Carotid intima media thickness in adolescents with increased risk for atherosclerosis

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In this study, we aimed to analyze early-onset atherosclerotic changes in adolescents with risk of cardiovascular disease in comparison to healthy controls using carotid intima media thickness (CIMT), homocysteine and markers of endothelial function as indicators. Children aged 10 years or older, all pubertal, with type 1 diabetes mellitus (T1DM), obesity, or obesity with glucose intolerance and age- and sex-matched healthy controls were included in the study. Endothelial markers (von Willebrand factor [vWF], tissue plasminogen activator [tPA], plasminogen activator inhibitor [PAI]-1), CIMT, homocysteine, folic acid, and vitamin B12 levels were measured in all subjects. Mean CIMT of the obese subjects were significantly higher than that of lean diabetic children and healthy controls (p=0.024). There was an independent relationship between CIMT and homocysteine level (b=0.76, p<0.0001). Further, homocysteine was negatively correlated with vitamin B12 (r=-0.20, p<0.001) and folic acid (r=-0.44, p<0.001). Homocysteine is an independent risk factor for early atherosclerosis in adolescents, which may be controlled by supplementation with vitamin B12 and folic acid.

Key words: adolescents, atherosclerosis, carotid intima media thickness, endothelial dysfunction, homocysteine.

Cardiovascular disease and stroke remain the most common cause of death in men and women of all ethnic backgrounds despite developments in treatment[1]. Although atherosclerosis manifests clinically in middle and late adulthood, it is now accepted that the disorder has a prolonged insidious course, and has its onset early in life[2,3]. The finding of fatty streaks in coronary arteries of half of the autopsy specimens from children aged 10-14 years attests to the ubiquity of the atherosclerotic process[4]. Identification of individuals at risk for atherosclerosis early in life to enable early intervention using preventive measures may slow the atherosclerotic process and delay cardiovascular disease. However, it is not easy to identify early atherosclerosis when the process is subclinical. Several markers or methods, some of them noninvasive, may be used to identify early changes. Endothelial dysfunction is the common shared pathway for vascular pathology[5,6], and occurs early in the development of atherosclerosis, even before the formation of plaque, making it the core process in the development of atherosclerosis[7]. Thus, demonstration of endothelial dysfunction could possibly lead to an early diagnosis of atherosclerosis[8]. Hence, biomarkers for endothelial function such as von Willebrand factor (vWF), tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), and thrombomodulin may prove to be useful as noninvasive markers to identify atherosclerosis[9]. Carotid intima media thickness (CIMT) is also a noninvasive method to detect and follow atherosclerosis. It is superior to other methods for the investigation of the coronary anatomy since it is noninvasive, devoid of side effects, and reproducible. It also enables evaluation of the arterial wall rather

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than the lumen and provides a useful tool for early detection of arterial plaques even in children[10,11]. It can show premature thickening of the arterial wall allowing identification of subclinical atherosclerosis, and thus gives the opportunity to take preventive measures before an actual cardiovascular event[12].

Hypertension, obesity, diabetes mellitus (DM), hyperlipidemia, smoking, sedentary life style, and family history of early-onset vascular disease are the major risk factors for cardiovascular disease[13]. Since obesity and hyperglycemia are two important risk factors for atherosclerosis, we hypothesized that they may induce subclinical atherosclerotic changes during childhood. Thus, in this study, we aimed to analyze CIMT and endothelial function markers as indicators of early atherosclerosis in children and adolescents with risk of cardiovascular disease (obesity with or without glucose intolerance and type 1 diabetes mellitus [T1DM]) in comparison to age- and sex-matched healthy controls. In addition to these classical risk factors, it has been shown that hyperhomocysteinemia is an independent risk factor for cardiovascular disease and atherosclerosis[14-17]. Supplementation with vitamin B12 and folic acid, which regulate homocysteine metabolism, may have a protective role against atherosclerosis by decreasing homocysteine levels[18]. Hence, we also measured homocysteine and its metabolic regulators in the study population to analyze the relationship between homocysteine and early atherosclerosis in childhood as determined by CIMT.

Material and Methods

Subjects

Children aged over 10 years, all pubertal, with known risk factors for atherosclerosis were included in the study. Four study groups were formed including: (1) T1DM, (2) obesity, (3) obesity with glucose intolerance (OwGI), and (4) control group. Each group comprised 20 individuals. Initially, OwGI patients (Group 3) were selected by a search of patient records among admissions over the last five years to reach and recall obese children with a diagnosis of GI. During their recall visit, they were reassessed, and those fulfilling the study criteria (children >10 years of age with a body mass index (BMI) >95th percentile for age and sex, and 2nd hour glucose between 140-200 mg/dl in oral glucose tolerance test [OGTT]) were recruited. The remaining groups were recruited consecutively among the admissions to the outpatient clinic of the Department of Pediatric Endocrinology. Both T1DM (Group 1) and obese (Group 2) patients were selected to match the initial recruits (Group 3) for age and sex. Patients with T1DM (Group 1) had a disease onset of at least six months before inclusion into the study. Obese patients (Group 2) had a BMI >95th percentile for age and sex with normal glucose tolerance on OGTT. Lastly, control subjects were recruited among age- and sex-matched healthy adolescents who were admitted to the outpatient clinic of the Adolescent Unit for normal follow-up or acute disorders. None of the subjects in the control group had chronic disease, especially celiac disease and/or malabsorption, and those on supplemental vitamins were excluded from the study. All subjects in the control group were questioned for smoking and family history of smoking, diabetes, obesity, hypertension, and coronary artery disease.

Patients with T1DM had a mean follow-up of 5.9±3.4 years (range: 1.5-12 years), and mean glycated hemoglobin (HbA1c) within the last year was 8.47% (±1.1). Mean glomerular filtration rate (GFR) was 138.9±62.2 ml/min/1.73 m^2 in the group with T1DM, and 5 patients had microalbuminuria. At the time of the investigations, all subjects were free of ketosis and hypoglycemia. Direct funduscopic examinations were performed in the DM group by an experienced pediatric ophthalmologist.

The study was approved by the local ethics committee. All parents and subjects received written information and provided their written informed consent.

Biochemical Assays

The following laboratory studies were performed in all subjects and controls: lipid profile, homocysteine, fibrinogen, vitamin B12, folic acid, tPA, PAI-1, vWF, and thrombomodulin. Venous blood samples were collected after an overnight fast in all subjects and before insulin administration in the subjects with diabetes. Plasma samples for tPA, PAI-1 and thrombomodulin were collected and frozen at

-80ºC. Twenty-four-hour urinary samples were collected from all patients with diabetes for the assessment of GFR and microalbuminuria.

Serum lipid profile was measured using MODULAR analytic system (Roche/Hitachi). Homocysteine was measured using fluorescence polarization immunoassay (FPIA) method.

Plasma vWF antigen level was measured using immuno-turbidimetric method (Diagnostica Stago, France), and intra- and interassay coefficients of variation (CV) were 2.7% and 4.5%, respectively. Plasma thrombomodulin was measured using a commercially available sandwich enzyme-linked immunosorbent assay (Diagnostica Stago, France), and intra- and interassay CV were 3.86% and 5.94%, respectively. tPA and PAI-1 were measured using ELISA (Asserachrom® and Diagnostica Stago, France, respectively). Intra- and interassay CV for tPA were 4.54 and 7.79, respectively, whereas those for PAI-1 were 6.53 and 8.69, respectively.

Measurement of CIMT

Carotid intima media thickness (CIMT) was measured using a real-time B-mode ultrasound imager. The same investigator performed all measurements of CIMT, in the anteroposterior projection with the patient lying supine after at least 30 minutes (min). The measurements were made within 1 cm above the bifurcation of the right common carotid artery. The CIMT of the artery was defined as the distance between two parallel echogenic (bright) lines of the vessel in the ultrasonography (USG) (Fig. 1).

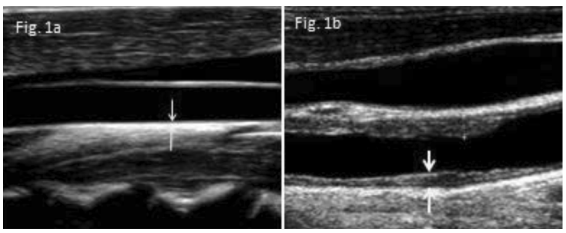

Fig. 1. Ultrasonographic examination of carotid intima media thickness (distance between arrows) at the wall of the common carotid artery is shown: a) normal CIMT b) increased CIMT.

Table I. Comparison of Demographic and Clinical Characteristics of the Subjects in the Four Groups (Type 1 Diabetes Mellitus, Obesity, Glucose Intolerance, Controls)

	Type 1 DM (n=20)	Obesity (n=20)	OwGI (n=20)	Controls (n=20)	p*
Sex (male) n (%)	9 (45)	10 (50)	9 (45)	10 (50)	1.000
Age (year)	14.8±1.5	14.9±1.6	15.0±1.6	14.9±1.6	0.989
Height (cm)	160.1±10.2	161.1±9.5	163.7±10.6	153.9±24.1	0.208
Weight (kg)	54.8±13.3	79.9±13.0	76.8±13.4	50.9±8.3	<0.001
BMI	21.0±2.5	30.5±2.9	28.7±4.6	20.0±1.3	<0.001
BMI z-score	0.58±0.67	2.61±0.37	2.26±0.53	0.24±0.29	<0.001
Tanner stage (median)	5	4	5	4.5	0.506
Systolic BP	110.2±10.2	117.5±14.6	116.0±15.5	103.0±22.9	0.028[a]
Diastolic BP	69.2±6.5	76.0±10.9	72.0±6.2	69.2±6.1	0.021[b]
Total cholesterol (mg/dl)	154.0±27.0	146.4±23.6	147.1±33.7	153.8±16.5	0.677
Triglyceride (mg/dl)	80.1±22.6	142.1±82.8	98.3±36.1	75.7±31.3	<0.001 [c]
HDL (mg/dl)	60.5±11.0	48.1±11.2	50.9±8.0	59.1±12.7	<0.001[d]
LDL (mg/dl)	77.2±23.9	69.5±19.1	76.3±30.9	74.1±19.6	0.744
VLDL (mg/dl)	17.8±5.2	28.4±16.6	19.6±7.1	15.1±6.3	<0.001[e]
LDL/HDL	1.2±0.5	1.5±0.6	1.5±0.6	1.4±0.5	0.252

BMI: Body mass index. BP: Blood pressure. DM: Diabetes mellitus. HDL: High-density lipoprotein. LDL: Low-density lipoprotein. OwGI: Obesity with glucose intolerance. VLDL: Very low-density lipoprotein. Values were given as mean ± standard deviation.

*p value between four groups

[a] group comparisons were not significant (p>0.01).

[b] group comparisons were not significant (p>0.01).

[c] p=0.0008 between groups 1 and 2; p= 0.0003 between groups 2 and 4.

[d] p=0.003 between groups 1 and 2; p= 0.011 between groups 2 and 4.

[e] p=0.002 between groups 1 and 2; p= 0.0003 between groups 2 and 4.

Table II. Mean Levels of CIMT, Homocysteine, Vitamin B12, Folic Acid, and Endothelial Function Markers of the Four Groups

	Type 1 DM (Mean±SD)	Obesity (Mean±SD)	OwGI (Mean±SD)	Control (Mean±SD)	p*
CIMT (mm)	0.39±0.05	0.44±0.08	0.42±0.07	0.40±0.05	0.136
Homocysteine (mg/dl)	8.4±2.2	13.2±5.5	12.2±7.7	10.5±3.5	0.004 [a]
Vitamin B12 (pg/ml)	435.0±223.6	298.9±126.0	334.4±138.3	341.0±158.9	0.070
Folic acid (ng/ml)	9.7±2.9	8.1±2.3	9.1±2.9	9.0±2.3	0.307
Fibrinogen (mg/dl)	289.7±50.3	317.5±47.9	330.1±53.8	291.4±43.8	0.025
vWF act. (%)	146.4±55.6	120.9±39.2	117.4±44.7	100.7±22.4	0.011 [b]
Thrombomodulin (ng/ml)	33.6±15.0	28.68±11.6	33.4±17.1	30.4±12.8	0.635
tPA (ng/ml)	17.9±6.8	20.3±7.3	19.8±5.7	18.2±5.9	0.588
PAI-1 (ng/ml)	143.6±82.8	238.5±105.5	224.8±96.5	181.8±91.6	0.009 [c]

CIMT: Carotid intima media thickness. DM: Diabetes mellitus. Mean±SD: Mean±standard deviation. OwGI: Obesity with glucose intolerance. PAI-1: Plasminogen activator inhibitor-1. tPA: Tissue plasminogen activator. vWF act: von Willebrand factor activity.
* p value between four groups.
[a]: p = 0.023 between groups 1 and 2.
[b]: p= 0.005 between groups 1 and 4.
[c]: p=0.011 between groups 1 and 2

The first line near the lumen is the intimal-luminal interface, and the second one close to the adventitia is produced by the collagen in the upper layer of the tunica adventitia. CIMT measurements were analyzed as quartiles. The lower quartile was between 0.31 and 0.36 mm, second quartile between 0.37 and 0.41 mm, third quartile between 0.42 and 0.44 mm, and upper quartile between 0.45 and 0.61 mm.

Statistics

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) (version 13) software. Differences between groups were assessed using one-way ANOVA for normally distributed data and Kruskal-Wallis for non-normally distributed data. Vitamin B12 and thrombomodulin levels showed lognormal distribution; thus, logarithmic transformation was carried out before the statistical analysis. Homocysteine, PAI-1 and TPA, on the other hand, were not normally distributed. Correlations were analyzed using the Pearson's correlation for normally distributed data and the Spearman's rank correlation for non-normally distributed data. Multiple linear regression analysis was undertaken to determine independent contributors to the CIMT such as BMI, BMI z-score, homocysteine, lipid levels, and high/low-density lipoprotein (HDL/LDL) ratio. Age, height and regulators of homocysteine were analyzed as covariates in the multiple regression analysis. Sex and pubertal stage were used in the analysis as factors that may affect CIMT. The relations of endothelial function markers with CIMT were also analyzed using multivariate linear regression. A value of $p<0.05$ was considered significant.

Results

Ages of the study subjects varied between 11.9 and 17.3 years, and the mean age was similar in all groups (Table I). Mean body weight, BMI and BMI z-scores were significantly higher in Groups 2 and 3 in comparison to Group 1 and the control group (Table I).

All subjects were pubertal - 4 were Tanner stage 2, 15 Tanner stage 3, 15 Tanner stage 4, and 46 Tanner stage 5. The distribution of the subjects in groups with respect to pubertal stage was tested using chi-square test. The groups were similar with respect to the pubertal status of the subjects included (p=0.671).

Mean CIMT did not show a statistically significant difference between groups (Table II, p=0.136); however, it was significantly higher in obese subjects (Groups 2 and 3, mean CIMT: 0.43±0.01 mm; 95% confidence interval [CI] 0.41-0.46 mm) in comparison to

Table III. Comparison of Homocysteine, Vitamin B12, Folic Acid and Endothelial Function Parameters in Subjects with CIMT in Upper and Lower Quartiles

	CIMT <25th percentile (n=21) (Mean±SD)	CIMT >75th percentile (n=17) (Mean±SD)	P
Homocysteine (mg/dl)	8.7±2.2	18.1±8.2	<0.001
Vitamin B12 (pg/ml)	362.0±196.2	267.6±107.8	0.173
Folic acid (ng/ml)	9.4±2.4	7.2±2.3	0.033
Fibrinogen (mg/dl)	315.8±52.7	309.8±50.1	0.983
vWF act. (%)	120.3±46.9	119.9±37.9	1.000
Thrombomodulin (ng/ml)	31.4±13.8	29.3±11.3	0.989
tPA (ng/ml)	19.1±6.7	19.9±7.7	0.977
PAI-1 (ng/ml)	233.6±86.2	236.8±99.6	1.000

CIMT: Carotid intima media thickness. Mean±SD: Mean±standard deviation. PAI-1: Plasminogen activator inhibitor-1. tPA: Tissue plasminogen activator. vWF act.: von Willebrand factor activity.

that of lean subjects in the study (Groups 1 and 4, mean CIMT: 0.40±0.01; 95% CI 0.38-0.41 mm; p=0.024). When study subjects were grouped into quartiles with respect to CIMT measurements, 15 subjects fell into the upper quartile and 21 were in the lower quartile. Nine patients (60%) from the obese group (Group 2) and 4 patients (26%) from OwGI group (Group 3) had CIMT measurements within the upper quartile. Only one child with T1DM and one from the control group were in the upper quartile for CIMT. Thus, obese subjects comprised 86% of the population with a CIMT in the upper quartile. This was significantly higher in comparison to the number of obese subjects (33.3%) in the lower quartile (p=0.03).

Among the markers for endothelial function, mean thrombomodulin and tPA levels did not show any statistically significant difference between groups (Table II). Plasma vWF antigen activity in Group 1 was significantly higher in comparison to the control group (p=0.005), but there was no significant difference between the other groups. Mean plasma PAI-1 levels in the obese groups were significantly higher in comparison to the group with T1DM (Table II, p=0.011).

Markers for endothelial function showed no significant difference between subjects with a CIMT measurement in the upper or lower quartile (Table III).

Comparison of homocysteine levels between groups revealed that obese subjects in Group 2 had a significantly higher mean homocysteine level than the group with T1DM (13.20±5.50 mg/dl vs 8.44±2.18 mg/dl, p=0.001, respectively, Table II). However, vitamin B12 and folic acid levels were statistically similar among groups (p=0.104 and p=0.307, respectively, Table II).

Comparison of subjects with CIMT in the upper quartile revealed a significantly higher mean plasma homocysteine level than that of subjects in the lower quartile (p=0.001, Table III). Subjects in the upper quartile also had a significantly lower mean plasma folic acid level in comparison to those in the lower quartile (p=0.033). There was a negative linear correlation between homocysteine and both folic acid (r= -0.444, p<0.001) and vitamin B12 levels (r= -0.197, p<0.001).

There was no difference in mean total cholesterol or LDL-cholesterol between groups. However, mean HDL-cholesterol level of obese subjects was lower than that of subjects with T1DM (60.5±11.0) and controls (59.1±12.7) (p<0.001). Furthermore, mean very low-density lipoprotein (VLDL) and triglyceride levels of subjects in Group 2 were higher than those of the other groups (Table I). Lipid profiles of subjects in the upper and lower quartiles for CIMT did not show any significant difference.

In the group with T1DM, mean CIMT of the 5 subjects with microalbuminuria was significantly higher than in the remaining subjects of the group (0.43±0.06 and 0.37±0.05, respectively, p=0.039). Also, median

Table IV. Univariate and Multivariate Correlation Analysis of Possible Risk Factors for CIMT

Independents	CIMT (Dependent variable)					
	Univariate			Multivariate		
	B	95% CI	P	B	95% CI	P
Age	0.009	(-0.001)-(0.019)	0.085			
Sex	-0.030	(-0.061)-(0.002)	0.065			
Height	0.001	(0.001)-(0.002)	0.125			
BMI	0.001	(0.001)-(0.005)	0.094			
Tanner	0.009	(-0.012)-(0.022)	0.579			
Homocysteine	0.008	(0.006)-(0.010)	<0.001	0.008	(0.006)-(0.010)	<0.001
Folic acid	-0.007	(-0.013)-(-0.02)	0.013			
Vitamin B12	-0.001	(0.001)-(0.002)	0.056			
tPA	0.001	(-0.001)-(0.004)	0.392			
PAI	0.001	(0.001)-(0.002)	0.452			
Thrombomodulin	0.001	(-0.001)-(0.001)	0.659			
vWF act.	0.001	(0.001)-(0.002)	0.702			
Cholesterol	0.001	(-0.001)-(0.001)	0.432			
TG	0.001	(-0.001)-(0.001)	0.974			
HDL	0.001	(-0.001)-(0.001)	0.785			

BMI: Body mass index. CI: Confidence interval. CIMT: Carotid intima media thickness. HDL: High-density lipoprotein. TG: Triglyceride. PAI-1: Plasminogen activator inhibitor-1. tPA: Tissue plasminogen activator. vWF act.: von Willebrand factor activity.

homocysteine level was significantly higher in microalbuminuric subjects in comparison to normoalbuminuric diabetics (9.90±2.17 and 7.95±2.01, respectively, p=0.049).

In multiple regression analysis, homocysteine was the sole independent variable related to CIMT ($\acute{\eta}^2$ =0.557, p<0.0001), and thus responsible for 56% of the variability in CIMT (Table IV). Among the endothelial markers, none showed a relation with CIMT, although the relation between PAI-1 and CIMT approached significance ($\acute{\eta}^2$=0.12, p=0.057). The variability explained by PAI would be 12%.

Discussion

In the current study, we aimed to analyze whether obesity and hyperglycemia, alone or in combination, have a role in the development of atherosclerosis during childhood. The principle marker for atherosclerosis was CIMT. We did not observe a statistically significant difference in CIMT when three study groups were compared to healthy controls; however, obese children had a significantly higher CIMT than the lean diabetics and healthy controls, and 86% of the children in the upper quartile for CIMT were obese. Moreover, mean serum level of homocysteine was higher in subjects from the upper quartile of CIMT in comparison to those from the other three quartiles, and multiple regression analysis revealed a strong relationship between homocysteine levels and CIMT, verifying the role of homocysteine in the development of early atherosclerosis. These findings suggest that obesity and homocysteine seem to play a role in the early development of atherosclerosis during childhood. Higher CIMT in obese children in comparison to controls as well as a positive association between CIMT and BMI during childhood was also reported by other researchers previously[19,20].

Our findings raise the question of whether there is a link between BMI and homocysteine levels. Although homocysteine levels and CIMT were correlated in the current study, and most of the patients in the upper quartile for CIMT were obese, suggesting a possible link between obesity and elevated homocysteine levels, no correlation was observed between serum homocysteine levels and BMI. Moreover, the

strong relation between homocysteine and CIMT in multiple regression suggests that increase in CIMT induced by homocysteine may be an independent one. Other studies analyzing the link between obesity and homocysteine levels show conflicting results. Some researchers reported increased homocysteine levels in obese subjects,[21, 22] whereas others did not[23]. Increased homocysteine levels may be one of the factors responsible for accelerated atherosclerosis in obese children; however, more comprehensive studies are necessary to analyze if there is an unprecedented link between obesity and homocysteine levels.

One important and expected finding is the negative correlation between homocysteine and both folic acid and vitamin B12 levels. Folic acid levels of subjects in the upper quartile for CIMT were significantly lower than that of subjects in the remaining three quartiles, suggesting that folic acid may have an indirect effect on CIMT by modifying plasma homocysteine levels. This was put to question by Till et al.[18], who observed a decrease in CIMT and homocysteine levels in patients supplemented with folic acid and vitamin B12, supporting the hypothesis that folic acid and vitamin B12 supplementation may have a protective effect against atherosclerosis by decreasing homocysteine levels.

All in all, the above findings suggest that CIMT may indeed be a valuable tool in the assessment of atherosclerosis during childhood, and obesity as well as hyperhomocysteinemia may be risk factors for the early development of atherosclerosis. Considering the ever increasing trend of childhood obesity all over the world in the last decades, these findings may have serious implications.

Interestingly, subjects with T1DM did not show a tendency to increased CIMT, suggesting obesity may be more important as a risk factor for early atherosclerosis than hyperglycemia. Previous data is conflicting in that respect as well. Some researchers reported higher CIMT in children with T1DM in comparison to controls,[24, 25] whereas others failed to show such a link[26,27]. In the current study, among the diabetics, CIMT was increased in children with microalbuminuria in comparison to those without microalbuminuria. Further, mean homocysteine level of microalbuminuric patients was elevated in comparison to those with normoalbuminuria. Another childhood study analyzing the relationship between microvascular complications and CIMT similarly showed increased CIMT in patients with microvascular complications in comparison to those without[28]. These findings suggest that early atherosclerosis in T1DM may more likely parallel the development of microvascular complications.

The primary process in the development of atherosclerosis is endothelial dysfunction. Thus, markers for endothelial function may reveal an alteration towards endothelial dysfunction in subjects prone to develop atherosclerosis. In order to analyze whether there is endothelial dysfunction in the study groups, we measured markers for endothelial function and compared them with controls. tPA, which is an activator of fibrinolysis, and thrombomodulin secreted from endothelia, which plays a part in coagulation, were similar in all the study groups when compared to the healthy controls. On the other hand, mean plasma vWF level was elevated in the subjects with T1DM in comparison to groups with obesity and healthy controls, suggesting endothelial damage in diabetics. Plasma vWF has been suggested to be an early indicator of endothelial damage in patients with T1DM in previous studies. Verrotti et al.[29] studied 102 children with T1DM for a minimum of eight years in a prospective case-control study, and showed that T1DM patients who developed microalbuminuria had significantly higher levels of vWF compared to their normoalbuminuric counterparts. In a similar study, Targher et al.[30] reported higher plasma vWF levels in young patients with T1DM in comparison to healthy controls. Increased vWF in diabetic subjects in the current study in the absence of similar findings in other endothelial markers as well as CIMT suggests that vWF may be an early marker for endothelial damage in subjects with a short duration of diabetes, good metabolic control, and without complications. Further studies in long-standing T1DM, including patients with a wider spectrum of metabolic control (both good and poor control patients) as well as patients with micro- and macrovascular complications are required to test this hypothesis.

Obese patients including those with glucose intolerance (Groups 2 and 3) had higher PAI-1

levels in comparison to subjects with T1DM (Group 1) in the current study. This may be caused by increased PAI-1 secretion from the adipose tissue in obese subjects, which may disrupt the balance of fibrin degradation by promoting anti-fibrinolytic activity. In other words, it may be speculated that the risk of thrombosis may be higher in obese patients in comparison to children with T1DM.

In conclusion, among the known cardiovascular risk factors, obesity may be a stronger correlate of early atherosclerosis than T1DM in childhood. CIMT is a helpful tool in the early detection and long-term follow-up of childhood atherosclerosis in obese children. It may also prove to be useful as a marker for early atherosclerosis in patients with T1DM who have microvascular complications. Moreover, the positive correlation between CIMT and homocysteine levels suggests that plasma homocysteine level plays an important role in the development of early atherosclerosis in children. In addition, the negative correlation between homocysteine and its natural metabolic regulators vitamin B12 and folic acid suggests that hyperhomocysteinemia and resultant atherosclerosis may be controlled by supplementation with vitamin B12 and folic acid. The link between homocysteine and obesity in the development of atherosclerosis, if any, remains to be established. Finally, more comprehensive studies are required to help establish the link between atherosclerosis and obesity during childhood.

REFERENCES

1. Fuster V. Epidemic of cardiovascular disease and stroke: the three main challenges. Presented at the 71st scientific sessions of the American Heart Association. Dallas, Texas. Circulation 1999; 99: 1132-1137.
2. McNamara JJ, Molot MA, Stremple JF, Cutting RT. Coronary artery disease in combat casualties in Vietnam. JAMA 1971; 216: 1185-1187.
3. Napoli C, Glass CK, Witztum JL, et al. Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) study. Lancet 1999; 354: 1234-1241.
4. Stary HC. Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. Arteriosclerosis 1989; 9: 119-132.
5. Bellamy MF, McDowell IF. Putative mechanisms for vascular damage by homocysteine. J Inherit Metab Dis 1997; 20: 307-315.
6. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci (Lond) 2005; 109: 143-159.
7. Quyyumi AA. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. Am J Med 1998; 105: 32S-39S.
8. Kinlay S, Ganz P. Relation between endothelial dysfunction and the acute coronary syndrome: implications for therapy. Am J Cardiol 2000; 86: 10J-13J; discussion 13J-14J.
9. Wu KK, Thiagarajan P. Role of endothelium in thrombosis and hemostasis. Annu Rev Med 1996; 47: 315-331.
10. Kastelein JJ, de Groot E. Ultrasound imaging techniques for the evaluation of cardiovascular therapies. Eur Heart J 2008; 29: 849-858.
11. Little WC, Constantinescu M, Applegate RJ, et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? Circulation 1988; 78: 1157-1166.
12. Cobble M, Bale B. Carotid intima-media thickness: knowledge and application to everyday practice. Postgrad Med 2010; 122: 10-18.
13. Berenson GS, Srinivasan SR, Bao W, et al. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998; 338: 1650-1656.
14. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 2002; 288: 2015-2022.
15. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002; 325: 1202.
16. Den Heijer M, Lewington S, Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. J Thromb Haemost 2005; 3: 292-299.
17. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969; 56: 111-128.
18. Till U, Rohl P, Jentsch A, et al. Decrease of carotid intima-media thickness in patients at risk to cerebral ischemia after supplementation with folic acid, vitamins B6 and B12. Atherosclerosis 2005; 181: 131-135.
19. Giannini C, de Giorgis T, Scarinci A, et al. Increased carotid intima-media thickness in pre-pubertal children with constitutional leanness and severe obesity: the speculative role of insulin sensitivity, oxidant status, and chronic inflammation. Eur J Endocrinol 2009; 161: 73-80.
20. Schiel R, Beltschikow W, Radon S, et al. Increased carotid intima-media thickness and associations with cardiovascular risk factors in obese and overweight children and adolescents. Eur J Med Res 2007; 12: 503-508.
21. Zhu W, Huang X, Li M, Neubauer H. Elevated plasma homocysteine in obese schoolchildren with early atherosclerosis. Eur J Pediatr 2006; 165: 326-331.

22. Huang XM, Zhang YY, Yu ZS, et al. [Early arterial atherosclerosis and level of plasma homocysteine in simply obese children]. Zhonghua Er Ke Za Zhi 2005; 43: 192-195.

23. Uysal O, Arikan E, Cakir B. Plasma total homocysteine level and its association with carotid intima-media thickness in obesity. J Endocrinol Invest 2005; 28: 928-934.

24. Yamasaki Y, Kawamori R, Matsushima H, et al. Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high-resolution B-mode imaging. Diabetes 1994; 43: 634-639.

25. Atabek ME, Kurtoglu S, Pirgon O, Baykara M. Arterial wall thickening and stiffening in children and adolescents with type 1 diabetes. Diabetes Res Clin Pract 2006; 74: 33-40.

26. Gunczler P, Lanes R, Lopez E, et al. Cardiac mass and function, carotid artery intima-media thickness and lipoprotein (a) levels in children and adolescents with type 1 diabetes mellitus of short duration. J Pediatr Endocrinol Metab 2002; 15: 181-186.

27. Yavuz T, Akcay A, Omeroglu RE, Bundak R, Sukur M. Ultrasonic evaluation of early atherosclerosis in children and adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2002; 15: 1131-1136.

28. Krantz JS, Mack WJ, Hodis HN, et al. Early onset of subclinical atherosclerosis in young persons with type 1 diabetes. J Pediatr 2004; 145: 452-457.

29. Verrotti A, Greco R, Basciani F, Morgese G, Chiarelli F. von Willebrand factor and its propeptide in children with diabetes. Relation between endothelial dysfunction and microalbuminuria. Pediatr Res 2003; 53: 382-386.

30. Targher G, Bertolini L, Zoppini G, Zenari L, Falezza G. Increased plasma markers of inflammation and endothelial dysfunction and their association with microvascular complications in type 1 diabetic patients without clinically manifest macroangiopathy. Diabet Med 2005; 22: 999-1004.