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Correlation between Upper Airway and Lower Airway Function in Current Smokers, Never Smokers and Former Smokers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Smoking is the major risk factor for the development of chronic lung disease and airway malignancy. The development of biomarkers for disease onset and early progression is hindered by the accessibility of the primary tissue in the lungs, so there is a need to evaluate alternative sites for surrogate biomarkers. The harmful effects seen in the lower and distal airways are also mirrored in the nasal epithelium as one airway and one disease.

Objective: To study the correlation between nasal mucosal cytology, mucociliary function, nasal airflow and lung function among the current smokers, never smokers, and former smokers.

Methods: Cross sectional, observational study from a tertiary care hospital.105 subjects were randomly distributed on the basis of smoking pattern into 3 groups, never smoker, current smoker and former smoker. Nasal mucosal cytology and function were assessed by saccharin transit time test (STT), peak nasal inspiratory flow(PNIF) and nasal ciliated cells & goblet cell ratio. The lower airway was assessed by spirometry.

Results: The increase in saccharin transit time was statistically significant (p <.001) in current smokers and former smokers compared to never smokers. The lower mean goblet cell count of the former smoker group was statistically significant when compared to the never smoker and current smoker groups, (p.023) while the change in ciliated cell/ goblet cell ratio remained statistically insignificant. The decrease in FEV1/FVC is statistically significant (p 0.036) in former smokers compared to both never smokers and current smokers.

Conclusion: Nasal mucociliary function is reduced in smokers and this reduction is permanent as cessation of smoking does not improve the mucociliary function.

Lay Summary: The study is focused to find out whether harmful effects seen in the lower and distal airways are also mirrored in the nasal epithelium as one airway and one disease in current smokers, never smokers and former smokers and thus to find out an early predictor of chronic lung disease so that intervention may be initiated to counsel and help the smokers taking part in the study to quit smoking. It was a Cross sectional, observational study from a tertiary care hospital.105 subjects were randomly distributed on the basis of smoking pattern into 3 groups, never smoker, current smoker and former smoker. Nasal mucosal cytology and function were assessed by saccharin transit time test (STT), peak nasal inspiratory flow (PNIF) and nasal ciliated cells & goblet cell ratio (CC/GC). Lower airway was assessed by spirometry.The increase in saccharin transit time is statistically significant (p <.001) in current smoker and former smoker compared to never smoker which implied that nasal mucociliary function is reduced in smokers. The lower mean goblet cell count of the former smoker group was statistically significant when compared to the never smoker and current smoker groups. (p.023) while the changes in ciliated cell and goblet cell ratio remained statistically insignificant. The decrease in FEV1/FVC is statistically significant (p 0.036) in former smokers compared to both never smokers and current smoker which lead us to the conclusion that this reduction is permanent and cessation of smoking does not improve the mucociliary function.

Keywords: Saccharin transit time; peak nasal inspiratory flow; nasal cytology; ciliated cell: goblet cell ratio; pulmonary function test; spirometry; current smokers; never smokers; former smokers.

ABBREVIATIONS

CC : Ciliated Cells;
COPD : Chronic Obstructive Pulmonary Disease;
CS : Current smokers;
FEV1 : Forced Expiratory Volume at 1st second;
FS : Former smokers;
FVC : Forced Vital Capacity;
GC : Goblet Cells;
NS : Never smokers;
PFT : Pulmonary Function Test;
PNIF : Peak Nasal Inflow meter;
Spo2 : Oxygen Saturation (%);
STT : Saccharin Transit Time Test.

1. INTRODUCTION

"Smoking is a major risk factor for the development of chronic lung diseases worldwide leading to significant morbidity and mortality. Cigarette smoke contains a number of toxicologically significant chemicals including

polycyclic aromatic hydrocarbons (Benzopyrene), tobacco-specific nitrosamines, aldehydes, carbon monoxide, hydrogen cyanide, nitrogen oxides, benzene, toluene, phenols, aromatic amines and harmala alkaloids" [1]. "A person's increased risk of disease is directly proportional to the length of time that a person continues to smoke as well as the amount smoked [1]. Pulmonary Function Tests (PFT) and various diagnostic techniques including the assessment of gas transfer and High-Resolution Computed Tomography (HRCT) assess the lung function and structural damage to it" [2,3].

"Although valuable, these methods do not allow for the identification of subjects at risk of developing lower airway disease early or those who have subclinical disease, when intervention might still be effective. The development of biomarkers for disease onset and early progression is hindered by the accessibility of the primary tissue in the lungs, so there is a need to evaluate alternative sites for surrogate biomarkers. The appreciation of the nasal epithelium as a surrogate for the lower airways has grown in recent years' [4]. "Not only is it the passage through which airborne toxicants travel to the lower airways, but it also mimics the bronchus with respect to cellular composition i.e., pseudostratified columnar ciliated epithelium. The harmful effects seen in the lower and distal airways are also likely to be mirrored in the nasal epithelium as one airway and one disease" [5,6].

2. REVIEW OF LITERATURE

The close relationship between upper respiratory tract (nose and paranasal sinuses) and the lower respiratory tract (tracheobronchial tree) are mentioned in the literature [7].

2.1 Upper Respiratory Tract

"The nasal vestibule is the anterior most aspect of the nasal cavity and serves as the entry point from the external nares into the nasal cavity" [8].

"The nasal cavity extends from the external nares to the posterior choanae, where it becomes continuous with the nasopharynx. The nasal cavity is divided into two passage ways by the nasal septum" [8].

"There are three different types of epithelium within the nasal cavity - squamous (nasal vestibule), olfactory (superior septum, superior turbinate and upper aspect of the middle turbinate) and respiratory (remainder of nasal cavity) epithelium" [8]. The CC is the most differentiated and represented cell of nasal mucosa [9]. "The normal ratio between CCs and GCs in nasal mucosa is 5:1, and it increases proceeding to the distal portion of the lower airways, where it can reach a ratio of 100:1 to 200:1.From a cytological point of view, irritants for nasal mucosa affect CCs and GCs, determining a rearrangement of the epithelium in favour of GCs (mucous-secreting metaplasia). This process has important pathophysiologic and clinical consequences: the increase of the number of GCs causes a mucous hyperproduction, whereas the decrease of CCs leads to a reduced efficiency of the mucociliary transport. These events favour stasis of mucous secretions in the nose, determining a major risk of inflammatory diseases. Considering that the turnover of a CC takes about 3 weeks, frequent inflammations do not allow the reestablishment of a normal ratio among the different cellular subsets" [10].

"At body temperature, cilia beat frequency ranges from 7 to 16 Hz. The frequency usually remains constant when the temperatures are between 32 and 40°C. The cilia can beat rapidly in a propulsive stroke or slowly during the recovery phase. Metachronous movement of cilia propels the mucus blanket backwards, thus only those (cilia) that are at right angle to the direction of the flow form the phase. Cilia that are in the flow-direction are out of phase until the cycle completes. From the front of the nose, mucus flows posteriorly" [11].

The parasympathetic nerve regulates nasal secretions, sympathetic nerve regulates vascular tone and turbinate congestion and the trigeminal nerve controls nasal cavity sensation [8].

The normal physiological phenomenon in which the nasal turbinates dilates and constricts every 0.5–3 hours is known as the 'nasal cycle' [8].

"The paranasal sinuses are paired structures lined by ciliated pseudostratified columnar respiratory epithelium identical to that in the lower airway. The cilia beat in a coordinated fashion to carry the mucous blanket which traps particles from the sinus into the nose through a series of well-defined pathways. The anterior functional unit of paranasal sinuses is comprised of the maxillary, anterior ethmoid and frontal sinuses that drain into the nose through the osteomeatal complex in the middle meatus. The

posterior functional unit is comprised of the posterior ethmoid sinus and drains into the nose through the superior meatus. The sphenoid functional unit is comprised of the sphenoid sinus and drains through the sphenoethmoid recess located medial and posterior to the superior turbinate" [8].

2.2 Lower Respiratory Tract

"The Trachea: It is the pathway for ventilation and clearance of bronchial secretions. It has D-shaped cross section with incomplete C-shaped cartilaginous rings. The lining of the lower airway is pseudo-stratified ciliated columnar epithelium with numerous goblet cells, resting on a broad basement membrane. The cilia beats the mucus blanket upwards towards the larynx and eventually the pharynx, where it is swallowed. The epithelium becomes thinner with increasing branching of the segmental bronchi. Eventually the epithelium becomes a single layer. Contraction of circular muscles form an interlacing network of fibres that shortens and constricts the segmental bronchi" [12].

Carina and Bronchi: Trachea bifurcates and narrows slightly with origin of Right and Left main bronchus which in turn branches further [12].

Most studies concerning smoking were directed to its effects on the lower respiratory tract. But the effects of smoking on nasal respiratory mucosa and its correlate with lower airway function have not been widely studied.

Spirometry is a frequently performed lung function test and an important tool in medical surveillance examinations of pulmonary diseases[1].

Nicola ML et al. [5], concluded that "young adult smokers have functional and inflammatory changes in the nasal and lower airways which correlate with smoking history. The asymptomatic smokers in the study showed no changes in pulmonary function, probably because spirometry is unlikely to detect early physiologic changes in the airways" [13].

Pagliuca G et al. [6] found that "tobacco smoke is a significant risk factor for respiratory diseases. They analyzed the cytological and functional features of nasal mucosa in smokers, non-smokers, and ex-smokers to evaluate if nasal alterations in smokers are permanent or reversible conditions after smoking cessation. Ninety healthy volunteers recruited from the staff of Alfredo Fiorini Hospital, Sapienza University of Rome was divided into 3 groups (smokers, non-smokers, ex-smokers) composed of 30 subjects each. Cytologic features of nasal mucosa and effectiveness of nasal mucociliary clearance were studied, focusing on 4 parameters: (1) nasal mucociliary clearance, assessed by saccharin nasal transit time; (2) ratio between the number of ciliated cells and goblet cells, analyzed by microscopic observation of cytologic specimens of nasal mucosa that had undergone May Grunwald Giemsa staining; (3) evaluation of ciliary motility; and (4) time of ciliary movement of ciliated cells analyzed by phase-contrast microscopy. All parameters were significantly reduced in the smokers compared to the non-smokers. But there were no statistically significant differences between the non-smoker and ex-smoker groups. The ratios between ciliated cells(CCs) and goblet cells(GCs) were 0.745 in smokers, 0.825 in ex-smokers, and 0.83 in non-smokers. Thus reduction of number of CCs compared to GCs[9]. Similar results were obtained through STT(Saccharin Transit Time) among the 3 groups (mean time: smokers, 15.6 minutes; non-smokers, 11.71 minutes; ex-smokers, 11.77 minutes) [10]. There was significant prolongation in STT (35%-120%) in long-term smokers and faster nasal ciliary beat frequency and transport in occasional smokers" [14].

"Smoking causes a reduction in the number of cilia and change in mucous viscosity. Studies have shown that eight hours after exposure to tobacco smoke the efficiency of mucociliary clearance had reduced, with heavier smokers having more marked impact" [11].

Xavier RF et al. [1] found that "smoking impairs mucociliary clearance and increases respiratory infection frequency and severity in subjects with and without smoking-related chronic lung diseases. This study evaluated the effects of smoking intensity on mucociliary clearance in active smokers. Seventy-five active smokers were grouped into light (1-10 cigarettes/day; n = 14), moderate (11-20 cigarettes/day; n = 34) and heavy smokers (≥21 cigarettes/day; n = 27) before starting a smoking cessation programme. Smoking behaviour, nicotine dependence, pulmonary function, carbon monoxide in exhaled air (exCO), carboxyhaemoglobin (COHb) and mucociliary clearance measured by the saccharin transit time (STT) test were all evaluated. An age-matched non-smoker group (n

= 24) was assessed using the same tests. Moderate (49 ± 7 years) and heavy smokers (46 ± 8 years) had higher STT (p = 0.0001), exCO (p < 0.0001) and COHb (p < 0.0001) levels compared with light smokers (51 ± 15 years) and non-smokers (50 ± 11 years). A positive correlation was observed between STT and exCO (r = 0.4; p < 0.0001), STT and cigarettes/day (r = 0.3, p = 0.02) and exCO and cigarettes/day (r = 0.3, p < 0.01). Smoking impairs mucociliary clearance and is associated with cigarette smoking intensity" [15].

Juliana T Ito et al., [16] a study on "nasal mucociliary clearance in subjects with COPD after Smoking Cessation showed the STT of smokers with COPD (16.5 [11–28] min, median [interquartile range 25–75%]), and current smokers (15.9 [10–27] min) was longer compared with ex-smokers with COPD (10.2 [6–12] min) and non-smokers (8 [6–16] min) (P .001). There was no difference in STT values between smokers with COPD and current smokers, and these values in ex-smokers with COPD were similar to those for the control group. This study demonstrated that smoking cessation, even in people with COPD, leads to an improvement in mucociliary clearance within 1 year of smoking cessation[16]. MCC impairment in patients with COPD leads to secretion retention, airway obstruction, and recurrent airway infections, mainly in smokers" [17,18].

Samy Elwany et al. did "a study on quitting smoking reverses nasal mucosal changes in which the mean duration of quitting smoking was 30.75 months (± 8.26). Examination of the electron microscopic sections before quitting smoking showed variable degrees of loss of cilia and columnar cells, oedema between the epithelial cells, few goblet cells, hyperplasia of seromucinous acini, and vascular congestion. The pathologic changes correlated positively with the smoking index of the participant. On the other hand, the sections after quitting smoking showed variable degrees of regeneration of the ciliated cells and decreased vascular congestion. Numerous goblet cells and seromucinous acini were seen. Less pathologic changes were observed with longer durations of cessation of smoking. The study showed an association between smoking and the nasal mucosa. Smoking has several injurious effects on the nasal mucosa. However, the nasal mucosa has excellent regeneration potentials and quitting smoking for sufficient periods of time may reverse these deleterious changes. Considering the established link between smoking and chronic rhinosinusitis, quitting smoking may help smokers to overcome their recalcitrant disease" [19].

PNIF is reported to be the best validated technique for evaluation of nasal flow through nose[7]. Measurement of PNIF may be useful for the assessment of large changes in nasal conductance such as those associated with nasal challenge and nasal decongestion [20] and for this type of work the measurement of PNIF compares well with rhinomanometry for the assessment of nasal patency.

"A study on Nasal peak inspiratory flow and clinical score in children and adolescents with Allergic Rhinitis. In this study, PNIF is affected by lower airway function and has been reported to positively correlate with peak expiratory flow(PEF) in healthy children and adults" [21].

Thomas Kjaergaard et al., observed in "a study on smoker's nose: structural and functional characteristics have clearly demonstrated that smokers exhibit lower minimal cross-sectional areas and nasal cavity volumes, achieve lower PNIF-values, and have a less compliant nasal mucosa than non smokers" [22].

Valin Rujanavej et al., concluded in "a study on validity of PNIF as a screening tool for nasal obstruction revealed good sensitivity and high negative predictive value but it had low specificity and positive predictive value. The nasal peak flow did not agree well with the subject's symptoms of blockage and sinonasal diseases" [23].

Sriram Sridhar et.al., observed in his study on "smoking induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium, it highlights the relationships between gene expression profiles in epithelial cells that line the intra and extra thoracic airway and identifies a common set of genes that are induced by tobacco smoke in buccal ,nasal and bronchial epithelium, supporting the concept that smoking induces a common field of injury throughout the airway. These similarities suggest that easily collected buccal and nasal epithelium can be used to measure an individual's physiologic response to tobacco smoke" [24].

Petitti and Friedman et.al. examined the "association between smoking cigarettes with low yield of tar and nicotine and respiratory diseases

by reviewing the medical records of 4610 current smokers and 2035 never smokers. They found that smoking low yield tar cigarettes was not associated with a lower risk for chronic obstructive pulmonary disease" [25].

Nawafleh HA et al. [3] found that "pulmonary function testing is a routine procedure for the assessment and monitoring of respiratory diseases. To estimate the values of peak expiratory flow rate (PEFR), forced expiratory volume in first second (FEV1), forced vital capacity (FVC) and ratio between FEV1/FVC among smoking and non-smoking students, staff and workers at Al-Zarqa Private University and to study the effect of age, gender and body mass index (BMI) on these variables. A cross-sectional research design was used. The study was conducted at Al-Zarqa Private University, Jordan. Two hundred and thirteen healthy smokers and non-smokers were approached through probability sampling among the students, staff and workers of Al-Zarqa Private University were screened through a questionnaire and spirometric test. Data from 213 subjects was used for analysis. Subjects were excluded if pregnant, or with cardiopulmonary disease body, mass index (BMI) not ranging from 17-25, FEV1/FVC% less than 70 or with no reproducible results. Mean FVC, FEV1 FEV1/FVC% and PEFR were found to be lower in smokers than the non-smokers, there were significant differences between mean spirometric values smoking and non-smoking in age 20-30 years and 30-39 and 40-49. The mean FVC, FEV1 and PEFR were lower in smoker. In order to generalize these reference values, a larger study following the ATS criteria is needed. Health education campaign needed to keep community aware of the risk of smoking" [26].

Kumar R et.al. 4(2017) from katihar , Bihar found that in "rural non-smokers, the observed value of pulmonary functions in mean ±standard deviation, FVC was 3.28±1.04 litres, FEV1 was 2.72±0.97 litres, FEV1% was 85.24±28.24, PEFR was 7.8±1.98 litres/minute, FEF25-75% was 4.28±0.99 litres. The observed value of pulmonary functions in rural smoker population in mean± standard deviation, FVC was 2.56±0.86 litres, FEV1 was 2.21±0.96 litres, FEV1% was 86.00±23.73, PEFR was 5.65±2.18 litres/minute, FEF 25-75% was 3.34±1.37 litres. : This study showed significant decreased value (p value < 0.05) in smokers of rural population. This study was done for a better understanding of effects of smoking in the rural population of Katihar" [27].

Yunus Çolak et al. studied the "importance of early COPD in young adults for development of clinical COPD. Findings from Copenhagen General Population for the Presence of Early COPD in smokers with ≥10 pack-years at baseline examination before age 50 yielded a sensitivity of 24%, a specificity of 96%, a positive predictive value of 21%, and a negative predictive value of 97% for predicting Clinical COPD at final examination 10 years later. In Early COPD sensitivity only dropped slightly from 24% to 18% without any noteworthy change in specificity, or in positive- or negative predictive values. Sensitivity dropped to 13% when never-smokers were also included without any large change in the other values. Results were similar when Clinical COPD was defined using lower limit of normal. A combination of baseline lung function and smoking exposure yielded a higher predictive capability for subsequent Clinical COPD development than lung function and smoking exposure separately" [28].

Elizabeth C Oelsner et.al. found in her study that out of 25 352 participants (ages 17–93 years) completed 70 228 valid spirometry exams. Over a median follow-up of 7 years (IQR 3–20), FEV1 decline at the median age (57 years) was 31·01 mL per year (95% CI 30·66–31·37) in sustained never-smokers, 34·97 mL per year (34·36–35·57) in former smokers, and 39·92 mL per year (38·92–40·92) in current smokers. With adjustment, former smokers showed an accelerated FEV1 decline of 1·82 mL per year (95% CI 1·24–2·40) compared to never-smokers, which was approximately 20% of the effect estimate for current smokers (9·21 mL per year; 95% CI 8·35–10·08). Compared to never-smokers, accelerated FEV1 decline was observed in former smokers for decades after smoking cessation and in current smokers with low cumulative cigarette consumption (<10 pack-years). With respect to current cigarette consumption, the effect estimate for FEV1 decline in current smokers consuming less than five cigarettes per day (7·65 mL per year; 95% CI 6·21–9·09) was 68% of that in current smokers consuming 30 or more cigarettes per day (11·24 mL per year; 9·86–12·62), and around five times greater than in former smokers (1·57 mL per year; 1·00–2·14). Among participants without prevalent lung disease, associations were attenuated but were consistent with the main results. In a large, US population-based sample, former smokers and low-intensity current smokers had accelerated lung function decline compared with never-smokers. Accelerated

decline in lung function persisted for decades after smoking cessation; was present in smokers with fewer than 10 pack-years; and was evident in current smokers reporting less than five cigarettes per day. These findings persisted in adults without prevalent lung disease. Our results therefore reinforce the view that there is no safe level of tobacco smoke exposure and that smoking cessation is the most effective means of harm reduction. Accelerated decline in lung function in former smokers is consistent with sustained pathophysiological abnormalities of the lung after smoking cessation [29].

Willemse BW et al. [9] found that "smoking is the main risk factor in the development of chronic obstructive pulmonary disease (COPD), and smoking cessation is the only effective treatment for avoiding or reducing the progression of this disease. Despite the fact that smoking cessation is a very important health issue, information about the underlying mechanisms of the effects of smoking cessation on the lungs is surprisingly scarce. It is likely that the reversibility of smoke-induced changes differs between smokers without chronic symptoms, smokers with nonobstructive chronic bronchitis and smokers with COPD. This review describes how these three groups differ regarding the effects of smoking cessation on respiratory symptoms, lung function (forced expiratory volume in one second), airway hyperresponsiveness, and pathological and inflammatory changes in the lung. Smoking cessation clearly improves respiratory symptoms and bronchial hyperresponsiveness, and prevents excessive decline in lung function in all three groups. Data from well-designed studies are lacking regarding the effects on inflammation and remodelling, and the few available studies show contradictory results. In chronic obstructive pulmonary disease, a few histopathological studies suggest that airway inflammation persists in ex-smokers. Nevertheless, many studies have shown that smoking cessation improves the accelerated decline in forced expiratory volume in one second, which strongly indicates that important inflammatory and/or remodelling processes are positively affected" [30].

Hurst JR et al. [21] found that this review presents "the evidence that chronic obstructive pulmonary disease (COPD) is associated with significant sinonasal symptoms, inflammation and airway obstruction. Upper airway symptoms in COPD cause impairment to quality of life. The severity of upper airway involvement relates to that present in the lower airway, suggesting that the nose may be used to model the lung in COPD. More importantly, relationships between upper and lower airway bacteria and inflammation, and the association between sinusitis and treatment failure at exacerbation raise the possibility that nasal intervention in COPD may not only improve health status but may also affect important clinical outcomes such as exacerbation frequency" [31].

Huvenne W et al. [26] found that "cigarette smoke (CS) is known to initiate a cascade of mediator release and accumulation of immune and inflammatory cells in the lower airways. We investigated and compared the effects of CS on upper and lower airways, in a mouse model of subacute and chronic CS exposure. C57BL/6 mice were whole-body exposed to mainstream CS or air, for 2, 4 and 24 weeks. Bronchoalveolar lavage fluid (BAL) was obtained and tissue cryosections from nasal turbinates were stained for neutrophils and T cells. Furthermore, we evaluated GCP-2, KC, MCP-1, MIP-3α, RORc, IL-17, FoxP3, and TGF-β1 in nasal turbinates and lungs by RT-PCR. In both upper and lower airways, subacute CS-exposure induced the expression of GCP-2, MCP-1, MIP-3α and resulted in a neutrophilic influx. However, after chronic CS-exposure, there was a significant downregulation of inflammation in the upper airways, while on the contrary, lower airway inflammation remained present. Whereas nasal FoxP3 mRNA levels already increased after 2 weeks, lung FoxP3 mRNA increased only after 4 weeks, suggesting that mechanisms to suppress inflammation occur earlier and are more efficient in nose than in lungs. Altogether, these data demonstrate that CS induced inflammation may be differently regulated in the upper versus lower airways in mice. Furthermore, these data may help to identify new therapeutic targets in this disease model|" [32].

Never-Smoker defined as a subject who has never smoked or who has smoked less than 100 cigarettes in his life time [33].

Current Smoker defined as a subject who had smoked X 100 cigarettes in his life time and who currently smoked at least one cigarette per day [33].

Former Smoker defined as a subject who smoked at least 100 cigarettes in his life time but who had quit smoking at the time of interview [33].

2.3 Objectives of the Study

1. Correlation between nasal mucosal cytology, mucociliary function, nasal airflow and lung function among the current smokers, never smokers, and former smokers.
2. To study the correlation between upper airway and lower airway function among current smokers, never-smokers and former smokers**.**

3. MATERIALS AND METHODS

Study Design: Cross sectional, Observational study.

Study Type: Observational study.

Target Population: Patients attending Pulmonary and ENT OPD satisfying the inclusion & exclusion criteria during the period of study.

Inclusion Criteria:

1. All participants will be over 19 years of age.
2. All participants will be free from any apparent nasal or paranasal sinuses disease.
3. Current Smoker participants
4. Never smoker participants
5. Former Smoker participants

Exclusion Criteria:

a) Nasal and paranasal sinuses disease
b) Nasal and paranasal sinuses trauma
c) History of Nasal and paranasal sinuses surgery
d) Exposure to occupation pollution
e) Subjects having high SNOT20 score (≥ 10)
f) Subjects on medications for nasal disease.

Study Area: OPD Of Otorhinolaryngology and Pulmonary medicine, COM & JNM hospital, Kalyani.

Study Duration: December 2019 to July 2021.

Sample Size: All persons attending OPD/IPD of Dept. Of Otorhinolaryngology and pulmonary medicine, COM & JNM Hospital, between a time period of January 2020 to July 2020 and fulfil the inclusion and exclusion criteria and also give proper consent to study.

Sampling (Recruitment of the participants): Sampling method will apply by recruiting all persons attending OPD/IPD of Dept. Of Otorhinolaryngology and Pulmonary medicine, COM &JNM Hospital staffs between a time period of December 2019 to July 2021 and fulfil the inclusion and exclusion criteria and also give proper consent to study.

Tools/Description of Procedure: All participants in this study will be subjected to the following after signing the informed consent:

1. History taking to satisfy inclusion and exclusion criteria
2. Examinations: Anterior rhinoscopy and nasal endoscopy to exclude local pathology such as rhinosinusitis and nasal polyposis.
3. Pulmonary Function: Spirometry will be performed according to the guidelines of the American Thoracic Society using a portable spirometer (RMS, Helios 702). Reference values are those specific for the Brazilian population [34].

Fig. 1. Portable spirometer (RMS, Helios 702)

4. PNIF will be measured with an In-Check inspiratory flow meter manufactured by Clement-Clark. Each subject will receive appropriate instructions and will take five measurements under supervision of a doctor. The highest of the five recorded measurements (PNIF MAX) was included in the analysis [35].
5. Nasal mucociliary clearance evaluation: Nasal mucociliary clearance will be measured by Saccharin test described by Andersen. Before performing the test, the nose will be examined endoscopically to remove any scabs or dried mucous. Two saccharin particles (each 1mm in size) are to be gently applied under endoscopic guidance one cm behind the anterior edge of inferior turbinate. Participants will be instructed to remain seated and to swallow every 30 seconds. They were also instructed to breathe normally and not to cough, sniff or blow their nose. The time from saccharin placement until the participant reports the sensation of sweetness is recorded with stopwatch. The test is supposed to be terminated if nothing had been tasted within 60 minutes [36].
6. Measurement of ratio between ciliated cells(CCs) and goblet cells(GCs) by nasal cytology. Each subject will be asked to blow his or her nose to get rid of any excess secretions before performing collection of nasal mucosa surface cells. Under direct visual control, in anterior rhinoscopy, specimens of ciliated epithelium are collected by scraping the nasal mucosa in the middle third of the inferior turbinate with a sterile nasal cytology curette. Samples will be carried out in all subjects on a different day than that of the SNTT. Samples will be uniformly smeared in the middle of a slide, fixed by air-drying, and stained by May Grunwald Giemsa quick stain. At the end of this procedure, the slides are washed in tap water, air-dried, and mounted in a synthetic resin with cover glass to increase its durability. Cytologic analysis is performed by a light microscope with a 3100 objective lens in oil immersion. Fifty microscopic fields will be examined. CCs and GCs were counted, and a measurement of the ratio between them was taken. The obtained data will be plotted on an appropriate evaluating sheet [37].
7. SNOT 20 score: It is a questionnaire for the measure of outcome in patients with sinonasal disorders.20 domains are given and the estimated completion time is 10 minutes.

Intervention (if any): not applicable.

Comparison (if any):

Group A: Never Smokers (NS)(Subject who never smoked or who has smoked less than 100 cigarettes in his life time) [33].
Group B: Current Smokers (CS)(Subject who had smoked X 100 cigarettes and who currently smoke at least one cigarette per day) [33].
Group C: Former Smokers (FS) (subject who smoked at least 100 cigarettes in his life time but who had quit smoking at the time of interview) [33].

Data Collection: It will be assessed by comparing the readings of PFT with PNIF, Saccharin Transit Time and Nasal cytology. Thus correlating both upper airway and lower airway functions in Current Smokers, Never smokers and Former smokers.

Fig. 2. In-Check inspiratory flow meter

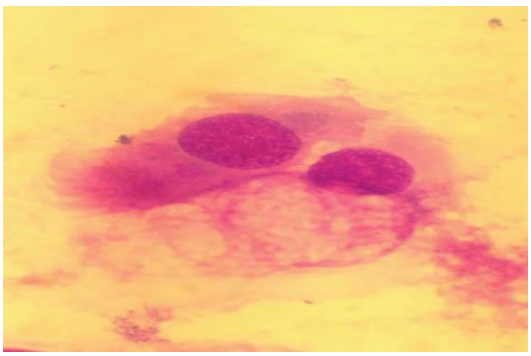
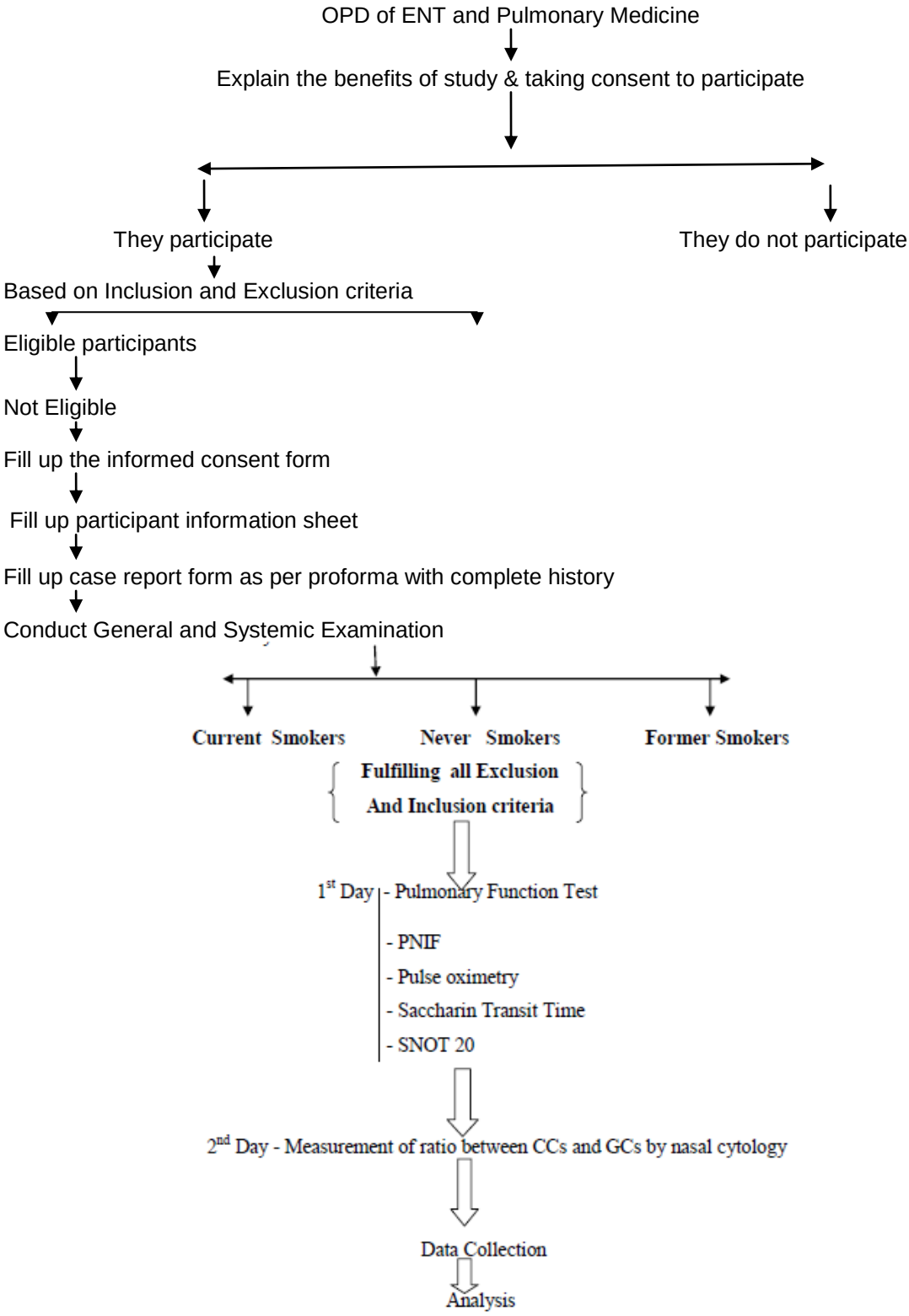


Fig. 3. Cytologic analysis

Methods (Preferably in a Flow Diagram):



After obtaining approval from the ethics committee of our institute and written consent from 105 subjects participating in the study, details and the involved procedures were explained to them and were randomly distributed on the basis of smoking pattern satisfying the

exclusion and inclusion criteria into never smoker, current smoker and former smoker groups of 35 each respectively. On first day of visit STT, PNIF, PFT was performed following which on the second visit, nasal smear was taken and stained for ciliated and goblet cell ratio as per the techniques described above. Data collection done and was subjected to analysis.

Plan of Data Analysis and Statistics: Statistical analysis will be done by appropriate statistical methods proposed for the study will be applied (SPSS Version 22).

Novelty of the Study: The study is focused to find out whether harmful effects seen in the lower and distal airways are also mirrored in the Nasal epithelium as One airway and One disease in Current Smokers, Never Smokers and Former Smokers and thus to find out an early predictor of Chronic lung disease so that intervention might be still possible.To Counsel and help the Smokers taking part in the study to quit smoking.

4. OBSERVATION AND RESULTS

The age of our subjects ranged from 20 to 52 years, 21 to 60 years and 23 to 62 years among never smokers (NS) group, current smokers (CS) and former smokers (FS) respectively (Table 1).

The mean age of the NS group, CS group and FS groups were 30.82(± 9.01) years ,31.69(± 10.69) years and 40.34(± 13.22)years respectively (Table 2). Mean age of NS and CS groups were in the same range while FS group had a slightly higher mean. However the mean age in 3 groups are within comparable limits.

88 males and 17 females participated in the study (Table 3).

In our study 56.2% of the population belonged to urban areas while 43.8% were of rural origin (Table 4).

The NS group had a mean STT of 921.48(± 46.96) seconds. The STT in CS and FS groups were comparatively elevated at 1338.11(± 67.82) seconds and 1322.50 (± 60.56) seconds respectively. This increase is statistically significant (p <.001) (Table 5). There isn't any statistically significant difference in STT between CS and FS groups. This indicates a strong correlation between smoking and elevated STT. Thus, there is statistically significant decrease in mucociliary function in CS/FS group compared to NS group manifested by an increased STT.

Table 1. Maximum and minimum age in different groups

Age	Never Smoker	Current Smoker	Former Smoker
Maximum Age	52	60	62
Minimum Age	20	21	23

Table 2. Mean age of different groups

Never Smoker	Current Smoker	Former Smoker
30.82 ± 9.01	31.69 ± 10.69	40.34 ± 13.22

Table 3. Gender distribution

Gender	Males	Females	Total
Frequency(Percentage)	88(83.81)	17(16.19)	105(100)

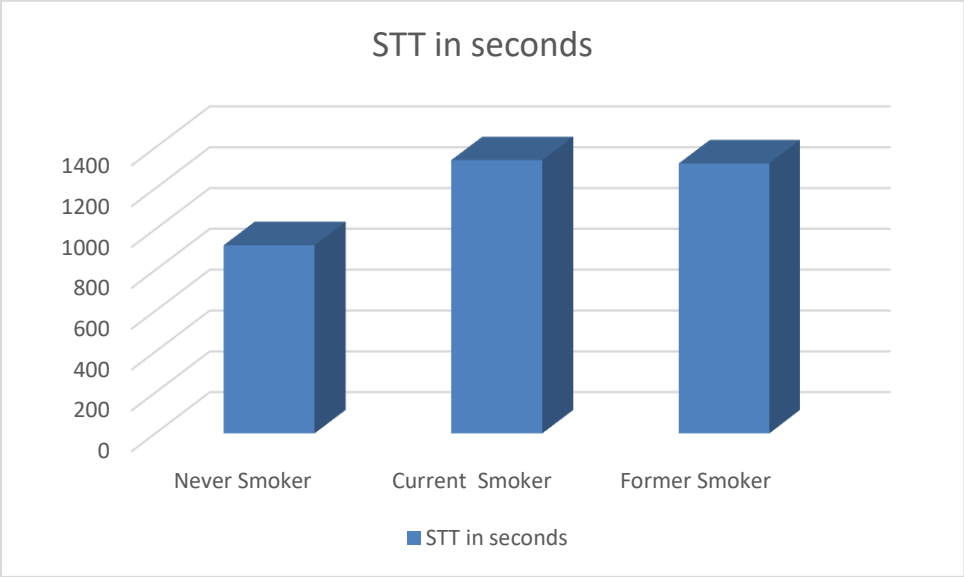
Table 4. Area wise distribution of study population

Area	Urban	Rural	Total
Frequency(Percentage)	59(56.2)	46(43.8)	105(100)

Table 5. Comparison of STT in seconds (Mean± Standard Error) between three groups

Never Smoker	Current Smoker	Former Smoker	P-value
921.48 ±46..96[b]	1338.11± 67.82[a]	1322.50 ±60.56[a]	<.001**

Different superscripts (a,b,c) differ significantly according to Tukey's HSD test.

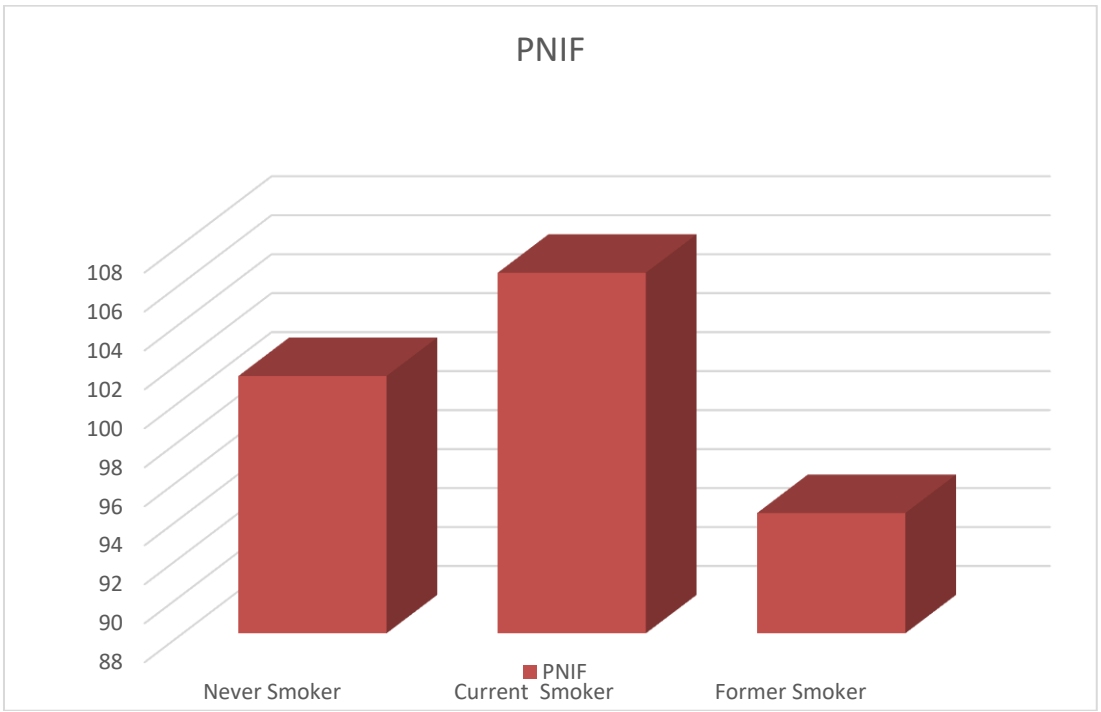


Graph 1. Comparison of STT in seconds

The mean PNIF value of NS group in our study was 101.2 L/min (± 4.07).In CS, it was higher at 106.05 L/min (± 4.11) but lower in FS group where it was 94.17 L/min (± 2.91) (Table 6). However, these differences were, not statistically significant. (p 0.083) indicating no significant nasal obstructive features in any of the groups.

Table 6. Comparison of PNIF in (Mean± Standard Error) between three groups

Never Smoker	Current Smoker	Former Smoker	P-value
101.2±4.07	106.05±4.11	94.17±2.91	.083



Graph 2. Comparison of PNIF between three groups

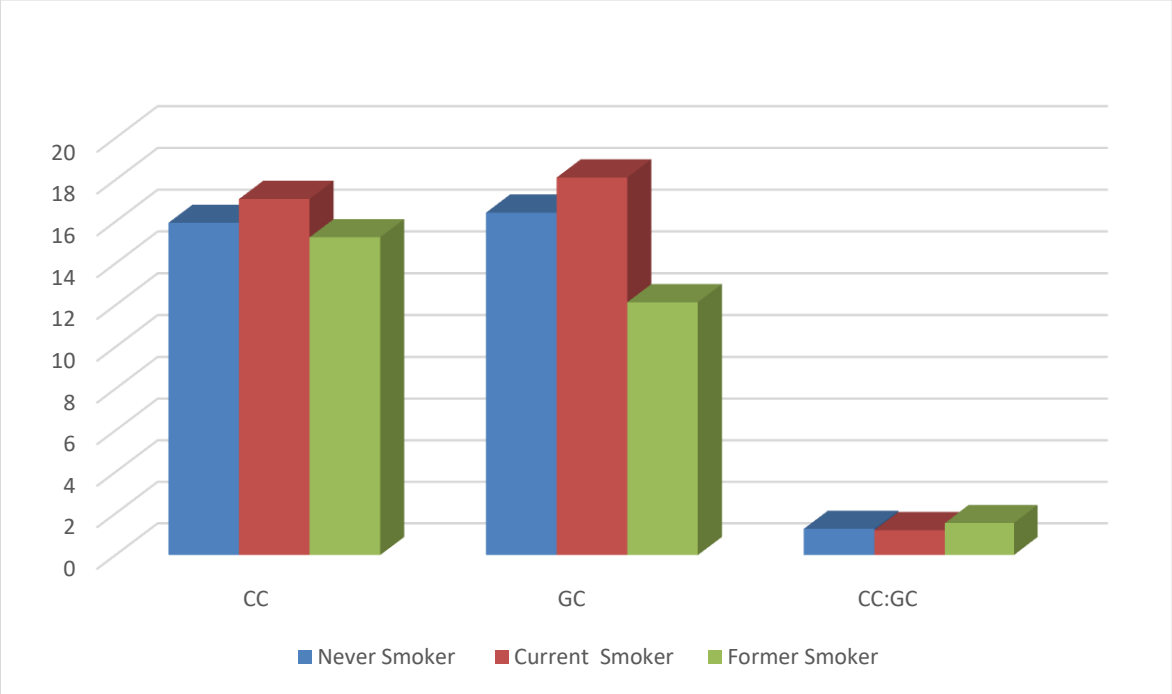
The mean ciliated cells/goblet cells (CC:GC) ratio in the NS group was 1.26 (± 0.17), while CS group had a mean ratio of 1.20 (± 0.19) and FS group had a mean ratio of 1.54(± 0.17). These differences were however, not statistically significant (p.327).

The mean goblet cell (GC) count of NS group was 16.43 (± 1.42) while in the CS group the mean GC count was 18.11(± 2.29). Interestingly, in FS group the mean GC count was lowest at 12.14 (± 1.11). This lower mean GC count of the FS group was statistically significant when compared to the NS and CS groups. (p.023) (Table 7) i.e. on an average the FS group had fewer GC per unit area compared to NS and CS groups. However, CC/GC ratio showed no statistically significant variations between the 3 groups (p 0.327) (Table 7).

Table 7. Comparison of CC: GC (Mean± Standard Error) between three groups

Group / Parameter	Never Smoker	Current Smoker	Former Smoker	P-value
CC	15.94±1.48	17.08±2.14	15.25±1.36	.703
GC	16.43^{ab} ±1.42	18.11^{a}±2.29	12.14^{b} ±1.11	.023*
CC:GC	1.26±.17	1.20±.19	1.54±.17	.327

Different superscripts (a,b,c) differ significantly according to Tukey's HSD test.



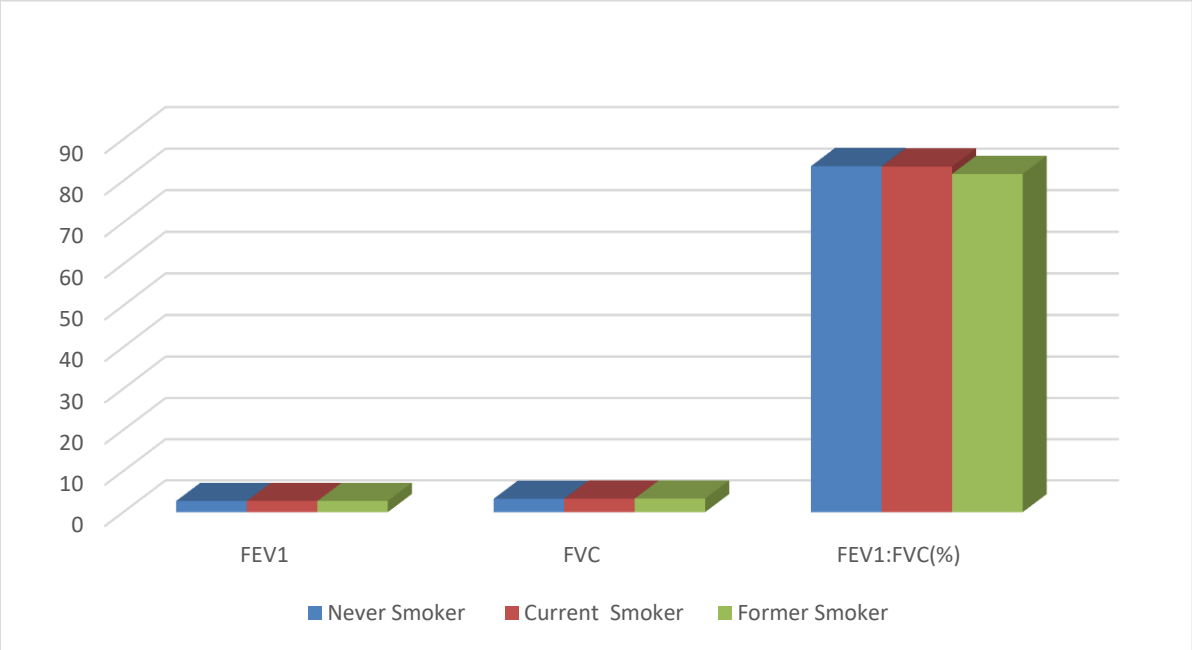
Graph 3. Comparison of CC:GC (Mean± Standard Error) between three groups

Table 8. Comparison of FEV1, FVC and FEV1:FVC (Mean± Standard Error) between three groups

Group / Parameter	Never Smoker	Current Smoker	Former Smoker	P-value
FEV1	2.71±.09	2.72±.07	2.70±.08	.988
FVC	3.23±.10	3.25±.08	3.29±.08	.854
FEV1:FVC(%)	83.50±.51	83.40±.54	81.62±.64	.036*

Forced Expiratory Volume at 1st second/ Forced Vital Capacity (FEV1/FVC) findings comparing the NS,CS and FS groups are 83.50% (± 0.51), 83.40%(± 0.54) and 81.62% (± 0.64) (Table 8) .There is statistically significant decrease in FEV1/FVC values of FS group compared to NS and CS group(p .036).

In our study, there wasn't any statistically significant difference in FEV1/FVC between NS and CS groups. However, the decrease in FEV1/FVC is statistically significant (p.036) in FS group compared to both NS and CS groups. Thus , there is a statistically significant decrease in FEV1/FVC in FS group compared to NS and CS groups which can mean that the FS group quit smoking after developing associated symptoms or complications. Adding to this observation, there is statistically significant reduction in goblet cell count in FS group compared to NS and CS groups which indicated that there is a change at cellular level in former smokers who quit after reaching a certain point.



Graph 4. Comparison of FEV1, FVC and FEV1:FVC (Mean± Standard Error) between three groups

Table 9. Comparison of Mean ± Standard Error of all parameters in rural and urban population among never smoker group

Area / Parameter	Rural	Urban	P-value
STT	907.13±80.07	933.57± 54.56	.781
PNIF	97.86± 5.56	104.25 ±5.27	.436
CC	18.62± 2.10	13.68± 1.92	.057
GC	17.06 ±2.40	15.89 ±1.76	.692
CC:GC	1.48 ±.28	1.07± .16	.193
FEV1	3.05± .14	2.85± .12	.098
FVC	2.98 ±.14	3.37 ±.11	.102
FEV1:FVC(%)	82.57 ±.92	84.67± .49	.201
SPO_2	98.57 ±.30	97.89 ±.15	.07

Table 10. Comparison of Mean ± Standard Error of all parameters in rural and urban population among current smoker group

Area / Parameter	Rural	Urban	P-value
STT	1384.25± 152.34	1324.78 ±65.33	.685
PNIF	112.25 ±8.05	104.22 ±5.27	.421
CC	15.37± 3.76	17.59 ±2.25	.634
GC	17.25± 2.45	18.37 ±2.55	.821
CC:GC	.93±.21	1.29±.22	.395
FEV1	2.57±.17	2.76 ±.08	.310
FVC	3.14±.16	3.28 ±.08	.446
FEV1:FVC(%)	81.61± 1.36	83.93 ±.56	.074
SPO_2	98.51± .47	98.37± .18	.731

Table 11. Comparison of Mean ± Standard Error of all parameters in rural and urban population among former smoker group

Area / Parameter	Rural	Urban	P-value
STT	1376.92 ±96.65	1296.22 ±90.28	.677
PNIF	101.33 ±5.72.	90.40± 3.09	.075
CC	15.58± 3.75	15.07± 1.29	.866
GC	12.08± 1.49	12.09± 1.37	.941
CC:GC	1.45± .31	1.58± .19	.715
FEV1	2.75± .10	2.67 ±.11	.617
FVC	3.36 ±.09	3.26± .09	.513
FEV1:FVC(%)	81.63 ±1.07	81.61± .82	.992
SPO_2	98.79 ±.48	98.5± .55	.116

In our study, the Mean ± Standard Error of all parameters in rural and urban population among NS group, CS group and FS group are not statistically significant. This indicates 3 groups are having no cellular level and structural changes in response to urban pollution and thus ruling it out as a confounding factor.

5. DISCUSSION

The nose plays a crucial role in warming, humidifying and filtering air before it enters the lower airways [7]. Impairment in nasal function can therefore impact the lower airways [7]. The upper and lower airways not only interact via their anatomical connection and common mucosal lining but there may also be neural reflexes and systemic mechanisms [38].

Considering the non invasive nature of nasal cytology, scientific review committee and institutional ethics committee granted the approval for our study.

The STT test was first described in 1974 by Anderson et al. [39]. It is a method for scientific research widely used to assess nasal mucociliary clearance as it is reproducible, simple and non-invasive, besides being low cost [15,40-44].

Studies done by Paglicua G et al. [14] and Xavier RF et al. [15] observed a positive correlation between STT and cigarette smoking i.e., nasal mucociliary transport time is significantly higher in smokers than non smokers. Meanwhile Nicole et al. [13] found a faster STT in young healthy smokers compared to healthy non-smokers. They speculate that young subjects who are early or light smokers may have a protective increase in ciliary beat frequency and transport in response to cigarette smoking. Juliana T et al. [16] studied nasal mucociliary clearance in subjects with COPD. They observed that after cessation of smoking, even in people with full blown COPD, there is an improvement in mucociliary clearance within 1 year of smoking cessation.

Our study showed STT of NS group close to the accepted upper limit of normal value of 10.99 min [45] compared to the CS and FS groups who had raised STT (Table 5). Paglicua G et al. [14] and Xavier RF et al. [15] studies tally with our study while comparing NS and CS.

Contrary to the studies done by Nicole et al. [13] and Juliana T et al. [16] our observations suggests a statistically significant decrease in mucociliary function and an increase in STT among current smokers and former smokers compared to never smokers due to the impaired mucociliary function following exposure to tobacco smoke($p < .001$). It was also noted that there is no improvement in STT with cessation of smoking as the values of former smokers was similar to that of current smokers. It concludes that the mucociliary function impairment is of a more permanent nature with no reversal to normal levels after cessation of smoking (in the FS group). Most of the current smokers had normal FEV1/FVC values.

Comparison of STT values between rural and urban population among the 3 groups was statistically insignificant. This probably indicates that urban pollution (smoke) doesn't have a significant impact on nasal mucociliary function unlike tobacco smoke.

Simple peak flow instruments such as the Wright, mini-Wright, and Youlten flow meters are often used to measure peak nasal inspiratory flow (PNIF) with the use of a face mask [46]. PNIF is reported to be the best validated technique for evaluation of nasal airflow [7]. Normal PNIF values of healthy individuals range from 130L/min to 140L/min [47]. In a study done by Thomas Kjaergaard et al. [22] observed that smokers exhibit lower minimal nasal cross-sectional areas and nasal cavity volumes, achieve lower PNIF-values and have a less compliant nasal mucosa compared to non smokers. Another study concluded that PNIF is affected by lower airway function and has been reported to positively correlate with peak expiratory flow (PEF) in healthy children and adults [21]. In our study, there are no statistically significant variations of PNIF values between the 3 groups (p 0.083) indicating no significant nasal obstructive features in any of the groups which contradicts the two former studies (Table 6). It was also noted that there was no statistically significant difference in PNIF values between rural and urban population among the 3 groups indicating no role for urban pollution and cigarette smoking on PNIF values.

Nasal cytology, being cheap, non-invasive and repeatable, can easily be considered as part of rhino-allergologic diagnostics [37]. A study done by Pagliuca G et al. [14] resulted in ratios between Ciliated Cells (CCs) and Goblet Cells(GCs) to be 0.745 in smokers, 0.825 in ex-smokers, and 0.83 in non-smokers. Thus, reduction of number of CCs compared to GCs [14]. Another study [19] showed variable degrees of regeneration of the ciliated cells and decreased vascular congestion post cessation of smoking. Numerous goblet cells and seromucinous acini were seen. It concluded that quitting smoking may help smokers to overcome their recalcitrant disease. But, we didn't observe any significant variations in the CC:GC ratio between the 3 groups (p value = 0.327) (Table 7). Interestingly there is a statistically significant reduction in Goblet cell count in FS compared to NS and CS.(Table 7). We would have expected an increase in CC/GC ratio in FS following the reduction in goblet cells. However CC/GC ratio is not significantly reduced which leads us to conclude that there was corresponding reduction in ciliated cells too among FS group.

There was no statistically significant difference on comparison of CC/GC Ratio values between rural and urban population among the 3 groups which signifies no cellular level change in response to urban pollution. Although nasal biopsy is a better method for assessing ciliated and goblet cells, in our study we used nasal scraping which is taken from the mid portion of inferior turbinate though inferior for nasal cytology. It is an effective and non-invasive method and introduces a good representative sample for cytology [37]. We also explained the procedure and took consent from our subjects before performing it.

Pulmonary function testing is an important diagnostic tool for assessing lower airway status particularly with regard to diseases such as COPD, asthma, and interstitial lung disease [48].A study done by *Nawafleh* HA et al. found that mean pulmonary function were found to be lower in smokers than the non-smokers, there were significant differences between mean spirometric values of smoking and non-smoking individuals in the age groups of 20-30 years and 30-39 and 40-49 years [26]. A comparative study of pulmonary function done by Kumar R et. al. between rural smokers and rural non-smokers

showed significant decreased value (p value < 0.05) in smokers of rural population [27]. Yunus Çolak et al. concluded that a combination of baseline lung function and smoking exposure yielded a higher predictive capability for subsequent clinical COPD development than lung function and smoking exposure separately [28].In an US based study, smokers had accelerated lung function decline compared with never-smokers and the accelerated decline in lung function persisted for decades after smoking cessation [29].

In our study the mean FEV1/FVC values were in the normal range for NS, CS and FS groups. There was no significant difference in FEV1/FVC findings among NS and CS groups. However, the decrease in FEV1/FVC is statistically significant (p value =0.036) (Table 8) in FS which tallies with the US based study. We do not have any conclusive evidence to explain this. But we can presume that it can be due to the quitting of smoking among FS group after developing significant associated symptoms or complications. Especially when the bulk of the subjects in our study consist of patients attending the pulmonology out patient department. It was also noted that, there is a statistically significant reduction in goblet cell count in FS compared to NS and CS which indicates that there was significant cellular damage. But the average CS seems to have better CC/GC population compared to an average FS. In FS, cell counts reduce due to structural damage after certain point where the patient is compelled to stop smoking. There is further scope of study in this. There was no statistical significance on comparison of FEV1/FVC values between rural and urban population among the 3 groups.

6. SUMMARY

The present study titled **"**Correlation between upper airway and lower airway in current smokers, never smokers and former smokers" is analytical study conducted in Department of Ear, Nose, Throat and Head and Neck Surgery of College of Medicine and JNM Hospital, Kalyani, Nadia on 105 Subjects visiting outpatient department for a period of 6 months that is December 2019 to July 2020

Salient features of our study:

1. The aims and objectives of our study are-
   a) To study the correlation between upper airway and lower airway function among current smokers, never-smokers and former smokers.
   b) Correlation between nasal mucosal cytology & function and lung function among the study groups.

2. After obtaining approval from the ethics committee of our institute and written consent from the subjects, details of the study and the involved procedures were explained to them and were randomly distributed into test and comparison group of 35 each respectively. STT, PNIF,CC/GC Ratio, spO2, PFT was performed in all subjects
3. The sample size was calculated as:

All persons attending OPD/IPD of Dept. Of Otorhinolaryngology and pulmonary medicine, COM & JNM Hospital, between a time period of January 2020 to JULY 2020 and fulfil the inclusion and exclusion criteria and also give proper consent to study.

a) Pulmonary Function: Spirometry will be performed according to the guidelines of the American Thoracic Society using a portable spirometer (RMS, Helios 702). Reference values are those specific for the Brazilian population.
b) PNIF will be measured with an In-Check inspiratory flow meter manufactured by Clement-Clark. Each subject will receive appropriate instructions and will take five measurements under supervision of a doctor. The highest of the five recorded measurements (PNIF MAX) was included in the analysis.
c) Nasal mucociliary clearance evaluation: Nasal mucociliary clearance will be measured by saccharin test described by Andersen. Before performing the test, the nose will be examined endoscopically to remove any scabs or dried mucous. Two saccharin particles (each 1mm in size) are to be gently applied under endoscopic guidance one cm behind the anterior edge of inferior turbinate. Participants will be instructed to remain seated and to swallow every 30 seconds. They were also instructed to breathe normally and not to cough, sniff or blow their nose. The time from saccharin placement until the participant reports the sensation of sweetness is recorded with stopwatch. The test is supposed to be terminated if nothing had been tasted within 40 minutes.

d) Measurement of ratio between ciliated cells(CCs) and goblet cells(GCs) by nasal cytology. Each subject will be asked to blow his or her nose to get rid of any excess secretions before performing collection of nasal mucosa surface cells. Under direct visual control, in anterior rhinoscopy, specimens of ciliated epithelium are collected by scraping the nasal mucosa in the middle third of the inferior turbinate with a sterile nasal cytology curette. Samples will be carried out in all subjects on a different day than that of the SNTT. Samples will be uniformly smeared in the middle of a slide, fixed by air-drying, and stained by May Grunwald Giemsa quick stain. At the end of this procedure, the slides are washed in tap water, air-dried, and mounted in a synthetic resin with cover glass to increase its durability. Cytologic analysis is performed by a light microscope with a 3100 objective lens in oil immersion. Fifty microscopic fields will be examined. CCs and GCs were counted, and a measurement of the ratio between them was taken. The obtained data will be plotted on an appropriate evaluating sheet[14] and results were calculated using software IBM SPSS 22.0.,www.spss.co.in,SPSS South Asia Pvt.LTD. Microsoft word and Excel have been used to generate graphs, tables etc.

4. Out of 105subjects, 35(33.33%) each in 3 study groups (NS,CS,FS groups).
5. The age ranged from 20 to 52 years, 21 to 60 years and 23 to 62 years among never smokers (NS) group, current smokers(CS) and Former smokers (FS) respectively.(Table 1).The never smokers (NS) group had a mean age of 30.82(± 9.01)years while current smokers(CS) had mean age of 31.69 (± 10.69) years .Former smokers (FS) had a mean age of 40.34 (± 13.22)years . (Table 2).
6. 88 Males and 17 Females participated in the study.(Table 3)
7. In our study 56.2% of the Population belonged to Urban Areas While 43.8% were of Rural origin.(Table 4).
8. NS are having a mean STT of 921.48 (± 46.96) seconds which is normal compared to the CS who is having an elevated STT of 1338.11 (± 67.82) seconds and FS with a mean STT of 1322.50(± 60.56) seconds (Table 5). There isn't any statistically significant difference in STT between CS and FS. However, the increase in STT is statistically significant (p <.001) in both these groups compared to NS. Thus, there is statistically significant decrease in mucociliary function among smokers manifested as an increased STT.
9. Mean PNIF value of NS in our study 101.2 L/min (± 4.07).In CS, it was 106.05 L/min (± 4.11). In case of FS it was found to be 94.17 L/min (± 2.91) (Table 6).PNIF values showed no statistically significant variations between the 3 groups (p value -.083). This indicates no significant obstructive features in any of the groups.
10. On comparison of CC:GC ratio between the three groups, NS mean CC;GC ratio was found to be 1.26 (± 0.17), while CS were having a mean ratio of 1.20 (± 0.19) and FS having a mean ratio of 1.54 (± 0.17).CC:GC Ratio showed no statistically significant variations between the 3 groups((p.327).(Table 7). Interestingly mean GC count of NS was found to be 16.43 ± (1.42).In CS the mean GC count was 18.11 (± 2.29) and in FS the mean GC count of 12.14 (± 1.11).There is statistically significant reduction in Goblet cell count in FS compared to NS and CS.
11. Pulmonary function test findings comparing 3 groups yielded FEV1/FVC in NS to be 83.50% (± 0.51) and in CS it was found to be 83.40% (± 0.54). In FS the value of FEV1/FVC was found to be 81.62% (± 0.64) (Table 8) .There is statistically significant decrease in FEV1/FVC values of NS and CS compared to FS (p.036).
12. There isn't any statistically significant difference in FEV1/FVC between NS and CS. However, the decrease in FEV1/FVC is statistically significant (p 0.036) in FS compared to both NS and CS. Thus, there is statistically significant decrease in pulmonary function in FS compared to NS and CS. This can be due to, the FS quit smoking after developing associated symptoms or complications. Adding to this observation, there is statistically significant reduction in goblet cell count in FS compared to NS and CS which indicated that there is a change at cellular level.
13. On comparison of STT, PNIF, CC/GC ratio and FEV1/FVC values between rural and urban population among the 3 groups, it was found to be statistically insignificant indicating no role of urban pollution as a confounding factor.

14. Our study concluded that nasal mucociliary function is reduced in smokers and is permanent because cessation of smoking does not improve the mucociliary function.

7. CONCLUSION

1. Nasal mucociliary function is reduced in smokers.
2. This reduction is permanent and cessation of smoking does not improve the mucociliary function.
3. Nasal mucosal cytology can be used as a reliable surrogate marker for assessment of lower airway function

CONSENT

Patient compliance may be an issue for which the initial consent will be taken from them before including them into study.

ETHICAL APPROVAL

Considering the non invasive nature of nasal cytology, scientific review committee and institutional ethics committee granted the approval for our study.

Ethics Clearance Number: F-24/PR/ COMJNMH/IEC/20/43.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ANNEXURE

Part IV
Proforma / Questionnaire/ Case Report Form
No.
Age (yrs):
Sex: 1. Male 2. Female
Education level:
Marital status: 1. Never married 2. Currently married 3. Separated 4. Others
Occupation: 1. Employed (Type of Job……) 2. Not employed
Frequency of use: 1. No smoking
2. Consuming upto 10 cigarettes per day
3. Consuming upto 10-20 cigarettes per day
4. Consuming more than 20 cigarettes per day
Age at onset of use:
Duration of use:
Cigarette use in family and friends (Specify):
1. Father 2. Mother 3. Sibling 4. Friends 5. Work place
Comorbidities a)Nasal and paranasal sinuses infections
b)Nasal and paranasal sinuses trauma
c)Nasal and paranasal sinuses surgery
d)Exposure to occupation pollutions
e)Hypertension
f)Allergic Rhinitis and Nasal polyposis
g)Asthma
h)Any medications
Height:
Weight:
BMI:

Part V
Participant/ Patient information sheet
Instructions - This is the patient information sheet. It should address the participant of this study. Depending upon the nature of the individual project, the details provided to the participant may vary. While formulating this sheet, the investigator must provide the following information as applicable in a simple language in English and Bengali which can be understood by the participant:

- Title of the project: CORRELATION BETWEEN UPPER AIRWAY AND LOWER AIRWAY FUNCTION IN CURRENT SMOKERS,NEVER-SMOKERS AND FORMER SMOKERS
- Name of the investigator: DR. ROJAN RAVI (1ST YEAR PRIMARY DNB PGT DEPT. OF OTORHINOLARYNGOLOGY
- Purpose of this project/study: To study the Correlation between upper airway and lower airway function in Current Smokers, Never Smokers and Former smokers.
- Expected duration of the subject participation: 3 days
- Benefits to be expected from the research to the participant or to others and the post-study responsibilities of the investigator: Benefit to the participant is counselling and helping the smokers taking part in the study to quit smoking.
- Any risks expected from the study to the participant: None
- Maintenance of confidentiality of records: Confidentiality regarding the identity will be maintained.
- Provision of free treatment for research related injury: No injury is expected as it is a non-interventional study.
- Compensation of the participants not only for disability or death resulting from such injury but also for unforeseeable risks: Not applicable.
- Freedom to withdraw from the study at any time during the study period without the loss of benefits that the participant would otherwise be entitled: Yes.

- Possible current and future uses of the biological material and of the data to be generated from the research and if the material is likely to be used for secondary purposes or would be shared with others: Not applicable
- Address and telephone number of the investigator :

S/O A.RAVI, SHARON, PLRA-41,820, PALLICHAL, NEMOM P O, TRIVANDRUM
KERALA, PIN CODE-695020
Mobile no. : 8304880144

The patient information sheet must be duly signed by the investigator

Informed consent form in English
Participant's name:
Participant's address:
Title of the project: CORRELATION BETWEEN UPPER AIRWAY AND LOWER AIRWAY FUNCTION IN CURRENT SMOKERS, NEVER-SMOKERS AND FORMER SMOKERS.

The details of the study have been provided to me in writing and explained to me in my own language. I confirm that I have understood the above study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the above study.

Signature of the participant: _____ **Date**: _____

Signature of the witness: _____ **Date:** _____

Note: Consent form should be appropriately worded for adults and children (less than 18 years), e.g. If the participant is less than 18 years of age, instead of 'my participation', 'my child's/ward's participation' needs to be written.

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