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The Association Between Respiratory Microbiota and Lung Function in Asthma Patients

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ABSTRACT

The study investigates the relationship between respiratory microbiota and lung function in asthma patients, shedding light on the microbial components contributing to asthma pathophysiology. A cohort of 100 participants, comprising 50 asthma patients and 50 healthy controls, was examined. The asthma group exhibited varying severity levels: mild (20 patients), moderate (20 patients) and severe (10 patients). Alpha-diversity indices (Shannon and Simpson) revealed significantly reduced microbial diversity in asthma patients compared to controls ($p<0.01$). Dominant microbial species were identified, with Haemophilus, Neisseria and Moraxella being prevalent in asthma patients, while Streptococcus, Prevotella and Veillonella were dominant in healthy controls. Correlation analysis demonstrated a significant negative correlation between Haemophilus abundance and lung function (FEV1) in asthma patients ($r = -0.65$). Notably, severe asthma patients displayed higher Haemophilus and Moraxella abundance, alongside reduced microbial diversity. Asthma patients exhibited impaired lung function, characterized by lower FEV1 and FVC, which correlated with specific microbial profiles. Corticosteroid treatment induced shifts in microbiota, including decreased Haemophilus and increased Streptococcus abundance. Elevated inflammatory markers (eosinophils and IGE) were observed in asthma patients, with positive correlations to Haemophilus and Moraxella abundance ($r = 0.55$ and $r = 0.60$, respectively). This study underscores the impact of respiratory microbiota on lung function in asthma, providing insights into the role of specific bacterial taxa and their associations with disease severity and treatment response. These findings suggest potential avenues for microbiota-based therapeutic interventions in asthma management, emphasizing the need for personalized approaches considering microbial composition and host inflammatory status.

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Key Words
Asthma, respiratory microbiota, lung function, microbial diversity, inflammatory markers

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INTRODUCTION

Asthma is a chronic respiratory disorder that affects millions of individuals worldwide, making it a significant global health concern. It is characterized by airway inflammation, bronchoconstriction and increased mucus production, leading to symptoms such as wheezing, shortness of breath, coughing and chest tightness[1,2]. While asthma is a well-studied condition, its pathogenesis remains complex and multifactorial. Recent advances in microbiome research have highlighted the potential role of the respiratory microbiota in asthma development, severity and exacerbations[3,4]. This study aims to explore the intricate relationship between the respiratory microbiota and lung function in asthma patients, shedding light on the microbial factors that may contribute to the clinical manifestations of asthma[5].

The respiratory microbiota: The human respiratory tract, once believed to be sterile, is now recognized as a dynamic ecosystem inhabited by a diverse array of microorganisms, including bacteria, viruses and fungi[6]. The composition of the respiratory microbiota can vary among individuals and is influenced by various factors, including age, environmental exposures and disease status. Emerging evidence suggests that alterations in the respiratory microbiota, known as dysbiosis, may play a pivotal role in the pathogenesis of respiratory diseases, including asthma[7]. Understanding the impact of the microbiota on asthma is essential for unraveling novel therapeutic strategies and personalized medicine approaches.

Asthma heterogeneity: Asthma is a heterogeneous condition and patients exhibit diverse clinical phenotypes and responses to treatment. This heterogeneity poses challenges in understanding the underlying mechanisms of the disease[8]. While airway inflammation and immune dysregulation are central to asthma pathophysiology, recent research has shifted attention to the potential role of the respiratory microbiota as a modulator of these processes. The concept of the "asthma microbiome" is gaining traction, emphasizing the importance of investigating microbial communities within the respiratory tract.

Aim of the study: The primary aim of this study is to comprehensively investigate the relationship between the respiratory microbiota and lung function in asthma patients. We aim to elucidate how specific microbial taxa within the respiratory tract may influence key lung function parameters, such as Forced Expiratory Volume in 1 second (FEV1) and Forced Vital Capacity (FVC). Our study will address several key objectives.

Microbiota diversity: We will assess and compare the diversity of the respiratory microbiota in asthma patients and healthy controls using alpha-diversity indices, specifically the Shannon and Simpson indices.

Dominant microbial species: We will identify the dominant microbial species present in the respiratory tracts of asthma patients and healthy controls, with a focus on variations in abundance.

Correlation with lung function: We will investigate correlations between the abundance of specific microbial taxa and lung function measurements (FEV1 and FVC) in asthma patients, aiming to uncover potential associations between microbiota composition and respiratory function.

Differences in microbiota with asthma severity: We will examine variations in microbial composition among asthma patients with different severity levels (mild, moderate, severe), seeking to identify microbial signatures associated with disease severity.

Response to treatment: We will assess changes in the respiratory microbiota following corticosteroid treatment in asthma patients, exploring the impact of medication on microbial composition.

Inflammatory markers and microbiota: We will investigate associations between inflammatory markers, such as eosinophil counts and IGE levels and the respiratory microbiota, providing insights into the complex interplay between inflammation and microbial dysbiosis in asthma.

MATERIALS AND METHODS

Study design and participants: This study employed a cross-sectional design and included a total of 100 participants. The study cohort comprised 50 asthma patients and 50 healthy controls. Asthma patients were recruited from the outpatient department of Guntur Medical College and the diagnosis was confirmed based on clinical assessment, medical history and spirometry results. Healthy controls were recruited from the general population in the same geographical area.

Asthma severity stratification: Asthma patients were categorized into three severity groups: mild, moderate and severe, based on internationally recognized criteria such as symptom frequency, nocturnal awakenings and lung function.

Data Collection

Clinical data: Demographic information, including age, gender and medical history, was collected for all participants. Disease-specific data, such as asthma duration, medication history and comorbidities, were recorded for asthma patients.

Microbiota sampling: Respiratory microbiota samples were collected from all participants using non-invasive methods, such as sputum induction or throat swabs. Strict aseptic techniques were followed during sample collection to avoid contamination. Sample collection was performed by trained healthcare professionals.

Microbial DNA extraction: Microbial DNA was extracted from the collected samples using standardized laboratory protocols. DNA quality and quantity were assessed to ensure the suitability of extracted DNA for downstream analysis.

Microbiota analysis: High-throughput sequencing techniques, such as 16S rRNA gene sequencing, were employed to characterize the respiratory microbiota. Bioinformatics tools and pipelines were used for taxonomic classification and determination of microbial abundance.

Lung function assessment: Lung function measurements, including Forced Expiratory Volume in 1 second (FEV1) and Forced Vital Capacity (FVC), were recorded for all participants using spirometry. Spirometry was performed by trained technicians following standard procedures.

Inflammatory marker analysis: Blood samples were collected to assess inflammatory markers, including eosinophil counts and Immunoglobulin E (IGE) levels. Blood samples were processed and analyzed in the clinical laboratory of Guntur Medical College.

Statistical analysis: Descriptive statistics were used to summarize demographic and clinical characteristics of the study participants. Alpha-diversity indices, including the Shannon and Simpson indices, were calculated to assess microbial diversity. Comparative analyses were performed to identify significant differences in microbial composition between asthma patients and healthy controls. Correlation analyses, including Pearson or Spearman correlation coefficients, were used to investigate associations between microbial taxa, lung function and inflammatory markers. Differences in microbial composition with asthma severity were evaluated using appropriate statistical tests. Treatment-related shifts in the microbiota were assessed through paired comparisons before and after corticosteroid treatment.

Ethical considerations: Ethical approval for the study was obtained from the Institutional ethics committee of Guntur Medical College, Guntur andhra Pradesh, India. Informed consent was obtained from all study participants and they were informed about the study's objectives and procedures.

Data analysis software: Statistical analysis and data visualization were performed using software packages such as R or SPSS and bio informatics tools were used for microbiota data analysis.

RESULTS

Microbiota diversity: Alpha-Diversity Indices: Asthma patients exhibited a significantly reduced microbiota diversity compared to healthy controls. Specifically the mean Shannon index for asthma patients was 2.5 compared to 3.8 in healthy controls. Similarly the mean Simpson index was 0.7 for asthma patients and 0.85 for controls. These differences were statistically significant ($p<0.01$).

Dominant microbial species: Prevalence in Asthma Patients: The bacterial profile of asthma patients was characterized by a higher prevalence of Haemophilus (15%), Neisseria (10%) and Moraxella (12%).

Comparison with healthy controls: In contrast, the dominant bacterial species in healthy controls included Streptococcus (18%), Prevotella (16%) and Veillonella (14%).

Correlation with lung function

Spirometry results: Lung function, as measured by spirometry, showed distinct differences between asthma patients and healthy controls. The average Forced Expiratory Volume in 1 second (FEV1) was 2.8 liters for asthma patients, ranging from 1.9-3.5 liters, compared to an average of 3.6 liters in healthy controls (range 3.2-4.0 liters). Forced Vital Capacity (FVC) followed a similar trend, with asthma patients averaging 3.2 liters (range 2.0-4.0 liters) and healthy controls averaging 4.2 liters (range 3.8-4.6 liters).

Correlation analysis: A significant negative correlation was observed between Haemophilus abundance and FEV1 values in asthma patients (correlation coefficient $r = -0.65$), indicating that higher bacterial presence is associated with poorer lung function.

Differences in Microbiota with Asthma Severity

Severe asthma subgroup: In patients with severe asthma, Haemophilus showed a relative abundance of 22% and Moraxella 18%, which was markedly higher compared to those with milder forms of asthma.

Microbial diversity in severe asthma: The Shannon Index averaged 2.1 and the Simpson Index was 0.65, indicating even lower diversity in severe cases.

Table 1: Alpha-Diversity Indices

Group	Shannon index (mean)	Simpson index (mean)
Asthma patients	2.5	0.7
Healthy controls	3.8	0.85

Statistical significance: $p<0.01$ for both indices

Table 2: Dominant Microbial Species

Group	Haemophilus (Relative Abundance)	Neisseria (Relative Abundance)	Moraxella (Relative Abundance)
Asthma Patients	15%	10%	12%
Healthy Controls	-	-	-
Group	Streptococcus (relative abundance)	Prevotella (relative abundance)	Veillonella (relative abundance)
Asthma patients	-	-	-
Healthy controls	18%	16%	14%

Table 3: Correlation with Lung Function

Group	FEV1 (Average, liters)	FVC (Average, liters)
Asthma patients	2.8 (Range: 1.9-3.5)	3.2 (Range: 2.0-4.0)
Healthy controls	3.6 (Range: 3.2-4.0)	4.2 (Range: 3.8-4.6)

Table 4: Differences in Microbiota with Asthma Severity (Severe Asthma Subgroup)

Severe asthma subgroup	Haemophilus (relative abundance)	Moraxella (relative abundance)	Microbial diversity (shannon index)	Microbial diversity simpson index)
Patients	22%	18%	2.1	0.65

Table 5: Response to treatment (post-corticosteroid treatment)

Response to treatment	Haemophilus (average decrease)	Streptococcus (average increase)
Asthma patients	5%	4%

Table 6: Inflammatory Markers and Microbiota

Group	Eosinophil count (average, cells µL)	IGE levels (average, IU mL)
Asthma patients	350	300
Healthy controls	150	85

Response to Treatment

Corticosteroid impact: Asthma patients undergoing corticosteroid treatment demonstrated a notable shift in their respiratory microbiota. Post-treatment, there was an average decrease in Haemophilus abundance by 5% and an increase in Streptococcus abundance by 4%.

Inflammatory Markers and Microbiota

Eosinophil and IGE levels: The average eosinophil count in asthma patients was significantly higher (350 cells µL) compared to healthy controls (150 cells µL). Similarly, Immunoglobulin E (IGE) levels were elevated in asthma patients (average 300 IU mL^{-1}) in contrast to controls (average 85 IU mL^{-1}).

Correlation with microbiota: A moderate positive correlation was found between eosinophil counts and Haemophilus abundance (r = 0.55) and between IGE levels and Moraxella abundance (r = 0.60). In this table, '-' indicates that the microbial species was either not detected or present in negligible amounts in the respective groups.

Correlation analysis: Haemophilus abundance vs FEV1 in asthma patients (correlation coefficient r = -0.65).

Correlation with microbiota: Eosinophils vs Haemophilus abundance (correlation coefficient r = 0.55) IGE vs Moraxella abundance (correlation coefficient r = 0.60).

DISCUSSIONS

Microbiota diversity and asthma: The findings of this study align with previous research, indicating that the respiratory microbiota in asthma patients differs significantly from that of healthy controls Bogaert *et al*.[8]. Reduced microbial diversity, as indicated by lower alpha-diversity indices (Shannon and Simpson), was observed in asthma patients compared to controls Bosch *et al*.[9]. This decrease in diversity is consistent with the concept of dysbiosis in the respiratory microbiota, which has been recognized as a characteristic feature of asthma Boutin *et al*.[10] . The diminished diversity may lead to altered immune responses and increased susceptibility to respiratory infections in asthma patients, contributing to the pathogenesis of the disease Budden *et al*.[11,12].

Dominant microbial species: The dominant microbial species identified in this study corroborate previous findings in asthma microbiome studies Cait *et al* .[13]. Haemophilus, Neisseria and Moraxella were prevalent in asthma patients, whereas Streptococcus, Prevotella and Veillonella were dominant in healthy controls Goldman *et al*.[18]. Of particular interest is the over representation of Haemophilus in asthma patients, as it has been associated with airway inflammation and exacerbations in previous studies Budden *et al*.[11,12]. These findings underscore the potential role of specific microbial taxa in the pathogenesis of asthma and the modulation of airway inflammation.

Correlation with lung function: The significant differences in lung function measurements between asthma patients and healthy controls are consistent with existing literature Bogaert *et al*.[8]. The negative correlation between Haemophilus abundance and FEV1 in asthma patients suggests a potential link between this microbial taxon and impaired lung function Caverly *et al*.[14]. This observation aligns with studies implicating Haemophilus as a driver of airway inflammation and broncho-constriction Budden *et al*.[11,12]. While our cross-sectional study cannot establish causality, these results emphasize the need for further research into the mechanistic interactions between microbial taxa and lung function.

Differences in microbiota with asthma severity: The identification of distinct microbial signatures associated with different levels of asthma severity highlights the potential for tailored therapeutic interventions Fujimura *et al*.[17]. Severe asthma patients exhibited higher Haemophilus and Moraxella abundance, coupled with lower microbial diversity. This observation supports the notion that severe asthma is characterized by a distinct microbial profile, potentially contributing to disease exacerbations and treatment resistance Cait *et al*.[13]. Understanding the

microbiota variations with disease severity may aid in personalized asthma management (Kirjavainen *et al*.[19]).

Response to treatment: The observed shifts in the respiratory microbiota of asthma patients undergoing corticosteroid treatment emphasize the potential impact of medication on the microbiota composition Budden *et al*.[11]. The decrease in Haemophilus abundance and increase in Streptococcus abundance post-treatment suggest the dynamic nature of the microbiome in response to therapy. Further research is warranted to elucidate the mechanisms underlying these shifts and their implications for treatment outcomes.

Inflammatory markers and microbiota: Elevated eosinophil counts and IgE levels in asthma patients are consistent with the inflammatory nature of the disease Budden *et al*[12]. The positive correlations between eosinophils and Haemophilus, as well as IgE levels and Moraxella, suggest potential associations between inflammatory markers and specific microbial taxa15. These findings underscore the complex interplay between inflammation and microbial dysbiosis in asthma, with implications for disease exacerbations and therapeutic strategies.

Clinical implications: The insights gained from this study have several clinical implications. First the identification of specific microbial taxa associated with asthma severity and lung function impairment may aid in the development of targeted therapies that modulate the microbiota to improve clinical outcomes. Second, understanding the microbiota's response to corticosteroid treatment highlights the potential for microbiome-based therapeutic strategies as adjuncts to conventional asthma management. Lastly the associations between inflammatory markers and microbial composition underscore the importance of considering both host and microbial factors in asthma management.

Limitations: This study has limitations, including its cross-sectional design, which precludes the establishment of causality. Additionally, environmental factors, such as air quality and allergen exposure, were not extensively assessed and may influence microbiota composition. Future longitudinal studies with larger cohorts are needed to validate and expand upon these findings.

CONCLUSION

This study provides valuable insights into the association between respiratory microbiota and lung function in asthma patients. The observed differences in microbial composition, correlations with lung function and variations with asthma severity highlight the relevance of the microbiota in asthma pathogenesis. These findings underscore the potential for microbiota-based interventions in asthma management and the need for personalized approaches considering both microbial and host factors. Further research is warranted to unravel the mechanistic underpinnings of these associations and translate them into improved asthma care.

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