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Evaluation of Salivary Oxidant-Antioxidant Statuschanges during Pregnancy

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Abstract

Introduction: Hormonal changes during pregnancy leads to changes in most of body organs including oral cavity and saliva. This study has been done in order to evaluate the changes of salivary oxidant-antioxidant system during pregnancy. Materials and methods: This cross-sectional study has been done on pregnant women referring to obstetrics and gynecology clinic of Rouhani hospital, Babol, Iran, during 2014-2015. Forty eight pregnant womenwere selected and divided in two groups of 24 pregnant mothers including: group 1: first trimester pregnant women, group 2: second trimester pregnant women. Saliva samples of all mothers were gathered with spitting method and recollected again after 3 months. Total Antioxidant Capacity (TAC) andMalondialdehyde (MDA) level were measured by FRAP(Ferric Reducing Antioxidant Power) andTBARS(Thiobarbituric acid Reactive substances), respectively. Cabonyl level was measured by carbonyl kit. Data were analyzed by SPSS-18. Results: Two of pregnant mothers in each group were excluded because of abortion and 22 pregnant mothers were remained. Oxidant-antioxidant serum levels of MDA (p=0.31), carbonyl (p=0.74) and TAC (p=0.61) were not significantly changed in second trimester comparing to first trimester in the first group. In the third trimester, TAC level was significantly higher than the second trimester (p=0.02), however, this was not applicable for MDA and carbonyl levels. Comparison of third trimester/second trimester ratio with third trimester/second trimester ratio was insignificant in all oxidant-antioxidant parameters. Discussion and conclusion: Results revealed that level of MDA and carbonyl oxidants do not have any significant changes during pregnancy and total antioxidant capacity is the only parameter which remarkably increases in third trimester comparing to second trimester.

Key words: Salivary Oxidant-Antioxidant System, Malondialdehyde, Carbonyl, Total Antioxidant Capacity.

Introduction

Balance of oxidant-antioxidant system is one of the most important defense mechanisms of body whichever disturbance of this balance will have three consequences: 1- increase in oxidant products 2- decrease of antioxidant capacity 3-combination of these two consequences, which this imbalance, leads to free radical formation (Potdar et al., 2009). Reactive oxygen species (ROS) is one of the important free radicals that their intracellular or extracellular increase over the physiologic rates, may causes oxidative stress (Arikan et al., 2009). Oxidative stress indicates imbalance of oxidant / antioxidant status (Sies, 1997) which is accompanied by various pathologic intracellular

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and extracellular effects due to its capability of changing intra and extracellular macromolecules such as protein, lipid, DNA and destruction of cellular function. Antioxidant is a substance that can prevent or delay oxidative damage to target molecules. When imbalance of free radicals and oxygen reactive species occurs, injury of immune cells happens that finally increase the risk of infection and inflammation (Potdar et al., 2009; Arikan et al., 2009; Sies, 1997; Çağlayan et al., 2008).

Pregnancy is one of the processed in which oxidant/antioxidant status has a great role in it and oxidative processes plays a fundamental regulatory role during pregnancy (Jenkins et al., 2000; Leal et al., 2011) which is secondary to increased metabolic activity in placental mitochondria (Fialova et al., 2006; Biondi et al., 2005). Hormonal changes and increase of estrogen and progesterone during pregnancy may lead to changes in most of body organs including oral cavity which qualitative and quantitative changes in saliva is one of them. Any change in amount and quality of saliva may causes oral health issues (SEIFI et al., 2011).

Malondialdehyde(MDA) is the main product of peroxidation of unsaturated fatty acids and used as the marker of oxidative stress. The most common method of evaluation of oxidative stress is the method that measures the concentration of peroxidation products (Çağlayan et al., 2008). Carbonyl is used to test proteins' peroxidation based on reaction of 2,4-Dinitrophenylhydrazine (DNPH) with carbonyl produced by protein peroxidation (Levine et al., 1994). Antioxidant system is one of the most crucial parts of salivary and serum defense systems. Saliva, as the first defense system, has various antioxidants that each of them is precious for the immune system. Therefore, average activity of all antioxidants together is more effective than one antioxidant alone (Çağlayan et al., 2008).

In most of studies on oxidant/antioxidant status during pregnancy, serum samples were used. Due to simple,non-invasive and relatively low cost salivary sample collection (SEIFI et al., 2011) and considering that saliva contains various oxidant/antioxidant systems and its MDA level clearly reflects the level of circulating MDA (Öztürk et al., 2010), evaluation of saliva may reveal multisystem oxidant capacity of the organism (Kolarzyk, et al., 2006). Since saliva is equipped with various antioxidants, then TAC is used in this study which shows average activity of all antioxidants together (Çağlayan et al., 2008). Therefore, due to important role of oxidant/antioxidant status in pregnancy, different changes during pregnancy months and its effects on mother and fetus status, various oral mucosa changes and oral pathologies during pregnancy which the oxidant/antioxidant status can be a risk factor for that, beside fewer studies on evaluation of salivary oxidant/antioxidant status comparing to studies on serum, we concentrated on salivary samples and evaluated the changes of oxidant/antioxidant status in pregnant women during different pregnancy trimesters.

Materials and Methods:

This cross-sectional study was held on pregnant women referring to obstetrics and gynecology clinic of Babol Rouhani hospital during years 2014-2015. Sample size was calculated by convenient sampling method and with confidence interval of 95% and power of 80%, 24 samples were considered for each group. First and second trimester pregnant women were included. Exclusion criteria were: having any systemic diseases, taking immunosuppressive drugs, NSAIDs, antioxidants, topical steroid in recent month and alcohol, smoking, active and extensive dental decays, chronic infections and abscesses, periodontal diseases above 3 millimeter pocket and other oral mucosal lesions. Patients were divided in 2 groups of 24 individuals (group 1: first trimester, group 2: second trimester). All the patients were informed about the process of the study and written consents were taken. General information of the patients including age (year) and medical and dental history were documented in a prepared form. All the individuals underwent oral examination to exclude presence of any active dental decay, periodontitis and any oral mucosal lesion. This survey was thoroughly done by a dentistry resident. Salivary sampling were done twice in both groups. First sampling were done in the first and the second group in first and second trimester, respectively. The second samples were taken after 3 months in both groups. Unstimulated saliva was gathered by spitting method.In order to do that, it was asked from the patients not to eat or drink or brushing their teeth in 90 minutes prior sampling. Samples were gathered during 9-11 AM. Patients, while sitting in a relaxed position and leaning forward, collected their samples during a 10 minute period and spitted 1 to 2 times per minute in test tubes (sterilized formerly). The samples were sent to biochemistry laboratory as soon as possible. In the lab, samples were centrifugedwithCelmet 2000 for 10 minutes at 2000 rounds per minute. TAC of saliva were measured by FRAP(Ferric Reducing Antioxidant Power) methodby which saliva was exposed to Fe3+. Revival of iron by means of salivary antioxidants indicated their activity. Result of the reaction is a blue colored pigment which was measured through Jenwayspectrophotometerin wave length of 593nm in comparison with FeSO4 standard curve.

After preparation of FRAP solution, 1.5 liter of this solution were heated to 37°C. Then, 50 microliters of saliva were added to this solution for initiation of reaction. Samples and standard solution absorptions were measured in wave length of 593nm and 37°C. Considering optical absorption of the standard solution, the standard curve was drawn. By comparison of optical absorption changes of the sample with standard solution in standard curve, antioxidants' concentration of the sample were measured.

MDA were measured by TBARS in a way that salivary MDA reacted with TBA (Thiobarbituric acid) in 90-100°C and in pH=2-3 during 15 minutes. The result of reaction between MDA and TBA is a pink pigment with a maximum absorption at 532 nm measured by spectrophotometry. One portion from sample was mixed with two portions from a solution including TBA(w/v) 0.375%, Thiochloroacetic acid15%(w/v) and hydrochloric acid 0.25N and this mixture was put in boiled water bath for 30 minutes. After cooling, the sample was re-centrifuged for 15 minutes at 3000 rounds per minute. Then, its absorption was measured in wave length of 593nm through spectrophotometer. Finally, MDA was calculated by formula of 1.56 x 105M-1CM-1 as molar absorptivity.

Measurement of carbonyl is based on 2,4-Dinitrophenylhydrazine (DNPH) reaction with carbonyl of proteins an formation of schiff bases which produced yellow color and has maximum of absorption in 405nm. In this study, a commercial carbonyl kit was used.

It should be mentioned that all of the chemicals used in this study –except from TPTZ (Sigma) and carbonyl (Stabiofarm)- were products of Merck factory. Data were coded and were statically analyzed with SPSS-18. Independent sample T-Test, Paired sample T-Test, Mann-Whitney test and correlation coefficients were used. P-value <0.05 considered statically significant.

Results:

In this study, 24 pregnant mothers were in each first and second group. Two patients in the first and two patients in the second group were excluded because of abortion. Overall mean age of mothers were 26.22 ± 3.87 and it was 26.40 ± 3.43 and 26.04 ± 4.34 in the first and the second group respectively. Serum level of oxidant/antioxidants of MDA, carbonyl and TAC in the second trimester was not significantly higher than the first trimester. Table 1 shows the salivary oxidant/antioxidant levels of second trimester in comparison with the first trimester in the first group. Salivary level of TAC in the second trimester was 226.30 ± 127.31 and it was 269.45 ± 120.27 in the third trimester (p=0.02). Table 2 demonstrates comparison of oxidant/antioxidant levels in second and third trimesters in the second group. Considering both groups, mean level of salivary MDA was 1.86 ± 0.71, carbonyl level 111.58 ± 21.21 and salivary TAC level was 263.99 ± 142.78 in the second trimester. Second/first trimester MDA ratio was 1.24 ± 0.68 and this ratio was 0.92 ± 0.28 for third /second trimester which did not have significant difference (p=0.19). Carbonyl ratio for second/first trimester and third/second trimester were 1.18 ± 0.88 and 0.99 ± 0.45, respectively (p=0.2). TAC ratio for second/first trimester was 1.05 ± 0.53, however, this ratio was 1.57 ± 1.11 for third/second trimester (p=0.06).

Table 1- Oxidant-antioxidant level in second trimester comparing to first trimester

Oxidant/antioxidant	Number of cases	Minimum	Maximum	Mean ±standard deviation	p-value
First trimester MDA	22	0.89	2.71	1.60 ± 0.51	0.31
Second trimester MDA	22	0.71	3.15	1.79 ± 0.65	
First trimester Carbonyl	21	29	162	112.03 ± 32.56	0.74
Second trimester Carbonyl	21	50	197	114.97 ± 33.66	
First trimester TAC	22	78.12	668.81	319.17 ± 166.95	0.50
Second trimester TAC	22	37.88	617.91	301.69 ± 150.19	

Table 2- Oxidant-antioxidant level in third trimester comparing to second trimester

Oxidant/antioxidant	Number of cases	Minimum	Maximum	Mean ±standard deviation	p-value
Second trimester MDA	22	0.97	4.05	1.93± 0.77	0.26
Third trimester MDA	22	0.64	3.07	1.70± 0.55	
Second trimester Carbonyl	22	52	160	108.39±26.72	0.13
Third trimester Carbonyl	22	30	148	97.74±29.70	
Second trimester TAC	22	36.69	465.21	226.30 ± 127.31	0.02
Third trimester TAC	22	76.94	583.58	269.30±120.27	

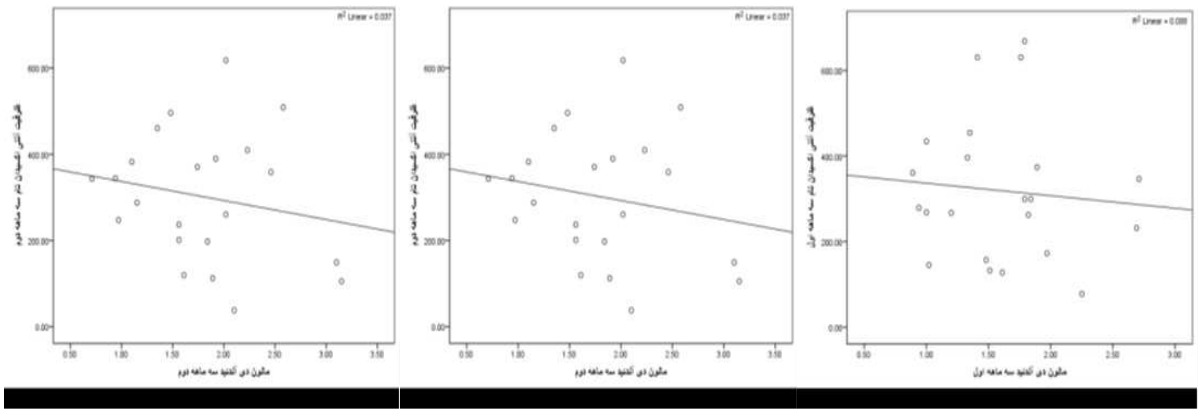

Chart 1-3. Correlation between MDA and TAC according to each trimester

Conclusion

In this study, 48 pregnant women referring to Babol Rouhani Hospital who did not have any systemic diseases, oral lesions and dental decays, smoking, alcohol consumption and taking any drug except pregnancy-related medications were evaluated. Twenty four patients were in the first trimester group as well as the second trimester group. In both two patients in both groups were excluded due to abortion, therefore, 22 cases remained in each group.

This study, which was designed to evaluate the changes of salivary oxidant/antioxidant status, revealed that salivary oxidant levels of MDA and carbonyl do not notably change during pregnancy and only TAC in third trimester had significant change comparing to the second trimester, however, this increase was insignificant in comparison of second trimester with first trimester.

Results of Seifi et al. study in Babol indicated that TAC level increased during first to third trimester of pregnancy comparing to before pregnancy. There was a statically significant difference in antioxidant levels of second and third trimester which is similar to our findings. In both studies, FRAP was used for measurement of salivary TAC. In Seifi study, maximum level of TAC was in second trimester, however, in our study minimum level of it was in the second trimester which this finding is similar to Kilarzyk study result in Poland. It was mentioned that pregnant women do not adapt with increase of antioxidants in second trimester.

Results of Loy et al. study in Malaysia showed that TAC in third trimester was remarkably higher than second trimester which this is the same as our findings. Although the method and condition of TAC measurement were the same in both studies, serum TAC level was measured in Loy study and that is responsible for the noteworthy difference of these two study results. In our study, salivary TAC level was 269.45 in the third and 263 micromoles in the second trimester which these were much fewer than the amounts found in Malaysia study (Silk et al., 2008; Loy et al., 2013).

Study of Yuksel et al in Turkey showed that level of MDA in third trimester declined comparing with second and first trimester which this was different with what we found (YÜKSEL and YİĞİT, 2015). Although the maximum level of MDA was in second trimester in our study, there were no significant difference in third/second trimesters' and second/first trimesters' ratios. We used salivary samples in our study while serum level of antioxidants were measured in study of Turkey. As it was mentioned previously, painfulness and stressfulness of sampling can affect the result. MDA is the main product of unsaturated fatty acid peroxidation. Loy study revealed that there is a relation between lipid profile of pregnant women and their antioxidant status. Unevaluated lipid profiles of pregnant women could be one of the probable hypothesis for these different results.

The study of Atiba et al in Nigeria demonstrated that serum MDA level in third trimester was significantly higher than second trimester which this finding is different from ours (Atiba et al., 2014). MDA level in third and second trimesters were 1.7 and 1.86 micromoles respectively, which were fewer than Nigeria results. One of the explanations could be the lower level of antioxidants in saliva(which we used for samples) rather than serumlevel of them. In Nigeria and some other studies such as Saikumar et al in India, mean MDA level in second trimester was higher than the first trimester. Serum level of antioxidants were measured (Saikumar et al., 2013).

In this current study, there were no correlation between salivary MDA level and TAC in any of trimesters. Probably small size of sample is responsible for this.

Salivary sampling is one of strength points of our study. Blood sampling is painful and this may affect oxidant/antioxidant status. Moreover, few studies have been done with salivary sampling for evaluation of oxidant/antioxidant status during pregnancy. One of the limitations of this study was inability to following up of cases in all trimesters. Thus, cases were divided in two groups and followed up for 3 months. The other weak point of this research was lack of measurement of weight and lipid profile of the cases. Some studies indicated that diet and lipid profile of pregnant women may affect the status of antioxidants during pregnancy.

The results of this study which aimed to evaluate the changes of oxidant/antioxidant status during pregnancy, demonstrated that salivary MDA and carbonyl oxidants do not alter significantly during pregnancy and only TAC increases notably in third trimester comparing to second trimester. Oppositely, this increase is not remarkable in comparison of second and first trimesters. It is assumed that some factors including sampling method (saliva or serum), diet, anthropometric criteria such as mothers' weight could have an effect on the results. Hence, performing more studies is suggested.

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