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Research article

Detection of anti-*Helicobacter pylori* antibodies in sera of women with recurrent spontaneous abortion

Rana Saady Abood[1], Anmar Saady Abood[2], Ghadah Mohommed Saleh[1]*

ABSTRACT

To determine the relationship between *Helicobacter pylori* infection and reproduction disorder (recurrent spontaneous abortion), twenty women patients who undergo spontaneous abortion during first trimester of pregnancy (20-38) years and have been investigated from 2015/12/1 -2016/3/1 and compared to fifteen healthy individuals. All subjects were carried out to measure anti-*H. pylori* IgA and anti- *H. pylori* IgG antibodies by enzyme linked immunosorbent assay (ELISA). There was significant elevation (p≤ 0.05) in concentration of anti- *H. pylori* IgG Abs (6.30± 0.99) compared to control group (4.48± 0.61) and IgA Abs (5.42 ± 0.90 U /ml) as compared to control group (3.92 ± 0.41 U/ml). The percentage of *H. pylori* IgG and IgA was 20% and 25%, respectively. There was high significant (P ≤ 0.01) difference between control and test groups. While, there was no significant differences (P > 0.05) in the concentration of IgA and IgG of *H. pylori* according to the age. These results indicate that infection with *H. pylori* play an important role in reproduction disorder of spontaneous abortion.

Keywords: abortion, *Helicobacter pylori*, IgA and IgG

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INTRODUCTION

Helicobacter pylori is a helix-shaped (classified as a curved rod, not spirocheate). Gram negative bacterium about 3 µm long with a diameter about 0,5 µm. It is microaerophlic; that is, it requires oxygen, but at lower concentration than is found in the atmosphere. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (H2) produced by intestinal bacteria It produces oxidase, catalase, and urease. It is capable of forming biofilm [1] as many other bacterial species such as *Pseudomonas aeruginosa* [2] and can convert from spiral to a possibly viable but nonculturable coccoid form, both likely to favor its survival and be factors in the epidemiology of the bacterium [3]. *H. pylori* represent one of the most common and medically prominent infections worldwide. Infection with this microaerobic, Gram-negative bacterium has been established as an etiologic factor in the development of peptic ulcer disease. In addition, *H. pylori* infection has been associated firmly with the development of gastric neoplase, including gastric adenocarcinomas and gastric mucosa-associated lymphoid tissue lymphomas [4]. Socioeconomic level seems to be the major determinant of risk of infection. In Australia, 25-30% of the population is infected, with the prevalence increasing with age. In some indigenous communities, prevalence is 2-3 times higher than in non indigenous communities [5]. Most individuals who are infected with *H. pylori* never suffer any symptoms related to the infection; however, *H. pylori* cause chronic active, chronic persistent and atrophic gastritis in adults and

*Correspondence: Saleh,GM.alquraishighadah@gmail.com.
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Full list of author information is available at the end of the article.

children [6]. Studies have looked at a possible role of *H. pylori* as a trigger for extra-intestinal diseases including cardiovascular diseases, neurological disorders, diabetes mellitus, ear and eye diseases, immunological and hematological disorders, liver and bile tract disease, gynecological and respiratory pathologies. In general, no conclusive evidence of a possible causal association has been found [7].

It has been shown in mice that *H. pylori* infected mice show decrease in implantation rates, and their offspring are of low birth weight [8]. Lanciers *et al* [9] found a significant increase incidence of pregnant subjects with high *H. pylori* IgM (marker for recently acquired infection) compared to non pregnant. *H. pylori* infection was tested before conception only in one prospective study, where early pregnancy loss was associated with maternal *H. pylori* CagA-strains seropositivity before intra-cytoplasmic sperm injection.

Prevalence of *H. pylori* infection in pregnant women varies according to geographic area, socioeconomic conditions and method used to detect *H. pylori* infection. For example, the prevalence of *H. pylori* infection among pregnant women is about 20%-30% in most European countries, Japan and Australia, while it is 50%-70% in Turkey, Mexico and in Texas, United States, more than 80% in Egypt and Gambia [10-12]. However, Rossi *et al* [13] observed higher number of fetal in *H. pylori* infected pregnant mice compared to non-infected control. Hajishafiha *et al* found an association between *H. pylori* CagA-strain maternal infection and early pregnancy loss in patients undergoing intra-cytoplasmic sperm injection. Recently, we found a significantly higher percentage of *H. pylori* seropositive women among prim gravidae with a miscarriage compared to controls, while the presence of maternal serum antibodies against *H. pylori* did not appear to be associated with recurrent miscarriage. These findings suggest a relationship between *H. pylori* infection and implantation/ placentation failure, possibly due to a cross-reaction between antibodies against *H. pylori* and placental tissue [14]. It is widely established that specific anti-*H. pylori* IgG antibodies are transplacentally transferred from mothers to fetuses and a close correlation between maternal and cord specific IgG levels was demonstrated These passively acquired antibodies decline over the first 3-4 more of life [15,16]. The aim of present study was to determine the relationship between *H. pylori* and reproduction disorder (recurrent abortion).

MATERIALS and METHODS

Blood samples collection

The study was carried out on twenty women patients suffering from recurrent spontaneous abortion during first trimester of pregnancy that introduce to Baghdad hospital, from 1, November 2015 to 1, February 2016. The ages of patient were ranged from 20 to 38 years old. The interviews were performed for each patient. Fifteen samples of healthy individuals female were studied as control groups of same age and sex. All samples were marked by the number of sample. Name of patient and day of sample collection were included in investigation report.

Blood samples (5 ml) were collection by disposable syringe into plane tubes and stand at room temperature until the coagulant was form. Then the samples were centrifuged at 3000 rpm for 5 min the sera were stored at -20 ^{o}C until carried out to detect anti- *H. pylori* IgA and IgG according to instructor's manufacture company.

Statistical Analysis

The statistical analysis system (SAS) [17] program was used to detect difference factors in study parameters. T-test was used to compare significance between means and Chi-square test was used to compare significance between percentages of groups that studied in present this study.

RESULTS and DISCUSSION

The results of the present study showed that there was a significant elevation ($P < 0.05$) in the concentration of *H. pylori* IgA (5.42 ± 0.90 U /ml) as compared to control group (3.92 ± 0.41 U/ml) (**Fig 1**).

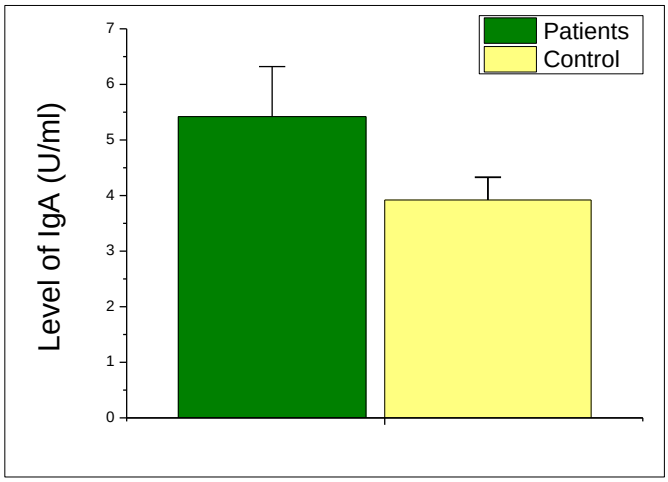

Fig 1. Mean of Level of anti-*H. pylori* IgA (U/ml) in sera of women with recurrent spontaneous abortion and control group.

There was significant elevation ($P < 0.05$) in concentration of *H. pylori* IgG (6.30 ± 0.99) as compared with control group (4.48 ± 0.61) (**Fig 2**)**.**

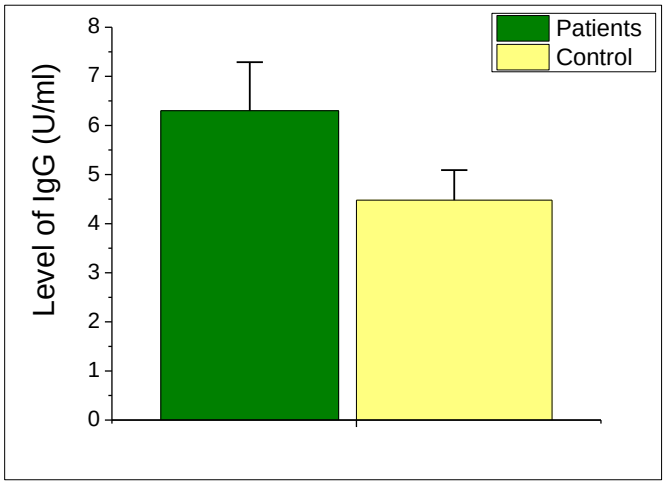

Fig 2. Mean of level of anti-*H. pylori* IgG (U/ml) in sera of women with recurrent spontaneous abortion and control groups.

The prevalence of anti-*H. pylori* antibodies IgG and IgA was (5/20) 25% and (4/20) 20%, respectively. There was high significant difference (P < 0.01) was found between test and control studied groups (**Table 1**).

Table 1. Percentage of anti-*H. pylori* IgG and IgA in sera of women with recurrent spontaneous abortion. *, P < 0.01.

Parameters	Positive	Negative	P- value
IgA (No. =20)	5 (25.00%)	15 (75.00%)	0.0025 **
IgG (No. =20)	4 (20.00%)	16 (80.00%)	0.0001 **
P- value	0.329 NS	0.329 NS	----

The results of the study showed that there was no significant difference (P > 0.05) in the concentration of IgG and IgA in terms of the age of groups (**Table 2** and **3**).

Table 2. Mean of level of anti-*H. pylori* IgA (U/ml) in sera of women with recurrent spontaneous abortion and control groups according to the age of groups.

Age group (year)	Mean ± SE of IgA	
	Patients	Control
Less than 30	5.34 ± 0.94	3.92 ± 0.86
More than 30	5.55 ± 0.81	3.97 ± 0.90
T-Test	1.327 NS	1.094 NS
P-value	0.517	0.482
NS: Non-significant.		

Table 3. Mean of level of anti-*H. pylori* IgG (U/ml) in sera of women with recurrent spontaneous abortion and control groups according to age of group.

Age group (year)	Mean ± SE of IgA	
	Patients	Control
Less than 30	6.33 ± 1.02	4.39 ± 0.78
More than 30	6.24 ± 0.96	4.52 ± 0.90
T-Test	1.407 NS	1.284 NS
P-value	0.549	0.492
NS: Non-significant.		

In this study we have tried to detect the relationship between *H. pylori* and reproduction disorder (spontaneous abortion). The results of the present study were agreed with previous several studies. One of them showed that 68.6% of pregnant women showed infection by *H. pylori* [18], another study revealed that *H. pylori* IgG Abs was positive among 23 and 28 (46 % vs. 48%) in women with aborted and normal pregnancy respectively [19]. Another study showed that women infected with CagA positive strain were more likely to have early pregnancy loss, and there was no associated between EPL and age [20]. The reasons of differences presented in this study from other studies may be due to differences in sample size, the methods that were used for detection of *H. pylori* IgG and IgA, age of aborted women, geographical area and the period when the samples were collected.

Infection with *H. pylori* was investigated not only in association with gastrointestinal manifestation during pregnancy but also with other severe pregnancy-related disorders [21]. Infection with *H. pylori* has a role in pathogenesis of these disorders through different mechanism: depletion of micronutrient (iron and vitamin B12) in case of maternal anemia and fetal neural tube defects; and oxidative stress in gastrointestinal disorders and pre-eclampsia; cross-reaction between specific anti-*H. pylori* antibodies and anti genes localized in placental and endothelial cell (pre-eclampsia, fetal growth restriction, and miscarriage [22].

Since, *H. pylori* infection is most likely acquired before pregnancy, it is widely believed that hormonal and immunological changes occurring during pregnancy could activate latent *H. pylori* with negative impact not only on maternal health [20]. Local and systemic induction of pro-inflammatory cytokines release such as interleukin (IL)-1β, IL-18 and others [23,24] play an important role in different diseases. It has been demonstrated that CagA positive strains of *H. pylori* can cause injury to different organs systems including male and female reproductive organs through the release of different inflammatory mediators such IL-8, IL-1β and tumor necrosis factor (TNF)-α [25]. TNF-α is multifunctional cytokine with a regulatory role in many fundamental processes such as immunologic response, angiogenesis or apoptosis, and is formed both by placenta and the uterus [21]. The CagA seropositivety in Iran has been reported to reach 72.8%. Infection with *H. pylori* CagA-positive strains has been shown to cause a severe inflammatory response and significant neutrophil infiltration in the gastric mucosa. Our finding of a statistically significant relationship between CagA-positive strain of *H. pylori* and EPL might be explicable on the basis of general inflammatory reaction to infection. Concentration of IL-1β, IL-8, and TNF-α were all significantly higher in *H. pylori* –positive gastric mucosa samples. These cytokines may cause systemic inflamm-ation that could affect the integrity of the fetoplacental unit and threaten the welfare of the fetus. It was shown that poor oral hygiene is associated with a history of miscarriage [26].

Serologic and stool antigen tests are the first choice for *H. pylori* infection diagnosis in pregnancy, since they are easy to perform and low cost non invasive diagnostic tests. Serologic tests are usually based on the detection of specific anti *H. pylori* IgG antibodies in the patient's sera by immune–enzymatic assay. Measurement of IgG antibodies against *H. pylori* reveals an immune response that could represent either a current infection of a previous exposure, since IgG antibodies disappear only several months after eradication of the microorganism [21].

The result indicated that infection with *H. pylori* may play an important role in reproduction disorders such as recurrent spontaneous abortion.

Conflict of interest

The authors declare that they have no conflict of interests.

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Author affiliation

1. *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*
2. *Department of Biology, College of Science, Al-Mustansiria University, Baghdad, Iraq.*