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Correlation between Gene Polymorphisms of Prostaglandin D Receptor and Severity of Asthma in Children

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ABSTRACT

Background: Patients, their families and the community suffer a heavy burden when it comes to asthma because it is both common and possibly life-threatening. It has been postulated that arachidonic acid's most prevalent cyclooxygenase metabolite, prostaglandin D2 (PGD2), is a mast cell activation marker in asthma. **Objective**: To evaluate association between PGDR-441 polymorphism with risk factors, laboratory characteristics, and severity of asthma in children. **Methods**: In this cross-sectional study, Forty Egyptian children were genotyped using allele specific polymerase chain reaction (AS-PCR) to assess single nucleotide polymorphism of PTGDR2 receptors. Selected cases were classified according to GINA guidelines and spirometrically assessed to evaluate pulmonary functions.

Results: There is significant difference between mild, moderate and severe asthma regarding total IgE level(P1<0.001). 68.0% of the studied patients had Homogenous PGDR2(TT) and 32% had Heterozygous PGDR2(TC). There were no statistically significant associations between PGDR2 Polymorphisms and both of asthma risk factors and laboratory characteristics. There was a statistically significant difference between PGDR2 Polymorphisms and bronchial asthma severity of the studied patients. Heterozygous PGDR2 was associated with more severe bronchial asthma.

Conclusion: Our study showed a strong relationship between polymorphism of PTGDR2 receptor and severity of bronchial asthma.

Keywords: Bronchial asthma, Prostaglandin D Receptor, gene polymorphism.

INTRODUCTION

People who suffer from asthma are burdened by the disease, as are their families and the community at large. Shortness of breath, tightness in the chest, and cough are common symptoms, which appear and disappear at varying intervals over time [(1)]. Environmental allergens such mites or house dust mites as well as smoking cigarettes and eating habits all play a role in asthma development. Social and economic factors have an impact on exposure to allergens and the availability of health care services, therefore they should not be neglected [(2)].

Activation of mast cells in asthma is suspected to be marked by the prostaglandin D2 (PGD2) metabolite of arachidonic acid, the most common cyclooxygenase enzyme metabolite. Genome 14q22.1 contains a gene for the Prostaglandin D2 Receptor in humans and the gene's protein sequence is codified by 359 amino acids, 40.276 kDa G protein-coupled receptor. Exposure to allergens increases PGD2 levels in asthma sufferers, which is known to cause bronchoconstriction in the airways, according to research [(2)]. Asthma patients' PGDR2 levels rise in response to allergen challenge, as do those with the most severe forms of the condition [(3)].

There have been numerous PGDR2 receptor polymorphisms discovered, a study of the American and African American populations found that the PGDR2 gene's 549T/C and 441C/T polymorphisms were linked to asthma [(4)].

We aimed at this study to evaluate association between PGDR-441 polymorphism with risk factors, laboratory characteristics, and severity of asthma in children.

PATIENTS AND METHODS

A cross-sectional study of children who visited the pediatrics general outpatient clinic at Zagazig University Hospitals was conducted on forty asthmatic patients (18 males and 22 females), their ages ranged from 5 to 12 years. With a mean value of (8.5± 2.4) years. This group was classified into 4 subgroups mild intermittent, mild persistent, moderate persistent and severe persistent.

As long as all parents of participants signed informed consent forms and submitted them to Zagazig University's research ethics committee, the study was allowed (ZU-IRB#6167/15-7-2020). We followed the World Medical Association's ethical code for human experimentation, the Helsinki Declaration.

Inclusion criteria:

Children aged from 5 to 12 years, having typical asthma symptoms in accordance with the Global Asthma Management and Prevention Initiative recommendations in 2016 [(1)], Variable expiratory airflow obstruction was validated and demonstrated by an increase in pre-administered leukotriene receptor antagonist FEV1 of >15 percent anticipated following montelukast (5mg per day) administration, in the last six weeks, had no history of corticosteroid treatment, Oral beta-adrenergic agonists should be taken within 1 week, inhaled beta-adrenergic

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agonists should be taken within 6 hours, antihistamines should be taken within 72 hours, and leukocyte modifiers should be taken within 4 weeks [5].

Exclusion criteria:

When parents refuse to participate their children in the study, Patients suffering from asthma who also have concomitant conditions such as cardiovascular disease or chronic pulmonary illness, additionally, a history of respiratory tract illness during the previous 4 weeks, as well as a history of allergy or dermatitis.

All participants in this study were subjected to the following:

1. History taking and clinical examination.
2. Before and after salbutamol administration, pulmonary function tests were performed (FEV1%, FVC %, and PEF %).
3. The determination of total serum IgE levels.
4. EDTA tubes were used to collect blood samples, which were then analysed by an automatic cell counter to determine the total leukocyte count and peripheral eosinophilic percentage.
5. Genetic study of the PGDR 2 gene to find polymorphisms in the PGDR-441 T/C gene was performed.

PGDR2 genetic analysis by allele specific polymerase chain reaction technique (AS-PCR):

EDTA-treated blood samples were obtained aseptically from each patient and placed in a tube that contained 0.5 mL of the anticoagulant EDTA. The samples were collected and maintained at –20°C until they were used for DNA extraction. Using the G-spin TM total DNA Extraction Mini Kit, we were able to extract DNA from our samples (Intron Biotechnology, Korea). The Taq polymerase and heat cycler were used to execute the enzymatic amplification, which was done in accordance with the methodology described by **Folwaczny *et al.*** [6].

Lyophilized primers were reconstituted to obtain an optimal concentration (30 pmole /Amplification) then stored at -20°C.

Forward Prime: 5’ cgagttcttggccaccccagttcaaacaccagcacaa - 3'

Reverse Primer: 5' ggagcaggccagtgaaga -3'

The thermal cycler Veriti 96 well from Applied Biosystems was utilized, which enables for continual heating and cooling of the block holding the PCR tubes. The detection of the amplification product was accomplished using Agarose Electrophoresis.

Interpretation:

It was discovered that bromide intercalated between the bases of the DNA and fluoreses when the gel was inspected under UV light. Bands situated at 35 bp and 195 bp were found in homogenous instances.

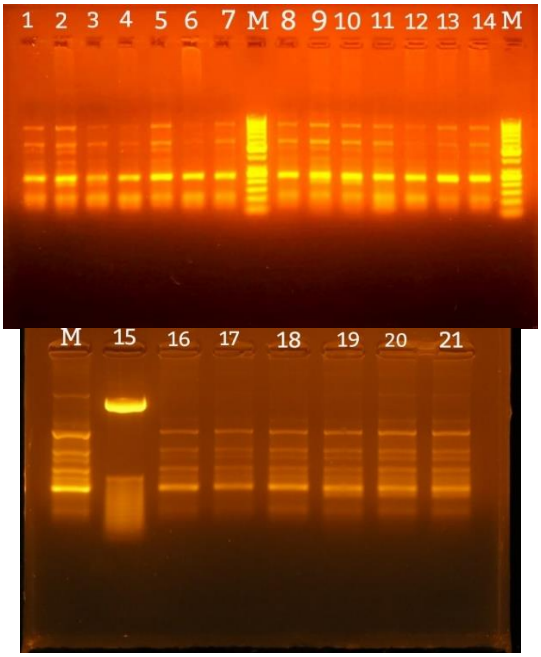

Figure (1); Agarose gel electrophoresis, Ethedium bromide staining for PCR visualization and identification of polymorphism of PGDR2 receptors:

Lane M: 200bp
TT Genotype: Lane 4,6,12
CC Genotype: Lane 15
TC Genotype: Lane 1,2,3,5,7,8,9,10,11,13,14,16,17,18,19,20,21
***The TT pattern** appeared as one band at 35 bp
***The CC pattern** appeared as one band at 195 bp
***The TC pattern** appeared as two bands at 35 and 195 bp

Statistical analysis

In order to analyse the data acquired, it was loaded into a computer and run via the Statistical Package of Social Services, version 25. (SPSS)**.** Tables and graphs were used to present the findings. The Shapiro–Wilk test was used to examine the distribution properties of variables as well as the homogeneity of variance. The quantitative data was reported in the form of the mean, median, standard deviation, and confidence interval. The frequency and proportions of qualitative data were used to present the information. For quantitative independent data, the student’s t test (T) and the Mann-Whitney test (MW) were employed to examine the data as needed. To examine qualitatively independent data, researchers employed the Pearson Chi-Square Test and the Chi-Square for Linear Trend (χ^2). P value equals or less than 0.05 was considered significant.

RESULTS

(Table 1) shows that the mean age of the studied patients was 8.5±2.4 years old, 55% were females and mean BMI was 22.8 ±4.9Kg/m^2. Regarding the distribution of common risk factors 75% of the studied patients had exercise induced asthma, 52.5% had history of hospital admission, 47.5% had upper respiratory tract infection, 45% had positive family

history, 40% had history of smoke exposure and 32.5% had history of life threating attacks.

(Table 2) shows that the most frequently distributed group was moderate persistent (37.5%), followed by mild intermittent (27.5%), mild persistent (22.5%) and severe persistent (12.5%).

(Table 3) shows that there is significant difference between mild, moderate and severe asthma regarding total IgE level(P1<0.001).

(Table 4) shows that 68.0% of the studied patients had Homogenous PGDR2(TT) and 32% had Heterozygous PGDR2(TC).

(Table 5) shows that there was no statistically significant association between PGDR2 Polymorphisms and asthma risk factors.

(Table 6) shows that there was no statistically significant association between PGDR2 Polymorphisms and laboratory characteristics of the studied patients.

(Table 7) shows that there was a statistically significant difference between PGDR2 Polymorphisms and bronchial asthma severity of the studied patients. Heterozygous PGDR2 was associated with more severe bronchial asthma.

Table (1): Demographic asthma risk factors characteristics of the studied patients.

Variables			Studied patients (No.=40)	
Age (years)	Range		7.5(5-12)	
	Mean ± SD		8.5±2.4	
Sex (No/%)	Male		18(45%)	
	Female		22(55%)	
BMI (kg/m^2)	Mean± SD		22.8±4.9	
	Median		23.3(10.7-32.8)	
Risk factors	Positive		Negative	
	No.	%		No.
Smoke exposure	16	40%	24	60%
Exercise induced asthma	30	75%	10	25%
Upper respiratory tract infection	19	47.5%	21	52.5%
Positive family history	18	45%	22	55%
Hospital admission	21	52.5%	19	47.5%
Life threating attacks	13	32.5%	27	67.5%

Table (2): Distribution of asthma severity among the studied patients.

Severity of asthma	Studied patients (No=40)	
	No.	%
Mild intermittent asthma	11	27.5%
Mild persistent asthma	9	22.5%
Moderate persistent asthma	15	37.5%
Severe persistent asthma	5	12.5%

Table (3): Comparison between severity of asthma and total IgE level.

Total IgE level	Severity of asthma			KW	P	Post-hoc
	Mild	Moderate	Severe			
IgE: Median	89.6	198.2	1261.4	175.7	<0.001 HS	P1<0.001 P2<0.001 P3<0.001

Table (4): Polymorphisms of PGDR2 in the studied patients.

PGDR$_2$	Studied patients (n=40)	
	No.	%
Homozygous(TT)	22	55%
Homozygous(CC)	5	12.5%
Heterozygous (TC)	13	32.5%

Table (5): Association between PGDR2 Polymorphisms and asthma risk factors.

Variables	$PGDR_2$				χ^2	P
	Heterozygous (TC) (n=13)		Homozygous (TT) & (CC) (n=27)			
	No.	%	No.	%		
Sex:						
Male(18)	5	27.7%	13	72.2%	3.4	0.06
Female (22)	10	45.5%	12	54.5%		
Exercise induced asthma:						
Positive(30)	10	75%	20	76.5%	0.01	0.9
Negative(10)	3	25%	7	23.5%		
Upper respiratory tract infection:						
Positive(19)	8	61.5%	11	40.7%	2.0	0.1
Negative(21)	5	38.5%	16	59.3%		
Positive family history:						
Positive(18)	5	27.7%	13	72.7%		
Negative(22)	8	36.3%	14	63.6%	0.4	0.5
Hospital admission:						
Positive|(21)	8	38.09%	13	61.9%	1.0	0.3
Negative(19)	5	26.3%	14	73.6%		
Life threating attacks:						
Positive(13)	5	38.4%	8	61.5%	0.3	0.6
Negative(27)	8	29.6%	19	70.3%		

Table (6): Association between PGDR2 Polymorphisms and laboratory characteristics of the studied patients.

Variables	$PGDR_2$		MW	P
	Homozygous (TT) & (CC)	Heterozygous (TC)		
WBCs: Mean ± SD	8.1 ± 3.4	9.1 ± 4.5	0.8	0.4
Eosinophil's count: Mean ± SD	0.41 ± 0.33	0.46 ± 0.27	0.8	0.4
Total IgE level: Mean ± SD	369.6 ± 494.3	393.4 ± 510.2	0.01	0.99

Table (7): Distribution of asthma severity among asthmatic children regarding PGDR2 polymorphisms.

Variables	$PGDR_2$				χ^2	P
	Heterozygous (TC) (n=13)		Homozygous (TT) & (CC) (n=27)			
	No.	%	No.	%		
Mild intermittent asthma	6	46.1%	3	11.1%	8.5	**0.004 S**
Mild persistent asthma	1	7.7%	8	29.6%		
Moderate persistent asthma	5	38.5%	12	44.4%		
Severe persistent asthma	1	7.7%	4	14.8%		

DISCUSSION

Breathlessness, chest tightness, and coughing are all common symptoms of bronchial asthma, which is an inflammatory condition that affects the airways and affects people more frequently at night or early in the morning. Widespread, but variable, airflow restriction is frequently associated with these episodes, and it's reversible either naturally or with treatment [7].

When it comes to asthma, there are many different phenotypes, each with its own symptoms, etiology, and pathophysiology. Genes, the environment, and the body's immune system all play a role in the development of an asthma phenotype. Having an asthmatic parent or sibling isn't a guarantee that you won't develop asthma [8]. Exercise and certain allergies, as well as irritants, can all be contributors to the symptoms [9]. PGD2 is a potent bronchoconstrictor in people with and without asthma, exerting its effects in a variety of tissues and organs [10], PGD_2 also induces mucus secretion [11], as well as being an inflammatory mediator [12].

These two polymorphisms in the promoter region of the PTGDR gene were found to be related with asthma, according to research on the American and African-American populations in the United States [4]. While the 197T/C promoter polymorphism has been found to be strongly related with asthma in a Spanish population [13], an additional 613C/T polymorphism in the PTGDR promoter region was found to exist [13].

Moderate persistent type was the most frequently distributed 37.5%, then mild intermittent type which was 27.5%, followed by mild persistent asthma 22.5%, the least frequent type was severe persistent asthma 12.5%.

So, our study is in agreement with **Ciprando *et al.*** [14] addressing the distribution of severe asthma. Asthma that is moderately persistent is the most common kind (36%) followed by asthma that is mildly intermittent (29%), mildly persistent (25%), and severe persistent asthma (10%).

In this study, the mean value of BMI was (22.8±4.9) kg/ $m^2$with a median range 23.3 (10.7-32.8) kg /m^2, so our study agrees with **Guerra *et al.*** [15] his study said that diagnosis of asthma is associated with low BMI of mean value (17.5±3.2) and median range was 18.5(9.6-28.4) kg/m^2.

As regarding risk factors distribution among asthmatic patients in this study, exercise induced asthma was the highest level of risk factors (75%) followed by hospital admission (52.5%),followed by upper respiratory tract infection (47.5%), then positive family history (45%), then smoke exposure(40%), which is confirmed by **Pamaja *et al.*** [16] how about 65% of people have exercise-induced asthma, 62.5% of them have been admitted to the hospital, 50% have had an upper respiratory tract infection, and 47.5% have had a positive family history of asthma? (40 percent).

In our study, there's significant positive relation between total IgE level and asthma severity, ($p<0.001$), which is matched with GINA guidelines [17]. And confirmed by **Davila *et al.*** [18] who confirmed our results that serum total IgE levels has significant relation with asthma severity ($p < 0.001$).

Our study discovered that there is a statistically significant association between polymorphism of $PGDR_2$ receptors and susceptibility to asthma, 55% of studied cases showed homozygous (TT) pattern, 12.5% of cases showed homogenous pattern (CC) and 32.5% of cases showed heterozygous pattern (TC).

Our study is found to be compatible with that study which has been performed in the university hospital of Salamanca (Spain) researchers studied the PGDR2 receptor polymorphism in 200 asthmatic children and discovered a strong link between the genes for asthma and the receptors. According to the findings of this study, 64% of instances had a homogeneous pattern, whereas 36% had a heterogenous pattern [13].

Another study carried out in Seoul, Korea, one hundred asthmatic children supported our findings that PGDR2 receptor polymorphism is associated with an increased risk of developing asthma. A homogeneous pattern was found in 57% of cases, whereas a heterogenous pattern was found in 43% of cases [19].

In 2014, a meta-analysis study found that in Europeans, Asians, and adults, the PTGDR -549 C/T polymorphism increases asthma risk. Asthma susceptibility and the PTGDR -441 C/T and -197 C/T polymorphisms or the CCC and TCT haplotypes were found to be unconnected [20].

A study conducted in North India on 992 asthmatic patients disagreed with our findings and determined that polymorphism of pgdr2 and asthma were not linked since 44% of cases were homogeneous and 54% were heterogenous [21].

We found that there is no statistical association between polymorphism of PGDR2 receptors and medical history of the studied cases, because the percentage of homogenous cases among males is 72.2% but among females is 22.7%,and the percentage of heterogenous cases among males is 27.7 but among females is 72.2%, the percentage of exercise induced asthma positive cases among the heterogenous group is 75% but negative cases are 25%, and exercise induced asthma positive cases among the homogenous group is 76.5% and negative cases is 23.5%,cases with upper respiratory tract infection in the heterogenous group is 61.5% and in cases without URTI are 38.5%, cases with URTI among the homogenous group is 40% and cases without URTI are 59.3%, cases with positive family history to asthma among the heterogenous group is 27.7%, but negative cases are 36.3%, the percentage of cases with positive family history among the homogenous group is 72.2% but negative cases are 63.6%, the percentage of hospital admission among the homogenous group is 61.9%.

But among the heterogenous group is 38.09%..the percentage of life threating attacks in the homogenous group is 61.5%,but is 38.4% among the heterogenous group, which is compatible with another

study made by **Lie *et al.*** [22] who stated that the percentage of males in the homogenous group is 25% but females are 28%, the percentage of exercise induced asthma positive cases is 75% among the heterogenous group but 76.5% among the homogenous group, and the percentage of cases with URTI among the heterogenous group is 61.5% but is 40.7% among the homogenous group, the percentage of cases with positive family history of asthma is 27.7% among the heterogenous group and 72.7% among the homogenous group.

Our study stated that there is a statistical significant relation between polymorphism of PGDR2 receptors and bronchial asthma severity in the studied patients, as the percentage of mild intermittent cases among the homogenous group is 11.1% and 46.1% among the heterogenous group, the percentage of mild persistent cases is 7.7% among the heterogenous group and 29.6% among the homogenous group, the percentage of moderate persistent asthma cases is 38.55 among the heterogenous group and 44.4% among the homogenous group, the percentage of severe persistent cases among the heterogenous group is 7.7% and 14.85 among the homogenous group which is in agreement with **Sanz *et al.*** [13] who stated that the percentage of mild intermittent asthma among the homogenous group is 10.4% and is 45% among the heterogenous group, the mild persistent asthma among the homogenous group is 33% and among the heterogenous group is 22%.

The current study stated that there is no statistical relation between polymorphism of PGDR2 receptors and laboratory characteristics of the studied patients, as the mean WBCs count among the homogenous group is 8.1±3.4 and is 9.1±4.5 among the hetrogenous group, the mean eosinophilic count among the homhgenous group is 0.41±0.33 and 0.46±0.27 among the heterogenous group, the mean total IgE level among the homogenous group is 369.6±494.3 and 393.4±510.2 among the heterogenous group, which is in consistence with **Kweon *et al.*** [19] who stated that the mean WBCs count among the homogenous group is 7.5±3.2 and 8.4±3.2 among the heterogenous group the mean oesinophilic count among the heterogenous group is 0.56±0.23 and 0.52±0.37 among the homogenous group, the mean total IgE level is 356.4±465.3 among the homogenous group and 325±534 among the heterogenous group.

CONCLUSION

Our study showed a strong relationship between polymorphism of PTGDR2 receptor and severity of bronchial asthma.

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