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IL-8 gene expression and bronchial asthma phenotypes in children

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Abstract

Asthma is a common chronic illness among school children, where different cytokines, including IL-8 play a role in its pathogenesis. IL-8 induces chemotaxis and migration of immune cells, especially neutrophils to the site of inflammation. IL-8 level was significantly increased in sputum of severely asthmatic patients, but can it be linked to some asthma phenotypes. Our aim of the study was to detect the IL 8 gene expression in different asthma phenotypes and to determine its relation to asthma severity. This case control study included 320 subjects (160 asthmatic and 160 matched controls) aged from 5 to 16 years old in Beni-Suef governorate. IL-8 gene expression was assessed by a real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and studied regarding its level in cases versus controls and its relations to severity, phenotype and other laboratory parameters. IL-8 gene expression was statistically higher in asthmatic cases (P<0.001) and was significantly correlated to the phenotype (presence of other allergy as urticaria and drug eruption) and degree of asthma symptoms (r=0.869, P<0.001), FEV_1(r=0.757, P<0.001) and serum IgE level (r=0.789, P<0.001). IL-8 gene expression level is increased with the degree of severity in asthmatic children and can be looked for in certain asthma phenotypes especially in presence of other atopic manifestation.

Keywords: Asthma, Atopic, Cytokines, IL-8 Gene expression.

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Introduction

Asthma is a heterogeneous disease, characterized by intermittent airway inflammation and respiratory symptoms that vary over time and in intensity, together with variable expiratory airflow limitation that may later become persistent.[1] Airway epithelium can produce chemokines and cytokines that recruit and activate immune cells at the site of injury.[2]

IL-8 initiates the acute and chronic inflammatory process; it has a role in the pathogenesis of several chest diseases like asthma, acute respiratory distress syndrome (ARDS), and respiratory syncytial virus infection.[3] Increased IL-8 level was noted in broncho-alveolar fluid, sputum, and

endobronchial biopsies of asthmatic patients, while a higher serum levels in severely asthmatic patients was reported, suggesting its role in asthma severity.[4,5] IL-8 is a strong chemotactic product that stimulates recruitment of neutrophils, mononuclear phagocytes, mast cells, and T cells.[6] It induces a series of physiological responses required for migration and phagocytosis such as increases in intracellular Ca^{2+} and exocytosis (e.g., histamine release).[7] Genetic studies showed association between IL8 and susceptibility to inflammatory diseases.[8] IL-8 level was one of the inflammatory markers that distinguished controlled from uncontrolled asthma patients and was inversely correlated to forced expiratory volume in the 1st second (FEV_1)[4], it increases in the serum[5]and sputum[9] of patients with severe asthma and was positively associated with sputum neutrophilia.[9] Martinez-Nunez et al., noted the relevant role of microRNA dysregulation in the control of IL-8 expression in asthma.[10]

Airway inflammation resulted in Airway remodeling that is a significant player in asthma pathogenesis. The absence of efficient epithelial wound healing mechanism lead to production of growth factors implicated in fibrosis, vasculogenesis and neurogenesis. Moreover chronically damaged/repairing epithelial mesenchymal trophic unit is the source of a range of growth factors and cytokines that support chronic inflammation.[11]

IL-8 is one of cytokines, also known as neutrophil chemotactic factor, which leads to chemotaxis and phagocytosis of neutrophils, it promote angiogenesis,[7] and has a proinflammatory role.[12] It can be secreted by any toll-like receptors cells such as macrophages that are the first cells to release IL-8 to recruit other cells.[13]

In Asthma T-helper type 2 (Th2)-cells produce IL-4 and IL-13, which induces the release of IL-8 from human bronchial epithelial cells. Various reports have also described the capacity of Th-9 and Th-17 cells to induce the release of proinflammatory cytokines including IL-8 in asthma.[14] The aim of our study was to evaluate the IL 8 gene expression in different asthma phenotypes and its relation to asthma severity.

Patients and Methods

Methods

This study was conducted at Beni-Suef University Hospital. The study protocol was reviewed and approved by the ethical committee of the Faculty of Medicine, Beni-Suef University (FWA00015574; July 3, 2018). Written informed consents were taken from guardians of both cases and controls for participation in this study. After explaining the study in simple language suitable for children, willing to provide a blood sample by a child was taken as his assent. The sample size of the patients was calculated using power and sample size calculator (version 3.0.0.34), with power of 80% and α error of 0.05.

Study population

This study included 320 subjects. Of these, 160 were clinically diagnosed symptomatic bronchial asthma patients, selected to fill 4 equal groups, 40 patients each [Mild intermittent (Group I) and mild (Group II), moderate (Group III), severe persistent attacks (Group IV) of asthma] according to Global Initiative for Asthma (GINA) (2017) recommendations[1]. In addition, 160 subjects were age and sex matched to serve as controls. Children of all groups were selected between 5 and 16 years of age, children with other chronic/recurrent chest problems or upper respiratory tract infection within the last 2 weeks before sampling were excluded. All the participants were subjected to full history (including age, sex, duration of illness, family history of allergic diseases, consanguinity and possible precipitating factors) taking from their guardian. They also were investigated by Spirometer to assess the degree of obstruction, Chest X-ray to exclude other chest conditions, complete blood count (CBC) to assess eosinophil count, IgE level using Electro Chemi Luminescence (ECL) technology for immunoassay analysis (Cobas e 411/ Roche, USA) and IL8 mRNA expression level.

RNA isolation and RTQ-PCR

Using RNA blood extraction kit (QIAamp®, Qiagen, Germany), RNA was isolated from 2 ml blood, collected on EDTA. For Complementary

DNA (cDNA) synthesis, high-capacity cDNA reverse transcriptase kit (Applied Biosystem, USA) was used according to the manufacturer's protocol, then cDNA stored at -20 °C until used.

The mRNA expression level of IL8 (CXCL8) gene was measured by quantitative RT-PCR using the pre-developed assay gene expression set for IL8, GenBank reference sequence number (Hs00174103_m1, Applied Biosystems, USA Catalog no.4453320) and B actin as the house keeping (internal control) gene (Applied Biosystems, USA) in combination with TaqMan™ Gene Expression Master Mix (catalogue no. 4370048, Applied Biosystems, USA). RTQ-PCR reactions were performed, in the final reaction volume of 25 µl., and the amplification reaction protocol used was by heating for 2 min at 50 °C then 10 min at 95 °C, followed by 40 cycles of amplification at 95 °C for 15 s and 60 °C for 1 min. Analysis was done using DTlite Real-Time PCR System instrument and DTmaster Sequence Detection Software (Agro Diagnostica , Russia). The expression levels of IL8 gene were expressed in the form of cycle threshold (CT) level, relative quantification (RQ) was calculated using the ΔΔCT equation, (RQ) = 2−ΔΔCT, and the results were normalized relative to B actin. A negative control without template was included in each experiment.

Statistical analysis

Statistical analysis was done using statistical package for social sciences (SPSS), computer software (version 22, IBM software, USA). All data were expressed as means (± standard deviation) or count (percent). Normality of continuous variables was tested using Shapiro test, showed that they were non-normally distributed. Nonparametric Kruskall Wallis test was used to elucidate significance among group means of IL8, followed by Bonferoni's post-hoc test to compare mean values pairwise. Chi square test was used to test differences between qualitative variables followed by Bonferoni's post-hoc test to compare mean values pairwise. Mann Whitney test was used to assess the differences between groups. Spearman rank correlation to assess the relation between IL8 Gene expression and other factors. Differences were considered significant at $p < 0.05$. Total p. values for Kruscall Wallis and Chi square tests were calculated and written.

Results

This study included 320 subjects, 160 of them were clinically diagnosed asthmatics [(mean age 9.44±3.6 years, 94 (58.8%) males, mean height 138.9±15.5 cm and weight 29.6±12.4 kg] and 160, age and sex matched controls [mean age 9.68±3.5 years, 84 (52.5%) males, mean height 142.65±14.5 cm and weight 32.3±9.8 kg]. (Table 1)

Spirometer was done for all patients and controls (Table 1); FEV_1 and FEV_1/FVC ratio were obtained. Ninety-two patients (57.5%) had normal results (FEV_1 > 80 % of predicted), 68 patients had FEV_1/FVC ratio below 70%.

Table 1. Characterization of asthmatic children compared to non-asthma control subjects.

Parameter		Cases	Controls	*P* value
Age		9.44±3.6	9.68±3.5	NS
Sex	Males	94 (58.8%)	84 (52.5%)	NS
	Females	66 (47.5%)	76 (47.5%)	
Height (Mean±SD), cm		138.9±15.5	142.65±14.5	0.025*
Weight (Mean±SD), Kg		29.6±12.4	32.3±9.8	0.027*
Peak expiratory flow		71.2%±22.2	90.3%±3.7	<0.001*
FEV_1> 80 %,		92 patients (57.5%)	100%	<0.001*

P>0.05 is not significant (NS).

The mean value of IL-8 gene expression was significantly higher in the patients group than the control group (*P* <0.001). The patients' 4 subgroups were studied separately, showed that IL8 gene expression level gradually increased with the severity of the condition with the highest levels in the severe persistent group and there was a significant difference between the tested groups (Table 2).

Regarding the patients' pattern of symptoms, 100 asthmatic patients had a paroxysmal pattern (repeated attacks of wheezy and dyspnea separated by periods without symptoms) whose IL-8 gene expression was (46.94±4.49) which was significantly lower (*P* <0.001) than the remaining 60 asthmatic patients who had a continuous pattern of symptoms (almost always wheezy) and IL-8 gene expression (186.77±15.36).

Table 2. IL8 Gene expression level in different groups.

Group	N	Mean	SD	Median	*P* value
Control	160	4.91	3.32	4	<0.001*
Patients	160	99.38[a]	10.48	63.500	
Mild intermittent	40	11.6[a]	8.62	8.5	
Mild persistent	40	54.53[ab]	2.78	54.5	
Moderate persistent	40	76.89[ab]	6.96	76	
Severe persistent	40	254.5[abcd]	100.33	257.5	

Test: Kruscall Wallis followed by Bonferroni's post hoc analysis for pairwise comparisons

a: significantly different from controls b: significantly different from mild intermittent

c: significantly different from mild persistent d: significantly different from severe persistent. *P*<0.05 is significant.

A significant higher level of IL8 expression (*P*<0.001) was noted with the presence of other atopic symptoms with the highest value with presence of drug eruption with rhinitis [Rhinitis (84.66), urticaria (148.62), eczema (166.23), conjunctivitis (159.43) and drug eruption with rhinitis (172) [Table 3], while this elevation was not noted with repeated infection, history of Animal contact, food allergy or exercise induced symptoms.

Regarding the pulmonary function test there was a significant negative association between FEV_1 and IL-8 gene expression level (Table 4).

Table 3. IL8 Gene expression level in different Asthma Phenotypes.

Atopic symptoms	N	Mean	Std. Error of Mean	Std. Deviation	Median	*P* value
No atopic symptoms	9	8.12	2.38	7.13	5.50	<0.001
Rhinitis	111	84.66[a]	8.28	87.20	56.00	
Urticaria	13	148.62[a]	30.60	110.34	88.00	
Eczema	10	166.23[a]	51.73	163.57	120.50	
Conjunctivitis	7	159.43[a]	60.54	160.17	77.00	
Drug eruption+rhinitis	10	172.00[a]	39.19	123.93	131.00	

Test: Kruscall Wallis followed by Bonferoni's post hoc analysis for pairwise comparisons

a: significantly different from no topic symptoms. *P*<.05 is significant.

Table 4. The relation between forced expiratory volume (FEV_1) and IL-8 gene expression**.**

FEV_1	N	Mean	SD	Median	*P* value
Normal (> 80% of predicted)	92	33.060	22.520	42.500	<0.001*
60-80% of predicted	30	76.893[a]	6.9596	76.000	
<60% of predicted	38	254.500[ab]	100.3325	257.500	

Test: Kruscall Wallis followed by Bonferroni's post hoc analysis for pairwise comparisons

a: significantly different from normal (FEV1>80% of predicted). b: significantly different from 60-80%. *P*<0.05 is significant.

This study revealed significant positive correlation between the degree of asthma severity regarding [severity of upper respiratory symptoms (r=0.77), pattern of symptoms (r=0.823), frequency of the symptoms (r=0.869), number of previous hospitalization (r=0.751), number of previous ICU admission (r=0.577), number of used controllers (r=0.866), presence of eosinophilia (r=0.788) and elevation of serum IgE (r=0.789)] and IL-8 gene expression level with *P*<0.001 for all] (Table 5).

Table 5. Correlation between the degree of asthma severity and IL-8 gene expression level.

Asthma severity parameters	correlation coefficient	*P* value
Severity of upper respiratory symptoms	0.770	<0.001*
Pattern of symptoms	0.823	<0.001*
Frequency of the symptoms	0.869	<0.001*
Number of previous hospitalization	0.751	<0.001*
Number of previous ICU admission	0.577	<0.001*
Number of used controllers	0.866	<0.001*
Eosinophilia	0.788	<0.001*
Elevation of serum IgE	0.789	<0.001*

P<0.05 is significant.

Discussion

In this study, IL-8 gene expression level showed a statistically significant increase in asthmatic patients as compared to the control group. Furthermore, there was a significant difference between patients with paroxysmal versus patients with continuous pattern of symptoms and those with mild versus severe symptoms regarding IL-8 gene expression level. As previous studies suggested, IL-8 might participate extensively in the development of airway inflammation and hyper-responsiveness in asthma.[15] Our findings are in concordance to Charrad et al., (2017),[6] who found that there was significant elevation of IL-8 expression at both protein and mRNA levels in asthmatic children compared to control subjects (*P*<0.0001, *P* = 0.004; respectively) and noted a higher level of IL-8 mRNA in subjects with moderate to severe asthma.[6] Also, one study on adult asthmatic patient reported that the circulating chemokines like IL-8 were significantly higher in asthmatics.[15]

Similarly, Shannon et al., (2008)[16] reported that IL-8 was increased in severe asthmatics compared to moderate cases (*P* < 0.001 for each comparison).[16] Moreover, Meyer et al., (2014)[16] compared serum level of IL-8 between two clusters; patients with higher eosinophil cationic protein (ECP) serum levels, higher numbers of circulating eosinophils, lower FEV_1 values, and better response to asthma therapy (as cluster 1) versus patients with lower ECP serum levels, lower numbers of circulating eosinophils, higher FEV_1 values, and poorer response to asthma therapy (as cluster 2) and noted that serum level of IL-8 was higher in cluster 1.[17]

Concerning the correlation between degree of asthma severity and IL-8 gene expression, the present study revealed a statistical

significance positive correlation between the degree of asthma severity regarding (Severity of upper respiratory symptoms, pattern and frequency of the symptoms, number of previous hospitalization and previous ICU admission, number of used controllers, presence of eosinophilia and elevation of serum IgE) and IL-8 gene expression level (*P*<0.001 for all).

Regarding different Asthma phenotypes, significant higher level of IL8 expression (*P*<0.001) was noted with the presence of other atopic symptoms with the highest value with presence of drug eruption with rhinitis, urticaria, eczema, conjunctivitis and drug eruption with rhinitis. This was also reported by other studies.[4,9,17,20] However, this elevation was not noted with repeated infection, history of Animal contact, food allergy or exercise induced symptoms.[17,20]

Regarding the correlation of IL-8 with pulmonary function parameters represented by forced expiratory volume (FEV_1), the current study showed that low $FEV_1$and lower FEV_1/FVC were significantly correlated with higher IL-8 gene expression (r= 0.757, *P*<0.001). our results are in agreement with these of Alwakil et al., (2011) who reported a negative correlation between serum IL-8 level and FEV_1 in patients with bronchial asthma,[18] and Huang et al., (2016) who reported that IL-8 was negatively associated with FEV_1, FEV_1 as a percent of predicted value, and FEV_1/FVC in patients with asthma, and acute exacerbation of other chronic obstructive pulmonary diseases.[19] In addition, Zhang and Bai (2017) reported that serum IL-8 level in uncontrolled asthmatics was significantly higher than that of the controls and showed a significant negative relationship with forced expiratory volume/forced vital capacity (FEV_1/FVC) ratio, and of FEV_1.[20]

Finally, a recent study by Marc-Malovrh et al., (2020) noted that asthmatic patients with accelerated FEV_1 decline (a decline > 30 ml per year) had increased IL-8 in induced sputum than controls [21]. However, whether IL8 is a driver of the inflammatory process in asthma or a result of this process or has a dual role with amplification loops still need to be determined.

In conclusion, IL-8 gene expression was significantly higher in pediatric asthmatics versus normal controls, significantly correlated to asthma symptoms, severity and some of the different asthma phenotypes. The increase in serum IL-8 gene expression was negatively correlated with spirometry parameters (FEV_1, FEV_1/FVC) and positively correlated with more continuous pattern of symptoms and significantly noted in presence of other atopic manifestations.

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Author Contributions

MH Interpret the patients data, design the experiment, writing and revising the manuscript. GT Design the experiment, interpretation of patients data and revising the manuscript. MM Revised the methodology part of manuscript andinterpretation of patients data. EM Design the experiment, interpretation of patients data, writing and revising the manuscript. NAD Design the experiment, interpretation of patients data, writing and revising the manuscript. All authors have read and approved the manuscript

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the ethical committee of the Faculty of Medicine, Beni-Suef University (FWA00015574; July 3, 2018).

Informed consent

Written informed consents were taken from guardians of both cases and controls for participation in this study. After explaining the study in simple language suitable for children, willing to

provide a blood sample by a child was taken as his assent.

References

1. Global Strategy for Asthma Management and Prevention report. (2019) Definition, description and diagnosis of asthma. 16-26.
2. Gandhi VD, Vliagoftis H. (2015) Airway epithelium interactions with aeroallergens: role of secreted cytokines and chemokines in innate immunity. *Frontiers in Immunology*, 6:147. doi:10.3389/fimmu.2015.00147.
3. Hieldebrand F, Stuhrmann M, van Griensven M, et al. (2007) Association of IL-8- 251A/T polymorphism with incidence of Acute Respiratory Distress Syndrome (ARDS) and IL-8 synthesis after multiple traumas. *Cytokine;* 37(3): 192–199
4. Hosoki K, Ying S, Corrigan C, et al. (2015) Analysis of a Panel of 48 Cytokines in BAL Fluids Specifically Identifies IL-8 Levels as the Only Cytokine that Distinguishes Controlled Asthma from Uncontrolled Asthma, and Correlates Inversely with FEV_1. *PLoS ONE* 10(5): e0126035. doi:10.1371/journal. pone.0126035
5. Silvestri M, Bontempelli M, Giacomelli M, et al. (2006) High serum levels of tumour necrosis factor-a and interleukin-8 in severe asthma: markers of systemic inflammation?. *ClinExp Allergy*. 36(11):1373–1381
6. Charrad R, Kaabachi W, Rafrafi A, et al. (2017) IL8 Gene Variants and Expression in Childhood Asthma. Lung. 195(6): 749-757.
7. Galvao KN, Pighetti GM, Cheong SH et al. (2011) Association between interleukin-8 receptor-α (CXCR1) polymorphism and disease incidence, production, reproduction, and survival in Holstein cows. *Journal of dairy science*. 94(4): 2083-91.
8. Li K, Yao S, Liu S, et al. (2009) Genetic polymorphisms of interleukin 8 and risk of ulcerative colitis in the Chinese population. *ClinChimActa*. 405(1): 30–34
9. Maes T, Cobos FA, Schleich F, et al. (2016) Asthma inflammatory phenotypes show differential microRNA expression in sputum. *Journal of Allergy and Clinical Immunology.* 137(5): 1433-46.
10. Martinez-Nunez RT, Bondanese VP, Louafi F, et al. (2014) A microRNA network dysregulated in asthma controls IL-6 production in bronchial epithelial cells. *PLoS One.* 9(10): 111659.
11. Stephen T. Holgate. (2009) Airway remodeling. Asthma and COPD 2nd In: Barnes P(ed) edn. *Elsevier*; (7) P: 83-97.
12. Dommisch H, Staufenbiel I, Schulze K, et al. (2015) Expression of antimicrobial peptides and interleukin-8 during early stages of inflammation: An experimental gingivitis study. *Journal of periodontal research.* 50(6): 836-45.
13. Hans M, Hans VM. (2011)Toll-like receptors and their dual role in periodontitis: a review. *Journal of oral science.* 53(3): 263-71.
14. Monteseirín J. (2009) Neutrophils and asthma. *J Investig Allergol Clin Immunol.* 19(5): 340-354.
15. Hasan HA, Hiba A, Ismaeel A. (2013) Risk factors that are associated with interleukin-8 level in Iraqi asthmatic patients. *Asian J Pharm Clin Res.* 6, 245-248.
16. Shannon J, Ernst P, Yamauchi Y, et al. (2008) Differences in airway cytokine profile in severe asthma compared to moderate asthma. *Chest.* 2008; 133(2): 420-6.
17. Meyer N, Nuss SJ, Rothe T, et al. (2014) Differential serum protein markers and the clinical severity of asthma. *J Asthma Allergy.* 7: 67-75.
18. Alwakil IM, Al Kabeer AM, Kabil TH, et al. (2011) Serum Interleukin-6 and Interleukin-8 in bronchial asthma. *AAMJ.* 9(3): 385-96.
19. Huang AX, Lu LW, Liu WJ et al. (2016) Plasma inflammatory cytokine IL-4, IL-8, IL-10, and TNF-α levels correlate with pulmonary function in patients with asthma-chronic obstructive pulmonary disease (COPD) overlap syndrome. *Medical science monitor: international medical journal of experimental and clinical research.* 22: 2800-8.
20. Zhang J, Bai C. (2017) Elevated Serum Interleukin-8 Level as a Preferable Biomarker for Identifying Uncontrolled Asthma and Glucocorticosteroid Responsiveness. *Tanaffos* 16(4): 260-269.
21. Marc-Malovrh M, Camlek L, Škrgat S, et al. (2020) Elevated eosinophils, IL5 and IL8 in induced sputum in asthma patients with accelerated FEV1 decline. *Respiratory Medicine*. 10: 105875.
22. Mahler DA, Huang S, Tabrizi M, et al. (2004) Efficacy and safety of a monoclonal antibody recognizing interleukin-8 in COPD: a pilot study. *Chest* 126(3), 926–934.
23. Gaga M, Nair PK, Hargreave F. (2010) SCH527123, a novel treatment option for severe neutrophilic asthma. *Am. J. Respir. Crit. Care Med.* 181, A6763.

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