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Relation between MBL2 Gene Polymorphism and Pediatric Bronchial Asthma at Zagazig University Hospitals

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ABSTRACT

Background: Among children, asthma is the most frequent chronic inflammatory illness with an airway hyper reactivity that leads to blockage and restriction of the airflow.

Objective: To detect relation between mannose binding lectin (MBL) genetic polymorphisms and childhood asthma among pediatric population at Zagazig University Hospitals.

Patients and methods: 116 individuals were studied in this case-control research, 1st control healthy group were 29 healthy non-asthmatic of matched age with patients in 2nd group who were 87 asthmatic children aged between 5 and 15 years old in Zagazig University Pediatric Hospital, Pediatrics Outpatient Clinic.

Results: There were statistically significant variations in IgE levels amongst the four groups investigated, with all the asthmatic groups significantly greater than the control group. There was significant difference found between cases and control group according to distribution of MBL2 gene polymorphism, and distribution of MBL2 gene alleles.

Conclusion: Our research on elevated levels of MBL and IgE in asthmatics suggests that these antibodies are part of the body's innate immunity, MBL may play a role in asthma pathogenesis by promoting airway inflammation or raising the likelihood of asthma development.

Keywords: Bronchial Asthma, MBL2 Gene.

INTRODUCTION

Asthma is a chronic inflammatory illness that frequently affects children and causes blockage and restriction of airflow because of the hyperresponsiveness of the airways. Coughing, wheezing, shortness of breath, and chest tightness are some of the recurring respiratory symptoms that are clinically defined by the presence of varying expiratory airflow limitation over time and intensity **(1).**

It's one of the 10 most common long-term illnesses among children 5 to 14 years old, according to international data. The global death rate for children with bronchial asthma ranges from 0 to 0.7 per 100,000 **(2).** The MBL2 gene, located on chromosome 10q11.2-q21 q21 and including four exons, encodes the protein mannose binding lectin **(3).** As one of the most important pathogen recognition molecules in the innate system, mannose binding lectin is considered an acute phase reactant that rises in production with any inflammatory stimuli **(4).**

However, there are few research that link MBL protein levels to asthma symptoms or severity, while some imply that MBL may act as a modulator of disease progression. Mannose binding lectin gene polymorphisms show high amounts of circulating MBL protein in both adults and children with bronchial asthma (BA). Since it is sensitive to severe disease, we can classify it as an asthmatic phenotype**(5).**

This study's purpose was to detect relation between mannose binding lectin genetic polymorphisms and childhood asthma among pediatric population at Zagazig University Hospitals.

PATIENTS AND METHODS

One hundred and sixteen subjects at Faculty of Medicine, Zagazig University Hospitals, were involved in this case control study.

Ethical consent:

Research Ethics Council at Zagazig University approved the study (ZU-IRB#6171) as long as all parents of participants provided informed consent forms. Ethics guidelines for human experimentation were adhered to by the World Medical Association's Helsinki Declaration.

Total number of children was divided into two groups: First group: included 29 non-asthmatic children, and **Second group:** included 87 asthmatic children. They were classified according to **GINA (1)** into: 29 asthmatic child who were well controlled, 29 asthmatic child who were partially controlled, and 29 asthmatic child with uncontrolled condition.

Inclusion Criteria:

Children with bronchial asthma, approval to share in the study, both sexes, and age from 5-15 years.

Exclusion Criteria:

Refusal to participate in the study, asthmatic children younger than 5 years old or older than 15 years old, children with accompanied inflammatory diseases as (inflammatory bowel, rheumatic fever, vasculitis), and children suffering from liver, renal or heart diseases or other chronic diseases rather than bronchial asthma.

All of the groups studied were subjected to the

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following:

1- **History taking:** Full history was collected and protocols of treatment of asthma, as well as family history.

2- Clinical examination: General examinations, vital signs, in addition to anthropometric measures; weight, height, body mass index (BMI) (BMI= weight/height m^2). Patients with BMI above 95th percentile were defined as obese (BMI >30), patients with a BMI of 25-30 were considered overweight, but those with a BMI of 75-95 were considered obese [(11)]. Waist circumference and blood pressure were also measured.

3- Laboratory investigations: Routine complete blood count, liver function test, C-reactive protein, kidney function test, and pulmonary function test: Asthmatic patients who had been diagnosed with asthma for at least five years underwent pulmonary function testing utilising D-97024 Hochberg, Germany's forced spirometry programme, which uses a tidal breathing analysis to quickly and accurately measure their respiratory resistance.; with assessment of forced expiratory volume in one second (EFV1), PEF, FVC%, FCV1% and PEF%.

Mannose-binding lectin-2 (MBL-2) gene polymorphism:

Identification of different genotypes of the MBL-2 gene codon 54-exon 1 employing the polymerase chain reaction-restricted fragment length polymorphism (PCR-RELP) approach.

Primers of MBL-2 gene were supplied as lyophilized agents by (ThermoFisher, scientific, USA). Forward primer sequence was 5'-GTAGGACAGAGGGCATGCTC-3' and reverse primer sequence was 5'-CAGGCAGTTTCCTCTGGAAGG-3'.

Primers were reconstituted with sterile deionized water to make stock. Then, dilutions were made from this stock to reach 10 µM concentration.

The PCR reaction was performed in a total reaction mixture of 25 µl, that contained 12.5 µl PCR Master Mix (iTaqTM, iNtRON, Korea), 1.25 µl forward primer, 1.25 µl reverse primer, 9 µl DNA extract, and 1 µl nuclease free water. There were 40 cycles of denaturation for 15 seconds (s) at 95^{o}C followed by 40 cycles of annealing for 30 s at 55^{o}C, and expansion for 30 s at 72^{o}C, all with a final extension for 5 minutes, all with the prescribed thermal cycling parameters.

This reaction yielded amplification products of 329 bp size that were analyzed using 1.5% agarose gel electrophoresis. Subsequent digestion of the obtained 329 bp products was done using restriction enzyme, BanI (BshNI) according to the manufacture's protocol (Fig. 1).

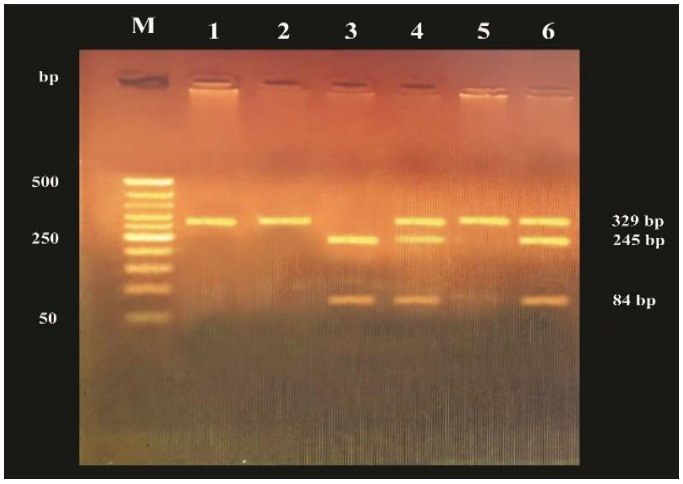

Figure (1): The BanI restriction profiles of mannose-binding lectin-2 (MBL-2) gene polymorphism. M: DNA ladder (50-500bp), Lane 1: PCR product at 329bp, Lanes 3: the wild genotype [A/A] at 245 bp and 84, Lanes 2 and 5 homozygous mutant genotype [B/B] at 329bp, Lanes 4 and 6: heterozygous mutant genotype [A/B] at 329, 245 and 84bp

Statistical analysis

In order to analyze the data acquired, Statistical Package for the Social Sciences (SPSS) version 20 was used to execute it on a computer. The quantitative data were presented in the form of the mean, and standard deviation (SD). The information was presented using qualitative statistics such as frequency and percentage. The student's t test and one-way ANOVA test were used to assess the data while dealing with quantitative independent variables. Pearson Chi-Square was used to assess qualitatively independent data. The significance of a P value of 0.05 or less was determined.

RESULTS

None of basic characteristics differed significantly between the 4 studied groups **(Table 1).**

Table (1): Characters of the four studied groups

			N. Control (29)	N. Case (87)	P
Age (years) mean±SD			**9.03±2.74**	**7.93±3.04**	**0.086**
Height (m) mean±SD			**134.34±16.98**	**129.40±17.76**	**0.192**
Weight (Kg) mean±SD			**31.37±9.43**	**32.19±10.05**	**0.750**
BMI (Kg/m^2) mean±SD			**17.85±4.36**	**18.02±3.66**	**0.837**
Sex	**Female**	**N**	**13**	**35**	**0.66**
		%	**44.8%**	**40.2%**	
	Male	**N**	**16**	**52**	
		%	**55.2%**	**59.8%**	

Eosinophil, CRP and IGE level differed significantly as they were lower in control groups **(Table 2)**.

Table (2): Lab parameters of studied groups

	Control Group (29)	Well Controlled Group (29)	Partial Controlled Group (29)	Uncontrolled Group(29)	P
WBC (n*1000/ul)	**7.94±1.38**	**8.66±1.78**	**8.14±1.95**	**8.68±1.86**	**0.632**
Hb (g/dl)	**11.83±0.76**	**12.04±0.75**	**12.22±0.90**	**12.06±0.76**	**0.338**
PLT (n*1000/ul)	**281.61±30.52**	**283.17±42.29**	**302.72±50.19**	**283.34±42.33**	**0.212**
Esino (%)	**222.86±65.36**	**2088.06±485.6**	**2162.17±763.3**	**2288.27±524.63**	**<0.01**
CRP (mcg/ml)	**1.04±0.07**	**1.20±0.21**	**1.05±0.09**	**1.20±0.3**	**0.044**
ALT (IU/L)	**16.12±3.63**	**18.87±4.39**	**16.33±3.80**	**18.65±4.88**	**0.204**
AST (IU/L)	**22.34±5.77**	**23.27±5.79**	**22.92±5.82**	**22.82±5.19**	**0.974**
Urea (mg/dl)	**10.71±2.31**	**10.24±1.45**	**11.52±2.58**	**10.23±1.46**	**0.054**
Creat (mg/dl)	**0.40±0.14**	**0.352±0.01**	**0.41±0.11**	**0.35±0.071**	**0.106**
IGE (IU/L)	**46.10±7.52**	**153.58±14.62**	**158.20±15.05**	**162.20±9.23**	**<0.001**

Data are presented as mean± standard deviation

There was a statistically significant correlation between AB polymorphism and the severity of asthma **(Table 3).**

Table (3): Comparison between cases and control groups as regards to mannose binding lectin 2 gene allele distribution

			Control	Case	P
Gene Allele	**A**	**N**	**54**	**129**	**0.002**
		%	**93.1%**	**74.1%**	
	B	**N**	**4**	**45**	
		%	**6.9%**	**25.9%**	
Total		**N**	**58**	**174**	
		%	**100.0%**	**100.0%**	

AB polymorphism and grade of asthma differed significantly between the groups **(Table 4).**

Table (4): Mannose binding lectin 2 gene polymorphism distribution among studied groups

			Control Group	Well Controlled Group	Partial Controlled Group	Uncontrolled Group	P
Gene polymorphism	**AA**	**N**	**25**	**20**	**17**	**10**	**0.005**
		%	**86.2%**	**69.0%**	**58.6%**	**34.5%**	
	AB	**N**	**4**	**7**	**11**	**17**	
		%	**13.8%**	**24.1%**	**37.9%**	**58.6%**	
	BB	**N**	**0**	**2**	**1**	**2**	
		%	**0.0%**	**6.9%**	**3.4%**	**6.9%**	
Total		**N**	**29**	**29**	**29**	**29**	
		%	**100.0%**	**100.0%**	**100.0%**	**100.0%**	

There was statistically significant difference between A and B allele and grade of asthma **(Table 5)**.

Table (5): Mannose binding lectin 2 gene allele distribution between cases and control

			Control Group	Well Controlled Group	Partial Controlled Group	Uncontrolled Group	P
Gene polymorphism	**A**	**N**	**54**	**47**	**45**	**37**	**0.002**
		%	**93.1%**	**81.0%**	**77.5%**	**63.7%**	
	B	**N**	**4**	**11**	**13**	**21**	
		%	**6.9%**	**19.0%**	**22.5%**	**36.3%**	
Total		**N**	**58**	**58**	**58**	**58**	
		%	**100.0%**	**100.0%**	**100.0%**	**100.0%**	

There was a statistically significant difference in the percentage of A and B alleles between the uncontrolled and partially controlled groups **(Table 6)**.

Table (6): Comparison of alleles and **genotypes between the uncontrolled group and partially controlled group**

	Uncontrolled (n=29)	**Partially controlled (n=29)**	**P value**
Genotype			
AA(n=27)	10 (34.5%)	17 (58.6%)	**0.18**
AB (n=28)	17 (58.6%)	11(37.9%)	
BB (n=3)	2 (6.9%)	1 (3.4%)	
Alleles	**(n=58)**	**(n=58)**	
A (n=42)	37 (63.7%)	45 (77.5%)	0.103
B (n=84)	21 (36.3%)	13 (22.5%)	

Uncontrolled and well-controlled groups differed significantly in terms of A and B alleles, with the uncontrolled group having a lower percentage of A alleles (63.7%) than the well-controlled group (81%) **(Table 7)**.

Table (7): Comparison of alleles and genotypes between the uncontrolled group and well controlled group

	Uncontrolled (n=29)	**Well controlled (n=29)**	**P value**
Genotypes			
AA (n=30)	10 (34.5%)	20 (69%)	**0.04****
AB (n=24)	17 (58.6%)	7 (24.1%)	
BB (n=4)	2 (6.9 %)	2 (6.9%)	
Alleles	**(n=58)**	**(n=58)**	
A (n=84)	37(63.7%)	47 (81%)	0.04**
B (n=32)	21(36.3%)	11(19%)	

In terms of genotypes and alleles, there was no statistically significant difference between the poorly controlled and the well-controlled groups **(Table 8).**

Table (8): Comparing genotypes and alleles between the partially and well controlled groups

	Partially controlled (n=29)	**Well controlled (n=29)**	**P value**
Genotypes			
AA (n=37)	17 (58.6%)	20 (69%)	**0.48**
AB (n=18)	11(37.9%)	7 (24.1%)	
BB (n=3)	1 (3.4%)	2(6.9%)	
Alleles	**(n=58)**	**(n=58)**	
A(n=92)	45 (77.5%)	47 (81%)	0.65
B (n=24)	13 (22.5%)	11 (19%)	

DISCUSSION

One of the most common illnesses in children is asthma, which frequently coexists with other conditions. Asthma frequency among youngsters worldwide has progressively increased during the past twenty years [(6)].

Increased mucus secretion and remodelling of the airways are caused by asthma immunopathological processes. When eosinophils, mast cells, and immunoglobulin E (IgE) are all activated, the result is allergic or atopic asthma, which is defined by the presence of ILC2 and a Th2 response that produces cytokines including interleukin (IL)-4, IL-5, and IL-13 [(7)].

As one of the most important pathogen recognition molecules in the innate system, mannose binding lectin is considered an acute phase reactant that rises in production with any inflammatory stimuli [(4)]. In a number of studies, MBL has been linked to asthma, however the majority of these were conducted on children with the condition. MBL deficiency increased infection risk in children and was linked to acute asthmatic exacerbations in children [(8)].

None of basic characteristics differed significantly between the 4 studied groups. In line with our results **Yalçın *et al.*** [(9)] involved 17 patients with asthma (mean age 7.8±1.7 years) and 26 control cases without any chronic disease (mean age 7.7±1.8 years).

There were no statistically significant changes in the distribution of laboratory data between cases and controls except for eosinophils, which were considerably greater in the asthmatic group than in the non-asthmatic group. The same result was obtained between different subgroups of patients. **Staley *et al.*** [(5)] stated that eosinophils and immunoglobulin E are two of the most critical immunological components in the

development of bronchial asthma.

Our results were in line with the findings by **Yalçın *et al.* [9]** who revealed that there were no statistically significant differences between cases and controls as regard laboratory data including hemoglobin, mean corpuscular volume, red cell distribution width, white blood cell and platelets. In disagreement with our results **Ardura-Garcia *et al.* [10]** reported that there were no statistically significant differences between cases and controls as regard eosinophil count (p=0.532).

Regarding IgE distribution between cases and control, our results revealed that cases had significantly higher levels of IgE than control; as well IgE was significantly lower in control than other groups with no significant difference among other subgroups. Our result was supported by **Wang *et al.* [11]** who found the difference in IgE levels between asthma patients and controls was statistically significant.

Genetic polymorphism was shown to be statistically significant in four groups, with 86.2 percent of the control group having the AA genotype and 55.6 percent of uncontrolled asthmatic patients having the AB genotype, according to our findings. The study by **Dogru *et al.*[12]** found that there was a highly significant difference in gene polymorphism between the analysed groups, which is consistent with our findings. While in the study by **Rantala *et al.* [13]** there were no statistically significant variations in the frequency of MBL2 genotypes in the whole population, those with asthma, and those without asthma, which agrees with the results of this study.

CONCLUSION

As a component of innate immunity, MBL may have a role in the pathogenesis of asthma by contributing to airway inflammation or raising the likelihood of developing asthma, as suggested by our findings of elevated MBL and IgE levels in asthmatics.

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Author contribution: Authors contributed equally in the study.

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