

Oxidative status among a group of pregnant women in relation to gingival health condition

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ABSTRACT

Background: pregnancy as a systemic condition causes changes in the functioning of human body as a whole and specifically in the oral cavity and it also is considered as a stressful condition. These changes may favor the increase of oxidative stress.

Aim: The aim of this study was to estimate the level of marker of oxidative stress (malondialdehyde) and antioxidant (uric acid) in saliva of pregnant compared to non-pregnant women and to assess the gingival health condition in both groups. Additionally, unstimulated salivary flow rate was determined in both groups.

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. Dental plaque and gingival indices were used for recording the oral hygiene and gingival health respectively. Salivary flow rate was measured then salivary samples were analyzed to determine the level of salivary antioxidant (uric acid) and lipid peroxidation biomarker of oxidative stress (malondialdehyde).

Result: The data analysis of the present study found that the level of salivary malondialdehyde was higher among pregnant women compared to non-pregnant controls with statistically significant difference ($p < 0.05$), while salivary uric acid was statistically significantly lower among the pregnant women compared to non-pregnant controls ($p < 0.05$). Salivary analysis demonstrated that the salivary flow rate was statistically significantly lower among the pregnant women compared to non-pregnant controls ($p < 0.05$).

The values of plaque and gingival indices were higher among pregnant women compared to non-pregnant controls with statistically significant difference ($p < 0.05$). The correlation of gingival index with plaque index in study and control group was significant ($p < 0.05$), while with others variables were non-significant ($p > 0.05$).

Conclusion: The current study showed an increase in oxidative status in saliva during pregnancy that could affect gingival health which was also affected by oral hygiene.

Keywords: lipid peroxidation, oxidative stress, pregnancy, gingival health, salivary antioxidant, salivary flow rate.

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INTRODUCTION

Periodontal diseases are generalized term for a range of pathological conditions affecting the supporting and investing structures of the tooth including bone, gingival tissue and periodontal ligament ⁽¹⁾. Generally, periodontal diseases have been divided into two major categories; gingivitis and periodontitis ⁽²⁾. Gingivitis is characterized by inflammation of marginal gingival tissues with no detectable loss of bone or connective tissue attachment ⁽³⁾. It may progress to periodontitis, which is gum disease that spread below the gum line and affects the tissue and bones that support the teeth ⁽⁴⁾.

The main etiological factor of gingivitis is dental plaque ⁽⁵⁾ which is defined as non-mineralized, sticky soft bacterial mass or deposits which is tightly adherence to the tooth surface and other solid in the mouth and resists removal by salivary flow or by water spray ⁽⁶⁾. The specific role of antioxidant is to neutralize rampaging free radical and thus reducing its capacity to damage ⁽⁷⁾.

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It was found that the salivary antioxidants inhibit the oxidation reaction and provide protection against reactive oxygen species induced damage of gingival tissues ⁽⁸⁾. Triveda et al. ⁽⁹⁾ and Zhang et al. ⁽¹⁰⁾ reported increased oxidative stress and MDA in periodontitis, also Canakci et al. ⁽¹¹⁾ Guentsch et al. ⁽¹²⁾ have shown a reduction in both systemic and local antioxidant capacity antioxidant concentration of gingival crevicular fluids in periodontitis patients.

Pregnancy, is a stressful condition in which many metabolic and physiological functions are changes to a considerable extent ⁽¹³⁾. So the main physiological and hormonal changes in the life of a woman occur during pregnancy ⁽¹⁴⁾, and the oral cavity is one of the target areas involved in these changes ⁽¹⁵⁾. Pregnancy gingivitis, defined as gingival inflammation initiated by plaque and exacerbated by endogenous sex steroid hormones ⁽¹⁶⁾. Clinical studies have reported an increase in the extent and severity of gingival inflammation during pregnancy, which abates postpartum with the fall in hormone production ^(17,18). The link between pregnancy and periodontal inflammation and the effect of pregnancy on periodontal health has been studied extensively and only recently

evidences indicated an inverse relationship to systemic health that periodontal disease may affect the wellbeing of the fetus by elevating the risk for low birth weight & preterm infant^(19,20). Pregnancy is a physiological state accompanied by a high metabolic demand and elevated requirements for tissue oxygen. This raised oxygen demand increases the rate of production of reactive oxygen species (ROS) and women with normal pregnancies experience increased oxidative stress (OS) and lipid peroxidation (LPO). The placenta is a major source of OS during pregnancy^(21,22).

Salivary physicochemical characteristics also affected during pregnancy. Several studies showed controversy regarding salivary flow Mutlak⁽²³⁾ reported increased in stimulated salivary flow rate, while, Sulaiman⁽²⁴⁾ reported opposite result. On the other hand, Al-Zaidi⁽²⁵⁾ reported no change in salivary flow rate among pregnant as compared with non-pregnant women. As far as it is known, there were no previous Iraqi studies concerned with the relation between reactive oxygen species, salivary antioxidant (uric acid) and gingival health condition among pregnant women; therefore, it was decided to conduct this study.

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). While the control group included 60 non-pregnant married nulliparous women (not being pregnant before) having a history of regular menstrual cycles (28-30 days), they were selected from companions of pregnant women; those women were matched with age. All women should have at least twenty teeth to be examined. Exclusion criteria in this study include 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. All participants signed informed consents, and the protocol of the study had been approved.

The collection of unstimulated salivary sample was performed under standardized

condition following the instructions cited by Navazesh and Kumar⁽²⁶⁾. Plaque index⁽²⁷⁾ (PII) was used for recording oral hygiene, while gingival index⁽²⁸⁾ (GI) for assessing the gingival health condition. Salivary flow rate was expressed as milliliter per minute (ml/min)⁽²⁹⁾. Then salivary samples were taken to the laboratory for biochemical analysis at the Poisoning Consultation Center/Gazi Al-Hariry hospital. The level of salivary antioxidant (uric acid) and lipid peroxidation biomarker of oxidative stress (Malondialdehyde) was determined calorimetrically using the spectrophotometer (Cecil CE 1011, UK). Salivary uric acid level was measured using a ready kit (Spinreact, Spain), while malondialdehyde (MDA) was measured using Malondialdehyde Assay kit (Chemical point Germany) according to the manufactured instructions. Data analysis was conducted by application of SPSS program (SPSS version 21). By using Shapiro-Wilk test to determine whatever the data was normally distributed or not. Normal distribution of data was not achieved, so following non-parametric tests were used. Wilcoxon-sum rank test was used to compare between two independent samples. Spearman correlation was used to evaluate the association between two non-parametric quantitative or ordinal variables. P-values less than 0.05 were recorded as statistically significant.

RESULTS

Table (1) shows the values of salivary MDA and uric acid among the study and control groups. It was found that the salivary MDA was higher among pregnant women as compared to non-pregnant women with statistically significant difference ($p < 0.05$). On the other hand, salivary uric acid was lower among pregnant women than in non-pregnant controls with statistically significant difference ($p < 0.05$).

Table (2) shows the values of salivary flow rate among the study and control groups. It was found that the salivary flow rate was lower among pregnant women than non-pregnant women with statistically significant difference ($p < 0.05$).

Table (3) shows the values of plaque and gingival indices among the study and control groups. It was found that the values of plaque and gingival indices were higher in the study group than that in the control group with statistically significant difference ($p < 0.05$).

Table (4) illustrates the correlations between GI with oral variable. A positive and statistically significant correlation was found between GI and dental plaque ($p < 0.05$) in both study and control group, while all others correlations were not statistically significant ($p > 0.05$).

Table 1: Salivary malondialdehyde and uric acid (median, mean rank) among the study and control groups.

Variable	Group				Wilcoxon sum rank test
	Pregnant		Non-pregnant		
	Median	Mean Rank	Median	Mean Rank	P-value
Malondialdehyde ($\mu\text{mol}/\text{dl}$)	0.07	74.39	0.04	46.61	0.000*
Uric acid (mg/dl)	2.45	51.55	2.55	69.45	0.005*

*Significant ($p < 0.05$)**Table 2: Salivary flow rate (median, mean rank) among the study and control groups.**

Variable	Group				Wilcoxon sum rank test
	Pregnant		Non-pregnant		
	Median	Mean Rank	Median	Mean Rank	P-value
Flow rate (ml/min)	0.10	55.35	0.10	65.65	0.005*

* Significant ($p < 0.05$)**Table 3: Plaque index and gingival index (median, mean rank) among the study and control groups.**

Variable	Group				Wilcoxon sum rank test
	Pregnant		Non-pregnant		
	Median	Mean Rank	Median	Mean Rank	P-value
PII	1.10	90.48	0.20	30.52	0.000*
GI	1.40	90.50	0.20	30.50	0.000*

* Significant ($p < 0.05$)**Table 4: Correlation coefficient between gingival index with oral variables among study and control groups.**

Variable	Pregnant		Non-pregnant	
	GI		GI	
	r	p	r	p
Malondialdehyde ($\mu\text{mol}/\text{dl}$)	0.186	0.154	-0.061	0.644
Uric acid (mg/dl)	-0.106	0.419	-0.041	0.757
PII	0.559	0.000*	0.879	0.000*
Flow rate (ml/min)	-0.037	0.782	-0.028	0.830

* Significant ($p < 0.05$)

DISCUSSION

Pregnancy is a normal physiological phenomenon with many biochemical changes⁽³⁰⁾. 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^(31,32). In the present study, data analysis showed that flow rate of saliva was lower among the pregnant women than among non-pregnant women, pregnant women are uncomfortable and distressed due to nausea in pregnancy and certain hormones contribute to this relationship (morning sickness). Pregnancy induces decreased gastroesophageal sphincter tone and prolonged gastric emptying times. These changes along with decreased esophageal tone lead to changes in the saliva flow^(33,34). This result was also reported by others^(35,36), while an opposite finding was reported by other studies^(33,37).

The result of current study showed that the concentration of lipid peroxidation (MDA) was higher among the study group than that among the control group. The same result was also reported by other studies^(38,39). This could be due to the fact that pregnancy is a physiological condition of stress and hyper-dynamic circulation⁽⁴⁰⁾. Furthermore, in a healthy placenta, there is an increase in oxidative stress levels, due to a high placental mitochondrial activity and increase partial pressure of oxygen in pregnant women resulting in an increase in ROS production^(41,42).

On the other hand, in the present study, the salivary concentration of uric acid was lower among pregnant women than that among the non-pregnant controls. The same result was also reported by other study⁽⁴³⁾. The result of the present study could be attributed to the fact that the renal system undergoes marked changes in function during pregnancy due to hormonal

effects, the increased metabolic load of the fetus and the outflow obstruction of the ureters by the enlarging uterus⁽⁴⁴⁾. Furthermore, the decreased salivary uric acid level among study group in comparison to the control group could be an indication of increased consumption and/or decreased production of antioxidants and the increased consumption of antioxidants is due to increased scavenging of oxidants⁽⁴⁵⁾. It was documented that salivary uric acid acts as a major salivary antioxidant which participate in 85% of the total antioxidant capacity in saliva and provide a protection against oxidative stress⁽⁴⁶⁾. It was found an association between exist serum and salivary uric acid concentration, so that, saliva testing may be a useful non-invasive approach for monitoring disease and health condition⁽⁴⁷⁾.

The current study showed that the value of the PII was found to be higher in the study group than that in the control group. The same result was also reported by others^(23,48). The higher value of dental plaque could be attributed to poor oral hygiene among pregnant women in comparison with non-pregnant controls and this may be explained by that pregnancy is a stressful condition and associated with many physiological and psychological events that sequentially lead to more self-neglect. While the young newly married non-pregnant women are more taking care of their appearance including their oral hygiene⁽⁴⁹⁾. Another explanation for the higher value of dental plaque among pregnant women could be due to a reduction in salivary flow rate among them. It was proved that the salivary flow rate may play an important role in relation to plaque accumulation since a decrease in salivary flow rate lead to a decrease of irrigation action of saliva⁽⁵⁰⁾, so dental plaque increased. The result of the present study showed an increase in GI 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⁽⁵⁾ and this fact is supported by a positive and statistically significant correlation between dental plaque and gingivitis in this study and the same correlation was also reported by others^(25,51).
2. Decreased salivary flow rate among pregnant women as compared to non-pregnant women. Saliva may affect periodontal diseases through its physiochemical properties⁽⁵²⁾, since decrease of salivary flow rate lead to reduce of washing action of saliva and oral dryness as well as protective constituents decreased with decreased flow rate

⁽⁵⁰⁾. Previous Iraqi studies also reported the same correlation^(23,48).

3. Lower antioxidant level which indicated by a decrease in salivary uric acid among pregnant women as compared to the controls since antioxidants enhance periodontal health by providing protection against ROS-induced damage of periodontal tissues⁽⁵³⁾.
4. The significant higher level of lipid peroxidation biomarker (MDA) among pregnant women and this supported by positive correlation between GI and MDA. Since reactive oxygen species damage periodontal tissues by causing peroxidation the lipid of the cell wall and hence cell death⁽⁵⁴⁾.

Furthermore, another explanation for higher level of GI 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⁽⁵⁵⁾. 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⁽⁵⁶⁾.

Pregnant women can be considered as an important target group with special periodontal health needs. Measuring of oxidative condition in saliva could be used as a mean for periodontal health monitoring and treatment success during the periodontal maintenance period. Furthermore, pregnant women are in need to public preventive programs to maintain good oral hygiene and improve their gingival condition.

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الخلاصة

الحمل كحالة وظيفية يسبب تغييرات في أداء الجسم البشري ككل وتحديدًا في تجويف الفم، ويعتبر أيضًا حالة مجهدّة. هذه التغييرات قد تعمل على زيادة الأكسدة. الهدف من هذه الدراسة هو تقدير المؤشر الحيوي لبيروكسدة الدهون (malondialdehyde) ومضاد الأكسدة (حامض اليورك) في لعاب الحوامل مقارنة بالنساء غير الحوامل ولتقييم الحالة الصحية التوتوية في المجموعتين. بالإضافة إلى ذلك، تم تحديد معدل تدفق اللعاب غير المتحفز. لموضوعات والمواد والأساليب: تألفت الدراسة من ستين امرأة حامل، تم تقسيمهن إلى ثلاث مجموعات متساوية حسب الثلث (20 امرأة حامل لكل ثلث)، وتم اختيارهن عشوائيًا من مراكز رعاية صحة الأم والطفل في مدينة بغداد، كانت الفئة العمرية 20-25 سنة. بالإضافة إلى 60 امرأة متزوجة حديثًا غير حامل كمجموعة ضابطة ومطابقة بالعمر. تم تجميع عينات اللعاب الغير محفز في ظل ظروف ثابتة. استخدام مقياس الصفيحة الجرثومية البلاك ومؤشرات التهابات اللثة لتسجيل صحة الفم وصحة اللثة على التوالي. تم قياس معدل التدفق اللعابي ثم تم تحليل العينات اللعابية لتحديد مستوى مضاد الأكسدة (حامض اليورك) والمؤشر الحيوي لبيروكسدة الدهون (malondialdehyde). النتائج: وجد تحليل البيانات لهذه الدراسة أن مستوى مالونديالدهيد اللعابي كان أعلى بين النساء الحوامل مقارنة مع الضوابط غير الحوامل مع وجود فرق ذي دلالة إحصائية ($p < 0.05$)، في حين كان حمض اليورك اللعابي أقل إحصائياً بشكل ملحوظ بين النساء الحوامل مقارنة إلى ضوابط غير حامل ($p < 0.05$). وأظهر التحليل اللعابي أن معدل تدفق اللعاب كان أقل إحصائياً بشكل ملحوظ بين النساء الحوامل مقارنة بالضوابط غير الحوامل ($p < 0.05$). كان مقياس الصفيحة الجرثومية البلاك ومؤشر التهاب اللثة أعلى بين النساء الحوامل مقارنة بالمجموعة الضابطة غير الحوامل مع وجود فرق ذي دلالة إحصائية ($p < 0.05$). استنتاج: أظهرت الدراسة الحالية زيادة في حالة التأكسد في اللعاب خلال فترة الحمل، التي يمكن أن تؤثر على صحة اللثة التي تأثرت أيضًا عن طريق النظافة الفموية.