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A Study on Diagnosis of Helicobacter Pylori Infectionby Culture and Molecular Methods from Gastric Biopsy Specimens and Serological Assays in Patients with Peptic Ulcer Disease

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Abstract
H.pylori are asymptomatic and it may play an important role in the natural gasric ecosystem. H. pylori infection is more prevalent in developing countries. The production of the nitrates by bacteria acts as a factor in the causation of mild gastritis to peptic ulcers. The study was conducted with the approval from the institutional Ethical Committee, Sree mookambika institute of medical sciences .Inclusion Criteria are Adults >18 Years and Patients resenting With Clinical features of Gastric Ulcer, Duodenal Ulcer, Antral Gastritis And Gastric Carcinoma. Exclusion Criteria are Patients Who Were On Antibiotics, Proton um Inhibitors Or Helicobacter Pylori Therapy Within 1 Month prior To This Study. In the present study, among the patients with peptic ulcer disaese, Epigastric pain was the predominant symptom (86%) dyspepsia in 80%, vomiting 64%, loss of weight 22%, haematemesis in 12% and melena in 5% of cases. Similarly Epgastric pain was the predominant symptom among the patients with gastric carcinoma (89%), followed by vomiting (65%). in patients with gastric malignancies loss of weight (62%) and loss of appetite (55%) were strikingly high when compared with the patients with non malignant lesions. A high preponderance of H.pylori infection was noted in patients with duodenal ulcer. The inexpensive Rapid urease test detected maximum number of cases Hence, it is a valuable screening test which could be used as an adjunct to endoscopy. HpIgG antibody assays could be employed as a useful screening assay with stringent quality control measures in place for the estimation of seroprevalence of Helicobacter pylori among the high risk population. However subsequent serum samples are required to follow up the severity of the disease.

INTRODUCTION

Helicobacter pylori was discovered by Drs. Marshall and Robin Warren[23] of Perth in 1982. Warren and Marshall were awarded the 2005 Nobel Prize in Physiology or Medicine. Helicobacter pylori is a Gram negative, microaerophilic bacterium found usually in the stomach. It is a curved motile rod found in the deeper portion of the mucous gel coating the gastric mucosa. It is extraordinary among bacteria in it's ability to colonize and persist among this niche for decades despite host defences and gastric acidity. However, over 80% of the individuals infected with the H.pylori are asymptomatic, and it may play an important role in the natural stomach ecology. H. pylori infection is more prevalent in developing countries. The nitrate conversion feature of the bacteria acts as a factor in the causation of infection ranging from mild gastritis to peptic ulcers[24] and even gastric malignancies, such that the International Agency for Research on cancer has declared this pathogen as an independent carcinogen. In addition to the gastric disorders, H.pylori is also associated with of this infection with cardiovascular diseases [25](due to virulence factor of Cag gene) and metabolic syndrome [26](due to the release of Interleukins) are being investigated. H. pylori infection is detected by several diagnostic modalities. These are classified into 1. Endoscopic or invasive tests and 2. Non Endoscopic or non-invasive tests. The Endoscopic tests are Rapid urease test, Histopathological examination, Culture (Gold standard) and Polymerase chain reaction(PCR). The non-invasive tests tests are antibody detection (IgG),Stool antigen test and carbon labelled (^{13}C or $^{1}4C$) Urea breath test. The selection of tests are based on the clinical condition and laboratory resources to diagnose the infection. H. pylori infection is detected immediately from gastric biopsy tissues by Rapid urease test (RUT) and Gram stain or Giemsa stain. The gastric biopsy specimens are also used for histopathological examination (HPE) .Though Culture is the gold standard, it is probably difficult to isolate the H.pylori in majority of the cases. But the advantages are its high specificity and additionally determination of antibiotic susceptibility test from the culture isolates. However, this organism being fastidious and microaerophilic in nature, culture methods has limited role in primary diagnosis. Detection of IgG antibodies in serum is used for diagnosis of H.pylori colonization. It induces both local and systemic immune response. Serum Anti-Helicobacter pylori IgG Antibody (HpIgG) titre was measured by using quantitative ELISA. Serology is sensitive for primary diagnosis but not useful for assessing post treatment status of the infection[27] The Urea breath test (UBT) relies on the urease activity of H.pylori which converts urea into carbon dioxide, which is detected in exhaled breath after 10 min^{1}6.The test has excellent sensitivity, because it represents the major portion of stomach. Unlike serology, it is useful in determining the success of eradication therapy. Hence it is useful screening test, additional advantage is that it is non invasive test. Limitations of tests are that it is non specific test and its high cost. Molecular methods like PCR (Polymerase chain reaction) are also very useful in identification of H. pylori in gastric biopsy samples. PCR also used to detect CagA and VacA virulence genes in gastric biopsy samples26,28. The potential advantage of PCR includes high specificity, quick results and ability to identify different strains of bacteria for pathogenic and epidemiologic studies. The major disadvantage of the molecular methods are they cannot be performed in resource limited settings. In the present study, the following parameters, namely rapid urease test(RUT), Gram stain, Giemsa stain, culture method and serology (HpIgG) were used to detect the presence of H.pylori in gastric biopsy samples which were subjected to HPE for pathological classification of gastric lesions. The diagnosis of H.pylori infection was supplemented with PCR in randomly selected 50 gastric biopsy samples. The analysis was done with the results of H.pylori infection by various methods in correlation with the results of histopathological examination.

Aims and Objectives of the Study: To identify the Helicobacter pylori in gastric biopsy samples from patients with clinical diagnosis of gastroduodenal disease. To compare the various tests like microscopic examination of Gram stain, Giemsa stain smears, Rapid urease test and Histopathology correlation with culture and molecular methods for identification of H.pylori. To evaluate antibody IgG response to H.pylori by ELISA. To perform the molecular detection of H.pylori from the gastric biopsy samples.

MATERIALS AND METHODS

The study was conducted with the approval from the institutional Ethical Committee, Sree mookambika college of medical sciences. Permission to conduct the study was sought from the respective hospital authorities. Informed consent was obtained from the patients before the enrolment into the study. Inclusion Criteria are Adults >18 Years and Patients With presenting With Clinical History Of Gastric Ulcer, Duodenal Ulcer, Antral Gastritis And Gastric Carcinoma. Exclusion Criteria are Patients Who Were On Antibiotics, Proton pump Inhibitors Or Helicobacter Pylori Therapy Within 1 Month prior To This Study. Biopsy Material Would Be Subjected To Rapid Urease Test, Histopathological Examination.

Specimen Collection and Transport: Patients were advised to fast 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% glutaraldehyde for 20 minutes and were thoroughly rinsed with sterile normal saline just before the collection of specimen. Six biopsy samples were taken from the antrum (2 cm from the pylorus) and were transferred to respective sterile leak proof container. One sample was kept in commercial RUT kit after placing one drop of normal saline. This is performed at the time of gastroscopy. There is colour change from yellow to red with in 5 minutes indicates positive reaction. Three specimens were tranported in normal saline, one is for culture and another two for PCR which should be kept in deep freezer. One is crushed between two frosted glassslides and used for gram stain and Giemsa stain. The last bit of sample was stored in formalin for histopathological examination. The specimens for culture were transported in ice to the laboratory and were inoculated on the culture media without delay. 3ml of venous blood was collected under aseptic conditions; serum was separated and stored at-20°C for further processing. The antral biopsy tissue was placed in a commercial RUT kit after placing one drop of normal saline. This is performed at the time of gastroscopy. There is colour change from yellow to red colour indicates positive reaction. Biopsy tissue was crushed between two sterile slides and the minced tissue was inoculated onto freshly prepared 5% defibrinated sheep blood and Skirrows supplement (selective media) and chocolate agar (non selective media). The plates were incubated at 37°C in a candle jar. The plates were examined for bacterial growth from 3-7 days. Characteristic small, translucent circular colonies were confirmed by gram stain, catalase, oxidase and urease. They were subcultured into chocolate agar and skirrow' s supplement (Vancomycin 10mg, Polymyxin B 2500 IU and Trimethoprim 5mg) till no growth was obtained. Another biopsy tissue was crushed between the two sterile glass slides and the minced tissue was used to make smears. One of the slide was air dried and heat fixed. The slide was covered with methyl violet for one minute, excess stain was poured off (primary stain) Grams iodine was added and washed after one minute (mordant). This was followed by acetone for 2-3 seconds (decolouriser). The acetone was washed and the slide was couterstained with dilute carbol fuchsin for 1 minute (counter stain), washed with water, blotted dry and observed under oil immersion objective. Helicobacter pylori appeared as gram negative spiral bacilli. One specimen was fixed in 10% formalin, paraffin sections were made and stained with Haematoxylin and Eosin (H&E) and examined for Helicobacter pylori. The serological detection ofIgG antibodies to cellular components of Helicobacter pylori was done using Calbiotech IgG ELISA KIT. Patients serum when added to wells coated with purified Hp antigen, H.pylori specific IgG antibody, if present, formed antigen-antibody complex which led to spectrometric reactions on subsequent addition of enzyme conjugate and substrate under suitable incubation conditions. The intensity of spectrometric reactions as determined by the spectrophotometer was proportional to the amount of IgG specific antibody in the sample. The assays were validated as per the Quality control criteria of the Kit insert. The results were calculated as Antibody index and were interpreted to be Negative, Borderline and Positive based on the Antibody Index. Serology was done for 120 cases with gastroduodenal symptoms. 1 Positive control, 1 negative control and 2 calibrated standards were available in the kit. Place the desired number of coated strips into the holder.1 Positive control, 1 negative control and 2 calibrated standards ready to use. Prepare 1: 21 dilution of test samples by adding 10µl of the sample to 200µl of sample diluents. Mix well. Dispense 100µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluents in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbency paper or paper towel. Dispense 100µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature. Remove enzyme conjugate from all wells. Wash wells three times with 300µl of 1X wash buffer . Blot on absorbance paper or paper towel. Dispense 100µl of TMB (tetra methyl benzidine) substrate and incubate for 10 minutes at room temperature. Add 100µl of stop solution. Read O.D at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600- 650 nm. Statistical analysis was done using the statistical package for social sciences (SPSS). Different statistical methods were used as appropriate. Mean±SD was determined for quantitative data and frequency for categorical variables. The independent t-test was performed on all continuous variables. The normal distribution data was checked before any t-test. The Chi-Square test was used to analyze group difference for categorical variables.

RESULTS AND DISCUSSIONS

Table 1: Symptoms and Gender Distribution in Relation to Study Population (N=120)

	Gastric carcinoma (n=29)			PUD (n=91)		
Symptoms	**Male**	**Female**	**%**	**Male**	**Female**	**%**
Epigastric pain	24	2	89	59	19	86
Vomiting	17	2	65	46	12	64
Dyspepsia	6	5	37.5	59	29	80
Appetite loss	13	3	55	12	9	22
Weight loss	14	4	62	15	18	35
Haemetemesis	6	1	24	7	4	12
Melena	5	5	31	3	2	5

Epigastric pain was the most common symptom (89%) among the study population.

Table 2: Categorization of Study Population Based on Endoscopic Diagnosis(n=120)

Endoscopic diagnosis	Total	Percentage
Duodenal ulcer	46	38
Gastritis	30	25
Gastric ulcer	15	13
Gastric carcinoma	29	24
Total	120	100

Duodenal ulcer was the most common (38%) endoscopic diagnosis among the study population. Rapid urease Test Positivity among Endoscopically diagnosed gastrodudenal diseases (n=120)

Table 3: Rapid Urease Test Positivity Among Endoscopically Diagnosed Gastrodudenal Diseases (n=120)

Endoscopic diagnosis	Total	Positive	Percentage
Duodenal ulcer	46	25	54.3
Gastritis	30	15	50
Gastric ulcer	15	10	66
Gastric carcinoma	29	15	51.7
Total	120	65	54

The overall positivity of RUT was 54%. but if the gastroduodenal diseases are analysed individually, RUT was positive in 54.3% of patients with duodenal ulcer disease.

Table 4: Positivity of Gram Negative Bacilli by Gram Stain (n=120)

Endoscopic diagnosis	Total	Positive	Percentage
Duodenal ulcer	46	12	28
Gastritis	30	6	14
Gastric ulcer	15	12	28
Gastric carcinoma	29	13	30
Total	120	43	35.8

35.8 % of Gram negative bacilli detected by Gram stain

Table 5: IgG ELISA among Endoscopically Diagnosed Gastroduodenal Diseases (n=120)

Endoscopic diagnosis	Total	IgG ELISA	Percentage
Duodenal ulcer	46	5	4.1
Gastritis	30	5	4.1
Gastric ulcer	15	5	4.1
Gastric carcinoma	29	18	15
Total	120	33	27.5

Seroprevalence in the study Population was 27.5%.

Table 6: Correlation of HpIgG and HPE Report (n=116)

	HpIgG Positive	HpIgG Negative
Malignant lesions	29 (25%)	32 (27.5%)
Non malignant lesions	0	55 (47.4%)

29 patients of histopathological finding of malignant lesions were positive for HpIgG.

Table 7: Results of Rapid Urease Test in Comparision with HPE Reports

Clinical status	No of case	RUT Positive	RUT Negative
Malignant lesions	64	50	14
Non malignant lesions	56	15	41
Total	120	65	55

50 and 15 patients were positive RUT in malignant and non malignant lesions respectively.

Table 8: Correlation of HPE results with various diagnostic tests for H.pylori(n=50)

	RUT	Gram stain	HPE	PCR
Malignant lesions	28	27	33	18
Non malignant	7	5	10	5
Total	35	32	43	23

Hispathological finding highly correlates with RUT of malignant (28) and non malignant (7) lesions.

Table 9: Correlation of PCR with Conventional Test in 50 Samples (n=50)

Test	Positive	Percentage
PCR	23	46
RUT	35	70
Gram stain	32	64
HPE	43	86
IgG ELISA	29	58

Histopathological findings consistent with 33 patients of gastric malignant lesions and 10 patients of non malignant lesions among randomly selected 50 samples.

Table 10: The Cases Among 50 Patients of Gastroduodenaldisease. PCR and RUT, Both Positive in 15 Cases

PCR	RUT Positive	RUT Negative
Positive	15	4
Negative	20	11

PCR was positive in 23 cases and RUT was positive in 35 cases among 50 patients of gastroduodenaldisease. PCR and RUT, both positive in 15 cases. Both were negative in 11 cases. RUT was positive in 35 cases in which 28 were malignant lesions and 7 were non malignant lesions. PCR was positive in 23 cases which includes 18 were malignant lesions and 5 were non malignant lesions. In this study, 120 patients with clinical diagnosis of gastroduodenal disease and endoscopic diagnosis of gastric lesions attending general surgery opd samples were obtained from the patients which were subjected to Gram stain, Giemsa stain, Rapid urease test, histopathological examination, bacterial culture and PCR with appropriate controls to diagnose H.pylori infection. Blood samples were obtained from these patients for serological work up. The results were analysed with SPSS (Statistical Package for Social Sciences) software in correlation with detailed clinical findings. Duodenal ulcer was the most common Endoscopic diagnosis among the study population. In this study, there was a male prepondarance 89(74.1%) among the study population and the maximum number of patients with were in third decade of life[1-5]. In the present study, among the patients with peptic ulcer disaese , the Epigastric pain was the predominant symptom (86%) dyspepsia in 80%, vomiting 64%, loss of weight 22%, 6haematemesis in 12% and melena in 5% of cases. Similarly Epigastric pain was the predominant symptom among the patients with gastric carcinoma (89%) , followed by vomiting (65%). But in patients

with gastric malignancies loss of weight (62%) and loss of appetite (55%) were strikingly high when compared with the patients with non malignant lesions. Haematemesis in 24% , dyspepsia 37.5% and melena in 31% of the patients in the study group. The endoscopic diagnosis of this study population revealed that duodenal ulcer accounted for 38%, suspected malinant lesions in 29%, gastritis in 25% and gastric ulcer in 13%[7-17]. All the 120 patients were subjected to Rapid urease test, Gram stain, Giemsa Stain, histopathological examination and bacterial culture . PCR was done for 50 Samples (randomly selected). Among 120 gastric biopsy samples, the overall positivity of rapid urease test was 54%. But if the correlation between RUT and gastroduodenal diseases are analysed individually, 66% of patients with gastric ulcer were positive for RUT, 54.3% of patients with duodenal ulcer 51.7% of patients with gastric carcinoma (Table - 4). The findings of the present study correlates with the study done by Sivaprakash *et al* in which Rapid urease test was positive in 38.7% of patients with gastroduodenal disease[11-15]. Among the various diagnostic methods to detect the presence of H.pylori in gastric biopsy samples, employed in this study, rapid urease test showed maximum positivity in 54% cases, HpIgG in 24% of cases. H.pylori was not isolated from any of these samples and Hence, culture was not contributory in the present study. In the study conducted by Anjana[11], in which 34 out of 47 cases were positive for Rapid urease test and culture positivity is 17.6%. This low isolation rate may be due to the patchy distribution of H.pylori in gastric mucosa, its fastidious nature, mucosal atrophy, administration of antibiotics (for some other infections) and proton pump inhibitors[7]. The direct gram stain showed gram negative bacilli about 35.8 % in which 17% of samples (21smears) revealed Gram negate ve bacilli morphologically consistent with H.pylori. These results were comparable with the study done by U. Arora *et al,* RUT was positive in 72%,direct microscopy of Gram stain in 20% of cases and 28% of culture positive cases. All cases which were positive by rapid urease test , had significant IgG levels (76%) (Table 6). In this study histopathological examination was consistant with malignant findings for 71(59.1%)cases and 49(40.8%) cases of non malignant lesions. (Table 11) This is compared with study conducted by Aarti *et al* that showed histopathological findings highly correlated with clinical diagnosis of gstroduodenal disease. Among the 120 patients included in the study, HpIgG antibodies were Positive in 33 (27.5%) patients, which includes 4 patients with borderline Positive in and negative in 87 (72.5%) patients. Among the 33 patients with Positive HpIgG (Titre=1.1and range between 1.1 and 2.7), 32 (96%) were diagnosed to have gastric malignancies by histopathological examination and 1 patient with non malignant lesions. Among 87 patients who had HpIgG titre $\leq$0.9 (ie) negative, 32 (36.7%) of them were diagnosed to have malignant gastric lesions and 55(63.2%) of them were having non malignant lesions as with HPE report[16-18]. Patients who tested borderline positive for HpIgG, were analysed separately, 3 of them were diagnosed with malignant and 1 with non-malignant gastric lesions on histopathological examination[19]. In the study conducted by Ruud J.L. F. Loffeld *et al* in dept of gastroenterology[1], free university hospital , Netherland in Jan 2000, correlation of H.pylori IgG antbody titre with malignant and non malignant gastric lesions, H.pylori colonization was found to be a risk factor in 28% of patients with gastric malignancy. Among the 64 patients with malignant gastric lesions, 29 were sero positive for HpIgG (45.3%). The prevalence of patients with H.pylori antibodies (HpIgG) was highest (80%) in the youngest age group (30-49 years) and in contrast to the prevalence of H pylori antibodies in the general population, showed the study conducted by P Sipponen[20]. In all age groups the prevalence of H pylori antibodies was higher in the patients with gastric carcinoma than in the controls without gastric carcinoma. In the PCR test, 23 cases were positive among 50 samples (randomly selected) which includes 18 cases of gastric carcinoma and 5 cases were peptic ulcer disease. Among the 50 gastric biopsy samples from patients with gastroduodenal disease which were subjected to PCR 23(46%) were positive for H.pylori with band at 294bp. Among the positive PCR samples, RUT was positive in 15 (65.2%), Gram stain was positive in 17 (21%). PCR was negative in 27 patients amongest whom RUT was positive in 20 (40%) samples. When the results of PCR are compared with serum HpIgG results in these 50 patients, 12 (24%) patients were positive by PCR as well as serum IgG, whereas 15(30%) patients were negative by both. 8 patients (16%) of gastric samples were positive for H.pylori gene by PCR in whom serum HpIgG were negative. In 13 patients (26%) HpIgG was positive but PCR was negative. Histopathological examination consistent with PCR in 18 cases of malignant lesions and 5 cases of non malignant lesions among 50 study population. P value is 0.7297 ,Since the sample size was very low in tertiary care centre, difficult to attain the p value<0.05. Among the 71 patients with malignant gastric lesions, 29 were positive for HpIgG (40.8%). 54% were positive for H.pylori by RUT. Among the 29 patients with malignant lesions who were subjected to PCR, 18 (46%) were positive by PCR. The study conducted by Parmar A *et al,* HPE results showed 66.7% cases were Adenocarcinoma which is the commonest tumour of stomach. HPE was the important diagnoatic tool for malignancy. Hospital sirio-Libanes[18] study shows the strong correlation between the H.pylori infection

detected by PCR and the histological findings from gastric biopsies noteworthy. PCR was positive in 86% of moderate to marked gastritis cases, 67% of adenocarcinomas and 100% of MALT lymphomas. Rapid urease test correlated with PCR hence, RUT was positive in 35 cases which includes 28 were malignant lesions and 7 were non malignant lesions. PCR was positive in 23 cases which includes 18 were malignant lesions and 5 were non malignant lesions. It shows the significant P value<0.024[19,20]. Jae-Sik Jeon *et al* 68 study showed that combination of RUT and PCR is a valuable diagnostic method and faster identification of antibitic resistance at the genetic level. The detection rate was 88.1% and 89.1% by RUT and H.pylori PCR respectively. RUT result is dependent on bacterial (at least 1X 105 copies) and has high sensitivity >95%. Thus in cases of samples with low bacterial load need to be tested with PCR for reliable results[19-22].

CONCLUSION

A high preponderance of H.pylori infection was noted in patients with duodenal ulcer. The simple and inexpensive Rapid urease test detected maximum number of positive cases among the conventional tests on endoscopy room. Hence, it is a valuable screening test which could be used as an adjunct to endoscopy. HpIgG antibody assays could be employed as a useful screening assay with stringent quality control measures in place for the estimation of seroprevalence of Helicobacter pylori among the high risk population. However subsequent serum samples are required to follow up the severity of the disease and a larger sample size for a point prevalence study. Isolation of organisms has varied sensitivity (2% to 26%) which are restricted only to reference laboratories. Hence PCR is a better alternative to culture, to diagnose H.pylori infection as Nucleic acid amplification methods are more sensitive, if appropriate primer probes are utilized. The combination test of rapid urease test with PCR serves as a rapid and appropriate diagnostic method for H.pylori infection. When compared with uninfected subjects, persons infected with H.pylori including those who had seroconverted were at high risk of developing gastric carcinoma. H.pylori infection is a chronic illness with added risk factors they play a definitive role in gastric carcinogenesis. Hence diagnosis of H.pylori infection should be routinely done in high risk population with the determination of their serological status, to plan for successful eradication of the infection and hence, reducing the risk of carcinogenesis.

Limitations: Since this is a single centered study with low sample size, the true prevalence of H.pylori infection could not be evaluated. Multi-centered studies with larger sample size are required to identify the overall prevalence of H.pylori infection in the community. Since the gastric biopsy sample is very precious, little bit inconvenience for getting consent from patient for sample collection. Symptomatic patients were only subjected to endoscopic diagnosis, so there is possibility of missing asymptamatic H.pylori infection cases. Molecular characterization was done only for the identification of H.pylori from gastric biopsy tissue. There are various genes responsible for detection of H.pylori infection, which were not done in this study. The combination tests of RUT and PCR should be performed for rapid and appropriate diagnosis of H.pylori and detection of antibiotic resistance. Hence, this is not possible to all cases due to expensive diagnostic method.

REFERENCES

1. Loffeld R.J.L.F., B.F.M. Werdmuller, J.G. Kusters and E.J. Kuipers., 2000. IgG antibody titer againstHelicobacter pyloriCorrelates with presence of cytotoxin associated gene A-positiveH. pyloriStrains. FEMS Immunol. and Med. Microbiol., Vol. 28: 10.1111/j.1574-695X.2000.tb01468.x.
2. Forman D. and F. Sitas., 2001. Association between infection with H.pylori and risk of gastric cancer., evidence from a prospective investigation., 342-345.
3. Steer H.W., P.R. Hawtin and D.G. Newell., 1987. An ELISA technique for the serodiagnosis of C.?ylori infection in ?atients with gastritis and benign duodenal ulceration. 143-145.
4. Laheij R.J.F., H. Straatman, J.B.M.J. Jansen and A.L.M. Verbeek., 1998. Evaluation of Commercially AvailableHelicobacter pyloriSerology Kits: A Review. J. Clin. Microbiol., Vol. 36: 10.1128/JCM.36.10.2803-2809.1998.
5. Shao L., Al L.Z. and D.L. Yan., 2003. Anti-Helicobacter pylori IgG antibody (HpIgG) and IgA antibody responsesand the value of clinical presentations in diagnosis of H.pylori infections in patients with precancerouslesions. Journal of Gastrenterology., 2502-2506.
6. Palli D. and F. Cipriani., 1993. Helicobacter pylori antibodies in areas of Italy at varying gastric cancer risk.,cancer epidemiolo., Biomarkers and Prevention., 2: 42-46.
7. Vagarali M., S. Metgud, H. Bannur, S. Karadesai and J. Nagmoti., 2015. Clinical significance of various diagnostic techniques and emerging antimicrobial resistance pattern of Helicobacter Pylori from Gastric Biopsy Samples. Indian J. Med. Microbiol., Vol. 33: 10.4103/0255-0857.167349.
8. Selvi T. and Y.G. David., 2012. Helicobacter pylori infection in india from a western perspective. Indian J Med Res., 136: 549-562.

9. Cruz-Herrera C.F.D., L. Flores-Luna, L. Gutierrez-Xicotencatl, L. Chihu-Amparan and M.A. Sánchez-Aleman et al., 2013. IgG2 response and low IgG titre specific to Helicobacter pylori CagA as serological markers for gastric cancer. J. Med. Microbiol., Vol. 62: 10.1099/jmm.0.050567-0.
10. Parsonnet J., G.D. Friedman, N. Orentreich and H. Vogelman., 1997. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection.. Gut, Vol. 40: 10.1136/gut. 40.3.297.
11. Sarma A., L. Saikia, R.K. Bhuyan and M.E. Hussain., 2017. Molecular Identification and Detection of Virulent Factors in Helicobacter pylori from Gastric Biopsy Samples of Patients Attended at Assam Medical College and Hospital, Dibrugarh, Assam, India. Indian J. Med. Microbiol., Vol. 35: 10.4103/ijmm.IJMM_17_232.
12. Araújo-Filho I., J. Brandão-Neto, L.A.M. Pinheiro, Í.M. Azevedo, F.H.M.A. Freire and A.C. Medeiros., 2006. Prevalence of Helicobacter pylori infection in advanced gastric carcinoma. Arquivos Gastroenterologia, Vol. 43: 10.1590/s0004-28032006000400009.
13. Vaira D., P. Malfertheiner, F. Megraud, A.T.R. Axon and M. Deltenre *et al.,* 2000. Noninvasive antigen-based assay for assessing Helicobacter pylori eradication: A European multicenter study. The Am. J. Gastroenterol., Vol. 95: 10.1111/j.1572-0241.2000.01931.x.
14. Cardinali L.D.C., G.A. Rocha, A.M.C. Rocha, S.B. de Moura and T.D. Soares *et al.,* 2003. Evaluation of [13 C]Urea Breath Test and Helicobacter pylori Stool Antigen Test for Diagnosis of H. pylori Infection in Children from a Developing Country. J. Clin. Microbiol., Vol. 41: 10.1128/jcm.41.7. 3334-3335.2003.
15. He Q., J.P. Wang, M. Osato and L.B. Lachman., 2002. Real-Time Quantitative PCR for Detection ofHelicobacter pylori. J. Clin. Microbiol., Vol. 40: 10.1128/JCM.40.10.3720-3728.2002.
16. Leite K.R.M., E. Darini, F.C. Canavez, C.M. de Carvalho, C.A.T.D. Mitteldorf and L.H. Camara-Lopes., 2005. Helicobacter pylori and cagA gene detected by polymerase chain reaction in gastric biopsies: Correlation with histological findings, proliferation and apoptosis. Sao Paulo Med. J., Vol. 123: 10.1590/S1516- 31802005000 300005.
17. Li S., 2003. Anti-Helicobacter pyloriImmunoglobulin G (IgG) and IgA antibody responses and the value of clinical presentations in diagnosis ofHelicobacter pyloriInfection in patients with precancerous lesions. World J. Gastroenterol., Vol. 9. 10.3748/wjg.v9.i4.755.
18. Sipponen, P., T.U. Kosunen, J. Valle, M. Riihelä and K. Seppälä, 1992. Helicobacter pylori infection and chronic gastritis in gastric cancer.. J. Clin. Pathol., Vol. 45: 10.1136/jcp.45.4.319.
19. Hiroaki S., M. Kozo,, K. Yusuke, M. Yuki, K. Hirohiko, M. Tomoyuki,F. Yoji, T. Shuichi, O. Tomohiro and F. Yoshiyuki., 2017. Decreased serum concentration of Total IgG is related to ?rogression in Gastric cancer patients. yonago Acta Medica., 60: 119-125.
20. Shuto M., T. Fujioka, O. Matsunari, K. Okamoto and K. Mizukami *et al.,* 2017. Association between Gastric Cancer Risk and Serum Helicobacter pylori Antibody Titers. Gastroenterol. Res. Pract., Vol. 2017: 10.1155/2017/1286198.
21. Warren J.R. and B.J. Marshall., 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. The Lancet., 1: 1273-1275.
22. Newell D.G., 1989. The serological diagnosis of campylopylori infection. erdiagnImmunther., Vol. 3.
23. Franceschi f., d. leo, l. fini, a. santoliquido and R. FLORE et al., 2005. Helicobacter pylori infection and ischaemic heart disease: An overview of the general literature. Digestive Liver Dis., Vol. 37: 10.1016/j.dld.2004.10.015.
24. Gunji T., N. Matsuhashi, H. Sato, K. Fujibayashi, M. Okumura, N. Sasabe and A. Urabe., 2008. Helicobacter PyloriInfection Is Significantly Associated With Metabolic Syndrome in the Japanese Population. The Am. J. Gastroenterol., Vol. 103:10.1111/j.1572-0241.2008.02151.x.
25. Lerang F., *et al.,* 1998. Accuracy of IgG serology and other tests in confirming Helicobacter pylori eradication. Scand JGastroenterol., Vol. 33: 10.1080/00365529850171648.
26. Rudi J., et al., 1999. Direct determination of H.?ylori Vac A genotypes and Cag A gene in gastric bio?sies and relation to gastrointestinal disease. Am J Gastroentrol., 94: 1525-1531.
27. Buckley M.J.M. and C.A. O'Morain., 1998. Helicobacterbiology-discovery. Br. Med. Bull., Vol. 52: 10.1093/oxfordjournals.bmb.a011681.