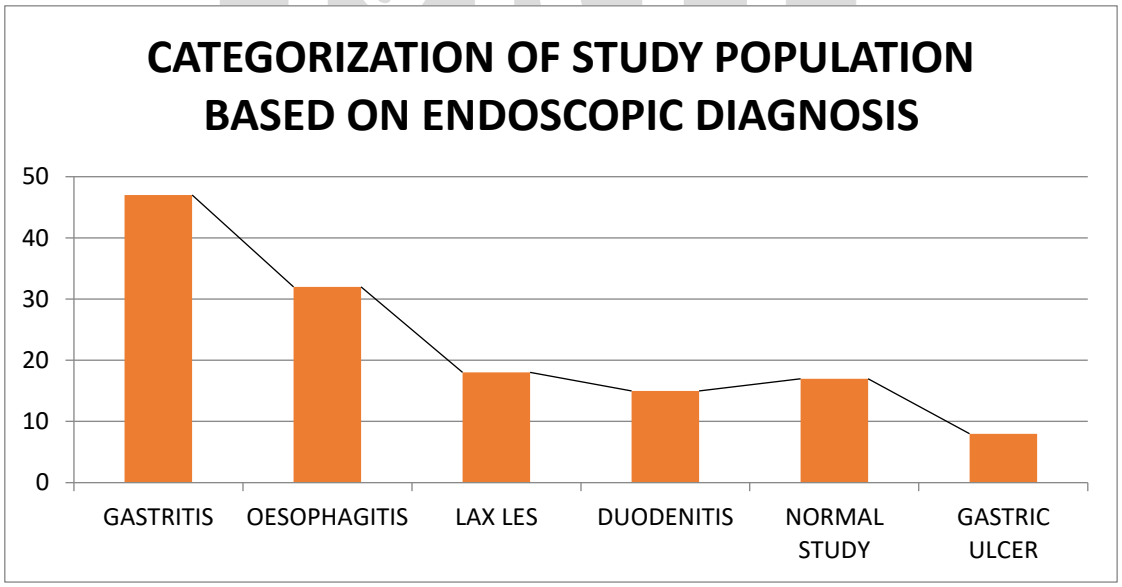


TABLE 4

EFFICACY OF DIFFERENT LABORATORY TESTS (N = 120)

TEST NAME	POSITIVE No (%)	NEGATIVE No (%)
ELISA	46 (38.3%)	74 (61.7%)
RAPID UREASE TEST	34 (28.3%)	86 (71.7%)
HISTOPATHOLOGY	24 (20%)	96 (80%)

TABLE 5
RAPID UREASE TEST POSITIVITY Vs ENDOSCOPIC DIAGNOSIS

ENDOSCOPIC DIAGNOSIS	TOTAL	RUT POSITIVITY	PERCENTAGE
Gastritis	56	24	42.86
Oesophagitis	38	8	21
Lax LES	22	2	9
Duodenitis & Duodenal ulcer	18	10	55.55
Gastric ulcer	10	6	60
Normal study	20	2	10

60% of gastric ulcer & 56% of duodenal ulcer patients are positive for Rapid urease test (RUT).

CHART 4

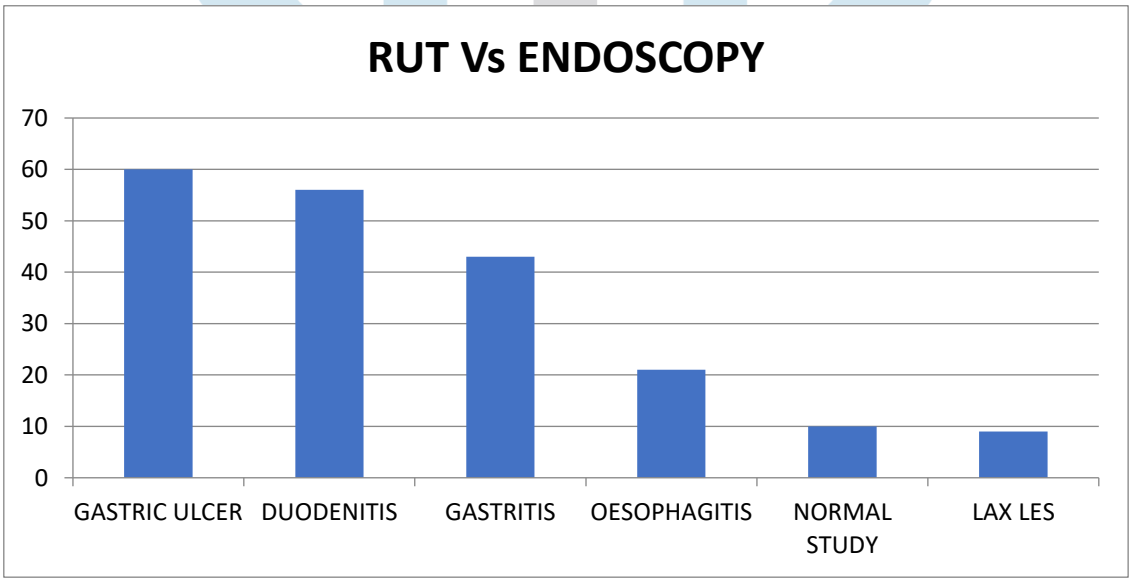


TABLE 6
HISTOPATHOLOGY Vs ENDOSCOPIC DIAGNOSIS

ENDOSCOPIC DIAGNOSIS	TOTAL	HISTOPATHOLOGY POSITIVITY	PERCENTAGE
Gastritis	56	16	28.6

Oesophagitis	38	6	15.8
Lax LES	22	2	9
Duodenitis & Duodenal ulcer	18	6	33.3
Gastric ulcer	10	4	40
Normal study	20	2	10

40% of gastric ulcer patients and 33% of duodenitis patients were positive for Helicobacter pylori by Histopathological examination.

CHART 5

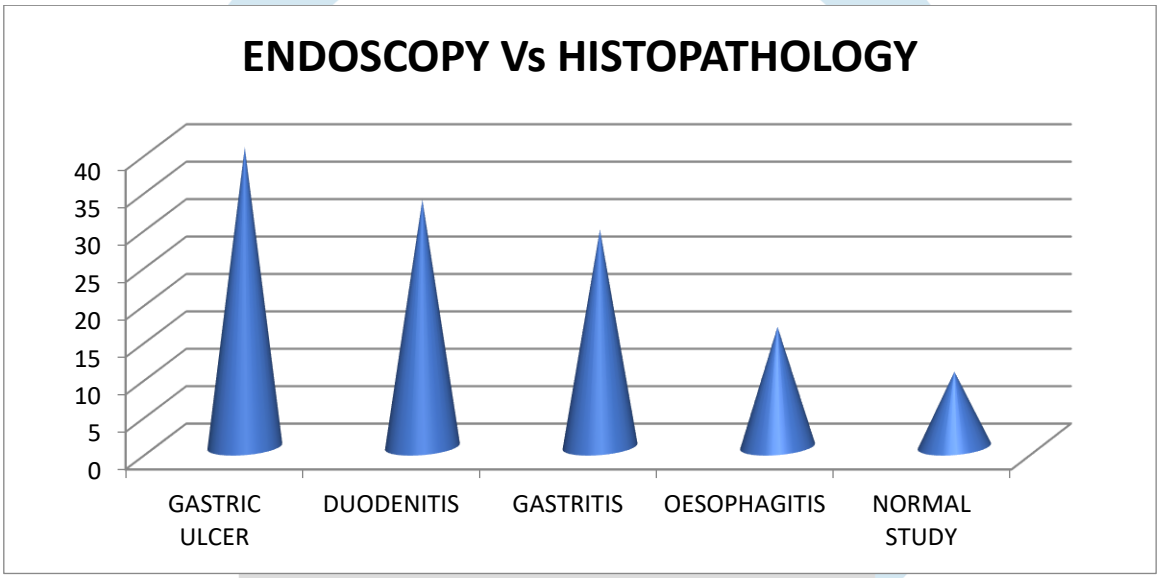


TABLE 7

ELISA POSITIVITY Vs ENDOSCOPIC DIAGNOSIS

	TOTAL	ELISA POSITIVITY	PERCENTAGE
Gastritis	56	32	57.14
Oesophagitis	38	16	42.1
Lax LES	22	8	36.36
Duodenitis & Duodenal ulcer	18	10	55.55
Gastric ulcer	10	4	40
Normal study	20	2	10

57% of gastritis patients and 56% of duodenitis patients are positive for HpSAg by Enzyme Linked Immunosorbent Assay.

CHART 6

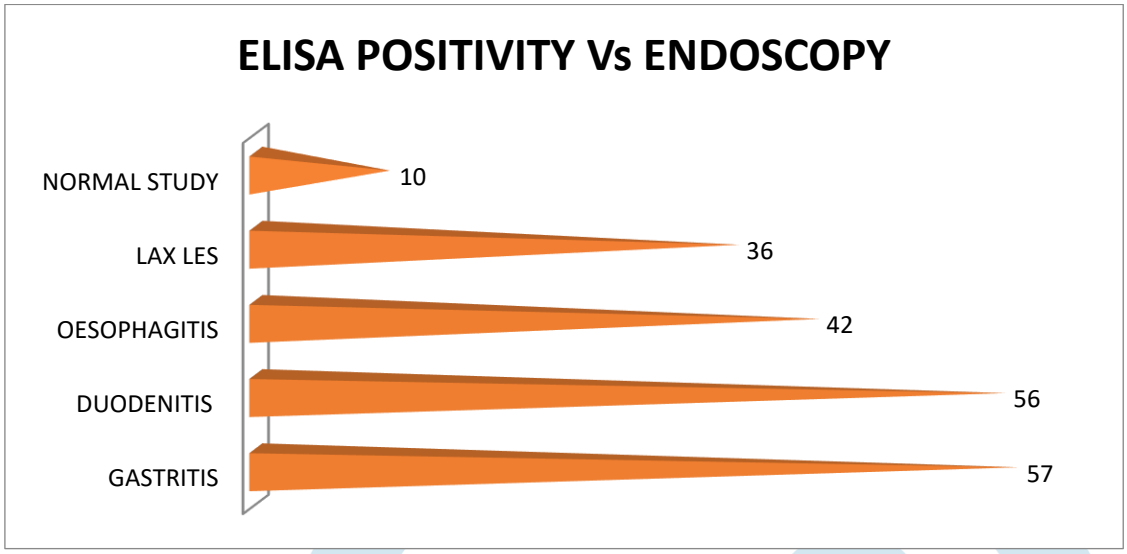


TABLE 8

CORRELATION OF RAPID URESE TEST WITH HISTOPATHOLOGY

n = 120

NAME OF TEST	POSITIVE	PERCENTAGE
RAPID UREASE TEST	34	28.3
HISTOPATHOLOGY	24	20

RUT was positive in 34 cases where as Histopathology was positive in only 24 cases.

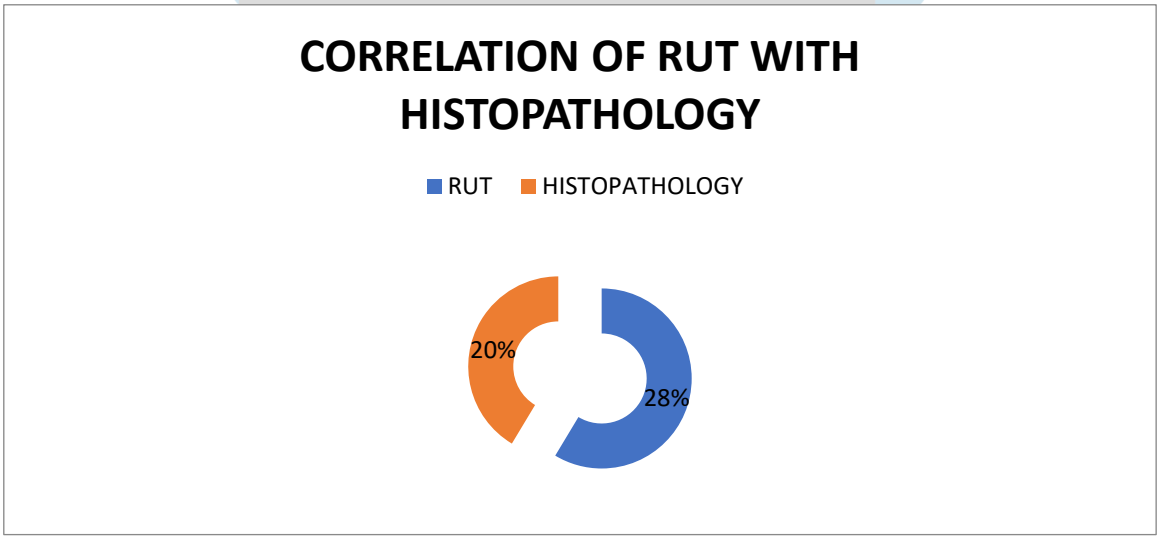


TABLE 9

CORRELATION OF ELISA WITH RAPID UREASE TEST

n = 120

NAME OF TEST	POSITIVE	PERCENTAGE

ELISA	46	38.3
RAPID UREASE TEST	34	28.3

ELISA was positive in 46 cases where as Rapid urease test was positive only in 34 cases.

CHART 8

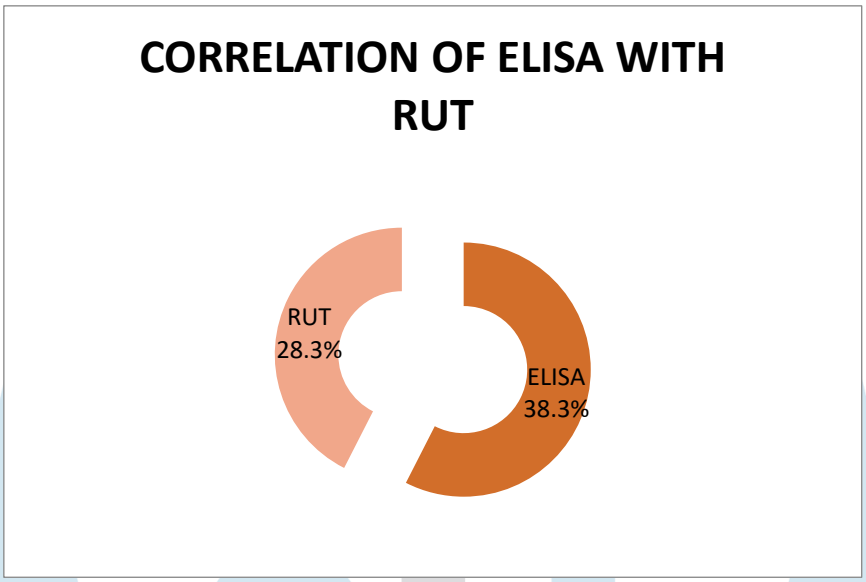
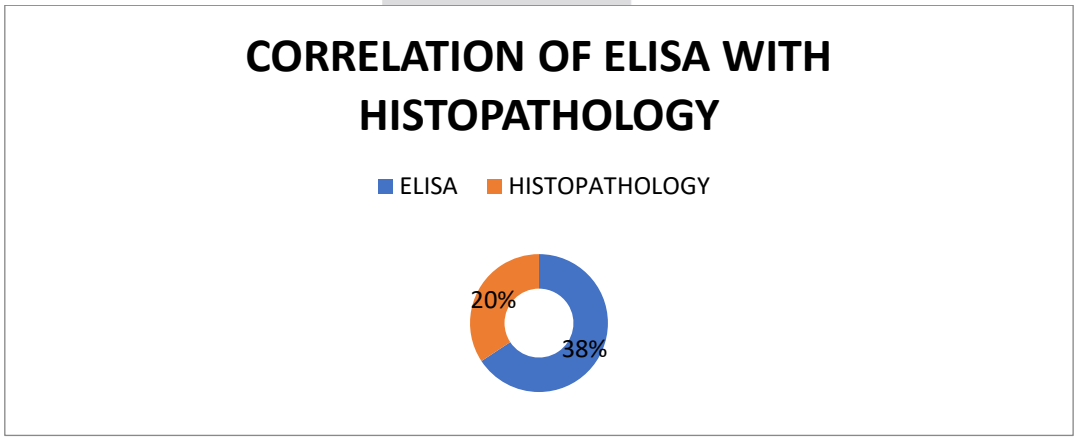


TABLE 10
CORRELATION OF ELISA WITH HISTOPATHOLOGY
n = 120

NAME OF TEST	POSITIVE	PERCENTAGE
ELISA	46	38.3
RAPID UREASE TEST	24	20

ELISA was positive in 46 cases where as Histopathology was positive in only 24 cases.

CHART 9



Comparison of results by ELISA & Gold standard methods:

A patient was classified as *Gold standard* positive when histopathological examination and urease test were both positive and *Gold standard* negative when both these tests are negative.

Out of the total 120 samples, 30 were positive by both ELISA and gold standard tests. 4 were positive by gold standard but negative by ELISA. 16 were positive by ELSA but negative by gold standard tests. 70 were negative by both ELISA and gold standard tests.

TABLE 11

Comparison of ELISA & Gold standard Test

ELISA	GOLD STANDARD		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	**24**	**22**	**46**
NEGATIVE	**0**	**74**	**74**
TOTAL	**24**	**96**	**120**

Sensitivity is the probability that a test result will be positive when the disease is present. Specificity is the probability that a test result will be negative when the disease is not present. By comparing with the gold standard method ELISA's sensitivity and specificity were calculated.

Formula Used

- Sensitivity is the probability that a test result will be positive when the disease is present (True positive rate) Sensitivity = a / (a+b)
- Specificity is the probability that a test result will be negative when the disease is not present (True negative rate) Specificity = d / (c+d)

TABLE 12

STATISTICAL COMPARISON OF ELISA WITH GOLD STANDARD METHOD

S.No	ELISA	GOLD STANDARD	IFA Positive
1	Positive	Positive	True positive (a = 24)
2	Negative	Positive	False Negative (b =0)
3	Positive	Negative	False Positive (c = 22)
4	Negative	Negative	True Negative (d = 74)
	Total		a+b = 24 c+d = 96

Comparing ELISA with the gold standard , the sensitivity of ELISA was found to be 100% and specificity of ELISA was 77 %.

TABLE 13
RESULTS FROM ELISA

S.No		Percentage (%)
1.	Sensitivity	100

2.	Specificity	77
3.	Positive predictive value	52
4.	Negative predictive value	100
5.	False positivity	48
6.	False negativity	0

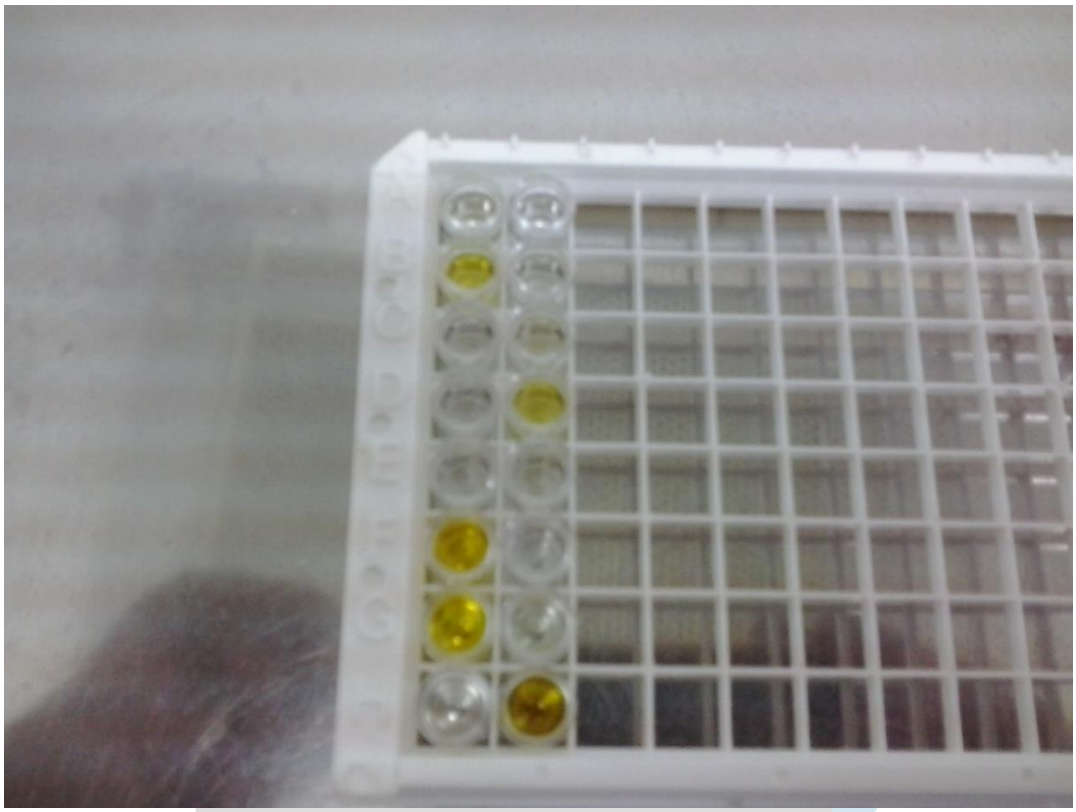


Fig 1: Stool antigen ELISA

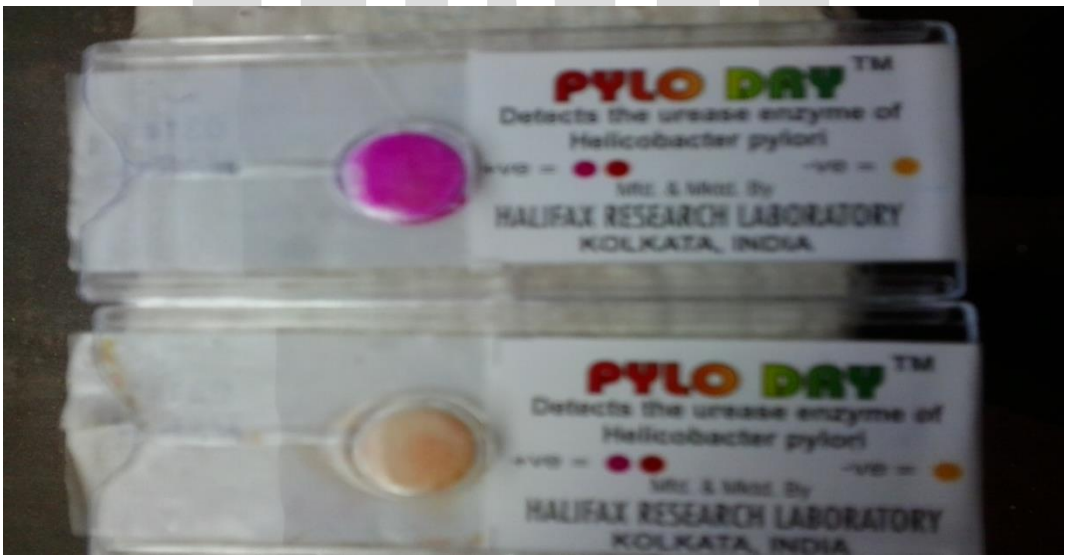


Fig 2: Rapid Urease Test

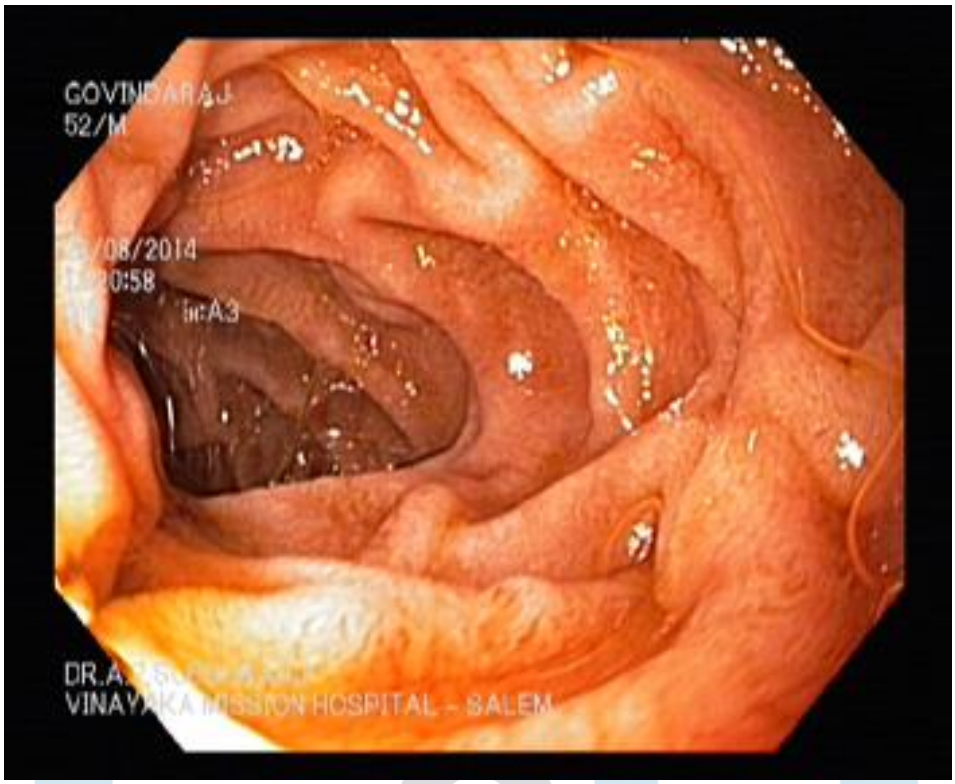


Fig 3: A case of Antral Gastritis due to H. Pylori infection

Discussion:

The present work is based on comparative evaluation of invasive and noninvasive methods of detecting *Helicobacter pylori* infection. Two biopsy based tests namely rapid urease test and histopathological examination and one stool antigen EIA were analysed. The conclusions from the study give fruitful thought about the relative merits and demerits of each methods. Patients undergoing upper gastro endoscopy for various GI disturbances were included in the study. A total of 120 patients with upper gastrointestinal symptoms were enrolled in the study. Among them 74 (61.67%) were males and 46 (38.33%) were females. (Table -1). The maximum number of patients in this study were in the age group 50-59. In the study conducted by D.Nair et al[13] out of the 136 patients, 116 were male and 20 were female is comparable to the present study which also showed males were more affected than females. Among the patients with peptic ulcer disease the predominant symptom was epigastric pain in 80% of cases, dyspepsia in 67%, vomiting in 48%, loss of weight in 28%, loss of appetite 18%, haematemesis in 10% and malena in 4% of the cases. Epigastric pain was the predominant symptom among patients with acid peptic disease. (Table -2). The endoscopic examination of the study population revealed that gastritis accounted for 47%, oesophagitis in 32%, lax LES in 18% and duodenal ulcer in 15% (Table -3). Out of 120 samples studied by Rapid urease test, 34 (28.3%) were positive. The overall positivity of RUT correlated well with reports by Sivaprakash et al[14] (38.7%), while it was lower than that reported by Maimooma et al[74] (65.8%). In the present study 96% of the cases were positive within the first 20 minutes. This is comparable to 95% reported by Sengupta et al[15]. Marshall et al using a CLO test reported that 75% of the positive tests are detected within 20 minutes, 92% at 3 hour and 98% at 24 hours. Histology has been considered by some to be the gold standard for detection of *H. pylori*. Unfortunately, histology is an imperfect gold standard as the detection of *H. pylori* relies upon a number of issues including the site, number, and size of gastric biopsies, method of staining, and the level of experience of the examining pathologist. In the present study, Histopathological examination by Haematoxyline and Eosin was positive in 24 (20%) cases.This correlates with a finding of 18% by Chitra et al. Out of 34 samples positive by RUT only 24 is positive by Histopathological examination. In the present study ELISA was positive in 46 (38.3%) cases.The sensitivity, specificity, postive predictive value and negative predictive value were 100%, 77%, 52 and 100% respectively. This correlates with a study done by Rani et al[16] in 2000 with a sensitivity of 100% and specificity of 57.9%. Syam et al[17] evaluated the Helicobacter pylori stool antigen (HpSA) for the detection of *H pylori* infection in 63 dyspeptic patients. The sensitivity and specifcity of HpSA test were 66.7% and 78.9% respectively. They concluded that HpSA stool test may be useful for the primary diagnosis of *H pylori* infection in peptic ulcer. A study conducted in Iran on 54 patients with gastrointestinal problems shows sensitivity and specificity of HpSA before treatment as 78.6% and 92.3% respectively. In 2005 a study was carried out on 100 children with dyspeptic symptoms in Tabriz, Iran. They compared three diagnostic methods (histology, serological test and HpSA). HpSA sensitivity and specificity was 54.8% and 79.4% respectively[18]. Arıkan *et al*[19] conducted a prospective study to examine the reliability of the HpSA test. The HpSA test had a sensitivity of 91% and specificity of 83%. HpSA test proved to be as reliable as pathological examination for confirming the existence of *H pylori* in humans. Thus, the HpSA test was a useful method for detecting *H pylori* in patients for whom endoscopy was not indicated. Inelmen *et al*[20] evaluated the accuracy of HpSA in the diagnosis of *H pylori* infection in 85 elderly patients affected by medication. Among 56 patients who were not taking Proton pump inhibitors (PPI), the sensitivity and specificity of the Helicobacter pylori stool antigen test were 76% and

93% respectively. Among 29 patients who had received pharmacological therapy with PPIs, the sensitivity and specificity of HpSA test were 82% and 83% respectively. They concluded that HpSA was a useful test in elderly people. The test was easy, simple to perform and non-invasive. Makristathis *et al*. (1998) in his prospective study, found that EIA has a sensitivity of 88.9% and a specificity of 94.6% to detect Helicobacter pylori stool antigen (HpSA) prior to eradication treatment. The study conclude that EIA is a satisfactory method to detect H. pylori infection in the feces, since it is just as sensitive as PCR, histology, and gastric biopsy culture[16]. Fanti *et al.* (1999) in his study to evaluate EIA for HpSA found that this method has a sensitivity of 98.2%, negative prediction value of 96.4%, specificity of 93.1% and a positive prediction value of 96.4%. Fanti *et al*[14] concluded that this test has a high sensitivity and specificity for the detection of H. pylori infection. Nevertheless, the accuracy of EIA in detecting antigen after eradication treatment requires further evaluation. The most recent reports demonstrate conflicting results, even though most studies report a satisfactory sensitivity and specificity even for HpSA testing after eradication treatment. Likewise, the precise point for the monitoring of H. pylori eradication treatment needs further evaluation. Vaira *et al.* (1999)[12], in a multi-center prospective study, found a sensitivity rate of 94.1% and a specificity rate of 91.8% for HpSA testing. The HpSA test and the UBT was conducted 4 weeks after eradication treatment. Sensitivity and specificity of HpSA test was 90% and 95.3% respectively and that of UBT was 90% and 98.9% respectively. Thus, unlike serologic testing that requires several months to achieve significant reduction in antibody titer, the HpSA and UBT with ^{13}C can be used to detect prognosis at 4 weeks after the end of the treatment. Forné *et al.* (2000) compared HpSA testing with histological methods, UBT and the rapid urease test for the diagnosis of H. pylori infection and to evaluate the use to determine H. pylori status after treatment. Before treatment, the HpSA test has a sensitivity and specificity rates of 89.5% and 77.8% respectively. The specificity is lower than that of UBT, histological evaluation and rapid urease test. Within 24 hours after treatment, the sensitivity for HpSA is 0%. Within 6 weeks after treatment, the sensitivity is 70.4% and 81.6%. Six months after treatment, the sensitivity and specificity is further reduced to50% and 79.3%[13]. Thus, they conclude that the HpSA test is beneficial for the primary diagnosis of H. pylori, with a similar sensitivity with other standard tests, but with a lower specificity. HpSA testing is not useful for early monitoring to determine the efficacy of treatment. Within 6 weeks and 6 months after treatment, for further evaluation of the result of eradication treatment, HpSA testing is not very accurate compared to the UBT. In this study we found that HpSA was positive in 57% of gastritis cases and 56% of duodenitis cases. These observations suggest that HpSA is a highly reliable diagnostic method for Acid Peptic disease at primary care level where endoscopy facility is not available. All comparative studies which were done since 2000, showed different results. User manual of this commercial kit specifies sensitivity of 96% and specificity of 96%. For explanation of these obvious differences, the following reasons are most likely to be considered:

1. These commercial kits are manufactured abroad, therefore storage and transportation can affect the quality of these kits (the best storage temperature is – 2 to – 8 c). On the other hand, HpSA kits typically expire at a maximum of two months after the date of manufacture. Considerable transport time can influence performance of the test.
2. According to the instruction, this commercial kit is able to detect 15ng/ml of H. pylori antigen. There may be a decrease in gastric bacterial load of the patients due to overconsumption of antibiotics that plays an inhibitory role on bacterial growth.
3. Quality control and human error in laboratory performance could also justify the difference.
4. There are a number of technical errors that can affect HpSA test result. Some of these problems are presented as:
    a. Cross contamination that can lead to false positive.
    b. Incorrect dilution.
    c. Insufficient homogenization after dilution.
    d. Fermented samples with PH values below 5 after suspension preparation may produce false negative results.
    e. The instruction cut-off is according to company protocol. A new cut- off may increase performance effectiveness.

Conclusion:

HpSA is suitable to use particularly in developing countries. Detection of *H. pylori* antigens using HpSA shows a high sensitivity and specificity and might be useful for non-invasive diagnosis of *H. pylori* infection in children and adult patients. HpSA may be useful particularly in selection of the cases requiring endoscopic examination, in monitoring the response to treatment and in epidemiological studies. We recommend using the stool antigen test as a diagnostic test for *H. pylori* infection.

References:

1. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;1(8336):1273–5.
2. Murray RP, Rosenthal SK, Kobayashi SG, Pfaller AM. *Campylobacter* and *Helicobacter*. In: Medical Microbiology.Mosby 2002; p. 288–93.
3. Aroori S. *Helicobacter pylori*. Gastroenterol Today 2001;5:131–3.
4. Gisbert JP, Trapero M, Calvet X, Mendoza J, Quesada M, Guell M, Pajares JM. Evaluation of three different tests for the detection of stool antigens to diagnose Helicobacter pyloriinfection in patients with upper gastrointestinal bleeding. *Aliment Pharmacol Ther* 2004; 19: 923-929

5. Peitz U, Leodolter A, Kahl S, Agha-Amiri K, Wex T, Wolle K, Gunther T, Steinbrink B, Malfertheiner P. Antigen stool test for assessment of Helicobacter pylori infection in patients with upper gastrointestinal bleeding. *Aliment Pharmacol Ther* 2003;17: 1075-1084
6. Lin HJ, Lo WC, Perng CL, Li AF, Tseng GY, Sun IC, Ou YH.Helicobacter pylori stool antigen test in patients with bleeding peptic ulcers. *Helicobacter* 2004; 9: 663-668
7. Inelmen EM, Gasparini G, Sergi G, Enzi G. Evaluation of Helicobacter pylori with a stool antigen assay in frail, elderly patients. *Scand J Gastroenterol* 2005; 40: 794-799
8. Lopez T, Quesada M, Almirall J, Sanfeliu I, Segura F, Calvet X. Usefulness of non-invasive tests for diagnosing Helicobacterpylori infection in patients undergoing dialysis for chronic renal failure. *Helicobacter* 2004; 9: 674-680
9. Gisbert, J. P., and J. M. Pajares. 2001. Diagnosis of *Helicobacter pylori* infection by stool antigen determination: a systematic review. Am. J.Gastroenterol. 96:2829–2838.
10. Konstantopoulos, N., H. Ru¨ssmann, C. Tasch, T. Sauerwald, H. Demmelmair, I. Autenrieth, and S. Koletzko. 2001. Evaluation of the *Helicobacter pylori* stool antigen test (HpSA) for detection of *Helicobacter pylori* infection in children. Am. J. Gastroenterol. 96:677–683.
11. Leodolter, A., K. Agha-Amiri, U. Peitz, C. Gerards, M. P. Ebert, and P. Malfertheiner. 2001. Validity of a *Helicobacter pylori* stool antigen assay for the assessment of *H. pylori* status following eradication therapy. Eur. J. Gastroenterol. Hepatol. 13:673–676.
12. Fan X G, Growth of H. pylori in candle jars, J Med Microbiol 1997; 46:3:545-5.
13. Nair D et al Immune response to H.pylori in gastroduodenal disorders Indian Journal of Medical Microbiology, 1997; 15:33-35.
14. Sivaprakash R, Rao U A, Indigenous, simple, sensitive and cost effective urease test in the diagnosis of H.pylori for the developing world, Indian J Med Microbiol 1994; 12:111-15
15. Sengupta S, Helicobacter pylori in duodenal ulcer disease and its eradication, Indian J Med Micro biol 2002; 20; 163-64.
16. Makristathis A, Pasching E, Schütze K, Wimmer M, Rotter ML, Hirschl detection of Helicobacter pylori in stool specimens by PCR and antigen enzyme immunoassay. J Clin Microbiol 1998; 36: 2772 – 4.
17. Fanti L, Mezzi G, Cavallero A, Gesu G, Bonato C, Masci E. A new simple immunoassay for detecting Helicobacter pylori infection: antigen in stool specimens. Digestion 1999; 60: 456 – 60.
18. Vaira D, Malfertheiner P, Mégraud F, Axon ATR, Deltenre M, Hirschl AM et al. Diagnosis of Helicobacter pylori infection with a new noninvasiveantigen-based assay. Lancet 1999; 354. p. 30 – 3.
19. Forne M, Domínguez J, Fernandez-Bañares F, Lite J, Esteve M, Gali N, et al. Accuracy of an enzyme immunoassay for the detection of Helicobacter pylori in stool specimens in the diagnosis of infection and post treatment check-up. Am Gastroenterol 2000; 95: 2200 - 5.
20. Arikan S, Kocakusak A, Barut G, Sengoz G, Yucel AF, Gokturk K. Helicobacter pylori stool antigen test: results of a prospective study. *Surg Today* 2004; 34: 318-322