Usefulness of Thiocyanate as a Biological Marker for Discrimination of Smoking Status

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Smokers have increased risk of death more than non-smokers due to its association with cancer, vascular and respiratory diseases, and tuberculosis. Epidemiological research has generally relied upon self-report information concerning smoking status but the validity is limited. Biochemical markers have been used in research on smoking are based on thiocyanate, nicotine, cotinine and carbon monoxide. Among them, thiocyanate is chosen as biomarker of smoking because of its long half-life. The aim of the study was to demonstrate the suitability of urinary, blood and salivary thiocynates (SCN^-) as indicators of smoking and to investigate the correlation among its content in salivary, serum and urinary SCN^-, and duration and amount of smoking. Thiocyanate levels were determined by spectrophotometric method in saliva, serum and urine samples to compare in smokers, passive smokers and non-smokers. The median saliva thiocyanate concentration of smokers [43.79(8.14-187.59 mg/ml)] was significantly higher ($p<0.001$) as compared to that of passive smokers [26.26(7.95-80.04 mg/ml)] and non-smokers [25.00(5.23-69.96 mg/ml)]. The salivary thiocyanate levels significantly correlated with duration of smoking in years ($r=0.366$) and number of cigarette smoking per day ($r=0.316$). Among three types of body fluids, saliva thiocyanate is the best biological marker for discrimination of smoking status. Heavy smokers can be distinguished from passive smokers and non-smokers by determination of thiocyanate level in saliva.

Keywords: Thiocyanate, Smoking, Saliva, Serum, Urine

INTRODUCTION

Among 1.3 billion smokers, over 80% resides in low- and middle-income countries (LMICs). Smokers have 2-3 times increased risk of death than non-smokers, leading to a reduction in lifespan of at least one decade. Smoking causes cancer, vascular and respiratory diseases, and tuberculosis. About 50% of the current five million smoking-related deaths world-wide occur in LMICs.[1] In 2012, an estimated 21.1% of people aged 15 or older were current-smokers (35.6% of males and 6.6% of females). By 2014, smoking prevalence dropped slightly to 20.5% (34.6% of males and 6.2% of females).[2] In Myanmar, a national survey on 8757 adults aged 24-64 years showed that the prevalence of current-smokers is 26.1% (43.8% for males and 8.4% for females) in 2014. Approximately 80% of the current-smokers were daily smokers and 28.1% of daily smokers used cigarettes. The per-centage of respondents who had been exposed to second-hand smoke in home on or more of the past 30 days was 39.1% and that in the workplace was 27.5%.[3] Research on cigarette smoking habits, smoking

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DOI: https://doi.org/10.34299/mhsrj.00948

prevention and cessation programmers needs accurate measurements of smoking behavior. Such research has generally relied upon self-report for information concerning smoking status but the validity of this measure is questionable.

Accuracy of data depends on many factors: bias of interviewer, the understanding and recall of the respondent, the degree of communication between the two individuals and accuracy of recording.[4] Thus, investigators recognized the need for biochemical validation of smoking behavior. A number of biochemical markers have been used in research work on smoking. These measures are based on thiocyanate, nicotine, cotinine and carbon monoxides.[5] Unfortunately, all these methods showed vary reliability, cost and accuracy.

Cyanide is a potent toxic agent present in the cigarette smoke and is metabolized to thiocyanate.[6] Higher concentrations of thiocyanate in the serum, urine and saliva of smokers were compared with those of non-smokers.[7, 8] Thiocyanate is chosen as a biomarker of smoking because of its half-life of 10-14 days which is longer than any other markers likes carbon monoxide, cotinine, and nicotine.[9]

Thiocyanate level of Myanmar people in relation to smoking status had never been studied. This study was undertaken to compare salivary, serum and urine thiocyanate levels in smokers, passive smokers and non-smokers. Then, it was investigated to find out the applicability of thiocyanate levels of body fluids to assess the smoking status.

MATERIALS AND METHODS

Study population

The study included 110 healthy adult subjects between 15 and 68 years of age and composed of 45 smokers, 36 passive smokers and 29 non-smokers. According to Ariyothai *et al.*[10] the following working definitions were used in current research.

1) Smoker: any person who smoked a tobacco product at the time of study or persons who used to smoke but had stopped smoking less than six months before the interview.
2) Passive smoker: any non-smoker who was exposed to tobacco smoke, more than three times per week, either at home, work, or in public places.
3) Non-smoker: any person that has never smoked (non-active smoker) or who has never or less than three times per week been exposed to tobacco smoke by others at home, work, or in public places (non-passive smoker).

The subjects recruited were staff of DMR (Department of Medical Research) and their relatives.

A questionnaire was administered regarding age, gender, smoking status, number of cigarettes consumed, duration of exposure, passive exposure of the participants, betel chewing status, and diet history. After an interview, blood samples were taken in sitting position and in a smoke free environment. Blood samples (5 ml) were obtained by venipuncture with syringes. The blood was allowed to clot and all samples were centrifuged at 1200xg for 5 minutes. Serum were collected and stored at -20°C until analyzed. About 3 ml of saliva were collected from each of subject by continuous spitting into a plastic sample bottle. Urinary sample was collected the mid-stream urine with sterile screw-cap bottle.

Determination of serum, urine and salivary thiocyanate levels was done in the Biochemistry Research Division of DMR using spectrophotometric method.[11] The body fluid sample of 100 µl was mixed with 150 µl of distilled water (DW) and 250 µl of 20% trichloroacetic acid solution. The mixture was kept at room temperature for 10 minutes and centrifuged at 1500 rpm for 15 minutes. The supernatant (250 µl) was mixed with 250 µl of ferric nitrate reagent (80 g of $Fe(NO_3)_3.9H_2O$ dissolved in 250 ml 2N HNO_3, made to 500 ml with DW). The

absorbance was measured at 455 nm within 15 minutes against the control.

The data were analyzed with SPSS Version 16. Independent Student's 't' test was used to compare the continuous variables. Pearson correlation (2-tailed) test was used to test the relation between variables.

Ethical consideration

The study proposal was approved by the Ethics Review Committee of Department of Medical Research and written informed consent was taken from each participant.

RESULTS AND DISCUSSION

Table 1 shows the median salivary, serum and urinary thiocyanate levels of smokers, passive smokers and non-smokers. Among biological markers in 3 types of samples, salivary thiocyanate is significantly higher in smokers [43.79(8.14-187.59 mg/ml)] than passive [26.26(7.95-80.04 mg/ml)] and non-smokers [25.00(5.23-69.96 mg/ml)] ($p<0.001$).

Table 1. Thiocyanate levels in different body fluids according to smoking status

	Smokers (n=45)	Passive smokers (n=36)	Non-smokers (n=29)	p value
Serum thiocyanate (mg/ml)				
Mean	5.56 ±5.96	3.47 ±5.99	4.54 ±3.16	
Median	3.37	1.99	4.02	0.0225*
Range	1.02-35.66	0.52-37.02	0.65-15.19	
Urinary thiocyanate (mg/ml)				
Mean	17.89 ±13.73	14.13 ±11.41	20.71 ±11.82	
Median	12	11.72	19.94	0.107
Range	1.55-65.47	1.86-55.64	4.48-61.85	
Salivary thiocyanate (mg/ml)				
Mean	58.57 ±50.84	28.79 ±16.84	26.76 ±14.47	
Median	43.79	26.26	25.00	0.000*
Range	8.14-187.59	7.95-80.04	5.23-69.96	

*Significant difference between two groups ($p<0.05$)

The results indicate that measures of thiocyanate in saliva perform better than serum and urine in discriminating the status of smokers. The levels of thiocyanate in saliva are also influenced by diet and smoking habits. However, the diet influence on the salivary thiocyanate level was not excluded because of the incomplete data set on dietary history of the subjects.

Table 2. Correlation between thiocyanate levels of different body fluids and amount of smoking (duration of smoking and number of cigarettes smoked per day)

	thiocyanate (mg/ml)		
	Serum	Urinary	Salivary
Duration of smoking (years)	0.295*	-0.032	0.366*
Amount of smoking (number of cigarettes per day)	0.053	0.255	0.316*

*Correlation is significant at the 0.05 level (2-tailed)

In Table 2, the serum thiocyanate (r=0.295) and salivary thiocyanate (r=0.366) levels increased according to duration of smoking in years. The salivary thiocyanate also showed a significant correlation (r=0.316) with number of cigarette smoking per day. However, urinary thiocyanate level showed no significant correlation with smoking habits. Thus, among 3 types of sample, salivary thiocyanate is the best biological marker of smoking status for the current population.

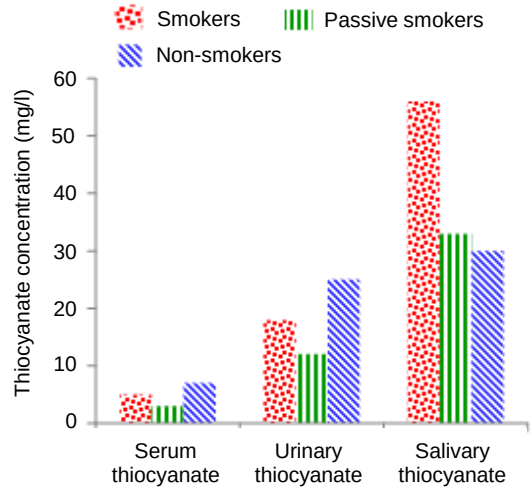

Fig. 1. Mean thiocyanate levels in different body fluids of male population according to smoking status

In Figure 1, the thiocyanate levels in 3 different body fluids have no significant

difference between the groups in male population (n=42). The median duration of smoking was 12(1-45) years and that for numbers of cigarette per day smoked was 4(1-20). However, in the female population (n=3) (Figure 2), there were significant differences in salivary and serum thiocyanate levels were found. The serum and salivary thiocyanate levels of smokers were significantly higher than those of the passive and non-smokers. The median duration of smoking of the female smokers was 29(20-35) years and that for number of cigarettes consumed per day was 4(2-15).

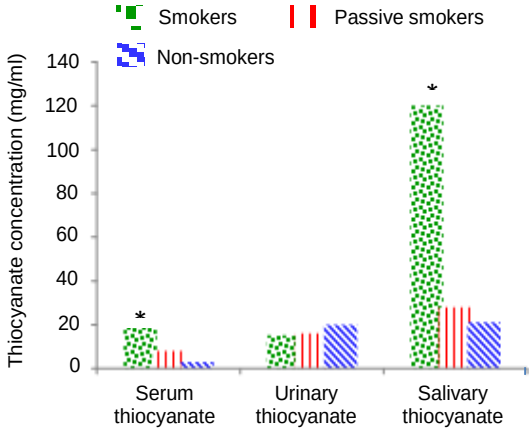

Fig. 2. Mean thiocyanate levels in different body fluids of female population according to smoking status. (*statistical significance between two groups)

Several studies of Luepker, *et al*., Muhammad, *et al.* and Rubaee, *et al.* showed that salivary thiocyanate level was significantly increased in smokers compared to non-smokers.[12, 13, 14] It is significantly higher as cigarette consumption increased. In this study, the thiocyanate concentrations in saliva were about 10 times higher than that in serum. This finding is supported by studies of Degiampietro and Peheim, and Riedel, *et al.* In the study of Degiampietro, the thiocyanate concentration in saliva was about 30 times higher than in plasma and the mean plasma thiocyanate concentration increased significantly according to the daily tobacco onsumption.[15] In Riedel's study, the thiocyanate levels in saliva were approximately 20 times higher than the corresponding levels in plasma. In addition, thiocyanate levels in saliva were found to be better precision than thiocyanate in plasma.[16]

In this study, thiocyanate level in serum is significantly correlated with duration of smoking rather than daily cigarette consumption. While thiocyanate levels in saliva showed significantly correlated with both duration and daily cigarette consumption. In general observation, the salivary thiocyanate level is significantly higher in smokers than non-smokers. When the analysis is done according to sex, both salivary and serum thiocyanate levels are higher in smokers than non-smokers in female population. Smoking females had a higher median value of serum/plasma thiocyanate than males which is in accordance with other studies of Degiampietro, *et al.* and Rubab, *et al.*[15, 17] For having statistically significant results in both sexes, the sample size needs to be larger.

In this study, the urinary thiocyanate level did not discriminate smokers from non-smokers nor passive smokers. The weak correlation between urinary thiocyanate to smoking habits was also not significant. This finding was similar to the study of Dhouha, *et al*.[18] Smoking increased the urinary thiocyanate concentration, but no close relationship between the measured values and the number of cigarettes smoked. The urinary thiocyanate values showed the great inter- and intra-individual variations among non-smokers, even greater extent among smokers.[19]

In contrast, in the study of Jain[7], urinary thiocyanate levels for smokers were 4.6 times higher than that for non-smokers. Generally, cigarette only smokers had statistically higher urinary thiocyanate levels than pipes only, cigars only, and mixed-use smokers. Twenty-four hours urinary analyses on 5 heavy smokers (20-30 cigarettes a day) showed that there were great differences in the excretion of thiocyanates in the urine in relation to the numbers of cigarettes smoked.[20] The type of smoke (cigars,

cheroots or mixed) used was unable to discriminate in the present study and it is one of limitations in the study.

Conclusion

Thiocyanate as an end-product of detoxification of hydrogen cyanide present in cigarette smoke was measured in different body fluids to distinguish the smokers from passive and non-smokers. Some foods including cassava, cabbage, broccoli, legumes, vegetables can elevate the thiocyanate level in body. In general, saliva thiocyanate level discriminates smokers from passive and non-smokers. It can reflect the habitual smoking status. According to sex group, female population showed significant high thiocyanate level in both saliva and serum. The serum thiocyanate level increased according to duration of smoking. The urinary thiocyanate level does not show any apparent difference between the study groups. Thus, salivary thiocyanate level is recommended to be used as a reliable, inexpensive and acceptable biomarker of smoking status.

Competing interests

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

The authors are grateful to subjects from Department of Medical Research for their voluntary participation in the research project. It is acknowledged to Dr. Khin Maung Maung, Former Deputy Director & Head, Radiation Toxicology Research Division and Daw Mee Mee Nwe, Former Research Officer, Biochemistry Research Division, Department of Medical Research, Yangon, Myanmar for sharing their knowledge and method implementing in this research.

REFERENCES

1. Jha P, MacLennan M, Chaloupka F, Yurekli A, Ramasundarahettige C, Palipudi K, *et al*. Global hazards of tobacco and the benefits of smoking cessation and tobacco taxes. In: *Disease Control Priorities. Cancer, Third ed*. International Bank for Reconstruction and Development/The World Bank, 2015; 175-193.
2. World Health Organization. Prevalence of Tobacco Use. In: *2016 Global Progress Report on Implementation of the WHO Framework Convention on Tobacco Control*. WHO 2016; 68-70.
3. Tint Swe Latt, Ko Ko Zaw, Ko Ko, Mya Ohmar, Moh Moh Hlaing, Ei Sandar Oo, *et al.* Tobacco Use. In: *Report on National Survey of Diabetes Mellitus and Risk Factors for Non-communicable Diseases in Myanmar (2014)*. MOH, WHO, WDF 2014; 26-48.
4. Densen PM, Davidow B, Bass HE & Jones EW. A chemical test for smoking exposure. *Archives of Environmental Health* 1967; 14(6): 865-874.
5. SRNT Subcommittee on Biochemical Verification. Biochemical verification of tobacco use and cessation. *Nicotine & Tobacco Research* 2002; 4(2): 149-159.
6. Tsuge K, Kataoka M & Seto Y. Cyanide and thiocyanate levels in blood and saliva of healthy adult volunteers. *Journal of Health Science* 2000; 46(5): 343-350.
7. Jain RB. Use of urinary thiocyanate as a biomarker of tobacco smoke. *Epidemiology: Open Access* 2016; 6(5): 1000268.
8. Zil-a-Rubab & Rahman M. Serum thiocyanate levels in smokers, passive smokers and never smokers. *The Journal of the Pakistan Medical Association* 2006; 56(7): 323-326.
9. Scherer G. Carboxyhemoglobin and thiocyanate as biomarkers of exposure to carbon monoxide and hydrogen cyanide in tobacco smoke. *Experimental and Toxicologic Pathology* 2006; 58(2-3): 101-124.
10. Ariyothai N, Podhipak A, Akarasewi P, Tornee S, Smithtikarn S & Thongprathum P. Cigarette smoking and its relation to pulmonary tuberculosis in adults. *The Southeast Asian Journal of Tropical Medicine and Public Health* 2004; 35(1): 219-227.
11. Bowler R. The determination of thiocyanate in blood serum. *Biochemical Journal* 1944; 38(5): 385-388.
12. Luepker R, Pechacek T, Murray D, Johnson C, Hund F & Jacobs D. Saliva thiocyanate:

A chemical indicator of cigarette smoking in adolescents. *American Journal of Public Health* 1981; 71(12): 1320-1324.

13. Muhammad I, Wasagu R & Abubakar M. Salivary level of thiocyanate in smokers and non-smokers: A case study taken at Usmanu Danfodiyo University, Sokoto Nigera. *Research & Reviews: A Journal of Life Sciences* 2017; 7(1): 1-5.
14. Rubaee DE, Kadhum HA & Hussaini NK. Toxic thiocyanate levels in saliva of healthy adult smokers and non-smokers from Baghdad city. *Al-Taqani* 2010; 23(5): 99-103.
15. Degiampietro D & Peheim E. Determination of thiocyanate in plasma and saliva without deproteinisation and its validation as a smoking parameter. *Journal of Clinical Chemistry and Clinical Biochemistry* 1987; 25(10): 711-717.
16. Riedel K, Hagedorn H & Scherer G. Thiocyanate in plasma and saliva, In: *The MAK-Collection Part IV: Biomonitoring Methods, Vol.13*. Wiley-VCH Verlag GmbH & Co. KGaA, 2013; 277-292.
17. Rubab Z & Rahman MA. Serum thiocyanate levels in smokers, passive smokers and never smokers. *The Journal of the Pakistan Medical Association* 2006; 56(7): 323-326.
18. Dhouha H, Asma E, Fadoua N, Wahiba D & Fadhel N. Comparison between plasma and urine thiocyanates and urinary cotinine determinations as indicators of cigarette smoking. *Journal of Health Science* 2009; 55(6): 895-900.
19. Heino J, Vilhunen R & Hernberg S. Urinary thiocyanate concentrations in smokers and non-smokers. *Scandinavian Journal of Work, Environment & Health* 1968; 5(1): 12-15.
20. Djuric D, Raicevic P & Konstantinovic I. Excretion of thiocyanates in urine of smokers. *Archives of Environmental Health: An International Journal* 1962; 5(1): 18-21.