Estimation and Comparison of Urinary Cotinine, Nitrite and Nitrate in Healthy Individuals with and without the Habit of Tobacco

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Abstract

Introduction: Among the leading causes of preventable death, tobacco use is a global pandemic. Most cancer deaths in developed countries are caused by tobacco products. Detection of tobacco exposure can be done using nicotine, cotinine, and No2+No3 in the urine of individuals who are exposed to tobacco products.

Aim: To assess tobacco exposure related biomarkers in urine like cotinine, nitrite and nitrate in patients with the habit of tobacco and in controls as well attending the OPD of oral medicine and radiology.

Objectives: Estimation and comparison of urinary cotinine, nitrite and nitrate levels in healthy individuals with the habit of tobacco usage and in individuals without the habit of tobacco usage.

Materials And Methods: The samples were selected from the patients attending the OPD of Oral medicine and radiology department. Individuals between the age group of 20 and 60 and above with a sample size of about 70 individuals were included. These individuals were those who gave a history of smoking and chewing smokeless tobacco. A sample size of 20 individuals without a smoking habit was considered as the control group.

Results: The cotinine levels in smokers and chewers showed increased p value of <0.01. Nitrite and Nitrate showed statistically significant p value of 0.01 for both smoking and chewing than smokers and chewers alone. As the frequency and duration increased the urinary nitrite and nitrate levels also increased which showed statistically significant p value of <0.01.

Conclusion: In conclusion, this study suggests that cotinine, nitrite, and nitrate can be used to determine whether a person has been exposed to tobacco.

Keywords: Urinary Cotinine, Tobacco, Nitrite and Nitrate, Oral medicine and radiology

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Introduction

As one of the leading causes of preventable death, tobacco use has become a global pandemic. Smokeless tobacco products are also used by millions of people, despite the fact that over one billion people smoke worldwide. Cancer can also be caused by exposure to environmental tobacco smoke [1]. In South-east Asia, approximately 90% of oral cancers are caused by tobacco use, according to the World Health Organization (WHO). Due to the habit of tobacco chewing prevalent in India, oral cancer accounts for 35-40% of all malignancies, making it one of the leading causes of death. Various forms of tobacco are consumed by millions of Indians [2]. In India, about one million people die as a result of tobacco use each year. People who smoke bidis or cigarettes die six to ten years earlier than those who do not. Oral cancer is one of the highest incidences of cancer in the world, which distinguishes tobacco-related morbidity in India. In terms of tobacco consumption and production, India ranks second and third, respectively. Various countries produce, manufacture, consume, and export tobacco to India, which poses a complex problem. Smoking and smokeless tobacco products are also widely available at low prices in India, contributing to tobacco use [3].

There are thousands of chemical constituents in these products, including nicotine [nicotine] and minor alkaloids [noricotine, anabasine, anatabine, etc.]. When they react with nitrite, these alkaloids form tobacco specific nitrosamines such as 4- [methy1 nitrosamino]-1-[3-pyridy1]-1-butanone [NNK] and 4- [methy1 nitrosamino] -1-[3-pyridy1]-1-butanol [NNAL]. Tobacco specific nitrosamines are formed from nicotine, cotinine, nitrate, and nitrite components. The smoke from tobacco and smokeless tobacco products also affects many people who don't consume tobacco. In tobacco-exposed individuals, the urine contains nicotine, cotinine, and NO2+NO3, three major components of tobacco [4] It has a long half-life, is stable in body fluids, and is a major metabolite of nicotine [5] In the body, nicotine doesn't stay for very long. About 60 minutes is its half-life. After 6 hours, approximately 0.031 mg of nicotine remains after inhaling 1 mg in a cigarette. It is for this reason that cotinine is used in the analysis of the body's nicotine levels. A major nicotine metabolite, it persists in the blood and urine for up to 21 days following nicotine usage. The liver and lungs metabolize nicotine primarily into nicotine oxide and cotinine [6].

They are mostly removed from our blood by our kidneys. Although most cotinine is excreted through the urine within a 24-hour period, some remain for up to three weeks before being completely eliminated. Therefore, cotinine levels can be detected in urine, enabling you to determine nicotine levels many days after you last used nicotine [7]. Polluted air (ambient air, indoor air, workplaces and tobacco smoke) and tobacco smoke contain nitrogen oxides. Food and contaminated drinking water are also sources of these contaminants [8]. Basically, smoking and chewing habits play a major role in oral cancer development. Tobacco-related cancers may ultimately be decreased in India through the use of this approach for the screening of oral cancer [9]

Estimation and Comparison of Urinary Cotinine, Nitrite and Nitrate in Healthy Individuals with and without the Habit of Tobacco

Aim

To assess tobacco exposure related biomarkers in urine which includes cotinine, nitrite and nitrate in patients with habit of tobacco and in controls

Objectives

Estimation and comparison of urinary cotinine, levels in healthy individuals with habit of tobacco and individuals without habit of tobacco.

Estimation and comparison urinary nitrite and nitrate levels in healthy individuals with habit of tobacco and individuals without habit of tobacco.

Materials And Methods

Urinary cotinine, nicotinine, nitrate and nitrite to be estimated as biomarkers of tobacco exposure.

Sample Size And Sample Population

Individuals presenting to the outpatient department were screened during the month of June, July and august. Individuals with age group of 20 to 60 and above with sample size of about 70 individuals were included in the study. Those who gave a history of smoking and smokeless tobacco were included in the study. Individuals without the habit of tobacco were considered as control and the sample size was 20

Approval From An Ethical Commission

An institutional ethical committee approved the application. All patients participating in the study provided written consent.

Inclusion Criteria

Participants in the study had to be at least 20 years old. For smoking and chewing, participants needed to be smokers or chewers and have a history of tobacco use without oral lesions for 1 year.

Exclusion Criteria

Patients with any systemic diseases and infectious diseases were not included in the study.

Methodology

All the enrolled subjects were interviewed and examined on the dental chair in the department. The details of patient like name, age sex occupation, address, chief complaint, personal history was recorded. Emphasis was made on the tobacco related habits which includes smoking and smokeless tobacco. Inquiry was made regarding frequency and duration of the habit

Sample Collection

Cotinine estimation is done by Direct ELISA Kit. For cotinine estimation o.o1 ml of urine is used. Nitrate estimation is done by GRIESS'S reagent method. For estimation of nitrites and nitrates .75ml of urine and .75 ml of Griess reagent was used

Cotinine Elisa

Assays for the measurement of cotinine in serum and urine can be performed using the Cal Biotech Cotinine Direct ELISA Kit (USA).

Testing Principles

Cotinine ELISA kit from Calbiotech is a solid phase competitive ELISA test. Using anti-cotinine antibodies, wells coated with samples and enzyme conjugates are added. Samples containing cotinine compete with an enzyme conjugate (HRP) to bind to it. This step is designed to remove unbound cotinine and cotinine enzyme conjugate from the system. Cotinine concentration in the samples is inversely proportional to the intensity of color following the addition of the substrate. In order to determine the concentration of cotinine, a standard curve is prepared.

PROVIDED MATERIALS	A 96-TEST ANALYSIS
Polyclonal Ab to cotinine coated in microwells	12 x 8 x 1
Standard- Ready to Use	0.5 ml
Enzyme conjugate of cotinine HRP- Ready to Use	12 ml
Substrate of TMB- Ready to Use	12 ml
Stop solution- Ready to Use	12 ml

Handing Of Specimens

Human urine or serum can be used with the cotinine Direct ELISA kit. There are a number of applications for this assay that have not been tested. The assay is affected by specimens that have been treated with sodium azide.

Procedure For Analysis

It is recommended to bring all reagents to room temperature before use (18-26^{o}C). In duplicate, pipette 10 ul each of standards, controls, and specimens into a selection of wells. Each well is added 100 ul of enzyme conjugate. Ensure that the plate is properly mixed by shaking it for 10-30 seconds. Suitable conditions include room temperature (18-26^{o}C) and dark incubation for 60 minutes. Take care not to cross-contaminate the wells by washing them six times in 300 ul of distilled water. Make sure all residual moisture is removed from wells by inverting and vigorously slapping them on absorbent paper. As a result, this step is crucial for avoiding skewing

of results caused by residual enzyme conjugates. On an automated system, ensure that each side of the well is aspirated on the final aspiration. Substrate reagent should be added to each well at a rate of 100 ul. After 30 minutes of incubation at room temperature, place the sample in the dark if possible. The control urine samples had a blue color, while the pathological urine samples had no color. The stop solution should be added to each well in a volume of 100 ul. Make sure the solution is well mixed by gently shaking the plate. The absorbance of the stopping solution should be read at 450nm within 15 minutes after it has been added to the ELISA reader.

Assessment Of Results

Standard curves are constructed as follows.

1. Standard vials are checked for cotinine standard values.
2. Standard curves are constructed by plotting absorbance for Cotinine standards (vertical axis) versus concentrations of Cotinine standards (horizontal axis). It is best to draw a curve through the points.
3. In the curve, the absorbance of each unknown sample and the control sample is read. Control samples and unknown samples are recorded according to their value.

Determination of nitrites and nitrates by Griess reagent method:

It is prepared by using 1% sulfanilamide, 1% naphthalene diamine dihydrochloride and 2.5% phosphoric acid 500mg of sulfanilamide in 50 ml of double distilled water. 500mg of naphthalene diamine dihydrochloride in 50 ml of double distilled water 1.25 gms of phosphoric acid in 48.75 ml of double distilled water. 75ml of urine was added to .75 ml of Griess reagent incubated at room temperature for 10 mins. urinary nitrite and nitrate were determined by the change in colour which is the purple color and readings were measured by spectrometer.

Statistics method

We compared levels between controls and patients with tobacco-related habits with the help of the statistical analysis.

Results

Table 1: One-way ANOVA to compare the mean age between different types of Tobacco usage

Tobacco usage type	No	Mean	Std. Dev	Min	Max	P-Value
Smoking	45	39.31	12.808	20	69	0.123
Chewing	18	38.22	13.863	23	60	
Smoking & Chewing	7	30.43	6.997	23	42	
Control	20	33.75	5.087	22	45	

Tobacco usage type	No	Mean	Std. Dev	Min	Max	P-Value
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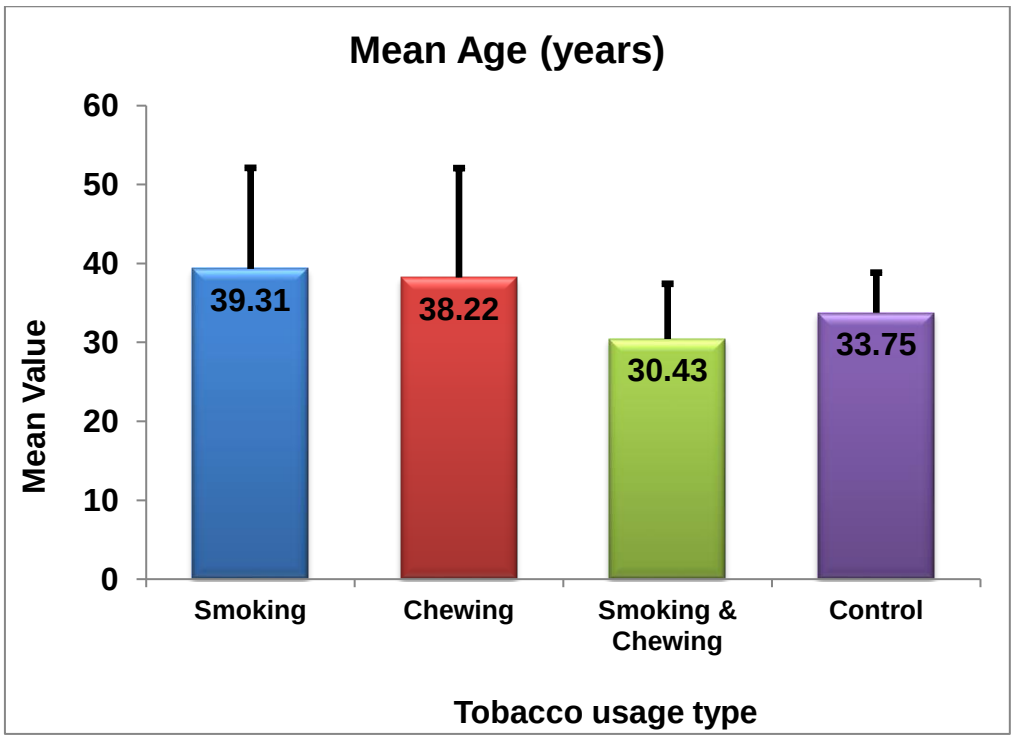
Smoking & Chewing	7	30.43	6.997	23	42	
Control	20	33.75	5.087	22	45	
Total	90	37.17	11.635	20	69	

(If P<0.05 then statistically significant

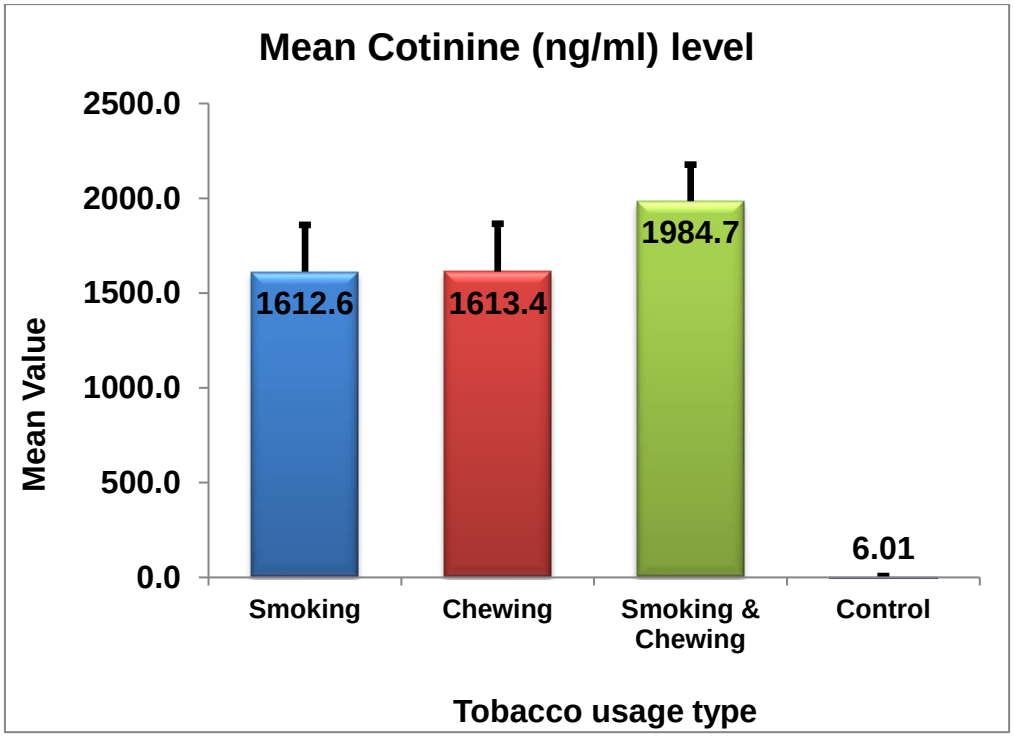


In the table 1 ANOVA method used to compare the mean age between different types of tobacco usage such as smoking, chewing and both smoking and chewing did not show statistically significant P value as the P value was 0.123.

Table 2: One-way ANOVA to compare the mean Cotinine (ng/ml) between different types of Tobacco usage

Tobacco usage type	No	Mean	Std. Dev	Min	Max	P-Value
Smoking	45	1612.64	246.89	1212.00	2234.00	<0.001
Chewing	18	1613.39	252.45	1210.00	1990.00	

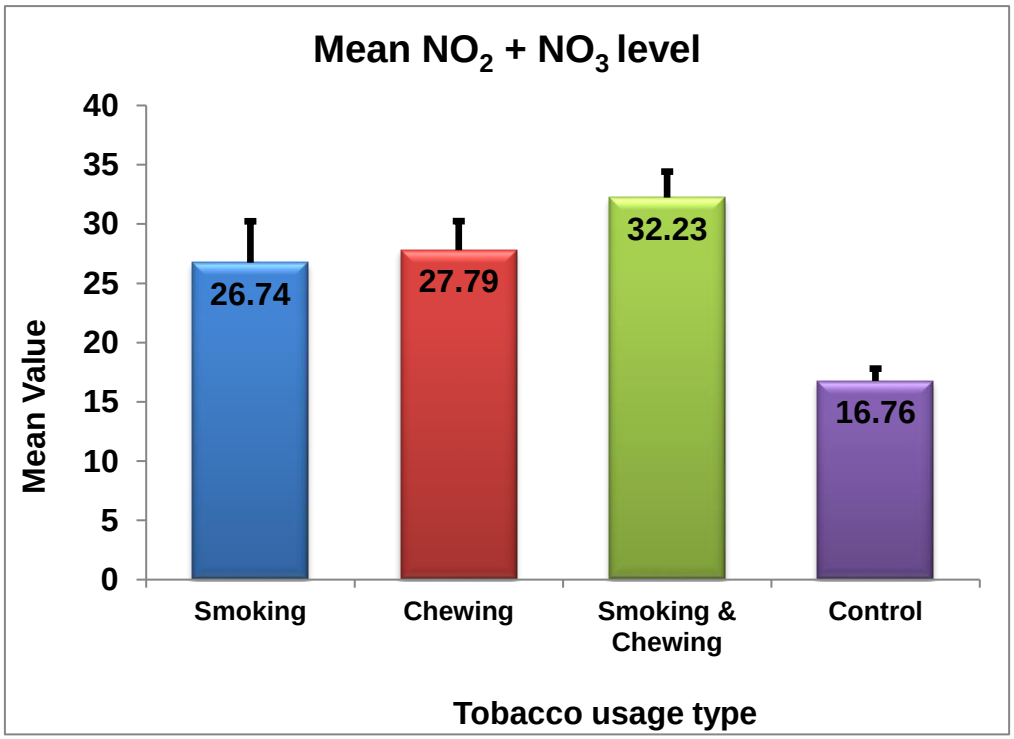
Smoking & Chewing	7	1984.71	192.90	1770.00	2370.00	
Control	20	6.01	1.04	4.70	7.90	
Total	90	1284.70	725.96	4.70	2370.00	



In Table 2 One-way ANOVA comparison of cotinine values revealed smoking and chewing showed statistically significant P value of <0.01 compared to smoking and chewing alone and also showed that control values were lower when compared to smoking, chewing and both smoking and chewing.

Table 3: One-way ANOVA to compare the mean NO2 + NO3 between different types of Tobacco usage

Tobacco usage type	No	Mean	Std. Dev	Min	Max	P-Value
Smoking	45	26.74	3.49	20.60	36.20	
Chewing	18	27.79	2.45	22.10	31.20	
Smoking & Chewing	7	32.23	2.19	29.60	36.00	<0.001
Control	20	16.76	1.04	15.20	18.44	
Total	90	25.16	5.50	15.20	36.20	



In Table 3 One-way ANOVA comparison of NO2+NO3 values revealed smoking and chewing showed statistically significant P value of <0.01 compared to smoking and chewing alone and also showed that control values were lower when compared to smoking, chewing and both smoking and chewing.

Table 4

	Study Groups	
	No	%
Smoking	45	50.0
Chewing	18	20.0
Smoking & chewing	7	7.8
control	20	22.2

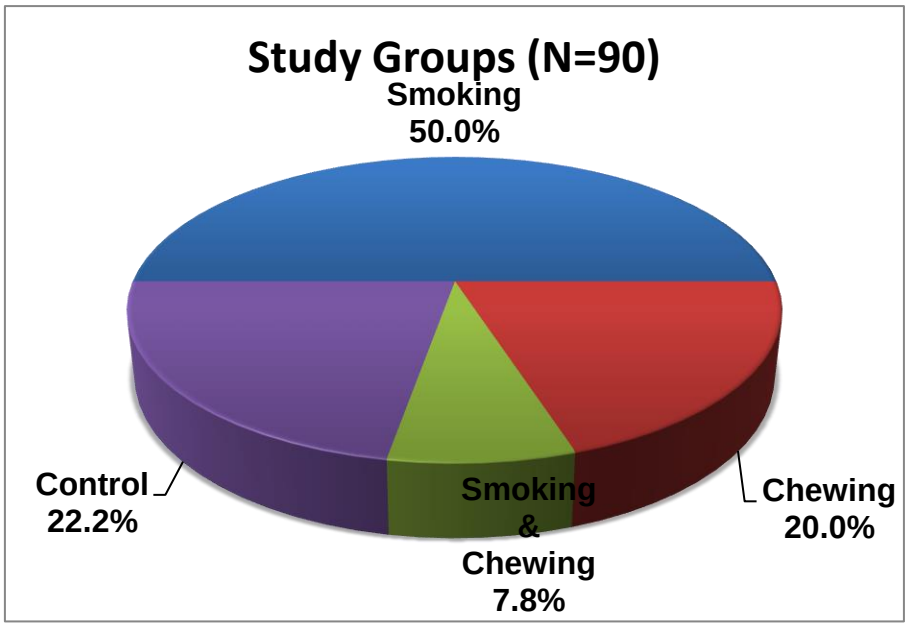


Table 5

	Smoking Habits	
	No	%
Beedi	2	4.4
Cigarette	43	95.6

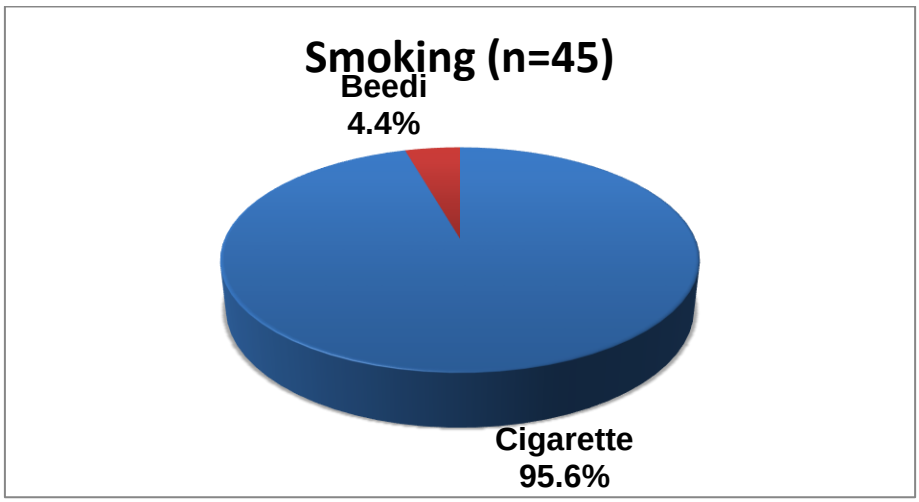


Table 6

	Chewing Habits	
	No	%
Pan Masala	5	27.8
Mawa	7	38.9
Super paak	1	5.6

Hans	4	22.2
Beetel quid	1	5.6

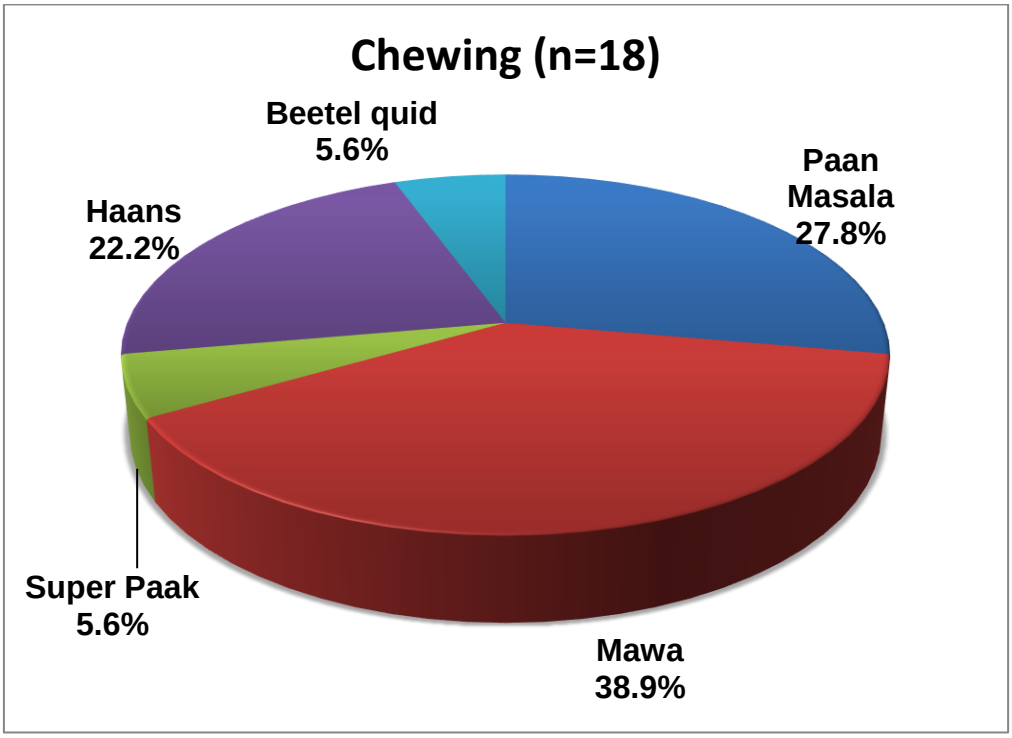


Table 7

	Smoking & Chewing Habits	
	No	%
Cigarette & Pan Masala	2	28.6
Cigarette & Mawa	1	14.3
Cigarette & Super Paak	1	14.3
Cigarette & Haans	1	14.3
Cigarette & Beetal quid	1	14.3
Beedi & Pan Masala	1	14.3

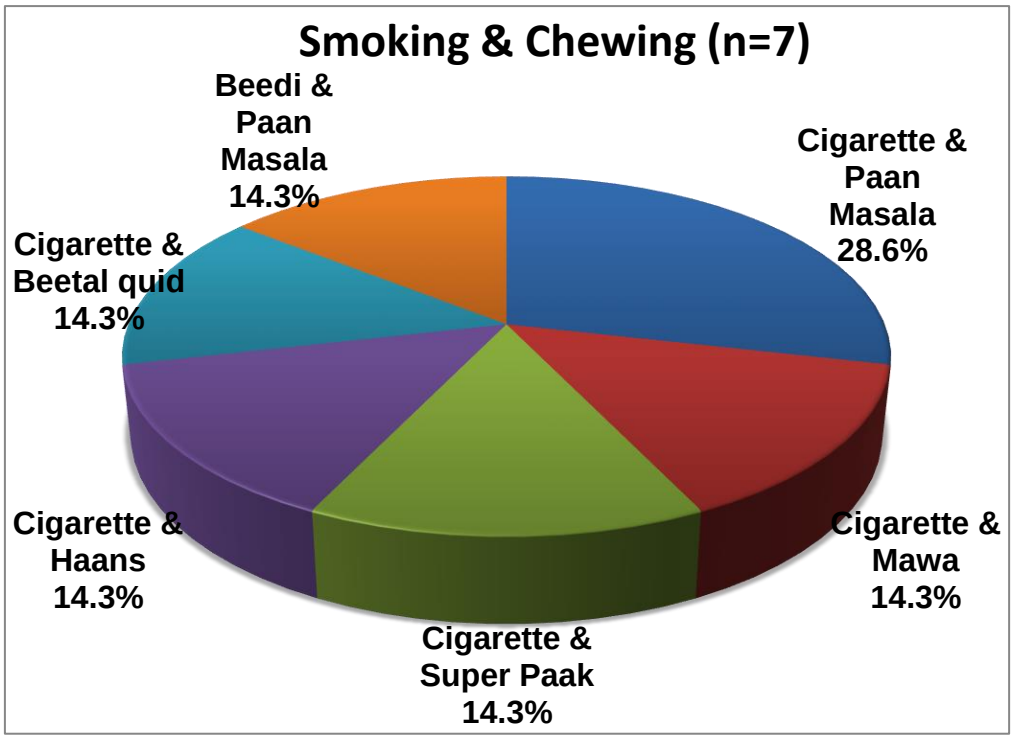


Table 8: Descriptive statistics for Cotinine ng/ml among subgroups among Smoking

Sub group	No	Mean	Std. Dev	Min	Max
Cigarette	43	1597.65	242.04	1212	2234
Beedi	2	1935.00	63.64	1890	1980
Total	45	1612.64	246.89	1212	2234

In table 8 the descriptive statistics for cotinine values among subgroups showed mean value for beedi was more than the cigarette smoking.

Table 9: Descriptive statistics for NO2 + NO3 among subgroups among Smoking

Sub group	No	Mean	Std. Dev	Min	Max
Cigarette	43	26.59	3.50	20.6	36.2
Beedi	2	29.90	0.99	29.2	30.6
Total	45	26.74	3.49	20.6	36.2

In table 9 the descriptive statistics for NO2+NO3 values among subgroups showed mean value for beedi was more than the cigarette smoking.

Table 10: Descriptive statistics for Cotinine ng/ml among subgroups among Chewing

Sub group	No	Mean	Std. Dev	Min	Max
Pan Masala	5	1688.20	269.90	1210	1860
Mawa	7	1572.71	299.64	1220	1990
Super Paak	1	1446.00	.		
Haans	4	1554.25	155.72	1326	1670
Beetel quid	1	1928.00	.		
Total	18	1613.39	252.45	1210	1990

In table 10 descriptive statistics for cotinine among subgroups for chewing revealed beetel quid had the highest mean value followed by pan masala, mawa, haans and super paak.

Table 11: Descriptive statistics for NO2 + NO3 among subgroups among Chewing

Sub group	No	Mean	Std. Dev	Min	Max
Pan Masala	5	26.64	2.94	22.1	29.2
Mawa	7	27.97	2.29	24.3	31.2
Super Paak	1	26.20	.		
Haans	4	28.48	2.05	25.6	30.4
Beetel quid	1	31.20	.		
Total	18	27.79	2.45	22.1	31.2

In table 11 descriptive statistics for NO2+NO3 among subgroups for chewing revealed betel quid had the highest mean value followed by haans, mawa, pan masala, superpaak.

Table 12: Descriptive statistics for Cotinine ng/ml among subgroups among Smoking & Chewing

Sub group	No	Mean	Std. Dev	Min	Max
Cigarette & Pan Masala	2	1833.00	89.10	1770	1896
Cigarette & Mawa	1	2370.00	.		
Cigarette & Super Paak	1	1845.00	.		
Cigarette & Haans	1	2010.00	.		
Cigarette & Beetal quid	1	2010.00	.		
Beedi & Pan Masala	1	1992.00	.		
Total	7	1984.71	192.90	1770	2370

In table 12 descriptive statistics for cotinine among subgroups for both smoking and chewing showed cigaarette and mawa had the highest mean value followed by cigarette and haans, cigarette and beetel quid which had same mean value and then the beedi and paan masala, cigarette and superpaak and finally cigarette and panmasala.

Table 13: Descriptive statistics for NO2 + NO3 among subgroups among Smoking & Chewing

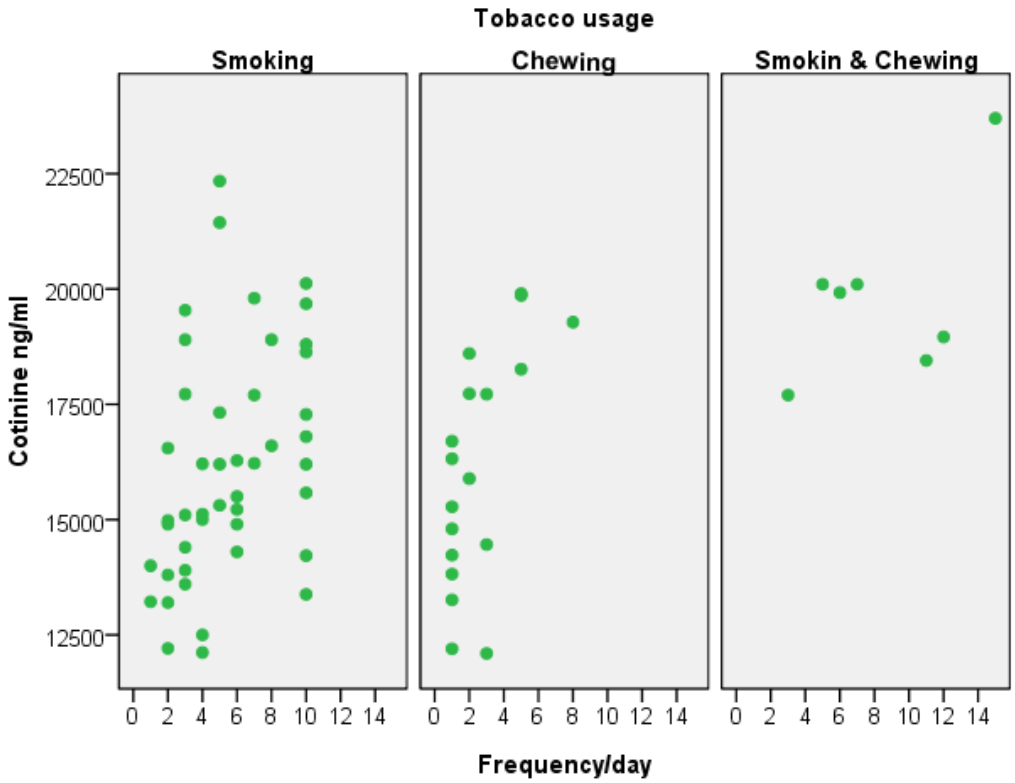
Sub group	No	Mean	Std. Dev	Min	Max
Cigarette & Pan Masala	2	30.30	0.99	29.6	31.0
Cigarette & Mawa	1	36.00	.		
Cigarette & Super Paak	1	30.20	.		
Cigarette & Haans	1	33.40	.		
Cigarette & Beetal quid	1	33.10	.		
Beedi & Paon Masala	1	32.30	.		
Total	7	32.23	2.19	29.6	36.0

In table 13 descriptive statistics for NO2+NO3 among subgroups for both smoking and chewing showed cigarette and mawa had the highest mean value followed by cigarette and haans, cigarette and beetel quid, beedi and panmasala.

Table 14: Pearson Correlations between Cotinine ng/ml and Frequency per day & Duration (years)

		Frequency/day	Duration (years)
Cotinine ng/ml	Correlation	0.487	0.596
	P-Value	<0.001	<0.001
	N	70	70

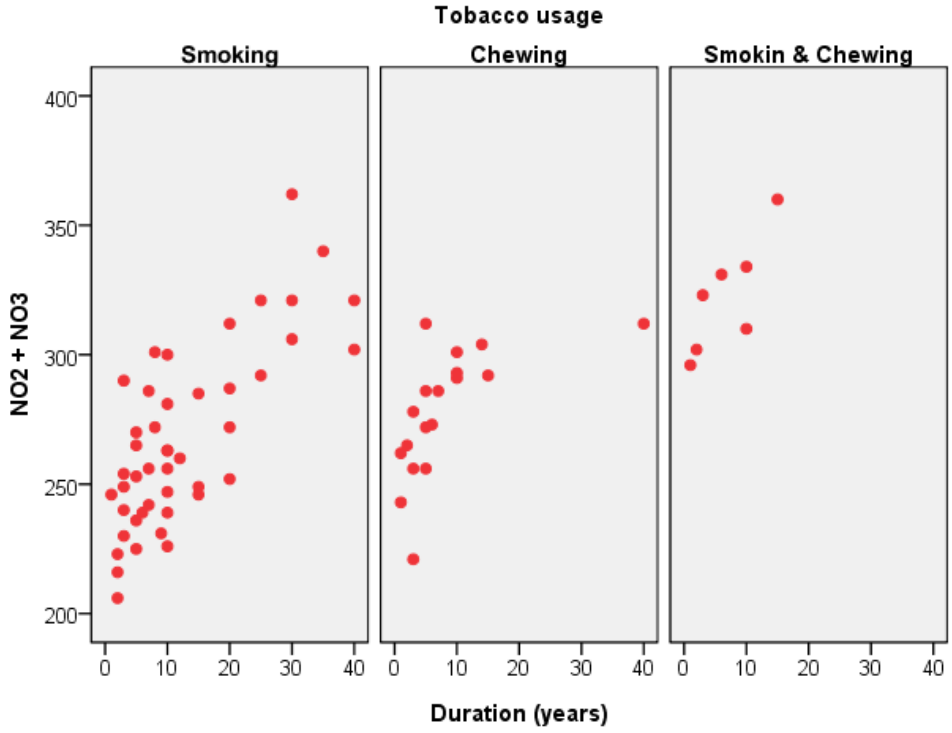
Scatter plots

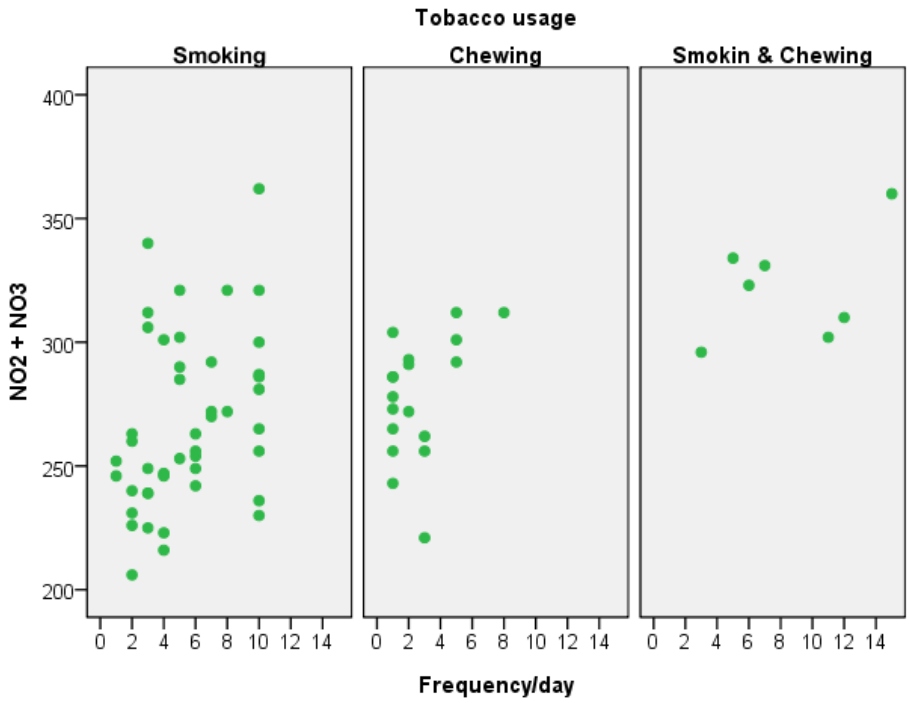


In table 14 the Pearsons correlation between cotinine and frequency per day and duration showed significant P value of <o.oo1 for both.

Table 15: Pearson Correlations between NO2 + NO3 and Frequency per day & Duration (years)

		Frequency/day	Duration (years)
NO2 + NO3	Correlation	0.377	0.536
	P-Value	0.001	<0.001
	N	70	70





In table 15 the Pearsons correlation between NO2+NO3 and frequency per day and duration showed significant P value of <o.oo1 for both.

Table 16: Descriptive statistics for frequency per day

Tobacco usage type	No	Mean	Std. Dev	Minimum	Maximum
Smoking	45	5.49	2.990	1	10
Chewing	18	2.56	2.007	1	8
Smoking & Chewing	7	8.43	4.315	3	15
Total	70	5.03	3.349	1	15

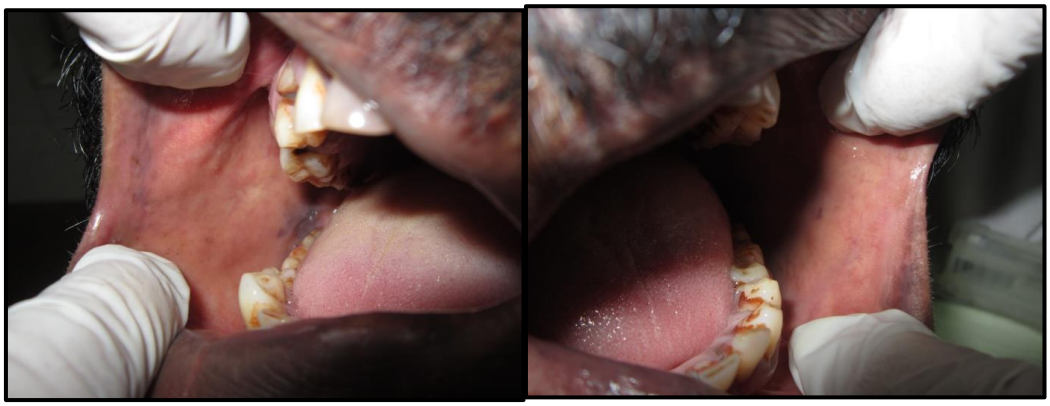
In table 16 the descriptive statistics for frequency per day the mean value was more for smoking and chewing followed by smoking and chewing because the frequency of using smoking and chewing forms of tobacco was more compared to smoking and chewing alone.

Table 17: Descriptive statistics for duration (years)

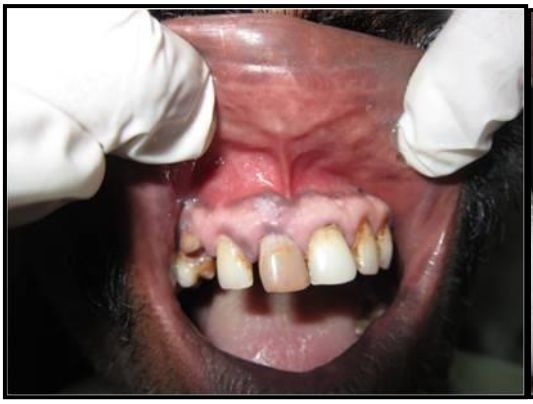
Tobacco usage type	No	Mean	Std. Dev	Minimum	Maximum
Smoking	45	12.69	10.578	1	40
Chewing	18	8.06	8.980	1	40
Smoking & Chewing	7	6.71	5.155	1	15
Total	70	10.90	9.976	1	40

In table 17 the descriptive statistics for duration (years) the mean was more for smoking followed by chewing and then smoking and chewing.

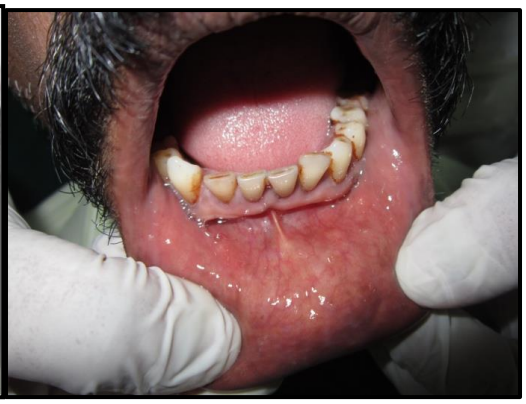
PATIENTS WITH TOBACCO RELATED HABITS WITHOUT LESIONS



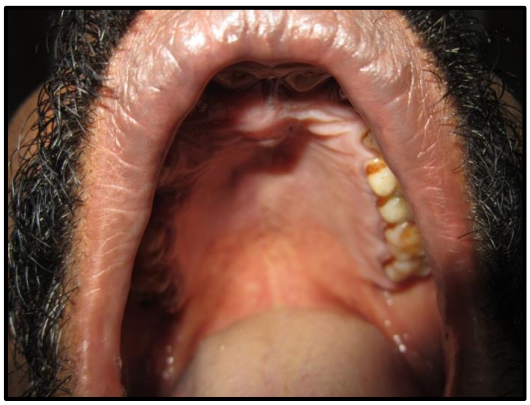
Right buccal mucosa Left buccal mucosa



Upper labial mucosa



Lower labial mucosa



Palate

Discussion

There are many countries around the world that use tobacco as a recreational substance. Lung cancer, heart disease, and other illnesses kill nearly 5.4 million people each year around the globe. Around 1 billion people around the world are expected to die from smoking in the 21st century, which is ten times as many as in the 20th century. It is mostly low- and middle-income countries that are affected by these deaths. Over 8 million people globally will die prematurely from tobacco by 2030, with 80% of these deaths occurring in countries with low- and middle-incomes. A million Indians die every year as a result of tobacco use. The life expectancy of those who smoke beedis and cigarettes is six to ten years shorter than that of those who don't. Individual smoking behavior determines the amount of smoke any smoker draws into the lungs, based on factors like puff volume, puff frequency, and depth of inhalation. There is a clear and statistically significant positive relationship between the amount of smoke constituents and their metabolites found in the urine and the number of cigarettes smoked. To determine whether someone is using tobacco and to estimate nicotine intake, nicotine and its metabolites are often measured in biological fluids. [10-14] Using nicotine gum, transdermal patches, nicotine inhalers or other nicotine medications does not allow nicotine and its metabolites to be used to assess tobacco use. In relation to smoking-related factors and socioeconomic status of smokers, urinary cotinine levels exhibited similar patterns of change. The number of cigarettes

smoked per day and the duration of smoking were associated with urinary cotinine levels. Variations in cotinine concentrations were observed within and between passive and active smokers.

Tobacco ribs and stems contain the largest percentage of nitrate, which is why they have the greatest effect on nitrosamine levels in tobacco products. There is a proportional relationship between nitrosamine yields and tobacco product nitrate content. As tar levels are reduced, so are tobacco-specific nitrosamines, and filtration greatly influences the main-stream smoke yields of cigarettes. During the present study, cotinine and NO2+NO3 levels were determined in healthy individuals who smoked and in individuals who did not smoke in order to assess whether tobacco exposure could be useful in reducing tobacco-related oral cancer in the future.

Age group:

The mean age group of different types of tobacco usage were for smoking 39.31, chewing 38.22 and for smoking and chewing 36.43 which was statistically not significant.

Cotinine:

Urinary Cotinine was analysed using calibotech ELISA kit and it was found. The cotinine levels in smokers No (45) the mean value was 1612. 64, in chewers No (18) was 1613.39 and for smokers and chewers No (7) was 1984. 71. Smokers and chewers showed increased P value of <0.01. Correlating the results between smoking and chewing was not significant but, in both smokers, and chewers it showed statistically significant results. The increased levels of cotinine in smokers and chewers was in accordance with study done. The mean values for subgroups for cotinine are for cigarette smoking n (43) is 26.59 and beedi n (2) is 29.90. Malson et al in their study found that nicotine content in Beedi found to be 37.7 mg/gm and that of cigarettes was 15mg/gm. The mean values for different types of chewing paan masala No (5) is 1668. 20, mawa No (7) 1572.71, super paak No (1) is 1446.00, Haans No (4) 1554. 25, beedi No (1928) statistically significant P values could not be obtained because the sample size of the subgroups was very small. The frequency and duration showed statistically significant P value which reveals as the frequency and duration increases that levels of urinary cotinine also increased. This was consistent with study [15], that urinary cotinine levels were associated with the No. of cigarette smoked per day which was also consistent. But the duration of smoking was not associated with cotinine levels.

NO2+NO3

The mean values for NO2+NO3 for smokers No(45) is 26.74,chewing No(18) is 27.79 and for both smoking and chewing No(7) is 32.23. It showed statistically significant P value of 0.01 for both smoking and chewing than smokers and chewers alone. The increased levels of NO2+NO3 in smokers and chewers was in accordance with study done. The mean values for sub groups of smoking i.e. for cigarette No (45) is 26.59, beedi No (18) is 29.90. The mean values

for subgroups of chewing for paan masala No (5) is 26.69, mawa No (7) is 27.97, super paak is No (26.20), super paak No(1) is 26.20, Haans No(4) is 28.48,beetel quid No(1) is 31.20.statistically significant P value cannot be obtained as the sample size for different sub groups were very small. As the frequency and duration increased the urinary NO2+NO3 also increased which showed statistically significant P value of<0.01. The present study showed that individuals with smoking and chewing were considered as higher risk group when compared to either smoking or chewing alone. Patients with both smoking and chewing showed higher urinary levels of cotinine and NO2+NO3 in both duration and frequency which was less when compared to that of smoking and chewing alone. The patients without habit of tobacco chewing and smoking had negligible levels of urinary cotinine and NO2+NO3. The present study reveals that patients with both smoking and chewing are considered to be higher risk which is responsible for the development of oral cancer in India. There are no reports on simultaneous evaluation of NO2+NO3 in tobacco and urinary biomarkers in healthy individuals and individuals with habit of tobacco. In the future urinary NO2+NO3 can be used as additional markers for tobacco exposure.

Summary

The present study was conducted to assess tobacco exposure related biomarkers in urine which includes cotinine, nitrite and nitrate in patients with habit of tobacco and in patients without habit of tobacco. Individual patients presenting to the outpatient department were screened during the month of June, July and august. Individuals with age group of 20 to 60 and above with sample size of about 70 individuals with minimum duration of 1 year were included in the study. Those who gave a history of smoking and smokeless tobacco were included in the study. Individuals without the habit of smoking and smokeless tobacco were considered as control and the sample size was 20. The levels of urinary cotinine, No2+NO3 were correlated with smoking, chewing and individuals with both smoking and chewing [16]. Statistically no significant P value was obtained for smoking and chewing alone whereas patients with both smoking and chewing showed statistically significant P value 0f >0.001. Patients with both smoking and chewing showed higher urinary levels of cotinine and NO2+NO3 in both duration and frequency which was less when compared to that of individuals with smoking and chewing alone [17]. So the study depicted that individuals with both smoking and chewing were considered as high risk factor for the development of oral cancer.

Conclusion

Several urinary biomarkers were positively correlated with tobacco exposure, including urinary cotinine and NO2 +NO3. In particular, urinary cotinine levels were positively correlated with urinary NO2 +NO3. In a study, urinary cotinine levels were also correlated with nitrate levels. TOBACCO has not been reported to evaluate NO2+NO3 levels in tobacco and urinary biomarkers simultaneously in healthy individuals. In this study, the authors observed that

tobacco-specific nitrosamine exposure (as produced by NO2+NO3) may be a major contributor to oral cancer cases in India. Indians are highly susceptible to oral cancer because they smoke tobacco and chew tobacco. It is possible to measure urinary NO2+NO3 as an additional marker for tobacco exposure. In this manner, the present approach may serve as a good assessment tool for assessing exposure to tobacco and tobacco-related habits among the general population.

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