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Original Article

Serologic and stool antigen assay of *Helicobacter pylori* infection in hyperemesis gravidarum: Which test is useful during early pregnancy?

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Abstract

Objective: To investigate the relationship between *Helicobacter pylori* infection and hyperemesis gravidarum (HG) during early pregnancy by using serologic and stool antigen tests in developing South Anatolia region of Turkey.
Materials and Methods: A prospective cross-sectional study was performed on 40 pregnant women with HG and 40 asymptomatic controls without gastric problems at 7–12 weeks of gestation. The sociodemographic characteristics were recorded. The presence of *H pylori* was analyzed in the sera of the study-group patients by serology-specific IgG test in serum and by a stool antigen test in fecal samples.
Results: The rates of serology-specific *H pylori* IgG positivity were 80% (32 of 40) in patients with HG and 35% (14 of 40) in control group. The difference between the two groups was significant [odds ratio: 6.9 (confidence interval: 2.2–22.1); $p < 0.01$]. The rates of *H pylori* stool antigen test positivity were 87.5% (35 of 40) in patients with HG and 62.5% (25 of 40) in control groups. The difference between the two groups was significant (odds ratio: 4.5, confidence interval: 1.09–18.5); $p = 0.028$.
Conclusion: Both serology-specific IgG and stool antigen tests seem to be good screening methods to identify *H pylori* in our pregnant patient population with HG during early pregnancy.

Keywords: Early pregnancy; *Helicobacter pylori*; Hyperemesis gravidarum; Serologic and stool antigen tests

Introduction

Nausea and vomiting affect up to 50% of pregnant women and diminish women's quality of life and social functions in early pregnancy [1]. In most women, these symptoms resolve by fluid and vitamin supplementation as well as dietary modifications. Hyperemesis gravidarum (HG) affects approximately 1% of pregnancies and causes severe and protracted vomiting that often results in dehydration, ketosis, and weight loss [1–3].

The onset of HG is always in the first trimester. The cause of HG, which still remains unknown, seems to be multifactorial and may be the end result of various unrelated conditions [3,4]. There is evidence indicating that, in HG, there are endocrine factors, such as elevated human chorionic gonadotropine, estradiol, and steroid hormone levels; gastrointestinal tract dysfunction; psychological causes; anatomical variations; genetic incompatibility; immunological factors; vitamin B6; and trace-element deficiency [2–4].

Helicobacter pylori is a spiral-shaped, gram-negative bacterium that is found in the gastric mucous layer or is adherent to the epithelial lining of the stomach. Most people who are infected by *H pylori* never suffer any symptoms related to the infection; however, *H pylori* is associated with chronic gastritis, gastroduodenal ulcers, duodenal structural and functional abnormalities, and gastric malignancies [5].

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Recently, the possible susceptibility to *H pylori* infection in pregnancy has been reported. Although there is an alteration for *H pylori* prevalence between different communities and considerable heterogeneity among studies, significant positive association between HG and *H pylori* infection has been demonstrated by a systematic review [2,6]. Indeed, endoscopic biopsy findings demonstrate that the severity of gastrointestinal symptoms in early pregnancy may be associated with the density of *H pylori* infection [7].

There is an increasing importance of treatment of *H. pylori* and a great need for simple, accurate, inexpensive, and noninvasive diagnostic methods. Serologic and stool antigen tests are the fast noninvasive techniques that should be recommended in the initial diagnosis of *H pylori* infection.

Serologic tests are based on the detection of specific anti–*H pylori* IgG antibodies in the patients' sera. These tests are not able to distinguish between active infection and a previous exposure to *H pylori*. Different commercial kits also have different levels of diagnostic accuracy (range, 68–82%) [8]. Stool antigen test is an enzyme immunoassay that detects the active presence of *H pylori* antigen in human feces. Stool test can be recommended for initial diagnosis in untreated patients with a pretreatment sensitivity and specificity of 63–100% [9].

The purpose of this study was to assess the clinical utility of noninvasive serologic and stool antigen tests for determining *H pylori* prevalence in patients with HG and to determine whether *H pylori* infection itself might contribute to hyperemesis during early pregnancy.

Materials and methods

A prospective cross-sectional study was designed and performed between January 2004 and June 2006 at Kahramanmaras Sutcu Imam University Hospital, Department of Obstetrics and Gynecology, in a developing South Anatolia region of Turkey. Institutional review board had approved the study. Forty women who were hospitalized with the diagnosis of HG and 40 healthy pregnant women without any gastrointestinal symptoms, attending the antenatal unit of the university, were included in the study. In total, 80 pregnant women with gestational age between 7–12 weeks confirmed by ultrasonographic measurement were recruited.

Severe nausea and vomiting in the first trimester with a frequency of more than three times per day, weight loss greater than 5% of pre-pregnancy weight, ketonuria and dehydration that require hospital admission, and intravenous fluid therapy were the criteria for the diagnosis of HG. The exclusion criteria of the study participants were uncertain gestational age; multiple pregnancy; urinary tract infections; previous treatment with *H pylori* eradication therapy; and endocrine, hepatic, gastrointestinal, and psychological disorders. Controls were those who had singleton uncomplicated pregnancies without HG symptoms.

After obtaining informed consent, sociodemographic data, such as educational level, working status, monthly family income, were recorded by a detailed questionnaire at first trimester. Maternal blood collected by venipuncture and stool samples from each patient were collected in clean cups. Blood samples were taken from 80 patients between 7 weeks and 12 weeks of gestation (study and control groups) and were kept at room temperature for 30 minutes till they coagulated. Then, they were centrifuged at 3,000 rpm for 10 minutes, and serums were obtained. These serum samples were stored at −20°C until studied. *Helicobacter pylori* IgG antibodies were investigated by enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun Lübeck, Germany). Serum specimens were analyzed as described by the kit procedure. Adsorbance was read at 450 nm by the threshold value of optic density. A threshold value greater than or equal to 20% was interpreted as a positive result, and a value less than 20% was interpreted as a negative result.

Helicobacter pylori antigens were investigated by ELISA (Connex GmbH, Martinsried, Germany) method in stool specimens that were stored at −80°C. The results were analyzed spectrophotometrically. Absorbance was read at 450/650 nm within 15 minutes of adding stop solution. The results were considered positive if the OD was greater than 0.12 and negative if it was less than 0.10.

Data were stored and analyzed using SPSS 11.0 statistical package (SPSS Inc., Chicago, IL, USA) and expressed as mean ± standard deviation or *n* (%). Demographic findings and clinical measures were compared with Mann–Whitney *U* test and *t* test. Comparison of serologic results for *H pylori* IgG antibody and stool antigen were assessed by Chi-square test. A *p* value less than 0.05 was considered statistically significant.

Results

A total of 80 patients were enrolled into the study. The sociodemographic data of these are presented in Table 1. There were no significant differences in gravidity, parity, body mass index, educational–working status, and family income, in women with HG and the controls. The maternal characteristics of age and smoking were associated with hyperemesis (Table 1). The mean age was 25.8 years in the study group and

Table 1
Sociodemographic characteristics of the study and control groups

Sociodemographic characteristics	Hyperemesis ($n = 40$)	Control ($n = 40$)	p
Age (yr)	25.8 ± 4.72	28.4 ± 4.21	0.025
Gravidity	1.9 ± 1.2	2.2 ± 1.2	0.14
Parity	0.5 ± 0.7	0.7 ± 0.9	0.17
Gestational age (wk)	10.2 ± 1.7	10.4 ± 1.7	0.84
Body mass index (kg/m^2)	23.8 ± 3.88	25.6 ± 3.54	0.066
Education ≤8 yr	21 (52.5)	18 (45)	0.22
Worker/student	4 (10)	6 (15)	0.28
Family income (TL/month)	648 ± 329	873 ± 956	0.16
Smoking	10 (25)	5 (12.5)	0.03

Data are presented as mean ± standard deviation or *n* (%).
1 TL = $0.66 and €0.46.
t test and Mann–Whitney *U* test were used for comparison.
TL = Turkish Lira.

Table 2
Positivity rates for *Helicobacter pylori*–specific IgG and stool antigen in hyperemesis and control cases

Helicobacter pylori tests	Hyperemesis ($n = 40$)	Control ($n = 40$)	p	Odds ratio (95% confidence interval)
Helicobacter pylori IgG	32 (80)	14 (35)	<0.001	6.9 (2.2–22.1)
Helicobacter pylori stool antigen	35 (87.5)	25 (62.5)	0.028	4.5 (1.09–18.5)

Data are presented as *n* (%).
Chi-square test was performed.

28.4 years in the control group ($p = 0.025$). Twenty-five percent (10 of 40) among patients with HG and 12.5% (5 of 40) among control group were smokers ($p = 0.02$).

In the HG study group, 52.5% women had less than or equal to 8 years of school education; 4% women were workers/students; and approximately 56% of mothers had given birth to their first child—only 6% of the mothers had a parity of at least three, 18% had a body mass index less than 20 kg/m^2 at the beginning of pregnancy, and 5% had a body mass index greater than 30 kg/m^2.

The rates of serologic *H pylori*–specific IgG positivity were 80% (32 of 40) in patients with HG and 35% (14 of 40) in control group. The difference between the two groups was significant [odds ratio (OR): 6.9, confidence interval (CI): 2.2–22.1; $p < 0.01$]. The rates of *H pylori* stool antigen test positivity were 87.5% (35 of 40) among patients with HG and 62.5% (25 of 40) in control group. The difference between the two groups was significant [OR: 4.5, CI: 1.09–18.5; $p = 0.028$] (Table 2). The Anatolia regions in Turkey and also the countries of the Europe/Middle East adjacent to Turkey are shown in the map in Fig. 1.

Discussion

The pathophysiology of HG is still controversial. However, pregnancy may be associated with an increased susceptibility to *H pylori* infection [6], and it has been hypothetically proposed that a shift in gastrointestinal tract pH during early pregnancy as a result of increased accumulation of woman's body fluid, steroid hormone changes, and immunologic tolerance, could lead the activation of latent *H pylori* infection, which can exaggerate the symptoms of nausea and vomiting [10]. In one controlled trial, endoscopic biopsy findings demonstrated that the severity of gastrointestinal symptoms in early pregnancy may be associated with the density of *H pylori* infection [7]. Shirin et al [11] reported recently that *H pylori* is associated with mild vomiting in early pregnancy but not with gastrointestinal symptoms later in pregnancy.

In earlier studies, detection of *H pylori* prevalences in patients with HG and the controls was performed by whole-blood or serum-based serologic tests, which show chronic infection. The main differences between our study and the previous studies are that we evaluated the association between *H pylori* and HG by both serologic and stool antigen tests and compared the clinical utilities of these noninvasive tests in early pregnancy. Also, this is the first study that was performed in the developing South Anatolia region of Turkey. The present findings support the possible relationship between *H pylori* and HG, and also suggest that there is an inverse correlation between socioeconomic status and prevalence rate of *H pylori* in developing areas.

A recent geographically stratified multinational European study performed by serology demonstrated that the overall

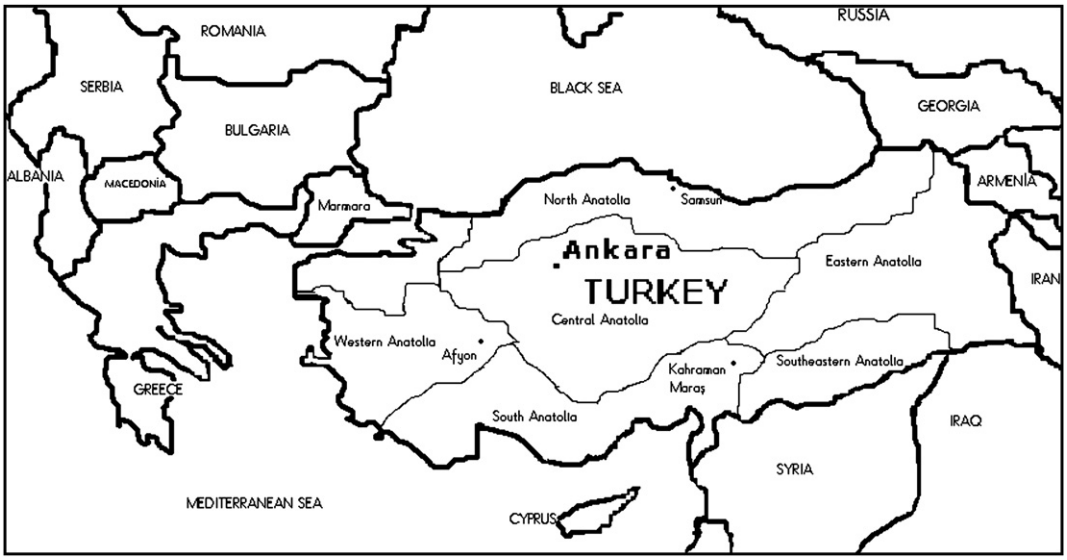

Fig. 1. The regions/cities in Turkey and countries of the Europe/Middle East adjacent to Turkey.

prevalence of *H pylori* in countries of southern Europe was significantly higher both in patients with HG and the controls (Yugoslavia: 96.4%; Austria: 83.3%; Poland: 84.6%; Turkish pregnant woman: 93.3%; and Arab countries: 86.7%) [12]. Two studies that were performed in different regions of Iran's pregnant population demonstrated statistically significant high and different *H pylori* seropositivity rates in HG cases (81.5% *vs.* 54.7% and 88.9% *vs.* 40.7%) [13,14]. In Chinese patients with HG, a prevalence rate of 88.9% for *H pylori* has been shown [15]. The overall prevalence of *H pylori* seropositivity rates in Israel [11], Japan [16], Norway [17], and Hispanic population [18] affected by HG are 45.9%, 47.5%, 43%, and 66% respectively. A study from Taiwan demonstrated a seropositivity rate of 69% in pregnant women with severe gastrointestinal symptoms during early pregnancy; however, no correlation was found between seropositivity and clinical symptoms [19]. Race affect the presence of *H pylori*, and this association was much stronger in Africans as compared with non-Africans [17]. First of all, the results of these studies give some clues about the effect of geography, economy, race, and country origin on *H pylori* prevalence.

Additionally, another similar study that was performed in developing North Anatolia region detected 91.5% seropositivity in patients with HG as compared with 44.8% in the controls [10]. The studies that were carried out in Iran and Turkey are along the same line as our study, but we detected a seropositivity ratio of 80% in the HG group and 36.7% in the control group; the difference between the two groups was significant [OR: 6.9, CI: 2.2–22.1; $p < 0.001$]. On the other hand, Jacobson et al [20] found no relationship between HG and *H pylori* by the serologic test in two American populations but showed the association among the risk of HG, young maternal age, and African American race. In this study, subjects and controls were not matched for maternal age. Another study that was performed in Central Anatolia region of Turkey could not demonstrate an association between *H pylori* seropositivity and HG [21]. Although, in this study, it was mentioned that all the study participants were from areas of low socioeconomic status, this situation was designated only by the education level.

To date, there are two studies in the literature evaluating the association between *H pylori* and HG by two noninvasive tests. These two studies were carried out in developed Western and Central Anatolia regions of Turkey [22,23]. Cevrioglu et al [22] reported that the frequencies of *H pylori* detection by serologic *H pylori* IgG and by stool antigen tests were about 2.1 and 5 times higher, respectively, in cases with HG than those in asymptomatic cases. The weak point of that study was the low number of cases ($n = 27$), and also, in that study, the education level affecting the *H pylori* prevalence was higher than that in our study population. Karadeniz et al [23] found no association between *H pylori* and HG by specific serologic and stool antigen tests. Another study that was performed in Central Anatolia region by Aytac et al [24] detected 42.3% *H pylori* stool antigen positivity in patients with HG as compared with 40% of controls. Our results are not in concordance with these studies; we found that *H pylori* detection was about seven times higher by serologic test and was 4.5 times higher by stool antigen test in cases with HG than those in controls. The prevalence of *H pylori* in our region was 87.5% by stool antigen test, which indicates active infection in HG cases, and this rate is higher than those in other studies. In all these studies, the socioeconomic status, such as income level, working status, has not been analyzed in detail. Moreover, the discrepancy between these studies and our study may be explained by multiple factors, such as geographic discriminations between regions of Turkey, as well as a population difference or a difference in methodology of socioeconomic level.

Moreover, we found that young maternal age and smoking were associated with increased risk of HG and *H pylori*. These findings are along the same line as previous studies [25,26]. Low socioeconomic level is a major risk factor for *H pylori* acquisition [27]. Although not statistically significant, in our study, there is a tendency of occurrence of HG and infection with *H pylori* in patients with lower socioeconomic level. In addition, there is a high prevalence rate of *H pylori* by stool antigen test, which shows active infection in our control group. This might be the result of low socioeconomic level of our study population or pregnant women's tendency to carry *H Pylori* infection.

In the light of these findings, our study provides new data that regional and geographic differences, and populations' sociodemographic variations affect the *H pylori* prevalence rates. Moreover, we suggest that *H pylori* diagnostic serologic and stool antigen tests could be a part of HG investigation during early pregnancy, especially in cases showing resistant or no response to treatment.

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