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ORIGINAL ARTICLE

Salivary Enzymes and Thiocyanate: Salivary Markers of Periodontitis among Smokers and Non-smokers; a Pilot Study

¹Balwant Rai, ²Simmi Kharb, ³S.C. Anand

¹*Editor in Chief Internet Journal of Dental Science USA*

²*Associate Professor, PGIMS, Rohtak*

³*Director, PDM Dental College and Research Centre, Bahadurgarh*

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ABSTRACTS

Objective: To determine the association between levels of salivary thiocyanate and enzymes and periodontitis. **Subjects and methods:** One hundred thirteen samples of periodontitis with smokers (n = 32), periodontitis in non-smokers (n = 31) healthy smokers (n = 28) and healthy non-smokers (n = 22) were recruited for the study and salivary thiocyanate and aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels were analyzed. **Results and conclusion:** Salivary levels of thiocyanate and ALT, AST, LDH were significantly higher in smokers with periodontitis as compared to others. Thus, measurement of salivary thiocyanate may prove to be useful in early detection of periodontal disease.

Keywords: non smoker, smoker, salivary enzymes, saliva, thiocyanate, periodontitis

Introduction

Periodontal disease is one of the common inflammatory disease within complex etiology and multifactorial in origin. Diagnosis of periodontal disease has been primarily based upon clinical and radiographic measures of periodontal tissue destruction. These parameters provide a measures of past destruction and are of limited use in early diagnosis[1]. Thiocyanate (SCN) ion is found in organic and inorganic compounds and is a normal constituent of body fluids such as serum, saliva, tears and urine. Hence saliva contains peroxidase enzymes and lysozyme among many other host innate defence systems. The complete peroxidase system in saliva has three components. Peroxidase enzyme, salivary peroxidase and myeloperoxidase, hydrogen peroxidase (H₂O₂) and an oxidizable substrate such as pseudohalide thiocyanate (SCN⁻). Thiocyanate reacts with H₂O₂ to produce hypothiocyanite which has

antimicrobial properties. Also, thiocyanate plays a role in peroxidase system by reducing toxicity of H₂O₂ produced by oral bacteria by reacting with it to produce less harmful hypothiocyanite[2,3]. It has been reported that salivary thiocyanate concentrations in both resting and stimulated saliva have an inverse relation with gingival inflammation and amounts of plaque[4].

Intra cellular enzymes such as aspartate aminotransferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH), acidic and alkaline phosphatase etc are increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid and saliva[5].

Total antioxidant activity has been reported to be reduced in saliva of patients with periodontitis as compared to those with healthy periodontium[6,8]. Since smoking is known to effect periodontium imbalance free radical induced injury from smoking and antioxidant defences resulting in gingival

Corresponding Author

Balwant Rai, Editor in Chief Internet Journal of Dental Science USA.
Mobile + 91- 9812185855 E.mail Address : raibalwant29@rediffmail.com

inflammation and plaque formation. Also, high salivary thiocyanate levels have been reported in recurrent aphthous stomatitis and the levels were higher in smokers as compared to non-smokers. The present study was planned to determine the relationship between salivary thiocyanate, AST, ALT, LDH levels and smoking in periodontitis.

Materials and methods

Thirty two smokers with periodontitis, non-smokers with periodontitis (n=31), smoker with healthy periodontium (n=28) and non-smoker with healthy periodontium (n=22), without any systemic disease, age 28-57 years attending Jain Dental Clinic, New Delhi and Bhagwan Dental Clinic, Jind (Haryana); India were selected for the study. This was a double blind randomized study. Periodontitis in patients was defined as the presence of at least seven teeth with probing depth > 5mm and demonstrable radiographic bone loss > 30 percent of tooth sites by full mouth intraoral radiographic series. All participants had chronic periodontitis who had not received any surgical therapy previously. All subjects were systemically healthy, with no medical conditions that would affect their participation in the study. The exclusion criteria was a course of anti-inflammatory or antimicrobial therapy within the previous 3 months, a history of regular use of mouth washes, use of any vitamin supplementation or mucosal lesions, chemotherapy, radiation therapy, or medications that cause xerostomia. Informed consent was obtained from the subjects. Patients were classified as current smokers i.e. regular daily smokers 18-20 cigarette (with periodontitis and without periodontitis), non smokers i.e. who had never smoked tobacco. Clinical measures of the severity of periodontal disease, such as bleeding on probing, probing depth (PD) and loss of clinical attachment level (CL) were determined using a conventional periodontal probe (Hu-Friedy, Chicago, IL). At six sites around each tooth (Meso-buccal, mid-buccal, disto-buccal, mesio-lingual, mid lingual, and disto-lingual, excluding third molars. The probe was directed parallel to the long axis of the tooth. Clinical loss of attachment measurements were made from the cemento enamel junction to the bottom of the sulcus. During the examination, paraffin wax stimulated whole saliva was collected, and samples were stored at -20° until analyzed. Saliva was centrifuged at 3800 rpm for 10 min and thiocyanate concentration was determined by colorimetrically[9]. The activity of AST, ALT and LDH in saliva was determined spectrometrically by the standard of the International Federation of clinical chemistry method with specific reagents[10]. Relationships between salivary enzymes and

thiocyanate; probing depths, clinical loss of attachment and bleeding on probing, were analyzed using a student's t-test. All statistical analyses were performed during SPSS (version 11.0, Chicago, USA).

Results and results

The mean salivary thiocyanate level in non-smokers without periodontitis and smokers without periodontitis were $0.32 \pm 0.3\text{mM}$ and $0.51 \pm 0.2\text{mM}$ respectively ($P < 0.005$). While in non-smoker with periodontitis and smoker with periodontitis were $1.22 \pm 0.4\text{mM}$ and $1.97 \pm 0.5 \text{mM}$ respectively (Table 1, $P < 0.05$).

The mean salivary AST level in non-smokers without periodontitis and smokers without periodontitis were $493 \pm 4.7 \text{U/l}$ and $59.7 \pm 6.24 \text{U/l}$ respectively ($p < 0.005$). While in non-smoker with periodontitis and smokers with periodontitis were $72.8 \pm 8.2 \text{U/l}$ and $79.2 \pm 6.3 \text{U/l}$ respectively ($p < 0.005$).

The mean ALT, LDH levels in non-smokers without periodontitis and smokers without periodontitis were $32.2 \pm 5.2 \text{U/l}$, $382.2 \pm 16.2 \text{U/l}$ and $42.3 \pm 6.7 \text{U/l}$, $412.3 \pm 16.3 \text{U/l}$ respectively (table I, $p < 0.005$).

While the mean ALT, LDH levels in non-smokers with periodontitis and smokers with periodontitis were $47.2 \pm 6.2 \text{U/l}$, $422.1 \pm 17.2 \text{U/l}$ and $59.7 \pm 6.3 \text{U/l}$, $472.1 \pm 18.9 \text{U/l}$ respectively (table I, $p < 0.005$) there was a significant positive correlation between the periodontal parameter's and salivary thiocyanate and enzymes (table - II, $r = 0.51$ to 0.61).

Discussion

To our knowledge, this is the first study to evaluate the salivary thiocyanate level in periodontitis in smokers of non-smokers and its relation with relation with salivary enzymes.

In the present study, high level of salivary thiocyanate were observed in periodontitis patients and level were still higher in smokers as compared to non-smokers (table I, $P < 0.05$). Previous reports have showed an inverse relationship between salivary thiocyanate and gingival inflammation and plaque[3,4]. The level of salivary thiocyanate in smokers was higher as compared to non-smoker. Our result is in agreement with those reported in literatures[11,12] and BP, CL and PD (Table I, II). These results indicate towards beneficial antimicrobial effects of salivary thiocyanate in these groups. In present study, smokers within chronic periodontitis exhibited greater BP, PD and CL as compared to non-smoker ($P < 0.05$, table I).

Table 1: Levels of salivary thiocyanate (in mM), AST, ALT, LDH (in U/l), probing depth, bleeding on probing in smoker with periodontitis (A), non smokers with periodontitis (B), healthy smokers (C), healthy non smokers (D).

Parameters	Mean ± S.D.			
	(A) (n=32)	B (n=31)	C (n=28)	D (n=22)
Salivary thiocyanate	1.97 ± 0.5	1.22 ± 0.4	0.51 ± 0.2	0.32 ± 0.3
Level (in mM)				
Probing depth (in mm)	5.63 ± 0.12	3.66±0.22	1.71±0.13	0.81±0.12
Bleeding on Probing (in%)	64.2±0.5	52.2±0.3	28.2±0.2	11.2±0.2
Clinical loss of Attachment (in mm)	4.32 ±0.17	2.3 ±0.12	1.32 ±0.14	0.81±0.13
AST (U/l)	79.2 ±6.3	72.8 ±8.2	59.7 ±6.2	49.3 ± 4.7
ALT (U/l)	59.7 ± 6.3	47.2 ± 6.2	42.3 ± 6.7	32.2 ± 5.2
LDH (U/l)	472.1±18.9	422.1±17.2	412.3±16.3	382.2±16.2

Table 2: Co-efficient correlation (r) between different parameter's.

Parameters	(r)
Salivary thiocyanate level and probing depth	r = 0.58
Salivary thiocyanate level and bleeding probing	r = 0.56
Salivary thiocyanate level and clinical loss of attachment	r = 0.61
AST and probing depth	r = 0.59
AST and bleeding on depth	r = 0.57
AST and clinical loss of attachment	r = 0.58
ALT and bleeding on probing	r = 0.58
ALT and probing depth	r = 0.56
ALT and clinical loss of attachment	r = 0.60
LDH and bleeding on probing	r = 0.54
LDH and probing depth	r = 0.53
LDH and clinical loss of attachment	r = 0.59
Salivary thiocyanate and AST	r = 0.57
Salivary thiocyanate and ALT	r = 0.56
Salivary thiocyanate and LDH	r = 0.54
AST and ALT	r = 61
LDH and ALT	r = 0.53
LDH & AST	r = 0.51

In the present study, higher level of salivary ALT, AST and LDH were observed in periodontitis patients and level were still higher in smokers as compared to non-smokers (table I, P <0.05). Previous reports have been showed that salivary ADH, AST and ALT activities correlated with PD and gingival index, and also decreased after oral hygiene instructions, scaling and root surface debridement and administration of antibiotics[13,14], it has been reported as community periodontal index of treatment needs code 4 than in those coded lower, which is detected by the evaluated diagnostic system[15] previously it has been reported that LDH activities in patients decreased at 2 to 3 months after scaling, corresponding to levels similar to those in subjects with healthy periodontism[15].

In the present study a positive significant correlation between periodontal parameter's and salivary thiocyanate and salivary enzymes (Table II r = 0.51 to 0.61).

From this study, it could be concluded that salivary defences are compromised and salivary enzymes activity is increased in patients with periodontitis. A weak salivary immuno system makes the periodontal ligament more vulnerable to the

destruction by enzymatically. This and other studies indicate that whole saliva may contain simply measured indicators of effect of thiocyanate and AST, ALT and LDH activity and may provide an important tool for monitoring and treating periodontitis. Hence salivary thiocyanate may have a protective role in periodontitis patients. In long run further studies are required on large samples to determine the relationship between salivary thiocyanate levels and periodontal disease.

Conclusion

Salivary thiocyanate, AST, ALT and LDH levels reflect inflammation and destruction of periodontal tissue, suggesting clinically useful markers.

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