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Maternal chronic oral infection with periodontitis and pericoronitis as a possible risk factor for preeclampsia in Egyptian pregnant women (microbiological and serological study)

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ABSTRACT

Background: Several studies have hypothesized that oral infection may increase the risk of preeclampsia. We explore the relationship between chronic oral infection and the risk of preeclampsia in Egyptian pregnant women.

Methodology: Forty preeclamptic women with periodontitis and/or pericoronitis (group I) and 40 control subjects having periodontitis and/or pericoronitis (group II) were subjected to microbiological assessment of subgingival plaque, pseudo-pocket and placental samples. TNF-α was determined in gingival crevicular fluid (GCF), saliva and serum by ELISA and real time PCR.

Results: There was no statistically significant difference between the two groups as regards to subgingival plaque and pericoronal pseudo-pocket organisms revealed by culture and PCR. The total number of anaerobes in blood and placental samples was higher in pre-eclamptic group than controls. There was a statistically significant difference between the two groups as regards to the level of TNF-α by ELISA in serum (P-value = 0.021).

Conclusion: There was a relationship between chronic oral infection and preeclampsia, so treatment of oral infection during pregnancy may represent a novel approach and preventive strategy that reduce oral bacterial load which would decrease the incidence of preeclampsia.

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1. Introduction

Preeclampsia is a disorder of worldwide significance and specific to pregnancy, it affects 5–10% of pregnancies. It is the third most common direct cause of maternal death in the United Kingdom and accounts for up to 40,000 pregnancy-related deaths per year in the developing world, also it may lead to obstetric morbidity and accounts for 25% of preterm deliveries which in turn result in adverse neonatal outcome [1,2].

It occurs usually after 20 weeks of gestation and is characterized by normal vascular response to placentation manifesting as generalized vasospasm, activation of coagulation system, reduced organ perfusion affecting the kidney, liver and brain. It requires both blood pressure exceeding 140/90 mmHg after the 20th week of gestation and proteinuria exceeding 300 mg in a 24 h urine sample [3,4].

Periodontitis is regarded as a chronic inflammatory oral infection that affects the tooth supporting structures and bone, in which bacteria of dental plaque and calculus and their byproducts are the principal etiologic agents. It is well known that teeth, gingival margins and periodontal pockets are places that could harbor bacterial colonization, and that one cubic millimeter of dental plaque contains about 100 million bacteria [5–7].

While pericoronitis is an inflammatory condition that may accompany the partially erupted teeth, the Gram-negative anaerobic bacterial growth that develops in the distally located coronal flap is the major cause of this condition. It is generally agreed that this process is potentiated by food debris accumulating in the vicinity of the operculum and occlusal trauma of the pericoronal tissues by the opposing tooth. Pregnancy and fatigue are associated with an increased occurrence of pericoronitis [8,9].

It is generally accepted that unhygienic oral conditions that results in inflammatory effects as in periodontitis and pericoronitis could negatively affect the general health of individuals. A cause and effect relationship between the health condition of the oral cavity and some systemic diseases is attributed to the presence of dental plaque, periodontal and pericoronal infections [10].

There is growing evidence that suggests an association between chronic oral infection and various systemic diseases, diabetes mellitus, preterm birth and low birth weight. Accordingly, a positive clinical association between preeclampsia and chronic periodontal or pericoronal infections has been hypothesized. Since both periodontal and pericoronal infections are chronic Gram-negative infections proposed to feature a chronic endotoxins burden that may result in transient translocation of the organisms to the placenta, thereby triggering placental inflammation [11–13].

In this respect, progress has been made for understanding the relationship, the following question is offered in this field that deserves vigorous attention and study: Is chronic oral infection itself (associated with periodontitis and pericoronitis) and the resulting bacterial byproducts can be considered a risk factor for the development of preeclampsia?

Here, it is completely clear that host tissues in periodontal and pericoronal infections could mount an immune-inflammatory response to bacteria and their byproducts by activating host-derived cytokines such as interleukins 1 and 6 as well as tumor necrosis factor-alpha (TNF-α) and prostaglandin E₂ (PGE₂) resulting in connective tissue destruction and bone loss [6,12].

It has been proposed that just as periodontal and pericoronal tissue trigger an immune-inflammatory response, there might be a systemic challenge with the resulting inflammatory cytokines including a vascular response in pregnant women, which may relate to various pregnancy complications like preeclampsia, preterm birth and low birth weight [4,12].

In this regard Herrera and coworkers 2001 established that the early identification of risk factors and the treatment of a symptomatic chronic infections lowered the preceding incidences of preeclampsia, they hypothesized that chronic infections may cause increased maternal cytokine levels sufficient to affect vascular endothelial function, thereby making pregnant women prime individuals for the subsequent development of preeclampsia [14].

Therefore, as an attempt to understand the initiating and risk factors for preeclampsia as a common pregnancy complication, this study was conducted to provide further evidence on the possible association between chronic oral infection (periodontitis or pericoronitis) and the risk of development of preeclampsia in a sample of Egyptian pregnant women. It is meant to evaluate the level of TNF-α in saliva; GCF and serum as a potent inflammatory cytokine that may be considered as a risk factor that may be contribute in the initiation of preeclampsia. As well as to explore the possible role of periodontal and pericoronal pathogens in the development of the placental lesion. Since proper understanding of the etiological risk factors may serve to properly design preventive and therapeutic strategies.

2. Subjects and methods

2.1. Selection of the studied population

This study was conducted at the hospital of obstetrics and gynaecology, Faculty of Medicine, Cairo University. Informed consent for the interview, examination as well as samples collection was obtained from all study participants in advance. The 80 selected subjects were of similar age (aged 25–35 years) and body mass index at the third trimester of gestation. They were divided by the study team into two groups:

Group I: consisted of 40 preeclamptic women having periodontitis and/or pericoronitis. Preeclampsia was defined according to the strict criteria recommended by ACOG, as high blood pressure (≥140/90 mmHg on two occasions at least 6 h apart after 20 weeks of gestation) and proteinuria (≥1+ by dip stick or ≥300 mg/24 h) [15].

Group II: consisted of 40 control subjects having periodontitis and/or pericoronitis. They were defined as women who delivered on the same day the women of group I did, but had no diagnosis of any hypertensive disorder of pregnancy by the obstetrician, and had no evidence of hypertension or proteinuria from medical record reviews.
All study participants were interviewed using a structural questionnaires at the first prenatal visit, reviews by a physician for demographic, health behavior and medical history. The included women were Egyptians, nonsmokers and not alcohol drinkers. The collected information concerned the participants age at delivery, education level, occupation, family income, pregnancy weight (kilograms), height (centimeters), body mass index (defined as the ratio of weight (kilograms) to the square of height (meters), blood group, consanguinity with partner, passive smoking status, coffee and tea consumption, and the newborn status. Additional information was collected on the number of previous deliveries, number or previous abortions, time interval between the last child and the newborn, history of preterm birth, history of low birth weight, history of preeclampsia and history of caesarian delivery. In addition to information related to the last pregnancy that should include the antenatal visits, maternal history of diabetes mellitus, self-reported emotional status during pregnancy, and history of urinary tract infection or Candida vaginosis during pregnancy. Maternal records regarding family history of cardio-vascular diseases, hypertension, cerebrovascular accidents, diabetes mellitus, preterm birth, low birth weight and preeclampsia were documented using a yes or no response. Women who had medical conditions that required antibiotic prophylaxis or consumption (3months before study inclusion), or had periodontal treatment, gestational diabetes, non-confirmed preeclampsia, hypertension before pregnancy, malaria, hemolytic anemia, elevated liver enzymes, and low platelet count syndrome, and any other infection other than periodontitis or pericoronitis were excluded from the study.

Clinical assessment of periodontal status were determined in all participants by one dental clinician as described by Drury et al., 1996 [16] using plaque index (PI) of Silness and Loé [17], gingival index (GI) of Loé and Silness [18], probing pocket depth (PPD) and clinical attachment level (CAL) [19]. Sterile dental mirrors and explorers were used to assess plaque accumulation and gingival status, whereas standardized Michigan O periodontal probes with Williams markings were used to measure (PPD) and (CAL). The teeth examined were 16, 22, 24, 36, 42 and 44 (Ramfjord index) teeth [20]. The sites examined on each tooth are mesiobuccal, midbuccal, distobuccal and lingual, none of the examined teeth had untreated periapical lesions [21,22].

Subjects were classified as having gingivitis if (PPD) < 3 mm, while chronic periodontitis was defined when at least one of the four sites per tooth with (PPD) ≥ 3 mm or (CAL) ≥ 2 mm chronic periodontitis was further classified according to severity as follows: mild with (PPD) ≥ 3 mm and ≤ 4 mm or (CAL) ≤ 3 mm, moderate with (PPD) > 4 mm but ≤ 6 mm or (CAL) ≥ 3 mm and ≤ 5 mm and severe when (PPD) > 6 mm or (CAL) ≥ 5 mm [19]. Accordingly, the selected women were suffering from moderate to severe chronic periodontitis with exclusion of subjects with healthy periodontium, gingivitis and mild periodontitis.

Asymptomatic chronic pericoronitis associated with the eruption of a third mandibular molar were diagnosed based on clinical evident of a dull pain or mild discomfort lasting a day or two, with remission lasting many months. They may also complain of a bad taste with dense and fibrous operculum forming a mean pseudo-pocket depth of (6.22 ± 2.79 mm). No dental radiographs were taken due to the special conditions of the participants.

2.2. Samples collection

1. Samples of subgingival plaque deposits were collected by using sterile periodontal curette.
2. While samples of deposits below pseudo-pocket of the operculum were collected by using small surgical curette. Samples were placed in tube containing 1 ml of VMGAIII (Viability Medium, Goteborg, and Anaerobically Prepared III) transport medium under anaerobic conditions, and immediately sent to the microbiological laboratory. After shaking by vortex for 60sec, 500 µl of the transport medium was used for culturing method and 500 µl for PCR.
3. After obtaining patient consent, placental tissue samples were obtained by dissection immediately following cesarean delivery. Sample collection was carried out in the operating room by a single physician using sterile methods. Once obtained, samples were numbered and coded. Samples of 1 cm³ diameter were taken from different sites of the organ, dissection was carried out on both the maternal and fetal sides using blade no 15 and a standard test tube. The samples should be placed directly into the BBL™ Port-Cul Transport system (balanced formula of reducing agents and resazurin in a buffered isotonic agar base) to maintain the viability of anaerobic, facultative and aerobic micro-organisms. Specimens were sent as soon as possible to the laboratory, avoid exposure to atmospheric oxygen, and inoculated onto blood agar, MacConkey agar, chocolate agar and selective media. Part of the specimens was subjected to PCR.
4. Ten ml of blood samples were collected aseptically from the women of both groups, each sample was divided into two halves, one half (5 ml) was centrifuged and serum was separated, while the other half was inoculated into signal blood culture system (oxoid) for aerobic and anaerobic micro-organisms. The bottles that give positive signal were subcultured on blood agar, MacConkey agar, chocolate agar and sabouraud dextrose agar media and selective media.
5. Whole unstimulated gingival crevicular fluid (GCF) specimens of all participants of both groups were collected by using micro-pipette or sterile paper point and stored as aliquots.
6. Unstimulated saliva samples were collected from participants of both groups; the samples were collected after oral cavity irrigation with water. After the irrigation, the subjects took 5 ml of water and held it for 5 min in the mouth. Saliva was obtained by passive drooling using plastic syringe. Serum, GCF and saliva samples were stored at −20 °C for tumor necrosis factor-alpha (TNF-α) determination by enzyme-linked immunosorbent assay (ELISA) technique and polymerase chain reaction (PCR).

2.3. Microbiological assessment by culture and PCR

- Subgingival plaque samples, samples of pseudo-pocket, blood samples and placental samples were subjected to...
Gram-stained smears for detection of pus cells and microorganisms.

- Specimens were subjected to a series of 10 fold dilutions (to $10^{-4}$) in 0.1 M phosphate buffer and aliquots of 100 μl from each dilution were spread onto blood agar, MacConkey agar, chocolate agar and sabouraud dextrose agar media or the following selective media:

1. CVE (trypticase soy agar, yeast extract 5 g/l, sodium chloride 5 g/l glucose 2 g/l, tryptophan 0.2 g/l, crystal violet 5 mg/l, erythromycin 4 mg/l, defibrinated sheep blood) [23] to assess Fusobacterium nucleatum the plates were incubated at 37 °C anaerobically.

2. KVLB-2 (Kanamycin 75 μg/ml, vancomycin 2 μg/ml, laked blood) to assess the black-pigmented Porphyromonas gingivalis and Prevotella intermedia [24] the plates were incubated at 37 °C anaerobically.

3. Wolinella agar (trypticase soy agar, vancomycin 9 μg/ml, ferrous sulphate 0.2 g/l, sodium thiosulphate 0.3 g/l, sodium fumarate 3 g/l, sodium formate 2 g/l) for isolation of Campylobacter rectus [25].

4. Brucella agar (BA) plates enriched with 5% defibrinated sheep blood and 5 μg/ml clindamycin to assess Eikenella corrodens.

5. TSBV (trypticase soy, bacitracin 75 μg/ml, vancomycin 5 μg/ml) as a selective media for Aggregatibacter actinomycetemcomitans [26] the plates were incubated at 37 °C microaerophilically.

All isolates were subcultured to obtain purified cultures then identified by API-ZIM System supplied by (BioMerieux, France).

### 3. Multiplex polymerase chain reaction

#### 3.1. DNA extraction

Subgingival plaque samples, samples of pseudo-pocket, and placental samples were vortex-mixed and centrifuged to collect the cells. The pellet was suspended in 300 μl of lyses buffer (50 mM Tris, 10 mM EDTA and 10% SDS) plus lysozyme (5 mg/ml) and incubated at 37 °C for 1 h. Then, 125 μg of proteinase-K was added and after 1 hour incubation at 65 °C, the DNA was extracted with phenol and chloroform-isoamyl alcohol treatment. Nucleic acids were precipitated in alcohol, washed with 70% (vol/vol) alcohol and suspended once more in sterile water. The DNA extracted from each sample was assayed by multiplex PCR for the detection of A. actinomycetemcomitans, E. corrodens, C. rectus, P. gingivalis and P. intermedia and F. nucleatum.

#### 3.2. PCR detection

Multiplex PCR was performed using specific primers for the 16S rRNA gene of each bacterium. PCR amplification reactions were carried out in a final volume of 100 μl consisting of 10 μl of DNA sample, and 90 μl of reaction mixture containing 30 pmol of each primer. Table 1, 200 μM of a mixture of deoxynucleoside triphosphate, 1.5 M MgCl2, 1 × PCR buffer (10 mM Tris-HCL, Ph 8), 50 mM KCl, 2.5 U Hot Start Taq TM DNA Polymerase (Qiagen). The PCR protocol was as follows: 98 °C for 15 min followed by 40 cycles of 95 °C for 30 s, 60 °C for 1 min, 72 °C for 1 min, and a final step of 72 °C for 10 min. Positive control was entered in the run. PCR amplification was performed in an iCycler System. Amplicons were detected by electrophoresis of 20 μl of samples from each PCR tube in a 2% agarose gel in TAE (Tris-Acetate- EDTA buffer) for 2 h at 80 V. The amplification products were visualized and photographed under UV light Trans illumination after staining by ethidium bromide (1 μg/ml).

#### 3.3. Assessment of TNF-α level by ELISA

TNF-α was determined in serum, GCF and saliva of both groups by ELISA according to the manufacture kit supplied by R and D system, Madison, USA.

#### 3.4. Assessment of TNF-α level by real time PCR

3.4.1. RNA extraction

- Total RNA was isolated from collected human samples using Qiagen cells/tissue extraction kit (Qiagen, USA)

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**Table 1 – PCR primers for periodontal and pericoronal pathogens.**

| Organism                        | Primer pairs (5′–3′)                                                                 | Amplicon size (bp) |
|--------------------------------|-------------------------------------------------------------------------------------|--------------------|
| Aggregatibacter actinomycetemcomitans | (1)GCT AAT ACC GCG TAG AGT CCG                                                        | 443                |
| Fusobacterium nucleatum         | (2)ATT TCA CAC CTC ACT TAA AGG T                                                    | 360                |
| Campylobacter rectus            | (1)JGG CAG CTT GCC ATG T                                                             | 598                |
| Eikenella corrodens             | (2)CTA AGT ACT GAC T                                                                | 688                |
| Porphyromonas gingivalis        | (1)AGG CAG CTG GCC ATG T                                                            | 404                |
| Prevotella intermedia           | (2)ACT GGT AGC ATC CGA T                                                            | 589                |
| Treponema denticola             | (1)TAA TAC CGA ATG T                                                                | 316                |
| Positive control                | (2)CTA AAG AGT GAC T                                                                | 122                |

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according to instructions of the manufacture. Total RNA was extracted from cells using SV Total RNA Isolation system. The purity (A260/A280 ratio) and the concentration of RNA were obtained using spectrophotometry (dual wave length Beckman, Spectrophotometer, USA).

- The extracted and purified RNA samples were treated with RNase free DNase at 37°C for 20 min and stored at –80°C for further use.

3.4.2. Primer design and selection
All primers were designed based on target sequences. For the selection of the ideal primer pair, the considered factors included melting temperature (Tm: 60–65°C), GC content (40%–60%), and amplicon length of about 90–200 bp (Table 2) [27].

3.4.3. cDNA synthesis
The total RNA (0.5–2 μg) was used for cDNA conversion using high capacity cDNA reverse transcription kit (#K1621, Fermentas, USA).

3.4.4. Real-time PCR (qPCR) using SYBR Green I
Real-time qPCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). The qPCR assays with the primer sets Table 2 were optimized at the annealing temperature. All cDNA including previously prepared samples (for VEGFR-2 and eNOS genes expression), internal control (for GAPDH gene expression), and non-template control (water to confirm the absence of DNA contamination in the reaction mixture), were in duplicate.

3.5. Statistical analysis
Numbers of organisms yielded in different samples were presented as frequencies and percentages. Chi-square test was used to compare between groups. TNF-α level by ELISA were presented as mean and standard deviation (SD) values. The cut off value for TNF-α in GCF, saliva and serum was 2.03 Pg/ml. Mann–Whitney U test used to compare between the two groups as regards TNF-α level. The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM SPSS (IBM corporation, NY, USA) (SPSS, Inc., an IBM company) statistics Version 20 for windows.

4. Results
This study was carried out on 40 preeclamptic Egyptian women as well as 40 control subjects.

4.1. Microbiological results

4.1.1. Subgingival plaque and pericoronal pseudo-pocket samples
Culture and PCR results of the subgingival plaque samples and pericoronal pseudo-pocket samples of the 40 preeclampsia patients and the 40 controls were shown in Table 3. There was no statistically significant difference between prevalence of different microorganisms in the two groups in both culture and PCR results.

4.1.2. Blood samples
Culture results of the blood samples of both groups showed that there was no statistically significant difference between prevalence of different microorganisms in the two groups Table 4. The total number of different anaerobes was higher in preeclamptic group (97) than control group (70) as shown in the table by culture.

4.1.3. Placental samples
Culture and PCR results of the placental samples of the 40 preeclampsia patients and the 40 controls were shown in Table 5. There was no statistically significant difference between prevalence of different microorganisms in the two groups except for E. corrodens where there was statistically significant difference between both groups since P ≤ 0.05. The total number of different anaerobes was higher in preeclampsia group (82) than control group (28) by culture. While PCR results of the preeclampsia group showed statistically significantly higher prevalence of Aggregatibacter actinomycetemcomitans, E. corrodens and Prevotella intermedia than control group.

4.2. B-TNF-α result: (Figs. 1–3)

4.2.1. TNF-α in GCF
The mean and standard deviation values of TNF-α in GCF were 0.37 ± 0.25 and 0.52 ± 0.06 in control and preeclampsia groups, respectively. There was no statistically significant difference between the two groups (P-value = 0.724).

4.2.2. TNF-α in saliva
The mean and standard deviation values of TNF-α in saliva were 0.60 ± 0.35 and 0.82 ± 0.13 in control and preeclampsia groups, respectively. There was no statistically significant difference between the two groups (P-value = 0.480).

4.2.3. TNF-α in Serum
The mean and standard deviation values of TNF-α in serum were 0.43 ± 0.08 and 1.47 ± 0.23 in control and preeclampsia groups, respectively. There was a statistically significant difference between the two groups (P-value = 0.021). Preeclampsia group showed statistically significantly higher mean TNF-α level than control.

5. Discussion
Chronic oral infections have been implicated as causative agents in a variety of systemic illness including...
Preeclampsia has an ill-defined multifactorial etiology involving both genetic and environmental factors and is associated with an underlying inflammatory dysfunction. Redman and coworkers [11] found that normal pregnancy was characterized by a physiological increase in systemic inflammation and that this response was exaggerated in patients with preeclampsia, they suggested this enhanced response may potentially arise from a decompensating of one or more maternal protective system. The inflammatory response associated with pregnancy and preeclampsia therefore can be assessed systemically by measuring circulatory cytokine profiles [11,13,31].

In this respect, infection of any kind in pregnant women represents a risk for adversities in the developing fetus. Here, it seems logic to hypothesize that chronic oral infection such as periodontitis and pericoronitis associated with increased maternal cytokine levels sufficient to affect vascular endothelial function, may have a significant role in the pathogenesis of preeclampsia, furthermore, in the last decayed several studies have identified the association between chronic oral Gram-negative infection and the development of atherosclerosis and thromboembolic events. In these studies, oral pathogens have been detected in atherosclerotic plaques where it can play a role in the initiation and progression of atherosclerosis leading to coronary vascular disease [28,32,33]. This was explained by the ability of oral Gram-negative pathogens to provide a chronic burden of endotoxins and inflammatory cytokines, which serve to initiate and exacerbate atherogenesis and thrombogenesis [34,35].

### Table 3 – The frequencies, percentages and results of Chi-square test for comparison between subgingival plaque samples and pericoronal pseudo-pocket samples in the two groups.

| Technique | Organism | Group | P-value |
|-----------|----------|-------|---------|
| Culture   | Aggregatibacter actinomycetemcomitans | Preeclampsia N = 40 | Control N = 40 | |
|           |          | N     | %      | N     | %      | 0.958   |
|           | Fusobacterium nucleatum | 16 | 13.8 | 16 | 14 | 6.868 |
|           | Campylobacter rectus | 20 | 17.2 | 22 | 19.3 | 0.478 |
|           | Eikenella corrodens | 18 | 15.5 | 14 | 12.3 | 0.679 |
|           | Porphyromonas gingivalis | 18 | 15.5 | 20 | 17.5 | 0.790 |
|           | Prevotella intermedia | 20 | 17.2 | 18 | 15.9 | 0.658 |
|           | Treponema denticola | 14 | 12.1 | 16 | 14 | 0.665 |
|           | Total | 116 | 100 | 114 | 100 | 0.665 |
| PCR       | Aggregatibacter actinomycetemcomitans | 20 | 14.3 | 18 | 13.6 | 0.877 |
|           | Fusobacterium nucleatum | 26 | 18.6 | 26 | 19.7 | 0.813 |
|           | Campylobacter rectus | 20 | 14.3 | 16 | 12.1 | 0.599 |
|           | Eikenella corrodens | 22 | 15.7 | 24 | 18.2 | 0.587 |
|           | Porphyromonas gingivalis | 26 | 18.6 | 24 | 18.2 | 0.934 |
|           | Prevotella intermedia | 16 | 11.4 | 16 | 12.1 | 0.859 |
|           | Treponema denticola | 10 | 7.1 | 8 | 6.1 | 0.720 |
|           | Total | 140 | 100 | 132 | 100 | |

Significant at P ≤ 0.05.

### Table 4 – The frequencies, percentages and results of Chi-square test for comparison between blood samples in the two groups.

| Organism | Group | P-values |
|----------|-------|----------|
|          | Preeclampsia N = 40 | Control N = 40 | |
| Aggregatibacter actinomycetemcomitans | 10 | 10.3 | 7 | 10 | 0.948 |
| Fusobacterium nucleatum | 16 | 16.5 | 13 | 18.6 | 0.727 |
| Campylobacter rectus | 14 | 14.4 | 5 | 7.1 | 0.143 |
| Eikenella corrodens | 9 | 9.3 | 7 | 10 | 0.876 |
| Porphyromonas gingivalis | 23 | 23.7 | 20 | 28.6 | 0.478 |
| Prevotella intermedia | 18 | 18.6 | 15 | 21.4 | 0.646 |
| Treponema denticola | 7 | 7.2 | 3 | 4.3 | 0.431 |
| Total | 97 | 100 | 70 | 100 | |

Significant at P ≤ 0.05.
Therefore, within the same scenario, it is possible that the placenta may be similarly burdened in pregnant women who develop preeclampsia. In our study, we tried to explore and clarify the evidence of periodontal and pericoronal pathogenic bacteria in human placental tissue for the first time in Egypt to provide further evidence on the association between oral Gram-negative chronic infection and the development of preeclampsia in a sample of pregnant Egyptian women. We have used the RamfJord teeth classification or half mouth examination procedure which is useful in providing maximum clinical information while conserving time and reducing patient and examiner fatigue [36,37].

In both groups we controlled for other variables like age, body mass index, race and gestational age. Here, there is a limitation in this study that should be acknowledged regarding the sample size of the study population, the sample size is relatively small when compared with other previous investigations concerning the adverse pregnancy outcomes, and actually we relied on the evidence of high prevalence of periodontitis and pericoronitis in the pregnant population.

In the present study, placental tissue samples obtained only from cesarean sections, also the dissection was carried out under sterile condition to reduce the possible vaginal and cervical contamination, even in the control group we tried to select the subject indicated for cesarean section due to

| Technique | Organism | Group | P-value |
|-----------|----------|-------|---------|
| Culture   | Aggregatibacter actinomycetemcomitans | N = 40 | N% N% |
|           | Fusobacterium nucleatum | 12 14.6 | 8 28.6 | 0.099 |
|           | Campylobacter rectus | 14 17.1 | 6 21.4 | 0.606 |
|           | Eikenella corrodens | 12 14.6 | 0 0 | 0.189 |
|           | Porphyromonas gingivalis | 14 17.1 | 6 21.4 | 0.606 |
|           | Prevotella intermedia | 8 9.8 | 0 0 | 0.086 |
|           | Treponema denticola | 8 9.8 | 0 0 | 0.086 |
| Total     | N = 82 | 100 N = 28 | 100 | |
| PCR       | Aggregatibacter actinomycetemcomitans | N = 40 | N% N% |
|           | Fusobacterium nucleatum | 14 14 | 11 29.7 | 0.034* |
|           | Campylobacter rectus | 14 14 | 8 21.6 | 0.281 |
|           | Eikenella corrodens | 14 14 | 0 0 | 0.016* |
|           | Porphyromonas gingivalis | 18 18 | 9 24.3 | 0.409 |
|           | Prevotella intermedia | 14 14 | 0 0 | 0.016* |
|           | Treponema denticola | 8 8 | 0 0 | 0.076 |
| Total     | N = 100 | 100 | 37 100 | |

Significant at P ≤ 0.05.

Fig. 1 – Mean TNF-α of GCF, saliva and serum in the two groups.

Fig. 2 – A quantitative Real time PCR curves to measure TNF-α gene expression through RQ method of Real Time-PCR. The fluorescence is plotted versus PCR cycle number for reaction and each sample is indicated.
prolonged pregnancy, previous cesarean section, suspected placental abruption and fetal distress. In addition, we tried to collect saliva beside GCF samples. Here it should be noted that current option for using saliva as a diagnostic marker has the following advantages: simple, safe and early detection of certain infections [38]. Suggesting that value of using saliva as a diagnostic marker, aided by current technological development will increase rapidly in the near future.

Several recent studies have provided provocative evidence for the role of oral infections in the initiation and/or progression of several important systemic conditions [9,39]. Also, the role of bacterial infections in pregnancy complications is well known, it has been shown that intrauterine infections were common among women who gave birth prematurely [40,41].

In this regard, it is worth noting that four possible mechanisms exist for microbes to spread to the uterus, which otherwise a sterile environment: 1) organisms from the vagina and the cervix ascend to the uterus, 2) organisms originate elsewhere in the body and infect placental tissues as a result of hematogenous spread, 3) organisms from the peritoneal cavity translocate retrogradely through the fallopian tube, and 4) organisms are inoculated accidentally in uterine tissues during invasive procedures, such as aminocentesis or chronic villous sampling [42].

Therefore, the possible putative link between chronic oral infection and pregnancy complications might be attributable to repeated exposures of the decidual tissues to periodontal or pericoronal pathogens through transient bacteremia. Previous studies in human showed that oral microorganism, including F. nucleatum and Capnocytophaga spuigena were detected in the amniotic fluid of women with intact membranes and in those with preterm labor [43],[45,46]. Moreover, Madians and Co-workers have assessed the umbilical cord serum for the presence of fetal immunoglobulin M (IgM) to oral pathogen Porphyromonas gingivalis, documenting a fetal humoral response to organisms distant from the intrauterine environment and suggesting that translocation of oral pathogens to the uteroplacental unit may occur [48]. These studies are supporting again the possibility that oral bacteria or bacterial products can spread through the blood stream to the placenta.

As regard to our work; the microbiological assessment of all samples revealed that the total number of different anaerobes of the blood samples was higher in preeclampsia group (97) than control group (70). Also, the total number of the same anaerobes was higher in the placental samples of preeclampsia group (72) than control (28) indicating the possibility of hematogenous spread of these organisms and repeated exposure of the placental tissues to it following transient bacteremia. Hence we recognized that several candidate pathogens were may be involved in the risk of development of preeclampsia instead of sole pathogen.

Thus, we assumed that oral pathogens gain access to the systemic circulation via local tissue inflammation and breakdown and might cause damage by affecting the placenta and possible the fetus itself. We have generally found that at least part of the association between pathogen burden and preeclampsia may be due to infection-induced inflammation.

A number of experimental studies have linked virulence factors of P. gingivalis to complications in pregnancy outcome. The cysteine proteinases produced by P. gingivalis, termed gingipains are considered to have potentially deleterious effects in activating coagulation factors and platelet aggregation and in altering the cytokine response in human umbilical vein endothelial cells. Fimbriae and Lipopolysaccharide are other important virulence factors of P. gingivalis that can activate peripheral blood monocytes resulting in the release of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α [49–52].

It is well known that the levels of systemic inflammation are regulated by cytokines such as TNF-α, which act as a key cytokine during inflammatory processes [53]. The results of our study were compatible with the results of many studies that showed that TNF-α contribute to pathologies such as periodontitis and periconitis, indicated by the high level of TNF-α in GCF and saliva of both groups [54,55]. We also found higher TNF-α concentration in the serum of patients with preeclampsia when compared to control with statistically significant difference by both ELIZA and PCR techniques.

The strong association of TNF-α with preeclampsia is well documented by many studies [53,56]. The increase in TNF-α level simply reflects the natural progression of the inflammatory cascade. Although studies have demonstrated evidence for an inflammatory response both in normal and preeclamptic pregnancies, in preeclampsia inflammation seems excessive [57,58].

Maternal infections may trigger release of pro-inflammatory cytokines into maternal circulation, which may further enhance the already heightened level of inflammation observed in women with preeclampsia, resulting in endothelial cell dysfunction and oxidative stress [59]. Similarly, in periodontal and pericoronal oral infection, monocyte-derived cytokines such as TNF-α and interleukins (IL-1, IL-6 and IL-8) may be released in response to oral infection with oral pathogens, resulting in major vascular response including an inflammatory cell infiltrate in the vessel walls, vascular smooth muscle proliferation, vascular fatty degeneration and intravascular coagulation which is manifested clinically by hypertension-related pregnancy disorders [60,61].
According to our present findings, this study has provided evidence supporting pathogens responsible in the etiology of periodontitis and pericoronitis could act as potentially risk factors in the development of preeclampsia. The determination of periodontal and peridental pathogens in placentas of women with preeclampsia may support a possible role of these microorganisms in the pathogenesis of preeclampsia, in which maternal oral infections exposure become a crucial phenomenon throughout pregnancy.

Also, the high level of TNF-α at sites affected by periodontitis and pericoronitis as well as the serum of preeclamptic patients might provide a potential pathogenetic mechanism and hence one therapeutic target, suggesting that this biomarkers could facilitate early prediction, screening and prevention of this condition. Obviously, there was a relationship between chronic oral infection and preeclampsia, so treatment of oral infection during pregnancy may represent a novel approach and preventive strategy that reduce oral bacterial load which would decrease the incidence of preeclampsia. Further studies are needed to focus on.

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