Identification of Possible Association between Helicobacter pylori Infection and Preeclampsia Incidence

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ABSTRACT

Background: Preeclampsia is a multi-systemic hypertensive disorder that adversely influences maternal, fetal and neonatal outcomes. The pathophysiology and primary triggers of this syndrome is not entirely understood. Recently, it was suggested that infection with certain pathogenic microorganisms can contribute to preeclampsia pathophysiological pathway as initial causative factor.

Objective: To investigate the possibility of an association between PE incidence and Helicobacter pylori infection and any adverse pregnancy outcome.

Methods: A case-control study at the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital from April to December 2017. We utilized immunochromatographic analysis to screen 40 pregnant women with preeclampsia (case group) and another 50 normotensive pregnant women with comparable gestational age (control group) for serum IgG antibodies against Helicobacter pylori. Serological screening was also carried out to examine blood samples collected from umbilical cord vessels for 24 pregnant women of preeclampsia group after delivery in order to diagnose transplacental transfer of anti- Helicobacter pylori IgG antibodies.

Results: the seropositivity of IgG antibodies against Helicobacter pylori was significantly higher in pre-eclamptic women (72.5%) than in control group (36%) (P value = 0.0006; Odds ratio = 4.687; 95% confidence interval = 1.9 - 11.56). No significant association had been reported between severity of preeclampsia and Helicobacter pylori IgG seropositivity (P value = 0.4507). Regarding blood samples analysis of umbilical cord vessels for 24 women of case group, most of seropositive pre-eclamptic women (positive maternal serum) also had positive cord serum results. This can further confirm the ability of Helicobacter pylori IgG antibodies to cross placental barrier.

Conclusion: A significant association had been observed in this study between infection with Helicobacter pylori and incidence of preeclampsia, but there was no remarkable relationship between this infection and severity of preeclampsia.

Keywords: Preeclampsia, Helicobacter pylori, Anti H. pylori IgG, type 1 helper cell, Anti-CagA Ab, Pregnancy.

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Pre-eclampsia (PE) is an idiopathic disorder of pregnancy characterized by protein uric hypertension that affects 5% to 8% of all pregnancies and is associated with significant morbidity and mortality to the mother and the fetus. The underlying pathophysiological process of PE is poorly understood hence early biomarkers are not fully identified[1]. However, it is obvious that abnormal placental development is the critical player behind it. Many theories are trying to explain this process.

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Imbalance between pro-angiogenic and anti-angiogenic factors. Such disparity has been reported early before the appearance of PE specific symptoms[2]. In normal pregnancy, vascular endothelium is maintained through the production of pro-angiogenic factors like vascular endothelial growth factor (VEGF) and transforming growth factor-β1 (TGF-β1), while in PE, there is over production of circulating anti-angiogenic factors by the placenta that will impair the endothelial homeostasis. Both soluble fms-like tyrosine kinase 1 (sFLT-1) and soluble endoglin (sEng) will bind and

neutralize VEGF and TGF-β1 respectively. The final result is endothelial cells dysfunction leading to a reduction in the production of nitric oxide and prostacyclin with concomitant release of procoagulant proteins[3].

There is a probable role for infection has been suggested as a possible initial trigger for poor placentation. Such assumption is based on the mechanism of molecular mimicry (epitope mimicry), where antibodies generated against microbial endotoxins can cross react with other host targets. In case of PE, these targets believed to be trophoblast cells[4].

Helicobacter pylori (HP) is a gram negative bacterium and its infection is associated with peptic ulcer and chronic gastritis and also considered a risk factor for gastric cancer[5]. One of the prominent pathogenic markers for HP is cytotoxin-associated gene-A (CagA), this gene encodes for a surface protein (antigen) that can elicit systemic inflammatory response[6]. Anti-CagA antibodies have been found to be able to react with certain antigens on the surface of endothelial cells; such interaction can explain the probable association between HP infection and endothelial cells impairment[7]. Since cytotrophoblast cells have endothelial origin, a recent hypothesis has been suggested a viable correlation between HP infection and impaired placentation observed in PE[4]. It has been observed that anti-CagA antibodies (IgG immunoglobulin) have the ability to cross react with β-actin protein on the surface of trophoblast cells culture. This modification of cytoskeleton is believed to adversely affect intercellular adhesion and impair placental anchorage[8]. Furthermore, anti-CagA antibodies have been shown to inhibit the activation of MARK/ERK pathway in the cell and the nuclear translocation of NF-kB. Inhibition of these signaling mediators is believed to diminish the proliferative capacity of trophoblast cells. These molecular evidences further promote the correlation between HP infection and placental impairment[4].

The objective of this study is to investigate the possibility of an association between PE incidence and HP infection.

Methods

This case-control study has enrolled 90 patients that was carried out in the department of obstetrics and gynecology at Al-Yarmouk Teaching Hospital between April - December 2017.

Ethical consideration: All participants were asked to provide their written consent form after they were fully informed about the nature of the study and its aim.

Ethical approval was also obtained from the Scientific Committee of the Research of the Obstetrics and Gynecology Department of Al-Yarmouk Teaching Hospital.

Inclusion and exclusion criteria: we utilized immunochromatographic analysis to screen serum IgG antibodies against H. pylori. This screen was done to 40 pregnant women with preeclampsia (case group) and another 50 normotensive pregnant women with comparable gestational age (control group).

Serological screening was also carried out to examine blood samples collected from umbilical cord vessels for 24 pregnant women of preeclampsia group after delivery in order to diagnose transplacental transfer of anti-H. pylori IgG antibodies.

The diagnosis of PE was based on the standards of the American College of Obstetricians and Gynecologists (ACOG); mild preeclampsia can be diagnosed when hypertension (two reading of systolic blood pressure ≥ 140 mmHg and/ or diastolic blood pressure ≥ 90 mmHg separated by a period of 4-6 hours) and proteinuria (≥ 300mg/ 24 hours or dipstick reading of 1+) are well evident after 20 weeks of gestation in a previously normotensive woman. Severe PE can be recognized through having sustained elevation in blood pressure (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg) or other signs of end organ damage like renal deterioration (proteinuria ≥ 3g/ 24 hours)[3,4,6].

The inclusion criteria: Having single viable fetus, gestation age between 37^{+0} weeks and 40^{+0} weeks, any maternal age and parity status.

The exclusion criteria were patients with medical diseases like chronic hypertension, diabetes mellitus, any previous history of having HP eradication treatment, smoker patient.

A full history, general examination and obstetric examination were also carried out for all participants.

Mid-stream urine sample was collected from each participant for the measurement of protein level in urine. Blood samples (5 ml) were collected for full blood count, renal function test, liver function test, and serological tests. Patients were followed till time of delivery and cord blood samples were collected from 24 patients with PET for serological screening. Blood samples were collected into clean sterile vacutainer tubes without any anticoagulant; and the blood was allowed to clot at room temperature. The sera were separated by centrifugation and stored in frozen (-20°C) until tested for HP Ab. The test included screening the collected specimens for IgG antibodies against H. pylori by using the H. pylori Ab combo rapid test (Labstix Diagnostics Pty Ltd) as a qualitative immunochromato-graphic analysis and the interpretation of the assay results as follow:

- Negative result: if the color is only visible within C band area.

- Positive result: if both test and control lines are developed.

- Invalid result: if no color is seen in the control line region, then the result should be neglected whether or not the test line has been developed.

Each participant was assigned a serial identification number. The data were analyzed by using GraphPad Prism version 5. The differences in means between case group (PE group) and control group were assessed by using independent *t*-test. For groups with unequal variances, Welch's correction was applied to unpaired *t*-test. The categorical data were presented as frequencies (percentages), the association among these variables was evaluated by using chi-square test of independence and odds ratio (contingency table). For groups with small size, Fisher's exact test was used as a more precise alternative to chi-square test. *P* value less than 0.05 was considered statistically significant.

Results

In attempt to minimize the possible influence of any confounding factor on the current association analysis, we have compared both demographic between PE group and control group, (Table 1). It is well obvious, from this table, there is no significant difference was reported between control women and PE patients in terms of maternal age and baby gender, but there was a significantly shorter duration of pregnancy and a lower neonatal weight had been reported for the PE women as compared to the normotensive pregnant women (for both variables, *P* value < 0.0001).The percentage of nulliparous women was significantly higher among PE patients (45%) than in control group (24%; *P* value = 0.0357).

Current association analysis between HP seropositivity and PE incidence is summarized in table 2. There were significantly more H. pylori IgG seropositive women in PE group (72.5%) as compared to control group (36%) (P value = 0.0006; Odds ratio = 4.687; 95% confidence interval = 1.9 - 11.56).

Table 3 reveals no significant association between the prevalence of H. pylori IgG antibodies and the severity of preeclampsia (P value = 0.4507).

To confirm the transfer of H. pylori IgG antibodies across placental barrier, blood samples were collected from umbilical cord vessels after delivery for 24 women in PE group. As shown in table 4, immunochromatographic assay had shown that 19 of the H. pylori seropositive women had positive cord serum results.

Table 1: The demographic characteristics for case group (PE group) and control group.

Variable	Control group Mean ± SD or No. (%)	PE group Mean ± SD or No. (%)	*P* value
Maternal age (years)	25.84 ± 4.344	24.28 ± 4.291	0.0912 (NS)
Gestational age (weeks)	38.88 ± 0.8485	37.45 ± 0.5524	< 0.0001
Nullipara	12 (24%)	18 (45%)	0.0357
Newborn weight (kg)	3.516 ± 0.3981	2.605 ± 0.2698	< 0.0001
Baby Gender: Male	20 (40%)	14 (35%)	0.6269 (NS)
Female	30 (60%)	26 (65%)	

Table 2: Seroprevalence of Helicobacter pylori IgG antibodies among PE and control groups.

Anti-HP IgG prevalence	PE group No. (%)	Control N (%)	Total	*P* value	Odds ratio (95% Cl)
Positive	29 (72.5)	18 (36)	47 (52.2)	0.0006	4.687 (1.9-11.56)
Negative	11 (27.5)	32 (64)	43 (47.8)		
Total	40 (100)	50 (100)	90 (100)		

Table 3: Association between seropositivity of H. pylori IgG antibodies and severity of preeclampsia.

Anti-HP IgG prevalence	Severe PE N= (12)	Mild PE N= (28)	Total	*P* value
Positive	10 (83.3%)	19 (67.9%)	29 (72.5%)	0.4507 (NS)
Negative	2 (16.7%)	9 (32.1%)	11 (27.5%)	
Total	12 (100%)	28 (100%)	40 (100%)	

Table 4: Seroprevalence of H. pylori IgG antibodies in umbilical cord serum among PE patients.

Anti-HP IgG prevalence	Maternal blood sample	Umbilical cord sample
Positive	21	19
Negative	3	5
Total	24	24

Discussion

PE is a worldwide leading cause of maternal mortality and morbidity with negative impact on perinatal outcomes. Although many of the details have been explained regarding PE pathophysiology, the primary trigger is still unknown. It has been suggested that IgG antibodies, formed against CagA antigen on the surface of HP, can cross react with β-actin protein on the surface of trophoblast cells. This proposed mechanism of epitope mimicry can inhibit the invasion of trophoblast cells leading to poor placentation[9].

It is well known that PE can adversely influence the duration of pregnancy and intrauterine growth rate[10]. In the current study, it was observed that pre-eclamptic women had a significantly shorter duration of gestation as compared to normotensive pregnant women (*P* value < 0.0001). In

addition, the weight of newborns was significantly lower among PE patients as compared to control women (*P* value < 0.0001).

In this study, we found a significant association between PE and HP infection (P value = 0.0006). Based on our results, it seems that exposure to this microorganism can increase PE incidence by more than 4 times (Odds ratio = 4.687). This association is consistent with those established by previous studies in the same trend as Ponzetto et al[11], Pugliese et al[12] and Cardaropoli et al[13].

Current study showed no significant association between severity of PE and HP infection (*P* value = 0.4507) and this may be due to small sample that was studied.

Several studies have proposed that PE pathologic mechanism is a systemic rather than a local one. It is believed that anti-H. pylori IgG antibodies play a crucial role in mediating such systemic pathological effect as these immunoglobulins possess the ability to cross placental barrier[9,11]. Our serological analysis on umbilical cord blood samples for PE patients indicates that only two HP seropositive women had negative cord results. This can be due to low titer of H. pylori IgG antibodies within cord blood sample for these two women. The transplacental transfer of anti-H. pylori IgG antibodies in most of seropositive PE women falls in favor of molecular mimicry (epitope mimicry) mechanism. However, the role of this mechanism and infection in PE pathophysiology need to be further elucidated.

In conclusions: A significant association had been observed between HP IgG seropositivity and PE incidence. The exposure to this pathogenic microorganism can increase PE frequency by more than four times. No significant association had been reported between severity of PE and HP infection.

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