ORIGINAL RESEARCH

Oxidative status and paraoxonase activity in children with asthma

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Abstract

Objective: To compare paraoxonase activity and changes in oxidative status in asthmatic children and healthy children by determining serum paraoxonase activity and total oxidative status, total antioxidant capacity and lipid hydroperoxidation.

Methods: Forty two asthmatic children were compared with 32 healthy children of similar age and sex. To evaluate the paraoxonase and oxidative status, total antioxidant capacity and lipid hydroperoxidation were examined. Serum paraoxonase activity was evaluated by measuring the rate of paraoxon hydrolysis. Oxidative status was evaluated by the method developed by Erel. Lipid hydroperoxide was measured by an iodometric method.

Results: In comparison with the healthy control group, the paraoxonase activity of the asthmatic children was found to be low (163.7 ± 73.0 (U/L) and 349.2 ± 153.9 (U/L), $P = 0.002$) and total oxidant status (9.0 ± 3.5 $\mu\text{mol H}_2\text{O}_2$ Eq/L and 13.4 ± 7.0 $\mu\text{mol H}_2\text{O}_2$ Eq/L, $P = 0.002$), total antioxidant capacity (5.5 ± 2.5 $\mu\text{mol Trolox Eq/L}$ and 1.0 ± 0.6 $\mu\text{mol Trolox Eq/L}$, $P < 0.001$), and lipid hydroperoxidation values (9.9 ± 3.4 $\mu\text{mol H}_2\text{O}_2$ Eq/L and 4.4 ± 1.5 $\mu\text{mol H}_2\text{O}_2$ Eq/L, $P < 0.001$) were found to be high. The high density lipoprotein (HDL) concentration of the asthmatic children was lower than that in the control group (40.1 ± 9.2 mg/dl and 54.5 ± 15.9 mg/dl, $P < 0.001$)

Conclusion: In asthmatic children, when total oxidant status, total antioxidant capacity and lipid hydroperoxidation levels increase, paraoxonase activity decreased.

Coronary heart disease (CHD) is a major killer and the most common cause of mortality and morbidity in the world.¹ It usually involves middle and older age groups but, recently, the incidence of CHD in younger individuals has been increasing.² Atherosclerosis is a process that starts in childhood and has a long pre-clinical phase before leading to clinical manifestations which usually appear in middle age.³ Changes in the peripheral vascular endothelium are the earliest signs of atherosclerosis and CHD.⁴ This has been demonstrated in asymptomatic children and young adults who are healthy but carry risk factors for atherosclerosis (e.g., high cholesterol, smoking, diabetes).⁵ HDL retards the accumulation of lipid peroxides on LDL cholesterol⁶ apparently due to paraoxonase which is an enzyme located on HDL particles.⁷ Paraoxonase has two functions. First, it contributes to detoxification of organophosphorous compounds, including the pesticide paraoxon. Secondly, it hydrolyzes lipid peroxides and prevents LDL oxidation.⁸

In both asthma and atherosclerosis, leukotrienes, which are potential inflammation mediators, play a role.^{9,10} Some studies have suggested that asthma itself could be a risk factor for stroke and heart

disease.^{11,12} In addition, allergic disorders such as allergic rhinitis or asthma may also contribute to enhanced risk for atherosclerosis.¹³

Asthma is a chronic inflammatory pulmonary disease related to increased oxidative stress.¹⁴ The association between chronic inflammation and oxidative stress is well documented. Elevated levels of reactive oxygen species (ROS), such as hydroxyl radicals, superoxides, and peroxides in inflammatory conditions have been reported previously.¹⁵

The cells infiltrating the bronchial mucosa in patients with asthma produce a variety of mediators including ROS.¹⁶ Increased production of ROS leads to an imbalance between the oxidative forces and the antioxidant defense systems favouring oxidative injury has been implicated in the pathogenesis of asthma.¹⁷ However, when the production of damaging ROS exceeds the capacity of the body's antioxidant defenses to detoxify them, a condition known as "oxidative stress" occurs.¹⁸ ROS may exert a number of toxic effects which have been demonstrated in many different biological systems. Oxidative stress leads to changes such as modification of receptor activity and signaling and release of endogenous mediators of inflammation. An imbalance in oxidant-antioxidant activity is involved in much free radical mediated pathology, e.g. ischemia-reperfusion and asthma.¹⁹

This suggests that an increase in oxidative stress and a decrease in paraoxonase activity may be important contributors to the acceleration of the progression of atherosclerosis and, also that, in asthmatic patients, atherosclerosis may start in childhood.

This study compared the paraoxonase activity in asthmatic children with that of healthy children by evaluating total oxidant status (TOS), total antioxidant capacity (TAC), lipid hydroperoxidation (LOOH) and lipid profile.

Materials and Methods

Study groups

The parents of all patients signed informed consent forms and the Ethics Committee of the Hospital approved the study. Forty-two subjects, 6-15 yr, who had been attending the Pediatric Allergy Unit of the Medical Faculty of Harran University for at least one year were included in the study. The clinical severity of the asthma was determined using the criteria (appropriate clinical and respiratory function tests) defined in the Global Initiative for Asthma guidelines (GINA).²⁰ A family history of atopy was considered positive if atopy was present in parents and/or siblings (bronchial asthma, allergic rhinitis, atopic dermatitis). In all patients, allergen sensitivity was performed with specific IgE (sIgE) and skin prick test (SPT) to aeroallergens. Patients with clinical signs of asthma who had a positive sIgE in addition to sensitivity against at least one aeroallergen on the SPT were included in the atopic asthma groups. Asthmatic patients were not receiving any controller medication and had not had any symptoms of lower or upper respiratory tract infection or asthma exacerbation within the previous 4 weeks. The control group consisted of 32 age-matched healthy children (6 - 16 yr). Healthy children were chosen from those referred to a pediatric outpatient clinic in Harran University Hospital, where all children periodically undergo check-ups for their growth and development. Control patients were evaluated with regard to chronic and/or severe infections, rheumatological and autoimmune disorders, and familial and personal history of atopy, and also by laboratory tests. Children were included in the control group if they had no personal and familial history of atopy and no signs of atopic disorder, and if they were negative for sIgE and SPT.

As smoking effects oxidative status, patients came from non-smoking households, and the control group was also selected from non-smoking households. All patients were weighed and measured and a calculation

was made according to the Body Mass Index Standard Deviation Score (BMI SDS).²¹

Study measurements

sIgE levels: Serum allergen sIgE measurements were performed using the CAP FEIA method (Pharmacia, Uppsala, Sweden) and evaluated with regard to the standard deviations listed in the user's manual according to age-sIgE levels reported by the World Health Organization. The sensitivity of the kits used for measurements was standardized to 0.35-100 kU/L for serum sIgE, which is used to detect the sensitization in the serum against inhaled allergens (house dust mite, yeasts, animal dander, grass pollen, trees and wild grass); the result was considered positive if the measured value was greater than 0.35 kU/L.

Skin prick test: Prick testing for aeroallergen sensitivity was done using lancets (Stallerpoint, Paris, France) providing a standard puncture of 1 mm. Commercial allergen solutions manufactured by Allergopharma (Joachim Ganzer KG, Reinbeck, Germany) were used for the skin test. Forty-four different allergens consisting of housedust mite, grass, wild grass, tree pollens, fungi, animal dander, and insects were tested. Test sites were evaluated 20 min after allergen application using European Academy of Allergy and Clinical Immunology criteria.²² The diameter of the induration on the volar aspect of the forearm was scored between 1 and 4 if it was at least half of the diameter of the induration caused by a positive control (1 mg/L histamine). A score ≥ 3 was considered significant.

Blood samples

Blood samples were obtained following overnight fasting. Blood samples were collected into empty tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples for

measurement of TOS and TAC levels and prolidase activity were stored at -80°C until they were used.

Measurement of paraoxonase and arylesterase activities

Paraoxonase and arylesterase activities were measured using paraoxon and phenylacetate substrates. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17,000 M⁻¹ cm⁻¹.²³ Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310 M⁻¹ cm⁻¹. One unit of arylesterase activity was defined as 1 μ mol phenol generated/min under the above conditions and expressed as U/L serum.²⁴ Paraoxonase phenotype distribution was determined by a double substrate method that measures the ratio of paraoxonase activity (with 1 M NaCl in the assay) to arylesterase activity, using phenylacetate.²³

Measurement of total antioxidant capacity (TAC)

The TAC of serum was determined using a novel automated measurement method, developed by Erel.²⁵ In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in Reagent 1 is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals, such as brown colored dianisidiny radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, the antioxidative effect of the sample against the potent-free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has excellent precision values of lower than 3%. The results are expressed as mmol Trolox Eq/L.

Measurement of total oxidant status (TOS)

The TOS of serum was determined using a novel automated measurement method, developed by Erel.²⁶ Oxidants present in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}$).

Measurement of total peroxide concentration of plasma (LOOH)

Serum LOOH levels were measured with the ferrous ion oxidation-xylenol orange (FOX-2) assay. The principle of the assay depends on the oxidation of ferrous ion to ferric ion via various oxidants and the ferric ion produced is measured with xylenol orange. LOOH are reduced by triphenyl phosphine (TPP), which is a specific reductant for lipids. The difference between with and without TPP pretreatment gives LOOH levels.²⁷

Measurement of Lipid profiles

Plasma triglyceride, total cholesterol, LDL, HDL, VLDL were measured by an automated chemistry analyser (Aeroset, Abbott, USA) using commercial kits (Abbott).

Exclusion criteria

Exclusion criteria included the presence of chronic disease, concomitant inflammatory disease such as infections and autoimmune disorders, immunocompromised patients, diabetes mellitus, familial hypercholesterolemia, major depression, neoplastic diseases, liver and kidney diseases and recent major surgical procedure. Patients suffering from heart disease, valvular heart disease, idiopathic hypertrophic and dilated cardiomyopathy, non-cardiac causes of chest pain or reflux esophagitis were also excluded. Patients taking antioxidant drugs, vitamins, diuretics, hormone replacement therapy and those who smoked were also excluded.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Qualitative variables were assessed by Chi-square test. Correlation analyses were performed using Pearson's correlation test or Spearman's correlation test. The differences between the different groups of controls and patients were analyzed by unpaired t-test or Mann–Whitney U test. A *P* value <0.05 was considered significant. Data were analyzed with the SPSS® for Windows computing program (Version 11.5).

Results

The demographic and clinical data of the study population are shown in Table 1. There were no differences between the two groups with regard to age, sex and BMI SDS (Table 1).

TABLE 1. Demographic data of asthma patients and healthy subjects

	Asthma patients (n: 42)	Controls (n: 32)	OR (95%CI)	<i>P</i> *
Age (yr)	9.0 $\pm$ 2.8	9.4 $\pm$ 3.8		NS
Sex (M/F)	20/22	10/22	0.50 (0.17-1.45)	NS
BMI SDS	0.77 $\pm$ 0.25	0.61 $\pm$ 0.20		NS
Atopy in family (%)	17 (40.4)	-		

NS: Not Significant

TABLE 2. Paraoxonase activity and oxidative-antioxidative parameters in asthma patients and control groups

	Asthma patients (n=42)	Controls (n=32)	P*
Paraoxonase (U/L)	163.7 ± 73.0	349.2 ± 153.9	0.002
TOS (μmolH ₂ O ₂ Equiv./L)	13.4 ± 7.0	9.0 ± 3.5	0.002
LOOH (μmol H ₂ O ₂ /L)	9.9 ± 3.4	4.4 ± 1.5	<0.001
TAC (μmol Trolox Eq./L)	5.5 ± 2.5	1.0 ± 0.6	<0.001

TOS: total oxidative status, TAC: total antioxidant capacity, LOOH: total peroxide concentration

The paraoxonase level in the asthma patient group was lower than in the control group (Table 2). TAC, TOS and LOOH levels in the asthma patient group were higher than the control group (Table 2).

There was no correlation between the TAC, TOS, LOOH level and paraoxonase activity between the patient and control group. The values for plasma triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) for both the patient and the control group are shown in Table 3.

SPT of the asthma patient group were 45% grass pollen, 30% polysensitization, 15% mites and 10% alternaria. No difference was determined in the paraoxonase activity and oxidative level between the mite positive group and patients positive for other allergens.

Discussion

In this study the paraoxonase activity level of the asthma patients was found to be lower than in the control group. In 3 studies in the literature^{28,29,30} paraoxonase activity in asthma patients were not different. Also, Can et al.³⁰ observed that asthmatic patients

had increased paraoxonase activity after treatment. Evidence of increased paraoxonase activity in asthmatic patients after treatment, may indicate that paraoxonase could play a role in asthma.

The study group of asthmatic children, particularly, consisted of those who were not experiencing an exacerbation of the asthma. A reduced level of paraoxonase activity in children with asthma could arise from including in the study those who had previously been under regular follow-up and where the asthma had not been able to be fully controlled. The reduced level of paraoxonase activity in our study may be related to regional and ethnic differences.

A previous study reported an increase in oxidative stress and a decrease in paraoxonase activity and also down regulation of paraoxonase expression.³¹ In addition, an inverse relationship between serum reduced paraoxonase activity and increased oxidative stress in patients with CHD has been seen.³² The acquired information about the relationships between oxidative stress and paraoxonase and paraoxonase and lipid metabolism indicates that paraoxonase may play a role in the development of atherosclerosis in asthma. Our study shows paraoxonase activity and the oxidative status outside of any treatment period.

TABLE 3. Asthma patients and control group lipid profile

	Asthma patients (n:42)	Controls (n:32)	P*
Triglyceride (mg/dl)	114.3 ± 59.3	117.9 ± 31.9	NS
Cholesterol (mg/dl)	150.7 ± 27.0	153.8 ± 24.7	NS
HDL-C (mg/dl)	40.1 ± 9.2	54.5 ± 15.9	<0.001
LDL-C (mg/dl)	85.2 ± 26.4	90.0 ± 17.2	NS
VLDL-C (mg/dl)	23.6 ± 13.8	28.0 ± 22.3	NS

The values represent the mean ± SD.

NS: Not Significant

The serum HDL concentration is inversely correlated with atherosclerosis risk.³³ The mechanism for this continues to be subject of considerable debate. However, recent studies have suggested more diverse mechanisms. HDL protects against oxidative modification of lowdensity lipoprotein (LDL)⁶, which is believed to be central to the initiation and progression of atherosclerosis.³⁴ The antioxidant activity of HDL relates to its enzymes, primarily paraoxonase and these can prevent lipid peroxide accumulation on LDL both in vitro and in vivo.³⁵ The lipid peroxidation products of the asthmatic patients in our study were seen to increase in a similar way to those of previous studies.^{28,36,37}

No difference was reported in the lipid values between the patient group and the control group in a previous study²⁹ whereas, in our study, a low level of HDL-C was determined in the patient group in comparison to the control group (Table 3). This low level of HDL-C may be due to these asthmatic children having been previously under regular follow-up and the asthma not having been able to be fully controlled. An increase in LDL oxidation arises from a low level of HDL and paraoxonase, thus possibly indicating an acceleration of the progress of atherosclerosis in asthmatic children.

The balance between oxidant-antioxidant system is impaired in patients with asthma.³⁸ Many observations suggest that levels of oxidative stress are increased in children and in adults with asthma, not only in the lungs but also in the circulation.³⁸ Several studies have suggested that, when the oxidant system increases, there is a decrease in the antioxidant system.^{28,39} In our study, TOS and TAC increased together. A similar previous study, reported⁴⁰ that, when there was an increase in the oxidant system, there was also an increase in the antioxidant system.

Oxidative stress is a key component of inflammation and inflammatory disorders. Host antioxidant systems are generally activated in response to the oxidant attack, but individuals have different capacities of antioxidant defense which are, in part, genetically

determined.⁴¹ Ercan et al.⁴² showed that there were genetic differences in the antioxidant response. Other studies have shown that as oxidative stress increases so the antioxidant capacity increases as a protective mechanism.⁴³ Due to the socio-economic conditions in the region, our cases were diagnosed with asthma at a late stage and were not receiving regular check-ups. In this situation they are vulnerable to long-term oxidative stress and so the development of the antioxidant system could be in response to that.

Limitations of this study were that there was no evaluation of the relationship between the severity of the disease and the treatment, and that paraoxonase gene polymorphism was not been taken into account.

In conclusion, we determined that the enzyme paraoxonase, which has an antioxidant function and is known to play a role in the development of atherosclerosis, was low in asthmatic children.

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