Bronchial asthma: clinical phenotypes and endotypes and their relation with glucocorticoids circadian rhythm and parasympathetic activity

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Introduction Asthma is a heterogeneous disease and presents in different clinical patterns 'phenotypes' as a result of diverse pathobiological background 'endotypes'.

Objectives The aim of this study was to study serum interleukin-13 (IL-13) levels and the frequency of (*IL-13*) *+1923C/T* gene polymorphism in Egyptian children with asthma and to study glucocorticoids circadian rhythm in nocturnal asthma.

Patients and methods The frequency of (*IL-13*) *+1923C/T* gene polymorphism genotypes was determined in 114 asthmatic Egyptian children and compared with a matched group of 152 healthy controls using PCR. Serum IL-13 and cortisol a.m. and p.m. concentrations in serum were assessed using enzyme linked immunosorbent assay.

Results Serum IL-13 was found to be significantly higher in asthmatic patients when compared with the control group ($P<0.0001$). In the asthmatic group, forced expiratory volume in 1 s showed a significant negative correlation with serum IL-13 ($\rho=-0.2$, $P=0.03$), whereas peripheral blood eosinophilic % showed a significant positive correlation with serum IL-13 ($\rho=0.18$, $P=0.05$). No statistically significant differences were found between asthmatic patients and controls in IL-13 C1923T genotype frequency. A significantly lower serum cortisol pm was found in asthmatic patients with nocturnal symptoms when compared with those without nocturnal symptoms ($P<0.0001$).

Conclusion Serum IL-13 is significantly higher in asthmatic patients when compared with controls. (*IL-13*) *+1923C/T* gene polymorphism is not a risk factor for development of asthma in Egyptian children. Nocturnal symptoms in some asthmatic patients can be partly attributed to lower serum cortisol level at night.

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Introduction

Asthma is a heterogeneous chronic inflammatory disease with reversible airway obstruction [1–3].

Asthma phenotype refers to 'observable characteristics' that can be observed and measured [4]. However, 'endotype' refers to the underlying pathophysiologic mechanism [5,6].

Studies of interleukin-13 (*IL-13*) *+1923C/T* gene in asthma showed diverse results [7].

Nocturnal asthma is attributed to the circadian rhythms of inflammatory mediators, hormones, and parasympathetic nervous system activity [8].

This study was conducted to investigate (*IL-13*) *+1923C/T* gene as a risk factor for asthma in children, to determine serum levels of IL-13 in asthmatic children, and to explore the relation between glucocorticoid circadian rhythm and nocturnal asthma.

Patients and methods

This cross-sectional study included 114 children with asthma, 68 male and 46 female, with a mean age of 10 years. They were recruited from allergy and pulmonology clinics, Mansoura University Children Hospital, Mansoura, Egypt, from 2013 to 2016. Asthma was diagnosed according to The Global Initiative for Asthma recommendations [1], based on clinical asthma symptoms and pulmonary function tests (PFTs). Patients with no history of corticosteroid treatment in the last 4 weeks, no history of antihistamine treatment in the last 3 months, no history of montelukast treatment in the last 2 weeks, and no history of respiratory tract infection in the last 4 weeks were enrolled in the study. The control group consisted of 152 healthy individuals, 85 male and 67 female, with a mean age of 10 years, matched in age and sex with patients and recruited from general pediatrics clinics Mansoura University Children Hospital, Mansoura, Egypt. All controls were healthy, free from history of asthma-like symptoms, having no positive family history of allergy

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or asthma, and having a normal forced expiratory volume in 1 s (FEV1). The characteristics of the studied groups have been presented in Table 1. The study protocol was approved by the Ethical Committee of Mansoura Faculty of Medicine, Egypt, on 11 July 2013 with code number MD/16. Informed consent was obtained from the parents of all patients.

Serum level of IL-13 was assessed using commercial enzyme linked immunosorbent assay (ELISA) kits from R&D Systems Inc. (Minneapolis, Minnesota, USA).

Serum cortisol was measured at 9 a.m. and 9 p.m. for patients and controls. It was measured with ELISA using the Calbiotech Inc. (CBI) cortisol ELISA kit (Calbiotech Inc., Spring Valley, CA, USA).

FEV1 was measured with a spirometer (Spirobank II basic, MIR Medical International Research, Roma, Italy); the highest of the three successive measurements was taken. For patients and controls, PFTs were performed at 9 a.m. and at 9 p.m. as well. Reference values were adjusted according to the recommendation of American Thoracic Society standards of acceptability and reproducibility [9]. The predicted values for each participant was calculated according to the data entered to the device before the maneuver, including age, sex, weight, and height.

Genomic DNA was extracted from whole venous EDTA blood using the GeneJET whole blood genomic DNA purification mini-kits (lot 00138029; Thermo Scientific, Lithuania, UK) and stored at −20°C until use. The genotypes of (*IL-13*) *+1923C/T* single nucleotide polymorphisms (SNPs) were analyzed using the PCR-restriction fragment length polymorphism according to the method of Li *et al.* [10]. The sequences of the sense and antisense primers were 5′-AAT GAG ACA GTC CCT GGA AAG-3′ and 5′-CCG CCT ACC CAA GAC ATT T-3′, respectively (prepared by Eurofins Genomics, Ebersberg bei München, Germany). The restriction enzyme used was BsaAI (Fig. 1).

The activity of the parasympathetic system was evaluated by performing ECG for all patients and controls twice, once at 9 a.m. and the other at 9 p.m., to assess heart rate variability.

Mean heart rate

Mean heart rate value is measured on the entire (ECG) recording. Mean heart rate is measured in beats per minute (bpm).

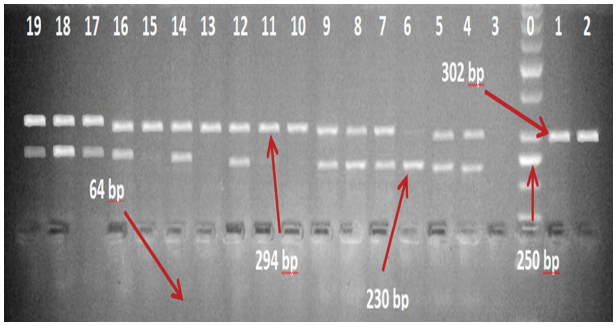
Mean NN

Mean NN is a mean heart beat interval value measured on entire ECG recording (NN means normal-to-normal beats). It is called the mean RR interval when it is derived from ECG recording (N-N interval=R-R interval). Mean NN is measured in milliseconds (ms) [11].

Statistical analysis

The Hardy–Weinberg equilibrium was estimated. Data were analyzed using Statistical Package for

Figure 1



Genotyping of *IL-13 C1923T* rs1295686 by agarose gel electrophoresis. Lane 1, 2: PCR amplified product at 302 base pair (bp). Lane 0: GeneRuler 50 bp DNA Ladder. Lanes: 4, 5, 7-9, 12, 14 and 16-19 and 12-15 = *CT* genotype (three bands at 294, 230, and 64 bp). Lanes: 6= *CC* genotype (two band at 230 and 64 bp), Lane 10, 11, 13 and 15=*TT* genotype (single band at 294 bp).

Table 1 Clinical characteristics of the study sample

	Asthmatic patients	Healthy controls	*P* value
Number of participants	114	152	
Mean age	10±2.3	10±2.8	0.9
Sex ratio (girls/boys)	0.67 (46/68)	0.78 (67/85)	0.5
Total frequency of female patients (%)	40.4	56	
Total frequency of male patients (%)	59.6	44	
Total serum IgE (IU/ml)	184.2±85	41.5±2	<0.0001*
Serum IL-13 (pg/ml)	74.6 ±31.82	14.8±6.64	<0.0001*
Peripheral blood eosinophilic (%)	3 (0.4–18)	1 (0.12–11)	<0.0001*
FEV1 (% predicted)	75.5±15.62	90.2±6.81	<0.0001*

FEV1, forced expiratory volume in 1 s; IgE, immunoglobulin E; IL-13, interleukin-13. Data are expressed as mean±SD or median and range. The Mann–Whitney test for comparison of FEV1, serum IgE, eosinophilic %, and serum IL-13 level between cases and controls.
*Statistical significance was defined as $P \leq 0.05$.

Social Sciences under Windows (version 22) (Armonk, NY: IBM Corp.). Numerical data, which showed a nonparametric distribution according to the Kolmogorov–Smirnov test, were expressed as median and range, whereas parametric data were expressed as median and SD. Categorical data were expressed as numbers and %. Differences in genotype and allele frequencies were evaluated using the χ^2-test. Odds ratio and 95% confidence interval values were calculated using Epi info 7 program (Developed by CDC, Atlanta, Georgia, US). A *P* value less than 0.05 was considered significant.

Results

Serum IL-13 was found to be significantly higher in asthmatic patients when compared with the control group ($P<0.0001$) (Table 1). In the asthmatic group, FEV1 showed a significant negative correlation with serum IL-13 ($\rho=-0.2$, $P=0.03$), whereas peripheral blood eosinophilic % showed a significant positive correlation with serum IL-13 ($\rho=0.18$, $P=0.05$) (Table 2).

No differences of statistical significance were found between asthmatic patients and controls in (*IL-13*) *+1923C/T* genotype frequency. However, results showed high prevalence of the CT heterozygote genotype (70% in asthmatics and 60% in controls) ($\chi^2=2.6$, $P=0.1$; odds ratio=1.5 and 95% confidence interval=0.9–2.6). The prevalence of TT genotype was lower in the control group and more common in the affected children (10 and 12%, respectively) (Table 3).

A significantly lower serum cortisol pm was found in asthmatic patients with nocturnal symptoms ($n=71$) when compared with those without nocturnal symptoms ($n=43$) ($P<0.0001$). However, there were no significant differences in PFTs, heart rate variability, and serum cortisol am between asthmatic patients with nocturnal symptoms and those without nocturnal symptoms (Table 4).

Discussion

Asthma is a complex clinical syndrome attributed to interplay between several factors: immunity, heredity, and environment [12]. More than 300 million people worldwide have asthma [13,14]. Asthma prevalence in the Delta region of Egypt was reported to be 7.7% [15]. As regards asthma pathogenesis, several pathophysiological effects have been associated with imbalanced T-cell activation and the presence or absence of distinct immune mediators. Thus, cytokines arouse an increasing interest for diagnosis and treatment [16].

IL-13 has been recognized as a key T helper 2 cell cytokine in asthma pathogenesis and airway remodeling as well. It switches B-cell antibody production from IgM to IgE, induces goblet cell differentiation, activates fibroblasts, and increases airway hyper-responsiveness [17]. In the current study, serum IL-13 level was significantly higher in the asthmatic group in comparison with controls, and, in asthmatic cohort, it was positively correlated to peripheral blood eosinophilic percentage and inversely correlated to FEV1. These results are in agreement with that reported by Lee *et al.* [18], who studied serum IL-13 level in 58 patients with acute asthma, 22 asymptomatic asthmatic patients, and 10 healthy individuals. They found that serum IL-13 level was significantly higher in patients with acute asthma; however, it was not correlated to FEV1. Another study was conducted on 32 atopic asthmatics and 22 nonatopic asthmatics and it concluded the same

Table 2 Correlation analysis between clinical and laboratory biomarkers and serum interleukin-13 in asthmatic patients

		FEV1 (% predicted)	Eosinophilic percentage	Serum total IgE (IU/ml)
IL-13 (pg/ml)	ρ	–0.20	0.18	–0.04
	P value	**0.03***	**0.05***	**0.6**

Depicted are correlation coefficients (Spearman's ρ) followed by *P* values (in bold). *Statistical significance was defined as $P\leq0.05$.

Table 3 Frequency of interleukin-13 (*IL-13*) *+1923C/T* genotype and allelic polymorphisms among asthmatic cases and controls

	Cases (*N*=114) [*n* (%)]	Controls (*N*=152) [*n* (%)]	OR (95% CI)	χ^2	*P*
(*IL-13*) *+1923C/T* genotype[a]					
CC	23 (20)	42 (28)	1.5 (0.9–2.6)	2.6	0.1
CT	80 (70)	91 (60)			
TT	11 (10)	19 (12)			
Allele frequency[b]					
C	126/228 (55.3)	175/304 (57.6)	0.9 (0.6–1.3)	0.19	0.7
T	102/228 (44.7)	129/304 (42.4)			

[a]The χ^2-test for comparison of CC genotype versus T-containing genotype (CT and TT) frequency in asthma patients versus controls. [b]The χ^2-test for comparison of C versus T allele frequency in asthma patients versus controls.

Table 4 Pulmonary function tests, heart rate variability, and serum cortisol levels in patients with nocturnal asthma in comparison with asthmatic patients without nocturnal symptoms

	Patients with nocturnal asthma (*n*=71)	Patients without Nocturnal asthma (*n*=43)	*P* value
FEV1 (a.m.) (% predicted)	75.4±14	75.8±18.11	0.9
FEV1 (p.m.) (% predicted)	68.8±14.63	75±18	0.1
FVC (a.m.) (% predicted)	71.1±14.21	69.7±16.42	0.6
FVC (p.m.) (% predicted)	66.2±13.73	69.1±16.43	0.06
FEV1/FVC ratio (a.m.)	75.3 (50.4–105.4)	75.6 (45.5–111.4)	0.9
FEV1/FVC ratio (p.m.)	70 (47–104)	74.3 (44.3–108)	0.14
Mean heart rate (a.m.)	99.6±11.92	100.6±12.83	0.7
Mean heart rate (a.m.)	95.8±13.41	92.2±13.64	0.17
Mean R-R interval (a.m.)	561±38.71	562±45.73	0.9
Mean R-R interval (p.m.)	575.5±56	573±45.45	0.8
Serum cortisol (a.m.)	62.8±17.73	59.1±19.41	0.24
Serum cortisol (p.m.)	28.7±13.74	55.4±19.22	<0.0001*

Data are expressed as mean±SD in FEV1, FVC, mean heart rate and mean R-R interval and median (range) in FEV1/FVC. ANOVA, analysis of variance; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity. *P* value based on the one-way ANOVA test.
*Statistical significance was defined as $P \leq 0.05$.

results with a trend of serum IL-13 to be elevated in moderate persistent asthma [19].

Siddiqui *et al.* [20] have found that IL-13 expression is increased in peripheral blood-derived T cells in asthma and that asthmatic serum upregulates IL-13 release from healthy peripheral blood mononuclear cells, which in part may be due to a proinflammatory stimulus in asthmatic serum. This can provide a comprehensive explanation of the high serum level of IL-13 encountered in asthmatic patients.

As regards the current study findings concerning the inverse correlation between serum IL-13 levels and FEV1, this may be due to increased airway hyperreactivity (AHR). In an experimental model of allergic asthma, IL-13 was found to be a major contributor to AHR [21]. In another animal study, IL-13 was involved in maintaining chronic asthma for 6 months after stoppage of allergen exposure in mouse model [22]. Furthermore, IL-13 was found to desensitize β2-adrenergic receptors in human airway epithelium, thus increasing bronchial hyper-responsiveness and may blunt the therapeutic effect of corticosteroids [23].

As regards the correlation between serum IL-13 level and peripheral blood eosinophilic percentage, Agache *et al.* [24] carried out a research to determine the predictors of blood eosinophilia in adults with asthma, and their final conclusion was that serum IL-13 and IL-5 are the best predictors of peripheral blood eosinophilic %.

In contrast, Pukelsheim *et al.* [16] have found no difference of statistical significance in the levels of serum IL-13 between asthmatic patients and healthy individuals. Similar results were reported by Davoodi *et al.* [25]. The explanation for this discrepancy in results between different studies could be the heterogeneity of asthma, which is not only at the clinical level but also at the pathophysiologic mechanisms level [26]. Moreover, the systemic serum pattern of T helper cell cytokines may not be the mirror image of the immune imbalance at tissue level. Concerning this dilemma, it has been recommended that findings concerning inflammatory mediators of asthmatic patients should be studied both in serum and bronchial lavage fluid [27].

Although asthma is a heterogeneous disease, a strong genetic basis has been firmly established [28]. Previous studies showed that gene polymorphisms of *IL-10*, *TNF-α-308*, *IL-6* and *IL-1Ra*, *IL4C-590T*, and *IL4RA 175V* play a role in the development of asthma in Egyptian children [29–31]. Genome-wide association studies in large populations have linked polymorphisms of IL-13 and its receptors with asthma prevalence [32].

In this study, no statistically significant differences were found between cases and controls as regards the frequency of (*IL-13*) *+1923C/T* genotype, thus indicating that this SNP is not a susceptibility factor for asthma development in Egyptian Asthmatic children. However, results showed high prevalence of the CT heterozygote genotype in asthmatic children. These results cope with that published by Li *et al.* [10], as they assessed the distribution of this SNP and asthma development in 192 asthmatic children and 192 controls in Chinese Han Nationality and they found no differences in statistical significance among the two groups.

However, (*IL-13*) *+1923C/T* locus was reported as a susceptibility factor for asthma in Mauritian Indian children [33]. A meta-analysis, which retrieved 26 articles including 17 642 asthma patients and 42 402 controls, found out that this (*IL-13*) *+1923C/T* polymorphism is a risk factor for asthma development [7]. Such diverse findings may be due to variable genetic makeup in different ethnic groups and also due to variable sample sizes. Further case–control studies with more ethnicities are still needed.

Nocturnal asthma is defined as worsening of asthma symptoms and airway responsiveness at night time resulting in poor quality of life [34]. Our primary hypothesis was to identify key predictors for the occurrence of nocturnal symptoms over time. A significantly lower serum cortisol pm was found in asthmatic patients with nocturnal symptoms when compared with those without nocturnal symptoms. However, there were no significant differences between the two groups as regards PFTs and parasympathetic activity (which were assessed by means heart rate variability).

According to the circadian rhythm of cortisol, it reaches its trough level during the early biological night, close to habitual bedtime [35]. Asthma is an inflammatory airway disease and this inflammation leads to AHR and if untreated it will lead to airway remodeling. Glucocorticoids being a potent anti-inflammatory agent, their circadian rhythm affect the diurnal variation of asthma symptoms [36]. This can explain why serum cortisol (at night) was lower in the studied nocturnal asthma cohort. Moreover, in a previous study, mometasone furoate dry powder inhaler (200 μg) was administered once/day at night time and it was found to significantly improve PFTs in patients previously using short-acting β2 agonists alone for asthma control [37]. Understanding the biological clock of cortisol release in cases of nocturnal asthma can justify the timing of administration of steroid therapy or what is called 'chronotherapy' for better symptom control.

Conclusion

We can easily recognize the complex nature of asthma syndrome, which led to a paradox in asthma management; we have effective treatments that are not biologically informative, and we have informative treatments that are less effective. Relating the observable phenotype to the underlying endotype, including physiological, biological, and genetic indicators, can provide better identification of asthma phenotypes, better preventative strategies, and more wise application of precision medicine approaches.

Genetic determinants are an attractive approach to detect susceptibility for asthma. However, further studies on different ethnic groups should be conducted to upgrade the level of evidence. Studying glucocorticoids circadian rhythm in nocturnal asthma is a promising approach for chronotherapy in such cases.

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Conflicts of interest

There are no conflicts of interest.

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