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Comparison between serological assay and stool antigen for Helicobacter pylori in primigravidarum with Hyperemesis gravidarum

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ABSTRACT

Background: Helicobacter pylori is one of the commonest bacterial infection worldwide and accepted as the cause of chronic active gastritis **Aim:** to investigate any possible association between Helicobacter pylori infection and cases of hyperemesis gravidarum (HG) by using both Helicobacter pylori Stool Antigen (HpSA) and Helicobacter pylori IgG (Hb IgG) serologic test and to compare the accuracy of the two tests in the diagnosis of HG.**Patients & Methods**: The present study was conducted prospectively between 1st of October 2012 and 1st of March 2013. A (50) pregnant primigravida women with hyperemesis gravidarum attending Baghdad Teaching Hospital and Nursing Home compared with a similar number of asymptomatic pregnant women as a control group were enrolled in the study. Demographic data of both groups were recorded. A full history , general and local examination was done for all participants. Ultrasound was done to exclude obstetric causes of hyperemesis as twin pregnancy, molar pregnancy or missed abortion. Serum H. pylori IgG antibody was measured by enzyme-linked immunosorbant assay (ELISA) method and stool samples for HpSA were done for all patients and control. The data of both groups were compared and analyzed statistically.**Results:** the study revealed that the rates of H pylori stool antigen test positivity were 52% (26 of 50) among patients with HG and 9% (18 of 50) in control group. The difference between the two groups was statistically significant, $p< 0.05$. The rates of serologic H pylori specific IgG positivity were 82% (41 of 50), 68% (34 of 50) in patients with HG and in control group respectively . The difference between the two groups was not statistically significant. **Conclusion:** The HpSA scan showed a statistically significant relation between H. pylori infection and Hyperemisis gravidarum. HpSA test gives more efficient, reliable and realistic results than specific IgG formed against H. pylori in the identification of H. pylori positivity in primigravida with Hyperemisis Gravidarum..

Key words: Helicobacter pylori, Hyperemesis gravidarum, Helicobacter pylori IgG antibody, Helicobacter pylori stool antigen tests.

Introduction:

:Helicobacter pylori is one of the commonest bacterial infection worldwide and accepted as the cause of chronic active gastritis (type B). Conservative estimates suggest that 50% of the world's population is affected(1). The diagnosis of H.pylori can be done through the following methods: 1-histological examination(2).2- urea breath test(1).3- stool test to detect the presence of HP proteins in the stool (HpSA) (1).4-Blood test for the detection of serum immunoglobulin G(IgG) antibodies to helicobacter proteins.H. pylori is highly susceptible to most antibiotics (3).Commonly used eradication regimes include a proton pump inhibitor or H2 receptors antagonist with any two of the following drugs, Metronidazole , Clarithromycin or Amoxicillin in appropriate dosage (4). Most patients continue through life with a chronic superficial gastritis while some develop either duodenal or gastric ulcer (5).

Helicobacter pylori is a gram negative , spiral shaped ,microaerophilic, organism,which can cause a non-invasive infection of the gastric epithelium and the mucous layer that coats this epithelium. It can cause duodenal ulcer, gastric ulcer, chronic gastritis, gastric adeno-carcinoma, mucosa associated lymphoid tissue lymphoma and a few other rare upper gastrointestinal disorders(6). It is reported that gastric mucosa infected by Helicobacter pylori almost always shows a combination of inflammation and epithelial changes. The classical feature caused by this organism is chronic active gastritis. The infiltrate generally consists of monocytes and neutrophils (7).The prevalence rate is higher in developing countries than developed countries 8).The possible transmission route may be oral oral, faecal -oral, iatrogenic transmission and vectorial spread (9) .

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One of the characteristic of the organism is its ability to hydrolyze urea resulting in production of ammonia, a strong alkali. The effect of ammonia on the antral G cells is to cause the release of gastrin which is responsible for modest but inappropriate hypergastrinaemia which in turn may result in gastric acid hypersecretion (4), Also H. pylori infection results in defective bicarbonate secretion, which normally occurs in response to duodenal acidification. The final result is an increased duodenal acid load in subject with H pylori infection (10).

Hyperemesis gravidarum

:Nausea is a very common symptom in humans but its pathophysiology remains extraordinary poorly understood.The nausea and vomiting of pregnancy are no exception (11) . Morning sickness is the common term for gestational nausea and vomiting. It is a complete misnomer, the uncomfortable symptoms occur throughout waking hours , not just in the morning and whereas sickness implies disease, healthy women experience the symptoms and bear healthy babies, nausea and vomiting of pregnancy (NVP) is more appropriate descriptive and objective. Within normal range of symptoms NVP is appropriately considered as adaptation , protecting the women and embryo from food-borne infections and toxins (12). Nausea and vomiting are the most common gastrointestinal symptoms affecting women during pregnancy. It is estimated that 50-80% of pregnant women experience NVP beginning by the 4th week and often ending by the 12th week after conception. Symptoms may persist throughout pregnancy in up to 20% of pregnant women , of this large group 1% to 3% experience a more severe form of NVP called hyperemesis gravidarum. Although there is no standard definition of Hyperemesis gravidarum , one frequently used is severe nausea and vomiting associated with weight loss, ketonemia , electrolyte imbalance,metabolic hypochloraemic alkalosis and elevated liver enzymes in pregnancy (13). Hyperemesis gravidarum with the incidence of 0.3-1.5% of all live births is the most common indication of hospitalization during the first trimester (14). The onset of HG is always in the first trimester. Little is known about the etiology of nausea and vomiting in pregnancy. Nausea and vomiting (morning sickness) is a major complaint in 70-80% of pregnancies (15,16).

HG has negative maternal effects on the social, professional, and family life to the extent that some women choose to terminate desired pregnancy when symptoms are severe (17).

The exact cause is not well known and is probably multifactorial in which psychological factors, alteration of gastrointestinal tract motility, hormonal changes, H. pylori infection, immunological, metabolic and anatomical factors appear to intervene (18,19).Hyperemesis gravidarum has been described as the "disease of theories"(20) .Newer studies have found a relationship between infection with HP and HG (21) ,especially when HG is unresponsive to medical and psychological therapy and or when haematemesis is evident , it is prudent to entertain a diagnosis of HP induced peptic or ulcer disease (22).The researchers hypothesized that in the early stage of pregnancy there is increased accumulation of fluid and electrolyte caused by elevated steroid hormones , a shift in PH may occur (23), which may in turn activate latent HP residing in the stomach (24),result in manifestation of subclinical HP infection which can exacerbate GI symptoms.Recently, the possible susceptibility toH 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 (25). Indeed, endoscopic biopsy findings demonstrate that the severity of gastrointestinal symptoms in early pregnancy may be associated with the density of H pylori nfection(26).

Although history and complete physical examination are important in evaluation of pregnant women with persistent vomiting (27),the defining symptoms of HG are gastrointestinal in nature (20). And even difficulty in reading because the eye ball shape is altered with dehydration (11).Other causes of nausea and vomiting unrelated to pregnancy must be ruled out before the diagnosis of HG can be made. Conditions such as diabetic gastroparesis, pancreatitis , gall bladder disease , polynephritis, uremia , degenerative uterine leiomyoma or torsion and hyperthyroidism can all be associated with nausea and vomiting (28).,so diagnosis of HG depends on clinical presentation and exclusion of other disorders (29).The purpose of this study was to assess the clinical utility of noninvasive serologic(IgG Ab) assay and stool antigen tests (HpSA) and their accuracy for the diagnosis of Helicobacter pylori in primigravidarum patients with hyperemesis gravidarum

Patients and Methods:

This present prospective case control study was conducted on (100) pregnant women who presented at Baghdad Teaching Hospital and Nursing Home (Department of Obstetrics and Gynecology) between 8 - 16 weeks of gestation during the period between (1st of October 2012 and 1st of March 2013). The study sample was divided into two groups (50 cases and 50 controls). The criteria for diagnosis of hyperemesis gravidarum included:severe vomiting

(more than 3 times a day) , weight loss (more than 5% of body weight or more than 3 kg) according to the patient gestational age, and ketonuria. All cases of hyperemesis gravidarum were admitted to hospital and their dehydration status was corrected by intravenous fluid therapy.Informed written consents were obtained from all women conducted in the study. Both groups were comparable for age , body mass index, gestational age, ultrasonographical age , educational level and smoking. 50 asymptomatic pregnant women who came for routine follow up in the antenatal clinic during the same period of time were involved in the study as control group. Demographic data of both groups were recorded. Gestational age was determined according to Neagle rule by the use of the date of last menstrual period and confirmed by ultrasonography. The participants eligible for the study had been informed about the study before blood samples and stool specimens were collected. The inclusion criteria are pregnant female ranged between the age of 17-40 years old (primiparity), gestational age was between 8-16 weeks, uneventful course of pregnancy, and weight within 20% of normal weight for height at the beginning of pregnancy, the same criteria of the study group were applied for control group except for the presence of hyperemesis gravidarum symptoms.The exclusion criteria of the study participants were uncertain gestational age , gestational trophoblastic disease , obstetric causes of hyperemesis as twin pregnancy, molar pregnancy or missed abortion , urinary tract infections, previous treatment with H pylori eradication therapy and patients on anti-acid drugs , patient with recent use of antibiotic (within the previous7 days) , endocrine, hepatic, gastrointestinal, and psychological disorders. Pregnant women in the control group were randomly selected among asymptomatic pregnant women (without nausea and vomiting) , and were interviewed regularly in the antenatal care clinic in our hospital to determine whether they are asymptomatic or not. Inpatient therapy for the study group consisted of the avoidance of oral nutrition and the administration of intravenous fluids (Ringers and glucose water), adding to that vitamin pyridoxine and antiemetic medication(e.g. metoclopromide) A full history was taken from all participants , physical examination and several investigations were done including complete blood picture, general urine examination (including ketones and specific gravity) , blood sugar, liver, renal and thyroid functions tests , and ultrasonography.Helicobacter pylori screening was done by the collection of venous samples on admission for the study group and in the antenatal care clinic as outpatient for the control group. The sample was obtained and stored at (-20°C) until use.

Specific serum IgG antibodies against HP were detected by ELISA test in Central Laboratory in Baghdad Teaching Hospital using H pylori kits produced by BIORAD company with sensitivity and specificity (100%,90%) respectively according to the documents of the manufacturing company. Interpretation of the serum sample was done according to the following values

>1.10 is considered positive antibody titer.

>0.90-<1.10 intermediate , is considered non significant antibody titer (equivocal)

< 0.90 is considered negative.

According to the above results the study group is classified into HP antibody sero-negative and HP antibody sero-positive. Concerning determination of HP stool antigen (HpSA), the specimen was collected in clean containers and the specimen should be tested as soon as possible after collection done either immediately after collection of the sample or may be stored refrigerated(2-8°C) for 72 hours. All specimen were tested for HpSA using SD BIOLINE Helicobacter Antigen rapid test kit Statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc, Chicago, III, USA) statistical software. Categorical variables were compared by Chi-square and Fisher's exact test. descriptive statistics were calculated and reported as mean± standard deviation or number (%). Categorical variables were described using frequency distribution. Student tests were used to compare demographic data between the study groups. Comparison between HpSa and IgG was assessed by Chi-Square test. value of less than 0.05 was considered as statistically significant

Results :

The demographic data of the study and control groups were summarized in Table (1).The study revealed that there was no statistically significant differences between the study groups with hyperemesis gravidarum and control groups in regard to age, gestational age (weeks), body mass index, educational level, Ultrasonographic gestational age (weeks) and smoking.

In the HG study group, (52.5%) of women had less than or equal to (7)years of school education. Mean duration of hospitalization in the Hyperemesis gravidarum group was 2.7±1.8 days.

The rates of H pylori stool antigen test positivity were 52% (26 of 50) among patients with HG and 18% (9 of 50) in control group. The difference between the two groups was statistically significant. P value < 0.05.(Table2).

The rates of serologic H pylori specific IgG positivity were 68% (34 of 50), 82% (41 of 50) in patients with HG and in control group respectively. The difference between the two groups was not statistically significant , P value 0.31(Table 2).

Table 1.The comparison of demographic data between the study group pregnancies with Hyperemesis gravidarum and the control group pregnancies

Characteristics	Study groups (n=50) %	Control groups (n=50) %	p-value
Age (year) <20 years 20-29 30+ Mean	 16 32 27 52 7 14 23.4±5.2	 13 26 28 56 9 18 23.1±5.1	NS
Gestational week	10.2 ± 1.7	10.4± 1.7	NS
Ultrasonographic gestational age (weeks)	9.3±2.9	9.6 ±2.5	NS
Body mass index	22.9±4.6	24.1±3.5	NS
Smoking	0	6(12%)	NS
Education <7 years	15 30	28 56	NS

Table (2)Positivity rates for Helicobacter pylori stool antigen and Helicobacter pylori specific IgG in Hyperemesis gravidarum and control group pregnancies.

Helicobacter pylori test	HG (No.50) %	Control (No.50) %	p
Helicobacter pylori stool antigen (HpSA) +ve -ve	 26 52 24 48	 9 18 41 82	 <0.05
Helicobacter pylori IgG Hb IgG Ab +ve -ve	 34 68 16 32	 41 82 9 18	 0.31
Total	50 100	50 100	

Discussion:

Hyperemesis gravidarum is a high risk disorder resulting in extreme nausea and vomiting in pregnancy and causes the mother and fetus to become severely dehydrated (28).Recently possible involvement of chronic HP infection in HG has been suggested in several investigations , based on serological studies (30).

In this study There was no statistically significant differences between the study groups with Hyperemesis gravidarum and control groups in terms of age, gestational age , body mass index , educational level , Ultrasonographic gestational age (weeks), and smoking and this is in agreement with that of Erdem et al (30) and Kocak et al (21)The study revealed that (36%) of HP were of younger age group(< 20 years) and this may explain that HG involves a younger age group and this is in accordance with that of Jacobson et al (29) who found that HG is reduced with increasing maternal age. In the present study we used both serological and stool test to investigate H. pylori infection in HG patients. The results was statistically significant between HG patients and the control groups for HpSA (52% vs. 18% respectively, p-value <0.05) , and was statistically insignificant in regard to IgG Ab seroposivity (68% vs.82% respectively, p-value >0.05.)In earlier studies, detection of H pylori prevalence 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 the use of both serologic and stool antigen tests and compared the clinical utilities of these noninvasive tests in early pregnancy. To date, there are two studies in the literatures that evaluate 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 (30,31).Cevrioglu et al (31). 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 (22), and also in that study the education level affecting the H pylori prevalence was higher than that in our study population.

Karadeniz et al (32) 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 (33).

detected 42.3% H pylori stool antigen positivity in patients with HG as compared with 40% of controls.

In agreement with our study Cevrioglu et al (31) reported that the rate of Hp SA positivity was statistically significantly higher in HG women compared to control cases (40.7% vs. 12.4%)respectively, P =0.001while the seropositivity rate of Hp IgG Ab were (85.2% vs.73.2%) in HG and asymptomatic cases with insignificant differences between both groups , P=0.198.

The HpSA test , which is an enzymatic immunoassay, detects bacterial antigens of an actual ongoing infection in the stool(34).There were a few studies which used HpSA tests to identify exposure to Hyperemesis gravidarum. The overall prevalence of HpSA was between(22.6-52.53 %) in pregnant women with Hyperemesis gravidarum (32,33,35).The Helicobacter pylori compared with control subjects (52%). HpSA test is a reliable non invasive marker in the primary diagnosis of H.Pylori (31,32,36).HpSA tests which are qualitative tests and show the presence of antigen determines colonization of H pylori in stool. It is easy ,simple to perform, rapid and less expensive.

Besides it becomes negative after 3-6 weeks after eradication treatment making it ideal as screening test to determine the prevalence of H pylori in both symptomatic and a symptomatic subjects (37).

Our results are not in concordance with that of Karadeniz et al (32). who found insignificant differences between HG and control cases by both diagnostic methods (22,7% vs.6.9%, P==0.08) for HpSA and (67.7% vs. 79.3%, P =0.31) for HpIgG Ab respectively and attributed the high seropositivity rates in both groups to the low socioeconomic status of their patients.

Moreover, the discrepancy between these studies and our study may be explained by multiple factors, such as geographic differences between regions, as well as a population difference or a difference in methodology.In agreement with the present study as regard seropositivity for HpIgG Ab ,Berker et al (38). reported insignificant differences between HG patients and control ones using HpIgG alone (70% vs. 61.3%) respectively. Serological testing is a primary screening approach for evaluation of H. pylori infection. It shows the IgG Ab status of patients infected with H pylori (33).

Our study must be interpreted taking into account the following limitations, first Although serological testing is non invasive , widely available, inexpensive simple and quick, yet it may not reflect current active infection because antibodies are positive several months or even years after infection (39,40).therefore this test has lower accuracy than HpSA test. Second HpSP test is for qualitative detection of H. pylori in stool sample and does not indicate the quantity of the antigen.

Third a negative results does not preclude the possibility of infection with H. pylori, other clinically available tests are required if questionable results are obtained. Finally as with all diagnostic tests , a definitive clinical diagnosis should not b e based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated

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