Relation between Gene Polymorphisms of Prostaglandin D Receptor and Responsiveness to Leukotriene Receptor Antagonist in Asthmatic Children

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Abstract

Background: Childhood asthma is a common public problem in Egypt that affects different aspects of lives and rate of hospital admissions. Many polymorphisms of 17q21 locus have been demonstrated especially PGDR2 polymorphism which have been showed to increase the risk of asthma exacerbations in children in spite of inhaled corticosteroids use.

Aim of the study: To evaluate the association between PGDR-441 polymorphism and bronchial asthma also the relation between it and asthmatic children response to leukotriene receptor antagonists.

Methods: Our study was a cross-sectional study of children who visited the pediatrics general outpatient clinic at Zagazig University Hospitals. PTGDR2 receptors were genotyped in forty Egyptian children using allele specific polymerase chain reaction (AS-PCR) in order to assess single nucleotide polymorphism in the PTGDR2 receptor. Selected cases were categorized according to GINA criteria, and their pulmonary functions were tested using a spirometry to determine their status.

Results: 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/m2. 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%). PEF%, FVC and FEV1 mean values pre-administrated leukotriene receptor antagonist were 72.3±10.7, 73.3±1.4 and 74.1±10.5. The mean values post –administrated were 71.2±13.1, 75.5±9.6 and 89.5±8.2 respectively. There was no statistically significant association between PGDR2 Polymorphisms and pulmonary functions of the studied patients.

Conclusion: Our study showed There was no statistically significant association between PGDR2 Polymorphisms and pulmonary functions of the studied patients.

Keywords: Prostaglandin D Receptor, Leukotriene Receptor Antagonist, Bronchial Asthma.

I. INTRODUCTION

According to WHO and the Center for Disease Control and Prevention, about 235 million people are suffering from asthma and 1 in 13 people have asthma (1). Data from several preclinical and clinical reports suggest a susceptibility for Covid19 in patients with asthma. This may be due to reduced expression of ACE2 and TMPRSS2 receptors. Hence may be also have a protective effect against infection with SARS-COV2 (2).
In Egypt, the prevalence of asthma is 12.5%. And there is a wide variety in different governorates in the prevalence of asthma. In Cairo the prevalence of asthma is 9.4% and in Nile delta is 7.7% (2).

Asthma is characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. This condition is due to inflammation of the air passages in the lungs. In an attack, the lining of the passages swell causing the airways to narrow and reducing the flow of air in and out of the lungs (1).

Prostaglandin D₂ (PGD₂) is the most abundantly produced cyclooxygenase metabolite of arachidonic acid in response to environmental allergens and has been proposed to be a marker of mast cell activation in asthma. The gene for the Prostaglandin D2 Receptor is located on chromosome 14q22.1 in humans and the protein encoded by the gene is a 359 aa, G-protein coupled receptor of 40.276 kDa. It has been observed that on exposure to the allergens, PGD₂ levels increase in asthma patients, and is known to induce bronchoconstriction in the lungs (3).

The number of PGDR2 receptor-positive cells within the submucosal tissue is also significantly higher in patients with severe asthma compared with healthy controls (4).

In a Spanish population, while the −197T/C promoter polymorphism has been significantly associated with asthma (5), novel −613C/T polymorphism in the promoter region of the PTGDR gene has also been identified (5).

We aimed at this study to evaluate the association between PGDR-441 polymorphism and bronchial asthma also the relation between it and asthmatic children response to leukotriene receptor antagonists.

II. PATIENTS AND METHODS

A cross-sectional study of children who visited the paediatrics general outpatient clinic at Zagazig University Hospitals was undertaken on forty asthmatic patients (18 males and 22 females), whose ages varied from 5 to 12 years. With a mean age of (8.5 2.4) years. A total of four subcategories were created from this group: those who were mildly intermittent, mildly persistent, moderately persistent, and severely persistent.

The Helsinki Declaration, a guideline of ethics for human testing developed by the World Medical Association, was what we followed.

Inclusion criteria:
1) Age between 5 to 12 years old.
2) The presence of typical asthma symptoms according to the established guidelines of Global Initiative for Asthma Management and Prevention in 2016 (6).
3) Confirmed variable expiratory airflow obstruction and evidenced by improvement in pre-administrated leukotriene receptor antagonist FEV1 of >15% predicted after montelukast (5mg per day) administration (6).
4) They have no history of corticosteroids treatment within 6 weeks, oral beta-adrenergic agonists within 1 week, inhaled beta-adrenergic agonists within 6 hours, antihistamines within 72 hours and leukotriene modifiers within 4 weeks (7).

Exclusion criteria:
1) Asthmatic patients with comorbidities such as, cardiovascular diseases and chronic pulmonary diseases.
2) History of respiratory tract infection within the past 4 weeks or history of allergy or dermatitis.

Methods

Patients underwent the following:
1. History taking and clinical examination.
2. Pulmonary functions test (FEV1, FVC%, and PEF%) before and after salbutamol administration.

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3. Total serum IgE level assessment.
4. Total leukocytic count and peripheral eosinophilic percentage: blood samples were collected on EDTA tubes and analyzed by automatic cell counter.
5. Genetic analysis of PGDR 2 to detect polymorphism of PGDR-441 T/C gene.

**Responsiveness to leukotriene receptor antagonist.**
To assess response to a leukotriene receptor antagonist
Montelukast (5 mg per day) was administrated for 8 weeks, and measurement of Pulmonary functions was performed before and 8 weeks after administration by the Spirometry. FEV1 % after montelukast administration was chosen as an outcome measure to drug receptor (8).
The response to montelukast was expressed as post-administration FEV1 % - pre-administration FEV1 % ×100%.
It was considered as responsive when the increasing of FEV1 % was 15% or more and irresponsible when the increasing of FEV1 % was less than 15% (9).

**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. (10)
Lyophilized primers were reconstituted to obtain an optimal concentration (30 pmole /Amplification) then stored at -20°C
Forward Prime: 5’ egagtttggccaccccagttcaaacaccagcacaa - 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.

**Statistical analysis**
The collected data was entered to and analyzed by computer using Statistical Package of Social Services, version 25 (SPSS) (IBM, 2017). Results were presented by tables and graphs. Shapiro–Wilk test was used to determine the distribution characteristics of variables and variance homogeneity. Quantitative data was presented as mean, median, standard deviation and range. Qualitative data was presented as frequencies and proportions. Student’s t test (T) and Mann-Whitney test (MW) were used to analyze quantitative independent data as appropriate. Pearson Chi square test and Chi square for linear trend (χ2) were used to analyze qualitative independent data. P value of ≤0.05 was taken as significant (Petrie & Sabin, 2009).

### III. RESULTS
The mean age of the studied patients was 8.5±2.4 years old, 55% were females and mean BMI was 22.8±4.9 Kg/m2. (Table 1).
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%) (Figure 1).
The mean WBCs in the studied patients was 8.8±4.2, mean Eosinophil’s count was 4.4±2.9 and mean IgE level was 385.8±500.2 (Table 2).
PEF%, FVC and FEV1 mean values pre-administrated leukotriene receptor antagonist were 72.3±10.7, 73.3±1.4 and 74.1±10.5. The mean values post–administrated were 71.2±13.1, 75.5±9.6 and 89.5±8.2 respectively (Table 3).

68.0% of the studied patients had Homogenous PGDR2(TT) and 32% had Heterozygous PGDR2(TC). (Figure 2).

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 (Figure 3).

There was no statistically significant association between PGDR2 Polymorphisms and pulmonary functions of the studied patients (Table 4).

Table (1): Demographic data among the studied cases:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Studied patients (No.=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Range 7.5(5-12)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD 8.5±2.4</td>
</tr>
<tr>
<td>Sex (No/%)</td>
<td>Male 18(45%)</td>
</tr>
<tr>
<td></td>
<td>Female 22(55%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean± SD 22.8±4.9</td>
</tr>
<tr>
<td></td>
<td>Median 23.3(10.7-32.8)</td>
</tr>
</tbody>
</table>

Figure (1): Distribution of asthma severity among studied patients

Table (2): Laboratory characteristics of the studied patients:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Studied patients (No=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCS</td>
<td>Mean±SD 8.8±4.2</td>
</tr>
<tr>
<td></td>
<td>Median (Range) 8.6(4-23.8)</td>
</tr>
<tr>
<td>Eosinophilic count</td>
<td>Mean±SD 4.4±2.9</td>
</tr>
<tr>
<td></td>
<td>Median(Range) 0.3(1-1.2)</td>
</tr>
<tr>
<td>Total IgE level</td>
<td>Mean±Sd 385.8±500.2</td>
</tr>
<tr>
<td></td>
<td>Median (Range) 178.7(11.3-16.94)</td>
</tr>
</tbody>
</table>

Table (3): The mean spirometric values among asthmatic children

<table>
<thead>
<tr>
<th>Variables</th>
<th>Studied patients (No=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22.5% Mild intermittent asthma</td>
</tr>
<tr>
<td></td>
<td>27.5% Mild persistent asthma</td>
</tr>
<tr>
<td></td>
<td>37.5% Moderate persistent asthma</td>
</tr>
<tr>
<td></td>
<td>12.5% Severe persistent asthma</td>
</tr>
</tbody>
</table>
Pre-administrated leukotriene receptor antagonist PEF%  
Mean±SD  72.3±10.7  
Median(Range)  49.3-93.2

Post administrated leukotriene receptor antagonist PEF%  
Mean±SD  71.2±13.1  
Median (Range)  71.4(51.8-108.1)

Pre-administrated leukotriene receptor antagonist FVC%  
Mean±SD  73.5±1.4  
Median(Range)  49.5-110.2

Post administrated leukotriene receptor antagonist FVC%  
Mean±SD  75.5±9.6  
Median(Range)  76.2(59.7-96)

Pre-administrated leukotriene receptor antagonist FEV1%  
Mean±SD  74.1±10.5  
Median(Range)  74.4(53.3-92.1)

Post-administrated leukotriene receptor antagonist FEV1%  
Mean±SD  89.5±8.2  
Median (Range)  92.4(74.2-100.6)

Percentage of improvement in FEV1  
Mean±SD  21.7±12.2  
Median(Range)  16.4(4.6-60.3)

**Figure (2):** Polymorphisms of PGDR2 in the studied patients

**Figure (3):** Distribution of asthma severity among studied patients regarding PGDR2 polymorphism
### Table (4): Association between PGDR2 Polymorphisms and pulmonary functions of the studied patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Studied patients (n=40)</th>
<th>Test of sig.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre -administrated leukotriene receptor antagonist FEV%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD;</td>
<td></td>
<td>T</td>
<td>0.2 0.3</td>
</tr>
<tr>
<td>Median(Range):</td>
<td>67.3±10.5</td>
<td>66.2±10.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.3-93.1</td>
<td>48.4-102.3</td>
<td></td>
</tr>
<tr>
<td>Post administrated leukotriene receptor antagonist PEF (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>69.8 ± 13.3</td>
<td>71.9 ± 13.2</td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>52.8 – 89.7</td>
<td>51.8 – 108.1</td>
<td></td>
</tr>
<tr>
<td>Test of sig.</td>
<td>T</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-administrated leukotriene receptor antagonist</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td></td>
<td>T</td>
<td>0.1</td>
</tr>
<tr>
<td>Median(Range)</td>
<td>62.1±9.5</td>
<td>63.4±10.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63.1-71.3</td>
<td>47.5-101.3</td>
<td></td>
</tr>
<tr>
<td>Post administrated leukotriene receptor antagonist FVC%:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>77.3 ± 5.4</td>
<td>74.7 ± 11.0</td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>78.1 (68.0 – 84.3)</td>
<td>75.3 (59.7 – 96.0)</td>
<td></td>
</tr>
<tr>
<td>Test of sig.</td>
<td>T</td>
<td>0.8 0.3</td>
<td></td>
</tr>
<tr>
<td>Pre-administrated leukotriene receptor antagonist FEV1 (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>73.8 ± 9.2</td>
<td>74.2 ± 11.2</td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>75.7 (58.2 – 83.3)</td>
<td>74.4 (53.3 – 92.1)</td>
<td></td>
</tr>
<tr>
<td>Test of sig.</td>
<td>T</td>
<td>0.1 0.8</td>
<td></td>
</tr>
<tr>
<td>Post-administrated leukotriene receptor antagonist FEV1 (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>89.3 ± 5.3</td>
<td>89.6 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>91.2 (82.1 – 95.5)</td>
<td>93.5 (74.2 – 100.6)</td>
<td></td>
</tr>
<tr>
<td>Test of sig.</td>
<td>T</td>
<td>0.1 0.8</td>
<td></td>
</tr>
<tr>
<td>Percentage of improvement in FEV1:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>22.6 ± 16.8</td>
<td>21.2 ± 9.7</td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>15.4 (7.6 – 60.3)</td>
<td>18.5 (4.6 – 43.3)</td>
<td></td>
</tr>
<tr>
<td>Test of sig.</td>
<td>MW</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**IV. DISCUSSION**

Asthma is estimated to affect 300 million people worldwide with an expected increase to 400 million worldwide by 2025 (11).

In a population of children and adolescent, bronchial asthma occurs with frequency of 5.10% causing 0.25 million deaths annually and substantial socioeconomic burden around the globe (11).

The symptoms of asthma can be nonspecific and varied, making the diagnosis difficult. Patients often present with wheezing, shortness of breath, and cough that occur more frequently during the night and early morning (12).

Genetic factors have a strong impact on the risk of developing asthma (13), in particular, childhood asthma is strongly associated with the 17q21 locus alleles. Analyzing 14 different populations of asthmatic patients from 12 different countries, showed that 17q21 polymorphism is related to an increased risk of exacerbations in children with asthma despite ICS use (14).

Prostaglandins modulate several physiological systems, as well as being involved in a wide range of disease states. They are synthesized sequentially from arachidonic acid by cyclooxygenase-1 and
cyclooxygenase-2 and prostaglandin synthase enzymes (15). Altered levels of hematopoietic PGDS (H-PGDS) may have a role in the pathophysiology of asthma (16).

PGD2 mediates a number of physiological effects in a range of tissues and organs, It is a powerful bronchoconstrictor, in people with and without asthma (Hadry et al.,2004), PGD2 also induces mucus secretion (17), as well as being an inflammatory mediator (15).

Many polymorphisms of PGDR2 receptor have been identified, The −549T/C and −441C/T polymorphisms in the promoter region of the PTGDR gene have been suggested to be associated with asthma in a study conducted on American and African American population (Oguma et al.,2004). In a Spanish population, while the −197T/C promoter polymorphism has been significantly associated with asthma (5), novel −613C/T polymorphism in the promoter region of the PTGDR gene has also been identified (5).

In this study, the mean value of BMI was (22.8±4.9) kg/m²with a median range 23.3 (10.7-32.8) kg/m², so our study agrees with Stefano et al., (18) 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/m².

Pre-administrated leukotriene receptor antagonist FEV1 % was 74.1±10.5, and the post-administrated leukotriene receptor antagonist FEV1 %was 89.5±8.2 and the percentage of improvement in FEV1 %was 21.7±12.2.

Our study discovered that there is a statistically significant association between polymorphism of PGDR2 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).

A study was performed in Spain on 637 Caucasian children and showed that there's no significant relation between polymorphism of PTGDR2 and susceptibility to asthma. In this study 23% of cases were of the homogenous pattern and 77%of cases were heterogenous pattern (19).

But our study is not compatible with the Chinese study carried out in Weifang asthma hospital and Shandong provincial hospital on 336 asthmatic children and concluded lack of association between polymorphism of PGDR2 receptors and susceptibility to asthma. In this study 33% of cases were of the homogenous pattern and 67% of the studied cases were of the heterogenous pattern (20).

The American study that studied polymorphism of PGDR2 receptors and its relation to asthma in 3 ethnic groups and studied asthmatic children ,Americans, Africans and Mexicans, from the San Francisco Bay area (SF), New York City (NY), Puerto Rico (PR), and Mexico City (MX).this study showed that there's no significant relation between polymorphism of PGDR2 and susceptibility to asthma , because 28% of cases were of the homogenous pattern and 72% of cases were of the heterogenous pattern (21).

This study did not find positive statistical relation between polymorphism of PGDR2 receptors and the spirometric values of the studied patients as the mean pre-administrated leukotriene receptor antagonist FEV1% among the homogenous group is 67.3±10.5 and 66.2±10.1, the post administrated one is 69.8±13.3 among the homogenous group and 71.9±13.2 among the heterogenous group which is in consistence with Li et al., (22) who stated that the mean pre-administrated leukotriene receptor antagonist FEV1% among the homogenous group is 65.3±10.2 and 65.4±10.8 among the heterogenous group and the post administrated one is 49.4±8.7 among the homogenous group and 70.3±9.6 among the heterogenous group (22).
V. CONCLUSION

Our study showed a strong relationship between polymorphism of PTGDR2 receptor and severity of bronchial asthma and susceptibility to asthma. There was no statistically significant association between PGDR2 Polymorphisms and pulmonary functions of the studied patients.

**From this study we recommended that:**
- To consider the possible relationship between asthma phenotypes and genotypes in classifying asthmatic children into different categories.
- Determining a strategy of treatment is to be taken into account, moving towards personalized medicine, is a more specific and accurate line for managing diseases.
- To conduct further pharmacogenomics studies on PGDR receptors on Egyptian asthmatic children to reach a more genotype guided practice, and to compare more than one drug category in relation to SNP on large sample size.
- To combine more than on locus of PGDR2 receptors in the same study, in order to be able to understand, haplotype – genotype interaction, where pure genotypic relation became less important in recent GWAS and sequencing studies, which depend mainly on haplotype. Honestly saying, it is one of the biggest limitations to any genetic study.

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