Unique Journal of Medical and Dental Sciences
Available online: www.ujconline.net
Research Article

ASSESSMENT OF TOTAL ANTIOXIDANT STATUS, IN PERIODONTAL DISEASE CASES AROUND RURAL AREAS OF KANCHEEPURAM DISTRICT, TAMILNADU.

Bhuvana Karthikeyan*[1],Thayappan.R[2], Suresh Babu Kondaveeti[3]

[1]Final year BDS, Adhiparasakthi College Of Dental Sciences & Hospitals, Melmaruvathur, 603319, Tamilnadu.
[2]Professor & Head, Department of Periodontics, Adhiparasakthi College Of Dental Sciences, Melmaruvathur, 603319, Tamilnadu.
[3]Assistant Professor, Department of Biochemistry, Anna Medical College & Research Center, Mauritius.

Received: 12-08-2013; Revised: 15-09-2013; Accepted: 14-10-2013

*Corresponding Author: **Suresh Babu Kondaveeti**
Assistant Professor, Department of Biochemistry AMCRC, Mauritius
E-mail: sureshbabu_kondaveeti@yahoo.com, Mb: +230-8204769

ABSTRACT

Background: The human inflammatory periodontal disease is amongst the most common of chronic diseases to affect adults. Human gingivitis and periodontitis are the results of complex interactions between pathogenic bacteria and the host's immune inflammatory responses. The main purpose of this research was to investigate the TAOC levels in gingival crevicular fluid of periodontitis patients.
Materials and Methods: This was an experimental study conducted on 100 patients with chronic peridontitis. They were randomly assigned to two groups as follows: Group-1, 50 patients with generalized chronic periodontitis were treated with SRP supplemented orally with vitamin C (1gm/day) & vitamin E (400 IU/day) for 10 weeks with (SRP + AO) both prior & after the therapy.Group-2 consisting of 50 patient controls (Only SRP). Probing pocket depth (PPD), plaque index (TQHPI), sulcus bleeding index (SBI), & attachment level were measured and samples were analyzed for total antioxidant status (TAOC). Statistical analyses were performed using oneway ANOVA
Results: Prior to therapy PI & SBI were observed to be higher, & TAOC to be lower. TAOC was inversely correlated with both PPD & SBI ($P<0.05$). In response to both SRP & SRP+AO therapies, clinical improvement was observed at all periodontal sites.
Conclusions: Our findings suggest that the importance of the antioxidant defense mechanism in periodontal diseases.
Keywords: Scaling/root planning, Antioxidant activity, Plaque index, Sulcus bleeding index.

INTRODUCTION

The human inflammatory periodontal disease is amongst the most common of chronic diseases to affect adults. Human gingivitis and periodontitis are the results of complex interactions between pathogenic bacteria and the host's immune inflammatory responses[1]. The destruction of periodontal tissue is thought to be mainly due to host-derived mediators[2,3,4] . Reactive oxygen species (ROS) have been implicated in the pathogenesis of many diseases, the imbalance between oxidative stress induced by ROS and the concentrations (or activity) of the antioxidant may result in tissue damage[5]. The main purpose of this research was to investigate the TAOS levels in gingival crevicular fluid of periodontitis patients.

As ROS and antioxidant defence mechanisms seem to act in concert rather than alone, an example being the re-cycling of a-tocopherol by vitamin C, studies of individual antioxidants in relation to inflammatory disease may not necessarily yield useful information. Research is now being directed towards assays for total antioxidant capacities of biological fluids, while not forgetting the importance of the constituent antioxidant compounds. Research workers have previously attempted to study biochemical parameters like Lipid peroxidation, super oxide dismutase, glutathione peroxidase & catalase,–Vitamin C, E, A (Beta-carotene) & reduced glutathione were studied individually or collectively in relation with periodontitis. It is likely that a comprehensive study of these parameters simultaneously will throw more light on the multidimensionality of this issue. Further more; data for rural population is scanty in literature. The fact that majority of Indian population lives in rural zone & many factors are to be identified. So a comprehensive study of various periodontal parameters & their association with periodontitis was conducted at the rural set up of Adhiparasakthi Dental College, Melmaruvathur Adhiparasakthi Institute of Medical Sciences & Research, Melmaruvathur, Kancheepuram District, TAMILNADU.

MATERIALS AND METHODS

Subject selection

seventy subjects were selected from those being treated at the Department of Periodontology, Adhiparasakthi Dental College, Melmaruvathur Adhiparasakthi Institute of Medical Sciences & Research, Melmaruvathur, Kancheepuram District, TAMILNADU. The study was carried out after the approval from the MAPIMS&R animal ethical committee(Regd. No. MAPIMS /1058/PO/ac/10/CPCSEA). Informed consent was obtained from all subjects. In addition to patients with periodontitis, fifteen subjects without evidence of periodontitis (healthy) were included in this study.

Group1: 50 patients all male with generalized chronic periodontitis treated with SRP alone & SRP + AO prior & after therapy for duration of 10 weeks.

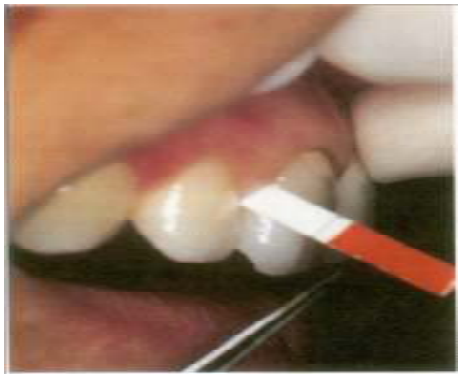
Group2**:** 50 patients healthy control treated with SRP alone.

The following criteria were adopted for selecting the patients:

Exclusion Criteria**:** Pregnancy or lactation, diabetes, HIV infection, bleeding disorders, immunosuppressive chemotherapy, and any condition necessitating antibiotic premedication for dental appointment. Smokers were excluded from this study.

Inclusion Criteria: No antibiotic usage within 3 months, and not a regular user of non-steroidal anti-inflammatory drugs.

Sample collection and preparation



1. Gingival crevicular fluid collection and clinical parameters

Each clinical evaluation was preceded by collection of gingival crevicular fluid from the mesiobuccal surfaces of maxillary teeth to avoid saliva contamination. Briefly, teeth were air dried and isolated with cotton rolls, supragingival plaque was gently removed, and gingival crevicular fluid samples were collected with Periopaper_ gingival crevicular fluid collection strips. Paper strips were measured for fluid volume with a calibrated Periotron, and then removed to separate microcentrifuge tubes containing physiologic saline 0.1% Tween 20. The tubes were stored at-70C until eluted. Following elution, each gingival crevicular fluid sample was analyzed separately. Clinical measurement was performed immediately after the gingival crevicular fluid collection. Probing pocket depth is the distance in mm from the most coronal margin of the free gingiva to the most apical penetration of the probe. Probing attachment level is the distance in mm from the cemento enamel junction to the most apical penetration of the probe. Probing pocket depth measured using a Williams Periodontal probe the diseased sites (including those with gingival recession and pseudo-pockets) had probing pocket depth 6 mm. The diseased were not bleeding on probing and did not show any signs of bone loss. A diagnosis of patients with periodontal disease was based upon the presence of at least one site with the criteria of diseased sites. The gingival index and the plaque index were recorded dichotomously during gingival crevicular fluid collection[6].

2. Ferric Reducing/Total antioxidant Ability of Plasma (FRAP Method)

The assay which measures the combined antioxidant effect of the non-enzymatic defenses in biological fluids is useful in providing an index of ability to resist oxidative damage. At low pH, when a ferric-tripyridyltriazine complex is reduced to the ferrous form by reductants (antioxidants) in the mixture, an intense blue color with an absorption at 593 nm develops. In this assay, excess ferric ion is used, and the rate-limiting factor of ferrous-tripyridyltriazine formation, and hence color, is the reducing/antioxidant of the plasma[7].

STATISTICAL ANALYSIS

Data is presented as means and standard deviations (SD). Significance of mean differences between healthy and diseased subjects was tested by paired t-test, Relationships among mean clinical periodontal parameters and gingival crevicular fluid, TAOS were tested by analysis of variance (ANOVA) followed by paired t-test. The associations between the levels of various index, gingival crevicular fluid and clinical parameters were calculated.

RESULTS

Table 1. General Characteristics of, Plaque index; Gingival index; Probing pocket depth; Probing attachment level; Gingival crevicular fluid in diseased & control subjects

	PERIODONTITS	HEALTHY CONTROL
	MALES(50)	MALES(50)
AGE (YRS)	57.4 ± 12.3	56.8 ± 13.4
GCF (ul)	0.72 ± 0.26	0.14 ± 0.06
PPD	6.82 ± 1.65	0.98 ± 0.10
TQHPI (PI)	2.91 ± 0.20	0.89 ± 0.02
SBI	7.26 ± 0.87	1.90 ± 0.06
PAL	6.87 ± 0.59	1.9 ± 0.01
GI	1.63 ± 0.34	0.03 ±0.01

Significantly different from diseased and healthy sites ($p < 0.0001$).

PI, plaque index; GI, gingival index; PD, probing pocket depth; PAL, probing attachment level; GCF, gingival crevicular fluid.

Table II. Summary of Plaque index; Gingival index; Probing pocket depth; Probing attachment level; Gingival crevicular fluid in diseased & control subjects before & after treatment ; in Males.

	Before Treatment	After Treatment	P Value
Plaque Index (TQHPI)	0.98	1.1	p < 0.001
Gingival Index	1.97	0.985	p < 0.001
Probing attachment level (PAL)	7.46	2.48	p < 0.001
Probing pocket depth (PPD)	8.47	2.82	p < 0.001
Gingival crevicular fluid volume (GCF)ul	0.98	0.49	p < 0.001
Total Antioxidant status	24.78	64.67	p < 0.001

The values are expressed as mean ± SD and were found to be significant (p<0.001)

Table III. Comparative improvement in ppd before & after srp alone vs srp+ao (vit C & vit E)

Procedure	PPD Before At Zero Week	PPD After At 8th Week	P Value
SRP alone (group 2)	8.47	3.88	P<0.05.
SRP+AO (group 1)	8.47	1.12	P<0.05.

The values are expressed as mean ± SD and were found to be significant (P<0.05).

DISCUSSION

The main findings of this study is that subjects with the worst periodontal health status tended to have greater oxidative injury, as indicated by the lower TAOC. This has been previously reported, but is wholly consistent with the hypothesis that there is enhanced reactive oxygen speciesmediated damage to tissues in the most advanced states of periodontal disease[8]. It is unlikely that oxidative processes play a causal role in the aetiology of periodontitis, but they are likely to contribute to disease progression unless abated through antioxidant action. Total antioxidant capacity (TAOC) reflects full spectrum of antioxidant activity against various reactive oxygen and nitrogen radicals. The total antioxidant assay measures predominantly the low molecular weight chain breaking antioxidants, such as urate, ascorbate, bilirubin etc. Generally, low total antioxidant capacity indicates oxidative stress or increased susceptibility to oxidative damage .

Ascorbic acid is the only endogenous anti-oxidant in plasma that can completely protect against peroxidative damage induced by aqueous peroxyl radicals and the oxidants released from activated neutrophils[9]. However, ascorbic acid seems to protect against peroxidation indirectly, by interacting with α-tocopherol[10]. There would appear to exist a concerted action of vitamins E and C, in which ascorbic acid regenerates vitamin E, thus maintaining its serum value at a constant level. When ascorbic acid is depleted, no regeneration of vitamin E is possible, and a decrease in its concentration is observed[11]. Chemically, ascorbic acid is an excellent reducing agent and most of its anti-oxidant properties are ascribed to this feature. Unfortunately, it is also able to reduce iron and copper ions. if H_20_2 is also present, ascorbate can drastically accelerate OH formation, according to the mechanism of $H_20_2 + Fe^{2+}$ (or Cu^{+}) $\rightarrow OH^{-} + OH^{\cdot} + Fe^{3+}$ (or Cu^{2+}), thus producing another pro-oxidant. The pro-oxidant effects of ascorbic acid do not usually occur in vivo, simply because, in the healthy state, free iron and copper are not available in extracellular fluids[12]. Finally, when acting as a pro-oxidant, ascorbic acid can modulate the expression of the procollagen gene through both lipid peroxidation production and stabilization; in this way, it may affect the secretion of collagen, thereby altering fibroblast differentiation through its effects on the extracellular matrix[13]. A vitamin-E-deficient diet as well as a vitamin E supplement can affect several periodontal parameters (in the rat)[14]. Vitamin E deficiency does not increase PE (in rats)[15]. and no significant difference in vitamin E levels in PD patients was found[16]. The authors agreed that their respective data did not provide any support for the treatment of PD with vitamin E. No variations in vitamin A or E levels in PD patients after zinc sulfate treatment, during pregnancy and during pregnancy in diabetic women, were found[17,18,19]. Gingival vitamin E concentrations decrease in males and females in proportion to the severity of pocket depth and gingival and bleeding indices, and in older subjects more affected by PE, vitamin E tended to increase[20]. Some positive effects after vitamin E administration on rat bone loss induced by stress have been observed, and vitamin E efficacy was detected only when the stress was introduced abruptly[21].

In common with other studies, we have found that smoking is the largest single risk factor for periodontal disease[22]. After adjusting for this factor associations were noted between periodontal disease status and plasma antioxidant status. Individuals with the lowest TAOC were nearly 4.5 times more likely to have periodontal disease, as defined by Community Periodontal Index of Treatment Needs (CPITN) below 18, than those with higher TAOC. Although it is unclear whether reduced antioxidant capacity is a cause or an effect of periodontal disease, it is certain that lower antioxidant concentrations in the gingival crevicular fluid will contribute to increased damage to the gingivae and surrounding structures by activated neutrophils. TAOC measured using FRAP in the present study may be a useful biochemical marker of disease progression and treatment. The potential use of this simple system for monitoring of treatment without dental examination may be attractive.

CONCLUSION

Periodontal disease is a systemic disease that manifests in the oral cavity. Periodontal disease has now been linked to oral cancer, heart disease, stroke, lung infections, pre-term and low birth weight babies, osteoporosis, and other chronic diseases. Free radicals play an important destructive role in the development and Progress of periodontiti. Antioxidants neutralize free radicals in body tissues. Patients with periodontitis tend to have lower antioxidant capacity – both

locally and systemically. Patients with low levels of certain vitamins and minerals have up to a 13-fold greater risk of periodontitis. Antioxidant vitamin supplements that work systemically to support the Immune system may help fight the disease and aid in the healing process.

RECOMMENDATION

On the basis of clinical evaluation and experiment setup we may conclude that the examined antioxidant vitamin's C & E has a beneficial effect on the periodontal tissue.

ACKNOWLEDGEMENT

The authors wish to express their acknowledgement to the Management MAPIMS&R, Adhiparasakthi Dental College & Hospital, For their constant help throughout the study.

REFERENCES

1. Page R. Milestones in periodontal research and the remaining critical issues.J Periodont Res,1999;34:331–339
2. Meikle MC, Atkinson SJ, Ward RV, Murphy G, Reynolds JJ. Gingival fibroblasts degrade type I collagen films when stimulated with tumor necrosis factor and interleukin 1: evidence that breakdown is mediated by metalloproteinases. J Periodont Res,1989 ;24:207–213.
3. Seguier S, Gogly B, Bodineau A, Godeau G, Brousse N. Is collagen breakdown during periodontitis linked to inflammatory cells and expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human gingival tissue? J Periodontol,2001; 72:1398–1406.
4. Suomalainen K, Sorsa T, Uitto VJ, Vauhkonen M, Lindy S. Characteristics of neutral proteases present in inflamed human gingiva. Scand J Dent Res,1989;97:346–354.
5. Sies H. Oxidative stress: from basic research to clinical application. Am J Med,1991; 91:31S–38S.
6. Benzie, I. F. F. and Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. Anal. Biochem. 1996;239: 70-76.
7. Brown LAS, lones DP. The biology of ascorbic acid. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker, Inc,1996, pp. 117-154.
8. Sculley, D. V. and Langley-Evans, S. C. Salivary antioxidants and periodontal disease status. Proc. Nutr.Soc,2002;61, 137–143
9. Buettner GR, Jurkiewicz BA. Chemistry and biochemistry of ascorbic acid. In: Handbook of antioxidants. Cadenas E, Packer L, editors. New York: Marcel Dekker,Inc,1996; pp. 91-115.
10. Roberfroid M, Buc Calderon P. Free radicals and oxidation phenomena in biological systems. New York: Marcel Dekker, Inc,1995.
11. Gutteridge IMC, Halliwell B. Antioxidants in nutrition, health, and disease. New York: Oxford University Press,1994.
12. Schneider HG, Pose G. Influence of tocopherol on the periodontium of molars in rats fed a diet lacking in vitamin E. Arch Oral Biol,1969;14:431-433.
13. Perish et al J, Kishan Z. Vitamin E and A and periodontium in pregnant diabetics. Acta Univ Palacki Olomuc Fac Med,1996;134:93-95.
14. Slade EW Ir, Bartuska D, Rose LF, Cohen DW . Vitamin E and periodontal disease. J Periodontol. 1976;47:352-354.
15. Cerna H, Vesely 1, Fingerova H, Pohanska. Bloodserum vitamin E and A levels during peroral therapy of periodontal disease with zinc sulphate. Acta Univ Palacki Olomuc Fac Med,1988;119:187-193.
16. Cerna H, Vesely J, Nastoupilova E, Lechner I, Fingerova H, Pohanka J. Periodontium and vitamin E and A in pregnancy. Acta Univ Palacki Olomuc Fac Med, 1990; 125:173-179.
17. Cerna H, Vesely J, Krikal Z. Vitamin E and A and periodontium in pregnant diabetics. Acta Univ Palacki Olomuc Fac Med, 1992;134:93-95.
18. Fujikawa K. Alpha-tocopherol content in the human gingiva in chronic marginal periodontitis. Nippon Shishubyo Gakkai Kaishi, 1983;25:178-184 lin Japanesel.
19. Cohen RE, Ciancio SG, Mather ML, Curro FA. Effect of vitamin E gel, placebo gel and chlorhexidine on periodontal disease. Clin Prev Dent, 1991;13:20-24.
20. Li KL, Vogel R, Jeffcoat MK, Alfano MC, Smith MA, Collins JG, et al. The effect of ketoprofen creams on periodontal disease in rhesus monkey. J Periodont Res,1996; 31:525-532.
21. Alpagot T, Wolff LF, Smith QT, Tran SD. Risk indicators for periodontal disease in a racially diverse urban population. J Clin Periodontol; 23:982–988. Lipid peroxidation in chronic periodontitis, 1996;383.
22. Calsina, G., Ramon, J. M. and Echeverria, J. J. Effects of smoking on periodontal tissues. J. Clin. Periodontol, 2002;29, 771–776

Source of support: Nil, Conflict of interest: None Declared