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Evaluation Of Salivary IL-1Beta And IL-6 Levels In Pregnant And Postpartum Women

Gebelik Ve Doğum Sonrası Tükürük IL-1Beta And IL-6 Seviyelerinin Değerlendirilmesi

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ÖZET

Amaç: Gebelikte ortaya çıkan fizyolojik değişiklikler lokal enflamatuvar medyatörleri etkileyebilir ve periodontal enflamasyonun şiddetini arttırabilir. Çalışmamızda aynı kadında gebelikte ve doğum sonrasında tükürük IL-1beta (interlökin-1beta) ve IL-6 (interlökin-6) düzeylerinin incelenmesi ve periodontal hastalık şiddeti ile ilişkisinin değerlendirilmesi amaçlanmıştır.
Yöntem: Toplam 96 gingivitis tanısı konan kadın çalışmaya dahil edilmiştir. Gebe kadınlar doğum sonrası 6. ayda tekrar değerlendirilmiş, gebe grubu ve doğum sonrası olmak üzere iki grup oluşturulmuştur. Tükürük IL-1beta ve IL-6 düzeyleri ELISA yöntemi ile belirlenmiştir. Parametreler arasında ki korelasyonlar Spearman's korelasyon analizi ile değerlendirilmiştir.
Bulgular: Tükürük IL-1beta düzeyleri doğum sonrası grubunda gebe gruba göre anlamlı olarak düşük bulunmuştur ($p=0.045$). Doğum sonrası grupta IL-6 seviyeleri gebe grubundan anlamlı olarak yüksek düzeydedir ($p<0.0001$). Gebe grubunda IL-1beta seviyesi plak indeksi, kanama indeksi ve sondalama derinliği ile anlamlı pozitif korelasyon göstermektedir. Doğum sonrası grupta IL-6 düzeyleri plak ve kanama skorları ile pozitif korelasyondadır.
Sonuç: Çalışmamızın bulgularına göre, lokal biyokimyasal parametreler gebelikten etkilenmektedir. Bu ilişkilerin anlaşılması diş hekimleri ve tıbbi uzmanlar açısından önem taşımaktadır.
Anahtar Kelimeler: Tükürük, periodontal hastalık, gebelik

ABSTRACT

Introduction: *Local inflammatory mediators could be affected from physiological changes in the pregnancy and exacerbate the periodontal inflammation. The aim of the present study was to evaluate pregnant and postpartum saliva levels of IL-1beta (interleukin1beta) and IL-6 (interleukin 6) levels in the same women and to investigate their association with periodontal disease severity.*
Methods: *A total number of 96 women that diagnosed as gingivitis were originally recruited for this longitudinal study, assigned to the following groups; pregnant and postpartum group: the pregnant group re-evaluated 6 months after giving birth. IL-1β[?]and IL-6 levels were detected by ELISA. Correlations between parameters were analyzed by Spearman's correlation test.*
Results: *Salivary IL-1beta levels were significantly lower in post-partum group than pregnant group ($p=0.045$). The post-partum group exhibited significantly higher IL-6 levels than pregnant group ($p<0.0001$). In the pregnant group, IL-1beta levels were positively correlated with plaque index, bleeding index and probing depths. In the postpartum group, IL-6 levels were correlated with plaque and bleeding levels.*
Conclusion: *According to our study, local levels of the biochemical data affected by pregnancy. The understanding of the mechanisms of these interactions are important for dental specialists and medical practitioners.*
Keywords: *Saliva, periodontal diseases, pregnancy*

INTRODUCTION

Periodontal diseases are a heterogeneous group of infectious/inflammatory diseases that may consequently result in tooth loss. A local elevation of pro-inflammatory mediators is caused in the periodontal tissues, which may lead to reversible inflammation (gingivitis) or to irreversible local tissue destruction (periodontitis).[1] This local inflammatory response is initiated by oral bacteria which is a distinct member of the microbial dental biofilm growing on the tooth surface.[2]

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Significant links have been elucidated between local inflammatory periodontal disease and systemic conditions, such as diabetes mellitus.[3] During pregnancy, the occurring physiological changes affect the maternal immune system. This may reflect on the clinical presentation of both systemic and local infections, such as periodontal diseases.[4] In this line, many studies supported a possible bi-directional relationship between periodontal disease and pregnancy with the link of mediators in immune system or antioxidants/reactive oxygen species.[4,5] The associations between adverse pregnancy outcomes and periodontal disease have also been supported by studies showing that preterm low birth weight (PLBW) are more common among patients with periodontal disease than individuals with periodontal health,[6,7] while others have failed to find such associations.[8,9]

The complex network of cytokines that intervene in the immune response of the host against external attacks include pro-inflammatory cytokines and anti-inflammatory cytokines plays important role in the pathogenesis of periodontal diseases.[10] Sex-steroid hormones have an effect on systemic and local inflammatory cytokines. Pregnancy may exacerbate gingival inflammation and local cytokine levels in saliva during pregnancy.

It is hypothesized that pro- and anti-inflammatory mediators could be affected from physiological changes in the pregnancy. The aim of the present study was to evaluate the salivary levels of IL-1β and IL-6 in pregnant and postpartum women and their associations with clinical parameters of periodontal inflammation and disease severity.

MATERIALS AND METHODS

Methods

Study Population

A total of 96 pregnant women (aged 19.0 to 40 years) were recruited for this study between March 2013 and March 2014, from the project of Municipality of Bornova, Dental Association and Ege University Medical School Department of Public Heatlth, Izmir, Turkey. General exclusion criteria were a) any known systemic disease and, b) periodontal treatment within the last six months, c) patients having less than 10 teeth, d) smokers, and e) individuals with BMI>30 kg/m^2. Further, additional exclusion criteria in the pregnant group gestational diabetes mellitus and preeclampsia. Pregnant women were re-evaluated 6 months after postpartum (n=96). The study was conducted in full accordance with the ethical principles of the World Medical Association Declaration of Helsinki. The study was approved by the Ethics Committee of Ege University (protocol number 13-3.3/8). The study protocol was explained to the participants, and written informed consent was obtained from each one of them prior to registering medical and dental histories, clinical periodontal examination and saliva sampling.

The study design included the following groups: 1) pregnant women (n=96) who were in their second trimester (weeks 16-24) or third trimester (weeks 25-34), 2) postpartum women (n=96) who were evaluated 6 months after delivery.

Saliva sampling

Whole saliva samples were obtained simply by expectorating into polypropylene tubes prior to clinical periodontal measurements or any periodontal intervention. This was performed during morning sessions, following an overnight fast during which subjects were requested not to drink (except water) or chew gum. The individuals were asked to rinse their mouth with tap water, before expectorating whole saliva into sterile 50 ml tubes for 5 min.

Clinical periodontal measurements

Subsequent to saliva sampling, clinical periodontal recordings, including dichotomous (+/-) plaque index (PI), pocket depth (PD), clinical attachment level (CAL) and dichomotous (+/-) presence of bleeding on probing (BOP) (occurring within 15 seconds after periodontal probing) were performed at 6 sites on each tooth present (except third molars) using a Williams periodontal probe (Hu Friedy, Chicago, IL, USA). CAL was assessed from the cement-enamel junction to the base of the probable pocket. All clinical measurements were performed by a single calibrated examiner (PG).

Biochemical analyses

Commercial enzyme-linked immunosorbent assay (ELISA) kits were purchased for the measurement of IL-1 and IL-6 (R&D Systems, Minneapolis, USA) and the analyses were performed according to the manufacturer's instructions.

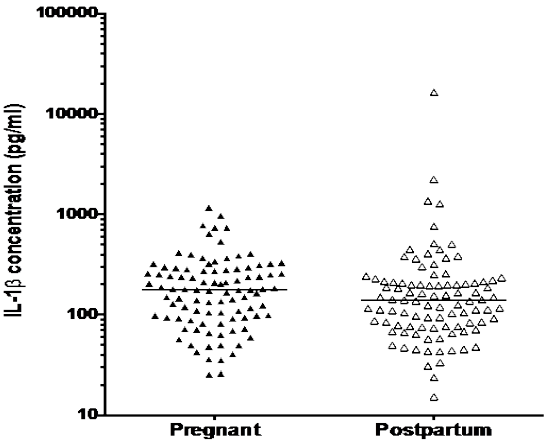

Figure 1: IL-1β levels in the study groups. Salivary IL-1β levels were significantly lower in post-partum group than pregnant group (*p=0.045*).

Statistical analysis

The distribution of the data was validated by D'Agostino-Pearson omnibus normality test and statistical analysis was performed using non-parametric

methods. Comparisons between the groups were made using the Wilcoxon test. Statistical analysis was conducted using the statistical software (GraphPad Prism version 6.00c for Mac OS X, GraphPad Software, La Jolla California USA), and statistical significance was considered at $p<0.05$. Correlations between parameters were analyzed by Spearman's correlation test.

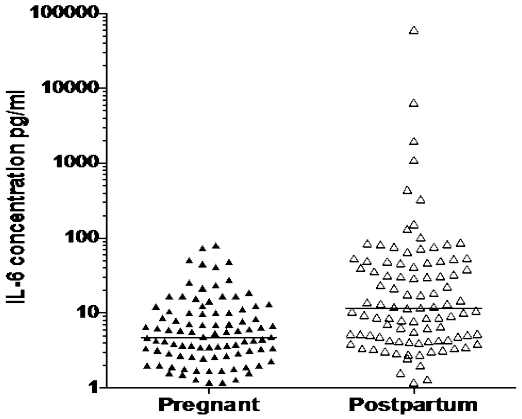

Figure 2: IL-6 levels in the study groups. Salivary IL-6 levels were significantly lower were significantly higher in post-partum group than pregnant group (*p<0.0001)*.

RESULTS

Clinical findings

Clinical periodontal measurements of the subjects among study groups are outlined in Table 1. PD, BOP, PI and CAL levels were similar between the two groups.

Biochemical findings

Salivary Il-1 β were significantly lower in post-partum group than pregnant group (p=0.045) (Figure 1). Regarding IL-6, the post-partum group exhibited significantly higher levels than pregnant group ($p<0.0001$) (Figure 2). The associations between biochemical and clinical periodontal parameters were evaluated in the study groups (Table 2). In the pregnant group, IL-1β levels were positively correlated with plaque index, bleeding index and probing depths. In the post-partum group, IL-6 levels were correlated with plaque and bleeding levels.

DISCUSSION

Physiological changes induced during pregnancy may alter the inflammatory response, amplifying the gingival inflammation.[4] Recent studies demonstrated that pregnancy gingivitis affects 36–100% of pregnant women and clinical parameters may increase during pregnancy, without concomitant increase in plaque index, which decreases after delivery.[11] Increased vascular permeability, depression of the immune system, and shifts on the composition of supra and subgingival microbiota act important role in the mechanisms underlying the increased inflammation in pregnant women.[11]

The present longitudinal study comparatively evaluated the saliva levels of IL-1β and IL-6 in pregnant women and in the same women after delivery. Immune response of the host against to the microbial dental plaque and products includes pro-inflammatory cytokines and anti-inflammatory cytokines. Cytokines play important role in the pathogenesis of periodontal diseases. Increased local inflammatory response in periodontal disease cause an exacerbation of the systemic inflammation in other systems of the body.[12,13] On the other hand, in pregnancy systemic inflammation of the body could exacerbate the local inflammation in oral tissues.[14,15] In this line, studies supported a possible bi-directional relationship between periodontal disease and pregnancy.[4] According to our results, we found that IL-1β levels were significantly lower; IL-6 levels significantly higher in the postpartum group. Sex-steroid hormones may have an impact on cytokines during pregnancy. They are able to modulate the activities of all the cells involved in the immune inflammatory response depending on their concentration.[16,17] Studies suggested that progesterone and estrogen have both immune-stimulatory and immune-suppressive effects depending on the concentration.[18]

GROUPS	Pregnant N: 96	Post-partum N: 96
	Median(IQR)	**Median(IQR)**
PD (mm)	2.0 (1.0)	2.0 (1.0)
CAL (mm)	2.0 (1.0)	2.0 (1.0)
BOP (%)	50.0 (42.5)	50.0 (40.0)
PI (%)	50.0 (50.0)	50.0 (40.0)
Baby Weight	-	3655 (636.25)

PD = Probing pocket depth (mm), **PI =** Plaque Index (%),
BOP = Bleeding on probing (%)
IQR = inter quartile range

Table 1. Clinical periodontal measurements of the women in the pregnancy and after birth.

Estrogen is particularly known to inhibit expression of IL-6, which are key mediators of the macrophages and several other cell types.[19,20] Sex-steroid hormones at physiological concentrations had an inhibitory effect on the secretion of pro-inflammatory cytokines, including IL-1β and IL-6.

These studies suggested an anti-inflammatory effect of sex-steroid hormones at high levels in vitro.[21,22] According to our results, we can speculate that hormonal changes during pregnancy have an impact on local biochemical parameters in saliva. The alterations of the

Pregnant Group		PD	BOP%	PI
Il-1β	r	0.383	0.440	0.448
	p	**<0.001**	**<0.001**	**<0.001**
Il-6	r	0.082	0.100	0.120
	p	0.424	0.330	0.242
Post-partum Group		**PD**	**BOP%**	**PI**
Il-1β	r	0.042	0.034	0.074
	p	0.682	0.736	0.471
Il-6	r	0.160	0.229	0.216
	p	0.117	**0.024**	**0.034**

Table 2. Correlation analysis between clinical periodontal parameters and salivary biomarkers in the pregnant and post-partum groups.

balance between anti- and pro-inflammatory markers could be the mechanism of bidirectional relationship in pregnancy and periodontal inflammation.

Figuero's et al.[23] found that the IL-1β levels in GCF showed no significant changes during pregnancy, though their concentrations were higher than those found in non-pregnant women. However, exacerbated gingival inflammation during pregnancy could not be associated with changes in GCF levels IL-1β. Bieri et al.[24] also found no significant differences in the expression of IL-1α, IL-1β and TNF-α in GCF between week 12 and postpartum. Otenio et al.[25] found no differences in the expression levels of IL-1β and IL-6 in pregnant women with and without periodontal disease. In our study, IL-1β levels in saliva were positively correlated with plaque index, bleeding index and probing depths in the pregnant group. After delivery, IL-6 levels were positively correlated with plaque and bleeding levels.

The levels of sex-steroid hormones in saliva are required to evaluate to better understand the relation between the clinical parameters and saliva sex-steroid hormone levels in saliva. This issue could be the limitation of our study.

CONCLUSION

It is clear that there are relationships between periodontal disease and various systemic conditions via the local and systemic interactions of the immune system. The understanding of the mechanisms of these interactions are important for dental specialists and medical practitioners which they have a greater responsibility for the overall health and well-being of their patients, including periodontal care. According to our study, local levels of the biochemical data affected by pregnancy, which may disrupt the balance of the periodontal tissue environment.

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