

# The relationship between carotid intima media thickness and oxidative stress in asthmatic children

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## Summary

**Background:** Asthma and atherosclerosis are both chronic inflammatory diseases. The progression of the inflammation in asthmatic patients is known to be similar to the increased development of atherosclerosis. **Objective:** The aim of this study was to research the relationship between the difference in carotid intima media thickness (CIMT) and oxidative stress together with difference in CIMT in asthmatic children and control group.

**Methods:** A total of 84 subjects between 6-15 years of age who had been attending the Pediatric Allergy Unit of the Medical Faculty were included in this study.

Asthmatic patients and a control group were evaluated by ultrasonography for measurements of CIMT and oxidative status.

**Results:** In the asthmatic patient group, the CIMT was  $0.48 \pm 0.06$  mm (right side) and  $0.44 \pm 0.05$  mm (left side). In the control group it was  $0.42 \pm 0.05$  mm (right side) and  $0.42 \pm 0.04$  mm (left side). This difference is statistically significant ( $p < 0.0001$ ,  $p = 0.019$  respectively). In the asthmatic group a positive correlation was determined between the total oxidant status (TOS) value and the right and left CIMT ( $p = 0.007$ ,  $r = 0.44$  and  $p = 0.001$ ,  $r = 0.50$  respectively).

**Conclusions:** A significant correlation was determined between an increase in oxidative stress and CIMT in asthmatic children. This indicates that atherosclerosis, which is known

as an adult disease, may start in childhood. These findings show that it might be beneficial for children who are being followed-up from a diagnosis of asthma to also be evaluated in respect of the development of atherosclerosis. (*Asian Pac J Allergy Immunol* 2010;28:256-61)

**Key words:** Asthma, Atherosclerosis, Carotid intima media thickness, Children, Oxidative stress

## Introduction

Asthma and atherosclerosis are both chronic inflammatory diseases. Inflammation plays an important role in the impairment of endothelial functions. If the inflammation is chronic, this leads to an acceleration of the atherosclerosis<sup>1</sup>. An early sign of atherosclerosis is hypertrophy of the arterial wall. Increased carotid intima-media thickness (CIMT) is a non-invasive marker of arterial wall alteration, which can easily be assessed in the carotid arteries by high-resolution B-mode ultrasound<sup>2,3</sup>, and this correlates with the frequency of stroke and myocardial disease<sup>4,5</sup> and the spread of cardiovascular disease<sup>4,6</sup>. Previous studies have shown that when there is gene ordering in both asthma and atherosclerosis, there is also a relationship in both of them with leukotrienes, which are known to be potential inflammation mediators<sup>7,8</sup>. Some studies have even stated that asthma itself could be a risk factor for stroke and heart disease<sup>9,10</sup>.

Asthma is a chronic inflammatory pulmonary disease related to increased oxidative stress<sup>11</sup>. The association between chronic inflammation and oxidative stress is well documented. Elevated levels of reactive oxygen species, such as hydroxyl radicals,

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superoxides, and peroxides in inflammatory conditions have been reported previously<sup>12</sup>.

This study aimed to investigate in asthmatic children the relationship between changes in the carotid intima media thickness and oxidative stress, together with changes in the CIMT.

## Methods

### *Study groups*

A total of 84 subjects between 6-15 years of age who had been attending the Pediatric Allergy Unit of the Medical Faculty of Harran University for at least one year were included in this study. Asthma was diagnosed from a history of intermittent wheezing and the presence of reversible airway obstruction as defined by at least a 12% improvement in FEV1 following bronchodilator administration and therapeutic response to anti-asthma treatment. 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).<sup>13</sup> All the patients had intermittent mild asthma and only underwent treatment in the event of an attack.

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. Immuno-compromised patients, patients with a history of chronic inflammation/rheumatological disorder, and patients with autoimmune diseases were excluded. Asthma 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 194 age-matched healthy children (6 to 16 years). Healthy children were chosen from those referred to a pediatric outpatient clinic, 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.

As it is known to have an effect on oxidative status, the patients came from non-smoking households, and the control group was also selected from non-smoking households. All the patients and control group in the study were weighed and measured then a calculation was made according to the Body Mass Index Standard Deviation Score (BMI SDS)<sup>14</sup>.

### *Study measurements*

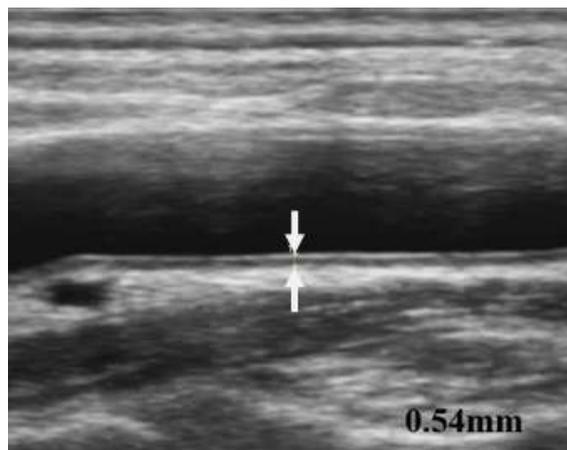
**sIgE levels:** Serum allergen sIgE measurements were performed using CAP FEIA method (Pharmacia, Uppsala, Sweden), which is used to detect the sensitization in the serum against inhaled allergens (house dust mite, yeasts, animal dander, grass pollen, trees and wild grass); the result was considered positive if the measured value was greater than 0.35 kU/L.

**Skin prick test:** Commercial allergen solutions manufactured by Allergopharma (Joachim Ganzer KG, Reinbeck, Germany) were used for the skin test. A total of 44 different allergens consisting of housedust mite, grass, wild grass, tree pollens, fungi, animal dander, and insects were tested and children with at least one positive test were considered atopic.

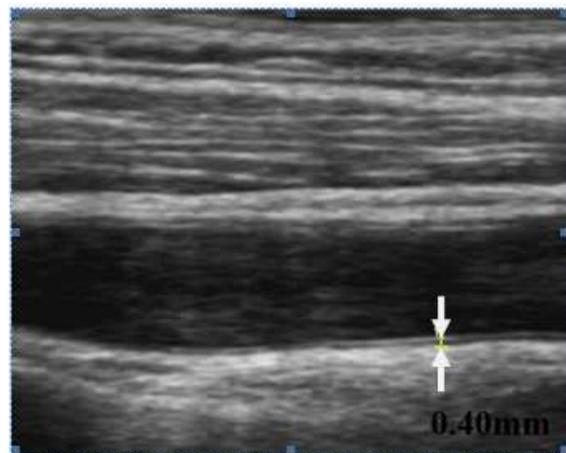
### *Carotid Intima Media Thickness Measurement*

All of the sonographic examinations were performed by the same examiner, who was unaware of the subject's clinical status throughout the study. Images were obtained by high-resolution Doppler ultrasonography (Logiq 7 Pro; General Electric, Milwaukee, WI, USA) with a 12-MHz linear-array transducer. Common carotid artery (CCA) intima media thickness were measured in both carotid arteries. (Figure 1, Figure 2) The average was taken of the measurements 1 cm above and below the largest measured place for the CCA intima media thickness.

Both groups were informed not to eat, drink or take any antioxidant medicine for 3 hours prior to the samples being taken. None of the subjects was taking any drug known to affect lipid and lipoprotein metabolism. Special care



**Figure 1.** Ultrasonic image of an asthma patient's increased carotid intima media thickness in the work group (0.54 mm)



**Figure 2.** Carotid intima media thickness ultrasonic image of a case in the control group (0.40 mm)

was taken to exclude subjects who were taking anabolic drugs, vitamins or other antioxidants, or who were smokers. None of the subjects was following a special diet.

Patients were excluded from the study if there were findings of septicaemia, pulmonary infection, hypoxia-anoxia, birth anomalies, chromosome anomalies, metabolic diseases, cephalhaematoma, ecchymosis or polycythemia or systemic disease apart from asthma or additional infection which would cause the presence of free radicals. Neither the patient nor control group were using vasoactive medication.

Parents of all patients signed informed consent forms and the study protocol was approved by the local Ethics Committee.

### Samples

Blood samples were withdrawn into heparinised tubes from a cubital vein then immediately stored in ice. Plasma was separated from cells by centrifugation at 3000 rpm for 10 min. Plasma samples were stored at  $-80^{\circ}\text{C}$  until analysis.

**Table 1.** Demographic data of asthma patients and control group

	Patients(n:84)	Controls(n:194)	<i>p</i>
Age (Years)	9.05±3.22	9.80±1.08	>0.05
Gender (M/F)	40/44	96/98	>0.05
BMI SDS	0.65±0.23	0.67±0.23	>0.05
Atopy in family (%)	34(40.4)	-	

The values represent the mean ± s.d.

Significance was defined as  $p < 0.05$ .

### Measurement of total oxidant status

Total oxidant status (TOS) of plasma was determined using a novel automated measurement method as previously described<sup>15</sup>.

### Measurement of total antioxidative capacity of plasma

The total antioxidant status (TAS) of the plasma was determined using a novel-automated measurement method, developed by Erel<sup>16</sup>.

### Measurement of total peroxide concentration of plasma (LOOHs)

The total peroxide concentrations of the plasma samples were determined using the FOX2 method<sup>17</sup> with minor modifications<sup>18</sup>.

### Statistical analyses

Data were analyzed using the SPSS® for Windows computing program (Version 11.5). The values are expressed as mean ± S.D. for the asthma patients and the control group, separately. The differences between the different groups of controls and patients were

**Table 2.** Asthma patients and control group; carotid intima media thickness and TOS, TAS, LOOHs values

	Patients(n:84 )	Controls(n:194)	p
RCIMT (mm)	0.48±0.06	0.42±0.05	<0.0001
LCIMT (mm)	0.44±0.06	0.42±0.04	<0.019
TOS	11.87±5.38	8.7±3.3	<0.004
TAS	5.45±2.66	1.01±0.6	<0.0001
LOOHs	9.84±3.56	4.3±1.5	<0.0001

The values represent the mean ± s.d.

Significance was defined as  $p < 0.05$ .

TOS: Total oxidant stress

TAS: Total antioxidant status

LOOHs: Total peroxide concentration

RCIMT: Right carotid intima media thickness (mm)

LCIMT: Left carotid intima media thickness (mm)

analyzed by Student's *t*-test or Mann–Whitney U test. Correlation analyses were performed using Pearson's correlation test. Statistical significance was defined for *p* values of less than 0.05.

## Results

The mean age of the asthma group was  $9.05 \pm 3.22$  years (40 male, 44 female), and of the control group  $9.80 \pm 1.08$  years (96 male, 98 female). There was no difference between the groups in terms of age, gender and BMISD (Table 1). In the asthma group the carotid intima media thickness was  $0.48 \pm 0.06$  mm (right side), and  $0.44 \pm 0.05$  mm (left side), and in the control group was  $0.42 \pm 0.05$  mm (right side), and  $0.42 \pm 0.04$  mm (left side). This difference is statistically significant. ( $p < 0.0001$ ,  $p = 0.019$  respectively) (Table 2)

In the asthma group TOS value was determined as  $11.8 \pm 5.3$   $\mu\text{mol H}_2\text{O}_2$  Eqv./L, and the control group was  $8.7 \pm 3.3$   $\mu\text{mol H}_2\text{O}_2$  Eqv. / L. ( $p = 0.004$ ) (Table 2)

The right and left carotid intima media thickness measurements, TOS, TAS and LOOH values, and statistical results for the patient and the control group are shown in Table 2.

A positive correlation was determined between the TOS value and the right and left CIMT in the asthma group. ( $p = 0.007$ ,  $r = 0.44$  and  $p = 0.001$ ,  $r = 0.50$  respectively) (Table 3)

No correlation was determined between TAS and LOOH values and right and left

**Table 3.** Asthma patients: correlation between right and left carotid intima media thickness and TOS, TAS, LOOHs

	r	p
RCIMT -TOS	0.44	<0.007
RCIMT -TAS	-	0.14
RCIMT-LOOHs	-	0.22
LCIMT -TOS	0.50	<0.001
LCIMT -TAS	-	0.50
LCIMT -LOOHs	-	0.95

RCIMT: Right carotid intima media thickness

LCIMT: Left carotid intima media thickness

Pearson correlation test

CIMT in the asthma group (Table 3). There was no correlation between TOS, TAS and LOOH values and right and left CIMT in the control group. There was no correlation between sIgE, TOS, TAS and LOOH values in the patient group.

## Discussion

When compared to the control group, the right and left CIMT of the asthmatic children was significantly higher. It is known that in adult patients an increase in the development of atherosclerosis is similar to the progression of inflammation in asthmatic patients<sup>19</sup>. However, this relationship has not been previously shown in asthmatic children. Early stage atherosclerotic changes in the blood vessel structure, particularly an increased thickening of the intima media, may be an early indication of atherosclerotic progression in asthmatic children. In a study by Berenson et al.<sup>20</sup> it is stated that when atherosclerosis starts in childhood, identification at an early stage has great importance in the prevention of progression of atherosclerosis. The increase in oxidative stress was also significant in the patient group when compared to the control group. A significant correlation was found between the increase in oxidative stress and CIMT. A correlation was determined in asthma patients between the measured increased CIMT and TOS as an indicator of oxidative stress. However, despite the increase in TAS and LOOHs being significantly different in the patient group when compared to the control group, the difference in CIMT showed no statistical correlation. Our observations suggest that there is a link between the increased levels of oxidative stress and increased CIMT seen in

patients with asthma, when compared with those seen in healthy controls.

While no relationship was found between asthma starting in childhood and the development of atherosclerosis, in a study on the effect of asthma on the development of atherosclerosis in adults, there was found to be a relationship between adult asthma and the development of atherosclerosis<sup>21</sup>. However in our study, the CIMT was found to be increased in the asthmatic patients. In the above-mentioned study by Onufrak et al., as a diagnosis of childhood asthma was given retrospectively, the authors do not place complete trust in this diagnosis and it is possible that some of these cases may not be asthmatic. Also, to make the level of asthma heterogeneous in the study group, some patients were regularly taking medication. Our study was prospective and comprised only cases with a definite diagnosis and who were not using any prophylactic medication. This may be the reason that a relationship was determined between asthma and CIMT. As there are no other studies in literature on this subject there has been no evaluation of the effects of regional and ethnic differences. Studies carried out in different regions could lead the way on this topic.

The proven CIMT in the asthmatic children is an indicator that atherosclerosis, which is known as an adult disease, could start in childhood. The most important limitation of this study is that it was performed at a single centre and with a small group.

Steroids, which are the most important medication used in the treatment of asthma, are known to have a cardiovascular effect.<sup>22,23</sup> Therefore, the patients included in the study were those with mild asthma who were not regularly using steroids. However, by only including mild asthma patients in the study group in order to avoid the effects created by prophylactic medication, it was not possible to evaluate the effect of the severity of the asthma on CIMT.

The relationship between the severity of the asthma and the CIMT, and the effect of non-treatment in the early phase on the risk of developing atherosclerosis in the long-term could be evaluated in a further study.

In conclusion, however, as this is the first time this relationship has been shown in children, we believe our findings are of value. A significant correlation was determined between an increase in oxidative stress and CIMT in asthmatic children and this increase in CIMT could be an indicator that atherosclerosis, which is known as an adult disease, could start in childhood. These findings show that it might be beneficial for children who are being followed-up from a diagnosis of asthma to also be evaluated in respect of the development of atherosclerosis. The proven CIMT in asthmatic patients could be evaluated in a multi-centre prospective study with a greater number of patients continuing into adulthood.

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