IN THE NAME OF GOD

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ISFAHAN UNIVERSITY OF MEDICAL SCIENCE
OBSTETRIC AND GYNECOLOGY DEPARTMENT

Thesis for obtaining the speciality degree in Obstetric and Gynecology

Title :

Is any difference between preeclamptic and healthy pregnant women regarding the presence of periopathogenic bacteria in the placenta?

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I PRESENT IT TO

MY FATHER AND MOTHER

&

MY DEAR HUSBAND

BECAUSE OF THEIR SUPPORTS

THANKS FOR MY MASTERS

SPECIALY DR.MOSTAJERAN

&

DR.MOHAJERI LABRATORY

&

MRS. MAHMOODI

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Dear Dr Bahareh Arbabi

I am pleased to inform you that your paper entitled: **"Is any difference between preeclamptic and healthy pregnant women regarding the presense of periopathogenic bacteria in the placenta?"**, with authors" Dr. Fateme Mostajeran, Dr Bahareh Arbabi (corresponding author: Dr Bahareh Arbabi)" is accepted for publication as an original article.

All the best

Prof.Roya Kelishadi,
Editor-in-Chief
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Is any difference between preeclamptic and healthy pregnant women regarding the presence of periopathogenic bacteria in the placenta?

Background:

Preeclampsia is an important cause of maternal morbidity and mortality with unclear cause. It is believed that inflammation plays an important role in the pathogenesis of preeclampsia. Periodontal disease is a chronic inflammatory infectious condition which commonly involves humans. Recently, chronic infection was linked to the atherosclerosis. Atherosclerosis shares some histopathological features with uteroplacental atherosis of preeclamptic women. This study was aimed to investigate presence of periopathogenic bacteria in the placental tissue of preeclamptic women, and compare it with women with normal pregnancy.

Methods:

Samples were obtained from 23 placentas of preeclamptic women and from 23 age-matched healthy pregnant women. Qualitative polymerase chain reaction was performed to detect five periopathogenic bacteria.

Results:

There was no significant difference between 2 groups regarding the relative frequency of women with different types of periopathogenic bacterial infection of the placenta. In addition, there was no significant difference in number of women with any type of infection of the placenta - regardless of the type of periopathogenic bacteria- (14 (61%) mothers with placental infection in the case group vs. 18(78%) mothers in the control group; p-value: 0.16).

Conclusion:

This study did not show any significant difference between preeclamptic women and healthy women with normal pregnancy regarding the periopathogenic bacterial profile of the placenta.

Introduction:

Preeclampsia is an important cause of maternal morbidity and mortality which complicates approximately 3% of all pregnancies (1-2). Although several theories including abnormal placentation, cardiovascular maladaptation to pregnancy, genetic and immune mechanisms, increased systemic inflammatory response, and

nutritional, hormonal, and angiogenic factors have been suggested to cause preeclampsia, the etiology of this serious disorder is still unclear (3-7).

Comparing to the normal pregnancy, preeclamptic women have a significantly greater systemic inflammatory response (4). It suggests an important role for inflammation in the pathogenesis of preeclampsia (4, 8). Infection is considered as an important source of inflammation that may play role in many adverse pregnancy outcomes including preeclampsia (9-11). Infection can either increase the risk of acute uteroplacental atherosis, and therefore initiate preeclampsia, or amplify the maternal systemic inflammatory response, and consequently potentiate preeclampsia (12-13).

According to the suggested association between infection and preeclampsia, periodontal infection which is one of the most common chronic infectious disorders in humans might be linked to preeclampsia (10, 14-16). Depending on the definition of periodontal disorders and the population, prevalence of periodontal disorders are reported to be between 10-60% (14-15).

A study has detected oral pathogens in atherosclerotic plaques. Uteroplacental atherosis shares some histopathological features with atherosclerosis (17). Therefore, some studies suggest an association between preeclampsia and periodontitis, and some believes that periodontal pathogens may be present in the placenta of women with preeclampsia (8, 17-20). However, it is still controversial due to some claims that deny such association (21).

Given the above evidence and the controversy on this topic, this study was aimed to investigate presence of periopathogenic bacteria in the placental tissue of preeclamptic women, and compare it with women with normal pregnancy.

Materials and methods:

After approval of the study by the ethic committee of Isfahan University of Medical Sciences (research project number 390058) and obtaining informed consent, this case-control study was performed on 46 women (23 preeclamptic women and 23 age matched healthy women with normal pregnancy) between June 2011 and February 2012 in Beheshti and Al-zahra hospitals, Isfahan, Iran.

We obtained placental samples only from cesarean section in order to avoid possible vaginal and cervical contamination.

Preeclamptic women who underwent cesarean section entered the case group. According to the American College of Obstetricians and Gynecologists (ACOG) guideline, preeclampsia was considered when a woman had hypertension (diastolic blood pressure of at least 90 mm Hg or a systolic pressure of at least 140 mm Hg) after 20th weeks of gestation plus proteinuria (urinary excretion of >300 mg protein/24 hours, or 2 random urine specimens obtained at least 4 hours apart demonstrating ≥1+ by dipstick testing or measured as >30 mg/dl) (8, 22).

Women who underwent cesarean section for reasons other than preeclampsia were assigned to the control group.

Excluding criteria were multiple gestation, diabetes mellitus, urinary tract infection, rupture of membrane, chronic hypertension and having other medical disorders. In addition, women who had received antibiotics over the 5 months prior to the study, or had been treated with calcium channel blockers, phenytoin or cyclosporine A for more than 3 months before the investigation were excluded from this study.

After qualifying patients according to the aforementioned criteria, demographic and clinical features of patients including age, parity, gravidity and gestational age at delivery were recorded. Besides, the labor phase at the time of delivery and the status of membranes were registered.

After the cesarean section, 4 placental samples were taken from both central and marginal areas of each placenta by a single physician under sterile condition. Two samples were obtained from the maternal side and 2 samples from the fetal side. To make the study blind, a code was assigned to each sample. Then, samples were sent to the laboratory for evaluation by qualitative polymerase chain reaction (PCR) regarding the presence of five periopathogenic bacteria (actinomycetem comitans, prevotella intermedia, porphyromunas gingivalis, treponema denticula, tannerella forsythenesis).

Data were analyzed by SPSS 16.5, and chi-square and independent T-test were used when appropriate. P-values less than 0.05 were considered statistically significant.

Results:

Baseline data:

There was no significant difference between 2 groups in mean of age, gestational age, gravidity and parity (table-1).

Table-1 Baseline characteristics

	Case group (N:23)	Control group (N:23)	p-value
Age (year)	29.21±1.50	28.30±2.60	0.15
Gestational age (weeks)	39.13±0.34	38.96±0.56	0.21
Gravidity	1.52±0.59	1.91±0.73	0.06
Parity	0.52±0.59	0.91±0.73	0.06

Data are presented as mean±SD

Indications of cesarean section in each group are presented in table-2.

Table-2 Indications of cesarean section in each group

group / CS indication	Case (N:23)	Control (N:23)	P value	Total (N:46)
CPD	3(13%)	6(26%)	0.31	9(20%)
Previous CS	9(39%)	15(65%)	0.22	24(52%)
Breech position	1(4%)	2(9%)	0.56	3(6%)
Severe preeclampsia	10(44%)	0(0%)	<0.0001	10(22%)

Data are presented as the number (%) of patients.

CS: Cesarean section, CPD: cephalopelvic disproportion

Periopathogenic bacterial infection of the placenta:

Two groups were compared regarding the number of cases with positive periopathogenic bacterial infection of the placenta, and no significant difference was observed (table-3).

Surprisingly, the number of women with placental periopathogenic bacterial infection was higher in the control group; however this difference was not statistically significant.

Table-3 Frequency of women with different types of periopathogenic bacterial infection of the placenta in each group

Group / Bacterium	Case group (N:23)	Control group (N:23)	p-value
Actinomycetem comitans	5(22%)	1١(4٨%)	0.06
Prevotella intermedia	6(27%)	5(22%)	0.50
Porphyromunas gingivalis	3(13%)	3(13%)	0.66
Treponema denticula	1(4%)	1(4%)	0.75
Tannerella forsythenesis	1(4%)	3(13%)	0.30

Data are presented as the number (%) of patients infected with different types of periopathogenic bacteria in each group.

Moreover, we compared the number of mothers who had positive placental infection -regardless of the type of periopathogenic bacteria- and found no significant difference between two groups (14 (61%) mothers with placental infection in the case group vs. 18(78%) mothers in the control group, p-value: 0.16).

Discussion:

The present study demonstrated no significant difference between preeclamptic women and healthy women with normal pregnancy regarding the relative frequency of placental infection with periopathogenic bacteria.

Surprisingly, we found a non-significantly higher frequency of placental infection with periopathogenic bacteria in healthy women which is inconsistent with findings reported by previous studies.

The previous study by Barak et.al was performed with a study design almost similar to this study. They found significantly higher frequency of infected placental samples in preeclampsia group compared with healthy women.

Having found T forsythensis, P gingivalis, A actinomycetemcomitans, P intermedia and F nucleatum in placenta, Barak et.al suggested that these pathogens

may have transmitted hematogenously, and played role in the formation of placental atherosis.

They also reported significantly higher bacterial count in the preeclamptic women (17); however, we do not have data about bacterial count due to qualitative PCR.

While all five investigated types of periopathogenic bacteria were found in both case and control groups of our study, they found all types in the case group and only three types in the control group. These dissimilarities between what we have found and findings of Barak et.al study could be attributed to some factors including racial and socioeconomic differences.

For instance, the difference could be caused by different levels of oral hygiene of studied women. In other words, it could be supposed that women who were investigated in the Barak et.al study had had better average level of oral hygiene than ours, so the difference between women with good oral hygiene and poor oral hygiene has been more prominent, and has led to significant differences between two groups. However, it is just a hypothesis not a definite reason because we do not have any idea about baseline level of oral hygiene of patients, neither from our study nor from the Barak et.al study.

Another investigation performed by Swati et.al confirms findings of Barak et.al (19). The important finding which could be helpful in interpretation of what we have found is that Swati et.al have detected periopathogenic bacteria in the placenta of two cases who did not have periodontal diseases. This finding shows that having infected placenta with periopathogenic bacteria does not necessarily mean having periodontitis. It was suggested that these pathogens may have gained access to the placenta through the genital tract as an ascending infection, or they may be translocated retrogradely from the peritoneal cavity through the fallopian tubes. Based on this condition, general hygiene could also play an important role in this regard, and can affect the probability of having placental infection with

periopathogenic bacteria in the absence of periodontitis. This emphasizes the role of socioeconomic status in presence of periopathogenic bacteria in the placenta.

Another study by Siqueira et.al on Brazilian women revealed that maternal periodontitis is a risk factor associated with preeclampsia (18). Their study design is completely different from this investigation.

They have defined clinical criteria for periodontitis, and then assessed the relationship between periodontitis and preeclampsia after controlling confounders.

Siqueira et.al did not study the bacterial profile of the placenta. They believe that maternal infections such as periodontitis accelerate cytokine release and endothelial dysfunction which increase risk of preeclampsia (18, 23-25).

The main difference between our study and the study of Siqueira et.al is that we investigated local effects of periodontitis on the placenta of preeclamptic women, while they investigated systemic effects of periodontitis on preeclampsia.

Therefore, although we did not find any evidence that support the role of placental infection with periopathogenic bacteria, we are not able to rule out the suggested association between preeclampsia and periodontitis at this stage.

Another study on a relatively large number of Jordanian women investigated the association between periodontal parameters and preeclampsia, and reported no association between them (21). This conclusion partly supports our findings. Khader et.al suggested that differences in study design, sample size, periodontal disease definition, and adjustment criteria may be reasons of their findings.

In summary, our study did not show any significant difference between preeclamptic women and healthy women with normal pregnancy regarding the periopathogenic bacterial profile of the placenta. We suggest further investigations on larger sample size with better control of confounding factors such as socioeconomic status, accompanied by simultaneous assessment of periodontal parameters to achieve more accurate results.

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