



“SERUM URIC ACID : EXPLORING THE LINK BETWEEN TOBACCO AND PERIODONTITIS”

Dental Science

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ABSTRACT

Background and objectives: Periodontitis results from an interplay between reactive oxygen species and antioxidants. The present study explores the possibility of using uric acid as a biochemical marker for periodontal disease.

Material and methods: 120 subjects were divided into 4 groups of 30 each of healthy, chronic periodontitis, smokers with chronic periodontitis and smokeless tobacco users with chronic periodontitis whose serum samples were collected and analyzed for uric acid. Values obtained were subjected to ANOVA and Post hoc test.

Results: Uric acid levels were found to be significantly lower in chronic periodontitis compared to healthy subjects. While tobacco usage was found to increase uric acid levels.

Conclusions: Serum uric acid level can be helpful estimator of oxidative stress produced by tobacco usage and periodontitis.

KEYWORDS:

Antioxidant, Oxidative stress, Periodontitis, Tobacco, Uric acid

INTRODUCTION

Tobacco be in any form has shown its potential to cause death and disease globally^{1,2}. Smoking related disease not only kills one half of all long term smokers but these diseases may also be contracted by non-smokers owing to the inhalation of second hand smoke. A 2007 report stated that about 4.9 million people worldwide each year dying due to smoking³.

Periodontal disease is one of the common chronic inflammatory diseases with a complex etiology and multifactorial origin². Periodontal diseases are one of the more prevalent oral diseases affecting more than 50% of Indian community.

Oxidative stress results due to an imbalance between the generation of highly reactive oxygen species and the antioxidant defense systems⁴. A basic hypothesis is that free radicals cause oxidative damage to macromolecules such as lipids, proteins, and DNA. Therefore, these radicals play an important role in the pathogenesis of diseases⁵. Smokeless tobacco has proven to generate highly reactive free radicals that act as initiators and/or promoters of carcinogenesis, causing DNA damage, activation of pro-carcinogens, and alteration of the cellular antioxidant defense system⁶. In accordance with this theory, antioxidants are believed to play important roles in resisting damage from oxidative stress resulting from cigarette smoking and smokeless tobacco. Antioxidants are those substances which when present at low concentrations compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate⁷. Hence the presence of antioxidants is essential to counteract the damage caused by free radicals⁸.

Uric acid is the end product of purine catabolism and contributes to the antioxidant capacity of blood and saliva⁹ contributing around 70-85% of the total antioxidative capacity of resting and stimulated saliva from healthy as well as periodontally compromised

subjects^{10,11}. It is the most abundant aqueous antioxidant, accounting for upto 60% of serum free radical scavenging capacity, and it is an important intracellular free radical scavenger during metabolic stress, including smoking^{12,13}. The measurement of its serum level, therefore, reflects the antioxidant capacity¹⁴. In lower species, allantoin is degraded by an enzyme called liver uricase resulting in very low serum uric acid levels. However, in humans, a mutation occurred in the evolutionary scale, probably in the late Miocene, making uricase not functional. It is believed that this selection has occurred because of the beneficial effects of uric acid as antioxidant¹⁵. However, with changing lifestyle and incorporation of a diet rich in salt and uric acid precursors such as fructose, it has been observed that uric acid is associated with hypertension, coronary artery disease, peripheral vascular disease, renal failure and strokes. Therefore uric acid appears to play a dual role in oxidative stress: antioxidant in the extracellular space and pro-oxidant within the cell¹⁶. Its free radical scavenging property is activated during metabolic stress including smoking¹³. therefore, measurement of its serum level reflects the antioxidant capacity.

The effects of smokeless tobacco on this marker have not been established yet. With this in mind the present study was designed to establish the reliability of uric acid as a biomarker in tobacco users with chronic periodontitis. This study contributes to the existing literature about serum uric acid levels in periodontal health and disease.

MATERIALS AND METHODS

The protocol for this cross-sectional study was approved by the Ethical Committee of P.M.N.M. Dental College and hospital, Bagalkot. Prior to enrolment in the study, a written consent was obtained from the candidates who fulfilled the inclusion criteria.

A total of 120 subjects, aged 18-60 years, of which 30 diagnosed as smokeless tobacco users with chronic periodontitis subjects, 30

diagnosed as smokers with chronic periodontitis subjects,30 diagnosed as chronic periodontitis subjects and 30 periodontally healthy subjects, were randomly selected from the Out-patient department, Department of Periodontics, PMNM Dental College, Bagalkot.

After clinical examination the subjects were divided in 4 groups. Group I consisted of healthy subjects showing absence of clinical and radiographic manifestations of periodontal disease, at least 20 teeth present, Group II comprised the subjects diagnosed as chronic periodontitis with at least 14 teeth in the mouth, Bleeding on probing and clinical attachment level of 3 mm or more at more than 30% of all sites in the mouth¹⁷. Group III consisted of subjects fulfilling the criteria of group II in addition to being smokeless tobacco users¹⁸. Group IV consisted of subjects fulfilling the criteria of group II in addition to being smokers¹⁹.

Subjects suffering from systemic conditions(rheumatic fever, heart problems, hypertension, diabetes, liver and kidney disease),any infection requiring prophylactic antibiotic therapy, pregnant, lactating women on hormonal contraceptives or on hormone replacement therapy, on steroids and NSAIDs (for previous 3 months) or on vitamin supplements, alcoholics and having undergone scaling and root planing in past 6 months were excluded from the study.

After proper grouping of the subjects, a full-mouth periodontal examination was performed by a single examiner. The following periodontal parameters were assessed using a Williams periodontal probe: Pocket probing depth, Clinical attachment level, Gingival index ((Loe and Silness 1963).

BIOCHEMICAL ANALYSIS

2ml of venous blood was drawn with aseptic precautions from median cubital vein into a serum separating tube. Serum obtained was immediately processed (centrifuged at 2500 rpm for 15 min) and transferred into eppendorf tubes which were then stored in the refrigerator. These were then transferred for analysis of uric acid on the same day by StatFax 3300semi automated dry chemistry analyzer.

PRINCIPLE²⁰

The series of reaction involved in assay system are as follows

Uric acid is oxidised to allantoin by uricase with the production of h2o2.

The peroxides react with 4-aminoantipyrine (4-AAP) and DHBS in the presence of peroxidise to yield a quinoneimine dye. The absorbance of this dye at 500 nm is proportional to uric acid concentration in the sample.

STATISTICAL ANALYSIS

Statistical analysis of the data was done by applying one way ANOVA and Post hoc analysis tests. It showed significant P value ($P < 0.001$).

RESULTS

The mean levels of serum uric acid of 5.15 ± 0.73 mg/dl, 4.11 ± 0.65 mg/dl, 5.63 ± 0.92 mg/dl and 5.77 ± 1.41 mg/dl was observed among periodontally healthy, chronic periodontitis, smokeless tobacco users with chronic periodontitis and smokers with chronic periodontitis, respectively. Comparison of mean serum uric acid levels in different groups by ANOVA test showed statistically significant difference $P = 0.001$ [Table 1]. The smokeless tobacco users group with chronic periodontitis showed the highest gingival index, clinical attachment level and pocket probing depth scores followed by the smokers group and then the chronic periodontitis group. (Table2,3,4)

TABLE 1: Comparison of values between cases and controls.

ONE WAY ANOVA	
URICACID	

	N	Mean	Std. Deviation	F	p
HEALTHY	30	5.1500	0.73614	17.71	<0.00
CHRONIC PERIODONTITIS	30	4.1133	0.65564	9	1
PERIODONTITIS + SMOKELESS	30	5.6367	0.92419		
PERIODONTITIS + SMOKER	30	5.7733	1.41736		

TABLE 2: Comparison of four groups (Healthy, Chronic Periodontitis and Smokeless tobacco users with chronic periodontitis, smokers with periodontitis) with respect to gingival index scores.

ONE WAY ANOVA					
GI					
	N	Mean	Std. Deviation	F	P
HEALTHY	30	0.6067	0.19640	632.8	<0.00
CHRONIC PERIODONTITIS	30	2.0967	0.24703	29	1
PERIODONTITIS + SMOKELESS	30	2.3600	0.11017		
PERIODONTITIS + SMOKER	30	2.2767	0.13309		

Table 3: Comparison of four groups (Healthy, Chronic Periodontitis and Smokeless tobacco users with chronic periodontitis, smokers with periodontitis) with respect to pocket probing depth scores.

ONE WAY ANOVA					
PPD					
	N	Mean	Std. Deviation	F	p
HEALTHY	30	0.0000	0.00000	658.2	<0.00
CHRONIC PERIODONTITIS	30	5.8667	0.81931	02	1
PERIODONTITIS + SMOKELESS	30	6.5000	0.77682		
PERIODONTITIS + SMOKER	30	6.3667	0.71840		

Table 4: Comparison of four groups (Healthy, Chronic Periodontitis and Smokeless tobacco users with chronic periodontitis, smokers with periodontitis) with respect to clinical attachment level scores.

ONE WAY ANOVA					
CAL					
	N	Mean	Std. Deviation	F	p
HEALTHY	30	0.0000	0.00000	277.3	<0.00
CHRONIC PERIODONTITIS	30	5.0333	0.99943	27	1
PERIODONTITIS + SMOKELESS	30	5.9000	1.09387		
PERIODONTITIS + SMOKER	30	5.7667	1.10433		

DISCUSSION

Periodontitis is a result of a complex interaction of various factors one of which is the inappropriate host response to periopathogens and their products resulting in tissue destruction. Recognition of markers of tissue destruction thus need to be incorporated in diagnosis of periodontal diseases²¹. Loss of balance between reactive oxygen species and antioxidant defense leads to generation of oxidative stress which has also been implicated as an etiologic factor for periodontal diseases⁷. It may manifest as an increase in oxidative stress, reduction of total antioxidant capacity, or decrease in individual antioxidant level.

The periodontal literature is filled with various biomarkers to evaluate periodontal destruction which includes enzymes, proteins, host cells, markers of cellular and humoral activity, ions, hormones, and markers of oxidative stress and antioxidants. Uric acid is one of the major antioxidants²² that has shown its presence in saliva, serum as well as gingival crevicular fluid⁹.

The presence of increased oxidative stress and thus decreased antioxidant levels explains the serum uric acid values being lower in

chronic periodontitis group ($p < 0.001$) compared to healthy group. Uric acid has been demonstrated to be an important antioxidant and a free radical scavenger in humans. It is one of the major radical-trapping antioxidants in plasma and is reported to protect the erythrocyte membrane against lipid peroxidation. Uric acid interacts with peroxynitrite to form a stable nitric oxide donor, thus promoting vasodilatation and reducing the potential for peroxynitrite-induced oxidative damage. Thus, uric acid could be expected to protect against oxidative stresses²³.

Tobacco usage demonstrated increased uric acid levels as compared to non users. The immediate precursor of uric acid is xanthine, which is degraded into uric acid by xanthine oxidoreductase. Xanthine oxidoreductase may attain two inter-convertible forms, xanthine dehydrogenase or xanthine oxidase²⁴. Xanthine dehydrogenase generates the reduced form of nicotinamide-adenine dinucleotide. The xanthine dehydrogenase form in vivo, is transformed into xanthine oxidase by irreversible proteolytic cleavage or reversible oxidation in specific environment including hypoxia²⁵. Smoking and smokeless tobacco has established itself as being detrimental to the periodontium by showing various effects such as vasoconstriction caused by nicotine as well as hypoxia, which may create a favourable subgingival environment for colonization by anaerobic bacteria²⁶. Nicotine leads to the up-regulation of HIF-1 α , the transcriptional activator hypoxia-inducible factor 1 (HIF-1) which plays a role in inflammatory processes²⁷. Tissue ischaemia and hypoxia deplete adenosine triphosphate and promotes degradation of adenine nucleotides to inosine, hypoxanthine, xanthine and uric acid, increased serum uric acid levels may reflect impaired oxidative metabolism^{28,29}. Contrasting results were obtained in various studies. Waring et al showed a low plasma uric acid in regular smokers³⁰, whereas other authors have shown a reduction of antioxidants, including uric acid, in smokers, indicating that oxidative stress increases each time a cigarette is smoked^{31,32}. Tobacco smoke was proved to cause upregulation of xanthine oxidase leading to increased production of uric acid³³. Uric acid administration has proved to increase circulating antioxidant defenses and allows the restoration of endothelium-dependent vasodilatation³⁴.

Our present study showed that there is no much difference existed in the uric acid levels between tobacco chewers and smokers. ($p = 0.032$) The periodontal destruction was seen high in smokers when compared to tobacco chewers. (Table 2,3,4) Tobacco chewers more often show periodontal destruction at localised areas i.e. the site where tobacco is placed whereas smoking produces a more pronounced generalised area of periodontal destruction³⁵. This owing to the prooxidant effect of uric acid explains the above findings. Contradictory results were seen in a similar to a study by Scully et al³⁶ wherein a decrease in concentration of uric acid levels in saliva was seen with increase in severity of periodontal disease. Antioxidant therapy is emerging as a promising new paradigm as prophylactic and therapeutic agents. Previously it had been reported that alteration may occur to concentrations of normal endogenous antioxidant including uric acid by chronic smoking, and recently the viability of administering uric acid in solution has been established³⁷. Various studies have reported the possible role of uric acid and other antioxidants in the initiation and progression of several inflammatory oral conditions including periodontal disease. Even they do not specifically address the question of a biological link between uric acid and mechanisms of endothelial dysfunction or atherosclerosis since inhibition of xanthine oxidase by allopurinol is likely to reduce the production of hydrogen peroxide and thereby ameliorate oxidative stress even in smokers³⁸ independent of its effects on uric acid³⁹. Furthermore, allopurinol has antioxidant properties⁴⁰. Therefore, raising serum uric acid concentrations protects against acute oxidative damage⁴¹. Use of antioxidant supplements will help prevent oxidative stress generation.

LIMITATIONS AND FUTURE DIRECTIONS

The limitations of the present study includes it having a smaller sample size so further studies are needed with increased sample size

to better our understanding of the biomarker. Further studies on the estimation of before and after SRP levels of uric acid will also be helpful in establishing its actual role in the pathogenesis of periodontitis.

CONCLUSION

From the present study it can be concluded that a chairside diagnostic test can be introduced in our practice. It is recommended for smokers and smokeless tobacco users to stop or reduce tobacco usage and introduce serum uric acid estimation as routine test since its cheap and simple to reflect their antioxidant level.

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