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Evaluation of serum levels of interferon beta and nucleotide binding and oligomerization domain 2 gene polymorphism in children with cough asthma phenotype: a case–control study

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Abstract

Background Individuals with asthma are thought to suffer from a variety of distinct disorders, or phenotypes, each of which is characterized by a unique combination of genetic and environmental factors. Syndromes that are exacerbated by allergens, non-allergic factors, and aspirin, as well as syndromes that are best differentiated by pathologic findings, response to therapy, and natural history, fall into this category. The best course of treatment for an individual patient with asthma can be determined by first determining his or her specific asthma phenotype and its underlying pathophysiology.

Aim of the work Explore clinical characteristics, serum INF-β in cough asthma phenotype and allergic march asthmatic children. Also, to assess the association of NOD2 (rs2066845) gene polymorphism among those asthma phenotypes in Egyptian asthmatic children.

Patients and methods The study included 64 cough phenotypic asthmatic children and 60 allergic march phenotypic asthmatic children in addition to 39 healthy controls (control group). The included children were subjected to full clinical history taking, full clinical examination, assessment of (total serum IgE, CBC for peripheral eosinophil percentage, cytokine profile (serum levels of INF-B), and genetic analysis: SNPs of NOD2 (rs2066845).

Results There was a significant increase in G allele frequency, in both homozygous (GG) and heterozygous (GC) states, among asthmatic children of cough and allergic march phenotypes compared to healthy controls, with no significant difference between the two phenotypes. In addition, serum INF-β was significantly lower in cough and allergic march phenotypic asthmatics with GG genotypes versus healthy controls of the same genotype.

Conclusions NOD2 (rs2066845) gene polymorphism is associated with both cough and allergic march asthma phenotypes in Egyptian asthmatic children. It was also shown that G allele may be implicated in asthma pathophysiology.

Keywords Asthma, Cough, Allergic march, Polymorphism, Allele, Genotype

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Background

Asthma is a chronic inflammatory disease. People of all ages can be affected by asthma, but children are the age group in which the disease is diagnosed at a higher rate [1]. It causes wheezing, shortness of breath, chest tightness, and cough, as well as activity restrictions and flare-ups (called "attacks") that may necessitate immediate medical attention and even prove fatal [2]. Previously Zedan et al., using a modified ISAAC questionnaire, 7.7% of Egyptian children in the Nile Delta schoolchildren had asthma [3].

Although a patient's history and physical are used to determine an asthma diagnosis, clinical phenotypes are not typically used to guide therapy [4].

Pathogenesis of asthma remains a matter of debate, despite the extensive research being conducted, with many risk factors being involved in its development such as genetic background, environmental exposure and infections showed the highest contribution in pathogenesis and were the subject of many researches [5].

The human NOD2 gene is located on chromosome 16, and it is responsible for the production of the protein known as protein with a nucleotide-binding oligomerization domain 2, or PONDO2 [6]. Inflammatory and immune responses are both significantly influenced by the protein NOD2 [7].

In response to NOD2 ligand, selective expression of thymic stromal lymphopoietin (TSLP) and interleukin (IL)-25 was observed, as was the TSLP-dependent induction of the TNF family costimulatory molecule OX40 ligand (OX40L). The activation of NOD2 signalling in the end led to a complete susceptibility to develop asthmatic disease, which could be prevented by targeting TSLP, IL-25, or OX40L [8]. Additionally, NOD2 is an essential factor in the development of T helper 2-type immunity, which results in the production of IL-4 and IL-5 [9].

Infections of the respiratory tract by viruses are the primary triggers for both the development of asthma and its exacerbations, yet the underlying mechanisms for this association remain unclear. One of these mechanisms is the improper activation of group 2 innate lymphoid cells (ILC2 cells). The activation occurs due to lack of type I interferon receptor signaling which resulted in type 2 immunopathology associated with infection [10].

The purpose of the present work was to investigate the relationship between allergic march and cough asthma phenotypes and serum levels of interferon beta and NOD2-gene polymorphism. Also, to evaluate total serum immunoglobulin E and peripheral eosinophilic percentage in these asthma phenotypes.

Methods

Study design

A case–control study.

Study location

The study was carried out at the outpatient clinic of the Allergy, Pulmonology, and Clinical Immunology unit, Mansoura University Children's Hospital, Egypt.

Duration

2.5 years.

The study participants

The study participants were distributed into three groups: 2 cases groups with asthma and control group. The asthma cases included 64 cough phenotypic asthmatic children (cough phenotype group) and 60 allergic march phenotypic asthmatic children (allergic march phenotype group).

Participants' ages ranged from 6 to 18 years old, and they were diagnosed with asthma on the basis of having typical asthma symptoms and the presence of variable expiratory airflow limitation, as evidenced by an increase in FEV1 that was greater than 12% predicted after inhaling salbutamol [11].

The enrolled asthmatic patients were subdivided into two clinical phenotypes depending on their predominant symptom (after validation of their symptoms) [3, 12, 13].

The cough. phenotype was identified in case of presence of cough as a predominant symptom [13, 14]. While the allergic march was identified in case of presence of typical asthma symptoms, in atopic phenotype (it is usually defined by the presence of sensitization to environmental allergens, had allergic rhinitis and atopic dermatitis as a predominant symptom defined according GINA 2021 guidelines) [13, 15].

The cases with the following criteria were excluded; asthmatic patients with co-morbidities and SOB clinical asthma phenotype, patients who were under immunotherapy or oral corticosteroids (OCS) as they alter cytokine profile [16] and the patients with a history of a disease altering cytokine profile including autoimmune rheumatoid arthritis, Hodgkin's disease, and Graves's disease as they influence cytokines levels [17].

The third group included 39 healthy controls who were free from history of asthma like symptoms and had no positive family history of allergy or asthma.

After obtaining of written consent from the legal guardians of the included children and approval of the institutional review board, Mansoura Faculty of Medicine (MS.20.07.1194), the children were subjected

to full clinical history taking, full clinical examination, assessment of (total serum IgE, CBC for peripheral eosinophil percentage, cytokine profile (serum levels of INF-B), and genetic analysis: SNPs of NOD2 (rs2066845).

Blood was drawn and placed in an EDTA tube to determine the eosinophil count and percentage. By using an automated cell counter, the total leucocyte count (TLC) and eosinophil percentage were measured.

To assess the total serum IgE level and serum interferon-beta, IgE ELISA KITS were used to measure total serum IgE from blood samples collected in plain tubes using immunoassay techniques (Bioactiva Diagnostic, Germany) or human INF-β ELISA KIT (NOVA, Bioneovan Co., China).

Genotyping of NOD2 (rs2066845) using step one real-time PCR [cat. no. (4,371,355)]

1-*DNA extraction* was performed using QIAamp DNA blood mini kit (provided by QIAGEN cat. no. 51104, USA) for DNA extraction from whole blood.

PCR amplification

For quantitative real-time PCR or allelic discrimination, TaqMan Assays have two probes and two preoptimized PCR primer pairs (qPCR). TaqMan Assays are used to amplify and detect specific variants in target genomic DNA (gDNA). DNA was amplified using *step one real-time PCR*, with the following thermal cycling conditions.

Detection of allele

Genotyping assays use the fluorescence shift of probe-associated dyes to establish the presence or absence of SNPs.

Statistical analysis of data

The software known as SPSS (version 27, distributed by IBM/SPSS Inc. of Chicago, IL) was utilized for the purpose of conducting the analysis of the data. At the outset of the investigation, the characteristics of the population that was going to be the subject of the study were summarized using frequency and percentage (percent) distributions, standard deviations, and median values (range).

It was decided whether to use Monte-Carlo test or the chi-square test to compare three independent sets of categorical data. To compare two groups with parametric and non-parametric quantitative data, independent samples *t* test or Mann–Whitney *U* test were used respectively. To compare three groups with parametric and non-parametric quantitative data, one-way analysis of the variance test or Kruskal–Wallis test used respectively. Spearman correlation was used to correlate continuous variables. Chi-square test (or Monte-Carlo test) were used. *P* values < 0.05 are considered significant.

Results

Table 1 shows that there was a significantly higher frequency of parental smoking among cough variant asthma cases in comparison to healthy control group ($P=0.027^{*}$). There was a significantly higher frequency of artificial feeding among cough phenotype cases, compared to allergic march phenotype ($P=0.003^{*}$). Cough variant asthma group showed a significantly higher frequency in exposure to both outdoor and indoor triggers compared to healthy control group ($P\leq0.001^{*}$, $P=0.006^{*}$, respectively). Allergic march group showed a significantly higher frequency in exposure to outdoor triggers compared to both cough phenotype and healthy control ($P=0.007^{*}$, $P\leq0.001^{*}$, respectively).

Allergic march phenotype group showed statistically significant elevated eosinophil percentage, total serum IgE, and lower serum INF-β compared to healthy control group ($P\leq0.001^{**}$, $P\leq0.001^{**}$, and $P=0.016^{*}$, respectively).

Cough phenotype showed statistically significant higher eosinophil percentage, total serum IgE and lower serum INF-β in a comparison healthy control group ($p=0.003^{*}$, $P\leq0.001^{*}$, $P\leq0.001^{*}$ for the three biomarkers).

Allergic march phenotype group showed statistically significant higher eosinophil percentage compared to cough phenotype group ($P\leq0.001^{**}$).

Table 2 shows that allergic march group showed a significantly higher frequency of the presence of atopic dermatitis and allergic rhinitis compared to cough phenotype ($P\leq0.001^{**}$). Regarding the severity of asthma, there was a statistically significant difference between the two asthma phenotypes that were studied, with severe cases being associated with the allergic march phenotype ($P=0.001^{*}$). Regarding level of control, there was no statistically significant difference between the two phenotypes ($P=0.544$).

Table 3 shows that in cases of allergic rhinitis, there was a positive correlation between serum INF-B and serum IgE ($r=0.266$, $P=0.0.04^{*}$). There was positive correlation between serum INF-B and serum eosinophil percentage in allergic march cases ($r=0.271$, $P=0.0.036^{*}$). Increased serum INF-B was associated with increased serum IgE and serum eosinophil percentage.

Table 4 significant association was observed between serum INF-β and degree of asthma severity in cough phenotype as serum INF-β was higher in moderate asthma in comparison to mild asthma ($P=0.03$). Significant association was observed between serum

Table 1 Demographic data and laboratory data of the studied groups (Allergic march, Cough variant asthma (CVA) and control)

Demographic data	Allergic march ($n=60$)	Cough variant asthma ($n=64$)	Control ($n=39$)	Test of significance		
				P1	P2	P3
Age (years)	9.30 ± 2.75	8.58 ± 2.66	9.53 ± 2.84	$t=0.416$	$t=1.716$	$t=0.416$
Mean ± SD				$P=0.679$	$P=0.089$	$P=0.679$
Gender						
Male	41(68.3%)	45(70.3%)	23 (59%)	$\chi^2=0.906$	$\chi^2=1.39$	$\chi^2=0.057$
Female	19(31.7%)	19(29.7%)	16 (41%)	$P=0.341$	$P=0.239$	$P=0.811$
Nutritional history						
Breast feeding	41(68.3%)	24(37.5%)	18(46.2%)	$\chi^2=4.91$	$\chi^2=0.866$	$\chi^2=11.8$
Artificial feeding	9(15.0%)	19(29.7%)	9(23.1%)	$P=0.086$	$P=0.649$	$P=0.003$*
Mixed	10(16.7%)	21(32.8%)	12(30.8%)			
Paternal smoking						
Positive	28(46.7%)	34(53.1%)	12(30.8%)	$\chi^2=2.48$	$\chi^2=4.90$	$\chi^2=0.517$
Negative	32(53.3%)	30(46.9%)	27(69.2%)	$P=0.115$	$P=0.027$*	$P=0.472$
Exposure to indoor trigger						
Positive	27(45.0%)	34(53.1%)	10(25.6%)	$\chi^2=3.78$	$\chi^2=7.48$	$\chi^2=0.82$
Negative	33(55.0%)	30(46.9%)	29(74.4%)	$P=0.052$	$P=0.006$*	$P=0.37$
Exposure to outdoor trigger						
Positive	58(96.7%)	52 (81.2)	16(41%)	$\chi^2=38.76$	$\chi^2=17.48$	$\chi^2=7.35$
Negative	2(3.3%)	12(18.8%)	23(59%)	$P\leq0.001$*	$P\leq.001$*	$P=0.007$*
Family history				–	–	
Positive	13(21.7%)	9 (14.1%)	0 (0%)			$\chi^2=1.23$
Negative	47(78.3%)	55(85.9%)	39 (100%			$P=0.268$
Serum eosinophilic percentage						
Median (min–max)	5.54 (0.53–13.18)	3.31 (0.01–19.24)	1.87 (0.15–10.61)	$Z=6.37$ $P\leq0.001$**	$Z=3.01$ $p=0.003$*	$Z=3.49$ $P\leq0.001$**
Total IgE (IU/ml)						
Median (min–max)	129.00 (3.30–532.00)	94.6 (1.36–688)	9.30 (2.88–45.6)	$Z=5.32$ $P\leq0.001$**	$Z=3.93$ $p\leq0.001$**	$Z=0.056$ $P=0.955$
Serum INF-B						
Median (min–max)	19.75 (5.70–64.00)	19.95 (1.76–109)	30.7 (8.8–434)	$Z=2.41$ $P=0.016$*	$Z=4.02$ $p\leq0.001$**	$Z=1.18$ $P=0.237$

P1 comparison between allergic March and control groups, *P2* comparison between cough and control groups, *p3* Comparison between allergic march and cough variant asthma groups

* significant $p\leq0.05$

** highly significant $p\leq0.001$

eosinophilic percentage and level of asthma control in allergic march group as serum eosinophilic percentage was higher in uncontrolled cases with (median = 6.91) in comparison to controlled cases, median = 4.53 ($P=0.02$*). In the cough phenotype group, there was no statistically significant correlation between laboratory biomarkers and asthma control.

Table 5 shows that GG genotype was statistically significantly higher among allergic march (84.4%) as compared to cough phenotype (62.5%) and control group (64.1%) while CC genotype was higher among control group (23.1%) as compared to 0% and 3.1 among allergic march and cough groups, respectively. There was no statistically significant difference in the frequency of NOD2 rs2066845 genotypes between the allergy march and cough groups.

G allele was statistically significantly higher among allergic march and cough groups (92.2%, 85.2%) as compared to 70.5% in control group while C allele was higher among control group (29.5%) as compared to 7.8%, 14.8% in allergic march and cough groups, respectively.

G allele increased 4.9 times with 95% CI ranged from 1.9 to 12.3 among allergic march group and 2.4 times with 95% CI ranged from 1.2 to 4.9 among cough group as compared to control. No statistically significant difference was observed between allergic march and cough groups regarding allelic polymorphisms.

Table 2 Characteristics of asthmatic cases (allergic march phenotype and cough variant asthma phenotype)

Characteristics of asthmatics	Allergic march ($n=60$)	Cough variant asthma ($n=64$)	*P* value
Duration of illness (years) Median (min–max)	5.0 (0.50–12)	4.25 (1–16)	0.882
Degree of asthma severity			0.001*
Mild	13 (21.7%)	9 (14.1%)	
Moderate	38 (63.3%)	55 (85.9%)	
Severe	9 (15.0%)	0 (0%)	
Atopic dermatitis			≤ 0.001**
Positive	60 (100.0%)	5 (7.8%)	
Negative	0 (0%)	59 (92.2%)	
Allergic rhinitis			≤ 0.001**
Positive	60 (100.0%)	15 (23.4%)	
Negative	0 (0%)	49 (76.6%)	
History of controller medications			0.631
ICS	2 (3.3%)	1 (1.6%)	
ICS and leukotriene	49 (81.7%)	56 (87.5%)	
Leukotriene modifier	9 (15.0%)	7 (10.9%)	
Level of asthma control			0.544
Controlled	22 (36.7%)	29 (45.3%)	
Partially controlled	13 (21.7%)	14 (21.9%)	
Severely uncontrolled	25 (41.7%)	21 (32.8%)	

* significant $p \leq 0.05$

** highly significant $p \leq 0.001$

Table 3 Correlation analysis between laboratory biomarkers in studied cases (allergic march, cough phenotypes)

Groups		Serum eosinophilic percentage		Serum IgE		Serum IFN-B	
		r	*p*	*r*	*p*	*r*	*p*
Allergic march	Serum eosinophil percentage	–	–	−0.084	0.522	0.271	0.036*
	Serum IgE	−0.084	0.522	–	–	0.266	0.04*
	Serum IFN-B	0.271	0.036*	0.266	0.04*	–	–
Cough phenotype	Serum eosinophil percentage	–	–	−0.039	0.831	0.048	0.712
	Serum IgE	−0.039	0.831	–	–	−0.313	0.076
	Serum IFN-B	0.048	0.712	-0.313	0.076	–	–
Control	Serum eosinophil percentage	–	–	0.640	0.006*	0.111	0.502
	Serum IgE	0.640	0.006*	–	–	0.264	0.307
	Serum IFN-B	0.111	0.502	0.264	0.307	–	–

* significant $p \leq 0.05$

Table 6 shows that there was a significantly higher frequency of GG genotype among asthmatic cases with positive history of parental smoking, patients with allergic rhinitis and patients with atopic dermatitis; accounted for 76.5%, 81.4%, and 85.7% as compared to 64.1% in control group, respectively with p value ≤ 0.05.

Regarding allelic polymorphisms, there was a significantly higher frequency of G allele among asthmatic cases with positive family history (94.7%), positive history of parental smoking (86.3%), associated allergic rhinitis (90.7%) and atopic dermatitis (92.9%) as compared to 70.5% in healthy controls p value ≤ 0.05.

Table 7 shows that no statistically significant association were observed between NOD2 *rs2066845* genotype, allelic polymorphisms and gender, family history, parental smoking, allergic rhinitis, and dermatitis p value > 0.05.

Table 8 shows that serum levels of INF-β were significantly lower in cough groups with GG genotype compared to healthy controls of the same genotype. Median

Table 4 Association between laboratory biomarkers and degree of asthma severity and degree of asthma control in different phenotypes (allergic march and cough phenotypes)

Groups		Serum eosinophilic percentage		Serum IgE		Serum IFN-B	
		Median (min–max)	*P* value	Median (min–max)	*P* value	Median (min–max)	*P* value
Asthma severity							
Allergic march	Mild asthma	5.64 (1.17–10.53)	KW = 2.7 *P* = 0.26	76 (3.30–532)	KW = 5 *P* = 0.08	18.80 (5.70–41.30)	KW = 0.62 *P* = 0.73
	Moderate asthma	5.08 (0.53–13.04)		181 (6.20–500)		20.20 (8.35–64.00)	
	Severe asthma	7.54 (4.14–13.18)		65.30 (32–154)		21.40 (11.30–46.80)	
Cough phenotype	Mild asthma	2.75 (1.69–9.03)	*Z* = 0.29 *P* = 0.76	440 (42.70–688)	*Z* = 1.0 *P* = 0.32	11.50 (7.330.8)	*Z* = 2.1 *P* = 0.03*
	Moderate asthma	3.45 (0.01–19.24)		83.70 (1.36–520)		21.60 (1.76–109)	
Asthma control							
Allergic march	Controlled	4.53 (1.78–10.49)[a]	KW = 7.45 *P* = 0.02*	164.00 (29.40–500.00)	KW = 4.01 *P* = 0.13	18.80 (5.70–62.20)	KW = 1.21 *P* = 0.54
	Partially controlled	5.45 (0.53–8.56)		231.00 (3.30–532.00)		23.60 (8.35–64.00)	
	Uncontrolled	6.91 (1.75–13.18)[a]		85.10 (6.20–483.80)		18.20 (9.50–46.90)	
Cough phenotype	Controlled	2.87 (0.01–19.24)	KW = 2.9 *P* = 0.23	46.00 (3.78–520.00)	KW = 4.1 *P* = 0.12	21.55 (4.43–48.90)	KW = 1.5 *P* = 0.47
	Partially controlled	4.45 (0.01–17.81)		72.80 (1.36–506)		21.50 (7.30–109)	
	Uncontrolled	3.31 (0.65–10.55)		391.00 (10.97–688)		13.15 (1.76–46)	

[a] Significant between controlled and uncontrolled

* significant $p \leq 0.05$

Table 5 Frequency of NOD2 *rs2066845* genotype and allelic polymorphisms among asthmatic phenotypes compared to controls

NOD2 *rs2066845*	Allergic march (*n* = 45)	Cough phenotype group (*n* = 54)	Control group (*n* = 39)	Test of significance		
				P1	P2	P3
Genotype frequency				**0.003***	**0.014***	0.277
CC	0 (0%)	2 (3.1%)	9 (23.1%)			
GC	7 (15.6%)	12 (18.8%)	5 (12.8%)			
GG	38 (84.4%)	40 (62.5%)	25 (64.1%)			
Allele frequency				**≤ 0.001***	**0.015***	0.123
C (r)	7 (7.8%)	16 (14.8%)	23 (29.5%)			
G	83 (92.2%)	92 (85.2%)	55 (70.5%)			
OR (95%CI)	4.9 (1.9–12.3)	2.4 (1.2–4.9)	(*r*)	–	–	–

* significant $p \leq 0.05$

serum INF-β was 14.9 ranged from 4.43 to 47.4 among cough patients with GG genotype group as compared to 24.30 ranged from 9.11 to 434 among control group *P* value ≤ 0.05.

In both cough and allergic march phenotypes, there were no statistically significant differences between serum INF-levels and various NOD2 rs2066845 genotypes. However, it was not significant, CC genotype was associated with higher serum levels of INF-β compared to GC and GG genotypes in cough phenotype (median = 36.40) as compared to 18.40 and 14.90, respectively.

Discussion

Inflammation of the airways and increased reactivity of those airways are hallmarks of the heterogeneous disease known as asthma. One percent to eighteen percent of people across the globe are affected by asthma. Patients with asthma often complain of respiratory symptoms such as shortness of breath, wheezing, coughing, and/or a tight chest. These symptoms become more frequent and severe as the disease progresses, and they are accompanied by a reduction in overall lung function and a restriction in the patient's ability to exhale freely [18].

Environmental and genetic factors interact in complex ways to cause asthma, which manifests itself in varying degrees of symptoms, inflammation, and even airway remodelling. A significant portion of asthma cases (around 60%) in both children and adults can be attributed to allergies [19].

Both the pathogenesis of asthma and its clinical manifestations are extremely complicated and diverse, displaying phenotypes and endotypes respectively. The terms

Table 6 Frequency of NOD2 *rs2066845* genotype and allelic polymorphisms among asthmatic cases compared to controls with their statistical significance

	Genotypes			MC (P)	Allele frequency		χ^2 (P)
	CC	*GC*	*GG*		*C*	*G*	
Family history				(0.066)			8.8
Positive (19)	0 (0)	2 (10.5)	17 (89.5)		2 (5.3)	36(94.7)	(0.003*)
Control (39)	9 (23.1)	5 (12.8)	25 (64.1)		23(29.5)	55 (70.5)	
Parental smoking				(0.017*)			6.7
Positive (51)	2 (3.9)	10(19.6)	39 (76.5)		14(13.7)	88(86.3)	(0.009*)
Control (39)	9 (23.1)	5 (12.8)	25 (64.1)		23(29.5)	55 (70.5)	
Allergic rhinitis				(≤ 0.001**)			13.3
Positive (59)	0 (0)	11(18.6)	48 (81.4)		11 (9.3)	107(90.7)	(≤ 0.001**)
Control (39)	9 (23.1)	5 (12.8)	25 (64.1)		23(29.5)	55 (70.5)	
Atopic dermatitis				(0.001*)			15.3
Positive (49)	0 (0)	7 (14.3)	42 (85.7)		7 (7.1)	91 (92.9)	(≤ 0.001**)
Control (39)	9 (23.1)	5 (12.8)	25 (64.1)		23(29.5)	55 (70.5)	

* significant $p \leq 0.05$

** highly significant $p \leq 0.001$

Table 7 Frequency of NOD2 *rs2066845* genotype and allelic polymorphisms among studied asthmatic cases

	Genotypes			MC (P)	Allele frequency		χ^2 (P)
	CC	GC	GG		C	GT	
Gender				(0.589)			0.78
Male (65)	2 (3.1)	13 (20)	50 (76.9)		17(13.1)	113(86.9)	(0.37)
Female (34)	0 (0)	6 (17.6)	28 (82.4)		6 (8.8)	62 (91.2)	
Family history				(0.427)			1.8 (0.17)
Positive (19)	0 (0)	2 (10.5)	17 (89.5)		2 (5.3)	36 (94.7)	
Negative (80)	2 (2.5)	17(21.2)	61 (76.2)		21(13.1)	139(86.9)	
Parental smoking				(0.60)			0.91
Positive (51)	2 (3.9)	10(19.6)	39 (76.5)		14(13.7)	88 (86.3)	(0.34)
Negative (48)	0 (0)	9 (18.8)	39 (81.2)		9 (9.4)	87 (90.6)	
Allergic rhinitis				(0.26)			1.49
Positive (59)	0 (0)	11(18.6)	48 (81.4)		11 (9.3)	107(90.7)	(0.22)
Negative (40)	2 (5.0)	8 (20.0)	30 (75.0)		12 (15)	68 (85)	
Atopic dermatitis				(0.126)			3.78
Positive (49)	0 (0)	7 (14.3)	42 (85.7)		7 (7.1)	91 (92.9)	(0.051)
Negative (50)	2 (4.0)	12 (24)	36 (72.0)		16 (16)	84 (84)	

"allergic asthma," "non-allergic asthma," and "delayed onset asthma" are all examples of common phenotypes. A better understanding of the severity of asthma, the duration of acute exacerbation, and other characteristics can be gained through the differentiation of phenotypes. The same phenotype may have various pathophysiological mechanisms, and by using "endotypes," phenotypes can be reclassified in accordance with pathogenesis [20].

Numerous diseases, such as Crohn's disease, early-onset sarcoidosis, Blau syndrome, autoimmune diseases, allergic reactions, and asthma, have been linked to the pathogenesis of NOD2 [21]. According to earlier research, asthma is linked to NOD2 polymorphism in the German population [22].

The current study's objectives were to investigate any associations between the NOD2 underlying gene polymorphisms, serum INF beta levels, and cough variant asthma and allergic phenotypes in Egyptian children, as well as to assess the relationship between NOD2 gene polymorphism and serum INF beta.

Table 8 The level of INF-β in individuals with different genotypes of the NOD2 *rs2066845* gene analyzed in the two studied asthmatic phenotypes and control

Allergic march (*n* = 60)		**Cough (*n* = 64)**		**Control**		**P1**	**P2**	**P3**
NOD2 rs2066845	**INF-β**	**NOD2 rs2066845**	**INF-β**	**NOD2 rs2066845**	**INF-β**			
C/C (*n* = 0)	–	**C/C *n* = 2**	36.40 (28.90–43.90)	**C/C (*n* = 9)**	31.20 (9.96–117.00)	–	*Z* = 0.23 P2 = 0.81	–
G/C (*n* = 7)	16.1 (11.5–46.9)	**G/C *n* = 12**	18.40 (1.76–109.00)	**G/C (*n* = 5)**	34.70 (8.80–100.80)	*Z* = 0.976 P1 = 0.343	*Z* = 1.07 P2 = 0.28	*Z* = 0.226 P3 = 0.82
G/G (*n* = 38)	19.1 (5.7–62.2)	**G/G *n* = 40**	14.90 (4.43–47.40)	**G/G (*n* = 25)**	24.30 (9.11–434.00)	*Z* = 1.48 P1 = 0.138	*Z* = 2.44 P2 = 0.015*	*Z* = 1.57 P3 = 0.12
	Z = 0.098 *P* = 0.754		KW = 1.89 *P* = 0.487		KW = 1.44 *P* = 0.487			

* significant $p \leq 0.05$

Cough variant asthma versus healthy control

The present study showed a significantly higher frequency of parental smoking (53.1%) and exposure to both outdoor (81.2%) and indoor (53.1%) triggers among cough variant asthma cases in comparison to healthy control group.

In the current study cough variant asthma group showed statistically significant higher eosinophil percentage, total serum IgE compared to healthy control group ($p = 0.003$). Osman and Elsaid discovered that the cough-predominant asthma phenotype is distinguished by prominent atopic features, which is consistent with our findings (allergic manifestations and elevated total IgE and increased eosinophilic percentage) [23]. Both the acute and recovery serum IgE levels in children with CVA were significantly higher than those in healthy controls, as demonstrated by wang et al. [24]. Additionally, Liu and colleagues found an increased number of eosinophils in the sputum of patients with CVA and mild asthma [25]. Patients with CVA have a high serum IgE level, which Nemat et al. showed to be a reliable marker for diagnosis and evaluation of disease severity [26].

Laboratory biomarkers (Serum eosinophilic percentage, serum IgE, serum IFN-B) were not significantly associated with asthma control in the cough phenotype group, according to the current study. Lai et al. found no correlation between the outcomes of the cough assessment and either the sputum eosinophil count or the blood eosinophil count, which is in agreement with our findings [27]. Contrarily to our results, previous studied showed that Peripheral eosinophils and serum eosinophil cationic protein levels were found to be sensitive markers for asthma [28] and assessment of asthma control [29, 30].

Allergic march asthma versus healthy control

The present study revealed that allergic march group showed a significantly higher frequency in exposure to outdoor triggers compared to both cough phenotype and healthy control group ($p < 0.001$).

In addition, there was a statistically significant distinction between the three groups in terms of the eosinophil percentage. In comparison to the cough group as well as the healthy control group, the allergic march phenotype was found to have the highest percentage.

Total serum IgE showed a statistical significance difference between allergic march group and healthy control group. In agreement with our results, Schatz and Rosenwasser, although total IgE levels are typically higher in patients with allergic asthma as opposed to those with nonallergic asthma, there is a significant amount of overlap between the two groups [31].

Positive association was observed between serum eosinophilic percentage and level of asthma control in allergic march group as serum eosinophilic percentage was higher in uncontrolled cases when compared to controlled and partially controlled cases. Previous research found, in line with our findings, that patients with eosinophilic asthma had a very low quality of life, and a significant number of them either suffered from frequent severe exacerbations or were dependent on oral corticosteroids [32]. Patients who suffer from moderate-severe asthma are significantly more likely to have an elevated peripheral eosinophil count, according to research conducted by Casciano and colleagues. This is in contrast to patients who suffer from mild asthma [33].

In the present investigation, there was a statistically significant increase in the incidence of artificial feeding among cough phenotype cases, compared to allergic march phenotype (15%, 29% respectively $p = 0.003$).

There was a statistically significant difference between the two asthma phenotypes that were studied in regard to the severity of asthma. Severe cases of asthma were associated with the allergic march phenotype ($P = 0.001$*). Regarding the level of asthma control, there was no

statistically significant difference between the two asthma phenotypes that were studied.

Allergic march group showed a significantly higher frequency of the presence of atopic dermatitis and allergic rhinitis compared to cough phenotype. In agreement with our results, Takejima et al. found that patients who were allergic to pollen had a higher incidence of atopic dermatitis and rhinoconjunctivitis, and, surprisingly, they had a more severe case of asthma than patients who were not allergic to pollen [34].

Also, in our study, there was statistically significant elevated *eosinophil* percentage compared to cough phenotype group (5.54%, 3.31 respectively $p < 0.001$).

The present study showed that serum INF-β was significantly lower in both allergic march phenotype and cough asthma phenotypes in comparison to healthy controls ($p = 0.016$ and < 0.001 respectively). In a previous study, Cai and colleagues discovered that the serum IFN levels of asthmatic children were noticeably lower than those of the children in the control group. ($p < 0.001$).

Also, positive association was observed between serum INF-β and degree of asthma severity in cough phenotype as serum INF-β was higher in moderate asthma in comparison to mild asthma ($P = 0.03$).

In allergic march cases, the current study demonstrated that there was a significant positive correlation between serum INF-B and both serum IgE and serum eosinophil percentage ($p = 0.04$ and 0.036 respectively). It is possible that having low levels of IFN- makes you more susceptible to developing severe asthma. In addition, previous research found that asthma patients had lower levels of IFN-β expression than healthy patients [35].

In the current investigation, there was not a statistically significant difference between the two phenotypes of asthma in terms of the patients' levels of control.

According to findings from earlier studies, the NOD1 + 32,656 polymorphism is linked to increased levels of serum IgE [36]. The findings of this study demonstrated that there was a significantly higher frequency GG genotype was statistically significantly higher among allergic march (84.4%) as compared to cough phenotype (62.5%) and control group (64.1%) while CC genotype was higher among control group (23.1%) as compared to 0% and 3.1% among allergic march and cough groups, respectively. Regarding the distribution of NOD2 rs2066845 genotypes, there was not a statistically significant difference in frequency between the allergic march and cough groups. There was a significantly higher frequency of GG genotype among asthmatic cases with positive history of parental smoking, presence of allergic rhinitis and AD compared to healthy controls (p 0.009, < 0.001, < 0.001).

There was a significantly higher frequency of G allele among asthmatic cases with positive family history compared to healthy controls ($p = 0.003$). Also, G allele was significantly elevated in asthmatic cases with positive history of parental smoking, presence of AD, and allergic rhinitis compared to healthy controls.

It was discovered that there were no differences that were statistically significant. Between the frequency of different NOD2 genotypes and allelic polymorphisms in asthmatic cases and their respective subgroups. Previous research has found, in line with our findings, that a genetic variant known as NOD2 polymorphism is linked to the development of asthma in the German population [22]. Additionally, it has been demonstrated that the polymorphisms of NOD1 and NOD2 are associated with Th2-mediated atopic diseases such as allergic asthma [37]. Wong and colleagues discovered that NOD1 and NOD2 ligands, on their own, were unable to have any effect on IgE level. However, the researchers also discovered that both types of ligands were able to significantly increase the production of total IgE serum. In a separate piece of research, it was demonstrated that both NOD1 and NOD ligands can raise the number of mucin-secreting goblet cells, as well as the level of bronchial subepithelial fibrosis and the amount of total IgE produced in vivo. The association between polymorphisms of NOD1 and NOD2 and asthma, as well as the participation of NOD1 and NOD2 activation in Th2-responses [38]. According to the findings of Belhaj and colleagues, the mRNA expression of NOD1, but not NOD2, was elevated in asthma patients in comparison to controls.

We found a statistically significant difference in the expression of NOD1 protein between asthma patients and healthy controls [39]. Weidinger and colleagues demonstrated that certain NOD1 SNPs are associated with an increased risk of atopic diseases [40].

As regards correlation between INF-β and NOD2 gene we found that serum levels of INF-β were significantly lower in cough phenotype with GG genotype compared to healthy controls of the same genotype ($p = 0.015$). Additionally, in the cough phenotype, the CC genotype was associated with higher serum levels of INF-β compared to both the GC and GG genotypes. However, no statistically significant differences between the cough and allergic march phenotypes were discovered in relation to the various genotypes of the NOD2 gene or the serum INF-levels. Cai et al. came to the same conclusion as we did and found that patients with severe asthma had lower levels of IFN than patients with less severe asthma. On the other hand, they were unable to find any association between the rs1077861, rs3135499, rs1861759, and rs2111234 and the serum level of IFN-β.

This is the first study that, to the best of our knowledge, investigates the clinical and laboratory characteristics of 2 distinct asthma phenotypes (cough-predominant asthma and March allergy asthma phenotypes) in children. These asthma phenotypes are distinguished by the predominant symptom of coughing. The relatively small number of participants, on the other hand, represents a significant limitation of this study. To validate the findings of the current study, therefore, it will be necessary to conduct additional research involving a significantly greater number of participants..

Conclusion

A polymorphism in the NOD2 gene known as rs2066845 is associated with both cough and allergic march as asthma phenotypes in Egyptian asthmatic children. In addition to this, it has been demonstrated that the G allele may play a role in the pathogenesis of asthma.

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Authors' contributions
SA designed research study and revised the paper. OE analyzed and interpreted the patient data regarding pulmonary function and skin testing and was a major contributor in writing the manuscript. ON collected the data. All authors read and approved the final manuscript.

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Availability of data and materials
All data and material are available.

Declarations

Ethics approval and consent to participate
The protocol was approved by Ethical Committee of Faculty of Medicine, Mansoura University at 23/7/2020, the code number (Ms.20.07.1194) confidentially and personal privacy will be respected. An informed verbal consent was obtained from caregivers of all patients.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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