Association between Porphyromanas Gingivalis Infection and Recurrent Early Pregnancy Loss

Thesis Submitted in Partial Fulfillment of the requirements for Master degree in Obstetric and Gynecology

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List of Abbreviations

ACOG : American College of Obstetricians and Gynecologists

APO : Adverse pregnancy outcome

ASRM : American society of reproductive medicine

BMI : Body mass index

BOP : Bleeding on probing

C I : Confidence interval

CPS : Capsular polysaccharide

CAL : Clinical attachment loss

GA : Geststional age

HCG : Human chorionic gondotropin Hormone

IVF : In vitro fertilization

Kgp : Arg-Gingipan

LH : Lutenizing Hormone

LPS : Lipopolysaccharide

MFaI : minor fimbriae

MV : Membrane vesicle

LMP : Last menstrual period

OMV : Outer membrane vesicle

PAI : Plasminogen activator inhibitor

PAD : Peptidyl- Arginine Deaminase

PCOs : Polycystic ovary syndrome

PCR : Polymerase reaction

PD : Periodontal disease

PMN : Polymorphonucler cells

PPP : Periodontal dental pocket depth

RAFS : Reproductive autoimmune failure syndrome

RCOG : Royal College of obstetricians and gynecologists

Rgp : Arg- Gingipan

REPL : Recurrent early pregnancy loss

RM : Recurrent miscarriage

SD : Standard deviation

SPSS : Statistical package for the social science

TFR : Transferrin

LPD : Luteal phase defect

TLR : Toll –like receptor

UPD : Unipaternal disomy

WHO : World Health Organization

ASSOCIATION BETWEEN PORPHYROMONAS GINGIVALIS INFECTION AND RECUURENT EARLY PREGNANCY LOSS

Protocol of thesis

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INTRODUCTION

Title :

Association between Porphyromonas gingivalis and recurrent early pregnancy loss (REPL)

Background:

Periodontal disease (PD) is a common chronic infectious and inflammatory disorder, which include gingivitis and periodontitis, with a prevalence of 10-60% depending on the definition and the population being studied. Maternal periodontitis is associated with an increased risk of pre-eclampsia, intrauterine growth restriction, preterm birth and low birth weight infants. PD is treatable with good oral hygiene and dental care, and consequently is a modifiable variable that may lead to improvements in adult health ***(Hart, Doherty et al., 2012;Kumar, Basra et al., 2013).***

Porphyromonas gingivalis (*P. gingivalis*), a periodontopathic bacterium, was found in placentae of women with preterm delivery and pre-eclampsia and in the amniotic fluid of pregnant women diagnosed with threatened preterm labour ***(Barak, Oettinger-Barak et al., 2007;Katz, Chegini et al., 2009;Leon, Silva et al., 2007)***.

P. gingivalis is a gram-negative anaerobic bacterium, and has bioactive properties including lipopolysaccharide (LPS), capsules and fimbriae on the cell surface. These properties induce the production of proinflammatory cytokines and could potentially disrupt homeostasis in the placental tissues, It is also potentially important that, during the first 10-12weeks of pregnancy, the placenta is in a state of physiological hypoxia, which would facilitate growth of anaerobes such as *P. gingivalis* ***(Holt, Kesavalu et al., 1999;James, Stone et al., 2006;Katz, Chegini et al., 2009)***.

Generally, it is recognized that Toll-like receptor (TLR)-4 is the receptor for gram-negative bacteria LPS and that TLR-2 is for gram-positive peptidoglycan and lipopeptides ***(Takeuchi, Hoshino et al., 1999;Underhill and Ozinsky, 2002).***

However, it has been shown that *P. gingivalis* LPS signals through both TLR-2 and TLR-4 ***(Kocgozlu, Elkaim et al., 2009) or TLR-2 (Diya, Lili et al., 2008;Hajishengallis, Tapping et al., 2006;Hashimoto, Asai et al., 2004;Hirschfeld, Weis et al., 2001)***.

P. gingivalis LPS induces interleukin-6 and interleukin-8 production via TLR-2 in chorion- derived cells ***(Hasegawa-Nakamura, Tateishi et al., 2011).***

Recurrent pregnancy loss (RPL) was defined by the American society for reproductive medicine as the presence of two or more failed pregnancies, proved either by sonographic examination or by histopathology (***Practice Committee of the American Society for Reproductive Medicine, 2013)***. RPL is one of the most common obstetrical complications. Multiple endocrine, anatomic, genetic, haematological and immunological aetiologies have been reported for this devastating disease; however over half of the cases remain unexplained. Inflammatory processes are often observed at the maternal-fetal interface as the final pathological assault in many cases of RPL, including those of unexplained aetiologies ***(Donckers, Scholten et al., 2012;Kwak-Kim, Yang et al., 2009)***.

RPL may be either a primary or a secondary process: primary RPL refers to those women with RPL who never had a live birth before ***(Ansari and Kirkpatrick, 1998;Paukku, Tulppala et al., 1999).***

Research hypothesis

Research question:

Is *Porphyromonas gingivalis* infection more prevalent in patients with primary unexplained recurrent early pregnancy loss?

Research hypothesis :

Exposure to *Porphyromonas Gingivalis*, in comparison to no exposure, does not increase the risk of recurrent pregnancy loss in early pregnancy .

Objective:

The aim of the current study was to evaluate the potential role of *P. gingivalis* in patients with primary unexplained recurrent early pregnancy loss.

Methodolgy

Study setting:

Patients will be recruited from women attending outpatient clinics of Ain Shams University Hosptial .

Study design:

ACase Control Study.

Patients and methods:

- The study population consisted of total of 100 patients will be recruited from women attending outpatient clinics of Ain Shams University Hosptial for Recurrent early pregnancy loss (REPL).
- It will be carried out after being approved by ethical and research committee of council of Obstetric and Gynecology Department, Ain Shams University.
- The study will be explained to all enrolled participants and a written informed consent was obtained from each participant .
- Demographic information collected included patient`s age, gravidity , parity, medical and surgical histories and gestational age
- The current study will include two groups of pregnant women who will be recruited from outpatient clinics of Ain Shams University Maternity Hosptial .

GroupI (Study Group) :

- This group will include about 50 Pregnant women who present with diagnosis of recurrent early pregnancy loss according the following inclusion and exclusion criteria.

Inclusion criteria :

- All ncluded women will be admitted to outpatient clinic between7-13 weeks gestation for termination of pregnancy due to absence of

fetal cardiac activity or absence of fetal pole on ultrasonographic examination .

- Included women with history of two or more consecutive unexplained first trimestric miscarriage and no live birth.
- Gestational age will be calculated from last menstrual period and confirmed by ultrasound examination performed at the time of enrollment.
- All included women had negative oral glucose tolerance test for diabetes mellitus , negative antiphospholipid antibodies , normal thyroid profile and normal parental karyotypes .

Exclusion criteria :

- Women who use antimicrobial drugs .
- Women with viral infection , autoimmune disease , diabetes and gestational diabetes .
- Women without history of recurrent pregnancy loss .

Group II (Control Group) :

This group will include 50 pregnant women who matching the study group with the same inclusion and exclusion criteria without being in recurrent early pregnancy .

- Such women will have live birth .
- Such women will be recruited from women attending outpatient clinics of antenatal care .

Procedures:

- Women who fulfill the eligibility will be subjected to :

1-History taking: with particular emphasis on past medical history, chronic medical disorders, past obstetric history as well as menstrual to calculate the gestational.

2-General examination.

3-Abdominal examination.

4-Pelvic examination.

5-Obstetric ultrasound to confirm the gestational age.

- Subgingival plaque sample and chorionic tissue biopsy will be obtained from each patient for further evaluation using PCR.
- A Cusco speculum will introduce into the vagina; the anterior lip of the cervix will grasped with ring forceps and a cotton-tipped swab was placed into the cervical canal and then into the posterior fornix (each for at least 1 min) to obtain an adequate amount of cervicovaginal secretions. Any sample found to be contaminated with blood was discarded.

sampling :

- Placental tissue will be collected by curettage , cleansed of decidual tissue, rinsed in HAMS F 10 medium and dissected under a stereoscopic microscope.
- DNA will be isolated from the placental tissue to investigate the presence of *Porphyromonas gingivalis* infection .
- Subgingival plaque samples will be obtained by inserting a sterilized paper point for 15 seconds into periodontal pockets of sites showing signs of gingival inflammation including gingival redness and swelling **.**
- Samples will be kept at 80º C until analysis.

- DNA of subgingival plaque, saliva and chorionic tissue samples will be measured by means of polymerase chain reaction (PCR) .
- Molecular analysis will be performed at Biotechnology Research Unit

DNA isolation :

- The placenta samples will be resuspended in lysis buffer (10 mM, Tris –HCL, PH 7.5 , 1 mM EDTA , PH 7.9 , 0.5% SDS) and treated overnight at 37 º C with proteinase K(100 µg ⁄ ml) .
- DNA will be isolated by phenol and chloroform extraction followed by ethanol precipitation and dissolved in distilled sterile water according to ***(Sambrook et al .,1998)***

Ethics and Patient`s Rights:

- **IRB approval :**

- The clinical research study will be conducted in accordance with the current IRB-approved clinical protocol ; ICH GCP Guidelines ; and relevant policies , requirements , and regulations of the Ain Shams University .

- **Consent procedure:**

-The investigator will make certain that an appropriate informed consent process is in place to ensure that potential research subjects , or their authorized representatives , are fully informed about the nature and objectives of the clinical study , the potential risks and benefits of study participation and their rights as research subjects .

- The investigator will obtain the written , signed informed consent of each subjects under study , or the subject`s authorized representative , prior to performing any study –specific procedures on the subject .

- The investigator retained the original signed informed consent form.

Subject Confidentiality:

All laboratory specimens , evaluation ,reports , vido recordings ,and other records that leave the site not include unique personal data to maintain subject confidentially .

Statistical analysis

Data were presented in terms of mean and standard deviation (SD), or frequencies (number of cases) and percentages (%) when appropriate. Mean difference with 95% confidence interval (95% CI) was calculated to measure the association between *P. gingivalis* DNA and the risk of missed miscarriage in both groups. Analysis of numerical variables was performed by using independent student's t-test. For comparing categorical data, chi-Squared Test was performed. Yates' continuity correction was applied to the chi-squared test when one or more of the expected values were less than 5. P value < 0.05 was considered statistically significant. All statistical calculations were done using SPSS version 15 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) for Microsoft Windows.

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