Estimation and correlation of salivary thiocyanate levels in periodontally healthy subjects, smokers, nonsmokers, and gutka-chewers with chronic periodontitis

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ABSTRACT

Aim: This study was conducted to estimate and correlate salivary thiocyanate (SCN) levels in periodontally healthy subjects, smokers, nonsmokers, and gutka-chewers with chronic periodontitis.

Methodology: The study population consisted of 40 systemically healthy subjects in the age group of 18–55 years that was further divided into four groups: Control, smokers, nonsmokers, and gutka-chewers with chronic periodontitis. Gingival index (GI) (Loe and Silness-1963), probing depth (PD), clinical attachment loss was assessed. Estimation of SCN was performed by ultraviolet spectrophotometer at 447 nm wavelength. Statistical analysis was performed using the one-way ANOVAs Welch test and Pearson’s correlation test using SPSS version 17 software.

Results: Results showed statistically significant increase in SCN levels in smokers as compared to gutka-chewers with chronic periodontitis, control, and nonsmokers with chronic periodontitis subjects. Significantly higher PD and loss of attachment were seen in smokers group compared with other groups. A negative correlation observed between the GI and thiocyanate levels.

Conclusion: The present study revealed a significant increase in SCN levels in smokers with periodontitis as compared to nonsmokers.

Key words: Salivary peroxidase, salivary thiocyanate, smokeless tobacco, smoking

Smoking is a major risk factor for chronic periodontitis. Periodontitis is a multifactorial disease which is initiated by plaque and influenced by other factors which also play a role in the pathogenesis and disease progression. Effect of smoking manifests as increased loss of attachment, development, and progress of inflammation, whereas smokeless tobacco leads to/associated with localized gingival recession at the site of tobacco placement. Smokeless tobacco is commercially available as gutka, which contains areca nut, slaked lime and spices and more nicotine content than cigarette. Periodontal disease has been primarily diagnosed by clinical and radiographic findings. Saliva can be a valuable source because it contains specific biomarkers for periodontal diseases.

Salivary thiocyanate (SCN) is a metabolic product of cyanide, found in organic and inorganic compounds. They prevent toxic accumulations of hydrogen peroxide (H₂O₂) and hypochlorite (ClO⁻), which may be carcinogenic or mutagenic.

SCN concentrations are variable; it ranges from 0.5 to 2 mM in nonsmokers, whereas it can be as high as 6 mM in heavy smokers.

The present study was taken up to estimate and correlate SCN levels in periodontally healthy subjects,
Thiocyanate levels in smokers, nonsmokers, and gutka-chewers with chronic periodontitis.

**METHODOLOGY**

Ethical clearance for the study was obtained from the Institutional Ethical Committee. The sample size was calculated at 80% of power and 5% significance level. The data were collected from the subjects visiting the out-patient section of Department of Periodontology of the institution. All the participants were provided a verbal explanation of nature of the study, and informed consent was obtained. In this cross-sectional, biochemical study, the study population consisted of 40 systemically healthy male subjects in the age group of 18–55 years that was further categorized into four groups comprising 10 in each group.

- **Group I** (control): Periodontally healthy subjects
- **Group II** (nonsmokers with periodontitis): Subjects with probing depth (PD) of ≥4 mm and attachment loss ≥2 mm and gingival index (GI) score of ≥1 in at least 30% teeth in the oral cavity
- **Group III** (smokers with periodontitis, nontobacco chewers): Subjects with PD ≥4 mm and attachment loss ≥2 mm and GI score of ≥1 in at least 30% teeth in the oral cavity. Subjects who smoked >5 cigarettes/beedis per day for the last 1 year but not chewed tobacco of any kind
- **Group IV** (gutka-chewers with periodontitis, without smoking): Subjects with PD ≥4 mm and attachment loss ≥2 mm and GI score of ≥1 in at least 30% teeth in the oral cavity. Subjects who have the habit of gutka chewing for the last 1 year, but not smoked what so ever.

Subjects who had received any periodontal treatment during the past 6 months, subjects with < 20 natural teeth, with any systemic disease, on any antimicrobial, or anti-inflammatory medication within the previous 3 months for any reason were excluded from the study.

The periodontal status was assessed by recording GI (Loe and Silness-1963), PD, clinical attachment loss (CAL). For biochemical analysis, unstimulated 3 ml of whole saliva samples were collected and centrifuged at 12,000 rpm for at least 10 min. For thiocyanate determination, a volume of 0.5 ml of supernatant saliva obtained after centrifugation was mixed with 9.5 ml Ferric nitrate (Fe [NO$_3$]$_3$) reagent; which leads to the formation of ferric thiocyanate (FeSCN$^{2+}$) complex by the following reaction: Fe$^{3+}$ (aq) + SCN (aq) $\rightarrow$ FeSCN$^{2+}$ (aq), which gives the solution a deep red colour.

Samples were measured with ultraviolet spectrophotometry (UV-1800®, Shimadzu Corp., Kyoto, Japan) at 447 nm wavelength. The thiocyanate concentration was calculated through Lambert and Beer’s law. Statistical calculation and data analysis were performed with one-way ANOVA Welch test and Pearson’s correlation test using SPSS version 17 software (SPSS v.17.0 for Microsoft Windows, IBM, Chicago). $P$ value of 0.05 or less was considered to be significant.

**RESULTS**

The mean age of subjects was 34.25 ± 10.04. In this study, mean thiocyanate levels 2.2938 ± 0.8130 mM found in smokers which were significantly higher ($P < 0.001$) as compared to gutka-chewers with chronic periodontitis 0.828 ± 0.3301, control 0.802 ± 0.336, and nonsmokers with chronic periodontitis subjects 0.927 ± 0.953 [Graph 1]. Significantly higher PD 3.41 ± 0.3247 and CAL 4.85 ± 0.6114 was shown in smokers group compared to other groups by using one-way ANOVA Welch test. A negative correlation observed between GI and SCN levels by using Pearson’s correlation test [Graph 2].

**DISCUSSION**

Intake of vegetables such as cabbage, turnip, kale, and foods such as milk and cheese produce low levels of thiocyanate in body fluids (e.g., serum, saliva, urine). Exposure to cyanide, inhalation of fires or tobacco smoke lead to higher levels of thiocyanate. Hence, it can be considered as a biomarker for identification of nonsmokers and smokers.

Tobacco use leads to an alteration in the production or composition of saliva along with an alteration in the mucosal permeability and predisposes to oral cancer.

SCN prevent toxic accumulations of H$_2$O$_2$ and ClO$^-$, which may be carcinogenic or mutagenic. Salivary SCN reacts with the H$_2$O$_2$ in the oral cavity, leading to the elimination of the H$_2$O$_2$. This reaction is catalyzed by the peroxidase. In the case of peroxidase inhibition, for example, when saliva is exposed to cigarette smoke, H$_2$O$_2$ is not removed and leads to the production of free radicals. The free radicals are highly reactive and immediately react with neighboring cellular macromolecules including DNA. This may result in malignant transformation and could be the

Graph 1: Comparison of thiocyanate levels between different groups
reason of cigarette smoke-induced role of free radicals in oral squamous cell carcinoma.\(^{[17]}\)

Insufficient levels of thiocyanate may manifest inflammatory diseases including atherosclerosis, neurodegeneration and certain cancers (e.g., gastric cancer, etc.)\(^{[10]}\)

In this study, smokers with chronic periodontitis exhibited lower GI score compared to other groups, which is in accordance with the study done by Kalburgi et al.\(^{[8]}\) Nicotine causes vasoconstriction of gingival blood vessels leading to less bleeding on probing in smokers. It is also noticed that smokers with chronic periodontitis showed a negative correlation between GI score and thiocyanate levels, which is in accordance with the study done by Jalil et al.\(^{[18]}\) There is a significantly higher PD, and loss of attachment was observed in smoker’s group compared to other groups.

The present study revealed a significant increase in SCN level in smokers as compared to gutka-chewers. The main source of thiocyanate is tobacco smoke which is absorbed in the lungs and later metabolized to thiocyanate. Thus, it can be estimated only in smokers and not in tobacco chewers. These findings are in accordance with a study done by Foss et al. and Aggarwal et al.\(^{[19,14]}\)

CONCLUSION

The present study revealed a significant increase in SCN levels in smokers with periodontitis as compared to nonsmokers. Within the limitations of this study, we can conclude that SCN can act as a reliable biochemical indicator for assessing smoking behavior and possible tissue damage. Further investigations/studies utilizing larger sample sizes and longer follow-up periods are recommended for supporting the finding of this study.

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Conflicts of interest

There are no conflicts of interest.

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