STUDY OF EPITHELIAL CELL GENES IN A SAMPLE OF EGYPTIAN CHILDREN WITH BRONCHIAL ASTHMA

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Abstract

Objective: to assess epithelial cell genes (TMEM178, FKBP5, CLCA1, SERPINB2 and Periostin) in childhood asthma and their utility in predicting asthma severity, level of control and atopic status. Study design: 70 stable asthmatic children included who were further subdivided into mild, moderate and severe persistent asthma, also subdivided into controlled and partially to uncontrolled asthma and 30 apparently healthy children. All children were subjected to medical history taking, clinical examination, complete blood count, serum IgE, and 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 with no statistically significant differences between different degrees of asthma severity. FKBP5 showed neither statistically significant difference with control group nor between different degrees of asthma severity. TMEM178, CLCA1, SERPINB2 and Periostin were significantly up regulated in controlled asthma. While, FKBP5 was significantly up regulated in partially to uncontrolled group. CLCA1, SERPINB2 and Periostin were significantly up regulated in atopic asthma while TMEM178 and FKBP5 showed no statistically significant differences between atopic and non-atopic asthma. Conclusion: TMEM178 expression gained attention as a predictor of asthma severity. CLCA1, SERPINB2 and Periostin expression were upregulated not only in airway epithelial cells of asthmatic children but also in controlled and atopic asthma, whereas FKBP5 was upregulated in partially to uncontrolled asthma.

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statistically significant up regulation in asthmatic children with no statistically significant differences between different degrees of asthma severity. FKBP5 showed neither statistically significant difference with control group nor between different degrees of asthma severity. TMEM178, CLCA1, SERPINB2 and Periostin were significantly up regulated in controlled asthma. While, FKBP5 was significantly up regulated in partially to uncontrolled group. CLCA1, SERPINB2 and Periostin were significantly up regulated in atopic asthma while TMEM178 and FKBP5 showed no statistically significant differences between atopic and non-atopic asthma.

Conclusion: TMEM178 expression gained attention as a predictor of asthma severity. CLCA1, SERPINB2 and Periostin expression were upregulated not only in airway epithelial cells of asthmatic children but also in controlled and atopic asthma, whereas FKBP5 was upregulated in partially to uncontrolled asthma.

Key wards; asthma control; Bronchial asthma; atopy; PCR

IV: Introduction

Asthma is a heterogeneous illness, characterized typically by persistent airway inflammation, history of respiratory symptoms such as shortness of breath, wheeze, chest tightness and cough which differ in severity over time along with variable restriction of expiratory airflow. The limiting airflow could later become persistent.[1] The inflammatory pathway starts with the development of type 2 (Th2) T helper cells 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 cytokines.[3]

While the majority of asthmatic children have minor to severe disease and can be adequately controlled with standard medicines, a minority (around 5%) of children with asthma suffer from a severe uncontrolled disease that carrying a significant health and socio-burden, and requires additional but still limited treatment options.[4] While asthma is already known to have 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 known actor in asthma pathogenesis.[6] Chloride pathway, calcium activated family member 1 (CLCA1) associated with mucus hyper-secretion,airway hyperreactivity, and hyper-eosinophilia in asthma[7] serpin peptidase inhibitor, clade B, member 2 (SERPINB2) (also known as plasminogen activator inhibitor-2) which belongs the serpin class of proteases and functions to inhibiting plasminogen activation and promoting fibrin formation and deposition. In turn, Periostin (POSTN) which is an integrin ligand and extracellular matrix protein with function in cell adhesion, cell motility, and matrix remodeling.[8] FKBP5 which is a hsp90 co-chaperone that control the sensitivity of glucocorticoid receptor (GR).[9]

Therefore, we aimed to study epithelial cell genes (TMEM178, FKBP5, CLCA1, SERPINB2 and Periostin) in childhood asthma and their utility in predicting asthma severity atopic status and level of control.

Subjects and Methods:

This was a cross-sectional case-control study with a total 100 children attending department of pediatrics in the University Hospitals of Benha during the period from November 2018 to December 2019. The study gained the approval of Benha University's 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 done according to principles of Helsinki Declaration.[10] Subjects were divided into two groups:

I – Patients group: 70 stable asthmatic children aged from 6-17 years who were diagnosed and graded by severity of asthma and control standard according to the Global Initiative for Asthma (GINA) Guidelines[1] into: mild, moderate and severe persistent bronchial asthma. Asthmatic children were further subdivided according to level of control into controlled and partially to uncontrolled asthma. **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.

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

Methods:

Enrolled children were subjected to full history taking with paying particular attention to intermittent cough attacks, expectoration, wheezy dyspnea and chest tightness. In order to detect tachypnea, signs of hyper-inflation, extended expiratory phase and expiratory rhonchi thorogh physical examination was carried out. lung functions assessment by Spirometry (performed by Erich jaejre 95 GmbH 1992-1997 for measurement of pulmonary function) were performed before and after bronchodilator therapy (administrating of total of 400 mcg of short acting B2-agonist salbutamol in four puffs at 30-second intervals by using a spacer device) [11] and measurement of Forced vital capacity (FVC), forced expiratory volume in 1st second (FEV1) and FEV1/FVC and post bronchodilator change in FEV1 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.

RNA extraction

Total RNA was extracted using Direct-zol RNA MiniPrep supplied by (Zymo Research, U.S.A) according to the manufacturer's instructions. Nanodrop 2000 (Thermofisher scientific, USA) was used for assessment of purity and concentration of the extracted RNA. Extracted RNA was then stored at -80degC for further processing.

Reverse transcription

Extracted RNA was reverse transcribed using high-capacity cDNA reverse transcription kit, Thermo Fisher Scientific, USA. Extracted RNA (1µg diluted to the correct volume 10 µl by Nuclease- free H2O) were added to 10 µL of 2X RT master mix. The 2X RT master mix contained 2 µL 10X RT Buffer, 0.8 µL 25X dNTP Mix (100 mM), 2 µL 10X RT Random Primers, 1 µL MultiScribe Reverse Transcriptase, 1 µL RNase Inhibitor 3.2 µL Nuclease-free H2O. The thermal cycler; Biorad, USA, program was 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes and 4°C for[?].

Real time PCR

PCR amplification of TMEM187, CLACA1, SERPINB2, POSTN and FKBP51 genes and the house keeping gene GAPDH were carried out in separate PCR tubes using gene specific primersas follows: TMEM187: forward 5'-ACTTATGCCGCCAGTATCTCG-3''; reverse 5'-AGGCGCAAAAGATGGACCAG-3'[12] ; CLACA1: forward 5'-ATGGCTATGAAGGCATTGTCG -3'; reverse 5'-TGGCACATTGGGGTCGATTG -3' [13] ; SERPINB2: forward 5'-GCTGGAGATGTTAGCATGTTCTTG-3'; reverse 5'-GGCTTGGTGGAACACTTCAGAAAG-3' [14] ; POSTN: forward 5'-GACTCAAGATGATTCCCTTT -3'; reverse 5'-GGTGCAAAGTAAGTGAAGGA-3'[15] ; FKBP51: forward 5'-GGATATACGCCAACATGTTCAA-3'; reverse 5'-CCATTGCTTTATTGGCCTCT-3'[16] ; and GAPDH: forward 5'-TGCACCACCAACTGCTTAGC-3'; reverse 5'-GGCATGGACTGTGGTCATGAG-3'[17] QuantiTect(r) SYBR(r) Green PCR kit supplied by Qiagen; Germany was used for amplification. The amplification reaction contained 10 µL of 2x QuantiTect SYBR Green PCR Master Mix, 2 µL of cDNA, 0.5 µL of the forward primer, 0.5 µL of the reverse primer and Nuclease-free H2O up to 20 µL final volume. The thermal profile of the PCR reaction was 95°C for 15 min., 40 cycles of 94°C for 15 sec, annealing temperature of the gene (55°C for TMEM187, 54°C for CLACA1, 54°C for SERPINB2, 47°C for POSTN, 51°C for FKBP51 and 57°C for GAPDH) for 30 sec and 72°C for 30 sec.

Melting curve analysis using StepOne software (Applied Biosystems, USA) was performed to ensure specificity of the amplification products. Relative expression of the target genes in each sample were finally detected after normalization to GAPDH expression and calculated as 2ˆ-ΔCt.[18]

Statistical Analysis

Statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) vs. 23 (IBM, Endicott, Broome_County, New York, United States). Numerical data were summarized using means and standard deviations; differences between groups were done using ANOVA or Kruskall-Wallis test. Categorical data were summarized as numbers and percentages, 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 cutoff point and the corresponding sensitivity and specificity and Area Under the Curve (AUC) were analyzed. 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, IgE in the asthmatic group were significantly higher than the control group (p<.001). FEV1, FVC and FEV1/FVC were significantly lower in moderate and severe persistent asthma when compared with mild persistent one **(Table 1).**

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-powered field (400×) was 285 ±103, with 98.2% epithelial cells, 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×) was 235 ± 75, with 95.4% epithelial 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×) was 214 ± 55, with 94.5% epithelial 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×) was 145 ± 85, with 91.22% epithelial cells, 8.28% PMNs, 0.15% squamous cells, and 0.35% eosinophils. Epithelial cells represented greater than 93% of the total cells isolated in all groups. The differences between analyzed groups were not statistically significant.

Relative gene expression levels of TMEM178 decreased in asthmatic children than control group (significant down regulation in asthma) and as regards to asthma severity, there was statistically significant decrease in TMEM178 expression levels with the increase of asthma severity. While, relative gene expression levels of CLCA1, SERPINB2 and Periostin showed statistically significant up regulation in the epithelial airway cells of children with asthma when compared with control, 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 control group (non-significant up regulation) and as regards asthma severity, there were no statistically significant differences between different groups. **(Table 2)**

Regarding performance characteristics of TMEM178 expression (2ˆ-[?]CT) as a predictor for asthma severity, the best cut off values for 2ˆ-[?]CT of the expressed TMEM178 gene with the highest specificity and sensitivity for discrimination between different degrees of asthma severity were determined and analyzed. Using 2.982 as a cut off value for 2ˆ-ΔCt of TMEM178 expression, the sensitivity and specificity of TMEM178 to discriminate mild asthmatics from severe asthmatics was 97.1 % & 100 %, respectively. Additionally, TMEM178 mRNA expression (2ˆ-ΔCt) at a cut off value of 2.617 discriminated moderate asthmatics from severe asthmatics with a sensitivity of 92.3 % and a specificity of 90 %. **(Table 3)**

Regarding to asthma control level, relative gene expression levels of TMEM178, CLCA1, SERPINB2 and

Periostin were significantly up regulated in controlled group. While FKBP5 was significantly increased (up regulated) in partially to uncontrolled group **(table 4)**

Regarding to atopic status, relative gene expression levels of CLCA1, SERPINB2 and Periostin were significantly up regulated in atopic than non-atopic asthma while TMEM178 and FKBP5 relative gene expression levels showed no statistically significant differences between atopic and non-atopic asthma.

(Table 5)

Discussion

The epithelium of the nasal airway is inhabited by basal, ciliated, and secretory epithelial cells Similar to epithelium of bronchial airway.[19] As such, the nasal airway provides an readily accessible counterpart to the bronchial airway and may represent much of the pathology present in the bronchial asthmatic airway. In the Support of this, roughly 2300 genes expression study in nasal and bronchial airway brushings revealed a close association between these 2 airway sites**.** [20]

The decision to perform our research on upper rather than the lower airway epithelium was inspired by its non-invasive sampling, which is readily applicable in young children. It helps recruiting true pulmonary disease-free controls, a circumstance which is simply not possible in researches using bronchoscopic specimen [21]**.**It also helps patients with severe or uncontrolled asthma phenotypes to be investigated without stopping their long-term inhaled corticosteroid therapy for asthma, because nasal deposition can be considered as negligible with the tools used in this study.

[22]

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 severity of asthma 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 (2ˆ-[?]Ct) for asthma severity prediction, the best cut off point for 2-ΔCt 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 asthma phenotypes, indicating that TMEM178 expression may be of considerable use in distinguishing between different asthma phenotypes**.**[23]

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 control. 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,**[24] who stated that inflammation of allergic airways in asthma was characterized by signature of an airway epithelial gene consisting of POSTN, CLCA1, and SERPINB2; this gene signature Th2 is suggested as a method for classification of asthma cases into phenotypes Th2- high and Th2-low**.** Also, **Woodruff et al.,**[8] found that CLCA1, serpinB2 and periostin, were up-regulated in asthmatics compared to healthy control.

Sterk and Lutter [25] found that gene expression for IL13, periostin, and CLCA1 were significantly up regulated in asthmatic patients compared with control subjects. Additionally,***Singhaniaet al.,*** [26] found greater expression (up-regulation) of the IL-13 response gene signature (CLCA1, POSTN, SERPINB2) in severe asthmatics compared to healthy controls.

Herein, FKBP5 expression was increased in asthmatic children compared to control, however; the difference didn't reach the border line of statistical significance. Likewise, FKBP5 showed no statistical variation between different asthma severity groups. In contrast,***Singhania et al*** ., [26]reported that FKBP5 was more

pronounced in severe asthma relative to healthy controls. In the present study, the asthmatic children were not on nasal or systemic steroid in order to induce FKBP5 expression, which explain this discrepancy.

Regarding to asthma control level, relative gene expression levels of TMEM178, CLCA1, SERPINB2 and Periostin were significantly up regulated in controlled group. While, FKBP5 was significantly increased (up regulated) in partially to uncontrolled group. **Woodruff et al.,**[8] found that the CLCA1, serpinB2 and Periostin were shown to have up-regulated in asthma. Corticosteroid therapy down-regulated expression of those three genes and dramatically up-regulated expression of FKBP51. Although high baseline expression of CLCA1, serpinB2 and Periostin was associated with good clinical response to corticosteroids, a weak response associated with high expression of*FKBP51* . By using cultured airway epithelial cells, they observed that IL-13 improved the CLCA1, Periostin, and serpinB2 expression, an effect that was inhibited by corticosteroids. Taken together, these finding indicate that airway epithelial cells in asthma have a distinct activation profile and recognize direct and cell-autonomous effects on airway epithelial cells relevant to corticosteroid therapy on that relate to treatment responses.

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 TMEM187 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.

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A clustering analysis by **Poole et al.,**[27] reported 70 genes expression distinguished TH2-high and TH2-low subjects, including IL13, IL5, Periostin, CLCA1, and SERPINB2. Compared with TH2-low subjects, TH2-high persons were more likely to be a topic, atopic asthma\sout, rhinitis, and elevated serum 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 finding indicated 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

Conclusion:

Our findings suggested the vital role of nasal airways epithelial cells in the pathogenesis of asthma by their differential gene expression in different asthma groups. Herein, TMEM178 expression gained attention as a predictor of asthma severity suggesting its significant value in the discrimination between different asthma phenotypes. CLCA1, SERPINB2 and Periostin expression were upregulated in airway epithelial cells of asthmatic children, however; no significant differences between different groups of asthma severity were observed. Regarding to asthma control level, TMEM178, CLCA1, SERPINB2 and Periostin were significantly up regulated in controlled group compared to partially to uncontrolled asthma group. Whereas, FKBP5 was upregulated in partially to uncontrolled asthma group. Regarding to atopic status, CLCA1, SERPINB2 and Periostin were significantly up regulated in atopic than non-atopic asthma while TMEM178 and FKBP5 showed no statistically significant differences between atopic and non-atopic asthma.

V: **Acknowledgement: NO**

Vi: Impact statement;

What is known about this subject:

- Asthma is a heterogeneous disease, characterized by chronic airway inflammation and airway hyper responsiveness and has a strong genetic component
- The airway epithelium is a complex physical barrier, playing an intrinsic role in innate and adaptive immunity and contributes to the natural history of asthma.

What this paper adds:

- TMEM178 expression gained attention as a predictor of asthma severity with a significant value in the discrimination between different asthma phenotypes.
- CLCA1, SERPINB2 and Periostin expression were upregulated not only in airway epithelial cells of asthmatic children but also in controlled and atopic asthma.
- Identification of nasal epithelial cell genes of childhood asthma may represent a step forward toward tailored management and therapy.

Vii: Refrences

1-**GINA:** Global initiative for asthma**.** Global strategy for asthma managements and prevention. 2019, available: (accessed march 1, 2020).

2- **Van Buul AR, Taube C.** Treatment of severe asthma: entering the era of targeted therapy. Expert Opin Biol Ther. 2015; 15(12):1713-25.

3-**Cockcroft DW, Davis BE.** Mechanisms of airway hyperresponsiveness.. J Allergy Clin Immunol 2006, 118:551–559.

4- **Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al.** International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014; 43:343–373.

5-**Mathias RA.** Introduction to genetics and genomics in asthma: genetics of asthma. Adv Exp Med Biol 2014; 795: 125–155.

6-Patel N.B, Lorena A. Ostilla L.A, Cuervo-Pardo L, Berdnikovs S and Chiarella S.E. Gene expression of *TMEM178* , which encodes a negative regulator of NFATc1, decreases with the progression of asthma severity. Patel *et al. Clin Transl Allergy 2019; 9(38*):1-3.

7-**Nakanishi A, Morita S, Iwashita H, Sagiya Y, Ashida Y, Shirafuji H, et al.** Role of gob-5 in mucus overproduction and airway hyperresponsiveness in asthma. Proc Natl Acad Sci USA. 2001; 98:5175–5180.

8- **Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al.** Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci USA 2007;104:15858–15863.

9-Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psycho neuroendocrinology **2009** ; 34: 186-195.

10- **Puri KS, Suresh KR, Gogtay NJ, Thatte UM.** Declaration of Helsinki, 2008: implications for stakeholders in research. J Postgrad Med 2009;55(2):131.

11**-Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al;** ATS/ERS Task Force. Standardization of spirometry. Eur Respir J 2005;26:319-38.

12-**Decker CE, Yang Z, Rimer R, Park-Min KH, Macaubas C, Mellins ED, et al.** Tmem178 acts in a novel negative feedback loop targeting NFATc1 to regulate bone mass. Proc Natl Acad Sci U S A. 2015; 112(51):15654-9.

13-**Mo Y, Zhang K, Feng Y, Yi L, Liang Y, Wu W, et al.**Epithelial SERPINB10, a novel marker of airway eosinophilia in asthma, contributes to allergic airway inflammation. Am J Physiol Lung Cell Mol Physiol. 2019; 316(1): L245-L254.

14-**Shijian Lv, Na Wang, Hong Lv, Jieqiong Yang, Jianwei Liu, Wei-Ping Li, et al.** The Attenuation of Trophoblast Invasion Caused by the Downregulation of EZH2 Is Involved in the Pathogenesis of Human Recurrent Miscarriage. Mol Ther Nucleic Acids. 2019; 14: 377–387.

15-**Herro R, Shui JW, Zahner S, Sidler D, Kawakami Y, Kawakami T, et al.** LIGHT-HVEM signaling in keratinocytes controls development of dermatitis. J Exp Med. 2018; 215(2):415-422.

16-**Shafi AA, Cox MB, Weigel NL.** Androgen receptor splice variants are resistant to inhibitors of Hsp90 and FKBP52, which alter androgen receptor activity and expression. Steroids 2013; 78(6):548-554.

17-**Cicinnati VR, Shen Q, Sotiropoulos GC, Radtke A, Gerken G, Beckebaum S.** Validation of putative reference genes for gene expression studies in human hepatocellular carcinoma using real-time quantitative RT-PCR. *BMC Cancer* 2008;8: 350.

18-**Schmittgen T and Livak K.** Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008; 3: 1101- 1108.

19-**Harkema JR, Carey SA, Wagner JG.** The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol Pathol 2006;34:252-69

20-Sridhar S, Schembri F, Zeskind J, Shah V, Gustafson AM, Steiling K, et al. Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. BMC Genomics 2008;9: 259.

21-Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, et al. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med 2007; 176: 858–864.

22-Glover W, Chan HK, Eberl S, Daviskas E, Verschuer J. Effect of particle size of dry powder mannitol on the lung deposition in healthy volunteers. Int J Pharm 2008; 349: 314–322

23-**Šimundić A..** Measures of Diagnostic Accuracy: Basic Definitions. EJICC **2009** ;19: 203- 211.

24-Mertens T.C, van der Does A.M, Kistemaker L.E, Ninaber D.K, Taube .C, Hiemstra P.S. *Cigarette smoke differentially affects IL-13-induced gene expression in human airway epithelial cells.*

Physiol Rep 2017; 5 (13):13347

25**-Sterk J. R and Lutter R.** Asthma phenotyping: TH2-high, TH2-low, and beyond . American Academy of Allergy, Asthma & Immunology 2014; 395-396

26-Singhania. A, Rupani .H, Jayasekera .N, Lumb .S, Hales .P, Gozzard .N et al. Altered Epithelial Gene Expression in Peripheral Airways of Severe Asthma PLoS ONE 2017; 12(1): 0168680.

27-Poole .A, Urbanek .C, Eng .C, Schageman .J, Jacobson .S, O'Connor.B.P et al. Dissecting childhood asthma with nasal transcriptomics distinguishes subphenotypes of disease. J allergy clin immunol 2014; 133(3):671-678.

Viii: Tables

Table (1): Global subject characteristics

Variables	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)	**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%)		

Variables	Variables	Control group (N=30)	Mild persistent (N=34)	Moderate persistent (N=26)	Severe persistent (N=10)	ANOVA	P
Eosinophils cells x10^9/L	**Eosinophils cells x10^9/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	**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	**FEV1\ FVC**	89.4 ± 1.44	87.5±4.02	78.4 ±2.89	65. 3 ±7.75	184.4	<0.001*
FEV1 %	**FEV1 %**	90.05 ± 2.09	84.4 ±1.56	70.2 ±4.14	52.0 ±6.51	352.9	<0.001*
FVC%	**FVC%**	92.7 ± 2.51	88.0 ±4.75	72.3 ±5.00	55.5 ±7.20	43.1	<0.001*

*P<0.05 is significant

Table (2): Expression of epithelial cell genes in the studied groups

Variables	**Control group (N=30)**	**Mild persistent (N=34)**	**Moderate persistent (N=26)**	**Severe persistent (N=10)**	**Κρυσκαλ Ωαλλις τεστ (χ2)**	**P**
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE		
TMEM178	6.251±0.149	4.703±0.12	3.521 ±0.181	1.293± 0.295	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	0.608 ±0.059	0.64± 0.087	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	0.049± 0.006	0.064 ± 0.014	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	2.33± 0.22	2.66±0.28	63.86	p1=0.001* p2=0.001* p3=0.001* p4=0.45 p5=0.06; p6=0.42

Variables	Control group (N=30)	Mild persistent (N=34)	Moderate persistent (N=26)	Severe persistent (N=10)	Κρυσκαλ Ωαλλις τεστ (χ2)	P
Periostin	0.022±0.005	0.061±0.016	0.115± 0.039	0.097 ± 0.038	19.3	p1=0.001*; p2=0.001* p3=0.006*; p4=0.17 p5=34; p6=0.97

*P<0.05 is significant, p1: mild and control, p2: moderate and control, p3: severe and control, p4: mild and moderate,

p5: mild and severe, p6: moderate and severe

Table (3): Performance characteristics of TMEM178 expression (2ˆ-[?]Ct) in discrimination between different asthma phenotypes

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

Table (4): comparison between controlled and partially to uncontrolled asthmatics regarding epithelial cell genes expression.

Variable	Controlled asthma (N=48)	partially to uncontrolled asthma (N=22)	MWU test (z)	P
	Mean± SE	Mean ± SE		
TMEM178	4.28±0.17	2.72±0.272	4.9	<0.001*
FKBP5	0.51±0.043	0.67±0.049	2.18	0.03*
CLCA1	0.056±0.005	0.039±0.007	1.97	0.048*
SERPINB2	2.59±0.128	1.415±0.084	5.07	<0.001*
Periostin	0.114±0.024	0.025±0.004	4.21	<0.001*

*P<0.05 is significant

Table (5): comparison between atopic and non-atopic asthma regarding 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

Variable	Atopic asthma (N=32)	Non atopic asthma (N=38)	MWU test (z)	P
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*

*P<0.05 is significant

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`tables.docx` available at https://authorea.com/users/323685/articles/452246-study-of-epithelial-cell-genes-in-a-sample-of-egyptian-children-with-bronchial-asthma