Original article

Evaluation of serum levels of interferon beta (INF-Ɓ) and nucleotide-binding oligomerization domain 2 (NOD2) gene polymorphism in relation to asthma phenotypes in children

Background: *Asthma is a heterogeneous airway disease resulting from an interaction between multiple factors. Interferon-beta (INF-β) induces robust antiviral and immunomodulatory response to interfere with viral replication. The implication of nucleotide-binding oligomerization domain 2 (NOD2) was highlighted in many allergic diseases.* ***Objective:*** *The purpose of this study was to investigate the serum levels of INF-β and NOD2 single nucleotide gene polymorphism (SNP) among Egyptian asthmatic children who presented with wheezy and cough phenotypes.* ***Methods:*** *A group of 131 Egyptian asthmatic children (67 wheezy phenotype and 64 cough phenotype) together with 39 controls were enrolled and analyzed for the genotypes of NOD2 (rs2066845) polymorphisms using real time PCR via TaqMan assays. Serum INF-β levels were determined by ELISA technique.* ***Results:*** *Serum INF-β levels were significantly lower in both wheezy and cough phenotypes compared to control group (Z=1.19, p=0.233). Concerning the studied NOD2 SNP (rs2066845), both GG and GC genotypes showed significantly higher frequencies among asthmatic cases compared to healthy controls (p= 0.002, 0.021, respectively). Also, serum INF-β levels were significantly lower in both wheezy and cough asthma phenotypes with GG genotype compared to controls (p= 0.012, 0.015, respectively) of the same genotype. No significant differences were observed between the two studied asthma phenotypes regarding serum levels of INF-β, genotypes or allele frequency of NOD2 gene.* ***Conclusion:*** *Asthmatic children have lower levels of INF-β compared to controls, which might indicate a potential role of IFNs-based therapies for asthma. The study also provided possible evidence of the impact of rs2066845 G allele on asthma development.*

Keywords: Childhood asthma, Wheezy, Cough, INF-β, NOD2.

Magdy M. Zedan, Engy Osman, *Nashwa K. Abousamra, Amal Osman, Hoda Rizq, Esraa Z. Samy

*Allergy and Pulmonology Unit, Department of Pediatrics, *Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.*

Correspondence:
Esraa Zeinhom Samy, Department of Pediatrics, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
Email: esraazeinhom @yahoo.com

INTRODUCTION

The term "asthma" is considered an umbrella diagnosis for a collection of several distinct endotypes and varying phenotypes.[1] A better understanding of those distinct phenotypes and endotypes would form the basis for personalized medicine.[2]

Presence of certain biomarkers is an important feature of specific asthma phenotypes.[3] Although still unconfirmed, administration of IFN-β by inhalation was previously found to enhance innate immunity and compensate for the IFN-β deficiency found in the epithelium of patients with moderate-severe asthma.[4]

Furthermore, many biological pathways, and genes in those pathways, have been implicated in asthma pathogenesis.[5] Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) plays an important role in inflammatory and immune responses and has been involved in asthma pathogenesis. NOD2 is an intracellular pattern recognition receptor that can trigger a strong antigen specific immune response with a Th2 type polarization profile. Moreover, NOD2 is a viral pattern recognition receptor that can sense viruses to activate IFN-β production and antiviral defense.[6] In the current study we aimed to investigate serum levels of IFN-β and NOD2 single nucleotide polymorphism (SNP) in two different clinical asthma phenotypes (wheezy and cough asthma phenotypes) in a sample of Egyptian asthmatic children.

METHODS

This is a cross-sectional controlled study comprising 131 asthmatic children and 39 controls of matched age and sex. The enrolled asthmatic children, aged 6-16 years, were categorized after validation of their symptoms according to the previously proposed clinical asthma phenotyping[7] into 67 children with wheezy phenotype and 64 children with cough phenotype. Bronchial asthma was diagnosed according to guidelines of Global

Initiative for Asthma Management and Prevention (GINA) 2021.[8] Asthmatics of wheezy phenotype are those presented predominantly with wheezes (as described by the cases or their parents as noisy breathing, creaking, whistling, rattling secretions in the throat or jingling), while asthmatics with cough phenotype are those who had cough as the predominant symptom.[9] Flow chart for the study participants is shown in figure 1. Patients who were receiving immunotherapy and those with a history of a disease altering cytokine profile as juvenile idiopathic arthritis, Hodgkin's disease, Graves's disease were excluded. Informed consents were taken from caregivers of all cases. The study was approved by the Institutional Review Board of our faculty (Code number: MS.19.12.948).

Data collection

Detailed clinical history and examination were evaluated. Patient's data included age, sex, residence, parental consanguinity, history of allergic rhinitis, atopic dermatitis, and family history of atopy. Asthma severity and control level were assessed according to *GINA, 2021*.[8]

Serum biomarker

Peripheral blood samples were collected from patients and healthy controls. Serum INF-β levels determination was done by immunoassay techniques using the NOVA, Bioneovan Co, China, human INF-β ELISA KIT following the manufacturer's instruction. Total serum immunoglobulin E (IgE) was detected by immunoassay techniques using the Bioactiva diagnostic, Germany, IgE ELISA KITs and eosinophils percentage was determined in peripheral blood counting by automated cell counter (Sysmex XM350).

DNA extraction and genotyping

Two milliliters of venous blood were withdrawn from subject into sterile Ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Genomic DNA was extracted from whole blood using the QIAamp DNA blood mini kit (provided by QIAGEN cat. No.51104, USA). The QIA amp DNA extraction procedure comprised 4 steps: lysis by QIAGEN proteases enzyme. DNA was adsorbed onto the QIA amp silica-gel membrane during a brief centrifugation step then DNA was washed in 2 centrifugations. Finally, purified DNA was eluted from the QIA amp spin column in a concentrated form in buffer AE. DNA was then stored at −80°C. The genotypes of NOD2 (rs2066845) polymorphisms were analyzed using step one real time PCR, Cat. No. (4371355) via TaqMan assays.

Statistical analysis:

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows version 25 (Armonk, NY: IBM Corp). The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test while Monte Carlo test was used when expected cell count is less than 5. Continuous variables were presented as mean ± SD (standard deviation) for normally distributed data and median (min-max) for non-normally distributed data. The two groups were compared with Student *t* test for normally distributed data and Mann Whitney test for non-normally distributed data while Kruskal Wallis test was used to compare more than two medians. The results were considered significant when *p* value was less than or equal 0.05.

RESULTS

Clinical characteristics of the study participants

The study comprised three groups of age and gender matched children: Asthmatic group with wheezy phenotype (n=67), asthmatic group with cough phenotype (n=64) and healthy children (n=39). Their clinical and demographic data are depicted in table 1.

Frequency of parental smoking was significantly higher among both wheezy and cough phenotypes in comparison to the healthy control group (χ^2 =5.93, 4.90, P =0.015, 0.027 respectively). Cough phenotype showed a significantly higher frequency in exposure to indoor triggers compared to healthy control group (χ^2 =7.48, P=0.006) and a significantly higher frequency of exposure to outdoor triggers compared to both wheezy phenotype and healthy control groups (χ^2 =11.2, 17.48, P=0.001, ≤0.001 respectively) (Table 1).

The median duration of asthma was 4.50 years and 4.25 years among wheezy and cough phenotypes respectively (z=0.042, p=0.967). No significant difference was observed between both groups as regard the frequency of associated allergic rhinitis and atopic dermatitis (χ^2 =2.98, 0.056, p=0.084, 0.814 respectively). A hundred and four cases had moderate asthma, 21 had mild asthma and 6 cases had severe asthma. All the severe asthmatic patients were of the wheezy phenotype (χ^2 =6.71, p=0.035). No significant differences were observed between both groups as

regard the level of asthma control (χ^2 =2.61, p=0.271). ICS represented the commonest controller medication among our patients, used in 91.1% among wheezy phenotype and 89.1% among cough phenotype. Comparison between characteristics of wheezy and cough phenotypes is shown in table 2.

Serum biomarkers of the study participants

Peripheral eosinophil percentages and total serum IgE levels were significantly higher in asthmatic children compared to healthy controls. However, serum INF-β was significantly lower in asthmatic cases compared to controls (Table 3). Regarding the two studied asthma phenotypes: patients with the wheezy phenotype showed significantly higher peripheral eosinophil percentages compared to those with the cough phenotype while cough phenotype showed significantly higher total serum IgE levels. INF-β serum levels were comparable between the two studied patients' groups (Table 4).

Genotypes and allelic frequencies

We observed a higher frequency of the genotypes GG and GC of NOD2 rs2066845 polymorphism in patients compared to controls. The frequency of the allele G was higher than the allele C and was associated with an increased risk of asthma (Table 5). However, there were no statistically significant differences between the wheezy and cough phenotypes regarding the frequency of NOD2 (rs2066845) genotypes and allelic polymorphism (Table 6).

Serum levels of INF-β was found to be significantly lower in asthmatics with GG genotype of NOD2 (rs2066845) in comparison with healthy controls of the same genotype (Table 7). Also, serum levels of INF-β was significantly lower in both wheezy and cough phenotypes with GG genotype of NOD2 (rs2066845) compared to healthy controls of the same genotype (Table 8).

Table 1. Demographic data of the studied groups (wheezy, cough and control groups)

	Wheezy (n=67)	**Cough (n=64)**	**Controls (n=39)**	**Test of significance**		
				p1	**p2**	**p3**
Age (years) Mean ± SD	8.59±3.05	8.58±2.66	9.53±2.84	t=1.568 p=0.120	t=1.716 p=0.089	t=0.022 p=0.982
Gender						
Male	38 (56.7%)	45(70.3%)	23 (59%)	χ^2 =0.051	χ^2 =1.39	χ^2 =2.61
Female	29 (43.3%)	19(29.7%)	16 (41%)	p=0.821	p=0.239	p=0.106
Nutritional history						
Breast feeding	9 (13.4%)	24(37.5%)	18(46.2%)	χ^2 =14.15	χ^2 =0.866	χ^2 =11.26
Artificial feeding	21 (31.3%)	19(29.7%)	9(23.1%)	p=0.001*	p=0.649	p=0.004*
Mixed	37 (55.2%)	21(32.8%)	12(30.8%)			
Parental Smoking						
Positive	37 (55.2%)	34(53.1%)	12(30.8%)	χ^2 =5.93	χ^2 =4.90	χ^2 =0.058
Negative	30 (44.8%)	30(46.9%)	27(69.2%)	p=0.015*	p=0.027*	p=0.810
Exposure to indoor trigger						
Positive	27(40.3%)	34(53.1%)	10(25.6%)	χ^2 =2.33	χ^2 =7.48	χ^2 =2.16
Negative	40(59.7%)	30(46.9%)	29(74.4%)	p=0.127	p=0.006*	p=0.141
Exposure to outdoor trigger						
Positive	36(53.7%)	52 (81.2)	16(41%)	χ^2 =1.59	χ^2 =17.48	χ^2 =11.2
Negative	31(46.3%)	12(18.8%)	23(59%)	p=0.207	p≤.001*	p=0.001*
Family history of allergy						
Positive	7 (10.4%)	9 (14.1%)	-	-	-	χ^2 =0.399
Negative	60(89.6%)	55(85.9%)				p=0.528

t: student t- test, χ^2: Chi square test, *p1*: comparison between wheezy and control groups, *p2*: comparison between cough and control groups, *p3*: comparison between wheezy and cough groups. * Statistical significance was defined as $p \leq 0.05$.

Table 2. Clinical data of the asthmatic patients (wheezy and cough phenotypes)

Parameters	Total (n=131)	Wheezy (n=67)	Cough (n=64)	Test (p value)
Duration of illness (years)	4.50 (0.5- 16)	4.50 (0.5- 15)	4.25 (1- 16)	Z=0.042 P=0.967
Degree of asthma severity Mild Moderate Severe	 21 (16.0%) 104 (79.4%) 6 (4.6%)	 12 (17.9%) 49 (73.1%) 6 (9.0%)	 9 (14.1%) 55 (85.9%) 0 (0%)	χ^2 =6.71 p=0.035*
Atopic dermatitis Positive Negative	 11 (8.4%) 120 (91.6%)	 6 (9.0%) 61 (91.0%)	 5 (7.8%) 59 (92.2%)	χ^2 =0.056 p=0.814
Allergic rhinitis Positive Negative	 23 (17.6%) 108 (82.4%)	 8 (11.9%) 59 (88.1%)	 15 (23.4%) 49 (76.6%)	χ^2 =2.98 p=0.084
History of controller medications ICS ICS & leukotriene Leukotriene modifier	 7 (5.4%) 111 (84.7%) 13 (9.9%)	 6 (9.0%) 55 (82.1%) 6 (9.0%)	 1 (1.6%) 56 (87.5%) 7 (10.9%)	χ^2 =3.59 p=0.166
Level of asthma control Well controlled Partly controlled Uncontrolled	 56 (42.7%) 37 (28.2%) 38 (29.1%)	 27 (40.3%) 23 (34.3%) 17 (25.4%)	 29 (45.3%) 14 (21.9%) 21 (32.8%)	χ^2 =2.61 p=0.271

z: Mann Whitney test, χ^2: chi square test; *significant p≤0.05

Table 3. Serum biomarkers in the patients and control group

	Patients (n=131)	Control group (n=39)	Test	p
Eosinophils (%)	3.46 (0.01- 38)	1.87 (0.15- 10.61)	z=4.83	≤0.001*
Total IgE (IU/ml)	75.15 (1.36- 688)	9.30 (2.88- 45.60)	z=3.89	≤0.001*
Serum INF-β (pg/ml)	17.60 (1.76- 125.30)	30.70 (8.80- 434)	z=3.50	≤0.001*

Data are expressed as median (minimum-maximum). Z: Mann-Whitney test. ***** Statistical significance was defined as $P \leq 0.05$.

Table 4. Serum biomarkers in the studied asthma phenotypes (wheezy and cough) and the control group

	Wheezy (n=67)	Cough (n=64)	Control (n=39)	Test of significance		
				p1	*p2*	*p3*
Eosinophils %	4.00 (0.06- 38)	3.31 (0.01- 19.24)	1.87 (0.15- 10.61)	z=4.76 p≤0.001*	z=3.15 p=0.002*	z=3.28 p=0.001*
Total serum IgE (IU/ml)	47.10 (3- 506)	94.6 (1.36- 688)	9.30 (2.88- 45.6)	z=3.92 p≤0.001*	z=4.02 p≤0.001*	z=3.01 p=0.003*
Serum INF-β (pg/ml)	16.70 (2.86- 125.3)	19.95 (1.76- 109)	30.7 (8.8- 434)	z=3.28 p≤0.001*	z=4.02 p≤0.001*	z=1.19 p=0.233

Data are expressed as median (minimum-maximum). Z: Mann Whitney test.
P1: Comparison between wheezy and control groups, *P2*: Comparison between cough and control groups, *P3*: Comparison between wheezy and cough groups. ***** Statistical significance was defined as $P \leq 0.05$.

Table 5. Frequency of NOD2 genotype and allelic polymorphisms among asthmatics compared to controls

NOD2 rs2066845	Patients group (n=109)	Control group (n=39)	*p* value	OR (95%CI)
Genotype frequency CC (r) GC GG GG+GC	 6 (4.6%) 17 (13.0%) 86 (65.6%) 103 (94.4%)	 9 (23.1%) 5 (12.8%) 25 (64.1%) 30 (76.9%)	 - *P*=0.021* *P*=0.002* *P*=0.003*	 1 5.1 (1.2-21) 5.2 (1.7-15.9) 5 (1.7-15.6)
Allele frequency C (r) G	 29 (13.3%) 189 (86.7%)	 23 (29.5%) 55 (70.5%)	 χ^2 =10.4 *P*=0.001*	 2.7 (1.5-5.1)

r: reference group, OR: odds ratio, CI: Confidence interval
Data are expressed as number (percentage); *p* value assessed via Chi-square test; ***** Statistical significance was defined as $p \leq 0.05$.

Table 6. Frequency of NOD2 genotypes and allelic polymorphisms among the studied asthma phenotypes (wheezy and cough phenotypes)

NOD2 rs2066845	Wheezy phenotype (n=55)	Cough phenotype (n=54)	*p* value	OR (95%CI)
Genotype frequency				
CC (r)	4 (6.0%)	2 (3.1%)	-	1
GC	5 (7.5%)	12 (18.8%)	*P*=0.12	0.2 (0.02-1.5)
GG	46 (68.7%)	40 (62.5%)	*P*=0.53	0.6 (0.1-3.3)
Allele frequency				
C (r)	13 (11.8%)	16 (14.8%)	χ^2=0.42	1.3 (0.6-2.8)
G	97 (88.2%)	92 (85.2%)	*P*=0.51	

r: reference group, OR: odds ratio, CI: Confidence interval
Data are expressed as number (percentage); *p* value assessed via Chi-square test; ***** Statistical significance was defined as $p \leq 0.05$.

Table 7. The serum level of INF-β between individuals with different genotypes of the NOD2 gene (asthmatics and healthy controls)

Asthma cases (131)		Controls (39)		Significance
NOD2 rs2066845	**Serum INF-β**	**NOD2 rs2066845**	**Serum INF-β**	
C/C (n=6)	36.40 (10.30-125.30)	**C/C (n=9)**	31.20 (9.96- 117.00)	Z=0.23 p= 0.81
G/C (n=17)	16.70 (1.76 -109.00)	**G/C (n=5)**	34.70 (8.80 - 100.80)	Z=1.07 p= 0.28
G/G (n=86)	15.90 (2.86- 95.70)	**G/G(n=25)**	24.30 (9.11- 434.00)	Z=2.74 p= 0.006*
	KW=4.18 p=0.124		KW=1.44 p=0.487	

KW: Kruskal Wallis test, Z: Mann Whitney test.
Data are expressed as median (range); p value assessed via Kruskal Wallis and Mann Whitney tests; ***** Statistical significance is defined as $p \leq 0.05$.

Table 8. Serum levels of INF-β variation according to different genotypes of the NOD2 gene in the studied asthma phenotypes (wheezy and cough) and control group

Wheezy Phenotype		Cough Phenotype		Control Group		*p*-value
NOD2 rs2066845	**INF-β**	**NOD2 rs2066845**	**INF-β**	**NOD2 rs2066845**	**INF-β**	
C/C (n=4)	38.90 (10.30- 125.30)	C/C n=2	36.40 (28.90 - 43.90)	C/C (n=9)	31.20 (9.96 - 117.00)	p1= 0.88 p2=0.81 p3= 1
G/C (n=5)	11.60 (3.30-105.00)	G/C n= 11	18.40 (1.76 -109.00)	G/C (n=5)	34.70 (8.80-100.80)	p1= 0.46 p2=0.28 p3=0.61
G/G (n=46)	16.90 (2.86 -95.70)	G/G n= 39	14.90 (4.43- 47.40)	G/G (n=25)	24.30 (9.11- 434.00)	p1=.012* p2=.015* p3=0.84
	KW=1.89 p=0.38		KW=3.17 p=0.21		KW=1.44 p=0.487	

KW: Kruskal Wallis test, Z: Mann Whitney test.
Data are expressed as median (range); p value assessed via Kruskal Wallis and Mann Whitney tests; ***** Statistical significance is defined as $p \leq 0.05$.
p1: wheezy versus control, p2: cough versus control, p3: wheezy versus cough

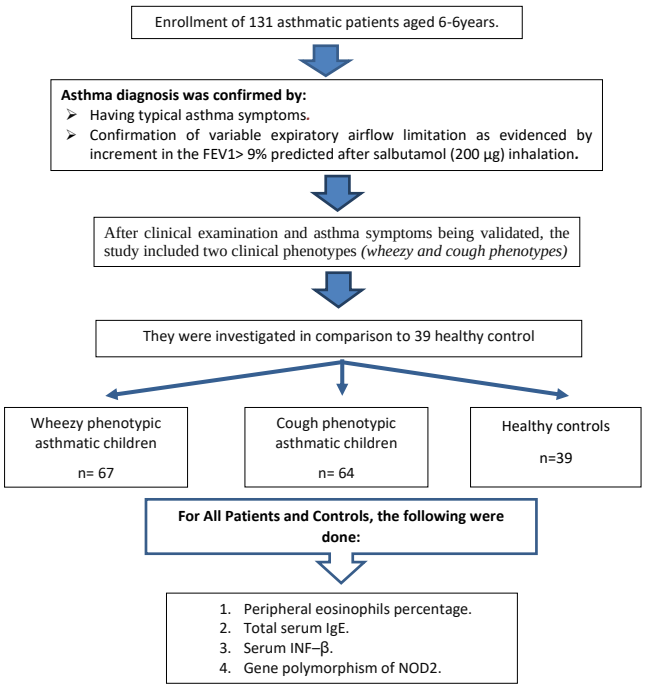

Figure 1. Flow chart of the study.

DISCUSSION

Childhood asthma remains a heterogeneous condition, and verification of its various presentations, risk factors, and outcomes is important because of its therapeutic and prognostic relevance. Further investigation into the immunopathology and genetic basis underlying childhood phenotypes is important so therapy can be tailored accordingly.[10]

The current study aimed to delineate serum levels of INF-β and analyze NOD2 gene polymorphism in a group of children with well-characterized asthma phenotypes (wheezy and cough phenotypes).

The IFN family represents a group of cytokines that play a central role in the protection against exacerbation of various infections and pathologies, including asthma. They play an indispensable role in the host immune system to fight off pathogens, which seems to be altered in both pediatric and adult asthmatics.[11]

Our results revealed that serum INF-β was significantly lower in asthmatic children compared to healthy controls with lack of significant difference between patients with wheezy and cough phenotypes. Several studies, *both in vivo and in vitro*, have investigated the levels of serum INF-β in asthma both in children and adults with contradictory results. However, none of them investigated serum INF-β in different asthma phenotypes.

A study by Zhu et al., compared bronchial mucosal IFN-β expression before rhinovirus infection and after rhinovirus infection in ten atopic asthmatic patients with mean age 23±1.4 and fifteen healthy controls with mean age 27±2.3, recruited from Imperial College London Healthcare NHS Trust (St Mary's Hospital). They observed IFN-β deficiency in the bronchial epithelium after viral infection in asthmatic patients in vivo and this was related to greater viral load, worse airway symptoms, airway hyper responsiveness, and reductions in lung function, together with lower frequencies of bronchial subepithelial monocytes/macrophages expressing IFN-β.[12] On the other hand, some studies observed that IFN response to viruses in airway epithelial cells in vitro was remarkably similar between subjects with and without asthma where the immune response was not deficient but rather modified by the atopic state.[13,14] For example, there are increased numbers of airway mucous cells in asthma and this subset of airway epithelial cells may have inherent differences in susceptibility to viral infection[15,16] as well as a distinct influence on innate and adaptive immune responses that indirectly impact viral clearance.[17,18]

This discrepancy among studies regarding antiviral IFN response may be related to several factors. First, it may be related to the type of virus being studied and the possibility that different viruses might elicit distinct types of IFN responses from the host cell. Second, it could be related to airway epithelial cell culture conditions. Third, it could depend on the types of asthmatic subjects selected for study considering the possibility that asthma severity might influence antiviral response given the differences in immune characteristics among mild, moderate, and severe asthma subsets.[13] Type I INF signaling is especially important for the

control of viral infections.[19] Several trials were carried out to evaluate the efficacy of inhaled IFN-β in combating viral induced asthma exacerbation, both in adults [4,19,20] and children[21,22] but results are yet to be validated.

Concerning NOD2 gene polymorphism (rs2066845), our study revealed that the heterozygous GC genotype and homozygous GG genotype were associated with a higher asthma risk. G allele frequency was significantly higher in total asthmatic cases when compared to healthy control group. Also, G allele showed significantly higher frequency among asthmatics with positive family history and with positive parental smoking. However, no significant difference in the genotype pattern was detected between the two included asthma phenotypes. Regarding the relation between the serum levels of INF-β and different genotypes of the NOD2 gene (rs2066845) we observed that serum levels of INF-β were significantly lower in patients with the homozygous GG genotype compared to controls of the same genotype. Also, in both studied asthma phenotypes (wheezy and cough) CC genotype was found to be associated with higher serum levels of INF-β compared to both GC and GG genotypes.

Genetic polymorphisms in NOD1 and NOD2 (Caspases Activation and Recruitment Domain 15"CARD15") genes were previously found to be associated with the pathophysiology of allergic asthma.[23] Three functionally relevant single nucleotide polymorphisms (SNPs) in NOD2, including rs2066844 (Arg702Trp), rs2066845 (Gly908Arg) and rs869147565 (Leu1007Pro), have been studied in school children in Germany. The authors suggested that these SNPs might be responsible for the development of asthma and allergies in children.[24] Also, a Chinese study have found that *NOD2* gene rs3135499 polymorphism genotype as a risk factor may influence the development of asthma.[7] Another study performed in a Caucasian adult population have found that the rs1077861 T allele decreased the risk of asthma, whereas the rs3135500 A allele was significantly associated with an increased risk of asthma.[25] On the other hand, a relatively recent study including asthmatic Tunisian children has found no association between NOD2 gene polymorphism and the development of asthma.[26]

Airway exposure to NOD2 ligand is suggested to prevent tolerance mechanisms from developing in the lung, suppressing the induction of antigen-specific CD4+forkhead box protein 3 (FOXP3) + regulatory T (Treg) cells while at the same time promoting interleukin 4 (IL-4)–secreting effector CD4 T cells. NOD2 ligand was reported to induce selective expression of the innate cytokines thymic stromal lymphopoietin (TSLP) and IL-25 and TSLP-dependent induction of the Tumor necrosis factor (TNF) family costimulatory molecule OX40 ligand (OX40L), with subsequent susceptibility to develop asthmatic disease.[27]

Viral infection results in significant induction of NOD2 expression, activating downstream Nuclear Factor Kappa-B (NF-κB) and IFN pathways.[28] In addition, during viral infections, NOD2 can also be relocated to the mitochondria by its interaction with the mitochondrial antiviral signaling (MAVS) protein inducing the production of type I IFNs.[29]

In conclusion, our study demonstrated lower serum levels of INF-β in asthmatic children compared to healthy controls which could highlight the potential role of IFNs-based therapies for asthma. Further, this study provided evidence that *NOD2* gene rs2066845 polymorphism genotypes differed between asthma and healthy controls in a cohort of Egyptian children. The rs2066845 G allele as a risk factor may influence the development of asthma. Eventually, no significant differences were detected between the two included clinical phenotypes regarding serum biomarker and the genetic pattern. Nonetheless, due to the limited sample size, further studies are needed to verify our results.

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