

Association between Prostaglandin Receptor Gene Polymorphism and Response to Anti Leukotriene among Children with Bronchial Asthma

Naser Miftah Asmaydeh Al Shehibi¹, Khalid Mohamed Salah², Mohamed Mohamed Abdel Salam³, and Rania Mohamed Mohamed Ibrahim⁴

¹M.B.B. Ch, of Medicine and Surgery, Benghazi University, Pediatrician, Al-Marg Teaching Hospital, Libya.

²Professor of Pediatrics, Faculty of Medicine Zagazig University.

³Professor of Pediatrics, Faculty of Medicine Zagazig University.

⁴Lecturer of Microbiology and Immunology, Faculty of Medicine Zagazig University.

Corresponding author: Naser Miftah Asmaydeh Al Shehibi

Email: nmofath55@gmail.com

Abstract

Background: Bronchial asthma is chronic respiratory diseases (CRDs) are form the most common causes leading to death worldwide, and bronchial asthma is rated the most common chronic disease affecting children. The function of prostaglandin D2 (PGD2) may affect the response of the leukotriene receptor antagonist (LTRA).

Aim of the study: This study aimed to identify PTGDR single nucleotide polymorphism and response to leukotriene receptor antagonist (LTRA).

Patients and methods: This study was a case control study including fifty asthmatic patients (25 males and 25 females). They were classified into 4 subgroups according to GINA, 2016 classification: mild intermittent, mild persistent, moderate persistent and severe persistent. Fifty clinically healthy control participants with matched age and sex were thoroughly evaluated, selected after careful clinical examination. All patient underwent to full history taking, clinical examination, assessment of pulmonary function (FEV1%, FVC% and PEF %) before and after montelukast (5 mg per day), Total serum IgE level assessment. Detection of SNP (PTGDR)—441T/C (rs803010) after PCR amplification of the target gene In all, 50 subjects with asthma performed an exercise challenge test twice both before and after receiving their daily dose of montelukast (5 mg per day) for 8 weeks.

Results: There were no statistically significant differences between patients and control regarding PTGDR polymorphism, so polymorphism at codon 22q14 can't be considered as a determinant factor for asthma susceptibility. This study revealed a strong positive association between SNPs at codon 22q14 and asthma severity. Patients carrying PTGDR T/C high risk of asthma severity while PTGDR homozygous can be considered as a protective risk factor for severe grades of asthma. patients having PTGDR T/C genotype are significantly responsive to antileukotriene, followed by PTGDR T/T Homozygous, while patients having PTGDR C/C genotype respond poorly to antileukotriene. no significant relationship had been found between PTGDR polymorphism of antileukotriene receptor and elevated levels of neither total serum IgE nor eosinophilic count for all studied genotypes.

Conclusion: Antileukotriene receptor polymorphism at codon 22q14 is not associated with asthma susceptibility; however, it can be a determinant factor for asthma severity and response to antileukotriene in Egyptian asthmatic children, to be confirmed by further Pharmacogenomic studies.

Keywords: Bronchial Asthma (BA), Anti Leukotriene, prostaglandin D2 (PGD2).

1.Introduction:

Asthma is a chronic inflammatory disease of airways in which many cells and cellular elements play a role in particular, mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells. Inflammation causes recurrent series of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread and variable airflow obstruction that is often reversible either spontaneously or with treatment **(1)**.

Bronchial asthma is chronic respiratory diseases (CRDs) are form the most common causes leading to death worldwide, and bronchial asthma is rated the most common chronic disease affecting children **(2)**.

Bronchial asthma is a chronic inflammatory disease of the airways that is associated with the development and progression of airway hyperresponsiveness which results from a combination of genetic and environmental factors. Clinically, it is characterized by recurrent attacks of wheezing, shortness of breath, chest tightness and cough. Its global prevalence has been on the rise over years **(3)**.

Asthma is one of the most common chronic diseases in the world. Genetic and environmental factors contribute to the development of asthma and its subsequent severity. Asthma is a complex syndrome with variable outcomes and this variability has made it difficult to appropriately monitor and control the condition **(4)**.

Asthma is often triggered by mast cells that are activated by an IgE- mediated allergic challenge. Asthma is recognized as a complex genetic disease that cannot be explained by single gene models, and in most cases it appears to result from the interaction of multiple genetic and environmental factors, socioeconomic factors, cultural factors, health maintenance behaviors, air quality and obesity **(5)**.

The disease etiology is poorly understood and the postnatal development is not well established. Genetic susceptibility believed to play a critical role in the development of asthma. Over 200 genes have been suggested to contribute to asthma occurrence. The high heritability and the co-occurrence of asthma within families highlight the importance of a genetic component in disease pathogenesis **(6)**.

Activated mast cells produce a variety of chemical mediators including prostaglandin D2 (PGD2), which is the major cyclooxygenase metabolite of arachidonic acid, in response to allergen exposure. PGD2 may contribute to pulmonary vasodilation, bronchoconstriction, and the recruitment of eosinophils, basophils and T-lymphocytes. PGD2 exerts its biological actions through the PGD2 receptor (PTGDR), which is localized to chromosome 14q22.1 and has been associated with asthma **(7)**.

The existence of genetic variants in the promoter region of the PTGDR (—549, —441 and —197) have been reported for several ethnic groups they may have a role in controlling PTGDR expression. Therefore, the PTGDR may serve as a therapeutic target for asthma.

Therefore, we presume that the function of PGD2 may affect the response of the leukotriene receptor antagonist (LTRA) **(3)**.

2. Patients and Methods:

This study was conducted in Zagazig University Hospital, Pediatric Pulmonology Unit, in cooperation with Zagazig Scientific and Medical Research Center during the period from Dec. 2017 to Dec. 2018.

This study is a case control study including fifty asthmatic patients (25 males and 25 females), their ages ranged from 5 to 14 years. This study subjects were classified into 4 subgroups according to GINA, 2016 classification: mild intermittent, mild persistent, moderate persistent and severe persistent (3).

Fifty clinically healthy control participants with matched age and sex were thoroughly evaluated, selected after careful clinical examination, they were completely free from any disease, not siblings to asthmatic patients.

Patients who were included in the study who were aged from 5 to 12 years who had typical asthma symptoms according to the established guidelines of Global Initiative for Asthma Management and prevention in 2016 and who underwent pulmonary function test for cases (FEV1%, FVC% and PEF %) before and after 8 weeks montelukast (5 mg per day).

Asthmatic patients with co-morbidities such as; cardiovascular diseases and chronic pulmonary diseases have been excluded and also, we excluded all patients with history of respiratory tract infection within the past 4 weeks or history of allergy.

Informed consents were obtained from all caregivers of patients and healthy control.

Methods:

Patients and controls underwent the following:

1. History taking and clinical examination.
2. Pulmonary function test for cases (FEV1%, FVC% and PEF %) before and after montelukast (5 mg per day).
3. Total serum IgE level assessment.
4. Detection of SNP (PTGDR)—441T/C (rs803010) by Restriction Fragment length polymorphism (RFLP) technique after PCR amplification of the target gene.

LTRA drug responsiveness study design

In all, 50 subjects with asthma performed an exercise challenge test twice both before and after receiving their daily dose of montelukast (5 mg per day) for 8 weeks. The standardized exercise challenge consisted of 8 min of free running outdoors.

The response to montelukast was expressed as:

Post montelukast FEV1% – Pre montelukast FEV1% x 50 %

It was considered as responsive when the increasing of FEV1% was 15% or more and unresponsive when the increasing of FEV1% was less than 15% (8).

Determination of total serum IgE by ELISA.

Specimen collection: 1-2 ml of venous blood was collected on serum collection tubes, stored in room temperature (20 – 27° C) for 30 minutes and centrifuged at 2000 x g.

Genotyping by PCR-restriction fragment length polymorphism:

1- Blood sample collection and processing:

Samples of peripheral venous blood were collected aseptically from every subject in a tube containing 0.5 ml EDTA. The collected samples were stored at -20°C until being extracted.

2- DNA extraction:

DNA extraction was performed using G-spin™ Total DNA Extraction Mini Kit (Intron

Biotechnology, Korea) according to the manufacturer's instructions.

3-PCR DNA amplification: Allele-specific PCR technique

Genotyping

PCR–restriction fragment length polymorphism was used to determine the polymorphisms. The primer pairs and annealing temperatures were as follows: 5¢-cgagttcttgccacccagttcaaaccaccagcaca-3¢ and 5¢-ggagcaggccagtgaga-3¢ and 57 °C for the PTGDR 441T/C (rs803010).

Statistical analysis

Data obtained from the present study were computed using statistical package for social sciences (SPSS) version 17 under the platform of Microsoft Windows XP, Professional Edition. Continuous data were expressed in the form of mean $\pm$ SD while categorical data were expressed in the form of count and percent. Comparison of continuous data were performed utilizing student t test, while categorical data were done using Chi-square test. P value less than 0.05 was considered statistically significant.

3.Results

No statistically significant difference was found between both groups regarding age (Table 1), There were no statistically significant difference between patients and control regarding smoke exposure (Table 2). Regarding distribution of severity among studies patients was mild asthma was (62%), followed by moderate persistent asthma (26%) and Severe persistent asthma was (12%) (Table 3).

The values of FEV1%, FVC% and PEF% values prior to montelukast administration, mean 78.29 $\pm$ 9.22, 77.26 $\pm$ 12.36 and 70.12 $\pm$ 13.21 respectively. The values of FEV%, FVC%, and PEF% after 8 weeks of montelukast administration were 92.26 $\pm$ 10.32, 95.5 $\pm$ 15.5, 101.45 $\pm$ 12.98, respectively (Table 4).

There was no statistically significant difference between patients and control regarding T/T homozygous and C/C homozygous polymorphism as T/T genotype represent 28% of asthmatic group, 20% of control group and C/C were 26% and 20% respectively. There was significant difference among T/C heterozygous polymorphism as represent 50% and 14% respectively (Table 5).

There was no significant difference between homozygous gene polymorphism T/T and C/C as regarding asthma severity but statistically significant difference as regard T/C polymorphism (Table 6). The values of FEV1% prior to the monoleukast administration T/T genotype 71.65 $\pm$ 11.58, T/C genotype 61.89 $\pm$ 9.54, C/C genotype 71.95 $\pm$ 18.54. after monoleukast administration T/T genotype 91.85 $\pm$ 25.64, T/C genotype 81.25 $\pm$ 18.74, C/C genotype 91.97 $\pm$ 21.54 (Table 7).

There was statistically significant positive correlation between total IgE level and Eosinophilic count (Table 8).

Table (1): Comparison between patients and control regarding age.

		Patients	Control	t	P
		N=50	N=50		
Age	Range	5-13	5-13	0.158	0.921
	Means $\pm$SD	8.12$\pm$1.66	8.32$\pm$1.87		

Table (2): Smoke exposure between studied groups

		Patients	Control	X²	P
		N=50	N=50		
Smoke exposure	Positive Exposure	N	27	26	0.04
		%	54.0%	52.0%	0.84
	Negative exposure	N	23	24	
		%	46.0%	48.0%	

Table (3): Distribution of asthma severity among studied groups.

		Sample	
		No.	%
Severity of asthma	Mild asthma	31	62.0
	Moderate persistent asthma	13	26.0
	Severe persistent asthma	6	12.0

Table (4): The mean spirometric value among asthmatic children.

Pre montelukast FEV1%	Range	62.35-91.25
	Mean $\pm$SD	78.29$\pm$9.22
Post montelukast FEV1%	Range	73.51-123.36
	Mean $\pm$SD	92.26$\pm$10.32
Pre montelukast FVC%	Range	51.87-113.55
	Mean $\pm$SD	77.26$\pm$12.36
Post montelukast FVC%	Range	78.5-124.6
	Mean $\pm$SD	95.5$\pm$15.5
Pre montelukast PEF%	Range	31.65-111.35
	Mean $\pm$SD	70.12$\pm$13.21
Post montelukast PEF%	Range	64.6-125.35
	Mean $\pm$SD	101.45$\pm$12.98

Table (5): Comparison between patients and control regarding gene polymorphism.

Group		X²	P	OR
Patient	Control			
(No. 50)				

T/C heterozygous	Mutation	N	25	7			
		%	50.0%	14.0%			
	NOT	N	25	43	14.89	0.00**	6.12 (1.8-8.65)*
		%	50.0%	86.0%			
T/T homozygous	Mutation	N	14	10			
		%	28.0%	20.0%			
	NOT	N	36	40	0.87	0.34	1.65 (0.57-3.54)
		%	72.0%	80.0%			
C/C homozygous	Mutation	N	13	10			
		%	26.0%	20.0%			
	NOT	N	37	40	0.508	0.47	1.4 (0.87-2.54)
		%	74.0%	80.0%			
Total		N	50	50			
		%	100.0%	100.0%			

Table (6): Distribution of asthma severity among asthmatic children as regard gene polymorphism.

			Asthma severity			X²	P
			Mild	Moderate	Severe		
Heterozygous T/C	Mutation	N	10	10	5		
		%	32.3%	76.9%	83.3%		
	NOT	N	21	3	1	10.33	0.006*
		%	67.7%	23.1%	16.7%		
Homozygous T/T	Mutation	N	9	2	3		
		%	29.0%	15.4%	50.0%		
	NOT	N	22	11	3	2.48	0.28
		%	71.0%	84.6%	50.0%		
Homozygous C/C	Mutation	N	8	2	3		
		%	25.8%	15.4%	50.0%		
	NOT	N	23	11	3	2.55	0.27
		%	74.2%	84.6%	50.0%		
Total		N	31	13	6		
		%	100.0%	100.0%	100.0%		

Table (7): Comparison between Pre- and post montelukast administration as regarding FEV1 among asthmatic children.

			Non mutation	Mutation	t	P
PTGR441T/T	FEV	Mean	73.5±12.58	71.65±11.58	1.35	0.131
	1 pre	±SD				
	FEV	Mean	94.84±15.87	91.85±25.64	1.29	0.113
	1 post	±SD				
PTGR441T/C	FEV	Mean	82.74±10.25	61.89±9.54	4.57	0.00*
	1 pre	±SD			8	*
	FEV	Mean	101.98±21.25	81.25±18.74	4.87	0.00*
	1 post	±SD			9	*
PTGR441C/C	FEV	Mean	72.88±14.65	71.95±18.54	0.87	0.389
	1 pre	±SD			4	
	FEV	Mean	93.87±20.54	91.97±21.54	0.78	0.457
	1 post	±SD			7	

Table (8): Correlation between serum IgE level and Eosinophilic count among asthmatic children.

Correlation	Pearson's correlation	
	r	p
Eosinophilic count * Total IgE level	0.412	0.0002*

4. Discussion

Bronchial asthma is a complex heterogeneous and multifactorial disease, whereas a reversible airway limitation represents one of the main important factors. Asthma is a complex polygenic disorder in which, many cells and cellular elements play a role (9).

WHO declared in December 2016 that, about 235 million people are suffering from asthma. There are 383 000 cases died in 2015 due to asthma (4). The prevalence of asthma among Egyptian children is 14.7%, where positive family history, overcrowding and bad housing conditions are the most commonly reported risk factors (10).

We conducted a case control study in Pediatric Pulmonology Department, Zagazig University, on fifty asthmatic children, with ages range from 5 to 12 years, with a mean of (8.12±1.66), who were selected and classified into 3 groups regarding the severity of their illness according to (3) into; Mild asthma, moderate persistent asthma, severe persistent asthma.

Where mild asthma was the most frequently distributed 62%, followed by moderate persistent asthma which was 26%. While, the least frequently distributed grade of asthma severity, was the severe persistent class 12%. So, our study is in agreement with (11) regarding distribution of asthma severity.

In this study, both patient and control groups were age and sex matched. The mean value of BMI was significantly lower among asthmatic patients than control group, which was (19.95±4.25) kg/m² among asthmatic patients, but it was (20.17±4.21) in control group, with a mean difference of about 3.74 kg/m².

So, our study agrees with (12), who stated that; diagnosis of asthma is associated with low BMI of about 18.5 kg/m².

Our study is found to be compatible with that study which has been performed in southwest China on 238 asthmatic patients and 265 control individual. This study revealed that, there is no association between PTGDR polymorphism and susceptibility to asthma (13).

Another Asian case-control study has been conducted on North Indian population, PTGDR polymorphism of antileukotrien was found also to be not associated with asthma risk (14).

One of the biggest meta-analysis carried out on Japanese population, demonstrated that, PTGDR does not contribute basically to susceptibility to asthma, but it is possible that these polymorphisms influence disease activity and drug responses in individuals with asthma (15).

In addition to all these studies, our study was consistent also with African American (16) and Hutterite populations (17).

Also we are in agreement with (18), who have found no statistically significant difference between cases and control group at codon 22q14 regarding asthma Susceptibility.

According to the present study, patients carrying PTGR T/C genotype can benefit more from using monoleukast accompanying bronchial asthma, on contrary with patients carrying other genotypes at codon 22q14, who respond more weakly to antileukotrein. This strategy is thought to decrease hazards and to reduce expenses of using needless antileukotrein in such non-responding cases.

In addition, (19) found that asthmatic subjects homozygous for PTGDR have less responsiveness to antileukotrein than asthmatic homozygous for PTGDR. So this study is found to be consistent with the previous Egyptian study of (18); which revealed that, total serum IgE was higher in codon 22q14 polymorphism but without a significant difference and showed nearly a similar level in different genotype in patients group. (20), detected also, no significant difference of the total serum IgE level with SNPs at position 22q14; moreover, when population was stratified according to the most common homozygous haplotype no association was found in relation to serum IgE.

The causal alleles that are common in most ethnic groups may have differential effects because of interactions with the environment and/or other genetic variants that are unique to certain ethnic groups. The interpretation of the findings of the genetic association studies of the PTGDR polymorphisms is complicated by the inadequate measurement of environmental exposures and differences in allele and haplotype frequencies of the PTGDR gene and asthma severity among different racial groups. The complexity of the observed genotype-response effects limits their clinical applications (21).

Diseases with a complex genetic origin, such as asthma, also may be characterized by pleiotropy (the same genotype has different phenotypes), genetic heterogeneity (the same phenotype results from different polymorphisms), and incomplete penetrance (the same polymorphism does not always produce the same phenotype (22).

Different findings in studies including ours, can be explained by; differences between centers in approach to clinical management, criteria for diagnosis of asthma may not follow guidelines, ethnic differences that can alter the genotypic frequency of polymorphisms, variations among enrolled patients (atopic and non- atopic), presence of non-reported comorbidities in addition to, variations between used Genotyping techniques (23).

5. Conclusion

Antileukotriene receptor polymorphism at codon 22q14 is not associated with asthma susceptibility; however, it can be a determinant factor for asthma severity and response to antileukotriene in Egyptian asthmatic children, to be confirmed by further Pharmacogenomic studies.

6.Conflict of Interest: No conflict of interest.

7.References

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