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Detection of *Helicobacter pylori* from Gastric Biopsy Samples by Culture, Polymerase Chain Reaction and Histopathological Methods in Eastern Turkey

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ABSTRACT

The aims of this study were to detect *Helicobacter pylori* (*H. pylori)* by culture, polymerase chain reaction (PCR) and histopathological methods, to determine the prevalence of active *H. pylori* infection in Elazig Province, East of Turkey and to evaluate the relationship between *H. pylori* infection and sex. Antrum and corpus samples of 184 Turkish patients (85 male and 99 female, age range 17 to 92 years, average 49) with gastrointestinal complaints attending the Gastroenterology Department of Firat University Hospital during 2009 and 2010 were used in this study and examined for the presence of *H.pylori* using culture, PCR and histopathological examination. Patients were grouped as gastritis (G) in 155 cases, peptic ulcer (PU) in 26 cases, gastric cancer (GC) in 3 cases at the time of endoscopy. *H. pylori* was isolated in 61 (33.2%) samples. By PCR, *H. pylori* was detected in 140 (76.1%) patients, 115 (74.2%) cases with G, 23 (88.5%) cases with PU and 2 (66.7%) cases with GC. Fifteen of 155 patients with G were excluded from the histopathological evaluation due to inadequate material given. Histopathological examination of 140 patients with G was detected to be *H. pylori* positive in 96 (68.6%). The prevalence of *H. pylori* was found to equal in male and female patients (50%) by using PCR. The prevalence of *H. pylori* in patients with PU and GC was found to be higher in men (60.9% and 100%, respectively) than in women (39.1% and 0%, respectively). However, the prevalence of *H. pylori* in patients with G was found to be higher in women (53%) than in men (47%). Our results exhibited that there was no significant difference between sex and *H. pylori*-positive patient groups ($p>0.05$).

1. Introduction

The diagnosis of *Helicobacter pylori* (*H. pylori*) infection involve both non-invasive (serology, urease breath test and stool test) and invasive techniques [culture, histological staining, urease test or polymerase chain reaction (PCR)] (Dunn et al., 1997; Logan and Walker, 2001). Currently, an optimal method for the diagnosis of *H. pylori* active infection does not available (Arismendi et al., 2011). The methods available have advantages and limitations related to factors such as level of

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technical difficulty, cost and extensive accessibility in the hospitals (Arismendi et al., 2011). Culture is not usually recommended for routine diagnosis of *H. pylori* infection because it is a tedious, time-consuming procedure, requiring and expensive skilled personnel (Chomvarin et al., 2006). Culture has been considered the "gold standard" when comparing the accuracy of non-invasive techniques (Megraud and Lehours, 2007). In addition, the isolation and identification of strains is important for the investigation of profiles of bacterial virulence and, particularly, antibiotic resistance monitoring (Yin et al., 2009). Histological examination using the Sydney System is used for classification and grading of gastritis (G) and *H. pylori* infection in worldwide (Dixon et al., 1996).

Helicobacter pylori specific target genes include the 16S rRNA gene (Ho et al., 1991), the random chromosome sequence (Valentine et al., 1991), 26-kDa species-specific antigen (SSA) gene (Hammer et al., 1992; O'Toole et al., 1991), the urease A (*ure*A) gene (Clayton et al., 1992) and the phosphoglucosamine mutase (*glm*M) gene [(previously named urease C (*ure*C)] (Bickley et al., 1993). A simple, reliable and specific PCR assay, using the *ure*C gene as a target, was developed for the rapid identification and discrimination of *H. pylori* (Lu et al., 1999).

The aims of this study were: 1) to detect *H. pylori* by culture, PCR and histopathological methods, 2) to determine the prevalence of *H. pylori* infection in Eastern Turkey, 3) to evaluate the relationship between *H. pylori* infection and sex.

2. Materials and Methods

2.1. Patients

All samples were obtained from the antrum and corpus of 184 Turkish patients [(85 males and 99 females) between 17-92 years of age (average 49)] who underwent gastrointestinal endoscopy at the Gastroenterology Department of Firat University Hospital during 2009 and 2010. Patients were grouped as G in 155 cases, peptic ulcer (PU) in 26 cases, gastric cancer (GC) in 3 cases at the time of endoscopy.

This study was approved by the Medical Ethics Committee of Firat University, we obtained informed consent from all patients prior to specimen collection. The gastric biopsy specimens were used for isolation and identification of *H. pylori* by culture, PCR and histopathological examination.

2.2. Bacterial Culture of H. pylori from biopsy samples

Culture of gastric biopsy samples was performed as previously described by Chomvarin et al., (2006). Biopsy samples were placed in sterile Eppendorf tubes containing Brain Heart Infusion broth (Oxoid, Basingstoke, UK) with 1.5% glycerol, kept immediately in an ice box and processed within a maximum of 2 h after sampling. Samples were immediately plated onto a selective media which was consisted of Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 7% laked horse blood (SR0048C, Oxoid, Basingstoke, UK) and *H. pylori* supplement SR0147E including 5 mg/l trimethoprim, 10 mg/l vancomycin, 5 mg/l amphotericin B and 5 mg/l cefsulodin (Oxoid) and incubated at 37ºC for up to 10 days in microaerophilic conditions by a gas generating kit (Campygen, Oxoid, Lot: 13L08-C25-14). Suspected colonies on selective media were examined by Gram-stained and biochemical tests including oxidase and catalase activities (Goodwin and Wesley, 1993). All isolates were kept at -80ºC in Brain Heart Infusion broth with 15% glycerol for any further examination. Reference *H. pylori* strains (some clinical strains) were supplied by Dr. Vildan CANER.

2.3. DNA Extraction and PCR for H. pylori identification

Bacterial genomic DNA was extracted from gastric biopsy asamples using QIAamp DNA mini kit (QIAGEN, Lot No: 11872534, Cat No: 51306) according to the manufacturer's instructions. The extracted DNA was immediately used for PCR amplification or stored at -20ºC for any further investigation.

For PCR reacrion, 2XPCR Master Mix (Fermentas, K01071) and forward primer, glmM-F (5′- AAGCTTTTAGGGGTGTTAGGGGTTT -3′), and reverse primer glmM-R (5′- AAGCTTACTTTCTAACACTAACGC -3′) (Lu et al., 1999) were used. Amplification was performed in touchdown thermal cycler (Hybaid, Middlesex, England) with 35 cycles at 93°C for 1

minute, 55°C for 1 minute and 72°C for 1 minute (Lu et al., 1999). The PCR product was analyzed by electrophoresis in 1.5% agarose gel with 0.5 μg/ml of ethidium bromide solution and visualized by UV transilluminator. Reference *H. pylori* strains (kindly supplied by Dr. Dilek OZTURK) and distilled water were used as positive and negative controls in all PCR tests.

2.4. Histopathologic diagnosis

Biopsy samples taken from antrum and corpus fixed in 10% buffered formalin and embedded in paraffin. Histological sections in 5 microns thick were stained by hematoxylin and eosin and examined under the light microscope and the grades of G were evaluated according to the Sydney classification system (Dixon et al., 1996).

2.5. Statistically analysis

Statistical analysis was performed by the χ^2 test. A p value of <0.05 was regarded as statistically significant.

3. Results

3.1. Culture results

Sixty one (33.2%) out of 184 gastric biopsy samples studied, isolated and identified as *H. pylori*. None of patients with GC identified as *H. pylori* while it was obtained in 51 (32.9%) isolates out of 155 patients with G and 10 (38.5%) isolates out of 26 patients with PU (Table 1).

3.2. Polymerase chain reaction results

Helicobacter pylori was detected in 140 (76.1%) patients [115 (74.2%) cases with G, 23 (88.5%) cases with PU and 2 (66.7%) cases with GC] by PCR (Table 1). A 294 bp amplification product was obtained, corresponding to expected product size.

In distribution according to endoscopic diagnosis groups of 140 *H. pylori*-positive patients by PCR, the *H. pylori* prevalence in patients with G, PU and GC were found to be 74.2%, 88.5% and 66.7%, respectively. The prevalence of *H. pylori* was statistically significant in patients with PU (88.5%) than in patients with G (74.2%). We did not determine relationship in these patients because the number of patients with GC is not sufficient (Table1).

We found that all of antral biopsy specimens by PCR were positive for *H. pylori*. One hundred and fifteen (82.1%) of antrum positive patients were also found positive samples in the corpus. Thus, antrum samples were found to be positive while corpus samples were negative in 25 (17.9%) patiens. Only 16 patients (13.9%) were positive for the antrum, whereas 99 (86.1%) of 115 *H. pylori* positive patients with G who were diagnosed with endoscopic examination, were positive for antrum and corpus samples. In addition, only 8 patients (34.8%) were found to be positive in antrum, while 15 (65.2%) of 23 *H. pylori* positive patients with PU who were diagnosed with endoscopic examination, were positive for antrum and corpus samples. Only one (50%) was found to be positive for the antrum, while one (50%) of 2 *H. pylori* -positive patients with GC who were diagnosed with endoscopic examination were positive for antrum and corpus samples. None of patients was found to be positive for only the corpus (Table 2).

In our evaluation of the prevalence according to sex of *H. pylori*-positive patients, the prevalence of *H. pylori* was found to equal in male and female patients (50%). In our evaluation according to sex of endoscopic findings, the prevalence of *H. pylori* in patients with PU and GC was found to be higher in men (60.9% and 100%, respectively) than in women (39.1% and 0%, respectively) while the prevalence of *H. pylori* in patients with G was found to be higher in women (53%) than in men (47%). Our results exhibited that there was no significant difference between sex and *H. pylori*-positive patient groups (P>0.05).

3.3. Histopathological examination results

Fifteen of 155 patients with G were excluded from the histopathological evaluation due to inadequate material given. Histopathological examination of 140 patients with G was detected to be *H. pylori* positive in 96 patients and 44 patients were detected to be negative. While there are 80 patients whose gastric biopsy specimens of both antrum and corpus regions were positive for *H. pylori* and 16 patients whose biopsy specimens of the antrum region only were positive

Table 1. Prevalence of *H. pylori* isolates obtained from patients with different clinical outcomes by culture and PCR tests.

	Endoscopic findings			
	G (%) (n = 155)	PU (%) (n = 26)	GC (%) (n= 3)	Total (%) (n = 184)
Culture	51 (32.9)	10 (38.5)	0 (0)	61 (33.2)
PCR	115 (74.2)	23 (88.5)*	2 (66.7)	140 (76.1)

G= Gastritis, PU= Peptic Ulcer, GC = Gastric Cancer

* significant $p<0.05$

Table 2. Distribution according to endoscopic findings of antrum and corpus biopsy samples of 140 *H. pylori*-positive by PCR.

Endoscopic findings	Antrum n (%)	Antrum+Corpus n (%)	Total n (%)
G	16 (13.9)	99 (86.1)	115 (82.1)
PU	8 (34.8)	15 (65.2)	23 (16.4)
GC	1 (50)	1 (50)	2 (1.4)
Total	25 (17.9)	115 (82.1)	140

Table 3. Inflamation form and number in gastric biopsies.

Form	Distribution		*H. pylori* (+)		Intestinal metaplasia	
	N	(%)	N	(%)	N	%
Chronic active G	84	(60)	61	(63.5)	13	(61.9)
Chronic non-active G	5	(3.6)	1	(1.1)	-	-
Chronic atrophic G	10	(7.1)	7	(7.3)	5	(23.8)
Chronic active non- atrophic G	30	(21.4)	27	(28.1)	3	(14.3)
Reactive G	11	(7.9)	-	-	-	-
Total	140	100	96	100	21	100

for *H. pylori*, there is no patient who has positivity for the corpus region only. Inflammatory changes in gastric biopsies are considered to be chronic active G in 84 (60%) of the patients; chronic atrophic G in 10 (7.1%) of the patients. Of the patients with inflammation along with *H. pylori*, 61 (63.5%) had chronic active G, 1 (1.1%) chronic non-active G, 7 (7.3%) in chronic atrophic G, 27 (28.1%) in chronic active non-atrophic G. Twenty one patients had inflammation of varying characteristics, along with intestinal metaplasia in the glands. The results obtained by the Sydney scoring system are summarized in Table 3.

4. Discussion

Accurate diagnosis of *H. pylori* is very important in Turkey where the prevalence of its infection are high. *H. pylori* prevalence varies between 53-87% (Sandikci et al., 1993; Nagiyev et al., 2009) in Turkey.

Differences in the prevalence of *H. pylori*, probably due to different factors showing regional variation, such as low socioeconomic status, poor sanitation, educational level, crowded living conditions, and also the diagnostic methods employed (Cesar et al., 2005). Our result (76.1% by PCR) are similar to those reported by Saribasak et al. (2004), Bolek et al. (2007), Nagiyev et al. (2009) (89%, 84.6% and 87%, respectively). *H. pylori* prevalence obtained from our study is higher than a study carried out in Elazig Province (Bulut et al., 2001). The reason of this is probably the low socio-economic conditions due to the density of population that occurred migrations from the surrounding cities in Elazig Province. It is noteworthy in this respect that the number of patients examined in present study was higher. In this study, *H. pylori* positivity (33.2%) by culture method found to be lower than studies conducted by Mishra et al. (2002), Moncayo et al. (2006), Chomvarin et al. (2006) (48%, 75.4% and. 87.5%, respectively). This is because *H. pylori* due to microbiological contamination, the difficulty of growth, the collection samples and transportation time.

The culture may give false negative results, when low density of *H. pylori* present in the specimens (Paoluzzi et al., 1999; César et al., 2005). It has been reported that transportation time and temperature can interfere with culturing (Veenendaal et al., 1993). Furthermore, coccoid forms may be induced by exposure to oxygen and high temperature is viable but is difficult to culture (Bode et al., 1993). The PCR technique has the advantage that bacterial DNA can be detected although the bacteria are not culturable and its high specificity and sensitivity in detecting genetic information of *H. pylori* (Fernando et al., 2002; Thoreson et al., 1999). We used the *glmM* (*ureC*) gene which is highly conserved and very specific to *H. pylori* as the PCR target (Bamford et al., 1998; Brooks et al., 2004).

Of the 184 gastric biopsies examined, culture was positive for *H. pylori* in 61 patients (33.2%), and the PCR in 140 patients (76.1%). Four of the patients which found negative for *H. pylori* by histological examination were detected to be positive by PCR. This situation may be explained that *H. pylori* colonizes the gastric mucosa in a patchy manner, or that the bacteria is present in very low numbers (i.e. fewer than 50 bacteria) or, more likely, by contamination of the biopsies by *H. pylori* DNA from the endoscopes (Thoreson et al., 1999; César et al., 2005). Our data supports previous studies (Kuipers et al., 1995; Lawal et al., 2007) which found that there was a significant association between PU and *H. pylori* infection.

Histological result of *H. pylori* in this study was found as 70%. It is obtained quite variable results reported histological evidence of *H. pylori* is 50%, 75.4%, 86.34% and 87% in studies conducted by Fabre et al. (1993), Kantarceken et al. (2004), Javed et al. (2010) and Arismendi et al. (2011), respectively. This variation may be due to improper collection of mucosal biopsy specimen, improper or delayed transport of the specimen to the laboratory, the techniques used and the experience of the pathologist, and also patchy localisation (Javed et al., 2010).

None of patients was detected in any of the only positive of corpus by histolpathology, culture, and PCR test. Our study confirms the results of other workers who reported that the biopsy site is very important, with a lower detection rate for the corpus than for the antrum (el-Zimaity et al., 1996; de Martel et al., 2010; Arismendi et al., 2011).

Regarding the sex, this study showed the prevalence of *H. pylori* equal in male and female patients (50%). *H. pylori* was found to be higher in female patients (53%) than male patients (47%) with G, while it was higher in male patients (respectively, 60.9% and 100%) than female patients (39.1% and 0%) with DU and GC. The results exhibited that there was no significant difference between sex and *H. pylori*-positive patient groups ($p>0.05$). It is highlighted that there is lower incidence of DU in young women until the onset of menopause and this led to the idea that somehow female hormones protect against development of duodenal ulcer (Andrecia et al., 1990; Javed et al., 2010).

In conclusion, our results confirm that PCR is the best method in which context for *H. pylori* detection, compared to the culture and histopathologic methods as mentioned in a study by de Martel et al. (2010). The differences between these two methods may be explained by changes in the gastric mucosa, leading to a decrease in the secretion of normal mucus, thus the altered environment is not very

appropriate for bacterial growth and the bacteria then migrate to another site of the stomach or the infection comes to an end (Cesar et al., 2005; Martel et al., 2010). In metaplastic regions, a minor affinity with the acid mucus is responsible for insufficient presence of *H. pylori* (Cesar et al., 2005). Further investigations are required to determine significant differences between these tests, as well as the development of more rapid and simple tests to detect *H. pylori.*

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