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A study of nasal epithelial cell gene expression in a sample of mild to severe asthmatic children and healthy controls

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Abstract

Background: Airway epithelium contributes to the natural history of bronchial asthma through the production of various cytokines and chemokines. The purpose of this study was to assess nasal epithelial cell genes (*TMEM178*, *FKBP5*, *CLCA1*, *SERPINB2* and *periostin*) in childhood asthma and their utility in predicting asthma severity, and atopic status. Seventy asthmatic children were included and further subdivided into mild, moderate and severe persistent asthma together with 30 apparently healthy children as a control group. All children were subjected to medical history taking, clinical examination. Nasal epithelial samples were collected for detection of epithelial cell genes (*TMEM178*, *FKBP5*, *CLCA1*, *SERPINB2* and *periostin*) by real-time PCR.

Results: *TMEM178* showed significant down-regulation in asthmatic children and its expression levels decreased significantly with the progression of asthma severity. *CLCA1*, *SERPINB2* and *periostin* showed statistically significant up-regulation in asthmatic children, whereas *FKBP5* was increased in asthmatic children but with non-significant up-regulation when compared with the control group. Regarding atopic status, relative gene expression levels of *CLCA1*, *SERPINB2* and *periostin* were significantly up-regulated in atopic asthma.

Conclusion: Our findings suggest the role of nasal airways epithelial cells in predicting asthma severity and atopic status, as *TMEM178* expression gained attention as a predictor of asthma severity. *CLCA1*, *SERPINB2* and *periostin* expression were up-regulated not only in asthmatic children, but also in atopic asthma.

Highlights

- *CLCA1*, *SERPINB2* and *periostin* expression were up-regulated not only in airway epithelial cells of asthmatic children but also in atopic asthma.
- *TMEM178* expression gained attention as a predictor of asthma severity.
- Identification of nasal epithelial cell genes of childhood asthma may represent a step forward toward tailored management and therapy.

Keywords: Bronchial asthma, Atopy, Children, PCR

Background

Asthma is a heterogeneous illness, typically characterized by persistent airway inflammation, history of respiratory symptoms such as shortness of breath, wheezes, chest tightness and cough. These characteristics differ over

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time with increased severity along with variable restriction of expiratory airflow, which could later become persistent [1]. The inflammatory pathway starts with the development of type-2 T helper cells (Th2), which are dependent on dendritic cells (antigen presenting cells) and certain cytokines such as IL-4 and IL-13 [2]. Additionally, airway epithelial cells and smooth muscle cells tend to play important roles in influencing or perpetuating the airway reaction to T helper type-2 (Th2) cytokines [3].

While the majority of asthmatic children have minor to severe disease, and can be adequately controlled with standard medications, a minority (around 5%) of children with asthma suffer from a severe uncontrolled disease that carries a significant health and social burden and requires additional, yet, limited treatment options [4]. While asthma is already known to have a large genetic component, several studies indicate that genotype variation contributes significantly to the heterogeneity of asthma phenotype and morbidity [5].

Epithelial genes directly induced in asthma include the transmembrane protein 178 (TMEM178), which is a negative regulator of the nuclear factor of activated T cells (NFAT). Inflammation caused by NFAT is a known actor in asthma pathogenesis [6]. Chloride pathway, calcium activated family member 1 (CLCA1), is associated with mucus hypersecretion, airway hyper reactivity, and hyper eosinophilia in asthma [7], serpin peptidase inhibitor, clade B, member 2 (SERPINB2) (also known as plasminogen activator inhibitor-2) which belongs to the serpin class of proteases and functions through inhibiting plasminogen activation and promoting fibrin formation and deposition. In turn, periostin (POSTN) is an integrin ligand and extracellular matrix protein which functions in cell adhesion, cell motility, and matrix remodeling [8]. FKBP5 is a hsp90 co-chaperone that controls the sensitivity of glucocorticoid receptor (GR) [9]. All these considered genes are markers of airway epithelial cells dysfunction and corticosteroids sensitivity.

Therefore, we aimed to study nasal epithelial cell genes (TMEM178, FKBP5, CLCA1, SERPINB2 and periostin) in childhood asthma and their utility in predicting asthma severity, and atopic status as expression of these genes in lower airway epithelial cells have been reported to be significantly associated with asthma.

Methods

This was a cross-sectional case-control study, with a total of 100 children attending the department of pediatrics in [Benha university], during the period from November 2018 to November 2020. The study gained the approval of [Benha university] local ethical committee. After explanation of the study, informed written consent

was obtained from the parents or caregivers of enrolled children, and the study was conducted according to the principles of Helsinki Declaration [10]. Subjects were divided into two groups:

I—Patient group: 70 asthmatic children (not in acute attack) aged from 6 to 17 years who were diagnosed and graded by severity of asthma [11] into: 1—Mild persistent asthma (symptoms >2 days/week but not daily, night awakenings 3–4/month, short-acting beta agonist use for symptom control (not to prevent exercise induced bronchospasm) >2 days/week but not daily and not more than once on any day, interference with activity is within minor limitation, FEV1 >80%, FEV1/FVC >85%). 2—Moderate persistent asthma (symptoms daily, night awakenings >1/week but not nightly, short-acting beta agonist use for symptom control (not to prevent exercise induced bronchospasm) daily, interference with activity is within some limitation, FEV1 60–80%, FEV1/FVC 75–80%). 3—Severe persistent bronchial asthma (symptoms throughout the day, night awakenings often 7 days/week, short-acting beta agonist use for symptom control (not to prevent exercise induced bronchospasm) several times per day, interference with activity is within extreme limitation, FEV1 <60%, FEV1/FVC <75%). Atopic asthma subjects determined to have atopy by elevated IgE and history of allergic rhinitis.

Exclusion criteria included: use of nasal or systemic steroids within the last 30 days, nasal malformations or tumors, parasitic infestation, acute infectious disease and chronic illness involving congenital heart or lung disease, liver and kidney diseases. The usage of inhaled corticosteroid was not interrupted for this study.

II—Control group included 30 apparently healthy non-atopic, age- and sex-matched children, without personal or family history of asthma, or other allergic conditions.

Study procedures

Enrolled children were subjected to full history taking with paying attention to intermittent cough attacks, expectoration, wheezy dyspnea and chest tightness. Thorough physical examination was carried out in order to detect tachypnea, signs of hyperinflation, extended expiratory phase and expiratory wheeze. Lung functions assessment by spirometry (performed by spirolab III ver. 4.3 SN 311860—Italy) for measurement of pulmonary function were performed before and after bronchodilator therapy (administering a total of 400 mcg of short acting B2-agonist salbutamol in four puffs at 30-s intervals by using a spacer device) [12]. Measurement of forced vital capacity (FVC), forced expiratory volume in 1st second (FEV1) and FEV1/FVC and post-bronchodilator

change in FEV1 that were automatically displayed by the apparatus.

Laboratory investigations were carried out, including complete blood count analyzed by Sysmex Kx-21N with microscopic manual differential count and total serum IgE level measurement by ELISA (DiaMed Eurogen, Turnhout, Belgium). Nasal epithelial cells were collected by brushing the inferior turbinate using a CytoSoft Brush (Medical Packaging Co., Camarillo, Calif, USA). The collected brush was submerged in nuclease-free H₂O and frozen at -80°C until extraction. Cells were identified by hematoxylin and eosin (H&E) staining viewed under light microscopy and counted manually.

RNA extraction

Total RNA was extracted using Direct-zol™ RNA Mini-Prep supplied by (Zymo Research, USA) and quantified by spectrophotometry. cDNA was synthesized using high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, USA). PCR amplification of TMEM178, CLCA1, SERPINB2, periostin and FKBP5 genes and the house keeping gene GAPDH were carried out in separate PCR tubes using gene specific primers [13–18] (Table 1). The relative expression levels were measured in step one real-time PCR system (Applied Biosystems, USA) using QuantiTect® SYBR® Green PCR kit supplied by (Qiagen; Germany). Relative expression of the target genes in each sample was finally detected after normalization to GAPDH expression and calculated as $2^{-\Delta\text{Ct}}$ [19].

Statistical analysis

Data management and statistical analysis were performed using the statistical package for social sciences (SPSS) ver. 23 (IBM, Endicott, Broome County, New York, USA). Numerical data were summarized using means and standard deviations; differences between groups were done using ANOVA or Kruskal–Wallis test. Categorical data were summarized as numbers and percentages, and differences were analyzed with χ^2 (Chi-square) test or Fisher exact when appropriate. Receiver

operating characteristics (ROC) analysis was performed to assess the performance characteristics of TMEM178 expression for asthma severity prediction. The best cut-off point and the corresponding sensitivity and specificity and area under the Curve (AUC) were analyzed. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant).

Results

Our study included 70 asthmatic children: 36 (51.4%) males and 34 (48.6%) females. They were divided according to severity of asthma into mild persistent asthma group that included 34 patients, moderate persistent asthma group that included 26 patients, and severe persistent asthma group that included 10 patients. **Control group** included 30 apparently healthy children: 15 (50%) males and 15 (50%) females. Eosinophils count and IgE in the asthmatic group were significantly higher than in the control group ($P < 0.001$). FEV1, FVC and FEV1/FVC were significantly lower in moderate and severe persistent asthma when compared with mild persistent asthma (Table 2).

For each subject, the cellular composition of the nasal respiratory epithelial sample was determined. For the control group, the mean number of cells per high-power field ($400\times$) was 285 ± 103 , with 98.2% pseudo-stratified columnar ciliated epithelium, 1.54% PMNs, 0.22% squamous cells, and 0.04% eosinophils. For the mild persistent asthma group, the mean number of cells per high-power field ($400\times$) was 235 ± 75 , with 95.4% pseudo-stratified columnar ciliated epithelium cells, 4.06% PMNs, 0.40% squamous cells, and 0.14% eosinophils. For the moderate persistent asthma group, the mean number of cells per high-powered field ($400\times$) was 214 ± 55 , with 94.5% pseudo-stratified columnar ciliated epithelium cells, 5.22% PMNs, 0.11% squamous cells, and 0.17% eosinophils. For the severe persistent asthma group, the mean number of cells per high-power field ($400\times$) was 145 ± 85 , with 91.22% pseudo-stratified columnar ciliated epithelium cells, 8.28% PMNs, 0.15% squamous cells, and

Table 1 Primers and annealing temperature of the genes included in the study

Gene	Forward primer	Reverse primer	An.Tm	References
TMEM178	5'-ACTTATGCCGCCAGTATCTCG-3'	5'-AGGCGCAAAGATGGACCAG-3'	55	[13]
FKBP5	5'-GGATATACGCCAACATGTTCAA-3'	5'-CCATTGCTTTATTGGCCTCT-3'	51	[14]
CLCA1	5'-ATGCTATGAAGGCATTGTTCG-3'	5'-TGGCACCATTGGGGTTCGATTG-3'	54	[15]
SERPINB2	5'-GCTGGAGATGTTAGCATGTTCTTG-3'	5'-GGCTTGGTGGAACTTCAGAAAG-3'	54	[16]
Periostin	5'-GACTCAAGATGATCCCTTT-3'	5'-GGTGCAAAGTAAGTGAAGGA-3'	47	[17]
GAPDH	5'-TGCACCACCAACTGCTTAGC-3'	5'-GGCATGGACTGTGGTCATGAG-3'	57	[18]

TMEM178: transmembrane protein 178, CLCA1: chloride pathway, calcium activated family member 1, SERPINB2: serpin peptidase inhibitor, clade B, member 2, GAPDH; glyceraldehyde 3-phosphate dehydrogenase

Table 2 Global subject characteristics

Variables	Control group (N = 30)	Mild persistent (N = 34)	Moderate persistent (N = 26)	Severe persistent (N = 10)	ANOVA	P
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Age (years)	10.7 ± 3.3	10.4 ± 3.2	11 ± 3.2	9.8 ± 2.4	0.417	0.741
Sex						
Male N (%)	15 (50%)	20 (58.8%)	10 (38.5%)	6 (60%)	$\chi^2 = 2.8$	0.419
Female N (%)	15 (50%)	14 (41.2%)	16 (61.5%)	4 (40%)		
Eosinophils cells × 10 ⁹ /L	213.5 ± 54.4	251.6 ± 87.0	412.4 ± 34.1	549.8 ± 44.2	96.6	< 0.001*
Serum IgE IU/mL	156.3 ± 34.6	482.8 ± 120.3	733.8 ± 147.8	1019.4 ± 137.0	119.4	< 0.001*
FEV1\FVC	89.4 ± 1.44	87.5 ± 4.02	78.4 ± 2.89	65.3 ± 7.75	184.4	< 0.001*
FEV1%	95.05 ± 2.09	84.4 ± 1.56	70.2 ± 4.14	52.0 ± 6.51	352.9	< 0.001*
FVC%	92.7 ± 2.51	88.0 ± 4.75	72.3 ± 5.00	55.5 ± 7.20	43.1	< 0.001*

FVC: forced vital capacity, FEV1: forced expiratory volume in 1st second, IgE: Immunoglobulin E

*P < 0.05 is significant

0.35% eosinophils. Epithelial cells represented greater than 93% of the total cells isolated in all groups. The differences between the analyzed groups were not statistically significant.

Relative gene expression levels of TMEM178 are decreased in asthmatic children when compared with control group (significant down-regulation in asthma). And as regards asthma severity, there was statistically significant decrease in TMEM178 expression levels with the increase in asthma severity. Meanwhile, relative gene expression levels of CLCA1, SERPINB2 and periostin showed statistically significant up-regulation in the epithelial airway cells of asthmatic children when compared with controls, but there were no statistically significant

differences between different degrees of asthma severity. Regarding FKBP5 relative gene expression, it was increased in asthmatic children but with no statistically significant difference when compared with the control group (non-significant up-regulation), and as regards asthma severity, there were no statistically significant differences between different groups (Table 3).

Regarding performance characteristics of TMEM178 expression ($2^{-\Delta Ct}$) as a predictor to detect asthma using 5.426 as cut off values show sensitivity of 86.7% and specificity of 92.9% and the best cut off values for $2^{-\Delta Ct}$ of the expressed TMEM178 gene with the highest specificity and sensitivity for discrimination between different degrees of asthma severity were determined

Table 3 Expression of nasal epithelial cell genes in the studied groups

Variables	Control group (N = 30)	Mild persistent (N = 34)	Moderate persistent (N = 26)	Severe persistent (N = 10)	Kruskal–Wallis test (χ^2)	P
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE		
TMEM178	6.251 ± 0.149	4.703 ± 0.12 (0.75 fold)	3.521 ± 0.181 (0.56 fold)	1.293 ± 0.295 (0.2 fold)	77.59	p1 = 0.001* p2 = 0.001* p3 = 0.001* p4 = 0.001* p5 = 0.001* p6 = 0.001*
FKBP5	0.47 ± 0.049	0.496 ± 0.047 (1.055 fold)	0.608 ± 0.059 (1.29 fold)	0.64 ± 0.087 (1.36 fold)	5.38	p1 = 0.747 p2 = 0.063 p3 = 0.104 p4 = 0.121 p5 = 0.17; p6 = 0.89
CLCA1	0.026 ± 0.006	0.047 ± 0.005 (1.81 fold)	0.049 ± 0.006 (1.88 fold)	0.064 ± 0.014 (2.46 fold)	13.58	p1 = 0.002* p2 = 0.003* p3 = 0.046* p4 = 0.77 p5 = 0.28; p6 = 0.34
SERPINB2	0.067 ± 0.01	2.01 ± 0.13 (30 fold)	2.33 ± 0.22 (34.78 fold)	2.66 ± 0.28 (39.7 fold)	63.86	p1 = 0.001* p2 = 0.001* p3 = 0.001* p4 = 0.45 p5 = 0.06; p6 = 0.42
Periostin	0.022 ± 0.005	0.061 ± 0.016 (2.77 fold)	0.115 ± 0.039 (5.23 fold)	0.097 ± 0.038 (4.41 fold)	19.3	p1 = 0.001* p2 = 0.001* p3 = 0.006* p4 = 0.17 p5 = 34; p6 = 0.97

p1: mild and control, p2: moderate and control, p3: severe and control, p4: mild and moderate, p5: mild and severe, p6: moderate and severe, TMEM178: transmembrane protein 178, CLCA1: chloride pathway, calcium activated family member 1, SERPINB2: serpin peptidase inhibitor, clade B, member 2

*P < 0.05 is significant

Table 4 Performance characteristics of TMEM178 expression ($2^{-\Delta Ct}$) in discrimination between different degrees of asthma severity

Variables	AUC (95% CI)	Cutoff points	Sensitivity (%)	Specificity (%)	P value
Control from asthmatics	0.967 (0.938–0.995)	5.426	86.7	92.9	< 0.001(HS)
Mild from moderate	0.870 (0.779–0.961)	4.176	79.4	80.8	< 0.001(HS)
Mild from severe	0.991 (0.970–1)	2.982	97.1	100	< 0.001(HS)
Moderate from severe	0.950 (0.877–1)	2.617	92.3	90	< 0.001(HS)

CI: confidence interval, TMEM178: transmembrane protein 178

Table 5 Performance characteristics of FKBP51, CLCA1, SERPINB2 and periostin expression ($2^{-\Delta Ct}$) to detect asthma

Variables	AUC (95% CI)	Cutoff points	Sensitivity (%)	Specificity (%)	P value
FKBP51	0.410 (0.291–0.529)	0.566	43.3	50	> 0.05
CLCA1	0.272 (0.150–0.393)	0.0747	20	70	< 0.001*
SERPINB2	0.083 (0.052–0.141)	1.220	20	20	< 0.001*
Periostin	0.234 (0.127–0.342)	0.0314	26.7	60	< 0.001*

CI: confidence interval, CLCA1: chloride pathway, calcium activated family member 1, SERPINB2: serpin peptidase inhibitor, clade B, member 2

*P < 0.05 is significant

and analyzed. Using 2.982 as a cut off value for $2^{-\Delta Ct}$ of TMEM178 expression, the sensitivity and specificity of TMEM178 to differentiate mild asthmatics from severe asthmatics was 97.1% and 100%, respectively. Additionally, TMEM178 mRNA expression ($2^{-\Delta Ct}$) at a cutoff value of 2.617 differentiated moderate asthmatics from severe asthmatics with a sensitivity of 92.3% and a specificity of 90% (Table 4).

Regarding performance characteristics of FKBP51, CLCA1, SERPINB2 and periostin expression ($2^{-\Delta Ct}$) to detect asthma; using 0.566, 0.0747, 1.220 and 0.0314 as cutoff values, respectively, shows sensitivity of 43.3%, 20%, 20% and 26.7% respectively and specificity of 50%, 70%, 20% and 60%, respectively (Table 5).

Regarding atopic status, relative gene expression levels of CLCA1, SERPINB2 and periostin were significantly up-regulated in atopic more than non-atopic asthma while TMEM178 and FKBP5 relative gene expression levels showed no statistically significant differences between atopic and non-atopic asthma (Table 6).

Regarding performance characteristics of TMEM178, FKBP51, CLCA1, SERPINB2 and periostin expression ($2^{-\Delta Ct}$) to detect atopic status; using 4.273, 0.573, 0.063, 2.325 and 0.041 as cutoff values, respectively, shows sensitivity of 50%, 50%, 53.1%, 84.4% and 81.3%, respectively, and specificity of 63.2%, 50%, 81.6%, 92.1% and 84.2%, respectively (Table 7).

Discussion

The nasal airway is lined by basal, ciliated and secretory epithelial cells similar to bronchial airway epithelium [20]. As such, the nasal airway provides a readily

Table 6 Comparison between atopic and non-atopic asthma regarding nasal epithelial cell genes expression

Variable	Atopic asthma (N = 32)	Non-atopic asthma (N = 38)	MWU test (z)	P
	Mean ± SE	Mean ± SE		
TMEM178	4.09 ± 0.24	3.53 ± 0.23	1.75	0.081
FKBP5	0.55 ± 0.057	0.57 ± 0.041	0.27	0.786
CLCA1	0.063 ± 0.006	0.039 ± 0.005	2.88	0.004*
SERPINB2	2.96 ± 0.13	1.59 ± 0.095	6.01	< 0.001*
Periostin	0.153 ± 0.034	0.03 ± 0.003	4.89	< 0.001*

TMEM178: transmembrane protein 178, CLCA1: chloride pathway, calcium activated family member 1, SERPINB2: serpin peptidase inhibitor, clade B, member 2

*P < 0.05 is significant

accessible counterpart to the bronchial airway and may represent much of the pathology present in the asthmatic bronchial airway. Supporting this was a study of roughly 2300 genes expression in nasal and bronchial airway brushings, which revealed a close association between these 2 airway sites [21]. In another study, differences in epithelial gene expression between the upper and lower airway epithelium largely disappear in patients with allergic rhinitis, with or without asthma [22].

The decision to perform our research on upper rather than the lower airway epithelium was inspired by its non-invasive sampling, which is more suitable in young children. It assists recruiting true pulmonary disease-free controls, which is not easy in researches using bronchoscopic specimen [23]. It also allows patients

Table 7 Performance characteristics of TMEM178, FKBP51, CLCA1, SERPINB2 and periostin expression ($2^{-\Delta C_t}$) to detect atopic status

Variables	AUC (95% CI)	Cutoff points	Sensitivity (%)	Specificity (%)	P value
TMEM178	0.622 (0.490–0.754)	4.273	50	63.2	0.081
FKBP51	0.481 (0.340–0.622)	0.573	50	50	0.786
CLCA1	0.701 (0.579–0.824)	0.063	53.1	81.6	0.004*
SERPINB2	0.919 (0.852–0.987)	2.325	84.4	92.1	<0.001*
Periostin	0.842 (0.742–0.941)	0.041	81.3	84.2	<0.001*

CI: confidence interval, TMEM178: transmembrane protein 178, CLCA1: chloride pathway, calcium activated family member 1, SERPINB2: serpin peptidase inhibitor, clade B, member 2

* $P < 0.05$ is significant

with severe or uncontrolled asthma to be investigated without discontinuing their long-term inhaled corticosteroid asthma therapy, as nasal deposition can be considered negligible with the tools used in this study [24].

In the current study, TMEM178 relative gene expression levels showed significant down-regulation in asthmatic children when compared with control. In addition, TMEM178 expression levels decreased significantly with severity of asthma progression. Consistent with our results, Patel et al. [6] stated that TMEM178 expression decreases with the increase in asthma severity giving the known role of TMEM178 as a negative regulator of nuclear factor of activated T cells (NFAT). They hypothesized that TMEM178 plays a significant role in NFAT-induced severe asthma inflammation.

To estimate the performance characteristics of TMEM178 expression ($2^{-\Delta C_t}$) for asthma severity prediction, the best cutoff point for $2^{-\Delta C_t}$ of the expressed gene and the responsiveness (sensitivity and specificity) were analyzed, to our knowledge, for the first time. The expression of TMEM178 had a strong potential to predict different degrees of asthma severity [25].

In the present study, relative gene expression levels of CLCA1, SERPINB2 and periostin showed statistically significant up-regulation in epithelial airway cells of children with asthma, when compared with controls. Regarding asthma severity, relative gene expression levels of CLCA1, SERPINB2 and periostin showed no statistically significant differences between different groups. These results run in accordance with Mertens et al. [26] who stated that inflammation of allergic airways in asthma was characterized by signature of an airway epithelial gene consisting of periostin, CLCA1 and SERPINB2; this gene signature is suggested as a method for classification of asthma cases into phenotypes Th2-high and Th2-low. In another study, CLCA1, serpinB2 and periostin were up-regulated in asthmatics compared to healthy controls [8].

Sterk and Lutter [27] found that gene expression for IL13, periostin and CLCA1 were significantly up-regulated in asthmatic patients compared with control subjects. Additionally, greater expressions (up-regulation) of the IL-13 response gene signature (CLCA1, periostin and SERPINB2) were found in severe asthmatics compared to healthy controls [28].

CLCA1 was part of the CLCA (calcium-activated chloride channel regulator) family and performs an important role in the formation of goblet cell mucus from the respiratory tract epithelium. CLCA1 is also a main regulator for innate immune responses, in addition to regulation of mucus secretion. The secreted form of CLCA1 can serve as a signaling ligand that activates monocytes and alveolar macrophages in a dose-dependent manner, increase the levels of pro-inflammatory cytokines and chemokines (IL-1 β , IL-6, IL-8 and TNF- α), and promote infiltration of inflammatory cells into the pulmonary epithelium and propria lamina. Accordingly, hCLCA1 in humans and its ortholog mCLCA1 in mice are suggested as a biomarker of inflammatory airway diseases (IAD) [29].

SERPINB2 is a protein coding gene which suppresses a serine protease and inhibits plasminogen activators and promotes formation of fibrin [30]. Indeed, SERPINB2 gene expression that occurs during T helper type 2 cell allergic inflammation contributes to goblet cell metaplasia of airway epithelium and can modify epithelial-mesenchymal signaling, resulting in increased sub epithelial fibrosis [8]. The overall impact is airway inflammation and bronchial hyper-responsiveness and epithelial remodeling, which are the main features of asthma [31].

Periostin is an IL-4 and IL-13 mediated extracellular matrix protein in epithelial airway cells and lung fibroblasts [32]. In airway biopsies, the immunolocalization of periostin to the sub-epithelial zone indicates that it is basolaterally secreted into the underlying matrix. Periostin may interact with matrix proteins from this position

to promote cell motility or epithelial–mesenchymal signaling that generate airway remodeling [8].

Herein, FKBP5 expression was increased in asthmatic children compared to control, however; the difference did not reach the border line of statistical significance. Likewise, FKBP5 showed no statistical variation between different asthma severity groups. In contrast, Singhanian et al. [28] reported that FKBP5 was more pronounced in severe asthma compared to healthy controls. In the present study, the asthmatic children were not on nasal or systemic steroids that induce FKBP5 expression, which explain this discrepancy.

Steroids cause expressions of FKBP5 by activating glucocorticoid receptor elements [33]. In addition, studies in vitro have shown that FKBP5 reduces the hormone binding affinity and decreases the amount of activated glucocorticoid receptor translocation to the cell nucleus [34]. In an in vitro bioassay the expression FKBP5 mRNA in the peripheral blood was used to assess human corticosteroids sensitivity. Furthermore, FKBP5 was also reported as a biomarker for glucocorticoid responsiveness and as a possible glucocorticoid resistant asthma mediator [8].

Regarding to atopic status, relative gene expression levels of CLCA1, SERPINB2 and periostin were significantly up-regulated in atopic asthma than non-atopic, while there were no statistically significant differences between atopic and non-atopic asthma regarding both TMEM178 and FKBP51 expression levels. In line with our findings, a previous study reported that SERPINB2 gene expression can serve as a back-up marker of Th2-driven inflammation in respiratory epithelial cells, which is considered as the main mechanism of atopic asthma pathogenesis [8].

Two major researches have highlighted apparently conflicting opinions on the association between periostin with eosinophilia. An observational analysis showed periostin would be a better predictor of inflammation of eosinophil in the airway [35], while another cross-sectional study [36] revealed that a strong correlation between blood eosinophils and sputum eosinophils was discovered, but the interaction between serum periostin and eosinophils in sputum was not demonstrated. The most likely clarification for these obvious variations is that the former study investigated the periostin sensitivity and specificity to identify airway eosinophilia using a composite score rather than specifically comparing the relationship between blood periostin and a sputum eosinophil count, as performed in the later study. This indicates that blood periostin may provide additional value as a diagnostic tool of Th2 inflammation, but is not directly comparable with either blood or sputum eosinophilia. Such discrepancy in the calculation of biomarkers in various studies further highlights the heterogeneity and complexity of airway inflammation and

the possible need of measuring several biomarkers in conjunction with one another to accurately try and determine a phenotype.

A cluster analysis reported 70 genes expression that distinguished Th2-high and Th2-low subjects, including IL13, IL5, periostin, CLCA1, and SERPINB2. Compared with Th2-low subjects, Th2-high individuals were more likely to be atopic and have elevated eosinophil counts. They stated that both atopy status and eosinophilic blood levels were closely related to Th2-high pattern of nasal gene expression, regardless of asthma status. These findings indicate that Th2 activation of atopic or systemically allergic airway is a part of the physiological foundation of asthma. Identifying of Th2-high subjects on the basis of nasal brushings is possible and may have a huge effect on both biomedical and clinical research [37].

Conclusion

The challenge of sampling the lower airway in children has been circumvented in this study by using noninvasive methods of sample collection. Our findings suggested the possible role of nasal airway epithelial cells in predicting asthma severity and atopic status, as TMEM178 expression gained attention as a predictor of asthma severity. CLCA1, SERPINB2 and periostin expression were up-regulated not only in airway epithelial cells of asthmatic children but also in atopic asthma. However, further large-scale studies are needed for validation of this study.

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Authors' contributions

OB contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results and contributed to the final manuscript. OM contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results and contributed to the final manuscript. RS contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results and contributed to the final manuscript. AS contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate

The current study was approved by the Medical Research Ethical Committee of the Faculty of Medicine, Benha University. All subjects were informed about the procedures and the aim of the study, and informed written consent was

obtained from the parents or caregivers of enrolled children. The committee's reference number not available.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare that they have no competing interests.

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