Salivary Protein Carbonyl Level in Relation to Gingival Health Status among a Group of Iraqi Pregnant Women

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ABSTRACT
Background: There is an increased oxidative stress during pregnancy that might affect their gingival health. This study was conducted to measure salivary protein carbonyl level of pregnant women and its relation with gingival health also compared the data obtained with those from healthy married non-pregnant women. Material and Methods: Thirty pregnant women during third trimester (i.e. study group) and thirty married non-pregnant women (i.e. control group) were selected from the Primary Health Care Centers in Baghdad city. The age range of both groups was 25-30 years. Stimulated saliva was collected and salivary protein carbonyl was analyzed using protein carbonyl assay kit. Plaque index (PII) was used for measuring dental plaque thickness while gingival index (GI) was used for diagnosis of gingival disease. Results: The mean values of salivary protein carbonyl, plaque and gingival indices were highly significantly higher among pregnant than non-pregnant women (P˂0.01). Person's correlation coefficient showed positive strong highly significant correlation (P˂0.01) between plaque accumulation and gingivitis among pregnant and non-pregnant women. While non-significant correlations (P>0.05) were recorded between salivary protein carbonyl levels with both gingival and dental plaque indices among both pregnant and non-pregnant women. Conclusion: Although dental plaque is the main etiological factor in pregnancy gingivitis, protein oxidation represented by salivary protein carbonyl level could play a role in gingival inflammation during pregnancy. Therefore examining the proteomic profile along with protein oxidation is recommended that helps in early detection and monitoring of periodontal infection during pregnancy.

Key words: pregnancy, oxidative stress, protein carbonyl, gingival health status


INTRODUCTION
Pregnancy is defined as a dynamic physiological process that affects profoundly even healthy woman. Many physiological and hormonal changes occur in pregnancy, to adapt requirements of the fetal homeostasis and growth. These physiologic changes may result in noticeable alterations in the oral cavity that may include pregnancy gingivitis, periodontitis, dental caries, pyogenic granuloma, dental mobility and erosion. There are several studies that recorded more severe periodontal diseases among pregnant women than control groups. The clinical manifestations of plaque-induced gingival inflammation are modulated by hormonal imbalances that occur during pregnancy. Normal pregnancy is associated with higher metabolic demand and elevated requirements for tissue oxygen that results in increased production of reactive oxygen species and increased oxidative stress. In fact in periodontal disease the local inflammatory response is initiated by oral bacteria in a form of biofilms growing on the tooth surface. In addition the increased production of oxidants and/or a decrease in protective antioxidants, namely oxidative stress, is postulated as one of the mechanisms responsible for periodontal tissue breakdown, since oxidative stress-mediated inflammatory pathway plays a role in periodontal disease pathogenesis.

Oxidative stress can cause periodontal tissue damage by a variety of mechanisms, including peroxidation of lipid membranes, massive protein oxidation and degradation, as well as stimulation of cytokine production. The observation of damaging actions of oxidized proteins accumulations in several pathological states such as neurodegenerative diseases, diabetes, atherosclerosis and ageing, highly increased the research in this field in the last decades of the 20th century. Furthermore, it had been recorded that individuals with periodontal diseases exhibit higher salivary protein carbonyl (PC) (i.e. biomarker of protein oxidation) levels than controls.
Also gingival crevicular fluid (GCF) PC levels were significantly higher in chronic periodontitis (CP) group than controls. Only one study could be found that considered the first study which evaluated oxidative stress biomarkers, in saliva of pregnant women, represented by thiobarbituric acid-reactive substances (TBARS), glutathione peroxidase (GPx) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), that were significantly elevated during pregnancy compared to controls. However, no studies could be found that measured salivary PC during pregnancy and their relation with periodontal diseases.

Thus, in view of the aforementioned findings and to address this gap this clinico-biochemical study was carried out to measure the PC levels in saliva of pregnant women in the third trimester and its relation with the clinical index of gingival health status also compared the data obtained with those from healthy married non-pregnant women.

MATERIALS AND METHODS

The sample:
In this comparative study, the study group included thirty pregnant women during third trimester with an age range of 25-30 years. In addition to thirty married non-pregnant women as control group that should be matched with age. All participants should be healthy without any systemic diseases, no smoking and not taking any medications or nutritional supplements. They were attending the Primary Health Care Centers in Baghdad city in AL-Russafa sector. An approval was achieved from the Ministry of Health for examining these women. The study protocol was explained to the participants and oral consent was obtained from them.

Collection of saliva and recording of dental plaque and gingival health:
Stimulated saliva was collected from pregnant and married non-pregnant women according to the instruction cited by Tenovuo and Lagerlöf. Each woman was seated in relaxed position without any heavy physical stress and was asked to chew a small piece of Arabic gum then to remove all saliva by expectoration and saliva collected in sterile screw capped bottle. Each salivary sample was then centrifuged by centrifugator at 3000 r.p.m. (revolution per minute) for 10 minutes. Salivary supernatant was stored at (-20°C) in polyethylene tubes for subsequent chemical analysis. Assessment of gingival health status was recorded according to Löe and Silness index. Dental plaque thickness was assessed and diagnosed according to Silness and Löe index. Clinical examination of gingival health status and dental plaque was conducted by using plain mouth mirror and periodontal probe.

Biochemical analysis of salivary sample and data:
Bio-chemical laboratory works were done in Poisoning Consultation Center at Gazi Al-Hariry hospital. Salivary protein carbonyl level was analyzed using protein carbonyl assay kit (SazaKits, India). The principal reaction of this kit is that, the content of protein carbonyl increased after oxidation, and the carbonyl group reacted with 2, 4-dinitrophenylhydrazine to form a reddish brown precipitate. The absorbance can be measured at 370 nm after the precipitation is dissolved. The carbonyl content can be calculated indirectly. Data were analyzed by using SPSS software version 19 (Statistical Package for Social Sciences) by application of both descriptive statistics including mean, standard deviation and inferential statistics including Student's t-test and Person's correlation coefficient. The confidence limit was accepted at 95% (P <0.05).

RESULTS
Total number of sample was 60 women, 30 of them were pregnant women in third trimester and the other 30 were married non-pregnant women. Normality distribution of sample was tested by application of Shapiro-Wilk test. Results showed that the mean values of dental plaque index, gingival index and protein carbonyl level in saliva were normally distributed among pregnant and non-pregnant women as shown in Table 1.

Results recorded in Table 2 and Figure 1 showed that the mean value of dental plaque was higher among pregnant (1.252±0.208) than non-pregnant women (0.882±0.356). So as the mean value of gingival index was higher among pregnant (1.305±0.225) than non-pregnant women (0.990±0.338). After application of Student's t-test the differences in the mean value of dental plaque and gingival indices were highly significant (P<0.01) between pregnant and non-pregnant women.

The mean value of salivary protein carbonyl was higher among pregnant (1.343±0.037) than non-pregnant women (1.125±0.035). By application of Student's t-test result showed that the difference in the mean value of protein carbonyl was highly significant (P<0.01) between pregnant and non-pregnant women as shown in Table 3 and Figure 1.

Person's correlation coefficient was applied and the results showed that there are strong positive highly significant correlations between plaque accumulation and gingival health status among pregnant and non-pregnant women that was stronger among non-pregnant than pregnant women. While non-significant correlations (P>0.05) were recorded between salivary protein carbonyl level with both plaque and gingival indices in both pregnant and non-pregnant women as shown in Table 4.
Table (1): Distribution of the samples regarding plaque index, gingival index and salivary protein carbonyl according to normality test.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Shapiro-Wilk</th>
<th>C.S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Statistic</td>
<td>df</td>
</tr>
<tr>
<td>PlI</td>
<td>Pregnant</td>
<td>0.937</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Non-pregnant</td>
<td>0.938</td>
<td>30</td>
</tr>
<tr>
<td>GI</td>
<td>Pregnant</td>
<td>0.940</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Non-pregnant</td>
<td>0.959</td>
<td>30</td>
</tr>
<tr>
<td>PC</td>
<td>Pregnant</td>
<td>0.942</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Non-pregnant</td>
<td>0.933</td>
<td>30</td>
</tr>
</tbody>
</table>

PlI=plaque index, GI= gingival index, PC=protein carbonyl, NS=non-significant.

Table (2): Mean values of plaque and gingival indices for pregnant and non-pregnant groups and the statistical differences between them.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant</td>
<td>Non-pregnant</td>
<td></td>
</tr>
<tr>
<td>PlI</td>
<td>Mean</td>
<td>±SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>1.252</td>
<td>0.208</td>
<td>0.882</td>
</tr>
<tr>
<td>GI</td>
<td>Mean</td>
<td>±SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>1.305</td>
<td>0.225</td>
<td>0.990</td>
</tr>
</tbody>
</table>

Df=58, SD= standard deviation, HS=highly significant.

Table (3): Salivary protein carbonyl level (M/L) for pregnant and non-pregnant groups and the statistical differences between them.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant</td>
<td>Non-pregnant</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean M/L</th>
<th>±SD</th>
<th>Mean M/L</th>
<th>±SD</th>
<th>t-Value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>1.343</td>
<td>0.037</td>
<td>1.125</td>
<td>0.035</td>
<td>23.609</td>
<td>0.000&lt;sup&gt;HS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Df = 58, M/L = mole per letter

Table (4): Correlations among plaque index, gingival index and salivary protein carbonyl for pregnant and non-pregnant groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GI</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P-value</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLI</td>
<td>0.502</td>
<td>.005&lt;sup&gt;HS&lt;/sup&gt;</td>
</tr>
<tr>
<td>GI</td>
<td>^</td>
<td>^</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLI</td>
<td>0.955</td>
<td>.000&lt;sup&gt;HS&lt;/sup&gt;</td>
</tr>
<tr>
<td>GI</td>
<td>^</td>
<td>^</td>
</tr>
</tbody>
</table>

HS = highly significant
DISCUSSION

Increased reactive oxygen species (ROS) production beyond the mother’s antioxidant potential leads to oxidative stress, which can affect both the maternal and fetal health \(^{(42,43)}\). It had been found that oxidative stress is present during all three trimesters of pregnancy and it is necessary to obtain normal cell function. However, it could give rise to different disease-states \(^{(44,45)}\). The cause of increased maternal oxidative stress during pregnancy is unknown but probably the placenta plays an important role in the production of oxidative stress \(^{(46,47)}\).

In the current study pregnant women in the third trimester only were selected as study group since during this trimester the female sex hormones, namely estrogen and progesterone, reach their highest level \(^{(48,49)}\). The same thing for oxidative stress as it was found that serum oxidative stress biomarker was higher in the third trimester \(^{(50)}\) also salivary oxidative stress biomarker reached their highest value in the third trimester \(^{(17)}\). Therefore studying gingival inflammation during this period will be more obvious.

Periodontal health in pregnant women had become a field of research since the 1960s, resulting in a flurry of studies to focus on it \(^{(8-19)}\). Similarly in the current study gingival inflammation was more severe among pregnant than non-pregnant women with highly significant difference although only third trimester pregnant women were recruited in the current study as mentioned previously. Higher gingival inflammation associated with pregnancy is initiated by dental plaque and exacerbated by endogenous steroid hormones \(^{(51)}\). Accumulation of dental plaque and
the hormonal changes during pregnancy affected the immune response and exaggerating gingival and periodontal inflammations (24, 25).

This is supported by the current study findings as dental plaque accumulations was highly significantly higher among pregnant than non-pregnant women in addition dental plaque revealed positive strong highly significant correlation with gingival index among pregnant women confirming the etiological role of dental plaque in gingival inflammation pathogenesis. However, the correlation of plaque accumulations with gingival inflammation was stronger among non-pregnant than pregnant women probably because dental plaque is the only causative agent for gingival inflammation among non-pregnant while during pregnancy there are hormonal changes (51).

Higher plaque accumulation during pregnancy, as found in the present study, might be related to oral hygiene negligence by pregnant women especially in the third trimester. Furthermore, during third trimester pregnant women may become anxious, restless and exhausted. It is important to mention that only two studies could be found that recorded non-significant differences between pregnant and non-pregnant women regarding plaque and gingival health status (52, 53). Few studies could be found that elucidated elevated salivary and GCF protein carbonyl level with increased gingival inflammation and periodontal tissues destruction in comparison to control groups (32-36).

Similarly in the current study PCs levels were highly significantly higher among pregnant than non-pregnant women. This might provide further explanation for more severe gingival inflammation during pregnancy in addition to poor oral hygiene, hormonal and immunologic changes (24, 25, 51). Although no studies could be found that measured salivary PCs and its relation to gingival disease during pregnancy, however, one study could be found that measured another oxidative stress biomarkers and their relation to gingival health during pregnancy confirming the role of salivary oxidative stress in pregnancy gingivitis (37). When proteins are exposed to ROSs, modification of amino acid side chains occurs that leads to functional changes disturbing cellular metabolism. Carbonyl (CO) groups (aldehydes and ketones) are produced on protein side chains (especially of Proline, Arginine, Lysine, and Threonine) when they are oxidized. These moieties are chemically stable, which is useful for both their detection and storage (54, 55). Protein carbonyl is used as biomarkers of oxidative stress due to its stability and early formation (29, 36, 56, 57).

However, weak positive non-significant correlations were recorded between both mean values of plaque and gingival indices with protein carbonyl level in saliva. First point these weak positive non-significant correlations are probably related to sample size since larger sample size is needed so that it would be amenable to correlation coefficient analysis. Second point is the type of the statistical test used as simple correlation coefficient test is only an assumption of the existence of a relation between two variables only with absence of other variables effect. Therefore, measuring other oxidative stress parameters and female sex hormones with other types of statistical tests that take into account factors interaction are needed (58), especially for periodontal diseases that are multifactorial diseases in which several factors play role in disease pathogenesis (59).

Third point have to be considered is when studying protein oxidation several factors have to be taken into account these are nature of the oxidants and protein composition since proteins revealed different proportion and structures in different tissues and some proteins are more susceptible to oxidation than others (56, 60, 61). In addition reversible (beneficial effect) and irreversible (detrimental effect) protein oxidation were noted through proteomic technologies that help in understanding the inflammatory process and tissue destruction associated with periodontal diseases (52).

Although salivary components are important determinants of oral health status, it is recommended that future studies measure PCs levels both locally in gingival crevicular fluid (GCF) and systemically in serum to further disclose the role of oxidative stress in gingival tissue damage during pregnancy. Moreover further studies are needed to examine the proteomic profile along with protein oxidation to elucidate the etiopathogenesis of periodontal diseases that help in early diagnosis and monitoring of periodontal infection during pregnancy.

REFERENCES


