Journal of Oral Biology and Craniofacial Research 9 (2019) 326–330

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

The interrelationship between urinary cotinine and nicotine dependence among tobacco users in an Indian de-addiction centre: A cohort pilot study

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ARTICLE INFO

Keywords:
Fagerstrom test for nicotine dependence (FTND) score
Urinary cotinine
Smoking tobacco
Smokeless tobacco (ST)
Beedi
ELISA

1. Introduction

Tobacco use is the largest preventable cause of high mortality and morbidity rate in the world.[1] Among the age group 30–69 year age, 1 in 5 males and 1 in 20 females die from tobacco-related diseases.[2] The cost high estimated to be US 200 billion annually worldwide.[3] Consumption of tobacco is associated with various cancers such as, lung, oesophagus, larynx, oral cavity, throat, kidney, bladder, pancreas, stomach, and cancer in cervix[4] Tobacco dependence is due to nicotine, it leads to an addiction and affect a person for development of tobacco related cancer[5]

There are several tools have been developed to assess the nicotine dependence level; out of that Fagerstrom Test to Nicotine Dependence (FTND) scale is widely accepted as a reliable method to determine the nicotine dependence level.[6] However, FTND score is used to cigarette smoking an FTND score is not validated to *Beedi* smokers and ST users, needs investigation.

Estimation of cotinine in human urine appears to be the most specific and the most sensitive biomarker and this strategy is rapid and relatively non-invasive when compared to the determination of this biomarker in blood serum. Furthermore, unlike nicotine, the urinary excretion of cotinine is rarely affected by urinary pH.[8–10]

The cotinine metabolite *trans*-3′-hydroxycotinine is also abundant in urine. Glucuronide conjugation is an important pathway of nicotine metabolism, and trans-3′-hydroxycotinine glucuronides represent is up to 30% of the total metabolites recoverable in the urine[9–11]

However, for estimation of *trans*-3′-hydroxycotinine, gas chromatographic and liquid chromatographic methods are not always suitable, since they both require a long duration of sample preparation, and also the equipment is expensive. They also require relatively large sample volumes. Therefore, the enzyme-linked immunosorbent assay (ELISA) serves as an improved alternative, since it is more rapid, highly sensitive, and requires only a small amount of sample[11–14]

By using the ELISA method, a correlation study was conducted by Heinrich-Ramm et al. (2002) to investigate the level of urinary cotinine, which was found to be associated with the severity level of nicotine dependency.[14,15]

The objective of this study is to investigate the inter-relationships

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https://doi.org/10.1016/j.jobcr.2019.07.002
Received 19 November 2018; Received in revised form 27 June 2019; Accepted 14 July 2019
Available online 15 July 2019

between the level of urinary cotinine and the severity of nicotine dependence ie FTND score amongst smokers and ST users.

2. Materials and methods

The study was conducted in a selected de-addiction centre of a hospital in India (KS Hegde Charitable Hospital of Nitte Deemed University, Mangalore, Karnataka, India). Before commencing the study, ethical approval was obtained from the central ethics committee of the Nitte Deemed University. Permission was also obtained from the hospital authorities, and informed consent was obtained from the participants. To check the reliability of FTND scoring system, the method was pre-tested amongst 25% of the sampled population. The total score of all the featured items was obtained and analyzed by using Cronbach's alpha reliability method. The result was r = 0.80, which justifies the use of the FTND scoring system in the selected sampled population.

A cohort pilot study with a sample size of n = 80 was included. Subjects who were diagnosed with any psychiatric illness were excluded from the study. A demographic profile and the FTND scoring were employed. Participants were instructed to collect 5 ml of urine into a sterilized specimen container. The collected samples were labelled and stored in a refrigerator at 4 °C. Urinary cotinine levels were determined using ELISA kits (Calbiotech, Direct ELISA, USA).

2.1. Statistical analysis

The demographic data/information collected was analyzed by descriptive statistics. Correlation between urinary cotinine concentrations and FTND scores were determined via the computation of a Pearson linear correlation coefficient. A statistical validation for sampling was determined by using the Kappa test, and this was performed to determine whether the inclusion of a sampling size of 80 participants would satisfactorily justify these groups in this study. The test result for sampling was ($r = 0.306$, $n = (0.84 + 1.96)^2/(0.306)^2/1-(0.306)^2 = 73.93$) for n = 80.

2.2. Analysis of the urinary cotinine by ELISA

Ten (10.0) µl of standards, controls, and specimens were selected using separate pipette wells in duplication, and 100 µl of the enzyme conjugate was added to each well before plates were shaken for 10–30 s for optimal mixing. Subsequently, samples were equilibrated for 60 min at ambient temperature (25 °C) in a dark place. Each well was washed 6 x with 300 µl of distilled water, and then the wells were inverted and vigorously slap-dried; absorbent paper was used to ensure removal of all residual moisture. One hundred (100.0) µl of the ELISA substrate (3,3′,5,5′- tetramethylbenzidine [TMB]) was then added to each well and the mixtures incubated for 30 min at ambient temperature, again in a dark place. One hundred (100.0) µl of stopping solution i.e. 0.16 M sulphuric acid with the ELISA substrate [TMB], was further added to each well, and the plate was gently shaken to ensure homogeneity. The values were read on the ELISA reader at the specified wavelength of 450 nm within 15 min following addition of the stopping solution, as recommended by previously documented methods.[17–20]

3. Results

Table 1 shows that 37 (46%) of participants were 31–40 years old, 19 (24%) were between the ages of 21 and 30 years, 14 (17%) were in the 41–50 year age group, and 10 subjects (13%) were aged 50 and above. The majority of participants (95%) were males, the remainder (5%) being females. Fifty-eight (73%) of participants were married, and 22 (27%) were unmarried. Out of 80 subjects, 34 (42%) had completed high school schooling, and 10 (13%) had completed higher secondary schooling, and 12 (15%) of them completed a full university education. Nine subjects (11%) were illiterate. Occupation-wise, out of 80 subjects, 59 (74%) were manual workers and 13 (16%) were office workers, 4 (5%) were businessmen, and 4 (5%) of participants were unemployed. With regard to monthly incomes, 32 (40%) of participants earned between 5001–10,000 Indian Rupee (INR) (average 7500 INR), and the income of 30 subjects (37%) was 10,000 INR. Eighteen subjects (23%) had incomes below 5000 INR. Out of the 80 recruited participants, 59 (74%) were smokers who also had the habit of ST chewing. Ten participants (12%) were *Beedi* smokers, of which 8 (10%) also had the habit of smoking cigarettes and ST chewing.

Table 1
Demographic characteristics among tobacco users.

Demographic characteristics	N (%)
Age	
21–30 years	19 (24)
31–40 years	37 (46)
41–50 years	14[17]
> 50 years	10[13]
Gender	
Male	76 (95)
Female	4[5]
Religion	
Hindu	65 (81)
Christian	6[8]
Muslim	9[11]
Marital status	
Married	58 (73)
Unmarried	22 (27)
Literacy status	
Illiterate	9[11]
Primary	15[19]
High school	34 (42)
Higher Secondary	10[13]
University or higher	12[15]
Occupation	
Physical workers	59 (74)
Business	4[5]
Employers	13[16]
No job	4[5]
Individual income per month	18 (23)
Up to 5000	32 (40
5001–10000	30 (37)
> 10000	
Type of addiction	
Beedi/cigarette/ST	10[12]
Beedi	8[10]
Beedi/ST users	3[4]
	59 (74)

Table 2 demonstrates that 42 (52%) of subjects expressed that they were relieved from stress while using tobacco, 20 (25%) of them explained that sleep disturbance is less intensive if they consumed tobacco, and 18 (23%) of them expressed that other problems such as, tiredness, boredom, and low confidence level are relieved whilst consuming tobacco. Out of the 80 participants, 14 (17%) had explained that they have less willpower and weak determination to quit tobacco. Sixteen participants (20%) explained that only stressful conditions render it difficult to cease tobacco smoking, but 34 (43%) enjoyed tobacco socially. However, 16 subjects (20%) expressed that they are more addicted to tobacco. Indeed, 60 (75%) did not try to cease tobacco smoking; on the contrary, 20 (25%) tried to cease, but failed to do so. However, 40 participants (50%) expressed the opinion that they were very confident regarding ceasing tobacco smoking, 30 (38%) were fairly confident, and 2 (12%) subjects are not at all confident about quitting their tobacco smoking habits. Forty-seven (59%) of participants responded that they would cease tobacco smoking immediately, although 33 (41%) did not want to do so.

The mean FTND score for *Beedi*/Cigarette smokers was 6, and their mean urinary cotinine levels were found to be 5 ng/ml. The mean FTND score of cigarette and ST users was recorded as 7, and their mean urinary cotinine level was 7 ng/ml. Notwithstanding, the mean FTND

Table 2
-Tobacco habits among tobacco users.

Details of tobacco habits	N(%)
When do you think tobacco habits help you most?	
Over stress	42(52)
Sleep Problem	20(25)
Others (tired, bored, low confident)	18(23)
Reason for not stopping tobacco use	
Less will power and fear of weight gain	14(18)
When stressed	16(20)
Enjoyment	34(42)
Addicted	16(20)
Have you tried to stop using tobacco in the past?	60(75)
Yes	20(25)
No	
How confident you are to stop tobacco use?	
Very confident	48(60)
Fairly confident	30(38)
No confident	2(2)
Are you ready to stop tobacco use immediately?	
Yes	47(59)
No	33(41)
If no, Why ?	33
Not interested to stop	19(58)
I don't know	14(42)

Table 3
Comparison of the nicotine dependence level (FTND score) and urinary cotinine level among different tobacco users.

Types of addiction	N (%)	FTND SCORE	Urine cotinine level
Beedi/Cigarette	10[12]	6	5 ng/ml
Cigarette/ST	8[10]	7	7 ng/ml
Beedi	3[4]	5	6 ng/ml
Beedi/ST users	59(74)	9	9 ng/ml

score amongst *Beedi* smokers was 5, and their mean urinary cotinine concentration was 6 ng/ml. For participants who had the habit of both *Beedi* smoking with ST, the mean FTND score was the highest i.e. 9, and their mean urinary cotinine level was also the highest i.e. 9 ng/ml in this study. These results are summarised in Table 3.

We found a significant relationship between nicotine dependence and the level of urinary cotinine amongst tobacco users for both the smoking and ST groups (Table 4). The scatter diagram shown in Fig. 1 also shows that there is a positive relationship between FTND score and urinary cotinine concentration amongst tobacco users. There was also a strong positive correlation between participants' nicotine dependence level and urinary cotinine concentration for any form of tobacco use by the cohort groups. However (Tables 3 and 4), shows that there was a significant relationship between nicotine dependence and urinary cotinine content, which was the highest in concentration amongst dual habits of *Beedi* smoking and ST-chewing participants. ($p < 0.005$, $r = 0.420$, $p < 0.01$).

Table 5 shows that amongst all the baseline variables, there was a significant association with gender, i.e. males were more dependent on smoking habit ($p = 0.000$). Again, participants in the low income group had the greatest dependency on tobacco smoking. However, no significant association was observed for the other baseline variables, i.e., age, marital status, literacy status, and occupation (p values < 0.05).

Table 4
Correlation between urinary cotinine level and nicotine dependence level (FTND Score) among tobacco users.

Nicotine dependence level (FTND score)	r value	*p* value
	0.420	0.000 *

Levels of significance (0.05) *Significant.

4. Discussion

From amongst the ST users, our results demonstrated that the nicotine dependence level i.e., the FTND score, was high for those who combined this habit with cigarette smoking (7 ng/ml).This finding was concorded with the study conducted by Divinakumar et al.(2017) that the highest prevalence of tobacco use was high among smoking and smokeless tobacco users.[21] This finding was supported by the study conducted by Ann et al. (2010) in which smokeless tobacco users showed symptoms of nicotine dependence at least as frequently as cigarette smokers[20,] However, the *Beedi* smokers with ST had the highest mean FTND value, i.e. 9, and their mean urinary cotinine value was 9 ng/ml. Symptoms of nicotine dependence, and those of withdrawal during quitting attempts, are particularly common amongst those who combine the smokeless tobacco chewing with smoking, and in this study results also agree with those factors of the study.[20,] However, we found that there was a significant interrelationship ($r = 0.412$, $p < 0.05$) between the urinary cotinine level and the FTND score of nicotine dependence amongst dual users (i.e., ST chewers and smokers of *Beedi* and/or Cigarette).

In a study by Jung et al. (2012), urinary cotinine levels were found to be significantly correlated to FTND scores ($r = 0.567$, $p < 0.001$). Similar results were found in this study (Table 4, Fig. 1).

Hyun et al. (2012) also detected a significant correlation between urinary cotinine levels and FTND scores. Our study revealed that urinary cotinine levels were increased with the higher scores of FTND.[7] (Table 4, Fig. 1) Our study results were similar to those of Hyun et al. (2012)[7,,8] However, Vlăsceanuam et al. (2012) found that the urinary cotinine level did not always correlate with the nicotine dependence level, measured as the FTND score.[2] Indeed, it may not always be possible to observe a significant correlation between cotinine and nicotine dependence, since it appears from this work that such correlations are occupation-dependent, i.e. there is a unique FTND score amongst manual workers, in our study we found that 59 (74%) of participants were manual workers. Indeed, it appears that strenuous manual labour is often associated with both smoking and smokeless tobacco consumption. In this cohort pilot study, we recorded that some participants also consumed alcohol together with their tobacco habits (Table 1). Most of the subjects had personal habits of *Beedi* (an indigenous form of smoking tobacco in India) and/or ST use was prone to stay with multiple personal habits (including ST, smoking and alcohol drinking). Forty-two participants (52%) responded that when they are overstressed, they commence tobacco use, both smoking and ST, and at least some of them required alcoholic drinks too. Additionally, some participants explained that other factors, such as weight gain after quitting tobacco were a reason for them to discontinue tobacco use. Forty-eight participants (60%) were very confident regarding their ability to terminate tobacco habits (Table 2).

Moreover, there was an association between tobacco consumption and gender, and also the employment income (Table 5), and similar findings were reported by Isam et al. (2002).[12,13] According to Table 3, we found that *Beedi* smokers with ST had the highest FTND score as well as the highest level of urinary nicotine amongst males, but there were only 4 females recruited to the study. However similar investigations like our study have not been previously reported.

Since cotinine is an alkaloid found in tobacco and is a metabolite of nicotine, its levels in urine help researchers to assess the severity of tobacco dependence when present as a combined form i.e.ST and smoking combination.[7] This study also emphasizes that intervention for tobacco cessation can be undertaken based on the level of urinary cotinine and FTND scores. Future studies, which would include smokeless tobacco (ST) products, will facilitate determinations of the relationship between the nicotine dependence i.e. FTND scores, and urinary cotinine concentrations.

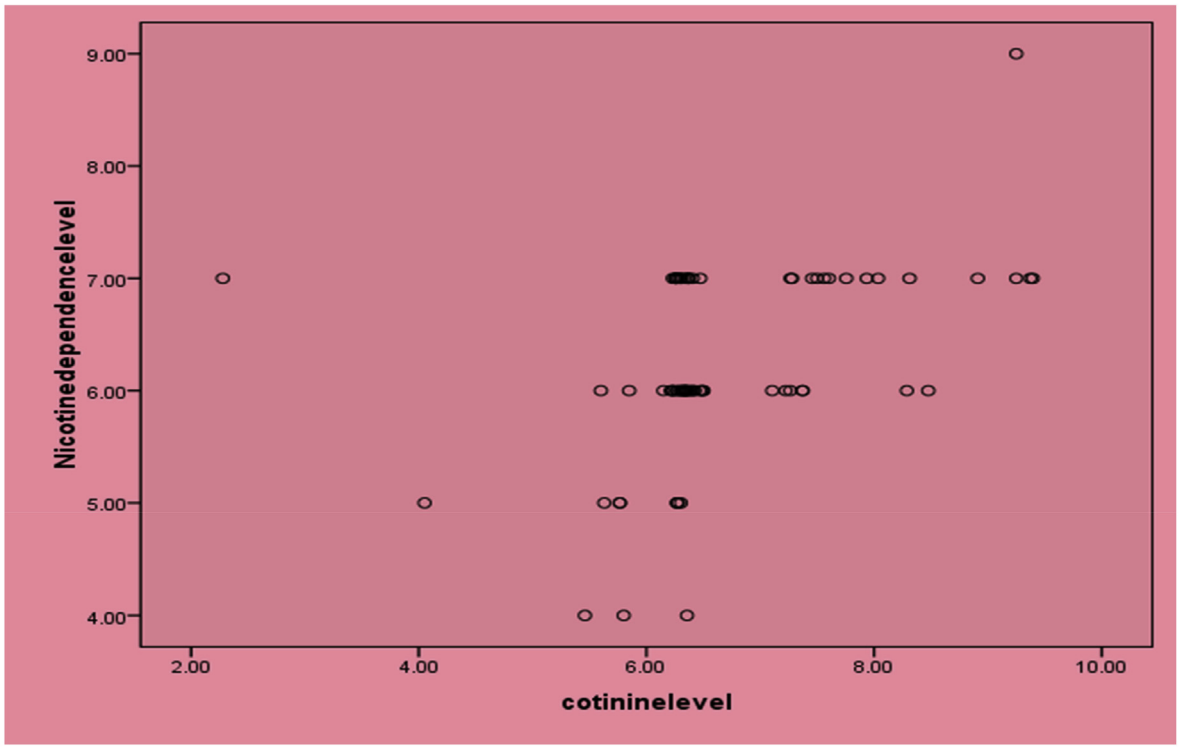

Fig. 1. Scatter Diagram on relationship with nicotine dependence level (FTND score) and urinary cotinine level among tobacco users.

Table 5
Association between nicotine dependence level (FTND score) and with selected demographic variables among tobacco users.

Demographic variables	Chi Square value	P value
Age in years	8.463	0.206
21–30		
31–40		
41–50		
> 50		
Gender	17.436	0.000*
Male		
Female		
Religion	0.999	0.910
Hindu		
Christian		
Muslim		
Marital status	0.000	1.000
Married		
Unmarried		
Literacy status	0.877	0.421
Illiterate		
Primary		
High school		
Higher Secondary		
University or higher		
Occupation	3.000	1.000
Physical workers		
Business		
Employers		
No job		
Individual income per month	0.054	0.000*
Up to 5000		
5001–10000		
> 10000		
Type of addiction:	0.63	0.000*
Beedi/cigarette		
Beedi/cigarette/ST		
Beedi and alcohol		
Beedi/ST users		

5. Conclusion

The Fagerstrom test for nicotine dependence (FTND) score values can be used to assess the severity level of nicotine dependence amongst smokers and smokeless tobacco users. Urinary cotinine level represents a valid biomarker to assess the severity level of nicotine dependence amongst dual users (both ST chewers and smokers). Indeed, urinary cotinine levels were the highest amongst participants who had the combined habits of smoking and smokeless tobacco when compared to those with a single habit. However, the highest FTND scores and urinary cotinine levels were also the highest amongst participants who combined *Beedi* smoking and ST chewing habit. This study also indicates that further investigations are required to explore the relationship between FTND score and urinary cotinine levels, for example the stratification of these datasets to allow for differing occupations, incomes and genders.

Conflicts of interest

There is no conflict of interest.

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