ORIGINAL RESEARCH

A Comparative Study of Respiratory Microbiota and Lung Function in Smokers vs. Non-Smokers

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ABSTRACT

Aim: This study aimed to compare respiratory microbiota composition and lung function parameters between smokers and non-smokers and to explore associations between microbial diversity indices and lung function. **Material and Methods:** A comparative cross-sectional study was conducted with 160 participants divided equally into smokers (n = 80) and non-smokers (n = 80). Sputum samples and nasopharyngeal swabs were collected for respiratory microbiota analysis using 16S rRNA sequencing. Lung function parameters, including Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 Second (FEV1), FEV1/FVC ratio, and Peak Expiratory Flow (PEF), were assessed using spirometry. Statistical analysis included t-tests, Mann-Whitney U tests, and correlation analysis using SPSS 16.0. **Results:** Smokers demonstrated significantly lower lung function parameters across FVC, FEV1, FEV1/FVC ratio, and PEF (p<0.001). Microbiota analysis revealed decreased alpha diversity (Shannon and Chao1 indices) and increased beta diversity (Bray-Curtis dissimilarity) in smokers (p<0.001). Smokers also exhibited lower relative abundances of *Streptococcus*, *Prevotella*, *Veillonella*, and *Neisseria* compared to non-smokers (p<0.05). Correlation analysis showed a positive association between alpha diversity and lung function and a negative association between beta diversity and lung function indices. **Conclusion:** Smoking significantly impairs lung function, reduces microbial diversity, and alters respiratory microbiota composition. The observed correlations between microbial diversity and lung function suggest that microbial dysbiosis may play a role in smoking-induced respiratory damage, highlighting the potential for microbiota-targeted therapies in respiratory health management.

Keywords: Respiratory microbiota, Lung function, Smokers, Microbial diversity, Smoking-related dysbiosis.

INTRODUCTION

The human respiratory system serves as a vital interface between the external environment and the internal body, facilitating oxygen exchange while constantly being exposed to environmental particles, pollutants, and microorganisms. Recent advances in molecular biology and next-generation sequencing techniques have revealed that the respiratory tract is not a sterile environment, as previously believed, but is instead inhabited by a complex and dynamic microbial ecosystem known as the respiratory microbiota. This microbiota consists of diverse bacterial, viral, and fungal communities that colonize the upper and lower respiratory tracts and play essential roles in maintaining respiratory health, immune regulation, and protection against pathogenic infections.[1]The composition and diversity of the respiratory microbiota are influenced by various factors, including genetics, age, geographic location, environmental exposures, dietary habits, and lifestyle choices. Among these factors, smoking remains one of the most significant and modifiable determinants of respiratory health. Tobacco smoke contains thousands of chemicals, many of which are known carcinogens, toxins, and irritants. These compounds not only cause direct damage to the respiratory epithelium but also create a hostile environment that can alter the composition, diversity, and functionality of the respiratory microbiota. Additionally, smoking suppresses mucociliary clearance, reduces local immune responses, and increases oxidative stress, further disrupting the delicate balance of microbial communities within the respiratory tract.[2] In smokers, the altered respiratory microbiota is often characterized by reduced microbial diversity, increased colonization by pathogenic or opportunistic

bacteria, and decreased abundance of beneficial commensal bacteria. These changes are thought to contribute to the heightened susceptibility of smokers to respiratory infections, chronic obstructive pulmonary disease (COPD), and other inflammatory lung conditions. Furthermore, smoking-induced microbial dysbiosis is associated with changes in host-microbe interactions, leading to persistent inflammation, impaired tissue repair, and compromised lung function.[3] Lung function, typically assessed using spirometry, measures key parameters such as Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV1), FEV1/FVC ratio, and Peak Expiratory Flow (PEF). These parameters provide insights into the mechanical efficiency of the lungs and are critical in diagnosing and monitoring respiratory diseases such as asthma, COPD, and chronic bronchitis. In smokers, lung function often deteriorates progressively, characterized by airflow obstruction, decreased lung volumes, and reduced pulmonary elasticity. The interplay between smoking-induced changes in the respiratory microbiota and lung function impairment remains an area of active investigation, with growing evidence suggesting a bidirectional relationship where microbial dysbiosis exacerbates lung dysfunction, and impaired lung function further disrupts microbial balance.[4] Non-smokers, in contrast, typically exhibit a more stable and diverse respiratory microbiota, with an abundance of commensal bacterial genera such as *Streptococcus*, *Prevotella*, *Veillonella*, and *Neisseria*. These bacterial communities are believed to contribute to immune homeostasis, reduce inflammation, and prevent colonization by pathogenic microorganisms. In non-smokers, the respiratory epithelium and mucosal barriers remain largely intact, maintaining an environment conducive to microbial stability and resilience. As a result, non-smokers are generally less susceptible to respiratory infections, chronic inflammation, and progressive lung function decline compared to smokers.Despite growing recognition of the importance of respiratory microbiota in lung health, significant gaps remain in our understanding of how smoking-induced microbial changes impact lung function over time. While cross-sectional studies have demonstrated differences in respiratory microbiota composition between smokers and non-smokers, the mechanisms underlying these differences and their clinical consequences remain poorly defined. Furthermore, the extent to which smoking cessation can reverse microbial dysbiosis and improve lung function is not fully understood.[5] Emerging research suggests that microbial diversity, richness, and composition are not merely bystanders but actively influence respiratory immune responses and lung physiology. For example, reduced microbial diversity has been linked to increased airway inflammation, impaired mucosal immunity, and heightened susceptibility to opportunistic infections. Conversely, a diverse and stable microbiota is thought to support homeostasis and enhance resilience against environmental insults, including tobacco smoke. Understanding these dynamics is crucial for developing novel therapeutic strategies aimed at modulating the respiratory microbiota to improve lung health in smokers.In addition to bacterial diversity and composition, the relative abundance of specific bacterial genera has garnered significant attention. Certain bacterial taxa, such as *Streptococcus* and *Prevotella*, are considered important markers of respiratory health, with shifts in their abundance often associated with disease states. Smokers frequently exhibit decreased abundance of these protective genera and increased colonization by potentially pathogenic bacteria. These microbial shifts may not only contribute to lung inflammation and damage but also serve as potential biomarkers for disease progression and therapeutic response.[6] Given the substantial burden of smoking-related respiratory diseases worldwide, understanding the relationship between the respiratory microbiota and lung function in smokers versus non-smokers holds significant clinical and public health implications. Insights gained from such research could pave the way for microbiota-targeted interventions, including probiotics, prebiotics, and other microbiome-modulating therapies, to prevent or mitigate smoking-related respiratory damage.[7] This study aims to compare lung function parameters and respiratory microbiota composition between smokers and non-smokers, exploring the associations between microbial diversity, specific bacterial taxa, and lung function indices.

MATERIAL AND METHODS

This study employed a comparative cross-sectional design to investigate the differences in respiratory microbiota composition and lung function between smokers and non-smokers. A total of 160 participants were recruited and divided into two groups:

- **Smokers Group (n=80):** Individuals with a history of smoking at least 10 cigarettes per day for more than 5 years.
- **Non-Smokers Group (n=80):** Age and sex-matched individuals who had never smoked.

Inclusion Criteria

- Adults aged 25–55 years
- No history of chronic respiratory diseases (e.g., asthma, COPD)
- No recent use of antibiotics or probiotics (within the last 3 months)
- No history of immunosuppressive therapy

Exclusion Criteria

- History of acute respiratory infections in the last 4 weeks
- Presence of chronic lung diseases or malignancy
- Pregnant or lactating women

- Individuals with occupational exposure to airborne contaminants

Participants provided written informed consent before enrolment. Ethical clearance was obtained from the Institutional Review Board (IRB).

Methodology

Respiratory microbiota sampling involved collecting sputum samples from participants using the induced sputum technique after inhalation of a 3% hypertonic saline mist and nasopharyngeal swabs using sterile flocked swabs under aseptic conditions. All samples were immediately stored in RNA preservation solution and transported on ice to the laboratory for analysis. Lung function assessment was performed using a spirometer (e.g., MIR Spirolab), with key parameters measured including Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 Second (FEV1), FEV1/FVC ratio, and Peak Expiratory Flow (PEF). Each measurement was repeated three times per participant, and the best result was recorded. For microbiota analysis, total microbial DNA was extracted using the QIAamp DNA Mini Kit according to the manufacturer's protocol. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using specific primers, and sequencing data were analyzed using QIIME2 software to determine bacterial diversity, richness, and relative abundance. Alpha and Beta Diversity Indices were calculated to compare microbial composition between groups.

Statistical Analysis

Descriptive statistics, including mean, standard deviation, and frequency distribution, were calculated to summarize the data. Comparative analysis was conducted to examine differences in lung function and microbiota diversity between smokers and non-smokers. For parametric data, an independent *t*-test was employed, while the Mann-Whitney *U* test was used for non-parametric data. Correlation analysis was performed using Pearson or Spearman correlation coefficients to assess the relationships between microbial diversity and lung function parameters. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS version 16.0.

RESULTS

The study included 160 participants divided equally into smokers (n = 80) and non-smokers (n = 80). Table 1 summarizes the baseline characteristics of the two groups. The mean age of smokers was 45.2 ± 6.5 years, while non-smokers had a mean age of 44.8 ± 5.9 years, showing no significant difference in age distribution. Gender distribution was also comparable, with a male-to-female ratio of 50:30 in smokers and 48:32 in non-smokers. Body Mass Index (BMI) was slightly higher in smokers (26.5 ± 3.2) compared to non-smokers (25.8 ± 3.0), though this difference was not statistically significant. Overall, the two groups were well-matched in terms of demographic and anthropometric parameters, ensuring comparability for subsequent analyses.

Lung function parameters, as shown in Table 2, demonstrated significant differences between smokers and non-smokers across all measured indices. Forced Vital Capacity (FVC) was significantly lower in smokers (3.2 ± 0.6 L) compared to non-smokers (3.8 ± 0.5 L), with a *p*-value <0.001. Similarly, Forced Expiratory Volume in 1 Second (FEV1) was reduced in smokers (2.5 ± 0.5 L) versus non-smokers (3.2 ± 0.4 L), indicating impaired lung function in the smoking group (p<0.001). The FEV1/FVC ratio, a critical indicator of airflow obstruction, was significantly lower in smokers (78.5 ± 5.3) compared to non-smokers (85.2 ± 4.8) (p<0.001). Additionally, Peak Expiratory Flow (PEF) values were also reduced in smokers (6.1 ± 1.2 L/s) versus non-smokers (7.0 ± 1.0 L/s) (p<0.001). These findings suggest that smoking is strongly associated with reduced pulmonary function across multiple parameters.

Table 3 presents the microbiota diversity metrics, revealing significant differences in both alpha and beta diversity between smokers and non-smokers. Alpha diversity, measured using the Shannon and Chao1 indices, was significantly lower in smokers (2.8 ± 0.5 and 150.2 ± 15.4, respectively) compared to non-smokers (3.5 ± 0.4 and 180.5 ± 18.3, respectively), with both comparisons showing *p*-values <0.001. Beta diversity, assessed using the Bray-Curtis dissimilarity index, was significantly higher in smokers (0.45 ± 0.08) than in non-smokers (0.30 ± 0.07) (p<0.001). These findings indicate that smoking reduces microbial richness and alters the overall microbial composition in the respiratory tract.

In Table 4, the relative abundance of key bacterial genera was compared between the two groups. *Streptococcus* was significantly less abundant in smokers (35.2 ± 5.6%) compared to non-smokers (45.3 ± 4.9%) (p<0.001). Similarly, *Prevotella* (25.1 ± 4.8% vs. 30.2 ± 5.1%; p<0.001), *Veillonella* (15.2 ± 3.5% vs. 20.4 ± 4.1%; p<0.01), and *Neisseria* (10.3 ± 2.7% vs. 14.8 ± 3.2%; p<0.05) were also significantly less abundant in smokers. These results suggest that smoking has a substantial impact on the relative abundance of key bacterial genera in the respiratory microbiota.

Correlation analysis, as shown in Table 5, revealed significant associations between microbial diversity indices and lung function parameters. Alpha diversity (Shannon Index) showed a positive correlation with both FEV1 (r = 0.45, p<0.001) and FVC (r = 0.41, p<0.001), indicating that higher microbial diversity is associated with better lung function. In contrast, beta diversity (Bray-Curtis dissimilarity) demonstrated a negative correlation with FEV1 (r = -0.42, p<0.001) and FVC (r = -0.39, p<0.001), suggesting that increased microbial dissimilarity correlates with poorer lung function. Furthermore, the relative

abundance of *Streptococcus* was negatively correlated with both FEV1 (r = -0.38, p<0.01) and FVC (r = -0.35, p<0.05). These correlations highlight the intricate relationship between lung function and the respiratory microbiota composition.

Table 1: Participant Characteristics

Group	Age (Mean ± SD)	Sex (M/F)	BMI (Mean ± SD)
Smokers (n=80)	45.2 ± 6.5	50/30	26.5 ± 3.2
Non-Smokers (n=80)	44.8 ± 5.9	48/32	25.8 ± 3.0

Table 2: Lung Function Parameters

Parameter	Smokers (Mean ± SD)	Non-Smokers (Mean ± SD)	p-value
FVC (L)	3.2 ± 0.6	3.8 ± 0.5	<0.001
FEV1 (L)	2.5 ± 0.5	3.2 ± 0.4	<0.001
FEV1/FVC Ratio	78.5 ± 5.3	85.2 ± 4.8	<0.001
PEF (L/s)	6.1 ± 1.2	7.0 ± 1.0	<0.001

Table 3: Microbiota Diversity

Diversity Index	Smokers (Mean ± SD)	Non-Smokers (Mean ± SD)	p-value
Alpha Diversity (Shannon Index)	2.8 ± 0.5	3.5 ± 0.4	<0.001
Alpha Diversity (Chao1 Index)	150.2 ± 15.4	180.5 ± 18.3	<0.001
Beta Diversity (Bray-Curtis Dissimilarity)	0.45 ± 0.08	0.30 ± 0.07	<0.001

Table 4: Comparative Analysis of Bacterial Genera

Bacterial Genus	Smokers (Relative Abundance %)	Non-Smokers (Relative Abundance %)	p-value
Streptococcus	35.2 ± 5.6	45.3 ± 4.9	<0.001
Prevotella	25.1 ± 4.8	30.2 ± 5.1	<0.001
Veillonella	15.2 ± 3.5	20.4 ± 4.1	<0.01
Neisseria	10.3 ± 2.7	14.8 ± 3.2	<0.05

Table 5: Correlation Between Microbiota Diversity and Lung Function

Parameter	FEV1 (r-value, p-value)	FVC (r-value, p-value)
Alpha Diversity (Shannon Index)	0.45, <0.001	0.41, <0.001
Beta Diversity (Bray-Curtis)	-0.42, <0.001	-0.39, <0.001
Streptococcus Abundance	-0.38, <0.01	-0.35, <0.05

DISCUSSION

This study explored the differences in lung function parameters and respiratory microbiota composition between smokers and non-smokers, highlighting significant associations between microbial diversity and lung health.

The baseline characteristics of smokers and non-smokers revealed no significant differences in age, sex distribution, or BMI. These findings ensure that the observed differences in lung function and microbiota are likely attributable to smoking rather than confounding demographic factors. Similar demographic matching has been reported in previous studies investigating smoking-related impacts on respiratory microbiota and lung function (Charlson et al., 2011; Morris et al., 2013). By minimizing confounding variables, this study strengthens the validity of its findings.[6,7]

The significant reduction in lung function indices, including FVC, FEV1, FEV1/FVC ratio, and PEF, in smokers compared to non-smokers aligns with previous findings (Lange et al., 2015; Scanlon et al., 2000).[8,9] Cigarette smoking is well-documented as a primary risk factor for reduced lung function due to chronic inflammation, oxidative stress, and structural damage to the airways. Lange et al. (2015) similarly observed decreased FEV1 and FEV1/FVC ratios in smokers, emphasizing that smoking contributes to airflow obstruction and impaired pulmonary mechanics. The consistent findings across studies suggest that smoking-induced pulmonary dysfunction is universally observed, regardless of demographic variations.[8]

Alpha diversity (Shannon and Chao1 indices) was significantly lower, and beta diversity (Bray-Curtis dissimilarity) was significantly higher in smokers compared to non-smokers. These results are consistent with previous studies, such as those by Charlson et al. (2011) and Erb-Downward et al. (2011), which reported reduced microbial richness and altered microbial composition in smokers.[6,10] The lower alpha diversity suggests that smoking reduces the resilience and stability of the respiratory microbiome, potentially favoring pathogenic bacterial overgrowth.

Additionally, the higher beta diversity indicates greater inter-individual variability in smokers' microbiota composition. These findings align with research by Sze et al. (2014), who reported increased microbial variability in smokers, reflecting environmental stress induced by smoking.[11]

In terms of bacterial composition, smokers showed significantly lower relative abundance of *Streptococcus*, *Prevotella*, *Veillonella*, and *Neisseria* compared to non-smokers . Similar trends have been observed in previous studies (Hilty et al., 2010). *Streptococcus*, a dominant genus in the healthy respiratory tract, plays a role in maintaining microbial homeostasis. Reduced abundance in smokers suggests a disruption of protective bacterial functions.[12] Hilty et al. (2010) reported a similar decline in *Streptococcus* abundance in smokers, attributing this change to smoke-induced alterations in the respiratory mucosa.[12] Likewise, *Prevotella* and *Veillonella* reductions have been linked to chronic inflammation and impaired mucosal immunity caused by smoking (Segal et al., 2013). The observed bacterial shifts reinforce the hypothesis that smoking reshapes the respiratory microbiota, potentially creating a niche for opportunistic pathogens.[13]

Alpha diversity was positively correlated with FEV1 and FVC, suggesting that higher microbial diversity is associated with better lung function. These findings are in agreement with those of Huang et al. (2014), who reported similar positive associations between alpha diversity and lung function in patients with chronic obstructive pulmonary disease (COPD).[14] Conversely, beta diversity was negatively correlated with lung function indices, indicating that increased microbial dissimilarity is linked to poorer pulmonary outcomes.

Additionally, the negative correlation between *Streptococcus* abundance and lung function parameters aligns with findings by Pragman et al. (2012). While *Streptococcus* is a key commensal genus, its dysbiosis in smokers may reflect pathogenic overgrowth or reduced protective functions. These correlations suggest a bidirectional relationship where altered microbial diversity contributes to impaired lung function, and smoking-related lung damage further disrupts microbial homeostasis.[15]

CONCLUSION

This study highlights significant differences in lung function parameters and respiratory microbiota composition between smokers and non-smokers. Smokers exhibited reduced lung function, decreased microbial diversity, and altered bacterial composition, with lower relative abundances of key genera such as *Streptococcus*, *Prevotella*, *Veillonella*, and *Neisseria*. Correlation analysis revealed strong associations between microbial diversity indices and lung function parameters, underscoring the critical role of the respiratory microbiota in maintaining lung health. These findings suggest that smoking-induced microbial dysbiosis contributes to impaired lung function, emphasizing the potential for microbiota-targeted therapies in mitigating smoking-related respiratory damage.

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