

Salivary Selenium and Glutathione Peroxidase among Group of Pregnant Women in Relation to Periodontal Condition

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Abstract: ***Background:** pregnant women experienced pronounced oral changes. Saliva composition particularly the antioxidants showed significant changes among pregnant women. **Aim:** The aims of this study were to assess the level of salivary antioxidants (glutathione peroxidase and selenium). Additionally, periodontal health was assessed among a group of pregnant women in comparison with non-pregnant women. **Subjects, materials and methods:** The study group consisted of sixty pregnant women, they were divided into three equal groups according to trimester (20 pregnant women for each trimester), and they were selected randomly from the Maternal and Child Health Care Centers in Baghdad city, their age range was 20-25 years. In addition to 60 newly married non-pregnant women as a control group and matched with age. Collection of unstimulated salivary samples was carried out under standardized conditions. Periodontal status was evaluated using the gingival index, periodontal pocket depth. The salivary samples were analyzed to determine the level of salivary antioxidants (glutathione peroxidase and selenium). **Result:** The data analysis of the present study found that the level of salivary antioxidants (glutathione peroxidase and selenium) were lower among pregnant women compared to non-pregnant controls with statistically highly significant difference between them ($P < 0.01$). Regarding trimester of pregnancy, the level of salivary antioxidants (glutathione peroxidase and selenium) were the lowest in third trimester with statistically significant difference for glutathione peroxidase ($P < 0.05$) and statistically highly significant difference for selenium ($P < 0.01$). The values of gingival index and probing pocket depth were higher among pregnant women compared to non-pregnant controls with statistically highly significant difference between them ($p < 0.01$). Regarding trimester of pregnancy, the values of gingival index and probing pocket depth were the highest in the second trimester with statistically highly significant difference among three groups ($p < 0.01$). **Conclusion:** The current study showed a decrease in antioxidant status increase in oxidative status saliva during pregnancy, that could affect periodontal health which was also affected by pregnancy.*

Keywords: periodontal diseases pregnancy glutathione peroxidase and selenium

1. Introduction

Periodontal diseases are a group of conditions that cause inflammation and destruction to the supporting structures of the teeth. These chronic oral infections are characterized by the presence of a biofilm matrix that adheres to the periodontal structures and serves as a reservoir for bacteria. Dental plaque biofilm is a complex structure of bacteria that is marked by the excretion of a protective and adhesive matrix⁽¹⁾. Within this matrix are gram-negative anaerobic and microaerophilic bacteria that colonize on the tooth structures, initiate the inflammatory process, and can lead to bone loss and the migration of the junctional epithelium, resulting in periodontal pocketing and periodontal disease⁽²⁾.

Pregnancy is a unique period during woman's life, characterized by complex physiological changes, which may adversely affect oral health⁽³⁾. During pregnancy, the most common dental problem for the mother is the periodontal disease⁽⁴⁾. The link between pregnancy and periodontal inflammation has been known for many years that was characterized by erythema, edema, hyperplasia, and increased bleeding⁽⁵⁾. On the other hand, gingival tissue physiology may be affected by hormones alteration, it become swollen, bright red in color and bleeds easily⁽⁶⁾.

Saliva as a minor host factor affects oral health through its physicochemical characteristics such as salivary flow rate, buffer capacity, pH, etc⁽⁷⁾. One of the important defense mechanism in saliva is the antioxidant system⁽⁸⁾, which defined as molecules that slow or prevent or inhibit the

oxidation of other (substrate) molecules⁽⁹⁾. The value of saliva as biological fluid for the detection of diagnostic and prognostic biomarkers have become well-established because of saliva contains locally produced as well as serum-derived markers that are useful in the diagnosis of a variety of systemic disorder⁽¹⁰⁾. Selenium behaves both as an antioxidant and anti-inflammatory agent. This is because selenium is its antioxidant role, can reduce hydrogen peroxide, lipid and phospholipids hydroperoxides, thereby dampening the propagation of free radicals and reactive oxygen species; reduce hydroperoxide intermediates in the cyclo-oxygenase and lipoxygenase pathways diminishing the production of inflammatory prostaglandins and leukotrienes. selenium with glutathione peroxidase reduces the radical-derived H₂O₂ content thus preventing the further deterioration by the radicals, which increase the proteolytic activity.

Glutathione peroxidase (GPx) is a selenium-dependent enzyme⁽¹¹⁾ and one of the most important enzymes for directly regulating ROS and is an important participant in antioxidant protection and detoxification⁽¹²⁾. As far as it is known, there were no previous Iraqi studies concerned with the assessment of the level of salivary antioxidant (selenium, GPx) and periodontal health condition among pregnant women with regard to trimester of pregnancy; therefore, it was decided to conduct this study.

2. Materials and Methods

The total sample for this study consisted of one hundred and twenty married women aged 20-25 years who were

attending Maternal and Child Health Care Centers in Baghdad city. They were divided into two groups: the study group which included 60 pregnant women who further subdivided into three subgroups according to trimester (20 women for each trimester of pregnancy) and the control group which included 60 non-pregnant married nulliparous women (not being pregnant before) having a history of regular menstrual cycles (28–30 days) they were selected randomly from companions of pregnant women; those women were matched with age. All women should have at least twenty teeth to be examined. women with systemic diseases that may affect oral health, or those who had medications which may affect periodontal health condition or had course of anti-inflammatory and antibiotic drugs during the last month before examination and those who were smoking, obese, used dietary supplement (vitamins, folic acid) or had a history of abortion, history of polycystic ovaries, hormonal disturbances, used of contraceptive, non-pregnant women on mensural cycle, wearing fixed or removable dental prostheses were excluded from this study. All participants signed informed consents, and the protocol of the study had been approved by ethical committee.

The collection of unstimulated salivary sample was performed under standardized condition following the instructions cited by Navazesh and Kumar⁽¹³⁾. Gingival index (GI)⁽¹⁴⁾ and probing pocket depth⁽¹⁵⁾ were used for recording the periodontal health condition. The salivary samples were taken to the laboratory for biochemical analysis at the Poisoning Consultation Center/Gazi Al-Hariry hospital. The level of salivary antioxidant (glutathione peroxidase) was determined calorimetrically using the spectrophotometer (Cecil CE 1011, UK). Salivary selenium level was measured by spectrophotometer system using atomic absorption spectrometer nov 350AA model (Analytikjena, Germany, 2012). Mode of action of system was absorption. This measurement was performed in Ibn Sina Labs, Ministry of Industry & minerals. Data analysis was conducted by application of SPSS program (SPSS version 21). Wilcoxon-sum rank test is non parametric test was used to determine whether two independent samples were selected from populations having the same distribution. Spearman correlation is non parametric test was used to evaluate the monotonic relationship between two non-parametric quantitative or ordinal variables. P-values less than 0.05 were recorded as statistically significant.

3. Results

Table (1) demonstrates the median and mean rank values of gingival index and the probing pocket depth for the study and control groups. It was found that GI and PPD were higher in the study group than that for the control group with statistically highly significant difference between them ($p < 0.01$).

According to trimester, Table (2) shows that the highest value for GI and PPD were the highest in the 2nd trimester with statistically highly significant difference among three groups ($p < 0.01$).

Table (3) shows the values of salivary antioxidants (Selenium, GPx) among the study and control groups. It

was found that the salivary antioxidants (Selenium, GPx) were lower in the study group than that of the control group with statistically highly significant difference between them ($P < 0.01$).

Regarding trimester of pregnancy, Table (4) shows the values of salivary antioxidants (Selenium, GPx) among pregnant women according to gestation age. It was found that the salivary antioxidants (Selenium, GPx) were lowest in the 3rd trimester with statistically significant difference for GPx ($P < 0.05$) and statistically highly significant difference for selenium ($P < 0.01$).

Table (5) demonstrates spearman correlation coefficient between GI with salivary antioxidants (Selenium, GPx) among study and control group. Negative correlations were found between GI and salivary selenium in both study and control groups with statistically significant difference ($P < 0.05$).

Regarding pregnancy trimester, Table (6) shows negative correlations between salivary selenium and GI in the 1st and 3rd trimester. However, all correlations were statistically not significant ($P > 0.05$).

Table (7) demonstrates the correlation between PPD and salivary antioxidants among the study group and control group. It was found that the correlations between PPD and salivary antioxidant (selenium) was negative with statistically no significant difference ($P < 0.05$).

Regarding pregnancy trimester, Table (8) demonstrates spearman correlation coefficient between PPD and salivary antioxidants among trimesters of pregnancy. Negative correlation was found between salivary selenium and PPD in 2nd trimester with statistically no significant difference ($P < 0.05$).

4. Discussion

Pregnancy is a physiological state accompanied by a high-energy demand and an increased oxygen requirement. Augmented levels of oxidative stress would be expected because of the increased intake and utilization of oxygen⁽¹⁶⁾. Evidence of increased oxidative stress in normal pregnancy in comparison with non-pregnant controls was estimated by measuring enzymatic antioxidants (Glutathione peroxidase and selenium). Selenium is an essential antioxidant trace mineral for the human body. It is a component of selenoproteins such as the antioxidant enzyme glutathione peroxidase (GPx), which protects human tissue from damage by hydrogen peroxide, lipid peroxides and free radicals⁽¹⁷⁾. Geographical location, soil content, intake of selenium in diet and its bioavailability significantly affect selenium status⁽¹⁸⁾. Saliva is considered as a mirror of the human body's health that reflects the normal internal characteristics and disease as most compounds found in blood are also present in saliva^(19,20).

The result of current study showed that the level of salivary selenium was lower among pregnant women than that of non-pregnant women. Several reasons may be responsible for the lower levels found in this study. Active transfer of

selenium from maternal blood to the tissues of the developing fetus had been advocated ⁽²¹⁾. Indeed, hemodilution due to increased plasma volume in pregnancy further depletes selenium concentration. Additionally, inadequate intake and storage in the maternal tissues and increased demand by the growing fetus invariably leads to low maternal levels during pregnancy ⁽²²⁾, furthermore, pregnancy is a stressful condition and there is increased oxidative stress ^(23,24) and selenium is an essential antioxidant trace mineral ⁽¹⁷⁾, thus, it is invaluable in the protection of the tissues against oxidative stress and this may give another explanation for the reduction in its level in comparison with the control. Regarding pregnancy trimester, the lowest level of salivary selenium was in 3rd trimester. The level decreased as gestation progressed, this may be due to its utilization as defense mechanisms against reactive oxygen species/free radical during the oxidative stress of pregnancy ⁽²⁵⁾, additional factors include hemodilution of pregnancy, when plasma volume may increase by 40-50% ^(26,27), and active transport of selenium from mother to fetus ⁽²⁸⁾. Furthermore, normal physiologic adjustments to pregnancy and response to hormonal changes may also contribute to the decline in selenium during pregnancy ^(29, 17).

Glutathione peroxidase is a selenium-dependent enzyme, reduces hydrogen peroxide and organic peroxides and leads to the oxidation of glutathione (GSH). Along with the other antioxidant enzymes, GPx protects cells and tissues from damage caused by reactive oxygen species by helping to maintain balance between pro-oxidant and antioxidant forces ^(30,31).

In the present study, salivary glutathione peroxidase level was lower among the pregnant women group as compared to the control group and the same result reported by ⁽³²⁾. The lower level of salivary GPx in comparison with controls could be due to either increase consumption and/or decrease production of antioxidants and the increased consumption of antioxidants is due to increased scavenging of oxidants among pregnant women ⁽³³⁾.

Regarding trimester, the result of the current study revealed that there was a progressive fall in activity of GPx as pregnancy advanced and as selenium, GPx is important antioxidant and it is utilized in defense mechanism against ROS/free radical during pregnancy ⁽³⁴⁾ and thus its level depleted in 3rd trimester, since pregnancy is a condition that favors oxidative stress. ⁽³⁵⁾.

The result of the present study showed an increase in gingival index value among pregnant women in comparison to the controls and this could be attributed to the followings:

- 1) Poor oral hygiene as indicated by the higher plaque accumulation among the pregnant women than non-pregnant women. It was proven that dental plaque is the main etiological factor of gingivitis ⁽³⁶⁾. It was obvious that pregnant women had poor oral hygiene and this may be explained by that pregnancy is stressful condition and associated with many physiological and psychological events that sequential lead to more self-neglect ⁽³⁷⁾.
- 2) Lower antioxidants level which indicated by a decrease in salivary (selenium, glutathione peroxidase) among pregnant women as compared to the controls since

antioxidants enhance periodontal health by providing protection against ROS-induced damage of periodontal tissues ⁽³⁸⁾ and this is supported by the inverse correlation between salivary selenium and gingivitis among pregnant women in present study.

Furthermore, another explanation for higher level of gingival index among pregnant women as compared to control could be the hormonal changes during pregnancy, the elevated levels of estrogen and progesterone in pregnancy could alter the connective tissue ground substance by increase fluidity and affect degree of keratinization of gingival epithelium, the decrease in the keratinization of gingiva, together with an increase in epithelial glycogen, result in decreased effectiveness of the epithelial barrier in pregnant women and make gingival more sensitive to injury ⁽³⁹⁾. When the female sex hormones act at high concentrations for prolonged periods, an increase in the permeability within the periodontal vascular system could occur. Additionally, it was reported that there was a significant connection between pregnancy-related vomiting and increased gingival inflammation and it was speculated that the main reason for this was impaired capability for proper brushing ⁽⁴⁰⁾.

Regarding the trimester of pregnancy, the highest value was found in 2nd and 3rd trimester, however, statistically no significant difference was found between 2nd and 3rd trimester. The same result was also reported by previous studies ^(41,42). A possible explanation of this result is that the difference in gingival inflammation is related to the time of pregnancy. According to many studies, it was found that the severity of gingival inflammation starts to increase from the 2nd month and rising later on ⁽⁴³⁾. This is supported by the result of this study in which a significant difference between 1st and 2nd trimester regarding gingival index the followings may explain the reason for the increased gingivitis with advancing pregnancy especially in 2nd trimester.

- 1) Poor oral hygiene as pregnancy advanced. During the first trimester of pregnancy the nausea and vomiting during the first week may be attributed to increase plaque and most the pregnant women reported that tooth brushing was nearly impossible, especially in premolar and molar areas because of the pregnancy-related nausea ⁽⁴⁴⁾, while during 3rd trimester pregnant women may become anxious, restless and exhausted.
- 2) In addition to poor oral hygiene, there can be other committing factors that vigor the gingival inflammation during pregnancy. This could be attributed to that estradiol and progesterone had higher concentrations during pregnancy with peak plasma level during the 2nd and 3rd trimesters and that these concentrations were positively correlated with the level of gingival inflammation during pregnancy ⁽⁴⁵⁾. As mention previously, the effect of these two hormones on the gingival health estrogen, increase cellular proliferation in blood vessels; decreases keratinization, while increasing epithelial glycogen; anabolic effect of estradiol in maintaining bone mass in periodontal region ⁽⁴⁶⁾. While progesterone, increase vascular dilation, thus increases permeability (results in edema and accumulation of inflammatory cells); increases proliferation of newly formed capillaries in gingival tissues (it increased

bleeding tendency); alters rate and pattern of collagen production (it reduced repair and maintenance by inhibition of fibroblast); increase metabolic breakdown of folate (a deficiency can inhibit tissue repair)⁽⁴⁷⁾. Also with increasing sex hormones level the defense mechanisms necessary for the maintenance of good oral health are compromised and certain bacteria harmful to oral environment are allowed to increase (overgrowth of *P. intermedia* is associated with increase gingival inflammation in 2nd trimester and increase in *P. gingivitis* and *T. forsythia* correlate with increase in gingival bleeding)^(48,49).

- 3) Lower antioxidant protection as indicated by negative correlation between gingival index and salivary antioxidants (selenium) in 3rd trimester in this current study and it was consistent with other⁽⁵⁰⁾. The antioxidants are utilized to maintain the balance and so the level of antioxidant decreases. it was reported that reduced antioxidant activities in saliva reflecting increased oxygen radical production or activity during periodontal inflammation⁽⁵¹⁾. Furthermore, antioxidants may be depleted probably because of overwhelming free radical's generation and elevated levels of ROS⁽⁵²⁾, and as recently has been hypothesized that pregnancy is proinflammatory condition associated with an inflammatory response characterized by leukocyte activation^(53,54).

In the current study the majority of women were with clinical pocket depth less than 4 mm. On the other hand, clinical pocket depth equal to 4mm was found only among pregnant women. This could be explained by that in the current study women examined were young since age is considered as one of the risk factors or indicators of periodontal disease⁽⁵⁵⁾.

Periodontitis represents pocket depth that is greater than 4mm, while false pocket if present usually do not exceed 4mm⁽⁵⁶⁾. This means that all of the pregnant women with pocket depth equal to 4mm had false pocket. This indicates that gingival alterations are more marked during pregnancy and none of them periodontitis.

Regarding trimester, majority of women with PPD equal 4mm were in the 2nd trimester followed by the 3rd one, this result was somewhat consistent with others^(56,45,57,58). This could be related to severe gingival inflammation with the associated gingival enlargement during pregnancy (especially in the second and third trimesters) which causes an increase in the depth of the gingival sulcus rather than periodontal destruction (false pocket).

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Table 1: Gingival index and probing pocket depth (median, mean rank) among the study and control groups

Variable	1st trimester		2nd trimester		3rd trimester		Kruskal-wallis		
	Median	Mean rank	Median	Mean rank	Median	Mean rank	Chi-square	df	Sig.
Selenium (mg/ml)	3.36	26.30	5.05	41.08	3.31	24.13	11.185	2	0.004**
Glutathione peroxidase (U/ml)	0.41	37.38	0.39	31.95	0.30	22.18	7.840		0.020*

** Highly significant (P<0.01)

Table 2: Gingival index and probing pocket depth (median, mean rank) and statistical difference among pregnant women according to gestation age

Variable	Groups				Wilcoxon sum rank test		
	Pregnant		Non-pregnant		W	Z	P-value
	Median	Mean rank	Median	Mean rank			
Selenium(mg/ml)	4.10	47.65	5.55	73.35	2859	4.047	0.000**
Glutathione peroxidase(U/ml)	0.39	38.53	0.59	82.47	2312	6.925	0.000**

** Highly significant (P<0.01)

* significant (P<0.05)

Table 3: Salivary antioxidants (glutathione peroxidase, Selenium) (median, mean rank) and statistical difference between study and control group

Variables	Groups				Wilcoxon sum rank test		
	Pregnant		Non-pregnant		W	Z	P-value
	Median	Mean Rank	Median	Mean Rank			
GI	1.40	90.50	0.20	30.50	1830	9.573	0.000**
PPD	0.00	67.00	0.00	54.00	3240	3.802	0.000**

** Highly significant (P<0.01).

Table 4: Salivary antioxidants (Selenium, Glutathione peroxidase) (median, mean rank) and statistical difference among pregnant women according to gestation age

Variables	Group			
	Study		Control	
	GI		GI	
	r	P	r	p
Selenium(mg/ml)	-0.124	0.344	-0.263	0.042*
Glutathione peroxidase (U/ml)	0.042	0.750	0.045	0.730

** Highly significant (P<0.01)

Table 5: Correlation coefficient between gingival index and salivary antioxidant (selenium, glutathione peroxidase) among study and control group

Variables	Pregnancy trimesters					
	1 st trimester		2 nd trimester		3 rd trimester	
	GI		GI		GI	
	r	p	r	p	r	p
Selenium(mg/ml)	0.359	0.121	0.229	0.332	0.083	0.728
GPx(U/ml)	0.371	0.107	0.226	0.338	0.327	0.159

Table 7: Correlation coefficient between probing pocket depth and salivary antioxidant (selenium, glutathione peroxidase) among study and control group

Variable	Group	
	PPD	
	r	p
Selenium(mg/ml)	-0.112	0.393
glutathione peroxidase (U/ml)	0.086	0.516

Table (8): Correlation coefficient between probing pocket depth and salivary antioxidant (selenium, glutathione peroxidase) among pregnant women according to gestation age

Variable	Pregnancy trimesters			
	2 nd trimester		3 rd trimester	
	PPD		PPD	
	r	p	r	p
Selenium(mg/ml)	-0.195	0.409	0.041	0.862
glutathione peroxidase (U/ml)	0.180	0.447	0.171	0.471

*Significant (P>0.05)