



Correlation between cotinine urinary levels & cardiovascular autonomic function tests among smokeless tobacco chewers: A cross-sectional study

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Received September 29, 2018

Background & objectives: Cardiovascular disease (CVD), the leading cause of death worldwide is responsible for over 17 million deaths globally, of which 10 per cent deaths have been expected due to consumption of tobacco. The association between CVD and chewing of tobacco is limited and remains arguable. The aim of this study was to find out the correlation between urinary cotinine level and cardiovascular autonomic function tests of tobacco chewers.

Methods: In the present study, 600 participants, 300 smokeless tobacco chewers (STC) and 300 non-tobacco chewers (NTC), between 18 and 65 yr were selected. Various parameters such as anthropometric, systolic blood pressure (SBP), diastolic blood pressure (DBP), autonomic function tests and urinary cotinine levels were measured.

Results: Significant difference ($P < 0.001$) was noted in STC and NTC for anthropometric parameters, SBP, DBP and urinary cotinine levels. Sympathetic and parasympathetic autonomic function test showed significant difference on comparison in STC and NTC groups, except in Valsalva ratio. Correlations with urinary cotinine levels were significant for sympathetic autonomic functions, SBP fall ($r = 0.138$, $P = 0.016$), DBP rise ($r = -0.141$, $P \leq 0.014$); parasympathetic autonomic function, heart rate (HR) response to standing ($r = -0.208$, $P \leq 0.003$), deep breathing ($r = -0.473$, $P \leq 0.001$) and Valsalva ratio ($r = -0.396$, $P \leq 0.0001$).

Interpretation & conclusions: Correlation between urinary cotinine levels and autonomic function tests elucidates the linkage involving autonomic nervous system damage which can be considered as an important associated relationship for early diagnosis of CVD health risk factors among smokeless tobacco (ST) users.

Key words Autonomic function test - cardiovascular - smokeless tobacco - urinary cotinine

It has been estimated that more than 300 million people worldwide are addicted due to consumption

of smokeless tobacco (ST)¹. Recent evidence shows that globally the ST consumption is responsible for an

estimated 6,52,494 all cause deaths across the globe annually. This constituted about 10 per cent of all the deaths that could be attributed to all forms of tobacco use². In 20th century 100 million premature deaths happened due to tobacco usage, if trends continues, at the end of 21st century it may rise upto one billion¹. The frequency of tobacco use is around 37.97 per cent in men, 12.5 per cent in the female in Belagavi, Karnataka, India, and the most common form of tobacco use is ST³. Depending on the type of ST products, nicotine content may vary, and therefore, the measurement of nicotine and its metabolites among ST users is important to understand the addictive potential of ST products. The half-life of nicotine is short, and its metabolites especially cotinine has a long half-life, which is a good biomarker of nicotine in urine (urinary cotinine level)⁴ and may serve as a useful marker to determine the effects of different forms of tobacco consumption⁵⁻⁷.

The epidemiological evidence on cigarette smoking in relation to cardiovascular disease (CVD) is well known, but the correlation between CVD and chewing of tobacco is limited⁸. ST is being used in dipping and chewing forms. Due to the high background rates of CVD, even small increase in risk may represent a large public health impact in countries that have a high prevalence of ST use. The present study was aimed to assess the correlation between the urinary cotinine levels and autonomic function among ST chewers.

Material & Methods

This cross-sectional study was done in the Belagavi region of Karnataka State in India from April 2013 to December 2016. The study participants were divided into two groups: group 1 - ST chewers (STC) and group 2 - non-tobacco chewers (NTC). Ethical clearance was obtained from the Institutional Ethical Committee of Belagavi Institute of Medical Sciences, Belagavi and written informed consent was obtained from the participants.

Inclusion criteria: A total of 300 STC aged between 18 and 65 yr were randomly selected from the outpatient department of the district (BIMS) hospital, attached to the college and 300 NTC participants were also randomly selected from the same hospital (including hospital staff) who were not consuming tobacco. The participants were brought to Clinical Physiology Laboratory, Department of Physiology, BIMS-Belagavi, where various parameters were

observed and measured.

Exclusion criteria: Participants associated with disease or conditions, known to affect autonomic function such as Guillain Barre Syndrome, poliomyelitis, diphtheria, tuberculosis, syphilis, amyloidosis, chronic renal disease, diabetes mellitus, bronchial asthma and giddiness on standing, evidence of hypertension, receiving drugs that are known to interfere with cardiac function or respiratory functions such as beta blockers, sympathomimetic drugs, vasodilators and diuretics were excluded from the study.

The sample size based on *r*-value of tobacco use, was calculated as 209 with 90 per cent power in each group⁹. However, the present study included 300 participants in each group to increase the power of the study.

Anthropometric parameters: Physical examination of the participants was done. Recording of anthropometric parameters such as height (in cm), weight (in kg), body surface area (BSA) (m²) and body mass index (kg/m²) was done. Recording of physiological parameters was also conducted. Respiratory rate (cycles/minute) heart rate (HR) (beats/min), systolic blood pressure (SBP) and diastolic blood pressures (DBPs) (mm of Hg) were measured using mercury sphygmomanometer. Electrocardiogram (ECG) recording was done with the help of 12-lead ECG Machine, Cardiart 6108T of BPL healthcare, India.

Recording of autonomic function parameters as recommended by the American Diabetic Association was performed as per Ewing criteria¹⁰.

Parasympathetic activity: The following parasympathetic autonomic tests were measured: (i) HR response to Valsalva manoeuvre (Valsalva ratio); (ii) HR response to deep breathing (maximum HR–minimum HR); and (iii) Immediate HR response to standing (postural changes in HR).

Sympathetic activity: The following sympathetic autonomic tests were measured: (i) blood pressure response to standing (postural changes in blood pressure) (orthostatic test); and (ii) blood pressure response to sustained handgrip test exercise (increased in DBP).

Collection of urine samples and analysis of cotinine: Fasting urine samples were collected from the participants. The collected samples were transferred

immediately to the laboratory at BIMS, Belagavi, and frozen at -20°C until analysis. Extraction of cotinine from the urine sample was done by gas chromatography-mass spectroscopy qualitatively and quantitatively by high performance liquid chromatography⁵.

Statistical analysis: The data in present study were not normally distributed. Hence, the statistical analysis of various parameters was applied for non-parametric test, *i.e.*, Mann–Whitney U-tests to compare between two different groups. The Spearman test was applied to find out correlation between the different variables of STC and NTC groups. The statistical analysis was conducted using SPSS v. 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Table I indicates the anthropometric characteristics of STC and NTC groups such as age, weight, body mass index, SBP and DBP. There were significant ($P<0.001$) differences between the STC and NTC group in terms of age, height, weight, BSA, body mass index, HR, relative risk and mean SBP and mean DBP. The urinary cotinine levels among STC ($1563.68\pm1198.97\text{ng/ml}$) was significantly higher ($P<0.001$) than that in NTC group ($23.48\pm11.08\text{ng/ml}$).

Sympathetic autonomic function tests (Table II) revealed that mean SBP fall during the orthostatic test was increased in case of STC when compared to NTC and was significant ($P<0.001$). The mean DBP rise during the handgrip test was significantly ($P<0.001$) decreased in case of STC as compared to NTC. Parasympathetic autonomic function test (Table III) showed that HR response to standing and deep breathing was decreased in case of STC as compared to NTC and was significant ($P<0.001$). However, HR response to Valsalva ratio was non-significantly decreased in case of STC in comparison with NTC.

On applying Spearman correlation coefficient test, mean SBP and DBP in case of STC did not show any significant correlation with urinary cotinine levels ($r=0.012$, $P=0.827$) and ($r=0.056$, $P=0.329$). Mean SBP fall during sympathetic autonomic function test (Orthostatic test) in case of STC showed weak positive significant correlation with urinary cotinine levels ($r=0.138$, $P=0.016$). Mean DBP rise during handgrip test in case of STC showed weak negative significant correlation with urinary cotinine levels ($r=-0.141$, $P\leq0.014$).

Table I. Description of samples based on mean anthropometric data of participants

Anthropometric parameters	NTC (n=300)	STC (n=300)
Age (yr)	37.00±16.05	37.10±15.99
Height (cm)	157.85±5.19	158.93±6.42**
Weight (kg)	59.49±7.24	52.00±4.96***
Body mass index (kg/m ²)	24.01±3.13	20.66±2.41***
BSA (m ²)	1.59±0.09	1.51±0.08***
HR (bpm)	76.64±6.05	91.98±7.29***
Respiratory rate (cycles/min)	15.67±2.29	17.03±2.69***
SBP (mmHg)	116.16±8.02	125.31±8.52***
DBP (mmHg)	75.61±5.43	80.56±5.77***

Values indicated mean±SD of 300 participants in each group. $P^{**}<0.01$ $^{***}<0.001$ compared to NTC group. NTC, non-tobacco chewers; STC, smokeless tobacco chewers; SD, standard deviation, SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; BSA, body surface area

Table II. Mean blood pressure response during autonomic function test for sympathetic function in smokeless tobacco chewers (n=300) and non-tobacco chewers (n=300)

Groups	Orthostatic test (SBP fall) (mmHg)	95% CI for mean
STC	19.02±8.24***	18.08-19.96
NTC	11.26±9.90	10.13-12.38
Hand grip test (DBP rise) (mmHg)		
STC	13.42±8.41***	12.47-14.38
NTC	20.14±8.95	19.13-21.54

Values are mean±SD $^{***}P<0.001$ compared to NTC group. NTC, non-tobacco chewers; STC, smokeless tobacco chewers; CI, confidence interval; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure

Table IV shows parasympathetic autonomic function test. HR response to standing in case of STC showed weak negative but significant correlation with urinary cotinine levels ($r=-0.208$, $P\leq0.003$). The HR response to deep breathing of STC showed moderate negative but significant inverse correlation with urinary cotinine levels ($r=-0.473$, $P\leq0.001$) The HR response to Valsalva ratio in case of STC showed weak negative but significant inverse correlation with urinary cotinine levels ($r=-0.396$, $P\leq0.001$).

Discussion

The urinary cotinine level range among STC and NTC was 100.84-810 and 10.01-59.02 ng/ml⁵. Similar results of urinary cotinine levels have been noted^{4,11-13}. Pandey *et al*¹⁴ reported that urinary cotinine levels

in non-smokers were always <100 ng/ml and with differences in cut-off values^{6,7}. The variations in the range of cotinine levels depends on tobacco chewer's dietary intake of nicotine, cotinine excretion, metabolic activity, passive smoking and environmental smoke⁴.

The difference in SBP and DBP may be due to population ethnicity, composition of tobacco products and diversity in sample size. Correlation between urinary cotinine levels with SBP and DBP was not significant¹⁵ except in one case¹⁶ which showed moderate correlation with the SBP.

Difference between SBP fall during the orthostatic test was significant which agree with one of the study¹⁷ but not with other studies^{18,19}. This may be due to regional difference, tobacco products and quantity of consumption of tobacco. The SBP fall during orthostatic test indicates loss of normal response due to vagal damage because of nicotine^{14,17}. The difference

between DBP rise during handgrip test of STC and NTC was significant and was consistent with other studies^{18,19}. It indicates a decrease in sympathetic reactivity of the individual and high content of sodium in tobacco products may contribute for DBP rise²⁰. A significant rise in the DBP is of major concern to note as an increase in DBP is indicative of hypertension¹⁴.

Differences in HR response to standing may be due to difference in inhabitants, tobacco product compositions, loss of normal response due to vagal damage by nicotine and reduced sample size¹⁸. Change in posture from supine to standing, autonomic nervous system acts to produce a rise in HR and vasoconstriction to maintain blood pressure which is mediated through sympathetic innervations to blood vessels²¹. The key process in tobacco use induces atherogenesis initiation²⁰, where the parasympathetic fibres being the longest fibres are affected first due to atherosclerotic changes of vasa nervorum²².

Urinary cotinine levels of STC group moderately correlated with the HR response to deep breathing (deep breathing, $r=-0.473$, $P<0.001$). HR response to deep breathing (sinus arrhythmia) is primarily due to the fluctuation of parasympathetic output to the heart²³. It is assumed that loss of R-R variation is probably due to vagal autonomic neuropathy. Parasympathetic efferents are responsible for mediating this effect²⁴. Difference in outcome may be due to age, resting HR, body mass index and administered medications²⁵.

The Valsalva manoeuvre tests the integrity of both parasympathetic and sympathetic divisions of autonomic nervous system¹⁷. The hemodynamic changes during the manoeuvre are mediated through baroreceptors. The absorption of nicotine affects sympathetic traffic to the skin, heart and adrenal glands, but reduces sympathetic traffic to muscle, which is mediated by increased baroreceptor activity triggered by arterial pressure increase, where it affects

Table III. Mean heart rate response during autonomic function test parasympathetic function in smokeless tobacco chewers (n=300) and non-tobacco chewers (n=300)

Groups	HR response to standing (bpm)	95% CI for mean
STC	1.25±0.17***	1.23-1.27
NTC	1.30±0.18	1.28-1.32
HR response during deep breathing (bpm)		
STC	1.25±0.11***	1.23-1.26
NTC	1.31±0.11	1.30-1.32
HR response during Valsalva manoeuvre (bpm)		
STC	1.58±0.27	1.55-1.61
NTC	1.62±0.26	1.59-1.65

Values are mean±SD; *** $P<0.001$ compared to NTC group. NTC, non-tobacco chewers; STC, smokeless tobacco chewers; bpm, beats/minute; CI, confidence interval; HR, heart rate; SD, standard deviation

Table IV. Correlation of urinary cotinine level of smokeless tobacco chewers (STC, n=300) with autonomic function test

Autonomic function test	Correlation coefficient (r)	95% CI for mean	P
SBP	0.012	-0.104 to 0.129	0.827
DBP	0.056	-0.060 to 0.172	0.329
SBP fall	0.138	0.022 to 0.251	0.016
DBP rise	-0.141	-0.253 to -0.024	0.014
HR response to deep standing	-0.208	-0.317 to 0.094	<0.003
HR response to deep breathing	-0.473	-0.559 to 0.377	<0.001
HR response to Valsalva ratio	-0.396	-0.490 to -0.293	<0.001

HR, heart rate; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure

baroreceptor control of sympathetic outflow to different vascular beds²⁶. In present study, Valsalva ratio was not significant in STC and this could be because of the difference in methodology.

In conclusion, the present study elucidates the linkage involving autonomic nervous system damage with correlation to urinary cotinine levels which is an important biomarker to predict the tobacco consumption exposure and might be constructive for diagnosis of health risk among tobacco users.

Acknowledgment: The authors thank the Head, department of Physiology, Principal and Director, Krishna Institute of Medical Sciences, Karad; Belagavi Institute of Medical Sciences, Belagavi, and Director, Indian Council of Medical Research-National Institute of Traditional Medicine, Belagavi, for providing necessary facilities and kind support.

Financial support & sponsorship: None.

Conflicts of Interest: None.

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