

Research Article

Salivary protein carbonyl and selected antioxidants in relation to dental caries among pregnant women

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Abstract: Background: Pregnancy is a physiological condition that affects the general and oral health. It is also associated with an increase in oxidative stress, which may predispose to oral diseases including dental caries. Aim of the study: This study aimed to measure salivary protein carbonyl, glutathione peroxidase and selenium levels of women who are pregnant and their association with dental caries in comparison to non-pregnant women, and to find out the mostly affected biomarker of oxidative stress during pregnancy. Subjects, materials and methods: A cross-sectional research was performed for a samples of 30 pregnant and 30 non-pregnant women who were chosen from city of Baghdad's Primary Healthcare Centers. Both groups aged 25-30 years. In unstimulated salivary samples protein carbonyl and glutathione peroxidase were determined colorimetrically using spectrophotometer by utilizing ready-made assay kits. Salivary selenium level was obtained by atomic absorption spectrophotometer. Plaque index had been used to determine the thickness of dental plaque. Caries was recorded using the Decayed, Missing, and Filled (DMF) index. described by WHO in 1997. Data was statistically analyzed using descriptive statistics method and Student's t-test, Wilcoxon sum rank test and Spearman's correlation in addition to Receiver Operating Characteristics Curve (ROC test) ($\alpha=5\%$). Results: The plaque index and salivary protein carbonyl values were significantly higher among pregnant while salivary selenium and glutathione peroxidase recorded significantly lower levels among pregnant women. Dental caries parameters were higher among pregnant with significant difference for MS fraction only. ROC area for protein carbonyl equal one with highest sensitivity and specificity. Conclusion: Pregnant women recorded higher dental caries severity with higher salivary protein oxidation but lowers salivary antioxidant defense mechanisms. Salivary protein carbonyl is more ideal, valid and mostly affected biomarker in revealing the oxidative stress status during pregnancy.

Keywords: dental caries, pregnancy, protein carbonyl, salivary glutathione peroxidase and selenium.

Introduction

Saliva has several merits like the presence of novel and soluble biomarkers, easy and non-invasive collection, continuous secretion and intimate contact with oral tissues reflecting physiological and pathological changes particularly those on the cellular molecular level. These merits make saliva a valid diagnostic fluid to screen, diagnose, and monitor disease progression (1). Saliva is a unique complex oral fluid produced by salivary glands and has a significant impact on keeping the maintainability of oral hard as well as soft tissue through its complex physical and chemical composition (2). The body's overall oxidative stress (OS) and antioxidant system are presented in saliva. Therefore, saliva is the first line of protection against OS (3). Salivary antioxidant system includes antioxidants with a low molecular weight as glutathione, vitamin E, ascorbic acid and uric acid in addition to peroxidase, catalase, superoxide dismutase, and glutathione peroxidase which act as an antioxidant enzymes (4).

The enzyme glutathione peroxidase (GPx) is selenium-dependent. It is one of most crucial enzymes in the regulation of reactive oxygen species (ROSs) by catalyzing the oxidation of other molecules (5).

Antioxidant trace elements as selenium (Se) are also found in saliva. It acts by reducing Hydroperoxides of lipids and phospholipids, as well as hydrogen peroxide, thus further halted the spread of ROS reactive oxygen species (6). Because of their abundance and responsibility, proteins were indeed main targets for ROS/RNS (Reactive Oxygen Species/Reactive Nitrogen Species) in most functional processes in the cell. Protein oxidation could occur at the single amino acid level residue causing polypeptide chains fragmentation or covalent crosslinking of two amino acids (7). When proteins are exposed to ROSs, modification of amino acid side chains occurs that leads to functional changes disturbing cellular metabolism. When protein side chains are oxidized, carbonyl (CO) groups (aldehydes and ketones) are formed. Because these moieties seem to be chemically stable, they can be detected and stored (8).

Under altered physiological conditions like pregnancy, higher metabolic demand and elevated requirements for tissue oxygen was reported during all three trimesters of normal pregnancy that results in an increased ROSs production (9). Excessive ROS production inside the oral cavity could result in oxidative stress (OS), which is an imbalance for both oxidant and anti-oxidant systems. Oxidative stress enhances an oxidative damaging of DNA, lipids and proteins consequently in cell predisposing to several oral diseases as dental caries and periodontal diseases (10, 11).

Salivary oxidant/antioxidant imbalance was recorded during pregnancy (12). Also Elevated salivary protein carbonyl levels indicate higher salivary protein oxidation, was found among pregnant women (13). Wagle et al (14) recorded higher oxidative damage (Malondialdehyde "MDA" content) and low level of total anti-oxidant capacity in saliva during pregnancy. Similarly higher salivary "8-hydroxy-2'-deoxyguanosine (8-OHdG)" level but lower glutathione peroxidase (GPx) were recorded among pregnant compared to controls. Such changes in salivary oxidants/antioxidants components of pregnant women make them susceptible to oral diseases as periodontal diseases (15). However, no studies could be found that relating salivary protein carbonyl (PC) and GPx and Se with dental caries parameters during pregnancy. Dental caries diagnosis based on clinical and radiographic examinations is of limited use in early diagnosis because it provides a measure of past dental destruction (16). The early detection of tooth decay has an essential role in pregnancy, as it permits establishing preventive measures in addition restorative therapy is more expensive and sometimes involves general health risks (17).

Therefore, in view of the aforementioned findings and to address this gap this clinico-biochemical study was aimed to measure the PC, GPx and Se levels in saliva of pregnant women during third trimester and their relations with the clinical index of dental caries. The data was also compared to that of healthy married non-pregnant women. In addition, this study aimed to find out which salivary constituent (PC, GPx, or Se) is the more affected oxidative stress biomarker during pregnancy. The null hypothesis was that salivary oxidants/antioxidants constituents remain stable throughout pregnancy and they have no relation with dental caries parameters. In addition, none of the measured salivary constituents was sensitive biomarker of oxidative stress.

Materials and Methods

Sample Size: Utilizing the Pilot survey of dental caries and G power 3.1.9.7 (a program created by Franz-Faul, University of Kiel, Germany) (DS) for both pregnant and non-pregnant groups about 10 subjects per individual with a mean±SD of 7.6±4.21 and 4.2±4. According to both groups, the power of the study is 85%, the effect size is 0.828, and the probability error is 0.05. According to all these circumstances Using 30 individuals for each is enough that will provide you the minimal sample size for each group, which is 28 people.

Study Design: A cross-sectional study was conducted in a convenience sample of 60 women recruited at the Primary Health Care Centers in Baghdad city in AL-Russafa sector. Thirty pregnant women with an age range of 25-30 years in addition to thirty non-pregnant women that matched with age were included in the current study. Only pregnant women in the third trimester were chosen. The non-pregnant women should be already married, nulliparous (never been pregnant before), and had a background of regular menstrual periods (28-30 days); they were chosen from pregnant women's companions. Both preg-

nant and non-pregnant women should be non-smoker, with no medical history that compromises salivary secretory mechanism, shouldn't take any medications with xerogenic effect or any nutritional supplementation, and shouldn't wear any fixed or removable dental prostheses. The study carried out in the period from June to August 2019.

Collection of unstimulated saliva and recoding of dental plaque and dental caries status: Unstimulated salivary specimens were collected under standardized condition according to the instructions listed by Navazesh and Kumar (2008)(18). The subject should avoid intake of food, chewing gum, beverage one hour before saliva collection. They should be seated on a chair, then irrigate their mouth with distilled water and relaxed for at least five minutes. Then subjects should reduce their movement and instructed to fix their forehead above and the test tube kept beneath it. After that, the subjects were instructed to keep their mouth opened to allow the drain of saliva into the tube for five minutes. At the end of the collection period, the subject was asked to collect any remaining saliva in the mouth and spit it very quickly into the test tube. The actual trial should last for five minutes. After the foam all disappeared, saliva was placed into cooler box and sent to the laboratory and centrifuged for 10 minutes at 3000 rpm (revolution per minute); then separation of the supernatant was done by micropipette and kept in deep freezing (-20 °C) in polyethylene tubes for the subsequent analyses.

Dental plaque thickness at the gingival third was assessed and diagnosed according to plaque index (PII) formulated by Silness and Løe (19). The PII was recorded on four dental surfaces (buccal, lingual, and proximal surfaces) of all present teeth. The values of four sites of each tooth were recorded in order to obtain the mean of PII for each tooth. The means of PII of all teeth of each volunteer was then calculated, followed by calculation of the mean PII of all volunteers. the Decayed, Missing, and Filled (DMF) index was used to capture the data of caries experience. Criteria of World Health Organization's description (WHO) (20). Clinical examination of dental caries status was conducted by single examiner using a No. 05 clinical mirror and CPI probe (recommended by the WHO) for dental caries measurement and plunget probe for plaque index. Repeating the measured data in ten patients yielded intra-examiner agreement of 0.950 and 0.958 Kappa coefficient for PII and DMFS respectively.

Biochemical analysis of salivary samples: Bio-chemical laboratory work was done at Poisoning Consultation Center at Gazi Al-Hariry hospital in Baghdad city. The protein carbonyl level in saliva was determined in (M/L) using a protein carbonyl assay kit. (SazaKits, India). The principal of reaction of this kit is that, after oxidation, the protein carbonyl content increased, as well as the carbonyl group tend to react with 2,4-dinitrophenylhydrazine to create a reddish brown precipitate. After the precipitate has been dissolved, the absorbance could be observed at 370 nm. It is possible to indirectly calculate the carbonyl content (21) (Salivary glutathione peroxidase concentration in (U/ml) was measured colorimetrically by the usage of GPx assay kit (Thomas Baker, India). Salivary selenium level was measured in (mg/ml) by a spectrophotometric system using spectrometer for atomic absorption nov 350AA model (22). The system uses absorption as its primary mode of action.

Statistical analysis: Utilizing IBM SPSS software version25, data are analyzed (Statistical Package for Social Sciences). Shapiro-Wilk test was used for testing the normality of data. When analyzing non-normally distributed variables, median and mean rank were utilized instead of descriptive statistics such as mean and standard deviation. Interferential statistics for normally distributed data was Student's t-test, while for non-normally distributed variables, Wolcoxon sum rank (W) test and Spearman's correlation tests were used.

Ethical Aspects: An approval was achieved from the Ministry of Health for examining those women in addition the The ethical committee at the University of Baghdad's College of Dentistry had accepted the study's procedure. (i.e. the ethical committee authorized this study, No. 223320). The study protocol was explained to the participants and all participants signed written informed consents.

Results

Shapiro-Wilk test was used to determine whether the sample's distribution was normal. Findings indicated that the mean value for decayed fraction DS of DMFS index was normally distributed among both not pregnant and pregnant women ($P=0.194$, 0.257 respectively). However, plaque index, salivary protein carbonyl, selenium and glutathione peroxidase were non-normally distributed among pregnant and non-pregnant women ($P<0.05$) except for selenium that was normally distributed among non-pregnant women ($P=0.752$).

Results recorded in Table 1 showed that the mean value of DS fraction was greater in pregnant women than non-pregnant women but with statistically non-significant difference after application of Student's t-test ($P=0.778$) between non-pregnant and pregnant women. Also that median value for MS fraction was higher pregnant women than non-pregnant women. with significant difference ($P=0.004$) after application of Wtest. Regarding FS fraction the median value was zero in both expectant and non-expectant women, however, the FS fraction's mean rank value was higher among pregnant women with non-significant difference ($P=0.793$) after application of Wtest. the median value of DMFS was also higher among woman with pregnancy compared to non-pregnant, but with no significant difference ($P=0.192$) after application of W test. For dental plaque thickness, it was recorded that the median value of PII was higher among pregnant women with significant difference ($P=0.000$) after application of W test.

In Table 2, data analysis revealed that the median value of salivary protein carbonyl compared to women who were not pregnant, was higher. By application of W test result revealed that the variation in median value of protein carbonyl was significant ($P=0.000$) between women who are pregnant and those who are not. However, salivary selenium revealed lower median value among pregnant compared to not pregnant with significant difference ($P=0.036$). Similarly salivary glutathione peroxidase (GPx) median value was lower pregnant women compared to non-pregnant women with significant difference ($P=0.000$).

Application of Spearman's correlation coefficient revealed no statistically significant relation ($P>0.05$) amongst salivary protein carbonyl, selenium and glutathione peroxidase levels with plaque index in both women who are and are not pregnant as illustrated in Table 3.

In Table 4 only salivary selenium recorded inverse weak correlations with dental caries parameters that were significant with MS and FS fractions ($P=0.047$, 0.042 respectively) and close to the confidence limit with DMFS ($P=0.06$) for pregnant women. Only salivary glutathione peroxidase recorded inverse weak non-significant correlations with dental caries parameters ($P>0.05$) for non-pregnant women. The remaining relations were non-significant for pregnant as well as not pregnant ($P>0.05$).

In Table 5 and Figure 1, pregnancy's impact on specific oral variables was analyzed by using the ROC test. The Curve's Underside (AUC) can be used in order for testing measurements according to their importance in discrimination between categories of women who are or are not pregnant (i.e. it can show which measurement are more affected by disease process (pregnancy) under study compared to non-pregnant). In the current study the area under the curve for salivary PC equal one with significant difference ($p<0.05$); therefore, it was the most affected oral variable by pregnancy (excellent for differentiation between study and control groups).

Table 1: Descriptive statistics of dental caries parameters and plaque index for pregnant and non-pregnant groups and the statistical differences between them.

Variables	Groups						df	P-value	
	Pregnant			Non-pregnant					
No.	Mean	±SD	No.	Mean	±SD				
DS	30	7.567	4.207	30	7.276	3.991	58	0.778 ^{NS}	
	No.	Median	Mean rank	No.	Median	Mean rank	W -test	Z-Value	P- value
MS	30	5.000	36.27	30	0.000	24.73	742.000	-2.861	.004
FS	30	0.000	30.87	30	0.000	30.13	904.000	-.262	.793 ^{NS}
DMFS	30	13.500	33.43	30	9.000	27.57	827.000	-1.305	.192 ^{NS}
PII	30	1.200	39.92	30	0.900	21.08	632.500	-4.193	.000 ^S

S significant P ≤ 0.05

Table 2: Descriptive statistics of salivary protein carbonyl and anti-oxidants for pregnant and non-pregnant groups and the statistical differences between them.

Variables	Groups						W -test	Z-Value	P- value
	Pregnant			Non-pregnant					
No.	Median	Mean rank	No.	Median	Mean rank				
PC (M/L)	30	1.348	45.50	30	1.126	15.50	465.000	-6.655	.000 ^{HS}
Se (mg/ml)	30	4.518	25.78	30	5.311	35.22	773.50	-2.095	0.036 ^S
GPx (U/ml)	30	0.275	16.57	30	0.660	44.43	497.000	-6.188	.000

S=significant, M/L=mole per letter, U/ml= Unit per milliliter, mg/ml=milligram/milliliter

Table 3: Correlations of plaque index with salivary protein carbonyl and antioxidants for pregnant and non-pregnant groups.

Variable	Pregnant		Non-pregnant	
	r	P-value	R	P-value
PC (M/L)	.056	0.770	0.070	0.713
Se (mg/ml)	-0.282	0.131	-0.092	0.630
GPx (U/ml)	0.098	0.608	-0.254	0.176

M/L=mole per letter, U/ml= Unit per milliliter, mg/ml=milligram/milliliter.

Table 4: Correlations of dental caries parameters with salivary protein carbonyl and antioxidants for pregnant and non-pregnant groups.

Dental caries parameter	Pregnant						Non-pregnant					
	PC (M/L)		Se (mg/ml)		GPx (U/ml)		PC (M/L)		Se (mg/ml)		GPx (U/ml)	
	r	P	R	P	R	P	R	P	r	P	r	P
DS	0.238	.205	-0.120	.526	.248	.187	0.287	.124	-.052	.785	-.166	.381
MS	-.173	.361	-.366*	.047	-.090	.635	.051	.788	.337	.068	-.064	.738
FS	-.105	.580	-.374*	.042	.014	.943	-.177	.350	-.192	.308	-.122	.521
DMFS	0.016	.933	-.340	.066	.121	.525	.154	.417	.030	.876	-.197	.296

Table 5: Effect of pregnancy on salivary variables (ROC test).

Oral variables	Area Under the Curve (AUC)	P-value	Optimal cut-off point
PC (M/L)	1.000	0.000 ^S	1.21
Se (mg/ml)	0.343	0.036 ^S	0.91
GPx (U/ml)	0.036	0.000 ^S	0.1

S= significant.

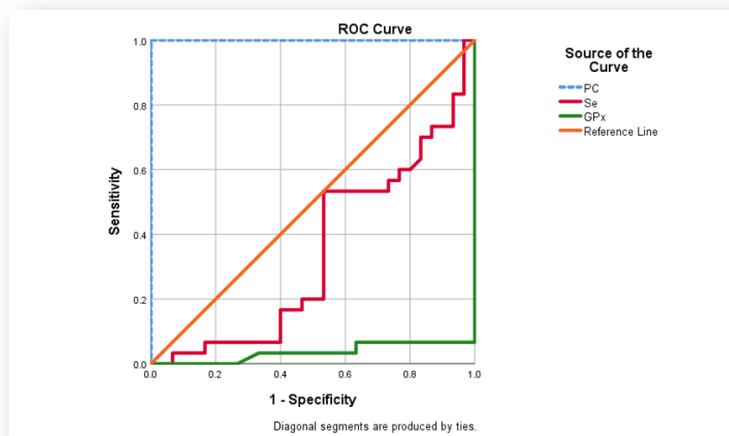


Figure 1: Roc curve for selected salivary variables.

Discussion

Salivary oxidative stress constituents reach their highest level during third trimester of pregnancy (23); therefore, the sample of this study included women in the third trimester.

Since unstimulated saliva is present all the time in the mouth, so it represents the major intra-oral condition. alivary stimulation process might also enhance the release of gingival crevicular fluid that contains antioxidants that might further increase antioxidants concentration in saliva (24).

The study results suggest that the null hypothesis was rejected since salivary protein carbonyl recorded significantly higher value among pregnant as compared to non-pregnant whereas salivary GPx and Se revealed significantly lower values among pregnant women. Therefore, the study results confirmed an increased salivary oxidative stress during pregnancy represented by higher salivary PC level but lower salivary GPx and Se levels. These results go with the findings of previous studies (12, 14, 15). The possible explanation for reduced salivary Se and GPx during pregnancy probably because these antioxidants were exhausted while counteracting reactive oxygen species (25).

Another result that was recorded by the current study was inverse correlations of salivary selenium with dental caries parameters that were significant in case of MS and FS among pregnant women. This result might partially explain the higher decayed surfaces (present caries experience) though statistical difference was non-significant, and higher missing and filled surfaces (past caries experience) during pregnancy with significant difference for missing surfaces only. Salivary oxidative stress has been found to affect the initiation and progression of dental caries (10). Less is known about how oxidative stress

affects the development of dental caries. However, it was found that salivary antioxidants perform a preventative function in the process of caries through inhibiting the inflammatory response occurs within dentine due to reactive oxygen species (ROSs) and high sucrose diet (26). Another possible explanation is that the oxidation of proteins in both enamel and dentine by reactive oxygen species might weaken the tooth structure thereby increasing the susceptibility to dental caries (27).

The main etiological factor in caries process is a complex biofilm (28). This is supported by the current study results as dental plaque accumulations was higher among pregnant than non-pregnant with significant difference.

Saliva has become a promising diagnostic fluid(1). Salivary protein carbonyl is used as biomarkers of oxidative stress due to its stability and early formation (5) Also this is confirmed in the current study as it was found that salivary protein carbonyl is the mostly affected biomarker of oxidative stress since the area under the curve for salivary PC equal one with significant difference ($p < 0.01$). Therefore, PC was the most affected oral variable by pregnancy as compared to salivary selenium and glutathione peroxidase.

Conclusions

In conclusion, pregnant women recorded higher dental caries severity with higher Protein oxidation, as indicated by the higher level of salivary protein carbonyl. with reduction in salivary antioxidant defense mechanisms (salivary selenium and glutathione peroxidase). Only salivary selenium recorded inverse correlations with dental caries parameters. Furthermore, salivary protein carbonyl could be considered a more ideal and valid biomarker in revealing the oxidative stress status during pregnancy.

The following study limitations should also be taken into account.:

Despite earlier estimates, the sample size was modest; only pregnant women who visited primary health care centers made up the sample., so this prevent generalizing the clinical findings to all pregnant women.

In addition, only the relation of salivary oxidative stress biomarkers with clinical index of dental caries was done, no mechanistic study was performed therefore, future both in vivo and in vitro researches are needed with larger sample size using panel of antioxidants and oxidative biomarkers to disclose the precise mode of action of these salivary oxidants/anti-oxidants constituents in caries process.

The comparison of data with other studies however, may not be completely valid due to variation in study designs used by different researchers.

Conflict of interest: None.

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العنوان: البروتين كاربونيل اللعابي ومضادات الأكسدة المختارة و علاقتهم مع تسوس الأسنان لدى النساء الحوامل الباحثون: بيداء حسين عون المستخلص:

الهدف: هدفت الدراسة إلى قياس مستويات البروتين اللعابي كاربونيل والجلوتاثيون بيروكسيداز والسيلينيوم لدى النساء الحوامل وارتباطهن بتسوس الأسنان مقارنة بالنساء غير الحوامل. كما هدفت هذه الدراسة إلى إيجاد أكثر المؤشرات الحيوية اللعابية حساسية للإجهاد التأكسدي أثناء الحمل
المواد وطريقة العمل: تم إجراء بحث مقطعي لعينات من 30 امرأة حامل و 30 امرأة غير حامل تم اختيارهن من مراكز الرعاية الصحية الأولية في مدينة بغداد. تتراوح أعمار كلتا المجموعتين بين 25 و 30 عامًا. في عينات اللعاب غير المحفزة ، تم تحديد بروتين كاربونيل والجلوتاثيون بيروكسيداز بطريقة لونية باستخدام مقياس الطيف الضوئي باستخدام مجموعات الفحص الجاهزة. تم الحصول على مستوى السيلينيوم اللعابي عن طريق مقياس الامتصاص الذري. تم استخدام مؤشر البلاك لتحديد سمك الصفيحة الجرثومية. تم تسجيل تسوس الأسنان باستخدام مؤشر المتسوس والمفقود والمعالج بحشوة أسنان (DMF). وصفتها منظمة الصحة العالمية (1997). تم تحليل البيانات إحصائيًا باستخدام طريقة الإحصاء الوصفي واختبار الطالب واختبار مان ويتني U وعلاقة سبيرمان بالإضافة إلى منحنى خصائص تشغيل جهاز الاستقبال (اختبار $\alpha = 5\%$ ROC).
النتيجة: كان مؤشر البلاك وقيم كاربونيل البروتين اللعابي أعلى بين الحوامل ($P = 0.00$) بينما سجل السيلينيوم اللعابي والجلوتاثيون بيروكسيداز مستويات أقل بشكل ملحوظ بين النساء الحوامل ($P = 0.03$ ، $P = 0.00$ على التوالي). كانت معاملات تسوس الأسنان أعلى بين الحوامل مع وجود فرق معنوي لكسر MS فقط ($P = 0.004$). منطقة ROC للبروتين كاربونيل تساوي واحدة مع أعلى حساسية وخصوصية.
الاستنتاج: سجلت النساء الحوامل شدة تسوس أسنان أعلى مع أكسدة البروتين اللعابي العالي ولكن يقلل من آليات دفاع مضادات الأكسدة اللعابية. يعتبر كاربونيل البروتين اللعابي علامة حيوية أكثر مثالية وصالحة وحساسة في الكشف عن حالة الإجهاد التأكسدي أثناء الحمل.