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ORIGINAL ARTICLE

Salivary and Serum Antioxidants in Women with Preeclampsia with or Without Periodontal Disease

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About the Author

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Abstract

Background and Objectives Preeclampsia is a challenging disease of human pregnancy that affects the mother and her foetus. It is a common obstetric syndrome of undefined aetiology affecting 7–10% of pregnant women. This study aimed to investigate the total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, malondialdehyde levels, uric acid, vitamin C, and vitamin E levels in serum and saliva of preeclamptic and normotensive pregnant women with and without periodontitis.

Methods Sixty pregnant women, divided into four groups, preeclamptic women with and without periodontitis, and normotensive pregnant women with and without periodontitis, were recruited between 26 and 36 weeks of gestation from the Outpatient Department of Obstetrics and Gynaecology, KS Hedge Hospital, Mangalore. Pocket depth, clinical attachment loss, and gingival index were measured in all subjects. Saliva and serum levels of TAC, SOD, and GPx activities, malondialdehyde levels, vitamin C, vitamin E, uric acid were determined using spectrophotometer.

Results Comparison of sociodemographic variables among the study groups showed no significant differences.

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Salivary malondialdehyde levels were highest ($p = 0.025$), levels of antioxidants vitamin C ($p = 0.027$) and uric acid ($p = 0.013$) being significantly lower in preeclamptic women with periodontitis. This group also had significantly lower serum levels of vitamin C ($p = 0.041$) and SOD ($p = 0.004$).

Conclusions The results of our study show that periodontal disease may be potential risk factor for severity, progression, and even initiation of preeclampsia because of reduced antioxidant capacity or increased oxidative stress.

Keywords Periodontitis · Antioxidants · Preeclampsia

Abbreviations

SOD	Superoxide dismutase
GPx	Glutathione peroxidase
TAC	Total antioxidant capacity
PP	Preeclamptic pregnant women with periodontal disease group
PH	Preeclamptic pregnant women with periodontal health group
NP	Normotensive pregnant women with periodontal disease group
NH	Normotensive pregnant women with periodontal health group
SD	Standard deviation
PD	Pocket depth
CAL	Clinical attachment level

Introduction

Preeclampsia is a challenging disease of human pregnancy that affects the mother and her foetus. It is a common obstetric syndrome of undefined aetiology affecting 7–10% of pregnant women and remains one of the two most common causes of maternal mortality in the developing world. Recently, an excessive systemic inflammatory response was found to be associated with preeclampsia. It has been postulated that maternal periodontal disease may burden the pregnant women systemically with endotoxin, inflammatory cytokines, and oxidative stressors at the maternal–foetal interface. Thus, it may be a vascular stressor that plays a role in the development of preeclampsia in pregnant women. Preeclamptic pregnant women were found to have higher level of cytokines in saliva, and gingival crevicular fluid, and also associated with some microorganisms [1].

There is increasing evidence that oxidative stress is an important contributing factor in pathogenesis of preeclampsia and periodontal disease. Malondialdehyde is an indicator of lipid peroxidation and one of the final decomposition products of lipid peroxidation, which has numerous deleterious effects on biologic systems. Free radicals released from poorly perfused fetoplacental unit initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes. The endothelial cells lining the high pressured arterial vessels are vulnerable to oxygen free radical initiated by lipid peroxidation. Various studies have observed increased lipid peroxidation in periodontal pocket and oral environment of subjects with periodontitis, and it contributes to pathogenesis of periodontal disease [2, 3]. Free radicals are neutralized efficiently by nutrient-derived antioxidant small molecules like vitamin C, vitamin E, carotenes, flavonoids, and uric acid. Uric acid is a predominant salivary antioxidant with albumin and ascorbate providing minor contributions. It was suggested in previous studies that estimation of uric acid in saliva may be a better index of uric acid production in body than uric acid concentration in blood or urine. Antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) protect the tissues against oxidative injury from free oxygen radicals generated by various metabolic processes. Antioxidant molecules are present in all body fluids and tissues. The function of SOD is to remove damaging reactive oxygen species from cellular environment by catalysing the dismutation of two O_2 to H_2O_2. Glutathione perioxidase-1 prevents cytotoxic peroxide-induced oxidative damage, lipid peroxidation, and protein degeneration [4].

Various studies have observed reduced total antioxidant capacity in pregnant women and explained that this could be associated with adverse periodontitis and pregnancy interactions [5]. A hypothesis has been observed that periodontal disease may be a risk factor for development of preeclampsia [6]. To our knowledge, there are not many studies on antioxidant status in pregnant women with or without periodontal disease. The aim of this study was to investigate the total antioxidant capacity (TAC), SOD and GPx activities and malondialdehyde levels, uric acid, vitamin C, vitamin E, levels in serum and saliva of preeclampsia and normotensive pregnant women with and without periodontitis.

Materials and Methods

The study population consisted of 60 pregnant women, divided into four groups; Group A—15 preeclamptic pregnant women with periodontal disease (PP), Group B—15 preeclamptic pregnant women with periodontal health (PH), Group C—15 normotensive pregnant women with periodontal disease (NP), Group D—15 normotensive pregnant women with periodontal health (NH), were

recruited between 26 and 36 weeks of gestation from the Obstetrics and Gynaecology Outpatient Department, KS Hedge Hospital, a Teaching Hospital affiliated to KS Hegde Medical Academy, Nitte University, Deralakatte, Mangalore, India. Necessary ethical clearance was obtained from Ethics Committee of the Hospital (reference number is ABSM/CC/1/2014, dated 08-01-2014). After informed consent, maternal sociodemographic and medical data were obtained using structured questionnaires. The inclusion criteria were women aged >18 years, carrying singleton, pregnancies >26 weeks gestation, and systemically healthy women. Exclusion criteria were twin pregnancies, overt diabetes and heart disease, women who have undergone oral prophylaxis for 6 months. The study is a cross-sectional study conducted between May 2014 and May 2015 at the study site.

Preeclampsia was defined as blood pressure ≥140/90 mmHg on more than two occasion 4 h apart and 1+ or more proteinuria by dipstick (Multistix® SG Reagent strips, Bayer Diagnostics India Limited) on a random urine sample. periodontal disease was defined as clinical attachment loss (CAL) ≥ 3 mm and a pocket depth (PD) ≥ 4 mm in total 30% sites examined, according to American Academy of Periodontology [7, 8]. The following oral health parameters were collected, gingival index [9], Plaque index [10], PD, and CAL. Clinical attachment loss (CAL) was measured from cemento-enamel junction to the base of the pocket, using UNC-15 periodontal probe (Hu-Friedy®, Chicago, IL, USA) at 4 sites per tooth on all teeth of dentition except third molars. Pocket depth (PD) was measured from gingival margin to base of the pocket. Gingival index (Loe and Silness) was recorded to assess gingival status.

The saliva sample was collected from 60 pregnant women in the morning after overnight fast [11]. Subjects were asked not to eat or drink anything in the morning. One to 2 ml of whole saliva was obtained by expectorating into disposable tubes which was centrifuged at 1000 g for 10 min at 4 °C. The sample was stored at −80 °C until the estimation of TAC, GPx, malondialdehyde, uric acid, vitamin C, vitamin E done using Spectrophotometer. Serum preparation—blood samples from 60 pregnant women were collected in plain tubes, without additives and centrifuged at 3500 g for 5 min to get serum. Aliquots were stored at −80 °C until analysis. All descriptive data were expressed as mean ± standard deviation (SD). Comparison of clinical parameter, gingival index (GI), and plaque index (PI), and various antioxidants in saliva and serum among the study groups was done using Kruskal–Wallis test. Difference in median values of PI, and GI, various antioxidants in saliva and serum among the groups was compared, and a p value of <0.05 was considered to be significant. Comparison of sociodemographic variables among the study groups was done by using Fisher's exact test. Pairwise comparisons among the intergroups were done for malondialdehyde, vitamin C, uric acid levels in saliva, vitamin C, and SOD levels in serum between different groups of pregnant women, using Mann–Whitney U test. A p value of <0.05 was considered to be significant. All data analyses were done using the statistical package SPSS version16 (IBM).

Results

Comparison of sociodemographic variables among the study groups is shown (Table 1). There were no significant differences in age, education, income, occupation, weight, parity among the various study groups.

There were significant differences in mean levels of malondialdehyde, vitamin C and uric acid in saliva, vitamin C level and SOD level in serum between different groups of pregnant women which are shown in Table 2. Pregnant women in PP (preeclamptic, periodontitis) group had highest level (1.31 ± 0.40) of malondialdehyde (μM/L) in saliva, and women in NH (normotensive, healthy periodontium) group had the lowest levels (0.93 ± 0.37). There was a significant difference in distribution of salivary malondialdehyde levels among the study groups ($p = 0.025$); however, the malondialdehyde level in serum did not differ significantly among the groups.

Significant difference in salivary vitamin C levels (mg/dL) was observed among different groups ($p = 0.027$). Lowest level was observed in PP (preeclamptic, periodontitis) group (1.23 ± 0.94) and highest vitamin C level was observed in NH (normotensive, healthy) periodontium group (1.56 ± 0.46). In serum, vitamin C levels were observed to be significant among groups ($p = 0.041$), lowest level was observed in PP (preeclamptic, periodontitis) groups (3.09 ± 1.26) and highest vitamin C level was observed in NH (normotensive healthy) groups (4.17 ± 0.77).

Uric acid levels (mg/dL) in saliva were observed to be lowest in PP (preeclamptic, periodontitis) group (1.11 ± 0.48) and highest in PH (preeclamptic, healthy) group (1.70 ± 0.58). The differences in the distribution of uric acid level among the pregnant women groups were statistically significant ($p = 0.013$).

SOD levels (U/mg) in serum were significantly different among the pregnant women groups ($p = 0.004$). Lowest SOD levels were observed in PP (preeclamptic, periodontitis) group (21.71 ± 4.84), and highest SOD levels were observed in normotensive healthy (NH) group (28.61 ± 4.82). Salivary SOD levels were not significantly different in intergroup pair analysis.

Table 1 Comparison of sociodemographic variables among the study groups

	Normotensive, healthy	Normotensive, periodontitis	Preeclamptic, healthy	Preeclamptic, periodontitis	*p* values	*p* value
Age						
20–25	6	2	6	2	5.93	0.435
26–30	5	9	5	8		
31–35	4	4	4	5		
Occupation						
Homemaker	10	11	11	14	3.574	0.359
Others	5	4	4	1		
Education						
Primary	1	6	4	7	7.115	0.313
High school	7	4	5	4		
College	7	5	6	4		
Income						
1000–3000	0	3	3	1	7.596	0.246
3000–5000	4	4	6	8		
>5000	11	8	6	6		
Parity						
Primi	10	10	10	10	0.146	1
Multi	5	5	5	5		
Weight						
45–55	8	8	8	7	0.312	1
≥56	7	7	7	8		

GPx (mg/dL), vitamin E levels (mg/dL) and total antioxidant capacity (mmol/L) in saliva and serum were observed to be higher in normotensive groups with healthy periodontium (NH) compared to preeclamptic groups; however, it was statistically non-significant.

Discussion

The results of this study indicate that serum vitamin C and SOD concentrations were least in preeclamptic women with periodontitis compared to normotensive pregnant women with periodontitis. In saliva vitamin C, uric acid concentrations were reduced in preeclamptic women with periodontitis compared to normotensive pregnant women with periodontitis. Malondialdehyde level was highest in preeclamptic pregnant women with periodontitis, signifying increased oxidative stress.

In saliva and serum, vitamin E, TAC, GPx concentrations were reduced in preeclamptic women with periodontitis group; however, the differences did not reach statistical significance. Our study also observed that among the various antioxidants used in this study significant association was observed only with vitamin C, malondialdehyde, uric acid concentration in saliva of preeclamptic, normotensive pregnant women with periodontal health and disease. Our study also suggests that above antioxidants levels are affected by periodontal disease in addition to impact of preeclampsia. Dahiya et al. [12] confirmed decreased SOD, catalase and glutathione peroxidase activity in periodontitis could be due to hyperlipid peroxidation in periodontal disease. Our study observed concentrations of serum SOD were least in preeclamptic women with periodontitis, which is consistent with the findings of these investigators.

Zang et al. reported that levels of TAC and SOD were significantly higher in chronic periodontitis patients than in patients with healthy periodontium and after periodontal treatment, there was a significant decrease in serum, saliva, gingival crevicular fluid, TAC, and SOD concentrations. This could be explained due to upregulation of antioxidant enzyme system in cells and tissues by oxidative stress [3].

Salivary malondialdehyde levels are found to be significantly higher in preeclampsia–periodontitis group in the current study. Malondialdehyde is an indicator of lipid peroxidation and one of the final decomposition products of lipid peroxidation, which has deleterious effects on biologic systems. Free radicals released from poorly perfused fetoplacental unit initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes [13]. Our study is also supported by Canakci et al. [13] and Khalili et al. [14] who assessed salivary malondialdehyde level in clinically healthy and periodontal diseased individuals concluded that increased concentrations of salivary

Table 2 Comparison of salivary and serum levels of malondialdehyde, vitamin C, uric acid, SOD, GPx, vitamin E, and TAC among the four subgroups of women

	Normotensive healthy (mean ± SD)	Normotensive periodontitis (mean ± SD)	Preeclampsia healthy (mean ± SD)	Preeclampsia periodontitis (mean ± SD)	*p* value
Malondialdehyde (μM/L)					
Saliva	0.93 ± 0.37	1.03 ± 0.33	1.30 ± 0.57	1.31 ± 0.40	0.025*
Serum	1.90 ± 0.93	1.64 ± 0.61	2.14 ± 0.74	2.17 ± 0.80	0.219
Vitamin C (mg/dL)					
Saliva	1.56 ± 0.46	1.33 ± 0.49	1.56 ± 0.34	1.23 ± 0.94	0.027*
Serum	4.17 ± 0.77	4.00 ± 1.20	4.16 ± 1.22	3.09 ± 1.26	0.041*
Uric acid (mg/dL)					
Saliva	1.57 ± 0.55	1.66 ± 0.57	1.70 ± 0.58	1.11 ± 0.48	0.013*
Serum	3.08 ± 0.90	3.16 ± 0.90	3.01 ± 1.36	2.92 ± 1.05	0.902
SOD (U/mg)					
Saliva	2.81 ± 0.78	3.02 ± 0.53	3.14 ± 0.74	2.95 ± 0.73	0.833
Serum	28.61 ± 4.82	22.74 ± 7.01	26.82 ± 7.88	21.71 ± 4.84	0.004*
GPx (mg/dL)					
Saliva	502.01 ± 197.52	659.82 ± 406.72	504.42 ± 159.98	424.92 ± 152.76	0.156
Serum	798.40 ± 346.46	705.60 ± 184.23	697.53 ± 134.33	683.67 ± 113.85	0.859
Vitamin E (mg/dL)					
Saliva	1.12 ± 0.30	0.95 ± 0.52	1.30 ± 0.56	1.43 ± 0.60	0.1
Serum	3.18 ± 0.85	2.73 ± 0.79	2.64 ± 0.79	2.45 ± 0.49	0.064
TAC (mmol/L)					
Saliva	0.56 ± 0.14	0.64 ± 0.26	0.92 ± 0.45	1.10 ± 0.66	0.078
Serum	0.96 ± 0.39	1.00 ± 0.53	1.38 ± 0.54	1.23 ± 0.46	0.055

*$p < 0.05$, statistically significant

Malondialdehyde in subjects with chronic periodontitis. Adithi et al. studied the effects of antioxidant therapy as an adjunct to treatment of periodontal diseases and concluded that salivary uric acid concentrations were lowest in individuals with periodontal disease, and after periodontal treatment was done salivary uric acid concentrations increased [15].

Our study is also supported by Kuzmanova et al. [16] who assessed vitamin C in serum and leucocytes in relation to periodontitis they concluded that a relationship existed between low plasma vitamin C levels and periodontitis. Study done by Park et al. [17] also supported an association between periodontitis and lower intake of vitamin C along with niacin and iron in women and nonsmokers. Vitamin E is fat soluble antioxidant; it transfers phenolic hydrogen to recipient free radical and gets converted into phenoxyl radical. Sobaneic et al. [18] found that there are significantly low levels of vitamin E and other antioxidants in saliva of epilepsy patients with periodontal disease.

In a study done by Canakci et al. to investigate the TAC, SOD and GPx activities in serum and saliva of preeclamptic women with periodontal disease, it was concluded that SOD, GPx in serum and TAC in saliva were lowest in preeclamptic women with periodontal disease. The findings of above study are in support of our study which concludes that SOD level was significantly low in preeclamptic women with periodontal disease [1].

Periodontal disease is chronic inflammatory condition, and oxidative stress may be an important contributing factor in pathogenesis of periodontal disease and preeclampsia. However, it is not clear whether this oxidative stress is the cause or result of this disease.

Conclusions

The results of our study indicate that periodontal disease may be potential risk factor for severity, progression, and even initiation of preeclampsia because of reduced antioxidant capacity or increased oxidative stress. Further studies with larger groups and analysis of oxidant/antioxidant status are required to address this hypothesis.

Preventive strategies may be developed that impact the prevalence of both diseases.

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Compliance with Ethical Standards

Conflict of interest There are no other conflict of interest for the P.I. Amitha Ramesh, Prasanna Kumar Shetty, Priyanka Agumbe declare that they have no conflict of interest.

Ethical Standard All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration of 1975, as revised in 2008(5).

Informed Consent Informed consent was obtained from all patients for being included in the study.

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