Investigating the Level of MUC5B Expression in the Plasma of Patients with Idiopathic Pulmonary Fibrosis (IPF) Compared to Healthy Individuals

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ABSTRACT

Introduction: Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease associated with high morbidity and mortality. Genetic factors, particularly the MUC5B promoter polymorphism, have been strongly implicated in disease pathogenesis. Given the shared embryological origin of the respiratory and oral epithelia, investigating systemic MUC5B expression may provide insights relevant to craniofacial and mucosal health. This study aimed to evaluate MUC5B gene expression in Iranian patients with IPF and compare the findings with healthy controls.

Materials and Methods: A case–control study was conducted involving 15 IPF patients and 15 age-matched healthy individuals. Peripheral blood samples were collected, RNA was extracted, and MUC5B expression levels were quantified using quantitative real-time PCR (qRT-PCR) normalized to a housekeeping gene. Statistical analyses included ANOVA, the Least Significant Difference (LSD) test, and Spearman correlation.

Results: MUC5B expression was detected in 80% of IPF patients compared to 40% of controls ($P < 0.001$). Relative expression analysis revealed that MUC5B mRNA levels were approximately 2.53-fold higher in the IPF group. No significant age difference was observed between groups.

Conclusion: Elevated MUC5B expression is significantly associated with IPF, supporting its potential role as a genetic biomarker for disease susceptibility. The systemic nature of this dysregulation suggests it could also serve as a model for understanding mucin-related pathologies in the aerodigestive tract, including the oral cavity. Further studies with larger cohorts are warranted to confirm these findings and explore their clinical utility.

Keywords: Idiopathic pulmonary fibrosis; Interstitial lung disease; Muc5b promoter polymorphism; Gene expression; Biomarker; Qrt-pcr; Iran; Genetic susceptibility; Pulmonary fibrosis pathogenesis; Mucin overexpression; Salivary mucins; Oral mucosa; Maxillofacial research; Aerodigestive tract.

Introduction

Interstitial lung diseases (ILDs) constitute a diverse group of pulmonary conditions affecting the lung parenchyma, often presenting with varying extents of inflammation and fibrotic remodeling. While certain forms of ILD arise secondary to known triggers—such as medications, autoimmune connective tissue disorders, hypersensitivity reactions to airborne organic agents, or systemic diseases like sarcoidosis—others fall under the category of idiopathic interstitial pneumonias (IIPs), in which no underlying cause can be identified [1]. Among the ILDs, idiopathic pulmonary fibrosis (IPF) is recognized as the most prevalent subtype. Reported incidence rates of IPF vary considerably depending on study design, data collection methods, and diagnostic criteria employed. A systematic review assessing the global incidence of IPF estimated annual rates between 2.8 and 9.3 per 100,000 individuals in North America and Europe, with markedly lower figures observed in regions such as Asia and South America. Even within individual countries, substantial geographic variation has been noted, likely influenced by differences in environmental and occupational exposures [2–6].

Recent data indicate that the incidence of IPF is on the rise [7]. An analysis conducted using a UK primary care database reported a 78% increase in IPF incidence between 2000 and 2012, alongside a two-fold rise in prevalence, reaching 38.8 per 100,000 individuals [5]. This growing trend has imposed a significant and escalating economic burden on global healthcare systems [8]. Furthermore, IPF is associated with a poor prognosis, with median survival following diagnosis ranging between two to three years. Previous survival estimates for idiopathic pulmonary fibrosis (IPF) were largely based on retrospective data sources [9]. Despite advancements in diagnosis and awareness, recent studies have not demonstrated any significant improvement in overall survival rates for patients with IPF [3,5,10]. In fact, mortality appears to be increasing—although this trend may partially result from heightened disease recognition and more accurate diagnostic practices [2,11,12]. Over the past several years, antifibrotic therapies have become more widely accessible; however, the extent to which these treatments impact long-term survival at the population level remains uncertain. Initial findings from an open-label extension of pirfenidone clinical trials suggested a median survival time of 77.2 months in treated individuals [13]. It is now widely acknowledged that IPF is a clinically heterogeneous disorder with an unpredictable progression. Forecasting the course of the disease remains a challenge, especially given that baseline pulmonary function tests alone are not reliable indicators of patient outcomes [14,15]. To enhance prognostic accuracy, composite indices such as the GAP model (Gender, Age, Physiology) have been developed, which incorporate demographic and physiologic parameters to provide a more comprehensive risk assessment Idiopathic pulmonary fibrosis (IPF) is believed to result from intricate interactions between genetic predispositions and environmental exposures, with cigarette smoking being one of the most well-documented external risk factors [17,18]. Substantial evidence supports a genetic basis for pulmonary fibrosis, as familial clustering has been observed in studies involving twins, siblings raised apart, and across multiple generations [22,23]. Several gene mutations have been implicated in the pathogenesis of pulmonary fibrosis, including those in surfactant protein C [24–26], surfactant protein A2 [27], and genes involved in telomere maintenance [19–22].

Among these, the MUC5B promoter polymorphism has emerged as the most robust and consistently replicated genetic risk factor for IPF. This variant appears to influence disease susceptibility by enhancing MUC5B expression in the distal airways—particularly within terminal bronchioles and honeycomb cysts—suggesting a potential pathogenic role. Under physiological conditions, mucus in the respiratory tract functions as a protective barrier that entraps inhaled particles, including pathogens, and facilitates their clearance via ciliary movement and coughing. Additionally, mucus contributes to the removal of senescent epithelial cells and immune debris. Mucins, which are large glycoproteins responsible for the viscoelastic nature of mucus, are encoded by approximately 20 identified genes—of which at least 11 are expressed in lung tissue. These include MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC13, MUC16, and MUC19 [28]. Among them, MUC5AC and MUC5B are the predominant secreted mucins. These are synthesized by goblet cells and submucosal glands throughout the upper and lower respiratory tract. While both are expressed in proximal airways supported by cartilage, MUC5B is the dominant mucin in the distal airways, where submucosal glands are absent. Its overexpression in these distal regions has been shown to impair mucosal host defense, thereby contributing to fibrotic changes observed in IPF [33]. Beyond its established role in pulmonary pathology, MUC5B is a critical component of the salivary mucin network, contributing to oral mucosal integrity, lubrication, and innate immune

defense within the oral cavity. Given the central role of MUC5B overexpression in disrupting airway homeostasis and promoting fibrosis, genetic variations in this gene—particularly within its promoter region—are considered major contributors to IPF pathogenesis [29–32]. Given that oral and respiratory epithelia share a common embryological origin and are part of a continuous aerodigestive tract, dysregulation of a key mucosal gene like MUC5B in the systemic circulation may reflect a shared pathogenic mechanism relevant to craniofacial and respiratory health. Accordingly, identifying blood-based biomarkers related to MUC5B dysregulation may offer valuable insights into disease onset and progression. In light of this, the present study aims to examine MUC5B gene polymorphism among Iranian patients with IPF and compare the findings with healthy individuals. Understanding the genetic mechanisms involved could pave the way for earlier diagnosis and more targeted therapeutic strategies, ultimately improving patient outcomes.

Materials and Methods

This case-control study was conducted on 30 individuals at a maxillofacial surgery center in Tehran. Prior to sample collection, informed consent was obtained from either the patients themselves or their legal guardians. Demographic and clinical data, including age, sex, and disease stage, were recorded using a standardized questionnaire. Peripheral blood samples were collected from both IPF patients and healthy control subjects, encompassing both male and female participants aged 30 to 80 years. To ensure sample integrity, all specimens were transported immediately to the pathology laboratory on ice following collection. Individuals with a documented history of chemotherapy, radiotherapy, or other adjuvant therapies were excluded from the study to eliminate confounding factors.

Total RNA was extracted from whole blood samples using a commercial RNA extraction kit, and microRNA was subsequently isolated utilizing the GENE miRNA extraction kit. Given the susceptibility of RNA to enzymatic degradation and the persistent activity of RNase, all extraction procedures were performed under RNase-free conditions. To minimize contamination, RNase-free microtubes and pipette tips were used throughout the process. Quantification and purity assessment of the extracted RNA were carried out using a Nanodrop spectrophotometer, which measures absorbance across a range of wavelengths using just 1–2 μL of sample, without the need for a cuvette. For gene expression analysis, complementary DNA (cDNA) was synthesized from the isolated miRNAs using a specific miRNA cDNA synthesis kit. In the next step, expression levels of the MUC5B gene were assessed by quantitative real-time PCR (qRT-PCR) using gene-specific primers. The expression was normalized against a housekeeping gene, which served as an internal control to ensure accuracy and consistency across all samples. The real-time PCR instrument was programmed based on the SYBR Green protocol provided by Pars Genome Company, with optimization tailored to the specific miRNA targets. The thermal cycling conditions were set as follows:

1. Initial denaturation at 95 °C for 5 minutes

2. 40 amplification cycles, including:

 * Denaturation at 95 °C for 5 seconds.

 * Primer annealing at an optimized temperature of 62 °C for 20 seconds.

 * Extension at 72 °C for 30 seconds.

3. A melting curve analysis was performed by gradually increasing the temperature from 60 °C to 95 °C to verify the specificity of the amplification products. Gene expression levels were quantified using the comparative Ct method ($2^{-\Delta\Delta Ct}$). Following the PCR reaction, the cycle threshold (Ct) values were obtained, and the relative expression of the target gene was calculated in comparison to a reference (housekeeping) gene, allowing normalization of the data. Statistical analysis was performed using SPSS software. To determine correlations and group differences, both analysis of variance (ANOVA) and the Least Significant Difference (LSD) test were applied. The Spearman correlation coefficient was used to assess the relationship between gene expression levels and other variables. The final evaluation of real-time PCR outcomes was conducted using the ΔCt method for relative quantification.

Results

The study sample comprised 30 individuals, including 15 patients diagnosed with idiopathic pulmonary fibrosis (IPF) and 15 healthy controls. The mean age of the IPF group was 46±10.19 years, while the healthy participants had a mean age of 43±11.72 years. Statistical comparison using an independent t-test revealed no significant difference in age between the two groups, indicating that age was not a confounding factor in this study. Following sample preparation, real-time reverse transcription PCR (RT-PCR) was performed, and the results were interpreted based on the melting curve

analysis, confirming amplification specificity. Out of the 15 IPF patients, 12 individuals (80%) tested positive for MUC5B gene expression. In contrast, only 6 out of 15 (40%) participants in the healthy control group demonstrated detectable MUC5B expression. The difference in biomarker positivity rates between the two groups was statistically evaluated using the two-sample binomial test, which demonstrated a significant association between MUC5B expression and the presence of IPF (P < 0.001). The fold change in gene expression was assessed by first determining the cycle threshold (Ct) values for each individual sample. The relative expression of the target gene between the two study groups was calculated using the $2^{-\Delta\Delta Ct}$ method, which compares the normalized expression levels of the gene of interest relative to a reference gene. Using this approach, it was found that the expression level of MUC5B mRNA in patients with idiopathic pulmonary fibrosis was approximately 2.53 times higher than that in the healthy control group.
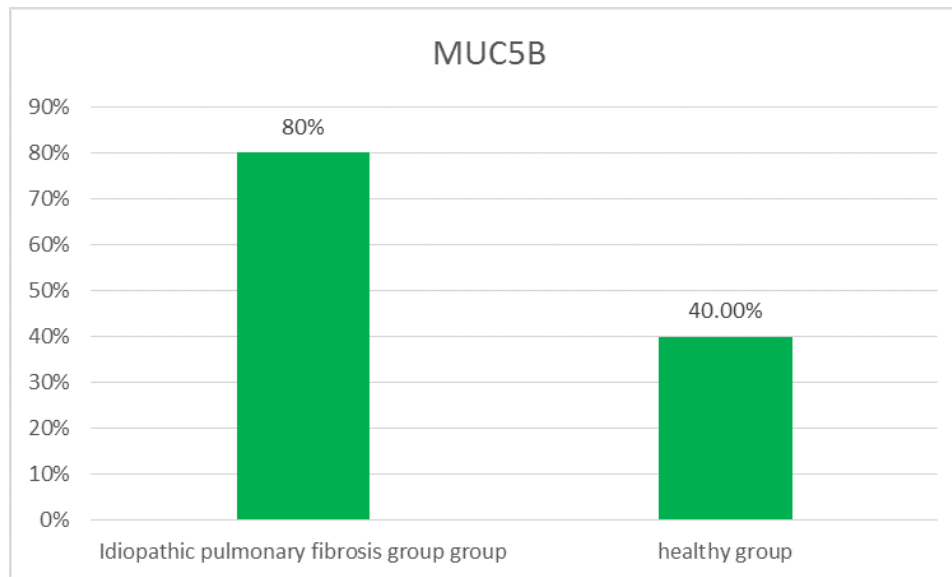
Figure 1. Proportion of individuals with detectable MUC5B mRNA expression in peripheral blood among patients diagnosed with idiopathic pulmonary fibrosis (IPF) compared to healthy controls.
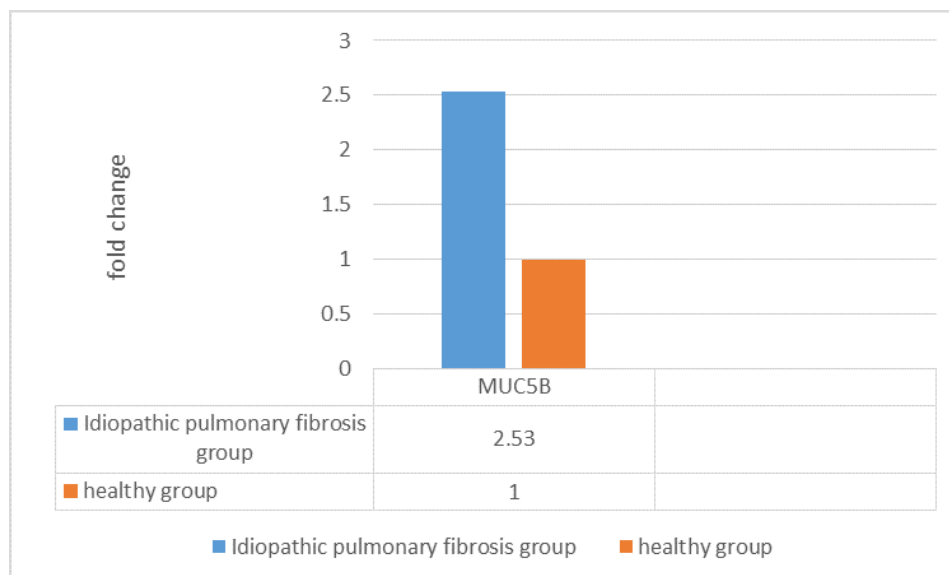
Figure 2. Comparison of MUC5B mRNA expression levels in peripheral blood between patients with idiopathic pulmonary fibrosis (IPF) and healthy controls.

Discussion

In this study, we reveal that the enhancer region contains an overlapping differentially methylated region (DMR) associated with the presence of the MUC5B promoter variant, its expression, and idiopathic pulmonary fibrosis (IPF). This enhancer region within the MUC5B promoter is dynamically occupied by the transcription factor FOXA2 and RNA polymerase II, strongly indicating its critical regulatory function in controlling MUC5B expression. The discovery of FOXA2 as a key component of the MUC5B transcriptional complex and the identification of relevant binding sites provide a foundation for further exploration of factors influencing MUC5B regulation and modulation of its expression. Given that many established risk factors for IPF, including male gender, aging, and cigarette smoke exposure, are known to impact DNA methylation patterns, epigenetic regulation remains a focal point in IPF research. Our findings demonstrate that increased MUC5B expression, the risk-associated T allele at rs35705950, and IPF are linked with elevated methylation levels. Although the positive correlation between methylation and gene expression was once viewed as contradictory, recent studies have clarified that enhancers tend to show a higher frequency of positive associations, while promoters generally follow the classic inverse relationship.

This phenomenon is often attributed to the binding preferences of certain transcription factors, such as the CCCTC-binding factor (CTCF), which preferentially binds methylated DNA, or the possibility that increased methylation reduces the recruitment of repressive protein complexes. The detection of significantly elevated MUC5B in peripheral blood suggests its potential as a readily accessible, systemic biomarker, which could be of particular interest for maxillofacial surgeons monitoring patients with fibro-inflammatory conditions affecting the oral and craniofacial region. This systemic overexpression of MUC5B may parallel similar mucin dysregulation phenomena observed in pathologies of the oral mucosa, such as oral submucous fibrosis or certain salivary gland disorders, suggesting a common theme of epithelial-mucin dyshomeostasis in fibrotic disease processes. In this study, the comparison of average age between groups was performed using a t-test, which revealed no significant difference. Therefore, it can be inferred that age is unlikely to be a confounding factor in the groups under investigation. Previous research has produced mixed results on this topic. While several pulmonary diseases—such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease—

are often associated with variables like age, sex, smoking exposure, and mucin regulation, the specific site of mucus production appears to play a critical role in determining the resulting pathophysiological characteristics [35]. For instance, in idiopathic pulmonary fibrosis (IPF) [36], mucus overproduction tends to be localized in the bronchioles. In contrast, airway-centered conditions such as cystic fibrosis [37,38] and chronic bronchitis [39] show increased mucus secretion predominantly in the proximal airways and submucosal glands. Our findings also indicate that MUC5B-deficient mice experience chronic impairment in mucociliary clearance, ongoing lung injury and inflammation, and ultimately succumb to bacterial pneumonia [40], suggesting that insufficient MUC5B levels may be detrimental as well.

This study has several limitations. The relatively small sample size may reduce the statistical power and limit the generalizability of the results. With fewer participants, findings may be less conclusive, and if the sample does not adequately represent the broader population of IPF patients or healthy individuals, the applicability of results may be compromised. Additionally, the cross-sectional design only captures MUC5B expression at a single time point, which does not reflect potential fluctuations during disease progression or in response to treatments. Variability in disease severity among IPF patients can further influence MUC5B levels, complicating interpretation. Other variables such as comorbid conditions, smoking history, and treatment protocols might confound outcomes if they are not thoroughly controlled or reported. The methods employed for measuring MUC5B may also have limitations in sensitivity and specificity, which could result in false positive or negative findings. MUC5B expression may vary with factors like infections or disease exacerbations, so measurements taken at different times could differ. Moreover, elevated MUC5B levels do not necessarily correlate with disease advancement, leaving the clinical relevance uncertain. Finally, if the study population is limited to a specific geographic region or demographic, the findings may not be fully generalizable to all individuals with IPF. Biological variability among subjects in MUC5B expression further adds complexity to data interpretation and may mask underlying patterns.

Conclusion

The presence of the MUC5B biomarker was more frequently observed in patients with idiopathic pulmonary fibrosis (IPF) compared to healthy controls. Statis-

tical analysis using a two-sample binomial test demonstrated a significant difference in biomarker positivity between these two groups. Future investigations should explore the correlation between circulating MUC5B levels and its expression in salivary or oral mucosal tissues to determine if systemic findings reflect local oral environment changes, potentially identifying a novel link between IPF susceptibility and oral health.

Acknowledgment

Conflict of Interest

There is no conflict of interest to declare.

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