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COMPARISON OF DIFFERENT METHODS OF *HELICOBACTER PYLORI* DETECTION IN SYMPTOMATIC GASTRODUODENAL PATIENTS OF KARACHI

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ABSTRACT

Helicobacter pylori is a principal cause of chronic gastritis (CG), gastric and duodenal ulcers and a major risk factor for gastric cancer in humans. The objective of this study was to determine and compare the prevalence of *H. pylori* by different detection methods in symptomatic patients of Karachi. A total of 227 biopsy samples were collected from patients having complaints of peptic ulcer disease (PUD), chronic gastritis and/or abdominal discomfort (AD) from three different health providing institutions of Karachi during 2004-2006 and analyzed for the presence of *H. pylori*. Comparative evaluation of three different conventional diagnostic techniques with that of a highly specific PCR amplification of *ureC* gene were carried out and found *H. pylori* in 62.5%, 64.7%, 68.7% and 59% by PCR, histology, rapid urease test and culture respectively.

Out of 227 biopsy samples collected, 120 (52.8%) were *H. pylori* positive by all methods comprising 62 male patients and 58 female patients. A significant association between *H. pylori* infection and gastro duodenal diseases was observed. Among 120 *H. pylori* infected patients 56 were comprised of PUD, 37 with CG, 19 with AD and 8 with Gastric cancer (GCa). The epidemiology of *H. pylori* infection is also discussed.

Key Words: *H. pylori*, duodenal ulcer, Gastro duodenal diseases, *ureC* gene.

INTRODUCTION

H. pylori persistently colonizes more than half of the global human population (Xue-Jun *et al*. 2005; Algood and Cover, 2006). Infection with *H. pylori* occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country (Feldman, 2001).

In developing countries, 70 to 90% of the population carries *H. pylori* and almost all of them acquire the infection before the age of 10 years. *H. pylori* colonize the gastric mucosa of humans and persist over decades if not treated. It causes chronic infection leading to gastritis. The infection is mostly asymptomatic, but a few individuals will develop severe diseases such as peptic ulcer disease, gastric adeno carcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma (Soltermann *et al*. (2007) Bickley *et al*. (1993), Marshall and Warren (1984).

The prevalence of *H. pylori* infection varies widely by geographic area, age, race, and socioeconomic status. The infection is acquired by oral ingestion of the bacterium and is mainly transmitted within families. The main source of transmission is the mother within families (Perez-Perez *et al*., 2004). The organisms can be cultured from vomits or diarrhea and stools, suggesting the potential for transmission among family members during periods of illness (Parsonnet *et al*., 1999). The overall prevalence of *H. pylori* is strongly correlated with socio economic conditions. Factors such as density of housing, overcrowding, number of siblings, birth order, sharing of bed and lack of running water have all been linked to a higher acquisition of *H. pylori* infection. In developing countries like India, Bangladesh, Pakistan and Thailand, infection with *H. pylori* is more frequent among general population and is acquired at an early age. There are several studies from India showing that *H. pylori* is acquired by most people in early childhood (Mazumder *et al*., 1997).

It is well established that *H. pylori* infection is very common in Pakistan in both the genders as well as in all age groups (Abbas *et al*., 1998). However, most of the studies were hospital based and conducted in symptomatic patients (Qureshi *et al*., 1996; Qureshi *et al*., 2000; Haq *et al*., 1991; Kazi *et al.,* 1990; Qureshi *et al*., 1995; Qureshi *et al*., 1999a;b; Arain *et al*., 1990).

In Pakistan, *H. pylori* exposure rate increases with advancement of age and lowering of socio-economic status (Qureshi *et al*., 1999). In a study, the overall exposure rate to *H. pylori* in children was 33% while in a group of adult dyspeptic patients undergoing upper gastrointestinal endoscopy, the prevalence of *H. pylori* infection investigated by histology and rapid urease test revealed that *H. pylori* was associated with 85% cases of duodenal ulcer (Qureshi *et al*., 1999a; Arain *et al*., 1990).

H. pylori infection can be diagnosed by noninvasive methods or by invasive endoscopic biopsy of the gastric mucosa; the selection of the appropriate test depends on the clinical settings (Howden and Leontiadis., 2000). Noninvasive methods include the urea breath test, serologic tests, and stool antigen assays.

When endoscopy is clinically indicated, the test of first choice is a urease test on an antral-biopsy specimen (Howden and Hunt, 1998). It permits cheap and rapid detection of urease activity in the biopsy material, with a sensitivity of 79 to 100 percent and a specificity of 92 to 100 percent (Graham and Qureshi, 2001). A number of rapid urease tests have been described; all these tests depend on the preformed urease enzyme secreted in large quantities by *H. pylori* (McNulty and Wise, 1985; Das *et al*., 1987; Arvind *et al*., 1988). Another non-invasive test is ^{14}C or ^{13}C breath test following the oral administration of ^{14}C or ^{13}C urea (Graham *et al*., 1986).

If the urease test is negative, additional biopsy specimens stored in fixative can be sent for histological examination. Culture of *H. pylori* with antibiotic-sensitivity testing is not routinely performed for the initial diagnosis of *H. pylori* infection, but it is recommended after the failure of second-line therapy (Bazzoli, 2001).
Recently, molecular biological methods like amplification of 16S rRNA by Polymerase Chain Reaction (PCR) (Solnick *et al*., 1993) and the follow up methods like Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), are the potential protocols to study genetic diversity of various *H. pylori* strains (Akopyanz *et al*., 1992).

In this study we have analyzed different detection methods specially the highly specific and sensitive PCR test with other conventional methods for the prevalence and diagnosis of *H. pylori* induced ulcers and other GI disturbances.

MATERILAS AND METHODS

Patient population:

Two hundred and twenty seven patients undergoing upper gastro duodenal endoscopy for diagnosis and treatment purpose in the gastroenterology departments of three different hospitals of Karachi *viz.*, Liaquat National Hospital (LNH), Pakistan Medical Research council (PMRC) and Jinnah Post Graduate Medical Centre (JPMC-Ward 7) were included in this study. The Institutional Review Board of KIRAN (Karachi Institute of Radiotherapy and Nuclear Medicine) and Pakistan Medical Research council (PMRC) approved the research protocol. All patients gave written consent for biopsy sampling.

Endoscopies, Biopsy sampling and Histopathology:

Four gastric biopsy specimens from antrum and corpus from each patient were collected for rapid urease test, culture, histopathology and PCR. Biopsy specimens for culture and PCR were taken into screw caped bottle containing 0.9% saline and transported to the Molecular biology Lab at KIRAN Hospital Karachi. The fourth biopsy specimen was directly used for DNA extraction for PCR analysis. Patients taking antibiotics, with bleeding ulcers or an acute hemorrhage from other sites in the upper gastrointestinal tract and patients who had recently undergone surgery of stomach were excluded. Two local strains with laboratory numbers 101 and 110 and one strain of *Campylobacter jejuni* (provided by Dr. Saleem Hafeez, Sind Institue of Urology and Transplantation (SIUT), Karachi) were used as the positive and negative reference controls. Biopsies were routinely processed and embedded in paraffin and assessed for *H.pylori* as described by Doglioni *et al*. (1997) by haematoxylin and eosin, and the HpSS stain.

Rapid Urease Test:

One biopsy specimen from each sample was introduced with a sterile medium in to a semisolid 2% urea agar and incubated at room temperature. Results were recorded up to 4 h after inoculation as described by Deltenre *et al*. (1989).

Culture:

Gastric biopsy specimens were ground with tissue homogenizer and then inoculated onto selective Columbia Blood Agar (Oxoid, CM331) and incubated under microaerophilic (5% O_2, 10% CO_2, and 85% N_2) conditions at 37 ^oC for up to 7 days. Organisms were identified as *H. pylori* on the basis of morphology on Gram stain and by oxidase, catalase and urease tests.

DNA extraction from biopsy specimens:

Each biopsy sample was ground in 1 ml of Brucella broth. The ground sample was then transferred to a microcentrifuge tube and centrifuged for 5 minutes at 10,000 X g. The supernatant was then discarded, and 300 µl of extraction buffer (20mM Tris HCl [pH 8.0], .0.5 % Tween 20) was added to the pellet. This pellet was then resuspended, 15 µl of a proteinase K solution (10mg/ml) was added, and the sample was incubated at 55 ^oC for 1 hour. Finally heating the sample for 10 min at 98 ^oC inactivated the *proteinase K.*

DNA extraction from *H. pylori* cultures:

Genomic DNA was extracted from each strain of *H. pylori* using commercially available kit (Gentra, PUREGENE USA) according to the manufacturer's instructions and dissolved in distilled water. The concentration of DNA was measured by absorption of light at 260 nm with the following conversion factor. 1 optical density (OD) unit of a double strand of DNA = 50 µg/ml.

Diagnostic PCR:

The presence of *H. pylori* DNA was confirmed by PCR amplification of the phosphoglucosamine mutase gene, *glmM*. Primers listed below were derived from the *H. pylori ureC* gene sequence (Labigne, *et al*., 1991) (accession numbers, EMBL X 57132 and GenBank M60398, respectively) which amplify a 294-bp DNA fragment and have already been tested for use in the diagnosis of *H. pylori* infection (Labigne, *et al*., 1991). PCR reactions were performed in a volume of 50 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide, 25 pmol of each primer and
2.5 units of *Taq polymerase.*

Primer sequence for *H. pylori ureC* gene fragment amplification:

Forward Primer (5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3')
Reverse Primer (5'-AAGCTTACTTTCTAACACTAACGC-3')

RESULTS

Out of 227 biopsy samples collected during endoscopies 120 were found *H. pylori* positive by four different diagnostic methods i.e. culture, rapid urease test, histopathology and *ureC* gene amplification by PCR (Fig 1) (table 1). Among 120 positive samples 62 were male patients out of 114 samples and 58 were female patients out of 113 samples (Fig 2). *H. pylori* infection and GI disorders were found in almost all age groups and the frequency of *H. pylori* colonization of gastric mucosa was highest in the middle age group (31years to 60 years) of these symptomatic adult patients (Fig 3) (Mean age 44 years; age range 18 to 78 years).

Endoscopic findings and *H. pylori* infections:

Among 120 infected patients 56 were that of PUD, 37 with CG, 19 with AD and 8 with GCa (Table 2) (Fig 5). Most common symptoms of the patients diagnosed by endoscopy were gastric and duodenal ulcers and gastritis. Similarly most of the patients carrying *H. pylori* infections had either gastric or duodenal ulcers or gastritis (Fig 4).

Cultural and Biochemical characteristics of *H. pylori* isolates:

Out of 227 biopsy samples inoculated on *H. pylori* selective medium (Columbia blood agar with supplement) 134 (59%) were found *H. pylori* positive after morphological, cultural and biochemical tests (Fig 1).
On Columbia blood agar medium supplemented with antibiotics and Laked horse blood (Dent's selective medium) characteristic growth of *H. pylori* appeared as discrete, translucent and non coalescent colonies. After 3-5 days of incubation colonies were small, convex and dew drop like with entire margin and after prolonged incubation (7-10 days) large size colonies with dark color were obtained.

All *H. pylori* isolates were found to be catalase, oxidase and urease positive. Results of urease test were observed within 30 minutes after inoculation. *H. pylori* isolates were differentiated from *C. jejuni* on the basis of urease and hippurate hydrolysis tests.

Rapid urease test:

The rapid urease tests were positive for 156 of 227 (68.7%) patients' biopsy specimens (Fig 1).

Histopathology:

All 227 biopsy specimens collected from different GI disorder patients at the time of endoscopy were examined for histopathological changes and for the detection of *H. pylori*. *H. pylori* organisms were detected by Giemsa and haematoxylin eosin stain in 147 out of 227 (64.7%) (Fig 1). The stained slides were first observed for the presence of *H. pylori* under oil immersion lens. In Giemsa stain slides *H. pylori* appeared violet colored, curved or comma shaped rods, attached to mucus and mucus secreting epithelial cell layers.

Amplification oF *ureC* gene by PCR:

The specific *ureC* gene fragment was amplified from biopsy specimens of 142 (62.5%) samples. The biopsy specimens of all culture-positive samples were positive by *ureC* PCR. Furthermore, biopsy specimen from one culture-negative samples yielded the expected 294-bp *ureC* fragment (Fig 6) following PCR amplification (Table 1). The specificity of PCR was determined by testing two local *H. pylori* strains with laboratory numbers 101 and 110 and one strain of *Campylobacter jejuni* (provided by Dr. Saleem Hafeez, Sind Institue of Urology and Transplantation), Karachi) with other bacterial contaminants recovered from gastric biopsy material.

Comparison of culture, histology, rapid urease test and PCR for the diagnosis of *H. pylori* infection:

The comparative results obtained by all the different methods are reported in Table 1. Comparative evaluation of three different conventional diagnostic techniques of 227 patients was carried out during this study. The prevalence rate of *H. pylori* infection was found to be 64 %, 68 %, 60 % and 62 % by histology, rapid urease test, culture and PCR respectively. *H. pylori* was observed in 68.7% by rapid urease test suggesting that it is more sensitive for this purpose. Moreover, it is less expensive, more rapid and sensitive test than culture and histology. The sensitivity, specificity, and positive and negative predictive values for *ureC* PCR were, respectively, 100, 97, 95, and 100%.

The frequency of *H. pylori* positivity confirmed by all four methods in 120 patients in relation to the clinical diagnosis as determined by endoscopy is shown in Table 1.

DISCUSSION

High prevalence of *H. pylori*:

The present study confirmed the high prevalence of *H. pylori* in symptomatic patients of Karachi previously reported by other authors as well (Abbas *et al*., 1998 and Kazi *et al*., 1990). High prevalence of *H. pylori* infection has been related to low socio-economic status, hygiene and sanitation conditions, which are still rather common among the majority of residents in Karachi, representing the overall condition of the rest of the population of Pakistan. As the biopsies were obtained from symptomatic patients, results reflect the findings in these groups of patients rather than entire population, however, the results obtained provided insight into possible distribution of *H. pylori* in this region.

In developing countries like Pakistan, India, Bangladesh and Thailand, infection with *H. pylori* is more frequent among general population and is acquired at an early age (Graham *et al.,* 1991; Ahmad *et al*., 1997; Perez-Perez *et al.,* 1990; Abbas *et al.,* 1998).

In our study *H. pylori* infection was found to be 64%, 68.7%, 59% and 62.5% of total collected samples by histopathology, rapid urease test, culture and PCR respectively (average 63%) (Table 1) (Fig1). Investigators from other parts of the world including Brazil, Korea, Japan, Canada and Turkey have also reported *H. pylori* infection in the range of 31-78% (Imazer *et al.,* 1988) indicating that the isolation rate from different parts of the world is quite variable, being more in under developed countries as compared to developed countries.

In more industrialized and developed regions of Asia like Japan, China and Singapore, frequency of *H. pylori* infection has been reported to be somewhat lower (Miwa *et al*., 2002). The prevalence of *H. pylori* in the United States has decreased to approximately 10% in the white middle and upper class population of 50 years of age or younger (Everhart *et al*., 2000). As *H. pylori* is transmitted by feco-oral route, overcrowding, poor sanitation, lower socioeconomic status and poor water supply are some of the major factors that result in higher frequency and lower age of acquisition of *H. pylori* in less developed Asian countries (Mazumder *et al.,* 1997).

Peptic ulcer disease and *H. pylori* infection:

In present study the frequency of *H. pylori* was found to be 62.5% and the many of these *H. pylori* positive cases had peptic ulcer disease PUD (56 out of 120). The results of our study confirm the strong association between *H. pylori* and peptic ulcer disease and gastritis, which is also similar to other report (Dooley *et al.,* 1989). Our observation regarding the frequency of *H. pylori* colonization of gastric mucosa in the middle age (in the age group 31-60 years) (fig 3) is also similar to the earlier reports from Dooley *et al.,* (1989) and Graham *et al*., (1988).

The frequency of *H. pylori* infection in patients with gastric ulceration is reported to vary from 56% to 96% (Crabtree *et al*., 1991). Karttunen *et al.,* 1991, identified *H. pylori* in 60 of 107 dyspepsia patients. Another report shows that the prevalence vary depending on the patient population selected (92% in Yemeni patients versus 46% in British patients (Shousha *et al*., 1993).

H. pylori was isolated from 54.3% from males and 51.3% from females and are slightly higher in males.

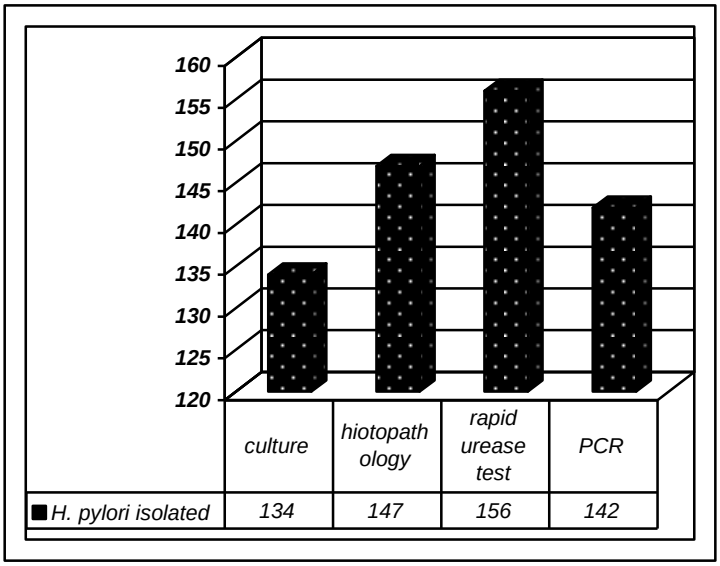

Fig 1.Number of *H. pylori* isolated by culture, histopathology, rapid urease test and PCR.

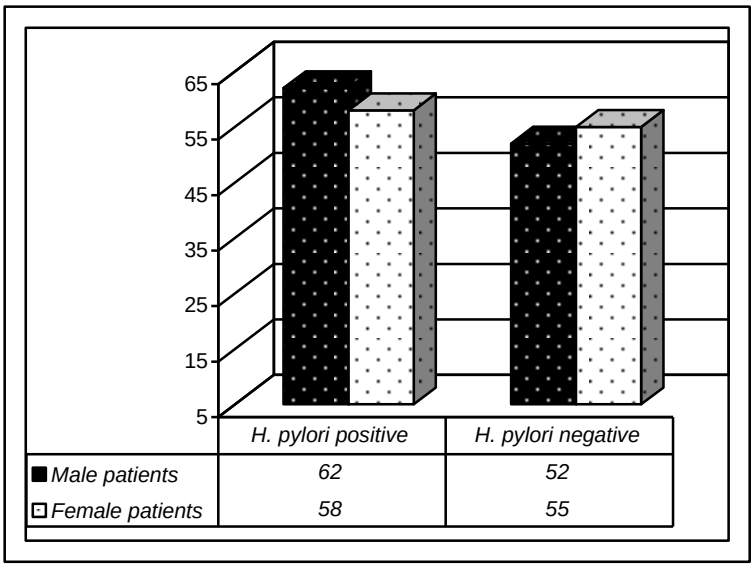

Fig 2. Prevalence of *H. pylori* in male and female populations.

Gastric cancer and *H. pylori* infection

Gastric cancer is the second most frequent cause of cancer-related deaths. There are very strong evidence that *H. pylori* increases the risk of gastric cancer. *H. pylori* has been classified as a type I (definite) carcinogen since 1994, mainly on the basis of large seroepidemiologic case control studies (Parsonnet *et al.,* 1991; Nomura *et al.,* 1991; Forman *et al.*, 1991). In the latest available epidemiological studies, based on the most accurate methodology, the presence of *H. pylori* infection combined with *cagA* antibody status increases the risk of gastric cancer 20-fold, compared with controls. One estimate attributed 70% of distal gastric cancers to *H. pylori* (Ekström *et al*., 2001), while the highest estimate claimed that *H. pylori* is a condition *sine qua non* for gastric cancer development (Brenner *et al*., 2004). In our study 6.6% of *H. pylori* positive samples were confirmed cases of gastric cancers. In a recent prospective study of 1526 Japanese subjects, (Uemura *et al.,* 2001) gastric cancer developed in 2.9 percent of

1246 patients with infection over 7.8 years, whereas no gastric cancer was observed in 280 non infected control subjects.

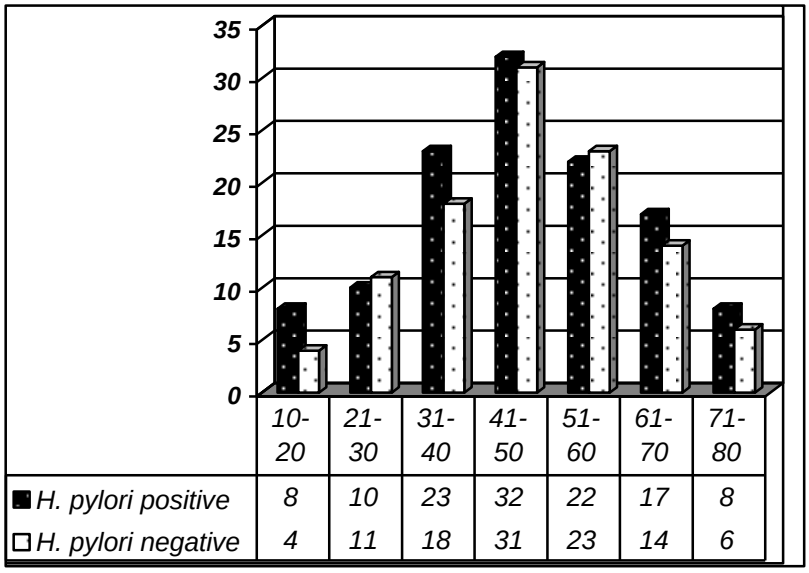

Fig 3. Relationship of *H. pylori* infection with different age groups

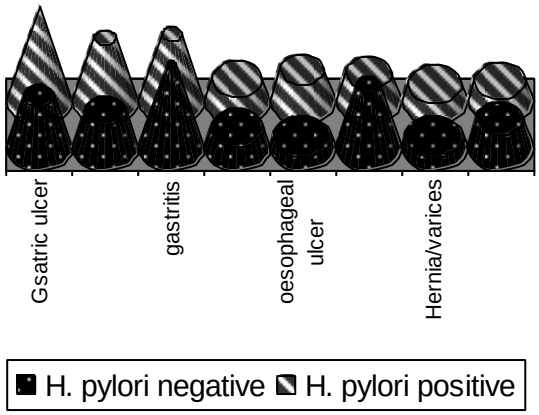

Fig 4. Most common symptoms recorded after endoscopy and among *H. pylori* positive patients

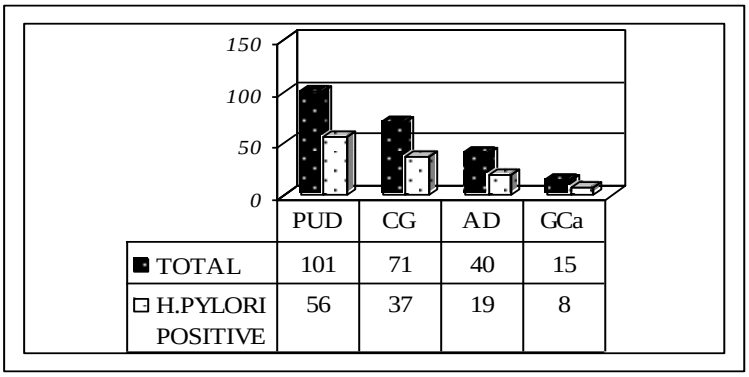

Fig 5. Number of *H. pylori* positive samples in various GI disorders

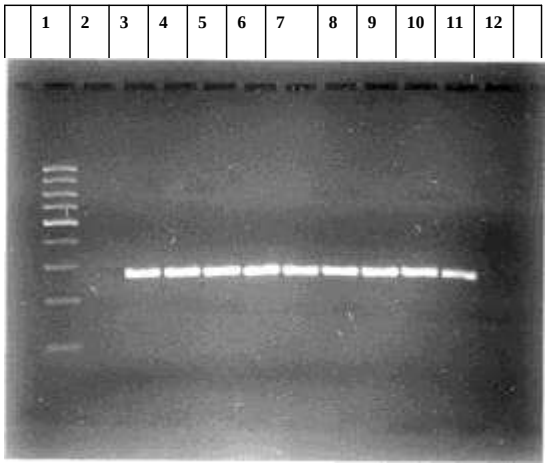

Fig 1a: Gel electrophoresis and PCR amplification of *ureC* gene
Lane 1 100 bp ladder (DNA marker)
Lane 2 Negative control
Lane 3 Positive control of 294 bp band for *ureC* gene
Lane 4-11 294 bp amplified *ureC* gene of *H. pylori* positive samples
Lane 12 Negative amplification control

Fig 6: 1.5% agarose gel electrophoresis of PCR products for *H. pylori ureC* gene; PCR reactions were performed in a volume of 50 ul containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM of each deoxynucleotides, 25 pmol of each primer and 2.5 units of *Taq DNA* $_{polymerase.}$

Table 1. Detection of *H. pylori* by four invasive diagnostic techniques.

Results of the diagnostic technique				**No. of patients**
Histology	**Rapid-urease test**	**Culture**	**PCR *ureC***	
+	+	+	+	**120**
-	+	+	+	**4**
+	-	+	+	**6**
+	+	-	+	**7**
-	-	+	+	**4**
-	+	-	-	**10**
+	+	-	-	**6**
-	-	-	+	**1**
-	+	-	-	**9**
+	-	-	-	**8**
-	-	-	-	**52**
147(64%)	**156(68%)**	**134(59%)**	**142(62%)**	**227**

Table 2. Distribution of *H. pylori* positive samples in various gastroduodenal diseases.

	Liaquat National Hospital (LNH)		**Pakistan Medical Research council**		**Jinnah Post Graduate Medical Center**	
	Total No. of samples	*H. pylori* Positive	Total No. of samples	*H. pylori* Positive	Total No. of samples	*H. pylori* Positive
Total	85	55 (64.%)	81	43 (53%)	61	22 (36%)
Peptic Ulcer Disease	35	25(71.4%)	40	23(57.5%)	26	8 (30.7%)
Chronic gastritis	30	21(70%)	20	8 (40%)	21	8(40%)
Abdominal discomfort	20	9(45%)	10	6 (60%)	10	4 (40%)
Gastric Carcinoma	0	0	11	6(54.5%)	4	2 (50%)

Most important, no case of cancer was detected in a subgroup of 253 infected patients who received eradication therapy early in follow-up. The results from ongoing intervention trials of the effect of *H. pylori* eradication on the incidence of gastric cancer may have major implications for global policies concerning the treatment and prevention of *H. pylori* infection. *H. pylori* infection significantly increases the risk of gastric MALT lymphoma, and 72 to 98 percent of patients with gastric MALT lymphoma are infected with *H. pylori* (Parsonnet *et al.*, 1994; Wotherspoon, 1998). Furthermore, eradication of *H. pylori* alone induces regression of gastric MALT lymphoma in 70 to 80 percent of cases (Bayerdorffer *et al.*, 1995).

Comparison of different detection methods:

H. pylori infection can be diagnosed by noninvasive methods or by invasive endoscopic biopsy of the gastric mucosa; the selection of the appropriate test depends on the clinical setting (Howden, and Leontiadis, 1998). Noninvasive methods include the urea breath test, serologic tests, and stool antigen assays. Patients with alarming symptoms, such as anemia, gastrointestinal bleeding, or weight loss, as well as patients more than 50 years of age, should undergo endoscopy for the diagnosis of *H. pylori* infection. When endoscopy is clinically indicated, the test of first choice is a urease test on an antral-biopsy specimen (Howden and Hunt, 1998). It permits cheap and rapid detection of urease activity in the biopsy material, with a sensitivity of 79 to 100 percent and a specificity of 92 to 100 percent (Graham and Qureshi, 2001).

In our study the highly sensitive, rapid and economical test was rapid urease test. We found that 156 out of 227 biopsies were positive for *H. pylori* infection by rapid urease test. Urease production is an important property of *H. pylori* and detection of urease production is important in the diagnosis of *H. pylori*. In our study it was found that rapid urease test is a rapid, economical and reliable test for the presumptive diagnosis of *H. pylori* infection as it can be easily performed in the endoscopy room, requiring less time period, not very expensive and easy to perform. Sensitivity can be improved by additional biopsies, but false negative results are observed in patients with active or recent bleeding and in patients taking antibiotics or antisecretory (H2 receptor antagonists and proton pump inhibitors, PPI) compounds.

One of the interesting aspects of the study was to determine and compare the sensitivity and specificity of the tests used for the diagnosis of *H. pylori* infection. Several investigators (Ormand and Talley, 1990; Debottgnie, *et al*., 1992) have suggested that culture of *H. pylori* should be the best method for the diagnosis of *H. pylori* infection but this is not true when we compare the prevalence of *H. pylori* infection by culture with other three methods i.e. rapid urease test, histopathology and PCR. This may be due to multiple factors during the endoscopy sampling, transportation and culture isolation of this fastidious organism. Since the survival rate of *H. pylori* decreases after the collection of biopsy specimen and delayed processing. The slightly lower prevalence rate of infection by culture in our study could be due to the fact that this organism is oxygen sensitive and some of the biopsies were processed more than two hours after collection which reduces the chance of isolation by culture method. The isolation rate was also less due to the fact that most of the patients included in our study were already on antibiotics and antiulcer drugs before reporting the physician, thus resulting in the suppression of growth and a negative culture test does not prove its absence from the case. Histology is also sensitive but expensive and requires experienced personal as

compared to rapid urease test. In our study 147 patient samples were detected *H. pylori* positive by histopathology out of 227 samples and proved to be least as sensitive as rapid urease test.

PCR offers great promise as a highly sensitive and specific technique for the detection and genotyping of *H. pylori.* The sensitivity, specificity and accuracy of PCR diagnosis of *H. pylori* is beyond any doubt and was established with the purpose of not only as a diagnostic tool but for genotyping as well. Serial dilutions of DNA from *H. pylori* positive control 101, starting from a concentration of 36.0 ng/µl in 10 fold dilution series were subject to amplification of *ureC* by PCR and it was found that the assay can detect at least 3.6 fg of bacterial DNA, which corresponds to approximately two *H. pylori* genomes (Taylor *et al*., 1992).

In this study we have found that PCR was more sensitive than culture and almost equal to histopathology in detecting *H. pylori*. Overall, our results are in good agreement with other recent data for comparison of different routine test for *H. pylori* in which Giemsa staining of histological sections (Weiss *et al*., 1994) or the agreement among all the tests was taken as the gold standard. We also found that *ureC* PCR was the most sensitive test for detecting *H. pylori* infection. *ureC PCR* was able to detect up to the equivalent of two genomes in a sample, which is in the same range of detection as that described for other PCR assays for *H. pylori* (Clayton *et al*., 1992; Hammar *et al*., 1992). However, our assay was more sensitive than the one reported in which the same *ureC H. pylori* primers but shorter steps in each cycle (1 min) were used (Bickley *et al*., 1993).

The results of PCR amplification directly from biopsy specimens and from the *H. pylori* strains subsequently isolated from them were perfectly correlated. No false-positive or false-negative results were found for biopsy specimens amplified by the *ureC* primers, suggesting the absence of inhibitory substances in gastric biopsy specimens. Overall, we found that the PCR assay targeting the *ureC* gene in biopsy specimens proved at least as sensitive as culture for detecting *H. pylori* infection in patients. In addition, although we do not yet have a comprehensive survey of post-treatment patients, we think that the *ureC* PCR could constitute a very useful diagnostic tool for treatment follow-up, at which time the number of bacteria in the gastric mucosa is usually small and may go undetected by culture or by other diagnostic methods.

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