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Salivary thiocyanate levels among tobacco users, non-users, and passive smokers: A biochemical study

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Original Article

Abstract

BACKGROUND AND AIM: The prevalence of tobacco use and its associated mortality is increasing worldwide. Biomarkers in saliva, such as thiocyanate levels, have been shown to be a suitable indicator for smoking. The present study is conducted to determine the thiocyanate levels in saliva and compare them among tobacco (smoking and smokeless) users, passive smokers, and non-smokers.

METHODS: A cross-sectional comparative study was conducted on 100 patients attending a dental college in South India. The patients were inquired about their tobacco habits and were divided into 4 groups: smokers (25), passive smokers (25), smokeless tobacco users (25), non-users (25). Saliva samples were collected from subjects in sterile containers, and thiocyanate levels were estimated. The results were tabulated and analyzed using SPSS software. Kruskal-Wallis test was used for the intergroup comparison of salivary thiocyanate levels. Mann-Whitney U test was used for the pairwise group comparison. If $P < 0.0500$, the results were reported to be statistically significant.

RESULTS: The results of the study showed that the salivary thiocyanate level was 79.46 ± 7.80, 50.16 ± 6.87, 50.16 ± 13.83, and 36.61 ± 5.84 mmol/l among smokers, passive smokers, smokeless tobacco users, and non-users, respectively. There was a statistically significant difference in salivary thiocyanate levels among various groups ($P \leq 0.0001$). All pairwise comparisons showed statistical significance ($P \leq 0.0001$) except the comparison between the passive smokers and smokeless tobacco users groups ($P \leq 0.9200$).

CONCLUSION: Salivary thiocyanate may be a diagnostic biomarker for differentiating tobacco users from the non-users.

KEYWORDS: Saliva; Thiocyanate; Smoking; Tobacco; Tobacco Smoke Pollution

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Globally, tobacco is the leading preventable cause of death, which causes one person to die every 6 seconds, and it is estimated to rise to more than 8 million deaths per year by 2030, most of who will be from the low- and middle- income countries.[1] Owing to the increasing tobacco availability and consumption in India, the World Health Organization (WHO) has considered it to be an 'epidemic'. Tobacco dependence has been classified as a disease by the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10). The tobacco consumption is rising by 3.4% every year and is the leading cause of head and neck cancers in India.[1] Hence, tobacco has become a significant public health concern in this country.

WHO has estimated that there are around 1.3 billion adult smokers globally (1/3rd of the global population), a vast majority of whom (about 84%) live in developing countries.[2] Every year, more than 5 million deaths are reported due to the use of tobacco.[3] The Global Adult Tobacco Survey (GATS)-India 2010 has reported that about 275 million adults, who account for 35 % of

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the Indian population, consume some form of tobacco, the most prevalent being smokeless tobacco. There are about 206 million users of smokeless tobacco in India, which is the highest rate in the world.[4]

Hydrogen cyanide and carbon monoxide are among the many gases present in tobacco smoke. Thiocyanate (SCN) present in the body fluids is partly because of the detoxification of hydrogen cyanide in smokers due to which the thiocyanate levels in the sera, urine, and saliva of smokers are found to be three times more in comparison to that of non-smokers. The half-life of thiocyanate is 10-14 days. Hence, the levels are less likely to vary in smokers within the span of a few days. Each cigarette delivers 30 to 200 µg of hydrogen cyanide into the mouth of the smokers.[5]

Smokeless forms of tobacco are commercially available as gutkha, khaini, mishri, paan, etc. They consist of mixture of slaked lime, areca nut, tobacco, and spices. Their role in the occurrence of oral cancers has been established in the literature.

Biomarkers in saliva such as thiocyanate levels have shown to be an acceptable indicator for smoking[6] and that its specificity to identify smokers is not affected by alimentation.[7] However, there was a paucity of studies on salivary thiocyanate levels in smokeless tobacco users; hence, the need for conducting the present study was felt. Therefre, the present study is carried out with an objective to determine the saliva thiocyanate levels and to compare them among tobacco users (smoking and smokeless), passive smokers, and non-users of tobacco.

Methods

This observational study was conducted on patients visiting the outpatient department of a private dental college and hospital in Mangalore, Karnataka, India. Institutional ethical clearance was obtained prior to commencing the study (ABSM/EC/31/2015 dated 23.04.2015).

The sample size was calculated based on the pilot study and a minimum sample of 22.5 was estimated in each group, which was rounded off to 25. A total of 100 study participants were selected by convenience sampling and categorized into the four groups based on the following criteria:

- Group I: Smokers
- Group II: Non-users (who do not use tobacco at all)
- Group III: Passive smokers (who are exposed to secondhand smoking)
- Group IV: Users of smokeless form of tobacco.

WHO classifies individuals as a smoker if they smoke any tobacco product either daily or occasionally at the time of the survey. Those who never smoked at all were termed as non-smokers and the ones who were exposed to tobacco smoke at workplace or home or during travel but never smoked were classified as passive smokers.[8] Smokeless tobacco products are tobacco products without combustion or pyrolysis at the time of use of gutkha, betel quid with tobacco and areca nut, khaini, mishri, etc.

The inclusion criteria were patients aged 20-40 years, systemically healthy patients, and who were willing to participate in the study and signed the informed consent. Patients with systemic diseases, such as myocardial infarction (MI), liver diseases, muscular dystrophy, and who were past smokers, were excluded from the study.

A self-administered, pretested questionnaire was administered to all the 100 study subjects. Questions included socio-demographic details, presence or absence of tobacco habit, and details on tobacco use (quantity, frequency, and duration of the habit) or passive exposure of the participants. Saliva samples were collected from the subjects between 9 a.m.-12 p.m. in sterile plastic containers according to the method proposed in the study by Navazesh.[9] This was to ensure that the variability in salivary flow rate and composition due to circadian rhythm was minimized. The subject was asked to rinse the mouth thoroughly with distilled water to remove any food debris and

then directed to spit into a sterile plastic container. Forcible spitting was avoided. The saliva samples were sent for biochemical analysis. Saliva was measured and analyzed by an independent scientist who was not aware of the study groups.

About 0.5 ml of 1 mol/l hydrochloric acid was added to 1 ml of saliva. After addition of two drops of a saturated solution of bromine water, the solution was mixed. Excess bromine was removed by adding three drops of arsenious oxide solution. About 1.8 ml of pyridine-p-phenylenediamine reagent was added, which resulted in a reddish-pink dye, the absorbance of which was read using a spectrophotometer at a wavelength of 520 nm against a reagent blank within 10 mm which is the isosbestic point.[10]

Data were entered in Microsoft Excel for Windows and analyzed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). Since the data collected followed a non-normal distribution, non-parametric tests were used in the analysis of the data. Comparison of salivary thiocyanate levels among the various groups was performed using the Kruskal-Wallis test. Mann-Whitney U test was performed for pairwise group comparison. If $P < 0.0500$, the difference was considered statistically significant.

Results

The present study was conducted to determine the thiocyanate levels in saliva and to compare them among tobacco (smoking and smokeless) users, passive smokers, and non-users of tobacco. Of the 100 subjects, 93 and 7 were males and females, respectively. The mean age of the participants was 36.5 ± 14.4 years. About 25 subjects were smokers, 25 used smokeless tobacco, 25 were passive smokers, and 25 non-smokers. Of the 25 smokers, 16 smoked daily and 9 occasionally. 18, 6, and 1 subjects smoked respectively cigarettes, hand rolled beedis, and cigar. Of the 25 smokeless tobacco users, 14 and 11 chewed tobacco daily and occasionally, respectively. Of the 100 subjects, 12, 10, and 1 reported that their family members smoked daily, weekly, and monthly, respectively. 57 and 43 of the subjects worked respectively outdoors and indoors, and 25 reported that their colleagues smoked at the workplace. Only 12 tried to quit smoking in the past, but it was unsuccessful, only 5 had visited a health care provider, and 4 were advised to quit tobacco use in the past 12 months by their health care providers.

In the current study, it was seen that the mean thiocyanate levels in saliva were 79.46 ± 7.80, 50.16 ± 6.87, 50.16 ± 13.83, and 36.61 ± 5.84 mmol/l among smokers, passive smokers, smokeless tobacco users, and non-users, respectively. A comparison of the salivary thiocyanate levels among various groups was conducted using the Kruskal-Wallis test, indicating statistically significant differences between various groups ($P \leq 0.0001$) (Table 1). Mann-Whitney U test was performed for pairwise group comparison. All pairwise comparisons among the groups showed statistically significant differences ($P \leq 0.0001$) except the passive smokers-smokeless tobacco users groups ($P \leq 0.9200$) (Table 2).

Discussion

The current study was conducted to determine the thiocyanate levels in saliva and to compare among tobacco users (smoking and smokeless), passive smokers, and non-users of tobacco.

Table 1. Comparison of the salivary thiocyanate levels among various groups using the Kruskal-Wallis test

Group	N	Salivary thiocyanate level (mmol/l) (mean ± SD)	Mean rank	Kruskal-Wallis χ^2	P
Smoker	25	79.455 ± 7.8043	87.40	70.553	≤ 0.0001
Tobacco non-users	25	36.610 ± 5.8352	19.04		
Passive smokers	25	50.590 ± 6.8743	49.88		
Smokeless tobacco users	25	50.155 ± 13.8320	45.68		
Total	100	54.202 ± 18.0962			

SD: Standard deviation

Table 2. Pairwise comparison of the groups using the Mann-Whitney U test

Group	N	Mean rank	Sum of ranks	Mann-Whitney U	P
Smokers-Nonsmokers	25	38.00	950.00	.000	≤ 0.0001*
	25	13.00	325.00		
Smokers-Passive smokers	25	37.92	948.00	2.000	≤ 0.0001*
	25	13.08	327.00		
Smoker-Smokeless tobacco users	25	37.48	937.00	13.000	≤ 0.0001*
	25	13.52	338.00		
Nonsmokers-Passive smokers	25	13.92	348.00	23.000	≤ 0.0001*
	25	37.08	927.00		
Nonsmokers-Smokeless tobacco users	25	18.12	453.00	128.000	≤ 0.0003*
	25	32.88	822.00		
Passive smokers-Smokeless tobacco users	25	25.72	643.00	307.000	P ≤ 0.92034, Not significant
	25	25.28	632.00		

*P < 0.5000 Significance level

The results of the study suggested that the mean thiocyanate levels in saliva were 79.46 ± 7.80, 50.16 ± 6.87, 50.16 ± 13.83, and 36.61 ± 5.84 mmol/l among smokers, passive smokers, smokeless tobacco users, and non-users, respectively. Intergroup comparison showed statistically significant difference in thiocyanate levels of saliva. The passive smokers-smokeless tobacco users groups did not show any statistically significant difference.

Previous studies have reported that salivary thiocyanate is a valid indicator of exposure to cyanide among smokers.[6,7,11] However, very few studies have reported its levels among smokeless tobacco users. Significantly higher salivary thiocyanate levels were reported among smokers than that in all the other groups, a finding which is similar to the findings reported in other studies.[5,11,12]

Zil-a-Rubab and Rahman reported that the thiocyanate levels in serum were higher in smokers than non-smokers and passive smokers.[13] A study reported higher levels of thiocyanate levels in serum and saliva in smokers compared to non-smokers and those who quit smoking.[14] However, their study did not include the smokeless tobacco users and passive smokers. Yet, another study demonstrated that salivary thiocyanate levels among e-cigarette smokers and cigarette smokers were not significantly different, but were higher compared to those who did not smoke.[15]

Additionally, a significant difference was present between passive smokers and non-smokers and between smokeless tobacco users and non-smokers. However, passive smokers-smokeless tobacco users did not show any statistical significance difference in the salivary thiocyanate levels. The mainstream cigarette smoke contains hydrogen cyanide, which is detoxified to thiocyanate (SCN) and excreted mainly in the urine. However, minor concentrations are also found in sweat and saliva.[12]

Tobacco use is not the only cause for increased serum or salivary thiocyanate levels. An increase in thiocyanate levels occurs due to occupational exposure in electroplating, refining of precious metal, steel, and gas manufacturing industries, etc. Thiocyanate is also produced in almonds and few vegetables such as garlic, cabbage family, turnips, etc. It is observed that the SCN levels among vegetarians are higher than the non-vegetarians. However, the dietary thiocyanate levels are not as much as those observed in tobacco users, especially smokers.[7,11,12] Zil-a-Rubab and Rahman in their study also emphasized that although there is considerable variation of dietary thiocyanate levels and other environmental exposure, the levels in smokers were much higher than the passive smokers and non-smokers.[13]

The threshold limit value of hydrogen cyanide for occupational exposure is 10 part per million (ppm). However, its concentration ranges from 40-70 ppm in mainstream smoke and is < 5 ppm in sidestream smoke.[12] This could be a possible explanation of why smokers have high levels of salivary thiocyanate, followed by passive

smokers compared to non-smokers and smokeless tobacco users. A similar finding has been observed in many other studies.[5-7,11]

To avoid age of initiation of smoking and smoking duration as a confounding factor in the study, only subjects between the age range of 20-40 years were included in the present study. Intergroup comparison of age distribution among the groups did not show any statistically significant difference. The assessment of smoking/passive smoking status of individuals was based on the questionnaire, assuming that all the answers were sincere. However, there may be information bias, which was a limitation of the present study.

Conclusion

Thiocyanate levels in saliva could be a biomarker for differentiating between tobacco users (smoking and smokeless) and passive smokers from tobacco non-users. It could be used as a tool in population studies to determine the prevalence of tobacco and also to monitor changes in tobacco usage among the population over time.

Conflict of Interests

Authors have no conflict of interest.

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