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Salivary Uric Acid and Protein Carbonyl in Relation to Gingival Health Condition Among Group of Pregnant Anemic Women

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ABSTRACT

Background: Anemia during pregnancy is a medical disorder that led to changes in the function of the human body and changes in the oral health condition, it considered stressful condition that led to oxidant –antioxidant imbalance as decrease in the salivary antioxidant and increase in the oxidative stress.

Methods: The study sample was consisted of 90 women divided into three groups including 30 pregnant anemic and 30 pregnant not anemic women (first pregnancy and in the second trimester), from the mothers and infant's health service center in Baghdad city, their age range was 20-25 years, in addition to 30 women as control group of newly married not-pregnant not anemic and match with age. Plaque index was used to assess dental plaque thickness, calculus index was used to assess dental calculus extension and gingival index was used to asses gingival health condition. Unstimulated saliva was collected then salivary uric acid and salivary protein carbonyl were analyzed.

Result: Data analysis of the current study revealed that the plaque index of pregnant anemic group was found to be higher than that of pregnant not anemic and control group. The difference was statistically highly significant between pregnant anemic and pregnant not anemic groups and significant between pregnant anemic and control groups. However the statistical difference between pregnant not anemic and control was not significant (P>0.05). The calculus index of pregnant not-anemic group was found to be higher than that of pregnant anemic and control group. It was found that statistical difference was significant only between pregnant anemic and control groups, while it was not significant between other groups (P> 0.05). The gingival index of pregnant anemic group was found to be lower than that of pregnant not anemic, but both the mentioned groups had gingival index higher than that of control group. The difference was statistically highly significant between different groups (P< 0.01). The level of salivary antioxidant (uric acid) of pregnant anemic group were found to be the lowest with statistically highly significant difference between each two groups. The salivary protein carbonyl of pregnant anemic group was found to be the highest with statistically highly significant differences between pregnant anemic and not anemic groups (P<0.01). There was a negative correlation between plaque index and (salivary antioxidants and protein carbonyl) in the pregnant anemic women but, it was a positive correlation in pregnant not anemic women. There was a positive correlation between calculus index and salivary antioxidant in the pregnant anemic and not anemic women. There was a positive correlation between calculus index and protein carbonyl in pregnant anemic women while, there was a negative correlation between calculus index and protein carbonyl in pregnant not anemic women. There was a positive correlation between gingival index and protein carbonyl in pregnant anemic women while, there was a negative correlation between gingival index and protein carbonyl in pregnant not anemic women. Highly significant positive correlation was found between gingival index and each of plaque index and calculus index in both pregnant anemic and not anemic groups .

Conclusion: This study reported decrease in the salivary antioxidant and increase in the oxidative stress status among pregnant anemic women which worsen the determintal effect of pregnancy on oral health.

Key words: PlI, CalI, GI, Uric acid, Protein carbonyl

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INTRODUCTION

Pregnancy is a special time through women's life. Provided by complex physiological changes, oral health adversely affected by these changes [1]. Pregnancy in humans adjusted by anatomic, physiological, and metabolic changes in the mother for providing her with nutritional and metabolic needs and concept of growing [2]. Some of systemic changes such as weight gain, renal systemic changes , changes in respiratory function, cardiovascular changes caused by physiological and metabolic changes [3,4]. Several conditions of oral health are in relation to pregnancy such as periodontal diseases. Periodontal disease are the common chronic inflammatory diseases of different etiological factor, the most common type of periodontal diseases that seen in pregnancy women is gingivitis [5]. There are conflicting suggestions about the main reason of gingival inflammation that occur in the pregnancy [6].

It may be due to poor oral hygiene, and hormonal changes during pregnancy. Many Iraqi studies measured the severity of gingivitis through pregnant women [7-10]. Anemia is the most common haematological condition that occurs in pregnancy, anemia occurs when the concentration of the peripheral blood hemoglobin (Hb) is less than or equal to 11 gm/ dl, according to the latest norm set by 'WHO' [11]. Iron deficiency is the commonest cause of anemia. One of causative factors of anemia is erythrocyte reactive oxygen species (ROS) which upraised either by activating the development of ROS or suppressing the antioxidative/redox mechanism [12].

Pregnancy caused many changes in the body's biochemical function that require high demand of energy and increase oxygen requirement, then increase oxygen utilization resulting in providing of ROS and rising the levels of free radicals and oxidative stress [13]. Pregnancy responsible for increase the oxidative stress, which defined as a difference in the peroxidant–antioxidant equilibrium [14]. The basic function of antioxidants is the neutralization of agitated free radicals thus reduce its potential to harm. Aksakalli et al. [15] found that Salivary antioxidants are oxidation inhibition protecting against reactive oxygen species that induced gingival tissue damage. Saliva contains many types of antioxidants such as uric acid, total protein and superoxide dismutase (SOD) [16]. As far as there was no previous Iraqi study available concerning estimation of antioxidant status in saliva and gingival health status among pregnant anemic women, consequently this study was conducted to explain whether the gestational anemia aggravate the maternal oxidative stress and gingival condition.

MATERIALS AND METHODS

The sample

The study was consisted of three groups including pregnant anemic and not anemic women (First pregnancy and in the second trimester), from the mothers and infant's health service center in Baghdad city, their age range was 20-25 years, in addition to control group of newly married not-pregnant not anemic women and match with age. The age was measured according to the last birthday [17]. Additionally, for the control group, the women were not examined during menstrual cycle. Salivary Unstimulated samples was collected under uniform conditions, it was conducted according to the instructions constructed via [18].

saliva was placed into cooler box and send to the laboratory and centrifuge for 10 minutes at 3000rpm; then separation of the supernatant was done by micropipette and keep in deep freezing (-20C) [19] for the subsequent analyses of selected salivary constituent using special kits and according to manufacturer's instructions. Salivary uric acid was assessed calorimetrically. Uric acid in the sample originates by coupled reactions, spectrophotometry used to measure the colored complex [20-24]. Salivary protein carbonyl was assessed by using biotin double antibody sandwich technology-based enzyme-linked immune sorbent assay (ELISA) to assay human sandwich technology protein carbonyl. Dental plaque thickness was assessed by using plaque index (PlI) of Silness et al. [23] using mouth mirror and dental explorer. Dental

calculus extent was assessed according to the criteria of calculus component of periodontal disease index (PDI) by Ramfjord et al. [22] using mouth mirror and dental explorer. Gingival index was assessed by using gingival index (GI) of Loe et al. [21] using mouth mirror and WHO (CPI) probe. The collected data were introduced into Microsoft excel sheet 2016, and loaded into statistical package for social science (SPSS version 24) for analysis. The confidence limit was accepted at 95% (P <0.05).

RESULTS

Result showed that the median and mean rank values of the plaque index of pregnant anemic group was found to be higher than that of pregnant not anemic and control group. The difference was statistically highly significant between pregnant anemic and pregnant not anemic groups and significant between pregnant anemic and control groups. However, the statistical difference between pregnant not anemic and control was not significant (P >0.05). The calculus index of pregnant not-anemic group was found to be higher than that of pregnant anemic and control group. It was found that statistical difference was significant only between pregnant anemic and control groups, while it was not significant between other groups (P> 0.05). the gingival index of pregnant anemic group was found to be lower than that of pregnant not anemic, but both the mentioned groups had gingival index higher than that of control group. The difference was statistically highly significant between different groups (P< 0.01).

Result showed that the median and mean rank of salivary uric acid of pregnant anemic group was the lowest with statistically highly significant difference between pregnant anemic and both groups.

The salivary protein carbonyl of pregnant anemic group was found to be higher than that of pregnant not anemic group, with statistically highly significant difference between them (P<0.01).

Result showed that no significant positive correlation between plaque index and protein carbonyl in pregnant not-anemic women, but negative not significant correlation for both anemic pregnant and control groups. No significant positive correlation between both calculus index and gingival and protein carbonyl for both pregnant anemic and control groups, but not significant negative correlation in pregnant not-anemic group.

Result showed that no significant positive correlation between protein carbonyl and salivary uric acid in pregnant anemic group and pregnant not anemic women. In control group a negative not significant correlation between protein carbonyl and salivary uric acid (Tables 1-6).

Table 1: Dental plaque, dental calculus, and gingival indices (median, mean rank) and statistical differences among different groups.

	Variables\Groups	Median	Mean Rank	Chi square	df	P value	Pair wise comparison		
							1-2	1-3	2-3
PII	Pregnant anemic	1	52.27	9.48	2	00.009**	0.003**	0.023*	0.781
	Pregnant not anemic	1	49.85						
	Control	0.93	34.38						
CalI	Pregnant anemic	0	46.72	7.198	2	00.027*	0.064	0.011*	0.261
	Pregnant not anemic	0.06	52.83						
	Control	0	36.95						
GI	Pregnant anemic	1	54.18	28.91	2	0.001**	0.001**	0.001**	0.001**
	Pregnant not anemic	1	56.17						
	Control	0	26.15						

*Significant **Highly significant

Table 2: Salivary uric acid (median, mean rank) and statistical differences among different groups.

Variables\Groups		Median	Mean Rank	Chi-square	df	P value	Pair wise comparison		
							1-2	1-3	2-3
Salivary uric acid (mg\dl)	Pregnant anemic	2.4	19.05	70.797	2	0.000**	0.001**	0.001**	0.001**
	Pregnant not anemic	3.2	42						
	Control	5	75.45						

**Highly significant

Table 3: Salivary protein carbonyl (median, mean rank) and statistical differences among different group.

Variables\Groups		Median	Mean rank	Chi-square	df	P value	Pair wise comparison		
							1-2	1-3	2-3
Salivary protein carbonyl (M\L)	Pregnant anemic	80.09	54.93	3.544	2	0.022**	0.005**	0.188	0.16
	Pregnant not anemic	72.46	45.3						
	Control	68.97	36.27						
* significant** highly significant									

Table 4: Correlation coefficient between (plaque index, calculus index and gingival index) and salivary uric acid.

Variable	Pregnant anemic PII		Pregnant not anemic PII		Control PII	
	r	P	r	P	r	P
Uric acid (mg\dl)	-0.124	0.514	0.118	0.534	-0.345	0.062
	CaII		CaII		CaII	
	0.178	0.346	0.008	0.966	0.041	0.83
	GI		GI		GI	
	0.139	0.464	-0.09	0.635	0.012	0.948

Table 5: Correlation coefficient between (plaque index, calculus index and gingival index) and salivary protein carbonyl.

Variable	Pregnant anemic PII		Pregnant not anemic PII		Control PII	
	r	P	r	P	r	P
Protein carbonyl(M\L)	-0.124	0.514	0.03	0.877	-0.306	0.1
	CaII		CaII		CaII	
	0.239	0.204	-0.205	0.277	0.152	0.422
	GI		GI		GI	
	0.028	0.882	-0.133	0.484	0.068	0.72

Table 6: Correlation coefficient between salivary uric acid and salivary protein carbonyl.

Variable	Pregnant anemic Salivary protein carbonyl (M\L)		Pregnant not anemic Salivary protein carbonyl(M\L)		Control Salivary protein carbonyl(M\L)	
	r	P	r	P	r	P
Uric acid (mg\dl)	0.018	0.927	0.044	0.819	-0.026	0.893

DISCUSSION

Pregnancy is a normal physiological condition accompanied by biochemical changes [25]. Pregnancy also is stressful condition and during it there is an increase in the oxidative stress because of high energy demand and there is an increase in the requirements for tissue oxygen [26]. The oxidative stress can be counteracted by the free radical trapping and antioxidant during normal pregnancy through enzymatic induction and activity and through non enzymatic free radical protectors and scavenging [27]. Including normal pregnant women, the concentration of hemoglobin decreases as the volume of blood in circulation increases. Ever after iron and folic acid are preferentially transported to the fetus in quantities appropriate for the fetus, anemia with iron deficiency and anemia with folic acid deficiency are likely to develop in the mother [28,29]. In this study, plaque index was higher in pregnant anemic than not anemic with statistically highly significant difference, explanation of this result attributed to the synergistic effect of both ROS–induced oxidative stress and aumanting inflammation owing to anemia. hypersegmanted neutrophils found in anemia can cause immune suppression by directly suppressing T-cell response by creation of an immunological synapse and the direct delivery of hydrogen peroxide resulting in the accumulation of plaque and tissue damage. Inhibition the activity of iron–dependent myeloperoxidase, which mediated the anti-bacterial activity of macrophage that increasing plaque index, the same result recorded by Sharma et al. [30]. The finding of the current study demonstrated that calculus index was higher among pregnant not anemic women as compared with pregnant anemic women, this may be related to reduced salivary calcium concentration in anemic patient [31] and higher salivary flow rate among pregnant anemic according to the result of this study. By increasing salivary flow rate, the concentration of salivary calcium and phosphorus decrease [32], there by calcification of dental plaque and

calculus formation decrease. This explanation is supported by inverse relation between calculus index and salivary flow rate among pregnant anemic women .In this study , gingival index in pregnant anemic lower than not anemic women with statistically highly significant difference , the explanation of this result may be related to pale gingiva in anemic patients because of low blood Hb concentration and therefore anemia masked the expression of gingival inflammatory signs which include redness and bleeding which were including in criteria of diagnosis of gingival index by Loe et al. [21] that was used in this study. This result agree with Sharma et al. [30]. In this study, salivary uric acid was lower in pregnant anemic women than not anemic with statistically highly significant difference, explanation of this result attributed to anemia that associated with decrease in hemoglobin concentration or decreased red blood cell and because the high energy demand and increased oxygen requirement in pregnant women that favors the imbalance between oxidants and antioxidants, the result of this imbalance is oxidative stress which could be more marked in anemic pregnant women and this lead to decrease in salivary uric acid [33]. The same result was reported by others [34,35]. In the present study , salivary protein carbonyl was higher in pregnant anemic than pregnant not anemic women with statistically highly significant difference, explanation of this result attributed to anemia that lead to decreased level of haemoglobin might be due to the fact that iron is an essential constituent of heme and when its levels are low, it may lead to decreased haemoglobin synthesis and this associated with increased level of PC in pregnant women with anemia , which may be attributed to over production of reactive oxygen species (ROS) or a deficiency of antioxidant defense [36]. Although previous studies have suggested that anemia may be related to increased protein carbonyl [37,38], however, its mechanism has not been completely clarified.

CONCLUSION

This study reported decrease in the salivary antioxidant and increase in the oxidative stress status among pregnant anemic women which worsen the determintal effect of pregnancy on oral health.

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