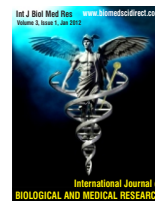


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Original Article

Peroxidative stress and nutrient antioxidant status in periodontal disease

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ABSTRACT

In the present communication, we report remarkably elevated levels of TBARS (Thio-barbituric reactive substances), serving as an index of lipid- peroxidation and thus free-radical mediated damage in blood of patients with periodontitis. Highly significant decrease in the levels of nutrient antioxidants- tocopherol and ascorbic acid has also been found in the patients. Our results indicate on the oxidant-antioxidant disturbances in periodontitis patients which can play an important role in pathomechanism of periodontal disease in these persons. Further study on role of antioxidants in preventing periodontitis will be continued.

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1. Introduction

The oral cavity is one of the most unhygienic sites because of our eating habits. In recent years, because of the change in life style, the consumption of different types of refined food such as candies, cakes, white bread, tinned foods has increased which lacks essential nutrients – vitamins and minerals including the antioxidants. In addition to these, stress and strain also plays an important role whose impact is visible in increased oral and other diseases.

Chronic Periodontitis is a chronic inflammatory disease that affects 10%-15% of developed world population and is major cause of tooth loss in adults (1). Periodontal disease has been proved to be associated with an imbalance between oxidants and antioxidants (2). Kwaitkowska et al reported that the products of lipid peroxidation diffuse from the site of inflammation & can therefore be measured in plasma (3). The antioxidant hypothesis proposes that it is the natural antioxidants in fruits and vegetables (vit. C, E, A and carotenoids etc.) That is protective and act by scavenging reactive oxygen derived free radicals. It is not well established whether oxidative stress found in patients with periodontal diseases results from an increased production from physiological system (4). So, this study was planned to measure oxidative stress that can be correlated with antioxidant levels on one hand and with diseases on the other.

2. Materials and Methods:

The present study was conducted in the Department of Biochemistry, Darshan Dental College & Hospital, and Udaipur. 100 patients irrespective of age & sex were selected from newly registered patients from Dept. of Periodontics, Darshan Dental College, Udaipur. Periodontitis was diagnosed on the basis of the pocket depth (>3mm) and gingival index. Present and past history of every patient was recorded on a pretested proforma. The healthy controls were matched in respect to sex, age and socio-economic status from the same region. Both the cases and controls were selected by a simple random method. After noting the details, venous samples were drawn from antecubital vein and were collected in double distilled washed plain vial. Serum was separated by centrifugation of blood sample. Alpha-tocopherol, ascorbic acid and TBARS were estimated in serum. TBARS, marker of index of oxidative stress was measured by Buege and Aust method (5), ascorbic acid by Natelson method (6) and alpha tocopherol by Baker and Frank method (7). The data after biochemical analysis was subjected to standard statistical analysis such as student's "t" test using the SPSS 11.5 software.

3. Results

The personal profiles & clinical parameters of all the subjects under study are shown in Table-1. The serum levels of TBARS, alpha –tocopherol and ascorbic acid in periodontitis cases and normal control subjects are shown in Table-2. Among 100 cases, there are 59 males and 41 females. The serum nutrient antioxidants (alpha- tocopherol and ascorbic acid) levels are significantly higher ($p < 0.001$) in periodontitis cases than controls. The nutrient antioxidants levels were significantly higher ($p < 0.001$) and TBARS was significantly lower ($p < 0.05$) in female

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patients than male patients (Table -3). When rural patients were compared to urban patients, the nutrient antioxidant values revealed significantly lower values ($p < 0.01$) and significantly higher values of TBARS ($p < 0.001$) in rural patients as compared to urban patients (Table 4).

Values are Mean \pm S.D; $p \leq 0.001$ highly significant, $p \leq 0.01$ significant.

Table -1: Personal profile and clinical details of healthy persons and periodontitis cases

Parameters	Healthy Controls	Periodontitis Cases
No. of Cases	60	100
Age (years)	36	45
Sex:		
i) Males	32	59
ii) Females	28	41
Residence:		
i) Rurals	23	30
ii) Urbans	37	70
Gingival Index:		
i) Low	45	19
ii) Moderate	09	69
iii) Severe	06	12
Oral Hygiene:		
i) Good	25	04
ii) Fair	33	42
iii) Poor	02	54
Gingival Recession:		
i) With	07	71
ii) Without	53	29
Pocket Depth:		
i) ≤ 2 mm	56	Nil
ii) $\leq 2-3$ mm	04	Nil
iii) ≥ 3 mm	Nil	100
Teeth mobility:		
i) With	04	44
ii) Without	56	56

Table - 2: Concentration of Nutrient antioxidants and TBARS in Periodontitis patients vs Controls.

Parameters	Control	Cases	Control vs Cases
TBARS (nmol/ml)	2.60 \pm 1.38	8.98 \pm 2.33	$P \leq 0.001$
Tocopherol (mg/dl)	1.16 \pm 0.23	0.85 \pm 0.24	$P \leq 0.001$
Ascorbic acid (mg/dl)	1.06 \pm 0.28	0.60 \pm 0.17	$P \leq 0.001$

Values are Mean \pm S.D; $p \leq 0.001$ highly significant.

Table - 3: Concentration of Nutrient antioxidants and TBARS sex-wise in Periodontitis patients vs Controls.

Parameters	Cases		Male vs Female cases
	Males	Females	
TBARS (nmol/ml)	9.34 \pm 2.12	8.25 \pm 2.31	$P \leq 0.05$
Tocopherol (mg/dl)	0.77 \pm 0.19	0.97 \pm 0.26	$P \leq 0.001$
Ascorbic acid (mg/dl)	0.55 \pm 0.17	0.70 \pm 0.16	$P \leq 0.001$

Values are Mean \pm S.D ; $p \leq 0.001$ highly significant , $p \leq 0.05$ not significant.

Table - 4: Concentration of Nutrient antioxidants and TBARS residence-wise in Periodontitis patients vs Controls.

Parameters	Cases		Rurals vs Urbans cases
	Rurals	Urbans	
TBARS (nmol/ml)	10.23 \pm 2.48	8.32 \pm 1.90	$P \leq 0.001$
Tocopherol (mg/dl)	0.75 \pm 0.16	0.90 \pm 0.26	$P \leq 0.01$
Ascorbic acid (mg/dl)	0.49 \pm 0.13	0.66 \pm 0.17	$P \leq 0.001$

Values are Mean \pm S.D ; $p \leq 0.001$ highly significant , $p < 0.01$ significant.

3. Discussion

Diseases of the periodontal tissues are among the most widespread inflammatory disorders worldwide & are a major cause of tooth loss in the adult population (8). It is well accepted that periodontitis is caused by imbalance between periodontal pathogens and the host defence. Host defence mechanisms may be influenced by genetic factors and nutrition (9). Few studies have considered the effect of the imbalance between oxidants and antioxidants in patients with periodontitis which in turn predisposes such individuals to the damaging effect of ROS in the periodontium (2). Previous studies revealed conflicting data for associations between antioxidant micronutrient intakes & periodontitis as assessed by dietary questionnaires (10,11). Our findings add to the existing body of evidence which suggests that the oral diseases are a result of imbalance between oxidants and antioxidants.

In present study, peroxidative stress was measured in terms of TBARS and nutrient antioxidants measured were Vit.C and Vit.E in serum. TBARS levels were in acceptable range and did not show any conclusive trend with regard to sex and residence in controls. In periodontitis patients, the mean TBARS level was 8.97 \pm 2.33 nmol/ml, thereby confirming that oxidative load was higher in periodontitis patients. Indirectly, it indicates that periodontitis is accompanied by increased ROS activity (12,13), resulting from activated neutrophils and macrophages in the active phase of disease. These presumptions are well supported by findings of

other workers (14,15,16). Kwaitkowska et al reported that the products of lipid peroxidation diffuse from the site of inflammation and can therefore be measured in plasma (3). Periodontopathogens and their products induce the generation of ROS by PMN leukocytes. PMN leukocytes are recognized as a particularly rich source of ROS, which in the absence of suitable antioxidants can lead to tissue damage (17,18). Free-radical-induced injury has been demonstrated to be increased in individuals with periodontitis (19). Enhanced lipid peroxidation was reported in the periodontal tissues of cats (20). Gutteridge reported that the extent of tissue damage could be assessed by measuring the concentration of lipid peroxidation products (21).

Since oxidation is an inevitable phenomenon of human life, it is reasonable to posit the vigilant and shielding role of antioxidants. Scrutinization of nutrient antioxidant in patients conspicuously showed that the levels of ascorbic acid (males- 0.55 ± 0.17 mg/dl & females- 0.70 ± 0.16 mg/dl) and alpha tocopherol (males- 0.77 ± 0.19 mg/dl & females- 0.97 ± 0.26 mg/dl) were definitely lower than recommended optimal nutrients in humans, thus suggesting that subtle deficiency of these antioxidants may make the periodontal tissue more susceptible to oxidation (16).

Vitamin E (α -tocopherol) is a lipid soluble peroxy radical scavenger in human cells and forms the first line of defence against the peroxidation of cell membrane PUFA. Reduced levels of Vitamin E may be due to increased requirement of vitamin E in per-oxidant milieu with enhanced free radical status, leading to increased lipid peroxidation, a resultant depletion of free radical scavenger and antioxidant reserves of the body. In our study, similar trend of decreased vitamin E level has been observed ($p < 0.001$). Antioxidant property of α -tocopherol is further enhanced by several antioxidants, the most prominent being ascorbic acid (22,23). Vitamin E transfers its electron to wandering vitamin C or other antioxidant in cytosolic medium where it has multiple options for transfer of its acquired additional electron for final disposal. We observed significant lower values ($p < 0.001$) of Vitamin C in patients as compared to controls. Patients with periodontitis were reported to have a low level of plasma ascorbic acid due to gingival bleeding (24). Rural patients had significantly lower value ($p < 0.001$) of ascorbic acid among patients. The rural patients having low socio-economic status and with a heavy work load, with low intake of fruits and vegetables, with smoking, drinking and chewing habits may therefore have lower levels of the vitamin as it is depleted due to its use in combating toxins by stimulating liver detoxifying enzymes. Alcohol can depress the concentration of ascorbic acid in plasma and increased urinary excretion of vitamin C (25). Vitamin C also has a remarkable immunomodulating action (26).

4. Conclusion

Concluding, it is observed that peroxidative stress increases and nutrient antioxidants levels (Vitamin C and Vitamin E) decreases in periodontitis patients as compared to normal subjects. Because of the role the antioxidants play in both the modulation of inflammation and the promotion of wound healing, more attention must be paid on the interface between nutrition including antioxidants and periodontal disease.

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