ABSTRACT

Systemic diseases and hormonal changes are risk factors for periodontal diseases. In diabetes mellitus patients periodontal destruction is more severe than systemic healthy patients. The increase of hormones during pregnancy causes more gingival inflammation and gestational gingivitis. In recent studies it was hypothesized that the increase of the level of progesteron in circulation stimulates the release of prostaglandin E$_2$ (PGE$_2$) which causes to gestational gingivitis. The aim of our study is to determine the level of PGE$_2$ in gingival crevicular fluid (GCF) of the pregnant individuals with type II diabetes mellitus and periodontitis. In addition it is aimed to investigate the probable correlations between GCF PGE$_2$ level and the clinical parameters and periodontal disease severity. A total of 40 pregnant individual in 24-32 weeks with periodontitis (20 pregnant individual with type II diabetes mellitus, 20 pregnant individual systemically healthy) were examined. To determine all the individuals periodontal status pocket depth (PD), plaque index (PI), gingival index (GI) and gingival bleeding index (GBI) scores were recorded. The volume of the GCF was also measured from the sampling site in addition to the other measurements. GCF PGE$_2$ level was determined by radioimmunoassy (RIA) method. The GCF PGE$_2$ level was determined as 38.27±26.08 pg/ml in type II diabetic pregnant group and 39.13±23.19 pg/ml in systemic healthy pregnant group. There was no important difference of GCF PGE$_2$ level among the groups (p>0.05). When the probable correlations between clinical parameters and GCF PGE$_2$ levels were investigated there was found no correlation in healthy pregnant group, but there was correlation determined in type II diabetic pregnant group. When the full mouth clinical parameters were compared there was important differences determined in the case of GI and GBI among the groups (p<0.01), but there was no important differences determined in the case of PD and PI (p>0.05). This study results establishes that there is no difference between type II diabetes mellitus pregnant patients with periodontitis and systemic healthy pregnant patients with periodontitis in the cases of GCF PGE$_2$ level and periodontal disease severity. Pays attention that GCF PGE$_2$ level can be used as a marker for determining the periodontal disease severity among type II diabetes mellitus pregnant patients as systemic healthy pregnant patients.

Introduction

In the gestational period sexual hormones reaches to their maximum level. As hormonal changes, metabolic changes can also effect oral metabolism and cause important changes in periodontium (1). Because of
this as if the period is temporary pregnant patients are accepted as high risk periodontal complication group (2, 3). It is reported that during pregnancy periodontal pocket depth, increase of bleeding during probing and brushing and GCF volume (2, 4, 5). Robinson and Amar reported that pregnancy causes 4 pathologic exists. These are: gestational gingivitis, gestational granuloma, periodontitis and caries (6).

Löe described the development of gestational gingivitis as; typically occurs in the second month, the incidence and severity increases until eighth month, heals a little in the last month (7). The prevalence and severity of gestational gingivitis is well investigated, occurs in 30-100% of all the pregnants. The difference of the prevalence score shows the heterogeneity of the diagnosis criterias (8, 9).

The increased level of progesterone in circulation, as a result gingival capillary dilatation, increase in capillary permeability, exudation like gingival microvascular effects may cause gestational gingivitis (10, 11). Progesterone can directly effect the endothelial cells and prostoglandines (12, 13). PGE\(_2\) which is a big arachidonic acid metabolite is released locally has many proinflammatory effects like vasodilatation, increase of vascular permeability in the inflammation site, collagenase release from inflammatory cells, activation of osteoclasts and increase of bone destruction (14, 15). It is believed that the main source of PGE\(_2\) in GCF is macrophages and PGE\(_2\) is a key proinflammatory mediator in periodontal diseases (16, 17, 18). During gestational period the increase of progesterone stimulates the release of prostoglandines and probably increases the gingival inflammation (12, 13, 18). Yalçın et al. (19) suggested that GCF PGE\(_2\) levels can be used as a marker for periodontitis existing during gestational period and the results of periodontal treatment.

Offenbacher et al. (18, 20) established that there is a correlation between periodontal disease during pregnancy and premature low birth weight (PLBW). The risk of PLBW increased 7.5 times more in the mothers whose attachment loss were more than 3 mm and the ratio with effected teeth as 60%. Periodontal infections effects on birth weight are more than alcohol and tabacco habits.

Diabetes mellitus is another important etiologic factor which reduces the reaction threshold and increases increases the inflammatory responce. Many investigations suggest that there is a correlation between diabetes and periodontitis (21), and periodontitis is defined as the 6. complication of diabetes mellitus (22).

By a long term study among Pima Indians, it was pointed out that the severity of periodontal disease in type II diabetes mellitus patients were much more than non diabetics (23, 24, 25).

Pregnant diabetics are accepted as high risk obstetrical patients. The increase of severity of periodontitis among diabetes the first attention is paid for presence of infection and and its effects on maternal and fetal structures. Diabetic pregnant patients are more exposed to bacterial infections and this may make more difficult to control of glucose (26). Guthmiller et al. compared the type I dibetic pregnant with non diabetic pregnanats and established that there was more gingival inflammation, more periodontal destruction, more attachment loss and plaque scores in type I diabetic pregnant (27).

We could not find any literature about type II diabetic pregnancies periodontal disease severity, GCF PGE\(_2\) level and its probable correlation with clinical parameters. This studies aim is to determine the GCF PGE\(_2\) level and in pregant type II dibetets mellitus patients. In addition it is aimed to investigate the probable correlation between GCF PGE\(_2\) level and clinical parameters and the severity of the periodontal disease.
Materials and Methods

Study Population
20 individual clinically diagnosed as type II diabetes mellitus with periodontitis (mean age 34.85±2.621) and 20 systemic healthy with periodontitis (mean age 30.55±3.082) a total of 40 women who applied to Dicle University Medicine Faculty Gynecology and Gestation Department were included to our study. By selecting the patients care was taken for the presence of periodontitis, not to have any systemic problem except diabetes mellitus type II, to have minimum ≥5mm PD and minimum 15 teeth, not to have an antibiotic, antiinflammatoar and peridontal therapy in last 6 months.

Any periodontal treatment was not performed to sampling site not to effect the present periodontal status. Also any order was not given to change their oral care. All individuals were informed about about the study and their approval were got.

Clinical Evaluation And Periodontal Examination
Clinical evaluation and GCF sampling procedures were made by one experienced clinician. To determine the patients periodontal status full mouth and sampling site plaque index (28) (PI), gingival index (2) (GI), pocket depth (PD), gingival bleeding index (29) (GBI) scores were determined by using a Williams probe and recorded. All measurements were performed on all teeth at 6 side which are distobuccal, buccal, mesiobuccal, disto lingual, lingual and mesiolingual sides and expressed and recorded as milimeter.

Getting Gingival Crevicular Fluid (GCF)
GCF samples were got with specifically manufactured paper ribbons (periopaper®)by the method of Rudin et al. (30). To prevent the contamination of the sampling material with saliva of upper jaws anterior teeth, the method is limited with vestibular sides. Care was taken into account to the pocket depth (PD) to be ≥5 mm.

The sampling method was performed always in morning among all individuals. Before sampling method was performed, the site was isolated with cotton rolls, plaques were eliminated and the teeth surface were dried with gentle air spray. The peripapers were placed in the entrance of sulcus by the help of o pressel. After waiting 30 minutes the peripapers were weightened on a sensitive scale and placed into eppendorph tubes. The tubes were kept at -20C untill analysis method was performed. All individuals sampling sites were recorded. Care was taken not to cause bleeding during sampling method, but if happens the samples were not included to the study. The GCF samples were taken at first not to effect the fluid flow and volume. Just before the analysis method was performed 1000ml sterile NaCl (9mg/ml) was added in the tubes and GCF was seperated at 3000g and +5C for 20min.

Laboratory Procedures
The analysis method of PGE$_2$ in GCF was performed in Dicle University Nuclear Medicine Department. The PGE$_2$ activity was determined by using commercial PROSTOGLANDIN E$_2$ [I$_{125}$] RIA KIT*. In the analysis method the guide of the kit was followed.

Statistical Analysis
‘Student t test’ was used to compare the groups. To compare full mouth clinical scores and sampling site clinical scores ‘Two pairs difference significany test’ was used. To compare the GCF volume, GCF PGE$_2$ levels and its probable relations with clinical parameters ‘simple correlation analyse’ was used. Statistically significany was taken from tyhe p<0.05 point.

Results and Discussion
The diabetic and systemic healthy pregnant full mouth and sampling site clinical measurements mean and standart deviation scores are shown in Table 1 in the case of full mouth measurements, the difference between GI and GBI found significant (p<0.01), while the other parameter scores were not differnt significantly (p>0.05).
TABLE 1

Data about full mouth and sampling site clinical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic pregnant</th>
<th>Sythemic healthy pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full mouth</td>
<td>Sampling site</td>
</tr>
<tr>
<td>PI</td>
<td>2.38±0.433</td>
<td>2.19±0.560</td>
</tr>
<tr>
<td>GI</td>
<td>1.39±0.316</td>
<td>1.74±0.313</td>
</tr>
<tr>
<td>GBI (%)</td>
<td>72.04±24.22</td>
<td>87.49±20.85</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>2.47±0.362</td>
<td>3.95±0.415</td>
</tr>
<tr>
<td>GCF (mg)</td>
<td>3.23±1.151</td>
<td>3.05±1.208</td>
</tr>
<tr>
<td>PGE2 (pg/ml)</td>
<td>38.27±26.08</td>
<td>39.13±23.19</td>
</tr>
</tbody>
</table>

Mean±Standard Deviation

TABLE 2

The results of ‘Simple Correlation Analyse’ in which the sampling site clinical parameters were compared

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic pregnant</th>
<th>Sythemic healthy pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>PI-GI</td>
<td>0.484</td>
<td>0.031*</td>
</tr>
<tr>
<td>PI-GBI</td>
<td>0.401</td>
<td>0.080</td>
</tr>
<tr>
<td>PI-PD</td>
<td>0.170</td>
<td>0.476</td>
</tr>
<tr>
<td>PI-GCF</td>
<td>-0.020</td>
<td>0.933</td>
</tr>
<tr>
<td>GI-GBI</td>
<td>0.694</td>
<td>0.001**</td>
</tr>
<tr>
<td>GI-PD</td>
<td>-0.106</td>
<td>0.111</td>
</tr>
<tr>
<td>GI-GCF</td>
<td>0.367</td>
<td>0.657</td>
</tr>
<tr>
<td>GBI-PD</td>
<td>-0.644</td>
<td>0.002**</td>
</tr>
<tr>
<td>GBI-GCF</td>
<td>0.160</td>
<td>0.499</td>
</tr>
<tr>
<td>PD-GCF</td>
<td>-0.005</td>
<td>0.982</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01

In the sampling site case, there was no significant difference between all the parameters among the groups (p>0.05). The GCF volume in the diabetic group was slightly more but it was not statistically important (p>0.05). In the GCF PGE2 case ‘Student t Test’ was used to compare the groups and it was determined that the difference between the groups was not statistically important (Table 1).

In Table 2 ‘Simple Correlation Analyse’ results are presented in which sampling site clinical parameters correlations were investigated. In the diabetic pregnant group there was determined an important correlation between PI and GI (p<0.05) while there was more important correlation between GI- GBI and GBI PD (p<0.01). In the sythemic healthy group there was determined important correlations between PI-GI and GI-GBI (p<0.01).

GCF PGE2 level and its correlations with clinical parameters of sampling site are shown in Table 3 in the diabetic pregnant group it was determined that there was an important correlation between PGE2 level and GCF volume (p<0.05), while there were no important correlations between PGE2 level and sampling site clinical parameters (p>0.05). Also in the group of sythemic healthy pregants there were no correlations between PGE2 level and sampling site clinical parameters (p>0.05).

Periodontal diseases have the potential to
TABLE 3

The results of ‘Simple Correlation Analyse’ in which the correlations between GCF PGE$_2$ level and the sampling site clinical parameters were investigated

<table>
<thead>
<tr>
<th></th>
<th>Diabetic pregnant</th>
<th>Sytheic healthy pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>PI- PGE$_2$</td>
<td>0.157</td>
<td>0.510</td>
</tr>
<tr>
<td>GI- PGE$_2$</td>
<td>0.121</td>
<td>0.613</td>
</tr>
<tr>
<td>GBI- PGE$_2$</td>
<td>-0.039</td>
<td>0.869</td>
</tr>
<tr>
<td>PD- PGE$_2$</td>
<td>-0.010</td>
<td>0.968</td>
</tr>
<tr>
<td>GCF- PGE$_2$</td>
<td>0.459</td>
<td>0.042*</td>
</tr>
</tbody>
</table>

* P<0.05

effect the results of gestation as gram (-) infection. Intraoral manipulations even tooth brushing may cause gram (-) bacteriamies and these bacteriamies mostly ob-served in individuals with gingival inflammation and bacterial plaques (31). Even their period is temporary pregnant patients are accepted as high risk group of patients (2, 3). Metabolic changes like hormonal changes may effecy the oral metabolism (11). It is established that PD, bleeding by probing or tooth brushing and GCF level increases are observed during pregnancy (2, 4, 5).

The effect of progesterone on capiilaries during pregnancy period may be as a result of poor oral hygiene (10, 11). This effect is PGE$_2$ release by the stimulation of progeste-ron (12, 13). Damare et al. compared the PGE$_2$ and IL-1B levels of GCF and amniotic fluid of the pregnant women in second tri-mester in their study and suggested that there is an important correlation between them (12).

Even the periodontal diseases development is different among sytheic healthy and diabetic individuals during gestational period; in both of the groups bacterial sourced mediators play role. Grossi and Genco suggested that these two diseases are related closely. According to their hypothosis; metabolic processes supports each other in this case and increase the destruction. This self destruction process may make the diabetic control more difficult and gestational results of diabetics. Because of this as a part of periodontal treatment to reduce the bacterial presensation may reduce the cytokin release and insulin resistance. In this way both the diabetic control and periodontal health may effected positively (32).

There are many studies which suggest that type II diabetes mellitus is a risk factor for severe periodontal diseases and is an important ethiologic factor which exaggerates the inflammatory response in periodontal diseases by reducing the reaction threshold in gestational period (1, 6, 8, 9, 22, 23, 33, 34, 35, 36, 37). But there is no literature about the GCF PGE$_2$ level in the pregnant type II diabetes mellitus patients with periodontitis. By the light of these data in this study we aimed to determine the GCF PGE$_2$ level of pregnant type II diabetes mellitus patients with periodontitis, to investigate its possible correlations with the clinical parameters and to establish the probable differences with the sytheic healthy pregnants.

Our study was performed on two groups, first the pregnant type II diabetes mellitus patients with periodontitis and second sytheic healthy pregnant with periodontitis as control group. In both of the groups sampling site mean PD and GBI scores were determined higher than mean full mouth CD and GBI scores. This can be
explained as we chose the sampling sites from the most destructed sites.

When the the correlations between clinical parameters were examined; in the diabetic group it was determined that there was positive correlation between GI-GBI and GBI-PD. Also in the healthy group positive correlation was determined between PI-GI and GI-GBI. Bleeding in gingiva after stimulation is accepted as the sign of gingival inflammation (38). Because of the bacterial plaque is main etiologic factor of gingival inflammation, the positive correlation between PI and GI in our study is an expected result.

When the clinical parameters were evaluated among the groups; it was determined that the difference of full mouth GI and GBI scores were important. But there was no important difference in the full mouth and sampling site clinical parameters. But there were important factors which limited this study. Variations like the small amount of the study population, tabacco habit, amount of calculus, level of glycemic control, length diabetes period, dental care and socio-economic status were not included to the study procedure. Multivariational and more studies should be performed on wide populations and their repeates should be investigated. The GCF volume in the diabetic group was higher than systemic healthy even this difference was not statistically significant. As it is known, one of the most important complication of diabetes is its effects on vascular system. In addition to the presentation of arteriosclerosis in wide wessels, the effects of diabetes is mostly seen in small arteries, arteriols, capillaries and venules. Endothelial proliferation, PAS positive accumulation and basal membrane thickening is seen of all the capillaries in whole of the body (microangiopathy) (39). The predisposition of diabetics to periodontitis especially to early onset form can be explained by the vascular permeability impairment (40). The higher volume of GCF in the diabetic group of our study can be explained by the capillary permeability impairment.

In our study we did not detect any difference in the case of GCF PGE2 level among the groups. Because of we could not find any literature about the level of GCF PGE2 of type II diabetus individuals with periodontitis we could not compare our results.

In addition, we investigated the probable correlation between GCF PGE2 level and clinical parameters. We did not determine any statistical correlation between GCF PGE2 level and the clinical parameters as PD, PI, GI and GBI. This result is adjusted with the study of Yalçın et al. (19).

As a summary, we did not detect any difference of periodontal disease scores and GCF PGE2 levels among systemic healthy and diabetic pregnant. This study can not compare the PGE2 level as a proinflammatory mediator and the activity of periodontal disease. In this study which we compared the two groups can not suggest any relation between PGE2 as a proinflammatory mediator and activity of periodontal disease. But our results can be tought as a different parameter which reflects the clinical periodontal status by GCF PGE2 level. Nowadays it is known that gestation and diabetes are important risk factors for periodontitis. Pregnants especially diabetic individuals should have their periodontal treatment performed before gestation. The cooperation of gestation physician and the periodontist by this way to educate the patients about preventive oral care, to prevent the dental problems which could not be detected by the patient, thge control of periodontal infection before and while gestation and diabetic control may minimise worse fetal results. In future periodontal evaluations may be added to diabetic pregants prenatal controls like ophthalic evaluations. By this way, oftenly asympthomatic periodontal diseases which is seen among diabetic individuals can be diagnosed in an easy way.
Our opinion is; long term multivariational studies on wide pregnant type II diabetes mellitus populations would be advantageous.

REFERENCES