RESEARCH ARTICLE

Estimation and Comparison of Salivary Thiocyanate Levels in Active and Passive Smokers

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Abstract

Introduction: Tobacco causes various adverse effects such as oral precancer, cancer, chronic obstructive pulmonary disease, hypertension, peripheral vascular disease, and myocardial infarction, etc. Passive smoking is inhalation of smoke called second-hand smoke or environmental tobacco smoke by persons other than active smoker. Tobacco smoke contains thiocyanate (SCN), which can enter the human body through ingestion or inhalation. Various adverse effects have been reported due to increased serum SCN concentration. Serum and salivary SCN levels have been used to evaluate toxicity among active smokers. However, screening of exposure to tobacco among passive smokers needs to be studied. Estimation of salivary SCN can be a useful indicator for the same.

Aim: Estimation and comparison of the salivary SCN levels in active and passive smokers.

Materials and methods: Twenty passive smokers and 10 active smokers aged between 18 years and 21 years, with the history of minimum of 1 cigarette/day, for a minimum period of 3 months were included in the study. Active smokers with history of chewable tobacco and passive smokers with history of chewable/nonchewable tobacco, patients under nitroprusside therapy, alcohol consumption, and any systemic diseases were excluded from the study. Whole unstimulated saliva was collected and was stored at −20°C. Salivary SCN levels were estimated.

Results: Study shows increased salivary SCN level in active and passive smokers with increased number of cigarettes and duration of exposure. Study revealed positive correlation with salivary SCN level and increase in number of cigarettes and exposure among passive smokers. Sensitivity and specificity are 70% and 80%, respectively.

Conclusion: Salivary SCN can be used for screening of passive smokers for the exposure of tobacco to monitor their associated toxicity.

Clinical significance: Passive smoking is inhalation of smoke called second-hand smoke or the environmental tobacco smoke by persons other than active smoker. Tobacco smoke contains SCN, which can enter the human body through ingestion or inhalation. Various adverse effects have been reported through increased serum SCN concentration. The present study significantly indicates that passive smokers are at equal risk to tobacco smoke as active smokers.

Keywords: Active smokers, Passive smokers, Salivary smoke, Thiocyanate, Tobacco smoke.

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Introduction

Saliva is considered to be analytical for the maintenance of healthy oral mucosa. Oral fluids provides noninvasive easy medium for the diagnosis in variety of diseases.[1] Quantitation in a smoker to exposure to tobacco is useful and necessary for the epidemiological studies to relate their smoking habits to pathology.[2] Literature shows various studies on quantitating the mean of cigarette consumption, that is, the rate of consumption, cumulative risk, that is number of packs of cigarettes per year, and the various types of smoking habits. Measuring nicotine and nicotinine levels in serum and urine samples requires elaborative methodology that is expensive and which may not be adequate for the validation in nicotine gum chewers.[3] One of the good markers for smoke inhalation is carbon monoxide. Carboxyhemoglobin levels can be observed for confirming the recently quit smoking habit as its half-life is only for few hours in the blood.[4]

Salivary SCN and serum SCN can be used to differentiate between smokers and previously exposed smokers. The same can be used with a high probability, especially in those who have quit habit of smoking for 14 days minimum, thus segregating them clearly from current smokers. Hydrogen cyanide is present in cigarette smoke. Smoke contains thiocyanate is produced as an end product of detoxification process of hydrogen cyanide.[5] The excretion of SCN in urine, saliva, and sweat can be utilized as an useful marker for the exposure in smokers and nonsmokers.[1]

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Passive smoking is inhalation of smoke called second-hand smoke or the environmental tobacco smoke by persons other than active smoker. Tobacco causes various adverse effects such as oral precancer, cancer, chronic obstructive pulmonary disease, hypertension, peripheral vascular disease, and myocardial infarction. Tobacco smoke contains SCN, which can enter the human body through ingestion or inhalation. Various adverse effects have been reported through increased serum SCN concentration. Serum and salivary SCN levels have been used to evaluate toxicity among active smokers.[6] However, for screening of exposure to tobacco among passive smokers, salivary SCN can be used.

Materials and Methods

Ten active smokers who were between 18 years and 21 years of age with history of minimum 1 cigarette per day for minimum period of 3 months and age-matched 20 passive smokers were included in the study. Active smokers with history of chewable tobacco and passive smokers with history of chewable/nonchewable tobacco, patients under nitroprusside therapy, alcohol consumption, and any systemic diseases were excluded from the study. After the informed consent, whole unstimulated saliva was collected and was stored at –20°C. Salivary SCN levels were estimated.

Reagents

Methodology was carried out based on the standard protocol.[1]

Stock Solution

To 2 g of KSCN, 1 liter of distilled water was added. Solution was then titrated against 20 mL of silver nitrate solution. 5 mL of concentrated nitric acid using 1 mL of saturated solution of ferric ammonium sulfate as an indicator was used. The stock solution contained 100 mg of SCN ions per 100 mL.

Working Standard Solution

10 mL of stock solution was mixed with 10 mL of distilled water. This solution contained 10 mg of SCN ions per 100 mL.

Ferric Nitrate Solution

50 g of crystalline ferric nitrate was added to 500 mL of distilled water. 25 mL of concentrated nitric acid was added to distilled water to make 1 L of solution.

Blank Solution

25 mL of concentrated nitric acid was added to 1 L of distilled water.

Calibration

1 mL of working SCN standard, 8 mL of distilled water, and 1 mL of ferric nitrate solution were poured into test tube. Reading was taken at the wavelength of 405 nm in spectrophotometer.

Average of four readings were recorded as the standard S. The value of S was recorded as 0.28. The absorbance readings were recorded as 0.10, 0.19, 0.32, and 0.5 at 1, 2, 3, and 4 mL of working solution, respectively. The absorbance readings were directly proportional to the SCN concentrations. Therefore, the standard reading S obtained from solutions contained 10 mg of SCN per 100 mL.

Method of Collecting Saliva

2 mL of whole unstimulated saliva was collected within 24 hours of exposure to tobacco smoke. Later saliva was stored at the temperature of –20°C. Saliva samples were taken for estimation within 24 hours. Samples were centrifuged at 3,000 rpm for 5 minutes and clear saliva was separated. One half of the saliva sample, i.e., 0.5 mL of clear saliva was poured into a test tube. 4 mL of distilled water and 0.5 mL of ferric nitrate were added. The other half of clear saliva, i.e., 0.5 mL of clear saliva was poured in test tube. 4 mL of distilled water and 0.5 mL of blank solution were added. After 5 minutes, the reading at 405 nm was taken on spectrophotometer.

Calculating SCN Content

Reading of blank solution was subtracted from the reading of unknown solution to obtain true reading. 10 mg per 100 mL solution was used, and readings were calculated as follows:

Reading of unknown—U × 10 mg = mg of SCN per 100 mL of saliva

Reading of standard—U/S × 10 = U/0.28 = mg of SCN per 100 mL of saliva.

Results

Discussion

In the present study, salivary SCN level increased in active and passive smokers with increase in the number of cigarettes and duration of exposure, indicating detoxification of cyanide.[7] Study revealed that salivary SCN level positively correlated with increase in the number of cigarettes and exposure among passive smokers which indicates that although SCN levels do vary according to diet and atmospheric exposure, the SCN levels in saliva exceeded in smokers than that of passive smokers.[7] Sensitivity and specificity of mg of SCN in 100 mL of saliva were measured ranging from 0.1 to 0.5 mg per 100 mL of saliva as per the absorbance readings found during calibration. Seventy percentage of sensitivity and 80% of specificity were noted at 0.3 mg/100 mL of saliva as 7/10 active smokers that showed >0.3 mg and 16/20 passive smokers that showed <0.3 mg per 100 mL of saliva (Tables 1 to 3).

Smoke contains thiocyanate is an end product of hydrogen cyanide present in cigarette smoke. Excretion of it in urine, saliva, and sweat provides as a useful tool for exposure in smokers and nonsmokers.[8] As salivary tests have advantages of being easy and noninvasive sampling technique, with good stability, even at nondemanding storage conditions. This can be considered for estimation of salivary SCN.

Increased salivary SCN levels can cause neurological alterations such as amblyopia, children suffered from infant strabismus as a

Table 1: Increased salivary thiocyanate level in active and passive smokers with increased number of cigarettes and duration of exposure

	Active	*Passive*	*p*
No. of cigarettes/day	6.9 ± 5.85 (1–20)	2.6 ± 2 (1–9)	0.027*
Duration in months	27.6 ± 19.43 (6–60)	9.4 ± 6.76 (3–36)	0.012*
Estimated SCN in mg/100 mL	1.32 ± 0.17 (0.38–2.14)	0.63 ± 0.35 (0.38–1.42)	0.002*

*Statistically significant

Table 2: Positive correlation with increased salivary thiocyanate level with increase in number of cigarettes and exposure among passive smokers

Cigarettes/day and duration: (Pearson correlation)	*SCN in active*	*SCN in passive*
No. of cigarettes/day	0.844 ($p = 0.002$*)	0.715 ($p = 0.001$*)
Duration	0.038 ($p = 0.918$)	0.140 ($p = 0.097$)
Exposure (no. of cigarettes and duration)	0.526 ($p = 0.119$)	0.567 ($p = 0.001$*)

*Statistically significant

Table 3: Sensitivity and specificity of mg of thiocyanate in 100 mL of saliva when measured ranging from 0.1 to 0.5 mg per 100 mL of saliva. Seventy percentage of sensitivity and 80% of specificity were noted at 0.3 mg/100 mL of saliva

SCN	*Value >0.3*	*Value <0.3*	*Total*
Active	7	3	10
Passive	4	16	20

result of their mothers with habits of smoking. Smoking is observed as one of the factors that is suggested in the delayed wound healing. Increased exposure of tobacco smoke leads to rashes, flushing, altered platelet aggregation increased intracranial pressure, altered ECG, and hypo functioning of thyroid gland.[8] Estimation of salivary SCN levels is used most commonly as a biochemical marker for establishing their incidence and prevalence in consumption of tobacco among the adolescents group.[9] The present study indicates that whole saliva contains indicators for SCN, which can be measured and can be utilized as a marker for assessment of tobacco exposure among the passive smokers.

Conclusion

Serum and salivary SCN levels have been used to evaluate toxicity among active smokers. However, salivary SCN can be used for screening of passive smokers for the exposure of tobacco to monitor their associated toxicity.

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