The levels of asymmetric dimethylarginine, homocysteine and carotid intima-media thickness in hypercholesterolemic children

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SUMMARY: Hasanoğlu A, Okur İ, Ören AC, Biberoğlu G, Oktar S, Eminoğlu FT, Tümer L. The levels of asymmetric dimethylarginine, homocysteine and carotid intima-media thickness in hypercholesterolemic children. Turk J Pediatr 2011; 53: 522-527.

The aim of this study was to examine the intima-media thickness (IMT) of carotid arteries and endothelial function parameters such as plasma asymmetric dimethylarginine (ADMA) and homocysteine levels in hypercholesterolemic children and to investigate the relations of these parameters with hypercholesterolemia. Fifty-seven hypercholesterolemic and 37 healthy children were included in the study. Hypercholesterolemia was defined as 155 mg/dl and above for low-density lipoprotein (LDL)-cholesterol. Plasma concentrations of ADMA and homocysteine were measured and the measurement of carotid IMT was determined. Both carotid IMT and plasma ADMA levels were significantly higher in hypercholesterolemic children than healthy children ($p<0.01$). No significant difference was determined in homocysteine concentration between hypercholesterolemic children and the control group ($p>0.05$). No significant correlation was observed between lipid profiles and the levels of ADMA and homocysteine. However, a significant positive correlation was found between carotid IMT and total and LDL-cholesterol levels and between the levels of ADMA and LDL-cholesterol. In conclusion, the progressive increase in ADMA levels and carotid IMT and the positive relationship between carotid IMT and serum cholesterol levels support that plasma ADMA levels and carotid IMT can be indicators of early atherosclerosis in hypercholesterolemic children.

Key words: asymmetric dimethylarginine, carotid intima-media thickness, hypercholesterolemic children.

Nitric oxide (NO) is an endogenous vasodilator released from the endothelium. It also inhibits platelet adherence and aggregation, reduces adherence of leukocytes to the endothelium and suppresses proliferation of vascular smooth muscle cells. Thus, NO is recognized as the most potent endogenous molecule against atherosclerosis. Accordingly, impairment of NO synthesis bioactivity may increase the risk of vascular disease. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthase that has been linked to endothelial dysfunction and atherosclerosis in the general population[1-3]. ADMA is formed endogenously by degradation of proteins containing arginine residues that have been methylated by S-adenosylmethionine-dependent methyltransferase[4]. ADMA is increased in the plasma of humans with hypercholesterolemia, atherosclerosis, hypertension, chronic renal failure, chronic heart failure, hyperhomocysteinemia, and other clinical conditions[5].

Bode-Böger et al.[6] and Böger et al.[7,8] demonstrated high levels of ADMA in plasma from hypercholesterolemic rabbits. Their later study showed that ADMA was elevated in young subjects with hypercholesterolemia and that elevation of ADMA was associated with impaired endothelium-dependent vasodilation. Intra-arterial infusion of ADMA causes

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endothelial dysfunction in humans[9]. Moreover, ADMA is a strong and independent predictor of cardiovascular events and atherosclerosis[10].

Another marker of early atherosclerosis is measurement of the carotid intima-media thickness (IMT) via high-resolution B-mode ultrasound. Several studies have shown that increased carotid IMT is a consistent predictor of the risk of future cardiovascular events and can also predict the presence of coronary artery disease[11]. Children with familial hypercholesterolemia are characterized by an increased IMT when compared with healthy controls[12]. Several investigators have looked for an association between various atherogenic risk factors and IMT of the carotid arteries. Most studies have been conducted among middle-aged and older subjects with hypercholesterolemia, while similar studies in children and adolescents are rare.

High serum homocysteine concentration is increasingly recognized as a new risk factor for atherosclerosis and other vascular diseases. The atherogenic effect of homocysteine is related to cytotoxin action on the endothelial cells and their function[13].

The aim of this study was to examine and compare IMT of the carotid arteries and endothelial function parameters such as plasma ADMA and homocysteine levels between hypercholesterolemic children and healthy controls, and to determine the relations between carotid IMT and atherogenic risk factors, such as lipid and plasma ADMA level.

Material and Methods

This study was performed on 57 hypercholesterolemic children, whose low-density lipoprotein (LDL)-cholesterol levels were >155 mg/dl (4 mmol/L). As the control group, 37 healthy children of similar age were included. The detailed medical and family history of all subjects was obtained and a complete physical examination, including the evaluation of height, weight and blood pressure, was performed. The patients were classified according to the total number of points calculated based on the Dutch Lipid Clinical Network Diagnostic criteria for familial hypercholesterolemia[14]. Patients who were scored as ≥3 points were classified as having familial hypercholesterolemia and those who were scored as <3 points were classified as having non-familial hypercholesterolemia. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2), and the subjects who were >95th percentile according to age and sex for Turkish children were not included in the study due to obesity[15]. None of the subjects suffered from any of the other risk factors for atherosclerosis and hypercholesterolemia, such as hypertension, diabetes mellitus, and renal, liver and endocrine diseases, and none was taking medication, such as lipid-lowering therapy, immunosuppressive therapy or vitamin supplements. The study was approved by the local ethics committee, and all patients and their family provided written informed consent.

The blood samples were obtained from the antecubital fossa vein in the morning after 12 hours of fasting and were immediately centrifuged at 2500 rpm for 10 minutes (min). Plasma and serum samples were stored at -80°C. The lipid profile (β-quantification) was analyzed on fresh samples. The subjects whose total cholesterol and LDL-cholesterol were >95th percentile according to age and sex were accepted as hypercholesterolemic. Total cholesterol, triglyceride and high-density lipoprotein (HDL) levels were determined by colorimetric-spectrophotometric Aeroset (Abbott) autoanalyzer at 500 nm according to the Trinder reaction. LDL levels were calculated according to the Friedewald formula (Total cholesterol – (HDL + very low-density lipoprotein [VLDL]).

Asymmetric dimethylarginine (ADMA), arginine and homocysteine concentrations of the plasma samples were determined with high performance liquid chromatography with fluorescence detector[16].

The measurement of carotid IMT was performed by the same sonographer (S.O.) using a General Electric® Logic 9 ultrasonography with a linear array probe of 7.5 MHz.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (version 11.5, SPSS, Inc., Chicago, IL). All values were expressed as mean and standard deviation (SD). The differences between the two groups were tested by Mann-Whitney testing. Linear

regression analysis with Pearson's coefficients was used to assess the strength of association between variables. Multivariate regression analysis was used to identify determinants of IMT of the carotid artery. The strength of these relationships was expressed as the β coefficient and p value. A $p<0.05$ was considered statistically significant.

Results

Age, sex, BMI, and laboratory parameters of the patient groups and control children are shown in Table I. No significant differences were observed between the patient groups and controls in terms of age and BMI ($p>0.05$). Total and LDL-cholesterol concentrations were statistically higher in the patient groups than in the control group ($p<0.001$), but no significant difference was observed between the patients and controls in terms of triglyceride concentration ($p>0.05$).

Both carotid IMT and plasma ADMA levels were significantly higher in all hypercholesterolemic groups than in normocholesterolemic children ($p<0.001$; $p<0.01$). The levels of plasma arginine were statistically higher in all patients and in children with non-familial hypercholesterolemia ($p<0.01$) than in the controls. No significant difference was determined in homocysteine concentration between hypercholesterolemic children and the control group ($p>0.05$) (Table I).

In the correlation analysis, no significant correlation was observed between lipid profiles and the levels of arginine, homocysteine and arginine/ADMA ratio. However, a statistically positive correlation was observed between the levels of plasma ADMA and LDL-cholesterol (Table II). A significant positive correlation was found between carotid IMT and total and LDL-cholesterol and triglyceride levels (Table III).

Discussion

Endothelial dysfunction has an important role in the atherosclerotic process, and the key mechanism of this loss of function is NO-based. In many studies, it was reported that there are defects in the biological activity of NO in hypercholesterolemia and atherosclerosis. The first step of the atherosclerotic process is endothelial dysfunction caused by leukocyte adhesion and platelet aggregation[17].

Recent studies have shown that ADMA is an agent for endothelial dysfunction and may be an indicator for atherosclerosis[7,18-20]. Several studies have demonstrated that plasma levels of ADMA are increased in conditions associated with atherosclerosis, including the risk factors of age, hypertension, diabetes, insulin resistance, hypercholesterolemia, hypertriglyceridemia, and hyperhomocysteinemia[2,7,17,20-24].

However, the study of ADMA in pediatric diseases has just begun. Unlike prospective data that indicate a direct and independent association between ADMA and cardiovascular endpoints in adult patients with different cardiovascular diseases, pediatric studies have been limited by inadequate power and small sample size[25]. Jehlicka et al.[26] showed that baseline levels of ADMA were significantly higher in children with familial hypercholesterolemia than in diabetes mellitus type 1 and healthy children. In our study, we found that ADMA levels were significantly higher in children with hypercholesterolemia when compared with controls. These findings support the fact that plasma ADMA concentration is a novel risk factor for atherosclerosis in hypercholesterolemic children in the future.

Zhu et al.[27] showed that plasma homocysteine concentrations are high in obese children with hypertension and dyslipidemia, and they suggest that homocysteine levels of these patients should be monitored. Szymczak et al.[28] found high homocysteine levels in hypercholesterolemic children with a family history for cardiovascular disease, and they indicated that high homocysteine level is a predictive risk factor for cardiovascular disease in these children. Sierakowska-Fijalek et al.[29] noted that high plasma homocysteine level is a risk factor for atherosclerosis. According to our findings, there was no significant change in homocysteine levels of the hypercholesterolemic children compared to the control group. However, the effect of homocysteine in the atherosclerotic process is proven[30]. We thus suggest the homocysteine levels may be an independent indicator in atherosclerosis in hypercholesterolemic patients.

Several studies have shown that high resolution B-mode ultrasound measurement of the carotid IMT is a feasible, direct and noninvasive

Table I. Baseline Characteristics of the Study Groups

	All patients		FHC		Non-FHC		Control children	
	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD
Sex	57	21M/36F	28	9M/19F	29	12M/17F	37	14M/23F
Age (years) [a]	57	9.86±3.64 (2-16)	28	9.55±3.72 (2-15)	29	10.17±3.59 (3.5-16)	37	9.68±2.93 (5-16)
BMI (kg/m^2)	57	18.08±2.91	28	17.81±2.87	29	18.33±2.97	37	17.14±2.92
Lipid profile (mg/dl) [a]								
Total cholesterol	57	298.75±127.00*** (223-840)	28	360.21±159.77*** (259-840)	29	239.41±14.93*** (223-293)	37	157.97±20.33 (120-198)
LDL-cholesterol	57	228.38±125.61*** (155-796)	28	290.21±157.46*** (189-796)	29	168.69±11.73*** (155-213)	37	77.47±22.16 (36-121)
Triglyceride	57	87.68±30.53 (47-164)	28	88.96±33.12 (47-164)	29	86.45±28.34 (48-148)	37	76.37±27.77 (13-127)
Carotid IMT (mm)								
Right IMT	57	0.676±0.350**	28	0.765±0.465**	29	0.587±0.122*	37	0.524±0.121
Left IMT	57	0.687±0.391**	28	0.789±0.525**	29	0.582±0.094*	37	0.522±0.132
Mean IMT	57	0.682±0.364**	28	0.777±0.487**	29	0.584±0.102*	37	0.523±0.123
ADMA (μmol/L)	54	2.20±0.79**	28	2.18±0.79*	26	2.22±0.80**	37	1.72±0.35
Arginine (μmol/L)	54	90.37±28.58**	28	85.41±28.22	26	95.71±28.53**	37	74.51±19.63
Arginine/ADMA	54	45.88±21.45	28	43.54±20.22	26	48.41±22.83	37	46.52±19.18
Homocysteine (μmol/L)	33	9.22±2.73	28	9.50±2.88	18	8.99±2.66	18	9.17±2.66

***p<0.001; **p<0.01; *p<0.05 vs. control children

SD: Standard deviation. FHC: Familial hypercholesterolemia. BMI: Body mass index. ADMA: Asymmetric dimethylarginine. IMT: Intima- media thickness. LDL: Low-density lipoprotein.

[a] Age and lipid profiles are expressed as range (minimum and maximum).

Table II. Correlation between ADMA, L-Arginine, L-Arginine/ADMA Ratio, and Homocysteine Levels and Serum Lipid Profile

Lipid profile (mg/dl)	ADMA (μmol/L)		Arginine (μmol/L)		Arginine/ ADMA ratio		Homocysteine (μmol/L)	
	r	p	r	p	r	p	r	p
Total cholesterol	0.200	0.06	0.185	0.84	0.071	0.51	-0.044	0.76
LDL-cholesterol	0.220	**0.04**	0.178	0.09	-0.086	0.42	-0.048	0.74
Triglyceride	0.025	0.81	-0.024	0.82	-0.081	0.45	0.026	0.85

r: Correlation coefficient.
ADMA: Asymmetric dimethylarginine. LDL: Low-density lipoprotein.

method for evaluating and detecting early atherosclerosis and preclinical lesions of the arterial wall. Increased thickness and stiffness of the carotid artery were noted as an early marker of impaired vascular health[2,10]. Previous studies that were performed in childhood showed significantly higher IMT in children with type 1 diabetes, obesity, hypertension, and familial hypercholesterolemia[31-34]. We demonstrated that carotid IMT is significantly higher in children with hypercholesterolemia when compared with controls and that carotid IMT is significantly correlated with total cholesterol, LDL-cholesterol and triglyceride levels.

There are a number of reports in the literature that have shown the association between plasma ADMA levels and carotid IMT in adults[3,35]. Our study is the first to investigate the association between plasma ADMA levels and carotid IMT in children with hypercholesterolemia. Our results showed that plasma ADMA levels and carotid IMT were significantly higher in children with hypercholesterolemia than healthy children, and also that there was a significant positive correlation between carotid IMT and total and LDL- cholesterol levels. However, no significant positive correlation was observed between plasma ADMA and cholesterol levels.

In conclusion, ADMA level and carotid IMT can be the early leading indicators in hypercholesterolemic children with atherosclerotic risks. The combination of ADMA and advanced imaging methods such as carotid IMT may play an important role in the prediction of cardiovascular risk in hypercholesterolemia in childhood.

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Table III. Correlation between C-IMT and Laboratory Measurements

	Right C-IMT (mm)		Left C-IMT (mm)		Mean C-IMT (mm)	
	r	p	r	p	r	p
Total cholesterol (mg/dl)	0.839	<0.01	0.860	<0.01	0.865	<0,01
LDL-cholesterol (mg/dl)	0.814	<0.01	0.823	<0.01	0.833	<0,01
Triglyceride (mg/dl)	0.343	<0.01	0.363	<0.01	0.360	<0.01
ADMA (μmol/L)	0.105	0.33	0.089	0.41	0.098	0.36
Arginine (μmol/L)	0.174	0.10	0.222	0.04	0.203	0.061
Homocysteine (μmol/L)	-0.032	0.82	-0.107	0.45	-0.081	0.57

r: Correlation coefficient.

ADMA: Asymmetric dimethylarginine. C-IMT: Carotid intima-media thickness. LDL: Low-density lipoprotein.

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