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# Investigating the Relationship between *FGF2* Gene Expression and Airway Remodeling in Severe Asthma

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## ABSTRACT

Severe asthma causes chronic airway inflammation and structural changes in the bronchial wall. Fibroblast growth factor 2 (FGF2) plays an inflammatory role in specific pathways in airway remodeling in asthma. Assessing the relationship between sputum pattern, bronchial thickness by high-resolution computed tomography (HRCT) scan, and *FGF2* expression level can evaluate the role of FGF2 in asthma remodeling.

The study aimed to investigate the correlation between airway wall thickness and *FGF2* gene expression in 100 participants with severe asthma. The method involved measuring airway wall thickness using HRCT and analyzing *FGF2* gene expression through real-time reverse transcriptase polymerase chain reaction. The participants were divided into 2 groups based on bronchodilator responsiveness and classified into different asthma phenotypes based on sputum cell count.

The baseline data did not show a significant difference between the groups. The study found significant differences in airway variables between different asthma subgroups. *FGF2* expression was associated with various characteristics of asthma, including body mass index, forced expiratory volume in 1 second (FEV1), and airway wall thickness. The receiver operating characteristic curve analysis showed that a fold change higher than 2.42 in *FGF2* expression indicated asthma.

Based on our research, FGF2 may play a critical role in airway thickness regardless of inflammation. We found increased FGF2 levels with disease severity and wall thickness in atopic severe persistent asthma patients with FEV1 below 60%. Further research is needed to understand FGF2's role across broader FEV1 ranges and other phenotypes.

**Keywords:** Asthma; Airway remodeling; Fibroblast growth factor 2; High-resolution computed tomography

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## INTRODUCTION

Asthma is a chronic airway inflammation characterized by recurrent episodes of wheezing, breathlessness, chest tightness, coughing, and variable expiratory flow limitation, which may progress to persistent airflow limitation or fixed airway obstruction in a subset of patients. One of the critical features of asthma is airway remodeling, which includes structural changes in the bronchial wall that contribute to persistent airflow limitation and worsen asthma symptoms.[1]

Asthmatic airway remodeling is a complex process that entails damage or loss of the typical pseudostratified structure of airway epithelium, an increase in the proportion of mucus-producing goblet cells, Fibrotic thickening of the subepithelial reticular basement membrane, increased vascularity, a proliferation of airway smooth muscle cells, and activation of fibroblasts within the cellular and extracellular matrix of both large and small airways. The airway remodeling process contributes to bronchial wall thickening, alterations in the physiologic consequences of smooth muscle contraction, or loss of airway-parenchymal interdependence and further exacerbates its symptoms.[1,2]

Release of profibrogenic and proliferative growth factors by airway structure and inflammatory cells is increased in remodeling asthma. One of the main factors that has received significant attention is fibroblast growth factor 2 (FGF2) for its involvement in airway remodeling in asthma (Figure 1). Some studies indicate that FGF2 can be released from both inflammatory cells including T lymphocytes, eosinophils, mast cells, macrophages, and myeloid dendritic cells, and also airway structural cells which include airway epithelial cells, airway smooth muscle cells, and endothelial cells. The increased release of FGF2 can enhance inflammatory cell recruitment, activation, and adhesion. Furthermore, studies have shown that FGF2 and its specific receptor FGFR1 are overexpressed in airway structural cells in a subset of patients. *FGF2* expression in the bronchoalveolar lavage fluid (BALf) of asthma patients is elevated after allergen stimulation, which may partly explain the inflammation.[3,4,5]

This suggests that FGFs may play a vital role in the immunomodulatory function of typical airway structural cells and their communication with inflammatory cells. As a result, they could be a promising therapeutic target for chronic airway inflammation.[4,6]

To assess the occurrence of airway remodeling, a high-resolution computed tomography (HRCT) scan of the lungs can be used for noninvasive evaluation and accessibility of small airways. The measurement of airway wall thickness by HRCT in patients with asthma has been demonstrated to correlate with the severity of asthma.[7,8]

This study aimed to investigate the changes in the structure of the upper right lobe bronchus by lung HRCT-determined values and their correlation with the *FGF2* gene expression to evaluate the potential role of FGF2 in the severity of airway remodeling.

This measurement could be a valuable method to assess the remodeling progression, and targeting FGF2 and its pathways may lead to innovative ways to prevent or reverse airway remodeling and enhance asthma management. Numerous preclinical trials have explored this potential, such as using FGF2 receptor inhibitors and FGF2-neutralizing antibodies, which have shown promising outcomes.

## MATERIALS AND METHODS

### Study Population and Design

This study was conducted to explore the correlation between *FGF2* gene expression and airway wall thickness in individuals with severe asthma. The Ethics Committee of the Central Organization of Islamic Azad University of Mashhad (IR.IAU.MSHD.REC.1399.088) approved this study. The study enrolled 100 participants over 18 years old with confirmed atopic asthma who had undergone lung HRCT either at the time of the survey or a month prior. Participants were excluded from the study if they were pregnant, breastfeeding, had emphysema, were current or former smokers, had lung infections, or had taken antibiotics within the past three months.

The diagnosis of asthma was confirmed based on clinical symptoms, the results of physical examination (wheezing on auscultation), and the results of the spirometry test (forced expiratory volume in one second [FEV1] < 60% or the ratio of FEV1 to forced vital capacity [FVC] < 75%). They were treated with Seroflo (manufactured by Cipla Company, Mumbai, India), an inhaled corticosteroid (ICS) and long-acting beta-agonist (LABA) combination containing fluticasone (250 μg) and salmeterol (50 μg).

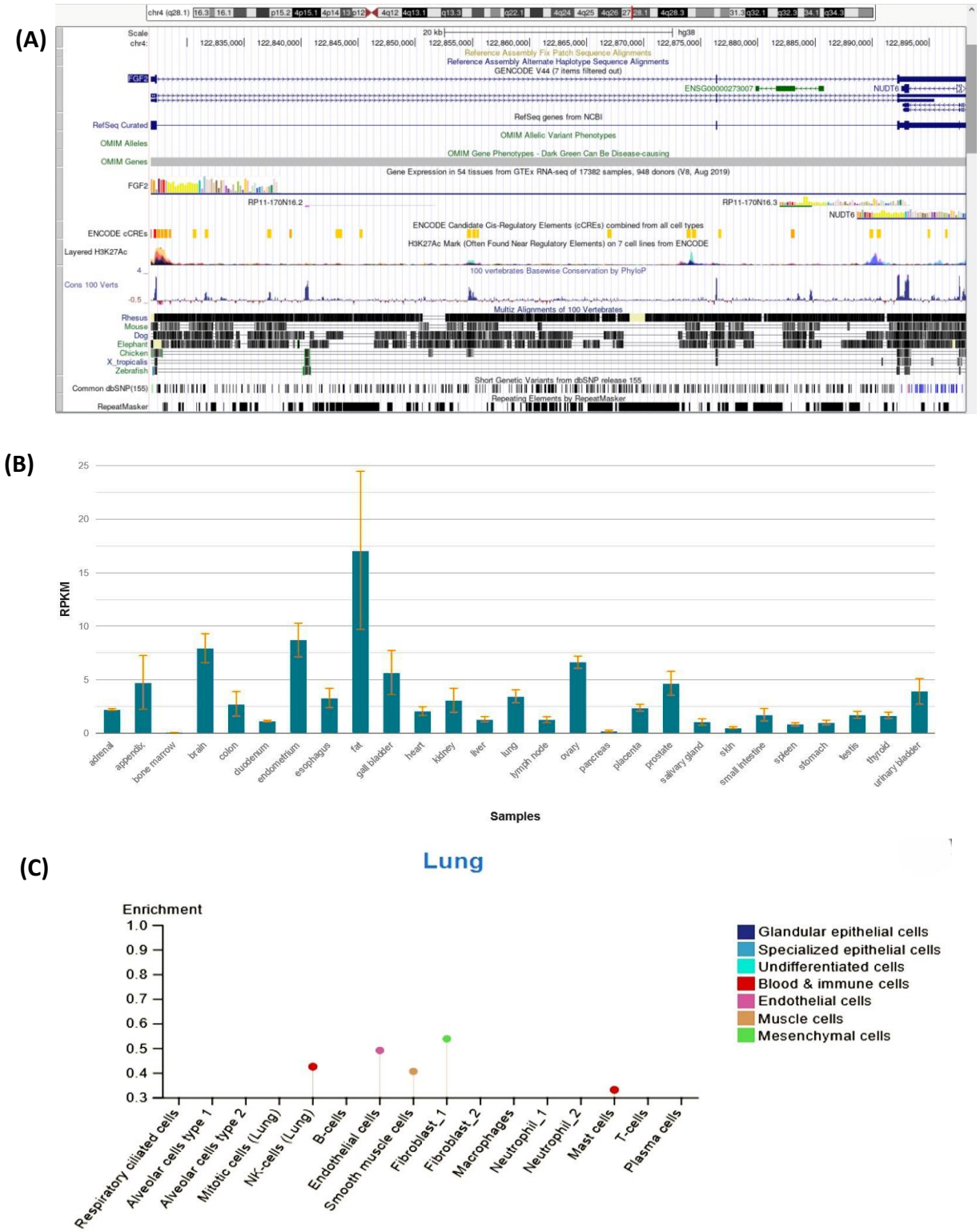


**Figure 1. (A) The fibroblast growth factor 2 (*FGF2*) genes conservation analysis among vertebrates from the University of California, Santa Cruz (UCSC) genome browser. (B) The protein levels of FGF2 in different tissues from National Center for Biotechnology Information (NCBI) Human Protein Atlas (HPA) RNA-sequencing normal tissues. (C) The single cell levels of FGF2 in the lung from HPA.**

If the initial treatment was ineffective, a high dose of ICS in Seroflo (500 μg) was administered. If the symptoms persisted, a second controller medication such as montelukast (10 mg, manufactured by Pursina Pharmaceutical Company, Tehran, Iran) or tiotropium bromide (Spiriva trademark, manufactured by Boehringer Ingelheim Co, Germany) might have been added.

The participants in the study were separated into 2 groups based on their response to the bronchodilator. They were given 2 puffs of a β-adrenergic agonist aerosol through a metered-dose inhaler, and those who showed an increase of 12% or 200 mL in FEV1 or FVC compared to their baseline were categorized in Group A (responsive to bronchodilator).[9,10] Those who did not meet these criteria were placed in Group B (nonresponsive to bronchodilator). Additionally, 18 participants in the control group were used to calculate the fold change in gene expression and compare the HRCT variables with the other groups.

Furthermore, participants were classified into different asthma phenotypes based on their sputum cell count, such as neutrophilic, eosinophilic, mixed granulocytic, and pauci-granulocytic asthma. Eosinophilic asthma was characterized by eosinophil counts above 2% to 3%, while neutrophilic asthma had neutrophil counts above 60% to 76% in induced sputum. Pauci-granulocytic asthma was defined as neutrophils< 76% and eosinophils< 3%, while mixed granulocytic asthma was defined as neutrophils> 76% and eosinophils> 3%. Participants provided written informed consent before being enrolled in the study.[11]

**Measuring Airway**

The participants in this study were administered a LABA before undergoing Lung HRCT scans to regulate their airway dimensions. Bronchial dimensions were evaluated using a 16-slice CT scanner with 32-slice reconstruction capability (Siemens, Germany). The HRCT scans were carefully evaluated by 2 highly qualified radiologists. They precisely measured the cross-sectional dimensions of the bronchus at the trunk of the apical bronchus of the right upper lobe (RB1) using 3D Slicer software from the esteemed Surgical Planning Laboratory at Harvard University in Boston, MA, USA (version 4.10.2) for each patient. The outer diameter (D), luminal diameter (LD), wall thickness (T), T/D, and percentage of wall area (WA%) were meticulously measured to ensure the highest level of accuracy. To account for potential variations due to height and weight, the parameters were adjusted based on body surface area using the following formula:

$$BSA\ (m^2) = \sqrt{((Height \times Weight) / 3600)}$$

(Height in cm, Weight in kg)

**Spirometry**

Micro Medical (SuperSpiro, London, UK) was used for the measurement of spirometry parameters, including FEV1, FVC, FEV1/FVC, and forced expiratory flow (FEF) 25-75, and FEF25- 75/FVC. This device has a mean error volume of 3%, a volume resolution of 10 mL, and a flow rate of 0.025 L/s.

**Sputum Processing**

To remove saliva contamination from sputum, a sampler was used to separate the liquid and removable parts. The sample volume was measured with a graduated cup before homogenization with dithiothreitol (DTT). Sputolysin 10% (Calbiochem Corp., San Diego, CA) was added to the sample in a Petri dish at 4 times its volume and mixed thoroughly for 15 minutes using a rotator mixer. The suspension was then filtered through a 48-μm nylon gauze to remove cell debris and mucus while preserving bronchial cells. After centrifugation at 1400*g* for 10 minutes at 4°C, a thin smear layer was made from the 25-μm cell pellet obtained. The smears were fixed with methanol and stained with hematoxylin-eosin (H&E) to distinguish blood cell types. An experienced laboratory technologist assessed the smears after rinsing. The remaining cell pellet was used for RNA extraction and PCR.[11,12]

**RNA Extraction and cDNA Synthesis**

The RNA was extracted using 100 μm of processed sputum (approximately $10^4$ cells) with the RNX-Plus Kit (CinnaGen Company, Tehran, Iran). To evaluate the RNA quality, the Epoch microplate spectrophotometer from Biotek in Santa Clara, CA, USA, was used with a 260/280 wavelength ratio. Only the RNA samples that met the necessary qualifications were utilized for cDNA synthesis, carried out with an Easy cDNA Synthesis Kit (Pars Tous Co., Mashhad, Iran).[11,12]

**Real-time PCR**

For this study, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as an internal control. The following steps were taken: heating for 15 minutes at 95°C, 40 cycles of PCR, 15 seconds of denaturation at 95°C, 30 seconds of annealing, and finally, elongation for 30 minutes at 72°C. RealQ Plus

2X Master Mix Green Denmark was used to amplify the genes. The expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences for GAPDH-human were ATGGGGAAGGT-GAAGGTCG (F) and GGGGTCATTGATGGCAACAATA (R). The primer sequences for *FGF2* were AGAGCGACCCTCACATCAAG (F) and CCAGTTCGTTTCAGTGCCAC (R).

**Statistical Analysis**

For reporting the results of descriptive analysis, mean, ratio, and 95% confidence interval were used. In our data analysis, we initially checked for normality using the Shapiro-Wilk test and Q-Q plots. As the data did not adhere to a normal distribution, we proceeded with the Mann-Whitney U test to compare differences between the 2 groups. To investigate the relationship between FGF2 expression and asthma characteristics, we conducted a univariate linear regression test and reported the coefficient and p-value. Additionally, we examined the potential clinical utility of FGF2 expression as a diagnostic biomarker through ROC curve analysis and determination of the diagnostic cutoff point. All tests were considered significant at a level of <0.05, and we utilized STATA software version 14.1 for statistical analyses.

## RESULTS

A study was conducted on 100 patients with severe asthma. The patients were divided into 2 groups: Group A, which included 46 patients with a positive response to bronchodilator, and Group B, which included 54 patients with no sufficient response and 15 individuals considered as the control group. The clinical and demographic characteristics of the patients examined are shown in (Table. 1). In Group A, 55% of the patients were women, while in Group B, 53% were men. The mean age of patients in Group A was $55.63 \pm 9.57$ and $56.78 \pm 11.31$ in Group B. The average duration of asthma in Group A was $10.25 \pm 2.4$ years and in Group B was $14.2 \pm 2.8$ years. The mean body mass index (BMI) of patients in Group A was $27.49 \pm 1.06$ kg/m$^2$, while in Group B, it was $26.32 \pm 1.42$ kg/m$^2$. FEV1% in Group A was $49.7 \pm 9.6$, while in Group B, it was $47.1 \pm 8.1$. The FEV1/FVC percentage in Group A was $60.3 \pm 9.1$, and in Group B, it was $59.6 \pm 8.3$ (Table. 1).

The predominant inflammatory pattern in the induced sputum was neutrophilic or mixed granulocytic respectively, Group A was classified into the following categories based on cell count: 16 (34%) neutrophilic, 7 (15%) eosinophilic, 11 (23%) pauci-granulocyte, and 12 (26%) mixed. Similarly, Group B was categorized into 20 (37%) neutrophilic, 7 (12%) eosinophilic, 8 (14%) pauci-granulocyte, and 19 (35%) mixed. There were no significant differences between Group A and Group B concerning the variables mentioned above (Table. 1). However, there was a considerable difference in the level of *FGF2* expression between Group B ($6.61 \pm 3.75$), and Group A ($1.69 \pm 2.39$) ($p = 0.06$).

Regarding airway variables, the overall results showed significant differences in T/D ratio and wall area ($p<0.05$), taking into account the asthma phenotype, outer diameter (D), luminal diameter (L), wall thickness (T), T/D, and percentage wall area (WA%) within the neutrophilic and mixed groups (p-value less than 0.05). However, there were no significant statistical differences in the eosinophilic and pauci-granulocytes subgroups. All airway data was adjusted by BSA's patients. (Table. 2).

The linear regression test showed that FGF2 expression (measured by fold change) was associated with various characteristics of asthma. Specifically, FGF2 was associated with BMI ($p = 0.025$, $r = 0.4326$), FEV1 ($p = 0.005$, $r = -0.4756$) (Figure 2A), wall thickness in the neutrophilic subgroup ($p = 0.049$, $r = 0.5941$) (Figure 2B), T/D ratio in the neutrophilic ($p = 0.043$, $r = 0.651$) and mixed subgroups ($p = 0.047$, $r = 0.698$), and wall area percentage in the neutrophil subgroup ($p = 0.035$, $r = 0.4581$) and mixed group ($p = 0.045$, $r = 0.4962$) (Table. 3).

According to the ROC curve of FGF2 in Group B and Group A in comparison to the control group, Group A's ROC is 0.549 and Group B's ROC is 0.889, the cutoff for fold change with 70% specificity and 80% sensitivity is higher than 2.42 patients and less than this amount is healthy (Figure 2C and D).


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**Table 1. Comparing the baseline characteristics**

| Variables | | Group A n = 46 | Group B n = 54 | $p^{\#}$ | Group C n = 18 | $p^{\$}$ |
|---|---|---|---|---|---|---|
| **Gender** | Men | 21 (45%) | 29 (53%) | 0.148 | 10 (55%) | 0.214 |
| | Women | 25 (55%) | 25 (47%) | | 8 (44%) | |
| **Age (years)** | | 55.63 ± 9.57 | 56.78 ± 11.31 | 0.425 | 53.8 ± 10.66 | 0.624 |
| **Duration of illness (years)** | | 10.25 ± 2.4 | 14.2 ± 2.8 | 0.084 | NA | NA |
| **BMI (kg/m$^2$)** | | 27.49 ± 1.06 | 26.32 ± 1.42 | 0.758 | 27.66 ± 0.89 | 0.627 |
| **BSA (m2)** | | 1.87 ± 0.54 | 1.68 ± 0.37 | 0.458 | 1.79 ± 0.48 | 0.357 |
| **FEV1** | | 49.1 ± 9.6 | 47.1 ± 8.1 | 0.054 | 96.47 ± 2.54 | 0.001* |
| **Before BD (%)** | | | | | | |
| **FEV1/FVC** | | 60.3 ± 9.1 | 59.6 ± 8.3 | 0.657 | 97.88 ± 1.21 | 0.001* |
| **Before BD (%)** | | | | | | |
| **Neutrophilic** | Number | 16 (34%) | 20 (37%) | 0.265 | 7 (38%) | 0.0691 |
| **Eosinophilic** | of patients | 7 (15%) | 7 (12%) | | 2 (11%) | |
| **Pauci-granulocytic** | | 11 (23%) | 8 (14%) | | 5 (27%) | |
| **Mixed** | | 12 (26%) | 19 (35%) | | 4 (22%) | |
| ***FGF2* (fold change)** | | 1.69 ± 1.39 | 3.61 ± 3.45 | 0.026* | 1.58 ± 1.32 | 0.019* |

BD: bronchodilator; BMI: body mass index; BSA: body surface area; FEV1: forced expiratory volume in 1 second; FGF2: fibroblast growth factor 2; FVC: forced vital capacity; NA: not applicable, Group A: responsive to BD, Group B: nonresponsive to BD, Group C: control. *Statistically significant ($p$ <0.05); #Mann-Whitney test between Groups A and B; $Mann-Whitney test between groups B and C

**Table 2. Comparison of bronchial dimensions in groups based on their sputum cell count**

| Parameter | Group | Overall | Eosinophilic | Neutrophilic | Pauci-granulocytic | Mixed |
|---|---|---|---|---|---|---|
| **Outer diameter/ BSA$^{1/2}$ (mm·m$^{-1}$)** | A | 6.92 ± 0.67 | 6.52 ± 0.54 | 7.29 ± 0.40 | 6.51 ± 1.25 | 7.37 ± 0.52 |
| | B | 6.67 ± 0.9 | 6.44 ± 0.61 | 6.86 ± 0.87* | 6.32 ± 0.91 | 7.06 ± 1.22* |
| | C | 6.93 ± 0.82 | 6.55 ± 0.53 | 7.33 ± 0.45* | 6.54 ± 1.27 | 7.4 ± 0.53* |
| **Luminal diameter/ BSA$^{1/2}$ (mm·m$^{-1}$)** | A | 4.78 ± 0.6 | 4.89 ± 0.54 | 4.72 ± 0.43 | 4.87 ± 0.62 | 4.65 ± 0.82 |
| | B | 4.95 ± 0.66 | 4.91 ± 0.58 | 4.95 ± 0.51* | 4.93 ± 0.75 | 5.02 ± 0.81* |
| | C | 4.75 ± 0.65 | 4.85 ± 0.51 | 4.69 ± 0.4* | 4.85 ± 0.6 | 4.65 ± 0.75* |
| **Wall thickness/ BSA$^{1/2}$ (mm·m$^{-1}$)** | A | 1.38 ± 0.89 | 1.23 ± 0.17 | 1.44 ± 0.18 | 1.18 ± 0.47 | 1.68 ± 0.28 |
| | B | 1.04 ± 0.23 | 1.08 ± 0.22 | 1.00 ± 0.14* | 0.99 ± 0.37 | 1.11 ± 0.2* |
| | C | 1.4 ± 0.87 | 1.25 ± 0.2 | 1.46 ± 0.21* | 1.2 ± 0.5 | 1.7 ± 0.31* |
| **Wall thickness/ outer diameter ratio** | A | 0.173 ± 0.05 | 0.156 ± 0.02 | 0.189 ± 0.023 | 0.158 ± 0.80. | 0.192 ± 0.035 |
| | B | 0.142 ± 0.14* | 0.148 ± 0.006 | 0.134 ± 0.028* | 0.139 ± 0.5 | 0.149 ± 0.038* |
| | C | 0.173 ± 0.08* | 0.158 ± 0.03 | 0.192 ± 0.04* | 0.158 ± 0.92 | 0.195 ± 0.041* |
| **Percentage wall area/ BSA (mm$^2$·m$^{-2}$)** | A | 23.45 ± 4.79 | 19.2 ± 3.9 | 24.8 ± 4.7 | 20.02 ± 4.39 | 23.9 ± 5.89 |
| | B | 16.94 ± 4.21* | 17.1 ± 3.4 | 17.3 ± 3.8* | 16.4 ± 4.82 | 16.99 ± 4.15* |
| | C | 23.45 ± 5.02* | 19.5 ± 4.3 | 25.36 ± 4.2* | 20.14 ± 4.33 | 24.38 ± 5.55* |

BSA: body surface area; D: outer diameter; L: luminal diameter; T: wall thickness; WA%: percentage wall area; Group A: responsive to BD; Group B: nonresponsive to BD; Group C: control.

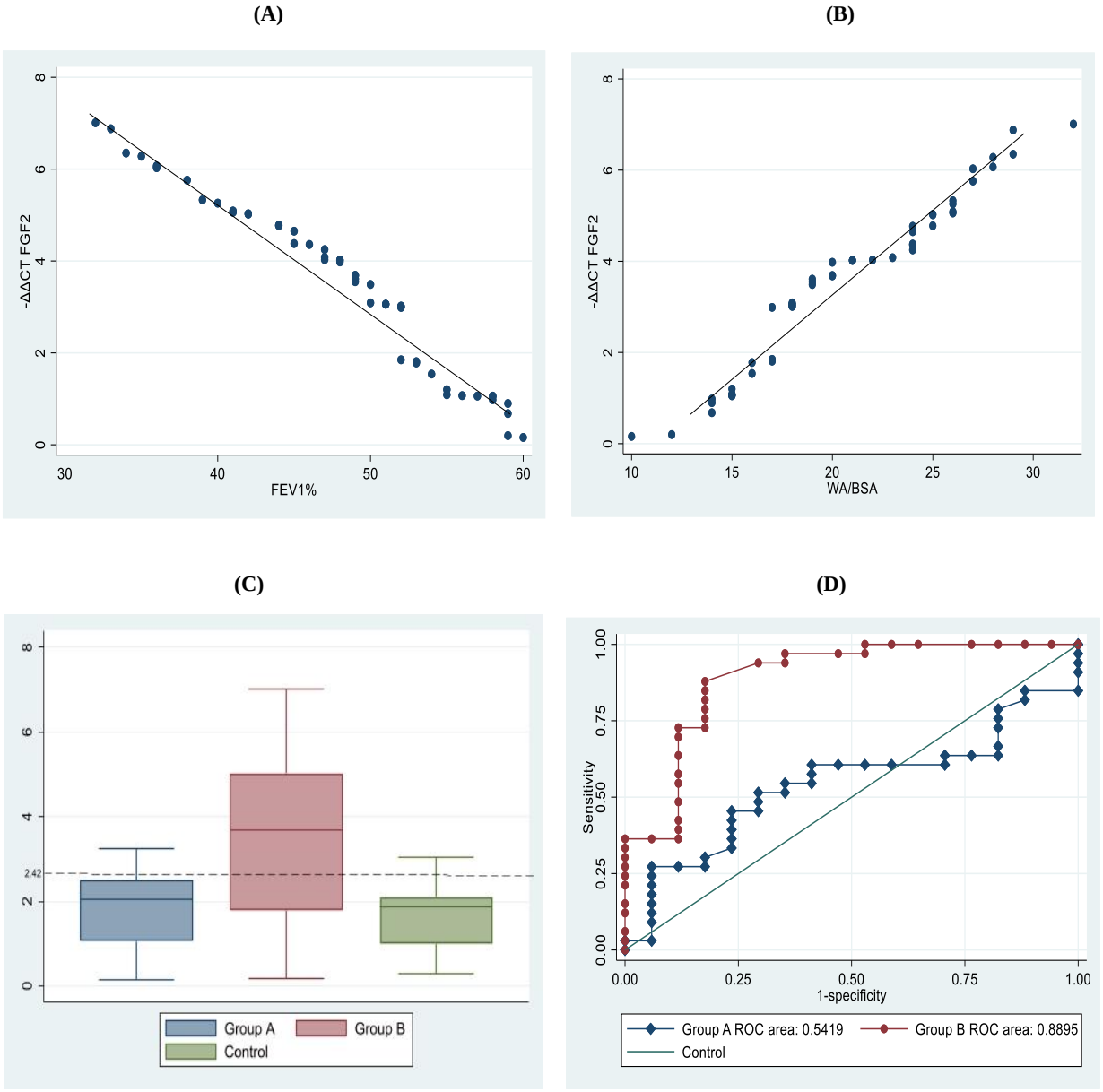


**Figure 2. (A) Linear regression test. There is a reverse correlation between *FGF2* expression and forced expiratory volume in one second (FEV1). (B) Linear regression test. There is a direct correlation between *FGF2* expression and wall area (WA) / body surface area (BSA). (C) The cutoff fold change with 80% sensitivity and 70% specificity is higher than 2.42 patients and less than this amount is normal. (D) Comparison of Groups A and B's area under the receiver operating characteristic (ROC) curve. Group A: responsive to BD; Group B: nonresponsive to BD**

**Table 3. FGF2 expression and asthma characteristics in Group B**

| Variable | Category | *FGF2* *p value* | Coefficient[#] |
|---|---|---|---|
| **Age** | | 0.065 | 0.2365 |
| **Gender** | | 0.325 | 0.2569 |
| **BMI** | | 0.025* | 0.4326 |
| **FEV1** | | 0.005* | −0.4756 |
| **FEV1 / FVC** | | 0.084 | −0.2354 |
| **D / BSA$^{1/2}$** | Eosinophilic | 0.354 | 0.3256 |
| | Neutrophilic | 0.463 | 0.3154 |
| | Pauci-granulocytic | 0.219 | 0.2985 |
| | Mixed | 0.251 | 0.3025 |
| **L / BSA$^{1/2}$** | Eosinophilic | 0.078 | 0.2341 |
| | Neutrophilic | 0.057 | 0.2546 |
| | Pauci-granulocytic | 0.564 | 0.3015 |
| | Mixed | 0.178 | 0.2647 |
| **T / BSA$^{1/2}$** | Eosinophilic | 0.325 | 0.4587 |
| | Neutrophilic | 0.049* | 0.5941 |
| | Pauci-granulocytic | 0.463 | 0.4021 |
| | Mixed | 0.845 | 0.5367 |
| **T / D ratio** | Eosinophilic | 0.284 | 0.0215 |
| | Neutrophilic | 0.043* | 0.0651 |
| | Pauci-granulocytic | 0.823 | 0.3254 |
| | Mixed | 0.047* | 0.0698 |
| **WA / BSA** | Eosinophilic | 0.584 | 0.3521 |
| | Neutrophilic | 0.035* | 0.4581 |
| | Pauci-granulocytic | 0.154 | 0.2986 |
| | Mixed | 0.045* | 0.4962 |

BMI: body mass index; BSA: body surface area; D: outer diameter; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity; L: luminal diameter; T: wall thickness; WA: wall area; Group B: nonresponsive to bronchodilator. *Statistically significant ($p$ <0.05). # Linear regression results

## DISCUSSION

The purpose of this study was to examine the relationship between the level of *FGF2* expression and CT measurements at the trunk of the apical bronchus (B1) of the right upper lobe in patients with severe asthma. The patients were separated into 2 groups based on their response to the bronchodilator. The study discovered an inverse correlation between *FGF2* expression and FEV1. Bissonnette et al findings also showed the exact inverse correlation between *FGF2* and FEV1/FVC ratio. Furthermore, FGF2 levels were higher in bronchial biopsies of severe asthma patients compared to patients with mild-to-moderate asthma and controls, indicating a correlation with asthma severity.[13] Our previous study also found the same result.[11] However, Cianchetti et al found a direct correlation with pulmonary function, which contrasts our findings. The differences in the inflammatory cell patterns of the patients and the methods of measuring the *FGF2* level may explain the conflicting results.[14]

We found a direct correlation between the level of *FGF2* and BMI and Hao et al suggests that *FGF2* may be associated with BMI and body weight regulation. We found that FGF2 levels are elevated in individuals with higher BMI or increased body weight. It has been

proposed that FGF2 may contribute to adipose tissue growth and the development of obesity.[15]

We found a direct correlation between *FGF2* expression in the T/D ratio and percentage wall area (WA%) in both neutrophilic and mixed groups and wall thickness only in the neutrophilic subset. Furthermore, we found that *FGF2* was overexpressed in patients with a thinner inner luminal diameter and thicker wall diameter and wall area. A study in a mouse model of induced asthma has suggested that FGF2 plays a significant role in the inflammation mechanism of the airway by promoting airway inflammatory cell infiltration and recruiting subepithelial neutrophils through an FGFR/MAPK/NF-κB-mediated pathway. The study also indicated that *FGF2* is overexpressed in both bronchial and alveolar areas in asthma patients and is localized in airway epithelial cells, subepithelial basement membrane, and inflammatory cells.[3]

FGF2 may also promote the proliferation or growth of airway smooth muscle cells. FGF2 can stimulate cell division and survival, and it is upregulated in the airways of individuals with asthma. This upregulation of FGF2 may contribute to the increased proliferation of airway smooth muscle cells observed in asthmatic airways.[16]

The exact mechanisms by which FGF2 promotes airway smooth muscle cell hyperplasia in asthma are not fully understood. However, it is believed that FGF2 activates signaling pathways that lead to cell division and growth. This can result in the thickening of the airway walls and contribute to the characteristic airway remodeling seen in asthma.[7]

In contrast, some studies have shown that FGF2 may have a protective effect on airways following lung epithelial injury. A study using a mouse model of bleomycin-induced pulmonary fibrosis found that FGF2 is crucial in repairing epithelial cells and maintaining their integrity. The study also found that *FGF2* expression increased in inflammatory cells, which facilitated its delivery to areas of injury. This led to the suggestion of a possible autocrine FGF2 feedback signaling mechanism that contributes to the resolution of inflammation as a result of sufficient epithelial recovery.[17] Another study suggested that FGF2 suppresses transforming growth factor-β (TGF-β) in vitro, which has a role in the differentiation of airway smooth muscle cells.[18] However, our findings suggest a direct correlation between the expression level of *FGF2* and an increase in wall thickness, and a decrease in luminal diameter. It's important to note that our study was a clinical study involving humans, and differences in quantification techniques may lead to different conclusions.

One of the limitations of our study was that the sputum characteristics of the patients showed a more neutrophilic pattern, which is indicative of a non-type-2 asthma phenotype. This may have resulted in the *FGF2* expression levels being more aligned with this phenotype in those resistant to corticosteroid therapy. Additionally, we explored only 1 biomarker in asthmatic airway cell hyperplasia, which needs more biomarkers and more concise measurements in translational studies. Understanding the role of FGF2 in asthmatic airway smooth muscle cell hyperplasia is essential for developing targeted therapies that can potentially modulate FGF2 activity and mitigate airway remodeling in asthma. Further research is ongoing to explore the specific mechanisms and potential therapeutic interventions related to FGF2 in this context.

Our study offers a unique comparison of the expression of the *FGF2* gene with the thickness of the right trunk bronchus. Our findings reveal an inverse correlation between FEV1 and the level of expression of *FGF2*. Additionally, we observed a higher expression level in patients with neutrophilic and mixed-type sputum, which was associated with a higher thickness of the right bronchus and a smaller bronchus lumen. It's worth noting that our study was limited to patients with atopic severe persistent asthma and an FEV1 below 60%, despite their positive response to short-acting bronchodilators. The dominant phenotypes were neutrophilic and mixed, which may have influenced the results. Therefore, we recommend further research to determine the true role of FGF2 in asthma, including a wider range of FEV1 and other phenotypes. Other studies have suggested that FGF2 could have a maintenance and treatment role in asthma, rather than a hypertrophic effect on the bronchus.

Our research findings suggest that FGF2 may have a pivotal role in determining airway thickness during the progression of airway remodeling in asthma, independent of inflammation. The observed increase in FGF2 levels with disease severity and wall thickness presents an opportunity for potential intervention. By measuring *FGF2* expression and targeting FGF2 receptors, we could potentially develop innovative approaches to prevent or reverse airway remodeling, thereby enhancing asthma management.

## STATEMENT OF ETHICS

The study was approved by the Ethics Committee of the Central Organization of Islamic Azad University of Mashhad (IR.IAU.MSHD.REC.1399.088) and registered in the Iranian Registry of Clinical Trials under the registration number IRCT20181225042116N1.

## FUNDING

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENTS

We are incredibly grateful for the patients who have generously volunteered to participate in our research.

## DATA AVAILABILITY

All necessary data will be readily available in an Excel file upon request. For any research reviews or contributions, please do not hesitate to contact Mahsa Manafi Varkiani at the following email address: mahsafall_2005@yahoo.com.

## AI ASSISTANCE DISCLOSURE

Not applicable.

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