Araştırma Makalesi (Research Article)

Astımlı Çocuklarda Atak Sırasında Plazma Paraoksonaz Düzeylerinin ve PON-1 Gen Polimorfizminin Önemi

The Importance of Plasma Paraoxonase Levels And PON-1 Gene Polymorphism During An Attack in Children With Asthma

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Özet

Amaç: Paraoksonaz 1 (PON-1) enzimi önemli bir antioksidandır. Astım atakları sırasında oksidatif stres ve antioksidan savunma mekanizmaları arasındaki denge bozulur. PON-1 enziminin aktivitesi, PON-1 gen polimorfizmi ile ilişkilidir. Bu çalışma astımlı çocuklarda PON-1 enzimi ve Q192R polimorfizminin aktivitesini değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Çalışma 2013-2014 yılları arasında 34 astımlı (çalışma grubu) ve 34 sağlıklı (kontrol grubu) çocuğun prospektif olarak değerlendirilmesiyle yapıldı. Serum PON-1 aktivitesi, çalışma grubunda astım atağının başlangıcında ve sonunda ve ayrıca kontrol grubunda değerlendirildi. Astımlı çocuklarda PON-1 enzim aktivitesini etkilediği bilinen Q192R gen polimorfizmi de araştırıldı.

Bulgular: Astımlı çocuklar ve sağlıklı kontroller arasında serum PON-1 enzim aktivitesi ve PON-1 gen polimorfizmi açısından anlamlı fark yoktu. Bununla birlikte, astımlı çocukların PON-1 enzim aktivitesinin astım atağı sonunda astım atağı başlangıcına göre önemli derecede arttığı gözlendi.

Sonuç: Astımlı çocuklarda PON-1 enzim aktivitesi ve Q192R genetik polimorfizmi sağlıklı çocuklardan farklı değildir, ancak PON-1 enzim aktivitesi astım atağının sonunda atağın başlangıcına göre daha yüksektir. Antioksidan mekanizmanın bir üyesi olan PON-1 enzimi, hem astım patofizyolojisini anlamada hem de yeni tedavilerin geliştirilmesinde önemli olabilir.

Anahtar kelimeler: Paraoksonaz, Genetik Polimorfizm, Astım, Çocuklar

Abstract

Objective: The paraoxonase 1 (PON-1) enzyme is an important antioxidant. The balance between oxidative stress and antioxidant defense mechanisms is disturbed during asthma attacks. The activity of the PON-1 enzyme is related to PON-1 gene polymorphism. This study aimed to evaluate the activity of the PON-1 enzyme and Q192R polymorphism in asthmatic children.

Material and Methods: During the 2013-2014 period, we performed a prospective study with 34 asthmatic (study group) and 34 healthy (control group) children. Serum PON-1 activity was evaluated in the study group at the onset of an asthma attack and at the end, and also in the control group. Q192R gene polymorphism of asthmatic children, which is known to affect the PON-1 enzyme activity, was also investigated.

Results: There was no significant difference in terms of serum PON-1 enzyme activity and PON-1 gene polymorphism between the asthmatic children and healthy controls. However, a significant increase was observed in the PON-1 enzyme activity of asthmatic children at the end of the asthma attack compared to its onset.

Conclusion: The enzyme activity and Q192R genetic polymorphism in asthmatic children are not different from those of healthy children, but PON-1 enzyme activity is higher at the end of an asthma attack compared to the onset. PON-1 enzyme, as a member of the antioxidant mechanism, may be important both in understanding the pathophysiology of asthma and in the development of new medications for asthma.

Keywords: Paraoxonase, Genetic Polymorphism, Asthma, Children

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INTRODUCTION

Asthma is the most common chronic disease in children, and there is a progressive global increase in the prevalence of asthma in recent decades (1). Recent medications are effective at keeping asthma in control, but a curative treatment modality is still absent due to the lack of an entirely understood pathogenesis.

Oxidative stress is known to be an important factor during asthma attacks (2). Pro-inflammatory cytokines are released from activated inflammatory cells and stimulate polymorphic leukocytes to produce reactive oxygen species (ROS). Antioxidants increase in an attempt to balance the increased oxidative stress (3). The paraoxonase1 (PON-1) enzyme is an endogenous antioxidant that plays a critical role in the defensive system against oxidative stress. This enzyme is synthesized in the liver and protects low-density lipoproteins (LDL) from peroxidation by free radicals. PON-1 has an anti-inflammatory effect, as it protects the cell membrane, which contains lipoproteins (4). The activity of this enzyme decreases when the high-density lipoprotein (HDL) cholesterol concentration is low (5).

The PON-1 gene is located in the q21-22 region of chromosome 7. L55M and Q192R are the most common polymorphisms. The activity of PON-1 is related by its Q192R single nucleotide polymorphism, which results in the glutamine to arginine substitution at position 192 (6).

Previous studies have demonstrated the role of PON-1 in asthma. However, studies on PON-1 activity during asthma attacks and its association with polymorphism yield conflicting results, and there is no study that evaluates the dynamic changes of PON-1 activity during asthma attacks. We aimed to evaluate the PON-1 activity at the onset and at the end of the asthma attacks in children. We also analyzed the association of PON-1 activity with Q192R genetic polymorphism in asthmatic children.

MATERIAL AND METHODS

After local ethics committee approval, this prospective study was performed between May 1, 2013, and May 1, 2014. The study group consisted of 34 asthmatic children aged 5 to 17 years. Asthma was diagnosed according to the "Global Initiative for Asthma" criteria. The control group consisted of 34 healthy children aged from 5 to 17 years who had no history of asthma and received routine care in the general pediatric outpatient clinic. Informed consent was obtained from the parents or legal guardian of each child.

The exclusion criteria were conditions that could affect the PON-1 activity, such as chronic diseases, inflammatory or autoimmune diseases, renal diseases, hepatic diseases, cardiac diseases, bronchiectasis, pulmonary anatomical problems, smoking exposure, and lipid metabolism disorders.

The same pediatric allergist managed the study group's asthma attacks. Asthma attack was evaluated as the patients' admission to the hospital with complaints as shortness of breath, cough, wheezing. Venous blood (5 mL) was collected from the healthy controls and study group (both at the onset and at the end of asthma attack). Blood samples were also collected from the control group.

Blood samples were drawn into a blood collection tube with ethylenediaminetetraacetic acid (EDTA) and a blood collection tube with gel. Blood in the tube with gel was centrifuged at 4000 rpm for 10 minutes to separate the serum. The serum was frozen at −80°C and stored in Eppendorf tubes. The activity of the PON-1 enzyme was measured by the western blot technique using monoclonal anti-paraoxonase 1 (Fitzgerald 10R-8414).

Blood in the tube with EDTA was stored at −20°C for 2–12 months for deoxyribonucleic acid (DNA) isolation. Genomic DNA was extracted from leukocytes using the ethanol precipitation method. Extracted DNA samples were amplified using the polymerase chain reaction (PCR). For amplification, the following primers were used: 5'-TAT TGT TGC TGT GGG ACC TGA G-3' (forward) and 5'-CAC GCT AAA CCC AAA TAC ATC TC-3' (reverse). Next, the PCR mixture was prepared for a reaction. Amplification steps were as follows: hot start (denaturation at 95°C for 5 min), annealing at 61°C for 1 min, and extension at 72°C for 1 min. After annealing and extension were repeated for 35 cycles, amplification was completed with a final extension time of 10 min. One restriction endonuclease was added to PCR products for Q192R polymorphism. Then, the digested products were separated by 3% agarose gel electrophoresis with 100 Volt for 40 min and identified in ultraviolet light. A normal QQ allele corresponds to the presence of a 99 bp fragment, while a heterozygote QR allele corresponds to three digestion fragments of 99, 66 and 33 bp, and a homozygote RR allele corresponds to two digestion fragments of 66 and 33 bp.

Statistical Analysis

Descriptive methods (frequency, percentage, mean, and standard deviation) were used to analyze the data. The Kolmogorov-Smirnov distribution test was used to analyze distribution normality. The paired sample t-test was used for comparison of qualitative data. The Mann-Whitney U test, Wilcoxon test, Chi-square test, and Fisher Exact tests were used for quantitative comparison. A p-value < 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS for Windows version 20.0 (IBM, USA).

RESULTS

The study group consisted of 19 boys (55.9%) and 15 girls (44.1%), with a mean age of 8.2± 3.6 years old. The control group consisted of 16 boys (47.1%) and18 girls (52.9%), with a mean age of 8.4± 3.1 years old. The groups were not significantly different in terms of gender and age. The mean body mass index was not significantly different between the study and control groups **(Table 1)**.

Table 1. Demographic characteristics of study population

	Asthmatic children	Healthy controls	p
Gender (male/female)	19 / 15	16 / 18	0.46 (X2=0.53)
Age	8.2±3.6	8.4±3.1	0.38
BMI (kg/m²)	17.51±2.22	16.89±3.09	0.34

HDL cholesterol (46.82±16.24 vs 45.5±12.58 mg/dL), LDL cholesterol (84.05±25.03 vs 90.08±23.76 mg/dL) and total cholesterol levels (149.02±28.46 vs 151.08±31.15 mg/dL) were not significantly different between the study and control groups (p=0.70, p=0.31 and p=0.77).

PON-1 enzyme activity of the study group (at the onset and at the end of the asthma attack) and the control group were evaluated **(Table 2)**.

Table 2. Comparison of paraoxonase 1 enzyme activity between the asthmatic children and the healthy controls.

	Asthmatic children		Healthy controls	p
	at the onset of the attack	at the end of the attack		
Paraoxonase 1 Activity (U/L)	86.05±47.88	110.71±75.83	87.79±56.25	0.04* 0.47** 0.17***

* p value between the enzyme activity levels of astmatic children at the onset and at the end of the asthma attack; ** p value between the enzyme activity levels of astmatic children at the onset of the asthma attack and healthy controls; *** p value between the enzyme activity levels of astmatic children at the end of the asthma attack and healthy controls

There was no significant difference between asthmatic children and healthy controls, but the *PON-1* enzyme activity of asthmatic children at the end of asthma attack were significantly higher than that at the onset.

There was no statistically significant difference between the asthmatic children and healthy controls in terms of PON-1 (Q192R) gene polymorphism **(Table 3)**.

Table 3. Comparison of asthmatic children and healthy controls in terms of paraoxonase Q192/R gene polymorphism

	Asthmatic children n (%)	Healthy controls n (%)	
QQ gene allele	24 (70.6)	27 (79.4)	X^2=1.42 p=0.49
QR gene allele	9 (26.5)	7 (20.6)	
RR gene allele	1 (2.9)	0 (0)	

DISCUSSION

Asthma is the most common chronic disease in children (1). Although the etiology and pathogenesis are not clear, it is commonly believed that asthma is caused by multiple interacting genes and environmental factors, of which some prevent attacks, and others contribute to the disease pathogenesis (7). Cytokines, reactive oxygen, and nitrogen materials are important in asthma pathogenesis due to their harmful effects on lipids, proteins, DNA, and cell membrane function (8).

The balance between oxidants and antioxidants is disrupted during asthma attacks (9). *PON-1* is an endogenous antioxidant that is primarily synthesized in the liver. Previous reports present insufficient and, in some cases, contradictory data about the clinical relevance of *PON-1* in a number of diseases, including cardiovascular disease, diabetes, neurologic diseases, and cancer (10). Plasma *PON-1*, total oxidant status/total antioxidant status ratio were reported as useful markers of inflammation in stable asthmatic children, and a ≥151 mmol/L for *PON-1*was found to reflect an uncontrolled disease (11).

PON-1 activity is associated with age, gender, race, weight, lipid levels, and some individual factors like vitamin D are directly correlated with *PON-1* and the total antioxidant status (12). *PON-1* activity is two times lower in neonates and reaches normal levels in the first year of life (13). Since our study was conducted in children only aged from 5 to 15 years, age does not seem to be an important variable in our

study. Enzymatic activity of *PON-1* varies according to the populations (14). *PON-1* activity was found to be lower in obese and overweight patients (15). In this study, there was no significant difference between asthma and healthy children in terms of age, gender, and body mass index.

It is known that serum *PON-1* activity decreases with low HDL-cholesterol levels (9). In our study, the groups were selected from children with normal lipid levels, preventing this condition from affecting our results.

In this study, we aimed to investigate whether there were any differences in *PON-1* activity in asthmatic children. Although there are some studies in literature in which *PON-1* activity was reported to be not different in asthmatic children (16), current researches have reported lower *PON-1* activity in asthmatic patients (17). In a study, *PON-1* was found lower in Egyptian asthmatic children than healthy controls. Furthermore, this study revealed a notably decreased serum *PON-1* activity with increasing severity of asthmatic attack and a positive correlation between *PON-1* activity and the percent predicted FVC and FEV1/FVC ratio (3). In our study, *PON-1* activity was not statistically different between the children with asthma and the healthy controls, both at the onset and at the end of the asthma attack. This result is inconsistent with the studies that reported decreased *PON-1* activity in children with asthma.Whether oxidative stress is the consequence of or the cause for chronic changes in asthma remains controversial (2).

We also compared the *PON-1* activity of asthmatic children at the onset and end of the asthma attack. The *PON-1* activity was significantly higher at the end of the asthma attack than at the onset. Our results showed that *PON-1* activity changes with the asthma attack. Presumably, *PON-1* increases to neutralize oxidative stress, weaken inflammation, and end the asthma attack. As asthma attack ends, it returns to normal levels until a new asthma attack. This result of our study suggests that *PON-1* and antioxidants might be a potential therapeutic target for the prevention and treatment of asthma attacks.

PON-1 gene is located at chromosome 7 and has two common functional polymorphisms: the missense single nucleotide at position 55 [leucine (L) to methionine (M)] and 192 [glycine (Q) to arginine (R) substitution] (18). Gene polymorphism distribution is known to differ extremely between races and regions (19). The hydrolytic efficiency of *PON-1* is strongly modulated by Q192R polymorphism (17). QQ genotype expresses the lowest enzyme activity, while QR and RR genotypes express moderate and highest enzyme activities, respectively (18). In literature, there are researches which reported that RR and QR polymorphisms to be risk factors of asthma while some reported that polymorphisms did not influence the susceptibility to asthma (16). In our study, gene polymorphism was not different between the asthmatic children (70.6% QQ, 26.5% QR, 2.9% RR) and healthy controls (79.4% QQ, 20.6% QR, 0% RR), and the QQ polymorphism was found to be higher in both groups. We think that Q192R polymorphism does not have a significant role in the asthma of children, but larger researches are needed to be done on different ethnic groups.

Our study has some limitations. We evaluated only one asthma attack, and it is known that a single measurement of enzyme activity may not provide certain information. The diagnosis of an asthma attack was made only with clinical findings, and a pulmonary function test was not performed. We did not evaluate the total oxidant status and total antioxidant capacity, types of medications, and the severity of asthma attacks. Also, our study group is small to obtain reliable and sufficient statistical data.

As a conclusion, Serum *PON-1* enzyme activity and gene polymorphism do not differ between asthmatic and healthy children. *PON-1* enzyme activity is not significantly low at the onset of the asthma attack, but it is increased at the end. Increase in the *PON-1* activity seems to be associated with the improvement of the asthma attack. The *PON-1* enzyme and antioxidants might be potential therapeutic targets for the prevention and treatment of asthma attacks. Further studies are needed to clarify the clinical importance of the *PON-1* in asthmatic children.

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Author Contribution: All authors contributed equally to the article.

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