ORIGINAL PROF-618

SERODIAGNOSIS OF HELICOBACTER PYLORI INFECTION

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ABSTRACT

Myocardial infarction, an increasing plaque of modern society, is a disorder of multifactorial nature. Many cases of MI have been reported with causes of established cardiovascular factors. This unexplained situation suggests that there are important unrecognized factors to play a role in the development of myocardial infarction. In this regard infective agents such as H.pylori is reported to increase the risk of myocardial infarction. Therefore this study was designed to observe the anti H.pylori antibodies (IgG) and role of H.pylori in myocardial infarction patients, and comparative study of ELISA technique and ICT (Kit) method, to see their sensitivity, specificity, positive and negative predictive values in diagnosing the H.pylori infection. The results showed significantly higher levels of IgG antibodies against H.pylori in myocardial infarction patients, 103 (68.7%) as compared to controls 22 (44%) showing significant difference (P<0.003). The positive and negative predictive values were 89.1% and 87.5% respectively. So the study demonstrated no link between H.pylori infection and myocardial infarction. The results of ELISA and ICT, when compared, are similar showing no significant difference both for patients and controls (P<0.445) and (P<0.424) respectively. Therefore we concluded that although, ELISA technique is considered goldstandard but it is time consuming, costly, and quite labour intensive while the ICT method is easy, cheap, give results with in ten minutes which makes it a fastest test in diagnosing H.pylori infection.

INTRODUCTION

H.pylori are gram negative, spiral (S-shaped) motile flagellate bacilli that reside beneath the gastric mucus layer adjacent to gastric epithelial cells. H.pylori are non-spore forming and measuring approximately 3.5 x 0.5 micrometer[1,2,3]. H.pylori grows under microaerophilic condition. If the humidity is high, it will grow at 37°C in standard CO_2 incubator containing 10% CO_2 and also produces a number of enzymes and virulent factors such as, phospholipase, protease, catalase, oxidase, alkaline phosphatase, mucinase, superoxide dismutase (SOD) etc[4,5,6].

Possible sources of infection may be water, house flies and animals (Abattoirs)[7,8,9]. Probable routes of

transmission are Iatrogenic Transmission/Nosocomial Transmission, evidence from person to person transmission.

Since its identification in 1983 considerable interest was focused on presence of H.Pylori on the surface of gastric antral epithelium in patients with active chronic gastritis.

The invasive characteristic of H.pylori plays an important role in its pathogenesis. The organisms are acid sensitive and appear to reside deep in the mucus coating layer that lines the stomach. They appear to invade the gastric mucosa in the regions of the intercellular junctions and produce large amounts of ammonium ions and carbon dioxide from the urea present at the site. Presence of the organisms on the surface, between enterocytes, deep inside antral pits, and inside enterocytes result in an inflammatory response that include polymorphonuclear leukocytosis. Loss of microvilli in parasitized regions occurs in some patients with chronic gastritis[4].

The host reaction to H.pylori may be an important cause of mucosal incompetence because large number of neutrophils and lymphocytes are attached to the bacterium. The attraction is related to the presence of chemotactic proteins liberated by H.pylori. Mononuclear cells release interleukins (IL_2), tumor necrosis factor (TNF), and oxygen free radicals in response to the bacterium. The inflammatory response seems ineffective at eradicating H.pylori infection. This may be due to production of superoxide dismutase (SOD) and catalase by the bacterium, which protect it from being killed in neutrophil phagocytic vacuoles[5].

The immunological response to H.pylori infection includes both the production of antibodies (local and systemic) and a cell mediated response, which is ineffective in clearing the infection. The first antibody to appear, is the IgM. Subsequently , IgG and IgA are produced and these persist, both systemically and at the mucosa, in high titre in chronically infected persons[1,10].

H.pylori infection is frequently asymptomatic, particularly in children and young adults. It is thought that H.pylori infection persists indefinitely once established. Some investigators suspect that self-resolving infection may occur in some cases. If an acute self resolving form of H.pylori infection exists, then it is necessary to distinguish between factors associated with the chronicity of the infection and those associated with its acquisition. The current level of interest in H.pylori infection is high because of its etiologic importance in peptic ulcer disease and a risk factor in stomach carcinoma[1,11,12].

H.pylori infection is associated with a number of pathological conditions including :

1. Chronic gastritis
2. Peptic ulcer
3. Gastric adenocarcinoma
4. Pancreatic adenocarcinoma
5. Skin diseases such as rosacea
6. Ischaemic heart disease
7. Ischaemic cerebrovascular disease.
8. Atherosclerosis.
9. Megaloblastic anaemia.
10. Lymphoma of mucosa associated lymphoid tissue (MALT)[5,13 to 16].

Among them, myocardial infarction is one of the commonest cause of mortality[17]. The classical risk factors for acute myocardial infarction fail to explain all the epidemiological variations of the disease. Among the new risk factors, recently reported, several infectious agents appear to increase the risk of acute myocardial infarction in particular H.pylori and chlamydia pneumonia seem to be strongly involved[12,18,19].

Various invasive and non-invasive techniques are available to diagnose. H.pylori infection.
In the invasive methods, endoscopy is required for the following tests to be carried out on biopsy material;

1. Urease biopsy test/Campylobacter like organism test (CLO).
2. Culture.
3. Histology, Giemsa's stain.
4. Polymerase Chain Reaction (PCR)

In non-invasive methods following test are carried out:

1. Serology:
2. Laboratory based - ELISA (Quantitative method) used to detect specific IgG antibodies against H.pylori.

3. Office based (Rapid test) immuno-chromatographic technique, (ICT) - (Qualitative test for IgG ntibodies).
4. Urea breath test C^{13}, C^{14} (invivo test)
5. H.pylori stool antigen test (HpSA test).
6. Culture of stool.
7. Quick saliva test[3,5,9,15].

At present, there is no universally accepted gold standard method for the diagnosis of H.Pyolri infection. Invasive test have been considered the gold standard, but infection may be patchy, culture is difficult and human errors in interpretation and reading may occur. As serology and urea breath test (UBT) are global rather than local, it is possible that these tests more accurately reflect H.Pyolri infection status. The non invasive urea breath test and IgG serology tests are as accurate in predicting H.Pyolri status in untreated patients as the invasive tests of Campylobacter Like Organisms (CLO)[20]. Among the non invasive tests C^{14}, C^{13} - urea breath test and H.Pyolri stool antigen (HpSA) test indicate active H.Pyolri infection. Both these tests have sensitivity and specificity of >90% and are the tests of choice to see the prognosis of the disease[3]..

The simplest test for H.Pyolri infection are serologic, involving the assessment of specific IgG levels in serum. Both standard quantitative (ELISA) and rapid office tests are available. The easiest way to diagnose H.Pyolri infection in a patient is by ELISA who is not undergoing for endoscopy. ELISA is most commonly used serological technique[21].

Serology has many attractions for the diagnosis of H.Pyolri infection. It is inexpensive, essentially non invasive, quick and easy to perform, requiring little specialized equipment and does not rely on the accuracy of the sampling technique to detect infection. Serology can be used to see the prognosis of disease. A drop in antibody titre between matched serum samples taken before and six months after treatment, accurately indicates that H.Pyolri infection has been eradicated[1,22].

This study was designed to observe the role of H.pylori in myocardial infarction and to compare the sensitivity and specificity of ICT with ELISA.

MATERIAL & METHODS

This study was conducted in the department of microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi between March to April 2001.

A total of two hundred subjects were included in the project. Subjects included in the study were divided into two groups:

GROUP-A
One hundred and fifty (150) patients suffering from myocardial infarction.

GROUP B
Fifty (50) apparently healthy, age matched subjects included as controls.

Patients with myocardial infarction were selected from, National Institute for Cardiovascular diseases Karachi. Patients clinically presenting with myocardial infarction, showing positive ECG changes were interogated about their age, sex, weight, height, BMI, social status, smoking habit, hypertension, diabetes mellitus, acid peptic disease and familial illness. The information so collected was recorded on a proforma specially designed for this study. Subjects of both groups were tested for lipid profile as well as for detection of IgG antibody against H.pylori.

Detection of IgG antibodies was being carried out by:

A. Enzyme Linked Immunosorbent Assay (ELISA)-quantitative test.
B. Immuno chromatography technique (ICT)-qualitative test, rapid method.

A. ELISA TEST

This test was carried out by using HpG screen ELISA kit of, G E N E S I S , Diagnostics Ltd (UK). The HpG kit uses the 96 test microtitre plate format. H.pylori antigens are coated onto the surface of the wells.

Serum specimens diluted (1:201) are added and incubated for 30 minutes to allow binding of antibodies directed against H.pylori antigens.

Method:
Test involved following four separate steps:

1. Initially test serum was diluted in specimen diluent buffer and allowed to react with H.pylori antigen bound to the microtitre well. Removal of the unreacted antibodies by washing allowed the specifically bound antibody to be detected by an enzymatic method.
2. Rabbit anti-human IgG conjugated with horseradish peroxidase (HRPO) was made to react with this bound patient's IgG. Unreacted conjugate was removed with a subsequent washing step.
3. Tetramethylbenzidine (TBM) substrate was added to be converted enzymatically to a blue colour. The rate of conversion of this substrate from colourless to a blue colour is being proportional to the amount of specific antibody bound.
4. Addition of stop solution (Reagent 5) gives a yellow colour and the absorbance of standards, controls and samples were measured using a microplate reader. The results were determined quantitatively.

B **Immunochromatography Technique (ICT) Qualitative test**

The test was carried out by ACON H.pylori one step test of ACON Laboratories, Inc (USA).

ACON H.pylori one step test device is a rapid chromatographic immunoassay for the qualitative detection of antibodies to H.pylori in serum or plasma to aid in the diagnosis of H.pylori infection.

Method:

A. The ACON H.pylori one step test device is a qualitative membrane strip based immunoassay for the detection of H.pylori antibodies in serum or plasma. In this procedure, anti-human IgG is immobilized in the test line region of the device. After a serum or plasma specimen is placed in the specimen well, it reacts with H.pylori antigen coated particles in the test strip. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized anti-human IgG. If the specimen contains H.pylori antibodies a colored line will appear in the test line region indicating a positive result. If the specimen does not contain H pylori antibodies, a colored line will not appear in this region indicating a negative result. To serve as a procedural control a colored line will always appear at the control line region if the test has been performed properly.

Interpretation of results

Positive: Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).

Note: The intensity of the red color in the test line region (T) will vary depending on the concentration of H pylori antibodies in the specimen. Therefore any shade of red in the test region (T) should be considered positive.

Negative: One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

STATISTICAL ANALYSIS

The data was fed in computer package "Microsoft Excel" and analysis was done on computer package "EPI-Info" ver 6.0 software of CDC (Center for Disease Control, Atlanta, USA). The result was given in the text as mean (X), standard deviation (S.D) and SEM for quantitative/continuous variable like age and lipids profile etc. and percentage for qualitative/categorical variables like gender, personal and family history and categorical variables and cut off point i.e. cholesterol <200 and >200, etc. To compare proportion /percentage of qualitative variables between groups (cases and control) was " Chi-square test" and the means fo quantitative variables between groups (cases and control) by Student's t test.

In all statistical analysis, only P values < 0.05 were considered to be significant.

RESULTS

Table I and Figure I show the seroprevalence of H.Pylori both in patients and control groups by standard

method i.e. ELISA. Among 150 patients, 68.7% were detected positive for anti H Pylori antibodies (IgG) while in the control group only 44% were positive for antibodies showing a significant difference (P= 0.003).

Table II summarizes the seroprevalence of H.pylori in both groups carried out by ICT (KIT) method. By this method a total of 73.3% of the patients and 54% of the controls were labeled positive for H Pylori infection indicating a statistically significant difference. Positivity with ICT method was more in males as compared to females.

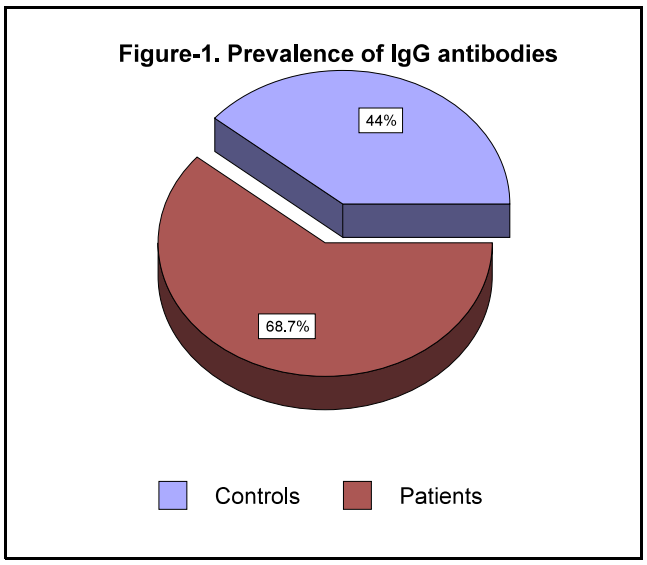


Table III and Figure 2 give a comparison of two methods employed for the detection of anti H.pylori antibodies (ELISA and ICT). The results of these two methods were quite similar showing no significant difference both for the patient and control groups, P=0.445 and P=0.424 respectively.

Table IV, Figure 3 show the sensitivity, specificity, positive and negative predictive values of ICT method both for the patients and controls. In the patients group sensitivity was 95.1% and with specificity of 74.5% respectively.

Table-I. Seroprevalence of H.Pylori in patients (Myocardial infarction) and controls by ELISA method

Gender	Patients (n=150)		Controls (n=50)		P value
	No	H.p+ve	No	H.p+ve	
Male	121	84 (69.4%)	38	16 (42.1%)	0.004*
Female	29	19 (65.5%)	12	6 (50%)	0.485
Total	150	103 (68.7%)	50	22 (44%)	0.003*

*shows significance as compared to controls.

Table-II. Seroprevalence of H.pylori in myocardial infarction patients & controls by ICT method.

Gender	Patients (n=150)		Controls (n=50)		P value
	No	H.p+ve	No	H.p+ve	

Table-II. Seroprevalence of H.pylori in myocardial infarction patients & controls by ICT method.

Gender	Patients (n=150)		Controls (n=50)		P value
Male	121	89 (73.6%)	38	20 (52.6%)	0.026*
Female	29	21 (72.4%)	12	7 (58.3%)	0.468
Total	150	110 (73.37%)	50	27 (54%)	0.018*

*shows significance as compared to controls.

Table-III. Comparison of ELISA & ICT method in Myocardial infarction patients and controls (n=200)

No of cases	ELISA (IgG antibody positive cases (%)	ICT IgG antibody positive cases (%)	P value
Patients (150)	103 (68.7)	110 (73.3)	0.445
Controls (50)	22 (44.0)	27 (54.0)	0.424

The positive and negative predictive values were 89.1% and 87.5% respectively.

Similarly in the control group the sensitivity and specificity were 100% and 82.1% respectively with 81.5% positive predictive value and 100% negative predictive value.

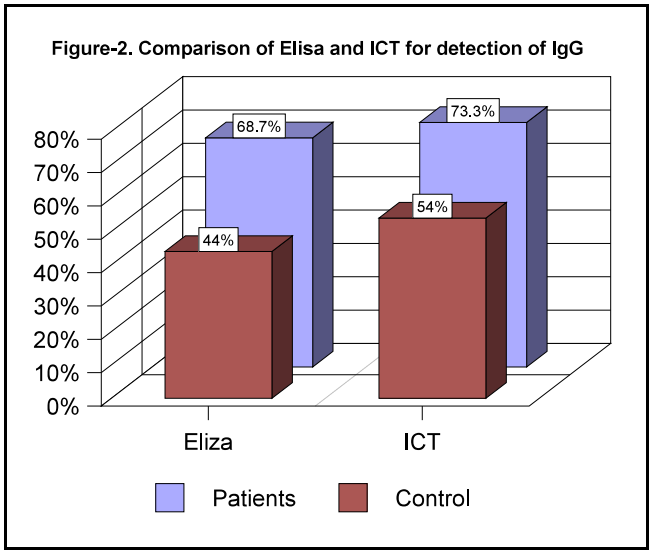
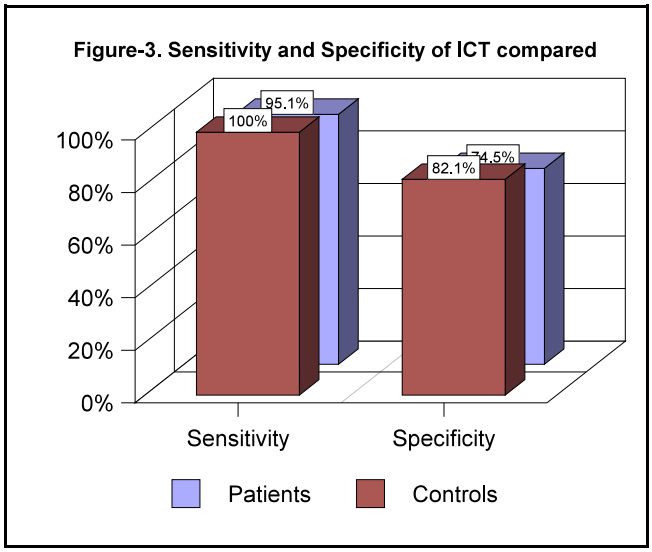


Table-IV. Sensitivity, specificity, positive & negative predictive values of ICT

	Pts (n=150)	Controls (n=50)
Sensitivity	95.1%	100%s
Specificity	74.5%	82.1%
Positive predictive value	89.1%	81.5%
Negative predictive value	87.5%	100%



The two test methods for the detection of anti H.pylori antibodies were compared for the reliability, time consumption and approximate cost per test, and the

results are shown in table V. The ELISA method, taken as standard took more than five hours, was costly and quite labour intensive.

While the ICT (kit) method gave results within 10 minutes making it a fastest test in diagnosing H.pylori infection. This method was also cheaper and easy to perform with a sensitivity and specificity of 95.1% and 74.5% respectively.

Table-V. Reliability , time consumption and approximate cost of test methods for detection of H.pylori infection

Test method	Reliability		Time consumption	Cost per test	Labor
	sensitivity %	Specificity%			
ELISA	standard		>5 hr	costly	Laborious
ICT	95.1	74.5	10 min	Cheap	Easy to perform

DISCUSSION

The role of H.Pylori is currently under debate in patients of ischaemic heart disease. Various studies have been conducted globally to observe the relation of H.Pylori infection in myocardial infarction patients[15,19,27,28].

In 1994, Mendall and colleagues reported that H.Pylori was twice as common in coronary heart disease patients as in control subjects[18]. Similar results in patients (73.21%) and controls (47.82%) were reported by Zuberi et al[15]. These findings are consistent with our study results, showing significant prevalence of antibodies to H.Pylori both in patients (68.7%) and controls (44%) as statistically significant difference was observed (P=0.003). Ridker also showed significant prevalence of antibodies in patients (62%) and controls (40%)[29].

The methods currently adopted for identification of H.Pylori infection in man include culture of the organism or its visual observation in mucosal biopsy specimens, use of an isotope based urea breath test (C^{13}-C^{14}) HPSA test and detection of serum IgG antibodies.

At present there is no universally accepted gold standard method for the diagnosis of H.Pylori infection. In this regard invasive tests have been considered the gold standard (Histology and culture techniques). But according to these methods, infection may be patchy, culture is difficult, human errors in interpretation and reading may occur and also there is danger of intake of radio labeled isotopes. The non invasive urea breath test and IgG antibodies serology test are global rather than local and are accurate in predicting H.Pylori status in untreated patients as the invasive tests of CLO[20,23].

Serology has many attractions for the diagnosis of H.Pylori infection . It is inexpensive, noninvasive, quick and easy to perform and also used to see the prognosis of the disease. A drop in antibody titre between matched serum samples taken before and six months after treatment, accurately indicates that H.Pylori has been eradicated[1,22].

In the present study antibodies against H.pylori were analyzed by ELISA (Standard one) and ICT (Kit) method. The seroprevalence of IgG antibodies to H.Pylori were determined by using a ELISA and ICT both in patients (150) and controls (50). In both patients and controls below 50 years of age, there was no significant difference observed in seropositivity. While significant difference (P=0.005) were observed after the age of 50 years. In case of male gender, 69.4% of patients and 42.1% of the controls were seropositive for H.Pylori infection. These figures show a significant difference.

In evaluation of a test method, it is necessary to observe the sensitivity, specificity, positive and negative predictive values fo the test. In the present study sensitivity , specificity, positive and negative predictive values of ICT were compared with ELISA test (Gold Standard Method) both in patients and controls. In the patients group sensitivity was 95.1% with 74.5% specificity. The positive and negative predictive values were 89.1% and 87.5% respectively. Similarly in the

control group the sensitivity, specificity, positive and negative predictive values were 100%, 82.1%, 81.5% and 100% respective.

The two test methods for the detection of H.Pylori infection were compared for the reliability, time consumption, labor and appropriate cost per test. The ELISA technique is time consuming, costly and quite labor intensive. While the ICT (Kit) office based test gave results within ten minutes, quite easy and marking it a fastest test in diagnosing H.Pylori infection. In other studies carried out by different researchers showed following results, Serum IgG (by ELISA) showed sensitivity 91.3%with specificity 91.6%. Positive and negative predictive values were 95.2% and 85.3% respectively. The sensitivity , specificity, positive and negative predictive values of (I C T) were 89.6%, 100%, 100% and 84.1% respectively[20].

Sensitivity and specificity by ELISA 98% and 94% respectively were observed by Mendall et al[24]. In another study sensitivity, specificity, positive and negative predictive values of ELISA (HEL_{-p} Test II) were 93.5% , 92.5%. 93.5% and 92.5% respectively. The Sensitivity, specificity, positive and negative predictive values of (ICT) test were 95.7%, 72.5%, 80% and 93.5% respectively[21]. Another study indicated the sensitivity and specificity of ICT as 95% and 85% respectively and sensitivity and specificity of ELISA as 95% and 95% respectively. Still another study[25] observed sensitivity, specificity positive and negative predictive values of ELISA as 89%, 98%, 94% and 96%. Study carried out by Gabriel et al[26], showed sensitivity, specificity PPV and NPV of ELISA, 99%, 100%, 96%% and 100% respectively, Kenneth and Mcquaid, showed sensitivity and specificity of ELISA over 90%.

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