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ORIGINAL ARTICLE

Evaluation of Lung Volume and Effect of Vital Staining as Motivation to Quit Tobacco among Nicotine Dependents of Kanpur, India

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ABSTRACT

Objective: To quantify and compare respiratory functions and further screen the oral mucosa of tobacco and non-tobacco users. **Material and Methods:** First control group, non-tobacco users (n=55); Second group, smokers' group (n=168) who currently smoked cigarettes; Third group smokeless/chewing type, tobacco group (n=81); Fourth group, both smokeless and smoking type tobacco users (n=46). Fagerstrom Test for Nicotine Dependences (FTND) and Fagerström Test for Nicotine Dependence-Smokeless Tobacco (FTND-ST) instruments were used to assess nicotine dependence. Subsequently, spirometry and Toluidine Blue (TB) vital staining were performed. Chi-squared and one-way analysis of variance (ANOVA) were used for statistical analysis. **Results:** Fagerstrom test resulted in 48.8% of subjects with low dependency, followed by an increase in nicotine dependency from low to moderate (29.2%), moderate (15.6%), and highly dependent (6.4%) groups. All respiratory function tests and oral screening confirmed significant changes amongst tobacco and non-tobacco users. The forced vital capacity of non-smoker group was significantly different from other tobacco users' group ($p<0.05$). **Conclusion:** Early effects of tobacco use can lead to complications with the respiratory system and oral cavity. Such data can be used to delineate the harm of tobacco and should be used to urge individuals to evade the utilization of tobacco.

Keywords: Lung Volume Measurements; Nicotine; Spirometry; Tobacco.

Introduction

Tobacco smoking and chewing impact practically every body part, significantly affecting the lungs and oral cavity. Smoking can cause lung disease by destructing airways and the small air sacs (alveoli) found in lungs. Lung diseases brought about by smoking integrate Chronic Obstructive Pulmonary Disease (COPD), which includes emphysema and chronic bronchitis [1]. Smokers are 12 to multiple times bound to pass on from COPD and 13 times more likely to die from COPD than non-smokers [1].

The respiratory capacity test may demonstrate worsening of respiratory capacity preceding clinical indications, and its outcomes can be utilized to forestall or decrease the rate of respiratory diseases [2]. The respiratory capacity test can be led in various manners; one of its methodologies is lung volume or flow with spirometry. Spirometry is a physiological test that determines how an individual breathes in or breathes out volumes of air as a function of time. It is significant as a screening trial of general respiratory health [3]. Some past assessments have exposed the effect of smoking on the pulmonary capacity of grown-ups. They showed that smoking diminished pneumonic capacity, including forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC, and forced expiratory flow at 25–75% ($FEF_{25–75\%}$) [4]. Cigarette smoking causes shortfalls in FEV_1/FVC and $FEF_{25–75}$, demonstrating airway obstruction and small airway disease in adult smokers [5]. A previous report detailed that more established indicative smokers with accounts of gigantic amounts of pack-years had lower FVC levels than non-smokers, while youthful grown-up smokers had FVC levels identical to or higher than age-comparable non-smokers [6].

The pulmonary function test with a spirometer was estimated in those investigations, revealing that FEV_1/FVC diminished in juvenile smokers. Hence, to explain the impact of smoking on the respiratory capacity of smoking and non-smoking, we performed the lung function test utilizing a spirometer to make tobacco users more aware of the threats of cigarette smoking.

Beginning phases are hard to recognize as the lesion may not be palpable, and colour changes are not different from the colour of the encompassing mucosa. In this way, recognizing clinically dubious/imperceptible lesions has acquired significance whereby findings can be affirmed by biopsy at a prior stage [7]. So, here we utilized toluidine vital stain to feature potentially malignant oral lesions and may identify early lesions, which could be missed out on clinical examination [8].

Toluidine blue (TB) (otherwise called tolonium chloride) is an acidophilic metachromatic color that specifically stains acidic tissue segments (sulfates, carboxylates, and phosphate radicals) [9]. TB has a liking for nucleic acids and hence binds to the nuclear material of tissues with a high DNA and RNA content [10]. It is a member of the thiazine group and is partially soluble in both water and alcohol [11].

It has been broadly utilized as a fundamental stain for mucosal lesions and has discovered applications in tissue areas to explicitly stain certain segments attributable to its metachromatic property. TB is utilized in light of the way that dysplastic and neoplastic cells may contain quantitatively more nucleic acids than typical tissues. Moreover, malignant epithelium may contain intracellular canals broader than typical epithelium, which may work with dye infiltration [9]. The different propositions about the take-up of TB in dysplastic and carcinomas incorporate the high thickness of nuclear material, loss of cell attachment, and increased mitosis [11]. In the current investigation, the use of TB staining was contemplated to recognize clinically doubtful oro-pharyngeal lesions and assess the impact of results of the Spirometry test and TB vital staining on tobacco users in motivating them to quit habit.

Behavioural change - smoking cessation intervention and motivational interviewing can increase the likelihood of our patients stopping smoking and thereby tackle the issue of nicotine dependency. Thus, the

objective of this investigation was to assess the impact of tobacco smoking on lung volume and oral cavity. This is done using a spirometer by recording lung volume and toluidine blue stain on oral cavity. In the current investigation, we have likewise attempted to utilize the results to educate and encourage tobacco users to quit using tobacco products by motivating them and showing their harmful effects on them.

Material and Methods

Study Design and Subjects

A cross-sectional study was conducted in Kanpur on 350 male subjects aged 18 to 50 years (mean age 32.7±3.2 years) from June to December 2018. Exclusion criteria were inability to perform spirometry, the individuals who couldn't comprehend and adhere to verbal guidelines, and those who were past smokers/chewers. Subjects were divided into four study groups; one control group, non-tobacco users (n=58); second group, smokers' group (n=168) who currently smoked cigarettes; third group, smokeless/chewing type, tobacco group (n=81); fourth group, both smokeless and smoking type tobacco users (n= 46).

Ethical Considerations

The study protocol was investigated and affirmed by the Institutional Ethical Committee of Rama Dental College and Hospital, Kanpur. All methods performed were as per the norms with the ethical standards of institutional ethical committee and with 1964 Helsinki declaration and its later revisions. Every study participant provided written informed consent.

Compilation of Study Participants

Considering the prevalence rate of 35% (tobacco consumers) as observed in a pilot study and with a power of 80% and a 5% alpha error, the sample size was 350. The study participants were enlisted through a convenient sampling technique from the patients visiting the Outpatient Department of Rama Dental College at Kanpur. The subjects were explained the study protocol, and their questions were cleared. Administration of questionnaires and testing of study participants were performed at the Tobacco Cessation Centre in the institution.

Data Collection Procedures

A survey proforma was prepared to acquire personal details such as age, and patterns of tobacco use (smoking, smokeless, and or a combination of both), specifying the frequency and duration. All subjects finished smoking-related questionnaires, including baseline characteristics, smoking attributes, and level of nicotine dependence. A current smoker is someone who has smoked greater than 100 cigarettes (including hand-rolled cigarettes, cigars, etc.) in the course of their lifetime and has smoked in the last 28 days [12]. Current smokeless tobacco use was characterized as individuals ever using either chewing tobacco or snuff 20 or at least multiple times in their life and utilizing either smokeless tobacco item consistently or some days [13].

Survey instruments - Fagerstrom tolerances test for nicotine dependence and Fagerström Test for Nicotine Dependence-Smokeless Tobacco (FTND-ST) were pre-tested in a sample of adults of the same age group as the study participants and, based on feedback provided by these participants; the instruments were determined to be acceptable (Cronbach's alpha = 0.75 for both). The current version of the Fagerstrom test was used for the assessment of nicotine dependence [14]. The Fagerström Test for Nicotine Dependence is a

standard instrument for assessing the intensity of physical addiction to nicotine. The test was designed to provide an ordinal measure of nicotine dependence related to cigarette smoking. It contains six items that evaluate the quantity of cigarette consumption, the compulsion to use, and dependence. In scoring the Fagerstrom Test for Nicotine Dependence, yes/no items are scored from 0 to 1 and multiple-choice items are scored from 0 to 3. The items are summed to yield a total score of 0-10. The higher the total Fagerström score, the more intense is the patient's physical dependence on nicotine. Scores of 0–2 indicate low dependence, scores 3–4 indicate low to moderate dependence, scores 5–7 indicate moderate dependence, and scores ≥ 8 indicate high dependence [14]. The Fagerström Test for Nicotine Dependence-Smokeless Tobacco (FTND-ST) measures the physical aspect of dependence. The good psychometric properties of FTND-ST and its orientation as a continuous scale indicate that FTND-ST is a useful measure of dependence among ST users. The total possible score on the FTND-ST is 10 [15].

Following this procedure, spirometry and TB vital staining was performed. Spirometry only for smoking-type tobacco users and TB vital staining for both smoke and smokeless tobacco users were performed and with an interpretation of the result, they were motivated to quit tobacco use. Spirometry was performed according to American Thoracic Society and European Respiratory Society guidelines [4,16], using PC spirometer (Vyntus SPIRO, Vyaire Medical, Inc., Mettawa, IL, USA). Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and FEF_{25-75} were recorded and reported in liters (L), %predicted, %, or liters per second (L/s).

Statistical Analysis

The data was analysed using SPSS version 21.0 (SPSS Inc., Chicago, Illinois, USA). Information obtained were appropriate for utilizing parametric tests since the outcomes were normally distributed, as seen by Shapiro Wilk test. Categorical data were presented as number (%) and continuous data as mean and standard deviation. Chi-squared test was applied to compare categorical variables between two groups. One-way analysis of variance (ANOVA) with post hoc Tukey's test was used to compare continuous data. For all tests, confidence interval and p-value were set at 95% and < 0.05, respectively.

Results

A total of 350 male subjects participated in the present study. There was a decrease in tobacco dependence with its increasing levels. Approximately half (n=144; 48.8%) of the study population showed a low dependency for nicotine, followed by low to moderate (29.2%), moderate (15.6%), and highly dependent (6.4%) groups. Among the 168 smoking tobacco users, 67 (39.8%) were identified with low dependence on tobacco, and 14 (8.3%) were reported with high dependence. Amongst 81 smokeless tobacco and 46 subjects who use both forms of tobacco, the nicotine dependence was almost similar; low among 49 (60.5%) and 28 (60.9%) and high in only 3 (3.7%) and 2 (4.3%) groups, respectively (Table 1).

Table 1. Distribution of study population according to Fagerstrom test results.

Grade of Dependency	Smoking Tobacco N (%)	Smokeless Tobacco N (%)	Combination of Tobacco N (%)	Total N (%)
Low	67 (39.8)	49 (60.5)	28 (60.9)	144 (48.8)
Low to moderate	49 (29.2)	24 (29.6)	13 (28.3)	86 (29.2)
Moderate	38 (22.6)	5 (6.2)	3 (6.5)	46 (15.6)
High	14 (8.3)	3 (3.7)	2 (4.3)	19 (6.4)
Total	168 (56.9)	81 (27.5)	46 (15.6)	295 (100.0)

The anthropometric characteristics of the study population are summarized in Table 2. Study participants were grouped into four main groups based on their FTND/FTND-ST test scores. The mean age was 32.7±3.2 years and Body Mass Index (BMI) was 24.97±2.54 kg/m². The prevalence of tobacco consumption among our study population was 35.9%. Smokers began smoking at the age of 17.12±2.31 years. The differences in the distribution of anthropometric characteristics according to the nicotine dependency between the groups were not statistically significant (p>0.05).

Table 2. Anthropometric characteristics of the study population according to nicotine dependence.

Anthropometric Characteristics	Control Mean±SD	Low Mean±SD	Low to Moderate Mean±SD	Moderate Mean±SD	High (n=19) Mean±SD	p-value
Age (in Years)	30.24±0.74	32.73±1.54	31.21±0.97	33.14±1.77	33.65±1.26	0.14
Weight (kgs)	69.12±2.14	65.78±1.63	66.45±1.82	64.65± 1.98	64.96±1.88	0.27
Height (cms)	165.34±1.65	164.63±0.25	164.96±0.87	163.98±1.35	164.21±1.36	0.19
BMI (kg/m²)	25.32±1.15	25.04±0.62	24.76±0.54	24.98±1.04	25.09±1.24	0.64

Spirometric observations of all selected four parameters were analysed, and the results showed a general reduction proportionate to the level of nicotine dependency, which was statistically significant across the groups (Table 3). Further, post hoc analysis using Tukey's test demonstrated clearly that the said parameters had a definite proportionate fall with increasing nicotine dependency compared to the control. The statistical significance of the results confirms the decrease in lung function with an increase in nicotine dependency.

Table 3. Nicotine dependence and its impact on lung volumes.

Parameters	Control Mean±SD	Low Mean±SD	Low to Moderate Mean±SD	Moderate Mean±SD	High Mean±SD	p-value
FEV_1(L)	2.91±0.24	2.02±0.22	2.31±0.17	2.39±0.16	2.63±0.21	0.001*
FVC(L)	3.12±0.12	2.80±0.28	2.46±0.26	2.58±0.07	2.68±0.23	0.002*
FEV_1/FVC	87.36±1.65	75.38±1.68	71.03±2.02	79.05±1.63	81.35±1.98	0.001*

*p<0.05: Statistically significant.

A toluidine test was done on all 295 tobacco users in which 54.3% of subjects with both forms of tobacco use were found to be positive, followed by 46.9% smokeless tobacco users and 7.1% smoking tobacco users. Among the controls, one subject was found to be positive, and the results were statistically significant (Table 4).

Table 4. Toluidine test results among the study population.

Type of Tobacco Users and Nonusers	N	Toluidine Test		p-value
		Positive N (%)	Negative N (%)	
Smoke Tobacco Users	168	12 (7.1)	156 (92.8)	0.001*
Smokeless Tobacco Users	81	38 (46.9)	43 (53.0)	
Both Smoke and Smokeless Tobacco Users	46	25 (54.3)	21 (45.6)	
Controls	55	1 (1.8)	54 (98.1)	

Discussion

This investigation assessed the relationship between respiratory function tests and toluidine staining among nicotine dependents. Nicotine dependency is the prime factor that leads a smoker to smoke again and again, not the number of cigarettes. Our study explored the volume changes in smokers with differences in their nicotine dependency. It is not the cost but the nicotine crave that is making these smokers cross even

their economic limits to continue the habit of smoking [17]. The forced vital capacity of the non-smoker group was fundamentally not quite the same as their particular qualities in the smoker group. Langhammer et al. [18] found that FEV_1/FVC diminished among young adult smokers.

It was found in our study that the spirometry test was normal among many smokers, but their FEF_{25-75} was found to be low or around 80%. Undoubtedly, by far, most of the subjects showed respiratory function values within the normal range. In this examination, FEV_1 and FVC of the control group were altogether more prominent than that of other groups. This proposes that cigarette smoking influences the lung capacity of smokers, making the volume related to the FVC test more modest than that of non-smokers. The outcomes for FVC may have been impacted by the directions given to subjects to perform maximal inhalation and afterward perform maximal exhalation as quickly and as wholly as expected. Thus, the FVC test depends on the strength of respiratory muscles. Cigarette smoking influences the respiratory muscles through the impact of free radicals on the vascular system [18], prompting a decrease in respiratory muscle blood supply unfavorably impacts respiratory capacity.

Our discoveries as far as the forced vital capacity of smokers in the early smoking time frame are steady with those of past examinations that have detailed that the beginning phase of smoking among young people lessens lung function [19]. Inhaled tobacco smoke has appeared to inspire intense changes in the respiratory capacity, including alterations in resistance to airflow, coughing, and irritation of the airways. Our study observations may support the execution of smoking cessation advice for youths.

Toluidine blue is a cationic metachromatic dye that stains deoxyribonucleic acid as well as might be held in intracellular spaces of dysplastic epithelium and clinically may show up as royal blue areas [20]. This study also evaluated the relationship between toluidine staining and changes in oral mucosa by tobacco use. The toluidine blue literature shows that it is a pragmatic, quick, cheap, and viable aide diagnostic instrument. TB is an essential metachromatic color that is known for its property of differentially staining harmful neoplasm; however, not ordinary epithelium. It is proposed that the expanded measure of DNA and RNA in neoplastic cells and the more extensive intercellular canals contrasted with ordinary epithelial cells are answerable for staining malignant cells [14]. Its clinical application in staining neoplastic cells was first portrayed by Richart in 1963, who utilized the color to stain cervical carcinoma *in situ*. From that point forward, it has arisen as a fundamental stain for the discovery of cervical dysplasia and carcinoma during colposcopy [21].

To the best of our knowledge, this is the first of its kind motivational study to quit the habit of tobacco by using spirometry and toluidine blue staining. However, the success rate was low (3.7%), but it can be used as an alternative method for motivating people to quit their tobacco habit. Those subjects whose spirometry test was mild and moderate were asked to quit tobacco by cold turkey method and were advised to visit a physician. For those subjects who were found with a moderate or higher dependency on tobacco, nicotine replacement therapy was advised to help them quit this habit.

Secondly, for subjects whose toluidine test was positive, their intra-oral photographs were taken. It was used as an educational method that tobacco chewing was causing damage to their oral mucosa; hence, they were asked to quit their tobacco habit. If the stain was present in a particular region of the oral cavity, a question was made to subjects whether at that particular region they used to keep their quid. The subjects then explained the reason for staining that particular region of the oral cavity where they used to keep the quid and which was causing damage to their oral mucosa.

Follow up after one month - Out of 295, only 138 reported, in which 17 participants mentioned that they had quit the habit by cold turkey method and are on nicotine replacement therapy, while 21 participants were still in a gradual reduction process and 12 participants said they had reduced the daily amount of tobacco they use. Almost 88 subjects didn't respond positively or confirmative in quitting of tobacco habit.

Follow up after 3rd month - Only 63 participants reported, out of which 11 participants had stopped using tobacco while 7 participants said they were still in a gradual reduction process and trying to quit, and 12 said they had reduced their daily amount of tobacco. Rest 33 participants didn't give any positive response in quitting of tobacco habit.

Finally, out of 295 subjects, we received 11 reported subjects who had stopped tobacco habits giving us a 3.7% success rate of quitting tobacco use by this present lung volume measurement and toluidine staining motivational approach.

This study has a couple of limitations, as we can't predict lung function changes later on in light of the fact that this investigation is a cross-sectional investigation. In this way, a planned report might be expected to regulate lung function changes among tobacco users.

Conclusion

The predominance of smoking among the study populace was moderately high, although nicotine habit was low and lung functions were still acceptable. This assists individuals in visualizing and realizing the harmful effect of tobacco by spirometry and toluidine blue staining and motivates them to quit this habit. A few procedures are expected to develop active prevention and intervention methods.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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