

## Serum Thiocyanate Levels in Smokers, Passive Smokers and Never Smokers

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### Abstract

**Objective:** To compare the serum thiocyanate levels in smokers, passive smokers and never smokers.

**Methods:** Serum thiocyanate was analyzed on 135 self reported smokers, never smokers and passive smokers by UV-Spectrophotometry (Manual method of Bowler).

**Results:** The mean serum thiocyanate concentration ( $112 \pm 36.6 \mu\text{mol/L}$ ) of smokers was significantly higher (p value  $<0.001$ ) as compared to passive ( $55 \pm 16.1 \mu\text{mol/L}$ ) and never smokers ( $41 \pm 6.6 \mu\text{mol/L}$ ). The serum thiocyanate of female smokers ( $133 \pm 28.7 \mu\text{mol/L}$ ) was higher (p value  $<0.05$ ) than male smokers ( $103 \pm 36.3 \mu\text{mol/L}$ ). Serum thiocyanate showed 91% sensitivity, 80% specificity and 84% accuracy at  $\geq 60 \mu\text{mol/L}$  for smokers.

**Conclusion:** Serum thiocyanate is significantly higher in smokers than passive and never smokers. Serum thiocyanate at  $\geq 60 \mu\text{mol/L}$  may be considered as cutoff value for distinguishing smokers from non-smokers for future studies (JPMA 56:323;2006).

### Introduction

Cigarette smoking accounts for 65-85% of global tobacco consumption according to a report published in 2000 by WHO. World wide about 4 million people die of smoking every year and this figure is likely to rise by the year 2030 when tobacco related disease mortality will be 10 million.<sup>1</sup> By the year 2020, 70% of all deaths from tobacco use will occur in the developing countries. Consequently, over the coming decades, developing countries will face increasing costs from tobacco use in terms of both health care expenditures and loss of productivity.<sup>2</sup>

Globally in 1995, 29% of the population aged greater or equal to 15 years were regular smokers or 1.1 billion people smoked daily and prevalence among males and females was highest among those aged 30-49 years (34%).<sup>3</sup>

In Pakistan, one out of every two to three middle aged men smoke cigarettes. The prevalence of smoking analyzed from the representative sample of 9442 individuals aged greater or equal to 15 years was 15.2% in the National Health Survey of 1990-94. The highest prevalence was reported in men aged 40-49 years (40.9%).<sup>4</sup> Moreover, smoking prevalence in medical students of a private medical university was 26% among males and 1.7% in females inspite of privileged awareness about health.<sup>5</sup>

Active smoking can cause respiratory disorders culminating in chronic obstructive pulmonary disease, emphysema and cardiovascular hazards by way of increased vascular spasm and atherosclerosis leading to acute and

chronic myocardial events, cerebral and peripheral vascular diseases and cancers of different types. Passive smoking can also lead to short as well as to long-term effects. It increases risk for respiratory tract illness, ischemic heart disease and lung cancer.<sup>6</sup> It has been estimated that secondhand smoke is responsible each year for 22,000 hospitalizations, between 150,000 and 300,000 cases of bronchitis and pneumonia, and between 8,000 and 26,000 cases of asthma.<sup>7</sup>

Cyanide is a potent toxic agent present in cigarette smoke that inhibits the activity of cytochrome oxidase. Cyanide is metabolized to thiocyanate through sulfuration with thiosulfate by mitochondrial rhodanase. In the physiological condition, blood cyanide is mainly distributed in erythrocytes, tightly binding to met-hemoglobin. Blood thiocyanate is mainly distributed in serum and its presence is regarded as evidence of cyanide detoxification.<sup>8</sup>

Thiocyanate levels in both plasma and saliva increase with the amount of cigarette smoke. It has a half-life of 10 -14 days and is thus less subjected to short-term variation in smoking.<sup>8</sup> Moreover, serum thiocyanate has been validated as a predictor of coronary mortality.<sup>9</sup> The concentration of thiocyanate in the serum, urine and saliva of individuals with little exposure to hydrocyanic acid (i.e. non-smokers) is very low but about three fold higher concentrations of thiocyanate are found in the sera, urine and saliva of smokers as compared with non-smokers, because of cyanide provided by cigarette smoke.<sup>10</sup>

Studies on different biochemical markers of tobacco smoke exposure are lacking in Pakistan. This study

compared serum thiocyanate levels in smokers, passive smokers and never smokers.

## Subjects and Methods

The study included subjects between 18 to 45 years of age and were divided into three groups: Group one were smokers, group two were never-smokers and group 3 were passive smokers. According to WHO, a smoker was a person who, at the time of survey, smoked any tobacco product either daily or occasionally. Never-smokers were those who either had never smoked at all or had never been a daily smoker and passive smokers were never-smokers but were exposed to cigarette smoke.<sup>11</sup> The subjects recruited were medical students and employees of Ziauddin Medical University, Karachi. The subjects who were ex-smokers were excluded from the study.

A questionnaire was administered regarding age, gender, number of cigarettes consumed, duration of exposure, brands of cigarettes, smoking status and passive exposure of the participants. After an interview, blood samples were taken in seating position and in a smoke free environment. Blood samples (5 ml) were obtained by venipuncture with BD vacutainers. The blood was allowed to clot and all samples were centrifuged for five minutes. Serum was collected and stored at -20°C until analyzed.

A manual method of Bowler was used with following alterations in Monica Manual, Part III.<sup>12</sup> In order to obtain higher absorbance, the amount of sample was

increased to 2 ml to give a final dilution of 1:3 in the reaction mixture. The volumes of diluents and TCA (Trichloroacetic acid) were decreased. The final ferric nitrate concentration was maintained at less than 10g/L, as at higher concentrations the absorption peak could be distorted at 455 nm.

The study was approved by the ethical review committee of Ziauddin Medical University and written informed consent was taken from each participant. The data was entered in "Microsoft Excel" and analysis was done on Epi-Info, Ver 6.0. Mean and standard deviation were calculated and p-values were obtained by student's t-test. However, p-values of number of cigarettes consumed, duration of smoking and brands of cigarettes used were obtained by analysis of variance (ANOVA). In all statistical analysis, only p-values <0.05 were considered significant.

## Results

A total of 135 subjects were included in the study. They were classified according to their self reported status as smokers (M = 30, F = 13), passive smokers (M = 33, F = 28) and never smokers (M = 9, F = 22). The subjects included as passive smokers were those who were exposed to cigarette smoke at home or workplace. Moreover they were also exposed to smoke during travel in public or private transport. There were some who possibly misreported their smoking as passive or never smokers. They were identified by discrepancy in their serum

Table 1. Serum Thiocyanate levels (µmol/l) according to different characteristics of Smokers, Passive Smokers and Never Smokers.

		Smoker (43)	Passive Smoker (58)	Never Smoker (21)
Age	< 25 years	116 ± 39.3 (27)	54 ± 16.9 (38)	40 ± 2.8 (8)
	> 25 years	104 ± 31.4 (16)	55 ± 14.8 (20)	39 ± 6.9 (13)
Gender	Male (69)	103 ± 36.3 (30)	54 ± 14.3 (33)	40 ± 1.5 (6)
	Female (53)	133 ± 28.7 (13)*	56 ± 18.4 (25)	39 ± 6.7 (15)
Cigarette Consumption (No. of cigarettes)	Upto 5	91 ± 26.8 (28)		
	6 - 10	85 ± 31.0 (7)		
	11 - 20	128 ± 39.9 (6)	-	-
	> 20	111 ± 32.5 (2)		
Duration (year)	< 3	97 ± 33.6 (11)		
	3 - 5	124 ± 39.1 (21)	-	-
	> 5	105 ± 27.8 (11)		
Brands	Gold Leaf	114 ± 42.9 (25)		
	Capstan	107 ± 29.1 (9)		
	Benson & Hedges	118 ± 32.4 (4)	-	-
	Others	107 ± 28.2 (5)		

The values are expressed as Mean & Standard Deviation (Number of subjects (n) is given in parenthesis

\* p value < 0.05 when compared with male

p value > 0.05 for age, # of cigarettes, duration of exposure and brands

**Table 2. Mean Serum Thiocyanate Level ( $\mu\text{mol/l}$ ) and 95% Confidence Interval according to smoking status.**

	Smoker (43)		Passive Smoker (58)		Never Smoker (21)	
	Mean $\pm$ S.D	Median	Mean $\pm$ S.D	Median	Mean $\pm$ S.D	Median
Thiocyanate ( $\mu\text{mol/l}$ )	112 $\pm$ 36.6 <sup>a,b</sup>	112	55 $\pm$ 16.1	52	41 $\pm$ 6.6	41
95% CI	101 - 123		51 - 59		38 - 44	

(The values are expressed as Mean, Standard Deviation and Median) (n is given in parenthesis)

- a P-value <0.001 significant compared with never smokers
- b P value <0.001 significant compared with passive smokers
- P value calculated by student t- test

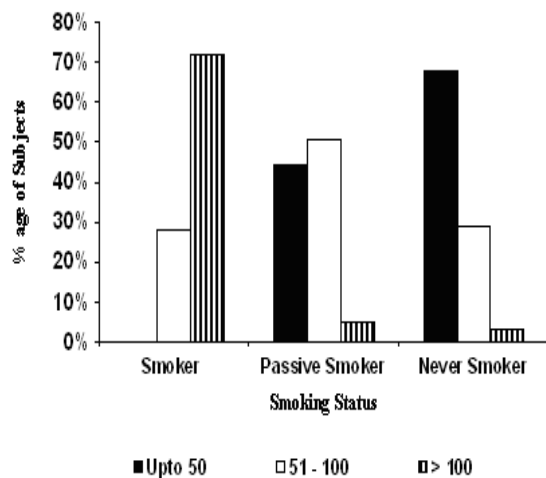


Figure 1. Serum Thiocyanate Level ( $\mu\text{mol/l}$ ) according to self reported smoking status.

**Table 3. Sensitivity, Specificity and Accuracy of Serum Thiocyanate Cut off Values for Smokers.**

Serum thiocyanate cut off value ( $\mu\text{mol/L}$ )	Sensitivity %	Specificity %	Accuracy %
$\geq 50$	100	61	75
$\geq 60$	91	80	84
$\geq 70$	84	86	85
$\geq 80$	77	94	88
$\geq 90$	72	97	89

Thiocyanate levels which does not match with the response in the questionnaire and excluded from the analysis (Passive smokers = 58, Never smokers = 21). The final results are given for 43 smokers, 58 passive smokers and 21 never smokers. Figure 1 shows the different levels of serum thiocyanate in smokers, passive smokers and never smokers. Serum thiocyanate levels in all smokers (100%)

were greater than 50  $\mu\text{mol/L}$ ; in never smokers 9(29%) had 51-100  $\mu\text{mol/L}$  and 1(3.2%) had levels of >100 $\mu\text{mol/L}$  respectively. Among passive smokers, 3(4.9%) had serum thiocyanate >100 $\mu\text{mol/L}$ .

Table 1 shows the mean serum thiocyanate levels of smokers, passive smokers and never smokers according to different characteristics. The female smokers (133  $\pm$  28.7  $\mu\text{mol/L}$ ) had higher values of serum thiocyanate than males (103  $\pm$  36.3  $\mu\text{mol/L}$ ) ( $p = 0.01$ ). The proportion of male smokers was 42% and female was 21% in this study sample. There was no statistically significant difference of serum thiocyanate in smokers regarding age group, cigarette consumption, duration of smoking and brands of cigarettes ( $p \geq 0.05$ ).

Table 2 shows the mean serum thiocyanate concentration and 95% confidence interval of the three groups. The mean serum thiocyanate concentration 112 $\mu\text{mol/L}$  (95% CI of 101-123  $\mu\text{mol/L}$ ) of smokers was significantly higher ( $P < 0.001$ ) as compared to passive (55  $\mu\text{mol/L}$ ) and never smokers (41 $\mu\text{mol/L}$ ).

Table 3 shows the sensitivity, specificity and accuracy values for identifying smokers at different cutoff points of serum thiocyanate. Serum thiocyanate is 91% sensitive and 80% specific with 84% accuracy at the cutoff value of  $\geq 60$   $\mu\text{mol/L}$ . This cutoff point may be used in future studies to distinguish smokers from non-smokers or to validate the questionnaires for self reported smoking status.

## Discussion

Serum thiocyanate has been validated as a marker to distinguish between smokers and non smokers in a number of studies.<sup>8,10,13</sup> In this study also, the mean serum thiocyanate was significantly higher in smokers than never

and passive smokers. Several factors other than tobacco smoke are known to affect serum Thiocyanate, which include industrial exposure to cyanides in electroplating, precious metal refining, case hardening of steel and gas manufacturing.<sup>14</sup> But this factor was not applicable in our study where subjects had no industrial background. Some foods also produce thiocyanate including the cabbage family, turnips, garlic, horseradish, mustard, almonds and beer. Vegetarians have slightly higher serum thiocyanate than non-vegetarians but the increase is not as large as that seen in smokers.<sup>15</sup> This factor does not effect the results in this study as Thiocyanate obtained by food is for less than produced by tobacco smoke. The reason for increased serum thiocyanate in smokers than passive and never smokers is explained by the fact that hydrogen cyanide in the mainstream smoke is as high as 40-70 ppm, which is beyond the threshold limit value for occupational health (10 ppm). The hydrogen cyanide level in the sidestream smoke is less than 5 ppm<sup>16</sup> and this study is in accordance with this fact in which the level of serum thiocyanate in passive smokers was less than smokers but higher than never smokers. This finding is fairly consistent with many other studies.<sup>17,18</sup>

In this study, females had higher serum thiocyanate than males which is in accordance with other studies.<sup>19</sup> Among smokers, the serum thiocyanate was on increase with the increased number of cigarettes consumed and duration of cigarette smoking but for statistically significant results, sample size should be larger. A large proportion of smokers (58%) used 'Gold Leaf' brand of cigarettes which is in accordance with another study done among Karachi population.<sup>20</sup>

The study has determined sensitivity, specificity and accuracy for identifying 'smokers' at different cutoff values of serum thiocyanate. It is recommended that  $\geq 60\mu\text{mol/L}$  may be considered to distinguish between smokers and non-smokers in future studies for validation of self reported smoking status in questionnaires. Serum thiocyanate levels have been shown to be useful in smoking cessation trials. It confirmed the smoking status of the non-smokers and showed that about one sixth of the ex-smokers had smoked in the last month and that the smokers on the average were heavy smokers smoking much more than the self reported number of cigarettes.<sup>21</sup>

It is recommended that carbon monoxide breath test would also be done in future studies in conjunction with serum thiocyanate. In many studies, measurement of exhaled carbon monoxide in combination with serum thiocyanate assay indicated higher specificity for carbon monoxide and higher sensitivity for serum thiocyanate.<sup>22, 23</sup>

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