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Association between SERPINB2 Gene Expression by Real Time PCR in Respiratory Epithelial Cells and Atopic Bronchial Asthma Severity

Nissreen E. ELBadawy¹, Randa S. Abdel-Latif¹, Hoda A. El-Hady²

Departments of ¹Medical Microbiology & Immunology and ²Internal Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Atopic bronchial asthma is chronic respiratory diseases of high frequency and high morbidity and mortality especially in patients refractory to the ordinary medical management. This study aimed to investigate the association between Serpin family B Member 2 (SERPINB2) gene expression and bronchial asthma severity. A total of 127 adult patients with asthma were enrolled in this study. Allergic respiratory symptoms were assessed and patients were classified according to Global Initiative for Asthma (GINA) criteria. The patients were subjected to skin prick test (SPT) by commonly encountered aeroallergens and pulmonary function tests. Sputum samples were subjected to RNA extraction and real time PCR amplification (q PCR) of SERPINB2 mRNA. The relative gene expression was determined by fold change ($2^{-\Delta\Delta Ct}$) after calculation of delta-delta Ct (Cycle threshold of patients- Cycle threshold of healthy control). Assessment of the q PCR results was done by Receiver operating characteristic curve (ROC). Patients with severe bronchial asthma constituted 44% of asthma patients and mild asthma 22% of asthmatics. SPT revealed that 23 % of the patients were mono-sensitized and 77 % were poly-sensitized. The mites and pollens were the most frequently sensitizing allergens detected by SPT (53%, and 47%, respectively). SERPINB2 gene expression in asthma group that discriminated them from healthy control was >0.01 . The highest increase of expression was found (>1.92 fold) severe asthma compared to the mild group. A negative correlation was found between SERPINB2 expression and pulmonary function tests FEV1/FVC % and FEV1% ($r=-0.921$ and -0.805 , $P<0.001$), respectively. While significant positive correlation was found between SERPINB2 expression and total IgE levels ($r=0.932$ and $P\text{-value}<0.001$), and SPT results ($r=0.923$ and $P\text{-value}<0.001$). In conclusion, the expression level of SERPINB2 gene significantly correlated with the severity of bronchial asthma.

Bronchial asthma is a very common long-term conditions characterized by respiratory obstruction, airway hyperresponsiveness and inflammation [1]. General clinical features of asthma are intermittent wheezing, breathlessness, and coughing. These symptoms are of a diurnal pattern with nocturnal cough and morning tightness and wheezing [2]. It was reported that asthma affects 5–10% of the population in developed countries [3].

Atopic bronchial asthma is characterized by high serum IgE levels and increased numbers of blood or sputum eosinophils [4]. Allergic patients should diagnosed by skin prick test (SPT) and specific IgE assay [5]. Also, lung function tests as forced vital

capacity (FVC) which is the exhaled maximum air volume, the forced expiratory volume 1 (FEV₁), and the ratio of FEV₁ to FVC are diagnostic for asthma as these patients have a lower values due to different degree of obstruction, especially in the patients with persistent severe asthma [6].

The severity of bronchial asthma could be assessed by key clinical features as asthma manifestations, corticosteroids doses, frequency, and hospital admission [7]. Also, inflammatory markers as TH2 cells are used for severity assessment [8]. Spirometer measures the severity as they provide a measure of airflow obstruction that may result from changes in airways structure due

to inflammatory process, which is known as airway remodeling [9].

Bronchial asthma is considered as severe if medication as inhaler corticosteroids of high dose (ICS) fail to control the symptoms according European Respiratory Society (ERS) guidelines and American Thoracic Society (ATS) [10]. Recently, severe asthma is considered as a risk factor of lung cancer in 1.4% of the patients [11].

Severe atopic asthma is frequently induced by mold allergens as these patients are often colonized with *Penicillium*, *Aspergillus*, *Curvularia* and *Candida* species [12, 13].

Bronchial asthma in most patients are controlled by regular medication and implementation of a stepwise escalation of treatment by international guidelines [14]. However, standard treatments fails to induce clinical improvement in many patients. As a result, other forms of treatment are important as allergen immunotherapy, neutralizing anti IgE, and other immunomodulatory drugs [15]

The inflammatory pathway starts with TH2 cells development which is dependent on dendritic cells (antigen presenting cells) and production of certain cytokines as IL-4, and IL-13 [16]. These cytokines induce allergic inflammation, epithelial and smooth-muscle alterations [3]

Airway epithelial cells have an important role in bronchial asthma due to production of inflammatory mediators which stimulate other inflammatory cells [17]. The TH2-secreted interleukin-13 (IL-13) up regulates the expression of the airway epithelial cells genes [18]. Epithelial genes that may have a role in asthma include periostin, chloride channel regulator 1, and serpin peptidase inhibitor, clade B, and regulated by IL-13 [19]. Thus, CLCA1 expression is associated with mucus hyper-secretion, airway

hyperreactivity, and hyper eosinophilia in asthma [20]. In addition, periostin gene product promotes subepithelial fibrosis [21].

SERPINB2 (serpin family B member 2) is a protein coding gene for a serine protease inhibitor [22]. It inhibits plasminogen activators, and stimulates formation of fibrin [23]. Thus, expression of SERPINB2 gene in respiratory epithelial cells may serve as a surrogate marker of Th2-driven inflammation in the lung which is considered as the main mechanism of atopic asthma pathogenesis [19]. Indeed, SERPINB2 gene expression that occurs during allergic inflammation mediated by T helper type 2 cell contribute to airway epithelial goblet cell metaplasia and may alter epithelial-mesenchymal signaling, leading to increased subepithelial fibrosis[22]. The net result is airway inflammation and development of bronchial hyperresponsiveness as airway inflammation and epithelial remodeling are two key features of asthma [24].

Currently, there is limited details about epithelial cell activation and SERPINB2 gene expression in patients with severe asthma. Indeed, this research is conducted to investigate association between SERPINB2 expression in respiratory epithelial cells of patients with bronchial asthma and disease severity.

Patients and Methods

Patients selection and classification

This is a case control study, conducted in Immunology & Allergy unit, and Molecular Biology unit in the Medical Microbiology and Immunology department, Faculty of Medicine, Zagazig University during the period between January 2016 and March 2017. Formal consents were signed by patients before study and IRB approval of this study was obtained.

A total of 127 adult patients with asthma were enrolled in this study together with 120 healthy controls. Allergic respiratory symptoms were

assessed from detailed history given by every subject. The cases were defined according to Global Initiative for Asthma (GINA) criteria [25]. The study groups were subjected to pulmonary function tests, blood and sputum samples were obtained for further analysis.

The exclusion criteria of the patients included; receiving antihistamines, or corticosteroids in the last 4 days, skin dermographism, urticaria, parasitic infection, and pregnancy.

Pulmonary functions of patients and control were tested using an electronic spirometer [26]. According to the pulmonary function, respiratory symptoms, and medication usage (inhaled corticosteroids), the severity of the diseases was assessed and patients were classified into 4 groups (table 1) [27].

Skin prick test DOLkiTen aeroallergens (Dermatophagoides farina, Derm. Pteronyssinus, cat, dog epithelial cells, mixed grass, tree, mold (Aspergillus, alternaria), sunflower seeds and cockroach mixes) with normal saline (0.9%, negative control) and histamine hydrochloride (1 mg/mL, positive control) (Omega diagnostics, UK) were used in skin prick tests (SPT) according to the European standards of skin prick test [28]. The results of SPTs were read after 15 minutes by measuring the two longest diameters of the wheal and a wheal > 3 mm larger than the saline was considered positive. SPT results scored as 1 if diameter 3-4 mm, 2: 5-7 mm, 3: 8-10 mm, and 4 if diameter > 10 mm [7].

Sputum sample collection and processing

Patients and control groups inhaled nebulized 3% saline through a mouthpiece for 12 minutes. Inhalation was interrupted every 2-minute to spit saliva and expectorate induced sputum into a sputum cup as previously described [29]. Induced sputum was processed in 10% solution of Sputolysin at a ratio of 1:1 g sputum/mL sputolysin and incubated in a water bath shaker at a 37°C for 15 minutes. Then, the mixture was centrifuged at 4°C at 2000 rpm for 10 minutes and cell pellet was then re-suspended in 600 µL of the lysis buffer (Qiagen, UK). Samples were stored at -80°C till used in RNA extraction, cDNA synthesis and q PCR process.

Total Ig E Assay

Serum was separated and stored at -20 °C and analysed for concentrations of total Ig E. Total IgE levels were assayed by enzyme immunoassay (Allergozyme® Total Ig E. Omega Diagnostics GmbH, Herrengarben; Germany).

Briefly, 10 µl of standard serums (1 – 5) and 10 µl of the serum samples and the diluents solution were pipetted into the well. An enzyme-labeled antihuman IgE was pipetted into the well. In the next step, a substrate solution was added. The quantity of bound and labeled anti-human-IgE was proportional to the quantity of the total IgE in serum. Evaluation was performed by means of a standard curve consisting of the extinction values of the measured standard wells. For each test run, values for quality control (positive control) was measured and was within the normal ranges. Negative control of 100 µl substrate solution was also included in each run. The extinctions of the coloured solutions were measured with ELISA reader at 405 nm

Isolation of Total RNA from sputum cells

Cell samples (1×10^7 cells) were subjected to disruption and homogenization by centrifugation in 600 µl of lysis buffer containing guanidine isothiocyanate for 5 min at 300 x g (The RNeasy Mini Qiagen, UK). Extraction of RNA was performed according to manufacture instruction. Sample mixtures were then applied to spin columns where the total RNA binds to the membrane and contaminants were washed away. High-quality RNA was then eluted in 30 µl water and stored in 75% ethanol at -20°C. Purity of RNA was spectrophotometrically tested and considered pure RNA if 260/ 280 ratios >1.7 [23]. Total RNA was analyzed by agarose gel electrophoresis. The RNA was considered to be intact, if both 18S and 28S rRNA appear as two sharp bands.

Real-time PCR of SERPINB2 Gene expression

RNA extracted from the sputum cells of 127 asthmatic patients and 120 healthy control subjects were subjected to c DNA synthesis and analyzed for expression of SERPINB2 genes by real time PCR [30].

RNA was reversely transcribed into cDNA by The Maxima First Strand cDNA Synthesis Kit (ThermoFisher scientific, USA). First, RNA extract was treated with double-strand specific DNase (dsDNase) to remove contaminating genomic DNA, incubated for 2 min at 37°C in preheated water bath, and Chilled on ice, with spin centrifugation. Treated RNA was directly subjected to reverse transcription by Maxima Enzyme Mix which contains the reaction components: reaction buffer, dNTPs, oligo (dT), random hexamer primers, reverse Transcriptase (RT) and RNase Inhibitor. The mixture was then incubated

for 10 min at 25°C followed by 15 min at 50°C. The reaction was terminated by heating at 85°C for 5 minutes. The reaction product of the first strand c DNA synthesis was stored at -70 °C.

Two µl of the first strand c DNA synthesis reaction was used as template for q PCR in a 25 µl total volume with the SYBR Green Maxima SYBR Green/ROX qPCR Master Mix (ThermoFisher scientific, USA) that contains hot start polymerase, SYBR green, Rox dye, reaction buffer, dNTPs. B-actin gene primers was used as normalizer for target gene expression [31].

SERPINB2 genes forward and reverse primers sequences were (5-3 sequences): (forward) GGGCTTTATCCTTTCCGTGTAA, (reverse): TTAGCTTTTCACGCAAGTACATCAT and b-actin primers sequence was CCTGGCACCCAGCACAAT (forward) and GCCGATCCACACGGAGTACT (reverse) [23]. A negative template free and RT enzyme free negative PCR control and human DNA for B-actin amplification as external positive control were included in each run. Dissociation curve analysis was performed and “no template controls” were analyzed to check for nonspecific products in the reaction. The PCR mixture was run on the Stratagene Mx3000P system (Agilent Tech., Washington, USA). The cycle conditions were as follow: 50° C for 2 min, 95° C for 10 min, and 40 cycles of 95°C for 15 s and 60° C for 1 min [23].

From each amplification plot, a threshold cycle (Ct) value was calculated, which was defined as the cycle at which a statistically significant increase in the fluorescence was first detected. For each sample, the quantity was derived from Delta Ct (ΔCt) = Ct (SERPINB2 gene) – Ct(b-actin reference). Healthy control gene expression was derived from Delta Ct (ΔCt) = Ct (SERPINB2 gene) – Ct(b-actin reference) [31]. Then $\Delta\Delta CT$ was calculated by subtraction of ΔCt of patients from ΔCt of healthy control. These values were log2 transformed and fold difference equals $2^{-\Delta\Delta CT}$ [23].

Statistical Analysis

Statistical analysis was performed with Statistical Package for the Social Sciences, version 15.0 for Windows (SPSS Inc., USA). Parametric data were summarized by mean calculation. Fisher exact, and Kruskal-Wallis tests (for nonparametric data) were used for comparison between groups. Correlations were determined by Spearman rank correlation coefficients. *P*-values < 0.05 were considered significant. Performance characteristics of SERPINB2 qPCR were determined by Receiver operating characteristic (ROC) analysis. ROC curve analysis determined a range of SERPINB2 expression cut off values to calculate the area under curve (AUC), sensitivity, specificity, and accuracy for different asthma groups and Interactive do diagram was done on Medcalc program. A *P* value of <0.05 was considered to be statistically significant.

Table 1. Classification of the Asthma Severity phenotypes

Severity parameters	Intermittent	Mild	Moderate	Severe
Symptoms	≤ 2 days/ week	> 2days/ week	Daily	all the day
Night arousals	≤ 2/ month	3- 4/ month	> Once/ week	7 times/ week
Short-acting beta agonist administration	≤ 2 days/ week	>2days/ week <1/ day	Daily	Several times/ day
Normal activity Interfering	No	Minor interference	Certain limitation	Extreme limitation
Lung function	FEV ₁ >80	FEV ₁ ≥ 80 normal FEV ₁ /FVC	FEV ₁ : >60 <80; reduced FEV ₁ /FVC by 5 %	FEV ₁ < 60; FEV ₁ /FVC reduced>5%

FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity.

Results

In this study, and according to GINA classification, patients with severe and moderate bronchial asthma constituted 44% and 34% of asthma patients; respectively and mild asthma constituted 22% of asthmatics.

Regarding age and sex distribution of the patients enrolled in this study in different classes of asthma, severe asthma was more frequently found in old male patients as mean age was 50 year ± 10.24 years, while the age of patients with mild asthma was 32 ± 5.8 ($P < 0.001$). Female patients mostly

presented with mild asthma (69%). However, male gender constituted 56.1%, and 54.4% of patients with moderate and severe asthma ($P < 0.001$)

FEV1 and FEV1/FVC % were significantly lower in patients than those in control group. In addition total IgE level and skin prick test wheal diameters were higher in patients than control. SERPINB2 mRNA expression in asthma group was significantly higher than control group. The highest increase of expression was demonstrated in severe asthma. The comparison between different patient groups and control was presented in table 2.

Table 2. Comparison between mild, moderate, severe asthmatic patients and control group as regard FEV1, FEV1/FVC, Total IgE, SPT and SERPINB2 gene expression.

	Mild (N=29)	Moderate (N=41)	Severe (N=57)	control (N=120)	P-value
FEV1					
mean $\pm$ SD	92.66 $\pm$ 2.29	73.57 $\pm$ 6.47	64.64 $\pm$ 7.98	105.5 $\pm$ 2.9	<0.001
Range	(89.5-95.5)	(60.0-81.3)	(51-79)	(99-110)	
FEV1/FVC					
mean $\pm$ SD	83.69 $\pm$ 2.0	69.46 $\pm$ 6.19	54.1 $\pm$ 4.58	96.7 $\pm$ 1.96	<0.001
range	(80-86.2)	(61-79)	(47 -60)	(93-99)	
Total IgE					
mean $\pm$ SD	142.72 $\pm$ 36.31	242.36 $\pm$ 84.0	449.25 $\pm$ 131.65	18.3 $\pm$ 5.51	<0.001
range	(97-210)	(103-378)	(279-787)	(10-28)	
SPT					
mean $\pm$ SD	5.05 $\pm$ 1.42	11.63 $\pm$ 1.79	15.9 $\pm$ 1.26	0.000	<0.001
range	(3.0-7.0)	(9.0-15.0)	(14-18)		
SERPINB2					
Gene expression $2^{-\Delta\Delta Ct}$	0.32 $\pm$ 0.017 (0.29-0.35)	2.46 $\pm$ 0.18 (1.87-2.46)	6.96 $\pm$ 1.04 (2.35-9.44)	0.015 $\pm$.013 (0.002-0.04)	<0.001

$P < 0.05$ is significant. FEV₁, forced expiratory volume 1; FVC, forced vital capacity; SPT, skin prick test (SPT) ; $2^{-\Delta\Delta Ct}$ delta-delta cycle threshold Gene expression: $2^{-\Delta\Delta Ct}$

SPT revealed that 23 % of the patients were mono-sensitized and 77 % were poly sensitized. The mites and pollens were the most frequently sensitizing allergens detected by SPT (53%, and 47%, respectively).

SERPINB2 mRNA expression ($2^{-\Delta\Delta C_t}$) at cut off point ($(2^{-\Delta\Delta C_t} > 0.01)$) discriminated patients with bronchial asthma from control with a sensitivity of 100 % and a specificity of 100 % (Table 3, Fig. 1). In addition, mRNA expression at cut off point ($2^{-\Delta\Delta C_t} > 1.9$) discriminated severe asthmatics from mild asthmatics with sensitivity of 100 % and a specificity of 100 % (Table 3, Fig.1, 2).

Moreover, results showed negative correlation between SERPINB2 mRNA and pulmonary function tests FEV1/FVC % and

FEV1% in asthmatic group ($r = -0.9$, $r=0.8$) respectively, p-value for both (<0.001) (Fig.3, 4).

While significant positive correlation between gene expression and serum IgE levels in asthmatic patients, ($r=0.93$ and p-value <0.001) (Fig. 5).

Gene expression and SPT correlation was assessed as shown in table 4 (Fig. 6).

In sever group, the correlation between SERPINB2 mRNA and pulmonary function tests FEV1/FVC % and FEV1% was stronger (Fig.7,8).

The correlation between SPT and total IgE from one side and lung function tests FEV1/FVC % and FEV1% in asthmatic group on the other side was assessed and presented in table 5.

Table 3. Validity of SERPINB2 mRNA expression in discrimination of asthmatics from control and differentiation between different asthma phenotypes:

SERPINB2 RNA expression*	Cut Off	AUC	P value	CI	Sensitivity	Specificity	PPV	NPV	Accuracy
Asthmatics from control	>.01	1.000	<0.001	0.97-0.99	90.5	100.0	100.0	100	100
Severe asthmatic from mild	> 1.9	1.000	<0.001	1.00-1.00	100.0	100.0	100.0	100.0	100.0
Severe asthmatic from moderate	> 3.6	1.000	<0.001	1.00-1.00	100	100.0	100.0	100.0	100.0
Mild asthmatic from moderate	> 0.5	1.000	<0.001	0.94- 1.0	100	100	100	100	100

*Gene expression: $2^{-\Delta\Delta C_t}$ $P<0.05$ is significant.

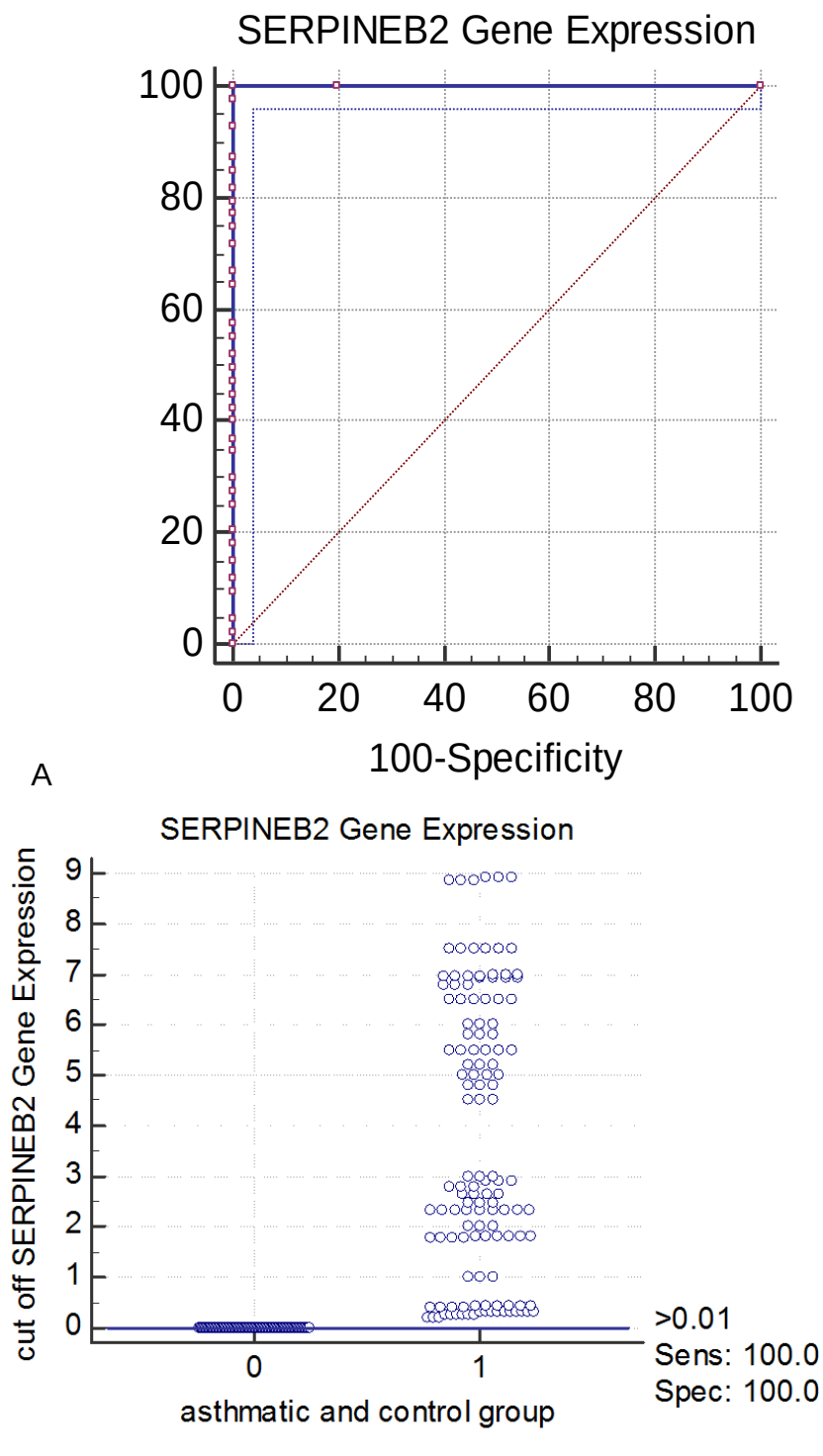


Figure 1. A, Ability of the chosen SERPINEB2 gene expression ($2^{-\Delta\Delta Ct}$) cut off point of >0.01 to discriminate between asthmatic patients and healthy control. B, Receiver operator characteristic curve analysis to determine the optimal SERPINEB2 gene expression cut off point that discriminates between asthmatic patients and healthy control with greater sensitivity and specificity.

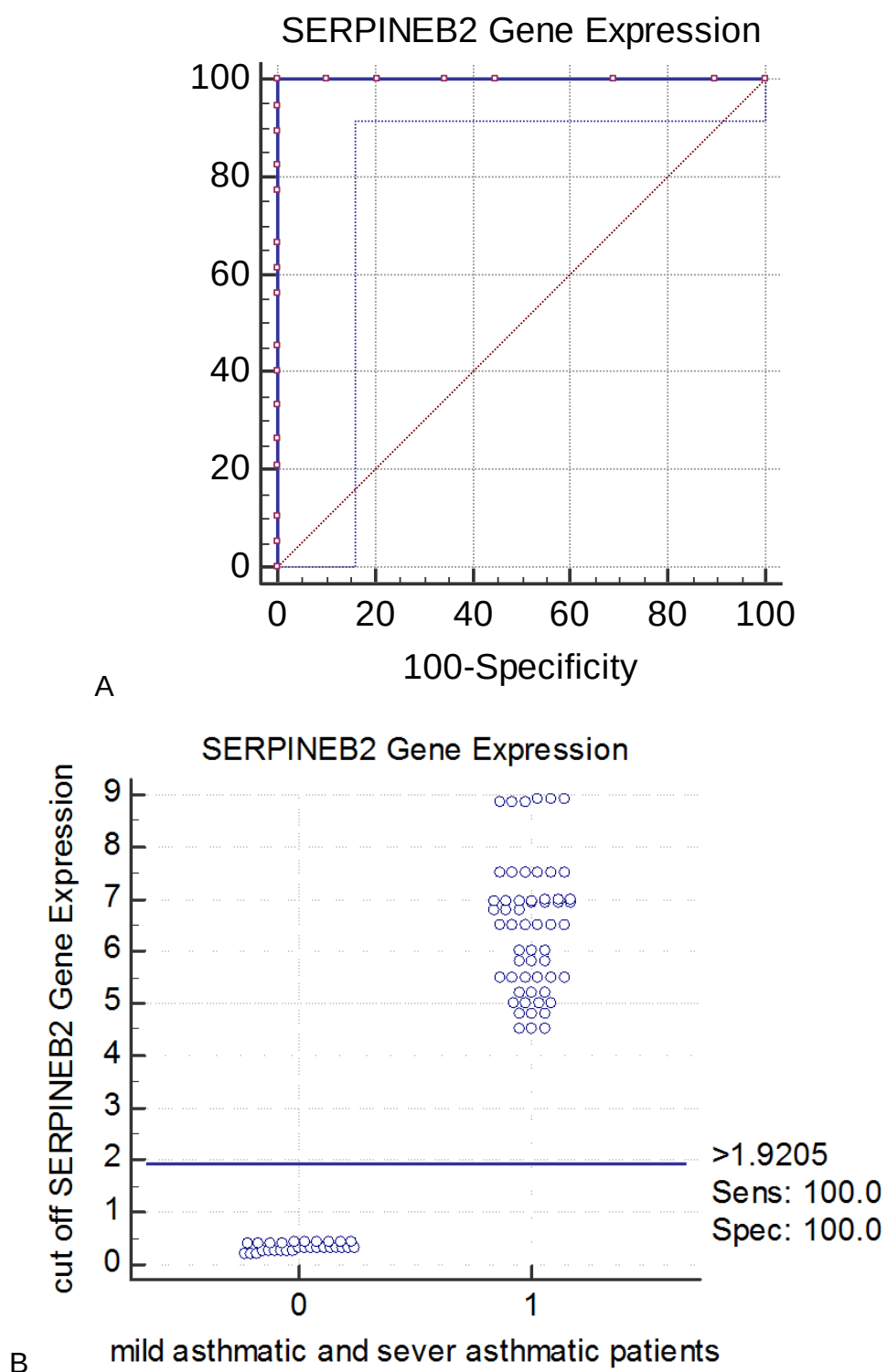


Figure 2. A, sensitivity, specificity of SERPINB2 gene expression ($2^{-\Delta\Delta C_t}$) and different cut off values and chosen cut point of >1.9 to discriminate between Mild bronchial asthma and sever cases. B, Receiver operator characteristic curve analysis to determine the area under the curve and optimal cut off point of SERPINB2 gene expression that discriminates between mild and sever cases.

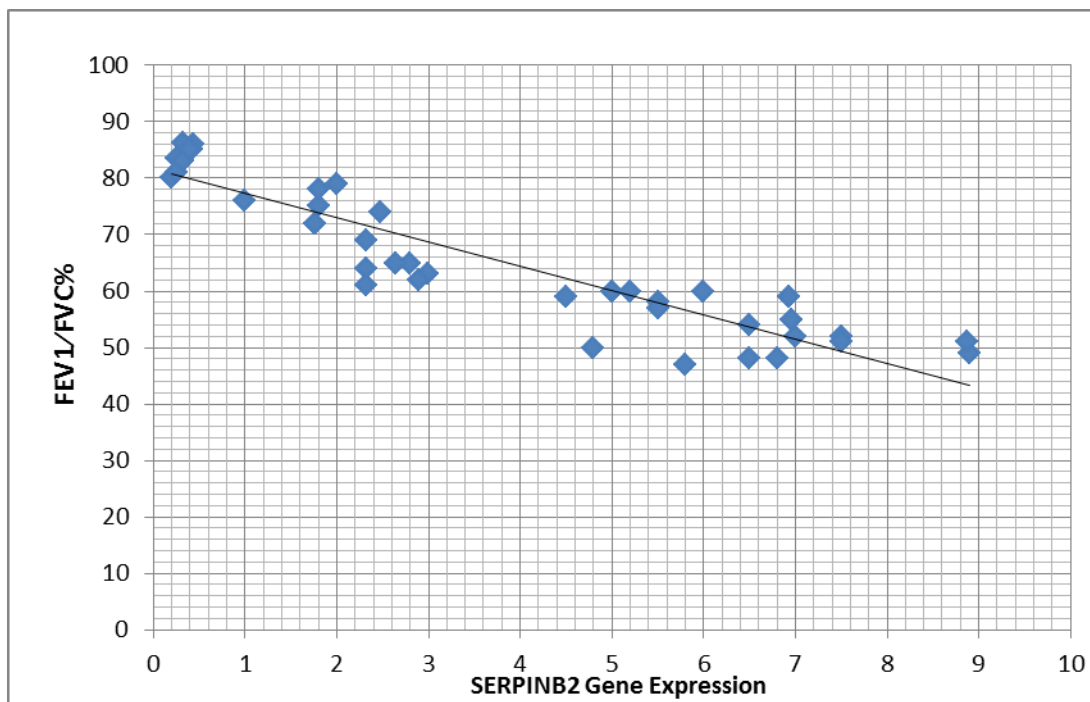


Figure 3. correlation between SERPINB2 mRNA ($2^{-\Delta\Delta C_t}$) expression and FEV1/FVC% in all asthmatic patients

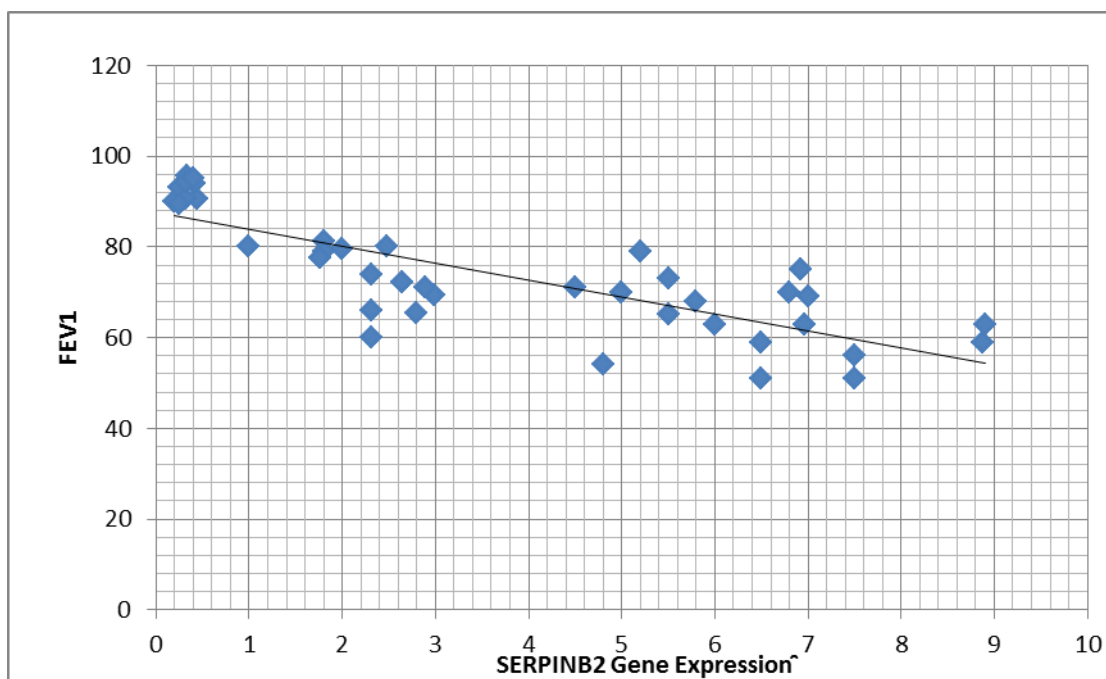


Figure 4. correlation between SERPINB2 mRNA expression ($2^{-\Delta\Delta C_t}$) and FEV1 in all asthmatic patients

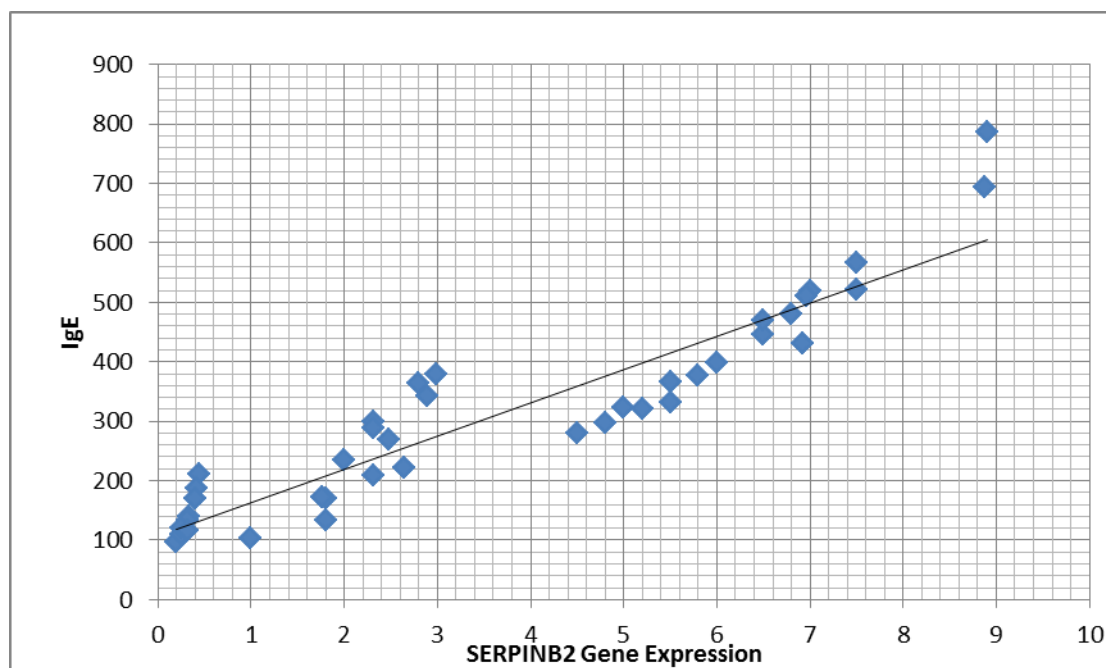


Figure 5. correlation between SERPINB2 mRNA expression ($2^{-\Delta\Delta C_t}$) and IgE in all asthmatic patients

Table 4. Correlation between SERPINB2 mRNA expression and (FEV₁/ FVC%, FEV₁%, IgE and SPT) in asthmatic patients groups.

Variable	SERPINB2 mRNA expression*	
	r	P value
FEV ₁ /FVC%	-0.921	<0.001
FEV ₁ %	-0.805	<0.001
IgE	0.932	<0.001
SPT	0.923	<0.001

P<0.05 is significant. FEV₁, forced expiratory volume 1; FVC, forced vital capacity; SPT, skin prick test (SPT); $2^{-\Delta\Delta C_t}$: delta-delta cycle threshold Gene expression: $2^{-\Delta\Delta C_t}$

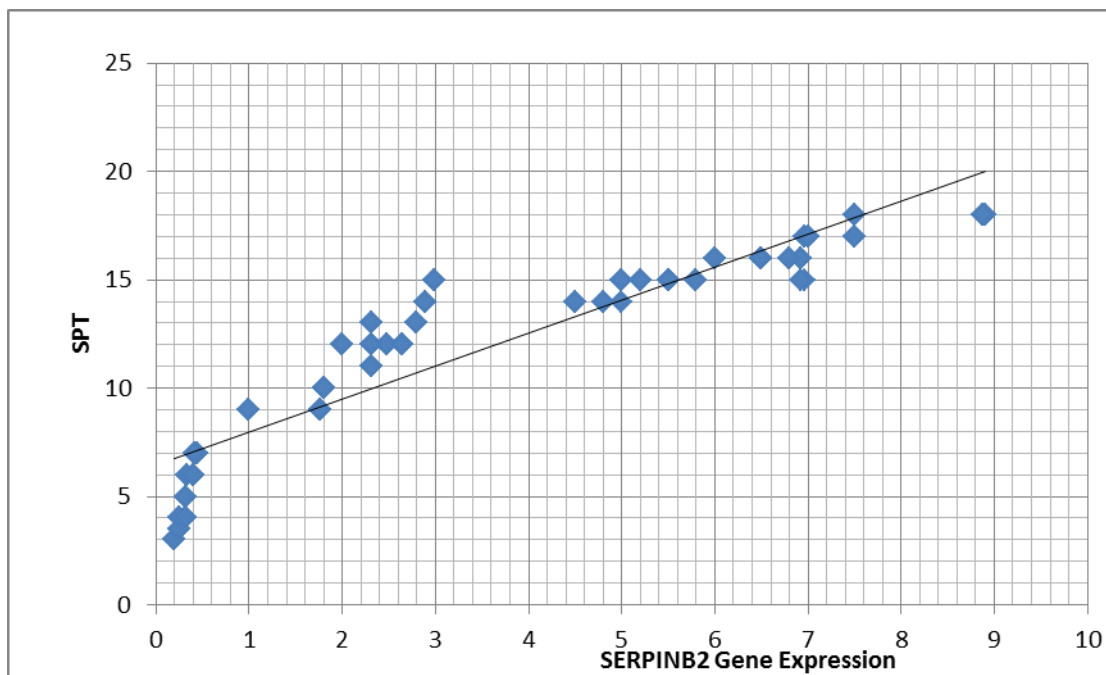


Figure 6. correlation between SERPINB2 mRNA expression ($2^{-\Delta\Delta C_t}$) and skin prick test (SPT) in all asthmatic patients

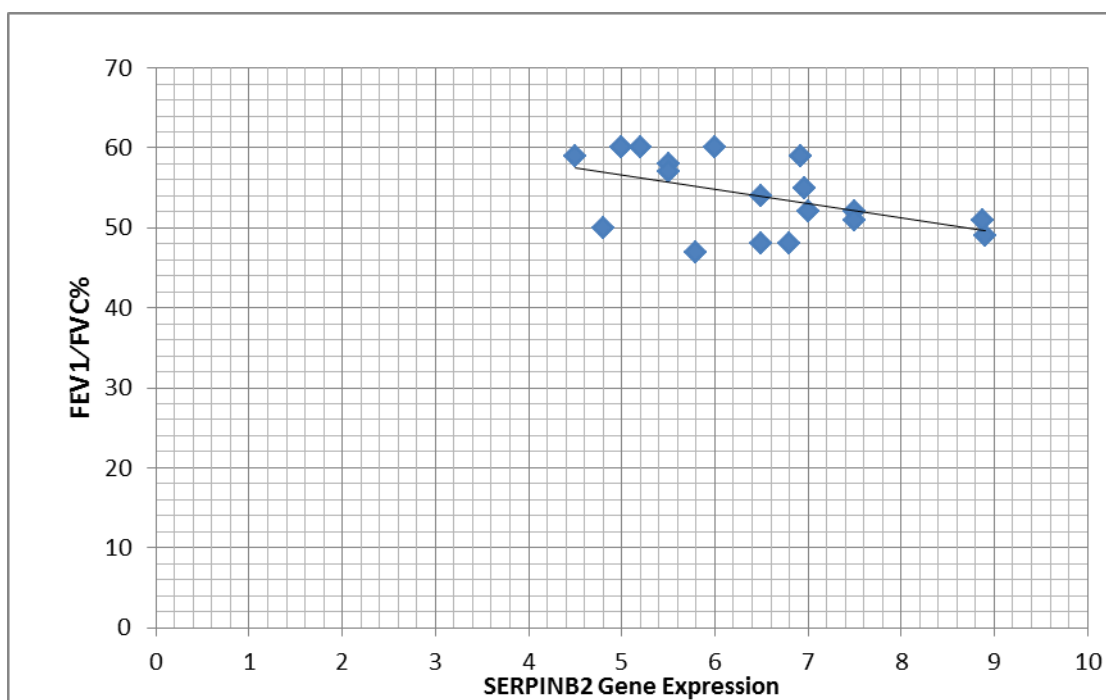


Figure 7. correlation between SERPINB2 mRNA expression ($2^{-\Delta\Delta C_t}$) and FEV1/FVC% in severe patients

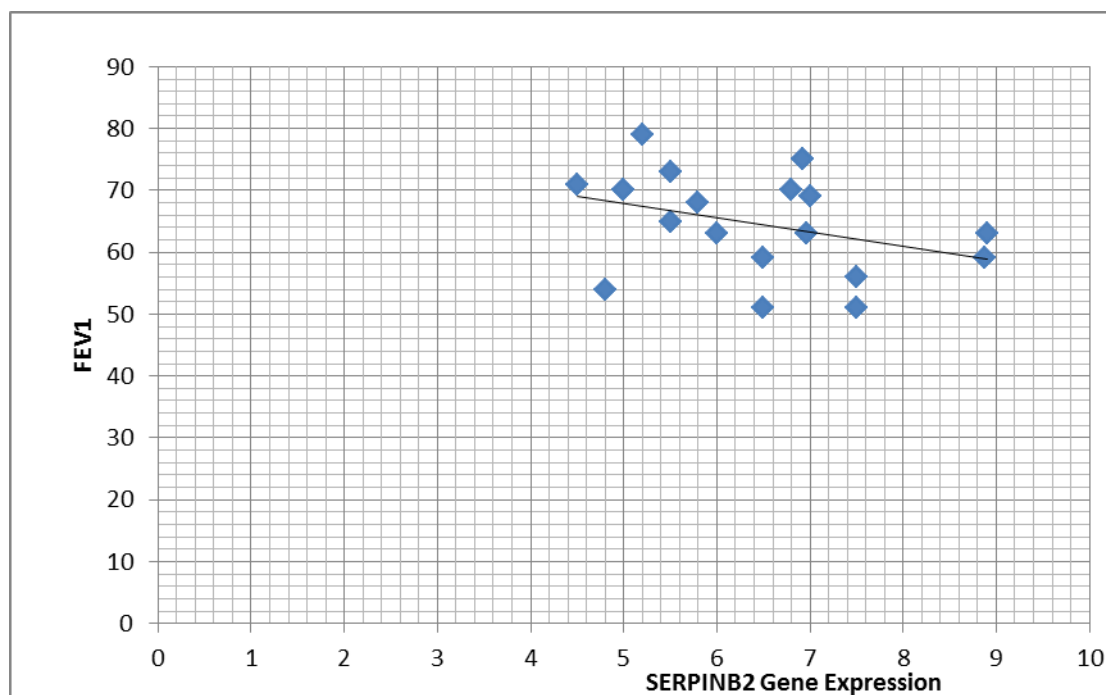


Figure 8. correlation between SERPINB2 mRNA expression ($2^{-\Delta\Delta C_t}$) and FEV1 in severe patients

Table 5. Correlation between SPT, total IgE and lung function tests in asthmatic patients groups

Lung function parameters	SPT (r, P)	Total IgE (r, P)
FEV1%	-0.863(<0.001)	-0.746 (<0.001)
FEV1/FVC%	-0.915(<0.001)	-0.835(<0.001)

$P < 0.05$ is significant. FEV₁, forced expiratory volume 1; FVC, forced vital capacity; SPT, skin prick test (SPT); $2^{-\Delta\Delta C_t}$ delta-delta cycle threshold Gene expression: $2^{-\Delta\Delta C_t}$

Discussion

Bronchial asthma is a diverse disease of different phenotypes. Genetic and environmental factors as variable allergens and medications usage are important factors determining the asthma phenotype and influencing serum IgE levels and bronchial hyper-reactivity [32]. Severe asthma phenotype is associated with more complications as high morbidity, with more work, and school absence, increasing the expenses of the disease as the costs for severe refractory patients are more than double those of patients with controlled asthma

[33]. These patients necessitate additional consideration and further treatment [16].

In this study, severe asthma frequency was high as 44% of asthma patients were severe. However, previous report suggested that severe asthma constitutes 10% of patients with asthma [10]. In another study [34] assessing asthma severity by scoring persistent symptoms revealed that more than 20% of their patients suffering from severe asthma despite their compliance. This difference indicated that severe asthma frequency is increasing over time and the prevalence of asthma varies according to the geographical factors.

The age and sex of patients enrolled in this study in different classes of asthma, were analysed and revealed that severe asthma was more frequently in old male patients. However, [16, 35] found that patients with severe asthma were mostly female. This difference may be insignificant due to rural and urban differences in the study groups.

The atopic bronchial asthma is diagnosed by allergy diagnostic tests as serum Ig E and skin prick test [36]. In this study total I gE was as higher as > 449 IU/ml than control group (18.3 IU/ml). Also, another study [36] observed that 71.43 % of asthmatics had total IgE>150 IU/ml. In addition to the previous study [37] that demonstrated that serum IgE was significantly higher in atopic asthmatics than others. These results indicate that IgE plays an important role in the pathogenesis of allergic asthma and proved the atopic nature of the study groups.

The current study revealed a significant inverse correlation between total I gE and asthma severity ($r -0.86$, $P<0.001$). Other studies, by Borish and Naqvi [38,39] found that the airway hyper responsiveness degree was related to total IgE level. In addition, Razi & Abbass study [40] suggested the use of total IgE level in the classification of asthma severity. In contrast, a previous study [41], revealed absence the correlation between total IgE level and the asthma severity. This might due to patients age variation among different studies [7].

In this study, Total IgE was significantly correlated with SERPINB2 gene expression as IL 13 response gene. This result is consistent with the other studies [36,42] that demonstrated a significant direct association between total IgE and one of allergy associated gene which is IL-4 in asthmatic subjects. These findings may result from the role of the two cytokines IL-4 and IL 13

which induce class switching of B-cells towards IgE synthesis [43].

This research proved a significant correlation between SPT wheal diameters and severity of asthma. This result agreed with the results obtained by a previous study [7] that demonstrated a direct correlation between SPT result and FEV1 value as asthma severity marker. Also, [44, 25] analysis demonstrated that wheal size correlated better with allergen reactivity. However, other studies [41, 45] found no correlation between the wheal size, and severity of asthma. The explanation for these inconsistencies may be the lack of standardized and confirmed measure of the disease severity [46,47].

Many human studies have concentrated their study on subjects with mild-to-moderate asthma [48, 49, 50]. Consequently, the IL-13 expression and its association with severe asthma is uncertain [51]. Thus there is a need to identify its role which may lead to detection of novel therapeutic targets in sever asthmatics patients.

There are several genes have the potential of being used as biomarkers of bronchial asthma discovered by several genomic studies. These include Serpin peptidase inhibitor (SERPINs), Chemokine ligands, mucin 12 (MUC12), proline rich 4 (PRR4) and Carboxypeptidase A3 [31, 52].

In agreement with Woodruff et al [22], IL-13 signature gene (SERPINB2) was upregulated and significantly higher in asthmatics than control group. Also, another study [53] found that SERPINB2 gene expression level was strongly correlated with asthmatic in addition to other genes expression including IL13, IL5, and periostin, as these genes are markers of T_H2-mediated bronchial asthma. However, previous study[30], revealed that the gene

expression of SERPINB2 was similar in asthmatics and healthy control.

In this study, the highest increase of expression of SERPINB2 gene was demonstrated in severe patients than those with mild and moderate asthma. This result is consistent with other studies [30, 31] that revealed by real time PCR a significant upregulation of SERPINB2 airways in patients with severe asthma.

These results confirmed indirectly by previous study [54] which revealed that The sputum IL-13 concentration was higher in patients with severe asthma than healthy control ($P < 0.05$) and SERPINB2 gene is one of IL13 response genes.

The association between SERPINB2 expression level and severity is explained by the action of this gene as it is a serine protease inhibitor [23, 55]. It suppresses plasminogen activators of both tissue and urokinase-types, so prevents plasmin activation and induces fibrin synthesis. The result is thrombin and fibrinogen accumulation of in the respiratory tract which leads to bronchial hyper responsiveness [24].

The finding is explained by the role of SERPINB2 genes in promoting airway inflammation, as Serpin peptidase inhibitor induces inflammatory process, and cell adhesion, which are characteristic features of bronchial asthma [56]. Indeed, SERPINB2 represents a novel mediator of the several airway remodelling induced by IL-13 cytokine [22].

In conclusions, our findings suggest the vital role of airways epithelial cells in pathogenesis of bronchial asthma by their differential gene expression in different asthma groups. In conclusion, gene expression of SERPINB2 gene is detected in sputum cells from patients, and its level of expression correlated with the severity of the

disease. Future studies of other genes profile in IL4/IL13 axis in severe asthma and its association with bronchial asthma control still urgent. In conclusion, the current findings in severe refractory asthma recommend a need for a novel therapy that will be effective in disease modification.

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