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SMOKELESS TOBACCO USERS SHOW DECREASED LUNG FUNCTION WITH A HIGH NEUTROPHIL TO LYMPHOCYTE RATIO – A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Smokeless tobacco products are highly addictive and contain over 4,000 chemical compounds with 30 known carcinogens. These products are known to be strongly linked with various systemic and localized health conditions. Smokeless tobacco users' pulmonary function hasn't been thoroughly documented yet. The purpose of the study was to assess the lung function tests associated with inflammatory markers among smokeless tobacco users.

Material and methods: 80 subjects were recruited, 40 from the smokeless tobacco user group and 40 from the control group with no tobacco use. Nicotine dependence was assessed using the Fagerstrom Nicotine Dependence scale (FTND-ST). Lung function tests, White blood cell counts, neutrophil lymphocyte ratio (NLR), and high-sensitivity C-reactive protein (hs-CRP) levels were measured in all participants.

Results: Lung function parameters, which include FVC, FEV1, FEV1/FVC%, PEFR, and FEF 25–75% were significantly reduced in smokeless tobacco users. Smokeless tobacco users had significantly higher levels of inflammatory markers, including WBC, NLR, and hs-CRP, than controls. Further, NLR levels were elevated in the significant nicotine-dependent group derived from FTND-ST compared to users with low and moderate dependence. However, no correlation was found between the inflammatory markers and pulmonary function test results.

Conclusion: This study concluded that smokeless tobacco use can cause lung function deterioration with an increase in inflammatory markers.

KEYWORDS. Smokeless tobacco users, pulmonary function tests, highly sensitive C-reactive protein, inflammatory markers.

INTRODUCTION

In India, chewing tobacco or smokeless tobacco (SLT) has been an extremely addictive habit for people of all ages and poses a serious risk to systemic health. According to recent data, 267 million people in India consume tobacco products annually, with 1.35 million deaths attributed to tobacco-related illnesses.[1] Among the Indian population, 10.38% of individuals are smokers, while 21.38% use smokeless tobacco products (SLT), demonstrating a higher prevalence of smokeless tobacco usage than smoking.[2]

Hans, gutkha khaini, betel quid with tobacco, and zarda are among the many forms of SLT products that are available. These products are typically placed in the oral cavity, allowing nicotine and other harmful substances to be absorbed into the bloodstream via the mucosal lining. Owing to their composition and mode of absorption, nicotine from smokeless tobacco (SLT) remains in the body longer than that from smoking, prolonging its harmful effects. Chronic exposure to smokeless harmful chemicals triggers the release of proinflammatory cytokines, including interleukin 1 & 6 and tumor necrosis factor-alpha (TNF-α). These cytokines stimulate the liver to release C-reactive protein (CRP), an established biological marker for systemic inflammation.[3] These items are linked to a variety of local and systemic health problems, such as cancers of the stomach, pancreas, esophagus, and mouth, as well as cardiovascular disorders

and stroke.[4]The physiological insults caused by smokeless tobacco have been well documented for inflammation, stroke, cancer, immune cells, as well as for cardiovascular disease.[5] However, its effects on lung function have not been explored yet.

Nicotine has deleterious effects on respiratory function by acting locally in smokeless tobacco users. However, in addition to local effects, nicotine stimulates the vagal reflex and parasympathetic ganglia as well as leading to increased bronchial secretions and airway resistance.[6] Over time, these changes contribute to a decline in lung function.

A critical indicator of inflammation and immune dysregulation is the NLR.[7] This ratio is increasingly being used to evaluate systemic inflammation and immune compromise. A higher NLR acts as a prognostic indicator for several pathologies, including colorectal, breast, and stomach cancers, and has been linked to several illnesses, including infections and pulmonary and cardiovascular disorders.[8]

Even though differences in chronic inflammatory markers in smokers and SLT users have been evaluated for cardiovascular health. [9] Yet there is a paucity of literature evaluating their effect on pulmonary function. Given the widespread prevalence of SLT use, systemic inflammatory effects of nicotine, and the indirect effects of nicotine on respiratory function, we hypothesized that smokeless tobacco can also have a deleterious impact on pulmonary function, which will be associated with a rise in inflammatory markers. Due to the limited research on this relationship in smokeless tobacco (SLT) users, this study aimed to address this gap.

MATERIALS AND METHODS

This analytical cross-sectional study was approved by the Institutional Ethics Committee which was carried out between April 2024 and January 2025. The study's sample size was determined by comparing the two groups' FEF25–75% mean and standard deviations from a prior study.[10] 40 subjects per group were sufficient to detect the determined effect size in the parameters at a 5% level of significance with 80% power of the study.

After taking informed consent, forty male participants aged 25–45 years were recruited in each group through purposive sampling. The study group comprised 40 volunteers who had been consuming any of the smokeless tobacco products for at least one year, as given by WHO guidelines.[11] The control group consisted of an equal number of non-tobacco users. Participants with respiratory diseases such as asthma, COPD, bronchitis, tuberculosis, or COVID-19, those working in dust-prone environments (such as textile mills, cement factories, and coal factories), suffering from any inflammatory or having recovered from any recent infection and those undergoing immunosuppressive treatments were excluded.

Physical parameters, age, height, weight, and BMI, were measured for all participants. Nicotine dependence among SLT users was evaluated using the FTND-ST questionnaire. FTND-ST is a well-established scale for quantifying nicotine dependence in SLT users with a good reliability score (Cronbach's $\alpha = 0.72$).[12] Significant dependence is indicated by a score of 5 or greater, which denotes a severe nicotine addiction. On the other hand, low to moderate dependence on nicotine products is indicated by a score of 4 or lower.

Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC ratio, peak expiratory flow rate (PEFR), and forced expiratory flow at 25–75% were all recorded with the MIR Spirobank Oxi spirometer for the pulmonary function test (PFT). According to the guidelines of the ATS/ERS.[13]

5 mL venous blood samples were drawn from each participant's antecubital veins for analysis of biochemical and immunological function. 2 mL was used for complete blood count (CBC), while the remaining 3 mL was stored at –20°C and used later for analysis of hs-CRP levels. hs-CRP levels were measured using the Diagnostic Biochem Canada Inc. (DBC) kit using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis:

The data were analyzed using SPSS 26. The Kolmogorov-Smirnov tests were used to determine whether the data were normal. Two-tailed tests of significance were used, and a p value of less than 0.05 was considered as significant. An independent t-test was used to compare the results of the pulmonary function test, and the Mann-Whitney test was used to compare the two groups' inflammatory marker levels

RESULTS

No difference was in physical parameters.

Table 1. Describe the baseline characteristics of the participants

Physical Parameters	**Smokeless tobacco users (n =40) Mean + SD**	**Control (n =40) Mean + SD**	**P value**
Age (yrs)	38.3 $\pm$ 7.1	39.1 $\pm$ 8.2	0.68

Height(cm)	168.2 ± 6.9	165.1 ± 7.6	0.1
Weight(kg)	69.8 ± 13.9	68.3 ± 13.6	0.6
BMI (kg/m^2)	24.3 ± 4.8	24.9 ± 3.5	0.5

Inflammatory markers significantly higher white blood cell count (p.value = 0.0001) and NLR (p.value = 0.003) in Smokeless tobacco users. hs-CRP level was also significantly higher (p value = 0.02) in the SLT group Table 2.

Table 2. Assessment of inflammatory markers among smokeless tobacco users and controls

Inflammatory markers	Smokeless tobacco users (n=40) Median (IQR)	Controls (n=40) Median (IQR)	P.Value
WBC (cells / cumm)	8700 (4130)	7200(1350)	**0.0001***
NLR%	1.5 (0.6)	1.3 (0.5)	**0.003***
hs – CRP (mg/dl)	2.2 (6.1)	1.2 (1.7)	**0.02***

WBC – White Blood Cells, NLR – Neutrophil Lymphocyte Ratio, hs- CRP- high–sensitive C-reactive protein.

FVC (p = 0.001), FEV1 (p value =0.040), FEV1/FVC ratio (p = 0.006), PEFR (p = 0.01) and $FEF_{25–75\%}$ (p = 0.004) were significantly lower in smokeless tobacco users. FVC no significant difference in percentage predicted. However, FEV1% (p=0.01) and FEV1/FVC% % (p=0.001), PEFR% (p=0.0001) and FER25-75% (p=0.004) were significantly lower in SLT users than the controls. The result is provided in Tables 3 and 4.

Table 3. Comparison of observed values of lung function parameters between smokeless tobacco users and controls

PFT Parameters	Smokeless tobacco users (n=40) Mean + SD	Control (n=40) Mean + SD	P value
FVC (L)	3.1+0.5	3.6+0.4	**0.001***
FEV1 (L)	2.8+0.5	3.0+0.3	**0.040***
FEV1/FVC %	78.1+7.6	82.4+2.9	**0.006***
PEFR(L/M)	460+108	512+60	**0.01***
FER25-75(L/S)	3.0+0.9	3.6+0.60	**0.004***

FVC, forced vital capacity; FEV1, Forced Expiratory Volume in the first second; PEFR - Peak Expiratory Flow Rate, FEF25-75, forced expiratory flow between 25% and 75%.

Table 4: Percentage predicted values of lung function parameters are compared between smokeless tobacco users and controls.

PFT Parameters	Smokeless Tobacco Users (n = 40) Mean ± SD	Control (n = 40)Mean ± SD	P-Value
FVC (%)	89.9 ± 11.3	90.4 ± 7.3	0.08
FEV1 (%)	82.6 ± 9.6	87.7 ± 7.8	**0.01***
FEV1/FVC (%)	92.9 ± 10.9	99.9 ± 6.1	**0.001***

PEFR (%)	87.6 ± 20.4	99.9 ± 6.1	**0.0001****
FER25–75 (%)	73.1 ± 24.8	86.2 ± 12.0	**0.004***

Given the differences in inflammatory and lung function parameters, further analysis was done to explore any association between inflammatory markers and lung function parameters in smokeless tobacco users. No correlation was found between hs-CRP, NLR and WBC count with lung function parameters.

Further evaluation was done by grouping SLT users based on nicotine addiction by using the FTND-ST scale. FTND-ST revealed that 23 participants in the SLT group had significant nicotine dependence, while 17 participants exhibited low to moderate level dependence. The NLR ratio was found to be significantly higher in SLT users with significant dependence (Fig. 1). However, the rest of the parameters, including pulmonary function and other inflammatory markers, were similar in both groups.

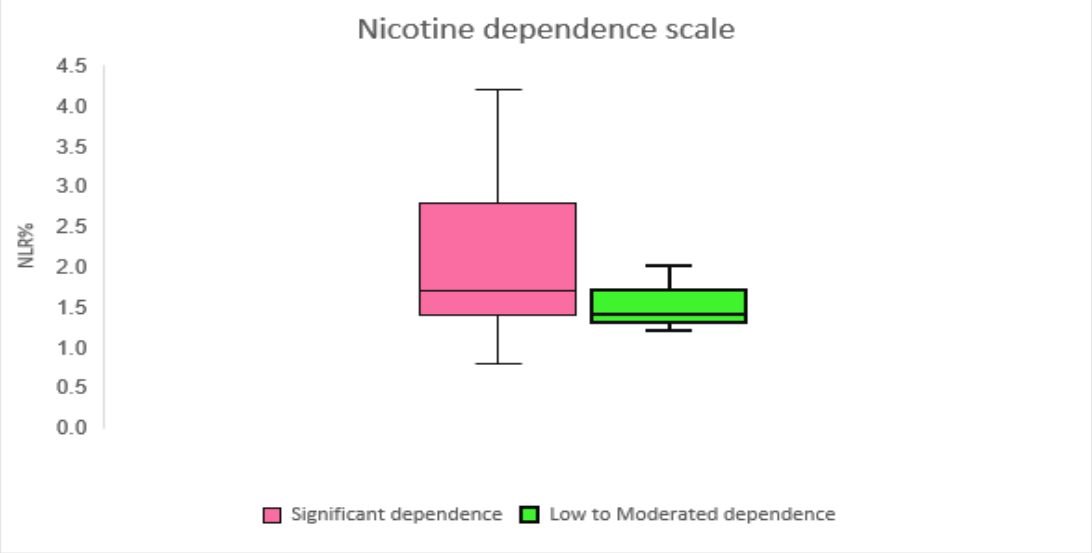

Figure: 1: Comparison of NLR based on nicotine dependence

DISCUSSION

The current study compared the lung function parameters and inflammatory markers of healthy smokeless tobacco users with those of people not using tobacco in any form. Previously, very few studies have assessed the pulmonary function in smokeless tobacco users. The current investigation's findings align with those of earlier research. [14 -16] However, Pramanik et al included subjects with a very wide age range of 18-70 years and did not comment on the age-normalized data of pulmonary function parameters.[10] It has been suggested before that smokeless tobacco products contain numerous toxic substances that cause oxidative stress and airway obstruction. Oxidative stress, caused by reactive oxygen species from tobacco, damages respiratory epithelial cells, disrupts the protease-antiprotease balance, and promotes airway remodeling. In addition, oxidative stress damages antioxidant defense systems and causes inflammation, direct cell damage, and the inactivation of antiproteases.[17]

The current study's findings on inflammatory markers were consistent with earlier studies that reported an elevated WBC count.[18-20] and hsCRP levels [21] in SLT users compared with non-tobacco users. While it is not the case for never smokers, a higher baseline hsCRP is associated with a higher risk of developing COPD in chronic smokers. hs-CRP is a well-established indicator of systemic inflammation. [22]

The NLR in peripheral blood is a reflection of systemic inflammation and immunity. Increased NLR is related to mortality caused by various lower respiratory tract diseases.[23] Our study found that SLT users had an increased NLR, which was reported in other studies. NLR was also considerably higher in SLT users with significant dependence than in those with low to moderate dependence, based on the subgroup analysis.

Although our study reveals that SLT users had higher levels of hsCRP and NLR, there was no association between these two parameters and lung function parameters. This is consistent with a few previous studies, which have not found any correlation.[24,25] However, other studies have found a negative correlation between hsCRP and pulmonary function tests.[26,23] Similarly reported that NLR is associated with a decline in lung function, particularly in conditions like COPD [27] and pulmonary fibrosis.[28] However, there are no studies available that have explored this relationship in SLT users.

In our study, it might be that NLR and hsCRP did not show any correlation with pulmonary function because even though there was a reduction in lung function on smokeless tobacco users, yet it was not of the severity in that can be

diagnosed as suffering from obstructive or restrictive disease. On the other hand, pulmonary function impairment in SLT users may be primarily due to localized inflammation and bronchoconstriction induced by the toxic components of smokeless tobacco, thus not associated with the systemic markers of inflammation. It has been found that nicotine stimulates nicotinic acetylcholine receptors (nAChRs) on sensory nerves in the airway, which can trigger neurogenic inflammation. This can increase bronchial reactivity and mucus production, indirectly affecting lung function.[29]
Nonetheless, NLR and hsCRP may be early markers of deterioration of lung function. Since NLR and PFT are non-expensive investigations, they can be used to counsel SLT users for the underlying harmful effects of SLT, which can later manifest as other grave diseases as well.
One strength of our study is that first to report which has analyzed the relationship between inflammatory biomarkers and lung function parameters in SLT users. Though previous studies have considered the parameters alone, none have analyzed the association. Also, the study used nicotine dependence to analyze the PFT and inflammatory markers. This is important since in people with significant nicotine addiction, the continuously elevated nicotine levels in blood affect the functions differently compared to people with low to moderate dependence, in whom the bodily defense mechanisms have some window period for repair.
The present study has certain limitations. First, we used a subjective questionnaire to categorize nicotine addiction. Had we assessed nicotine blood levels, it would have provided a better objective mechanism with less response bias. Secondly, we did not assess for oxidative stress markers which have been hypothesized to have a role in the deterioration of pulmonary function. We recommend that in future studies, these parameters be taken into account to elucidate the effects of nicotine on lung function.

CONCLUSION

Overall, suggests that even SLT can cause lung function deterioration with an increase in inflammatory markers. The observed nicotine addiction and the harmful health effects of smokeless tobacco on pulmonary function underscore the need for effective interventions to mitigate these risks.

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