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Association of Matrix Metalloproteinases-2, Metalloproteinases-9, and Endothelial Dysfunction with Carotid Intima-Media Thickness and Left Ventricular Mass Index in Children with End-Stage Renal Disease

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Abstract

Background and Aim: Cardiovascular disease (CVD) has been recognized as a major cause of death among children with end-stage renal disease (ESRD). Matrix metalloproteinases (MMP-2 and MMP-9) and endothelial dysfunction play a significant role in the development of CVD in these children. This study was conducted to assess the association of MMP-2 and 9 and endothelial dysfunction markers (sE-selectin and brachial flow mediated dilatation (FMD)) with carotid intima media thickness (c-IMT) and left ventricular mass index (LVMI).

Methods: Thirty-one pediatric ESRD patients and 18 healthy controls were included in this research. The case and control groups were matched in terms of sex and age. Serum levels of MMP-2, MMP-9, sE-selectin, and other biochemical parameters were measured. Brachial FMD, c-IMT and LVMI were also measured in the two groups.

Results: C-IMT had a positive correlation with diastolic blood pressure, MMP-2, MMP-9, low-density lipoprotein (LDL), triglyceride, phosphorus, PTH, and calcium × phosphorus (Ca × P) product, and a negative correlation with FMD, high-density lipoprotein (HDL), and calcium. LVMI demonstrated correlations with systolic blood pressure, MMP-2, FMD, cholesterol, triglyceride, HDL, phosphorus, PTH, and Ca × P product. The area under the curve (AUC) in ROC curve analysis was used to determine the abnormal c-IMT and LVH values for MMP-2, which was 0.76 and 0.71, respectively ($p<0.05$).

Conclusion: C-IMT and LVMI are two major CVD markers in pediatric ESRD patients. These two markers correlated with MMP-2, MMP-9 and endothelial dysfunction markers. According to this study, MMP-2 may determine abnormal c-IMT and LVH in ESRD children.

Keywords: End-stage renal disease; Cardiovascular disease; Left ventricular hypertrophy; Atherosclerosis; Matrix metalloproteinases.

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Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in children with end-stage renal disease (ESRD) (1). One of the common CVDs in this population is atherosclerosis, which is defined as arterial wall thickening (2).

Carotid intima-media thickness (c-IMT) is a useful indicator of atherosclerosis in these patients (3). Another cardiovascular problem in children with ESRD is left ventricular hypertrophy (LVH), which can be measured using the left ventricular mass

index (LVMI) on echocardiography (4). Traditional and uremia-related risk factors cannot completely explain the high prevalence of CVD in patients with ESRD; therefore, discovering new biomarkers and risk factors for CVD in this population is warranted (2, 5). Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that play a role in degrading extracellular matrix (6). Matrix metalloproteinases-2 and 9 (MMP-2, 9), which belong to the gelatinase family, can cleave gelatin, elastin, collagens type I, II, and IV (6). The contribution of MMP-2, 9 has been studied in many CVD and kidney diseases (7-9). Therefore, these two MMPs might make a connection between heart and kidney pathologies and their study can help clarify pathophysiological aspects of the simultaneous disease's occurrence in both systems. Endothelial dysfunction (ED) plays an important role in the pathogenesis of CVD in children with renal failure by contributing to atherosclerosis and arteriosclerosis in these patients (10).

In fact, ED could connect both chronic kidney disease (CKD) and CVD and probably clarify the high frequency of CVD in patients who suffer from renal failure (11). Several methods could help evaluate endothelial dysfunction, including flow-mediated dilatation (FMD) and measurement of blood biomarkers (12). Brachial artery FMD is a non-invasive test to measure brachial artery dilatation after relative ischemia; it represents the amount of nitric oxide released from the endothelium, which is one of the major products of the endothelium (13). Another indicator of endothelial dysfunction is soluble E-selectin (sE-selectin) that is expressed in the endothelium, especially in inflammation, and is classified as one of the non-traditional CVD risk factors in patients with ESRD (14, 15).

So far, most of the studies investigating the role of MMP-2, 9 and endothelial dysfunction in development of CVD are limited to adults (16-20). In a few studies of the association of MMPs with CVD conducted in children, the main focus was on interrelationships between MMPs and known CVD risk factors (21-25). On the other hand, some confounding variables such as obesity, smoking, and diabetes mellitus, which can affect the levels of both MMPs (26-28) and endothelial dysfunction markers (29) and also interfere with CVD (30), are less common in children. Therefore, evaluating the association of the above variables with CVD in children could elucidate the role of these markers in CVD more clearly. The present study was a supplement to our previous work, which evaluated the correlation of MMPs and endothelial dysfunction markers with several CVD risk factors in children with ESRD (21).

This study was conducted to evaluate the association of MMP-2, MMP-9 and endothelial dysfunction markers (sE-selectin and brachial FMD) with two indicators of CVD (c-IMT and LVMI) in children with ESRD.

Methods

Study Population

This analytical cross-sectional study was performed in children with ESRD who presented to Al Zahra and Imam-Hossein Hospitals, Isfahan, Iran. These hospitals are the main tertiary care centers for children with ESRD in Isfahan Province and are affiliated to Isfahan University of Medical Sciences. The subjects who met the following criteria were included in the study:

(i) age below 20 years; (ii) receiving hemodialysis or peritoneal dialysis treatment for at least 6 months, and (iii) complete and clear medical documents.

The subjects who had the following conditions were excluded from the study to minimize the probable confounding variables:

(i) any rheumatologic diseases including systemic lupus erythematous, Wegener's granulomatosis, and other vasculitis, (ii) congenital heart diseases, (iii) signs of systemic infection, (iv) diabetes mellitus, and (v) a history of surgery within one month before study.

Control subjects were selected from healthy children who were visited for routine examination by the hospital staffs. After enrolling the eligible subjects, the objectives and the protocol of the study were completely explained and written informed consent was obtained from their parents/caregivers. The Ethics Committee of Isfahan University of Medical Sciences approved the study protocol. The study adhered to the tents of the Helsinki Declaration.

The study protocol and clinical evaluation

Overall, 31 consecutive patients were included in the study. All the available patients in the above medical centers were enrolled in the study according to the inclusion and exclusion criteria. Twenty-six patients (84 %) were on hemodialysis and 5 patients (16 %) were on peritoneal dialysis. In

the first step of the study, demographic characteristics and clinical data of the eligible subjects, including age, gender, weight (kg), height (cm), body mass index (BMI; kg/m^2), and blood pressure (mmHg), were collected. In addition, the Z scores of the blood pressure and BMI were determined for every subject. Then, c-IMT, LVMI, and brachial artery FMD were measured by an expert pediatric cardiologist. In the next step, fasting venous blood samples were obtained from patients and controls. The blood samples were analyzed for MMP-2, MMP-9, sE-selectin, and other biochemical parameters.

Blood sampling and laboratory evaluation

Venous blood samples were collected from all participants after a 12-hour overnight fast. Immediately after blood collection, the samples were centrifuged at 3000 rpm for 20 minutes. After serum separation, the samples were aliquoted and stored at -80ºC until measurements. The serum levels of MMP-2, MMP-9, and sE-selectin were measured by commercial enzyme-linked immunosorbent assay kits (Abingdon, R&D system, UK). The parathyroid hormone (PTH) level was measured with the Cobas e411 auto analyzer (Roche Diagnostics International Ltd, Basel, Switzerland) using the electrochemiluminescence method. Common standard laboratory methods were used to measure other biochemical variables.

FMD measurement

FMD was measured according to the guidelines presented by the International Brachial Artery Reactivity Task Force (31). All measurements were done in the morning after 8 hours of fasting in a temperature-controlled room at 23ºC to 25ºC. The participants were required not to use caffeine-containing drinks and exercise for at least 12 hours before the procedure. The measurements were done in the supine position with the forearm positioned in a semi-open splint. A high-frequency (7 MHz) vascular transducer (EKO 7 by Samsung Medison Company) was stabilized with a stereotactic probe-holding instrument. To produce a flow stimulus by reactive hyperemia, a pediatric blood pressure cuff was fixed on the participant's wrist and then an image of the radial artery was obtained in a longitudinal plane 5 cm distal from the antecubital fossa. After collecting a baseline image, the Doppler signal based on time-averaging the mid artery sample volume was used to estimate the blood flow velocity. After cuff deflation, the images of the radial artery and Doppler signal were recorded alternatively at 5 minutes and 20 seconds intervals. Radial artery distance measurements were done at maximum systolic extension. Finally, the saved images in the system were analyzed.

C-IMT measurement

C-IMT was measured using a high frequency (7.5 MHz) grayscale vascular linear transducer. The participants were examined in the supine position with the head turned 45º away from the scanner. The c-IMT was measured at two points on the right and left side including 20 mm distal to the common carotid artery (CCA) and 20 mm proximal to the internal carotid artery (ICA). The measurements of these two points were performed at 2 mm intervals at the near and far wall and the max and mean values of c-IMT were calculated. The mean values derived from CCA and ICA on both sides were determined as c-IMT. The normal upper limit of c-IMT was considered 0.416 mm, which was previously used as a normal value in Iranian children (32).

LVMI measurement

Echocardiography was done using the Medison EKO-7 (Samsung, Seoul, South Korea) with 2-4 MHz probes. Echocardiographic measurements were done over at least three cardiac cycles according to the American Society of Echocardiography. M-mode echocardiography was performed in the parasternal long axis view for measuring LVMI. For this purpose, left ventricular measurements were performed at the tips of the mitral valve leaflets. Left ventricular mass (LVM) was calculated using the following equation (33):

$$LVM\ (g) = 1.04 \times ((LVDd + IVST + PWT)^3 - (LVDd)^3) - 13.6$$

Where LVDd is the left ventricular end-diastolic dimension, IVST is the interventricular septal thickness, and PWT is the posterior wall thickness. For calculating LVMI, the measured LVM was divided by $height^{2.7}$ in meters. This calculation was performed to minimize the effect of gender, race, age, and obesity on LVM and standardize the values. LVH was defined as LVMI value greater than 38 $g/m^{2.7}$; this value is above the 95^{th} percentile of male and female healthy children (34).

Statistical analysis

Continuous and categorical variables are expressed as the mean ± SD and percentage, respectively. To compare the mean values of variables between the two groups, independent sample *t*-test, and Mann-Whitney *U* test were used when appropriate. Chi-

square or Fisher's exact test were used to compare the frequencies of variables between the two groups. Correlation analysis was done to determine any associations between variables using Spearman's or Pearson's correlation coefficient as appropriate. Multiple linear regression analysis was performed to determine the independent predictors of c-IMT and LVMI. Receiver operating characteristic (ROC) curve analysis was performed to assess the performance of serum levels of MMP-2, MMP-9, and sE-selectin as diagnostic tests for abnormal c-IMT and LVH. Statistical analysis was performed using SPSS version 22.0 (SPSS Inc; Chicago, IL, USA) and MedCalc 15.8 software (*MedCalc*, Belgium). *P* values less than 0.05 were considered significant.

Results

Participants

The mean age of the patients and controls were 13.93 ± 4.81 (range: 7-19.5) and 13.08 ± 4.36 (range: 6.5-19.5) years, respectively. The demographic characteristics of the study participants are shown in Table 1.

There were no significant differences in age and sex between patients and control.

Table 1. Baseline characteristics of patients and controls

Variables	Patients (n = 31)	95% CI	Controls (n = 18)	95% CI	p-value
Gender (female/male)	19/12		10/8		0.92
Age (years)	13.93±4.81	12.16-15.7	13.08±4.36	10.91-15.25	0.54
Weight (Kg)	31.37±11.16	27.28-35.46	46.40±16.52	38.19-54.62	0.0001
Height (cm)	131.38±20.82	123.74-139.02	146.27±15.04	138.79-153.75	0.01
BMI (Kg/m^2)	17.81±3.62	16.48-19.14	21.07±5.01	18.58-23.56	0.01
BMI (Z score)	-0.88±1.61	-1.47- -0.28	0.44±1.24	-0.17-1.06	0.004
SBP (mmHg)	120.70±20.67	113.12-128.29	101.11±19.52	91.4-110.81	0.02
SBP (Z score)	1.06±1.24	0.61-1.52	-0.29±1.31	-0.94-0.36	0.001
DBP (mmHg)	72.48±20.61	64.92-80.04	58.05±10.02	53.07-63.03	0.008
DBP (Z score)	0.70±1.36	0.2-1.2	-0.34±0.92	-0.81-0.11	0.006
Duration of hemodialysis (months)	67.64±39.87	53.01–82.27			
ESRD etiologies Kidney cystic disease, n (%)	13 (42 %)				
Focal and segmental glomerulosclerosis, n (%)	9 (29 %)				
Tubulointerstitial disease, n (%)	4 (13 %)				
Chronic glomerulonephritis, n (%)	3 (9.5 %)				
Congenital anomaly of kidney and urinary tract, n (%)	2 (6.5 %)				

BMI: body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure; CI; Confidence interval.

Biochemical parameters, endothelial dysfunctio n markers, and CVD indicators

Table 2 summarizes the measured variables including MMP-2, MMP-9, endothelial dysfunction markers, CVD indicators including c-IMT and LVMI, and other biochemical parameters in the study subjects.

According to Table 2, the mean levels of serum MMP-2, MMP-9 and sE-selectin were significantly higher in patients compared to controls. In addition, brachial FMD was significantly lower in children with ESRD versus healthy subjects.

The mean values of c-IMT and LVMI were significantly higher in patients versus controls.

Table 2. Biochemical, cardiovascular and endothelial function characteristics of patients and controls

Variables	Patients (n = 31)	95% CI	Controls (n = 18)	95% CI	p-value
MMP-2 (ng/mL)	166.25±26.88	156.39-176.11	126.66±31.43	111.03-142.29	0.0001
MMP-9 (ng/mL)	347.41±104.01	309.26-385.57	277.77±168.68	193.89-361.66	0.01
sE-selectin (ng/mL)	60.58±18.04	53.96-67.19	48.72±20.01	38.76-58.67	0.01
FMD (%)	4.62±4.16	3.1-6.15	6.59±2.29	5.45-7.73	0.005
Hemoglobin (g/dL)	9.8±0.91	9.47-10.14	12.68±0.78	12.29-13.08	0.0001
BUN (mg/dL)	54.90±12.78	50.21-59.59	12.22±2.18	11.13-13.3	0.0001
Creatinine (mg/dL)	6.66±0.93	6.32-7.01	0.63±0.18	0.53-0.72	0.0001
Triglyceride (mg/dL)	162.45±128.12	115.45-209.44	62.55±17.06	54.07-71.03	0.0001
Cholesterol (mg/dL)	170.32±38.14	156.33-184.31	162.05±20.13	152.04-172.06	0.16
LDL (mg/dL)	95.80±26.59	86.05-105.56	84.05±2.07	83.02-85.08	0.0001
HDL (mg/dL)	42.38±8.89	39.12-45.64	49.66±3.02	48.16-51.17	0.0001
P (mg/dL)	5.67±1.58	5.08-6.25	2.70±0.25	2.57-2.83	0.0001
Ca (mg/dL)	8.26±1.27	7.79-8.73	8.88±0.33	8.71-9.04	0.01
P × Ca (mg^2/dL^2)	46.80±15.36	41.16-52.43	24.03±2.42	22.82-25.24	0.0001
PTH (pg/mL)	391.13±242.65	302.12-480.14	45.77±10.55	40.52-51.02	0.0001
Albumin (g/dL)	3.93±0.76	3.65-4.21	4.43±0.65	4.21-4.86	0.02
Homocysteine (µmol/L)	11.54±4.52	9.88 – 13.2	6.96±1.65	6.14-7.78	0.0001
c-IMT (mm)	0.47±0.08	0.44-0.5	0.38±0.05	0.35-0.4	0.0001
LVMI ($g/m^{2.7}$)	51.39±35.86	38.24-64.55	19.16±7.09	15.63-22.69	0.0005
Abnormal c-IMT, n (%)	25 (80.6)		0		0.0001
LVH, n (%)	17 (54.8)		0		0.0001

MMP-2: Matrix metalloproteinase-2, MMP-9: Matrix metalloproteinase-9, sE-selectin: Soluble E-selectin, FMD: Flow mediated dilatation, BUN: Blood urea nitrogen, LDL: Low density lipoprotein, HDL: High density lipoprotein, P: Phosphorus, Ca: Calcium, PTH: Parathyroid hormone; C-IMT: Carotid intima media thickness; LVMI: Left ventricular mass index.

Correlation analysis

In correlation analysis, c-IMT had a direct correlation with MMP-2, MMP-9, diastolic blood pressure (DBP), triglyceride, low-density lipoprotein (LDL), phosphorous (P), P × Ca product, and PTH and an indirect correlation with FMD, hemoglobin, high-density lipoprotein (HDL), and calcium.

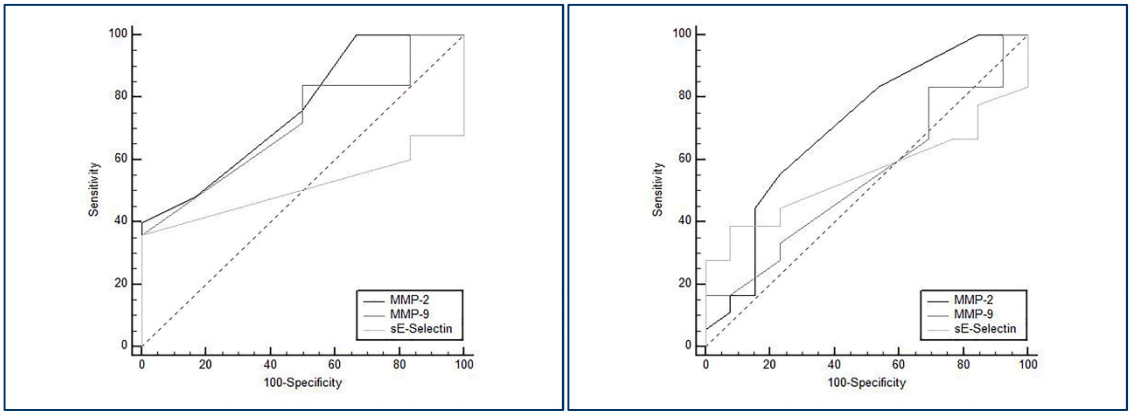
LVMI had a positive correlation with MMP-2, systolic blood pressure (SBP) and its Z scores, DBP, triglyceride, P, P × Ca product, and PTH and a negative correlation with FMD, hemoglobin, and HDL. Table 3 shows the results of correlation analysis between measured variables and c-IMT and LVMI in details.

Multiple linear regression analyses

The statistically significant variables in univariate analysis were included in multiple regression analysis to determine the independent predictors of c-IMT and LVMI (Table 4). According to this analysis, triglyceride, calcium, and P × Ca product were independent predictors of c-IMT. Furthermore, multiple regression analysis showed that HDL was an independent predictor of LVMI.

ROC curve analysis

Serum levels of MMP-2 and MMP-9 were of acceptable accuracy for diagnosis of abnormal c-IMT with AUC values of 0.76 ($p<0.05$) and 0.72 ($p<0.05$), respectively. In addition, MMP-2 had an acceptable diagnostic value for LVH (AUC = 0.71, $p<0.05$). AUC values for MMP-9 for detecting LVH did not reach a significant threshold (AUC = 0.54, $p>0.05$). In addition, this analysis regarding serum sE-selectin was not statistically significant in determining abnormal c-IMT and LVH values. Figure 1 shows the ROC curves of measured variables for diagnosing abnormal c-IMT and LVH values in patients.



1A 1B

Figure 1. Receiver operating characteristic (ROC) curve analysis of MMP-2, MMP-9, and sE-selectin for predicting abnormal c-IMT (A) and LVH (B) values in children with ESRD.

Table 3. Correlation of c-IMT and LVMI with the measured variables in children with ESRD

Variables	c-IMT (mm)		LVMI ($g/m^{2.7}$)	
	r	p-value	r	p-value
Age (year)	-0.29	0.1	-0.45	0.01
Duration of dialysis (months)	-0.15	0.39	-0.06	0.73
BMI (Kg/m^2)	0.01	0.93	0.09	0.61
BMI (Z score)	0.29	0.1	0.3	0.1
SBP (mmHg)	0.23	0.19	0.43	0.01
SBP (Z score)	0.06	0.73	0.36	0.04
DBP (mmHg)	0.42	0.01	0.43	0.01
DBP (Z score)	0.19	0.28	0.07	0.67
MMP-2 (ng/mL)	0.6	0.0001	0.45	0.009
MMP-9 (ng/mL)	0.59	0.0001	0.16	0.36
sE-selectin (ng/mL)	0.22	0.22	0.02	0.91
FMD (%)	-0.36	0.04	-0.36	0.04
Hemoglobin	-0.36	0.04	-0.63	0.0001
BUN (mg/dL)	0.12	0.51	0.11	0.54
Creatinine (mg/dL)	-0.003	0.98	0.06	0.71
Triglyceride (mg/dL)	0.39	0.02	0.68	0.0001
Cholesterol (mg/dL)	-0.27	0.13	-0.18	0.32
LDL (mg/dL)	0.47	0.007	0.29	0.1
HDL (mg/dL)	-0.39	0.03	-0.46	0.008
P (mg/dL)	0.58	0.001	0.43	0.01
Ca (mg/dL)	-0.37	0.03	0.09	0.62
P × Ca (mg^2/dL^2)	0.59	0.0001	0.5	0.004
PTH (pg/mL)	0.4	0.02	0.54	0.001
Homocysteine (µmol/L)	0.15	0.41	0.13	0.48
Albumin (g/dL)	-0.007	0.91	0.03	0.85

MMP-2: Matrix metalloproteinase-2, MMP-9: Matrix metalloproteinase-9, BMI: body mass index, SBP: Systolic blood pressure, DBP: Diastolic Blood Pressure, sE-selectin: Soluble E-selectin, FMD: Flow mediated dilatation, BUN: Blood urea nitrogen, LDL: Low density lipoprotein, HDL: High density lipoprotein, P: Phosphorus, Ca: Calcium, PTH: Parathyroid hormone; C-IMT: Carotid intima media thickness; LVMI: Left ventricular mass index.

Table 4. Statistically significant predictors of c-IMT and LVMI using multivariate linear regression analysis in children with ESRD.

Variables	Predictor	B coefficient	Standard error	p-value
c-IMT (mm)	Triglyceride (mg/dL)	0.4	0.0001	0.03
c-IMT (mm)	Ca (mg/dL)	-1.21	0.02	0.007
c-IMT (mm)	P × Ca (mg^2/dL^2)	1.92	0.004	0.01
LVMI ($g/m^{2.7}$)	HDL (mg/dL)	-0.43	0.91	0.04

C-IMT: Carotid intima media thickness; LVMI: Left ventricular mass index; FMD: Flow mediated dilatation; LDL: Low density lipoprotein, HDL: High density lipoprotein; DBP: Diastolic Blood Pressure; P: Phosphorous; Ca: Calcium.

Table 5. ROC curve analysis of MMP-2, MMP-9 and sE-selectin for predicting abnormal c-IMT and LVH values in children with ESRD

Predicting abnormal c-IMT									
Variables	**AUC**	**95 % CI**	**Cut-off values**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**LR+**	**LR-**
MMP-2 (ng/mL)	0.76¶	0.6-0.86	≥168	40 %	100 %	100 %	28.6 %	∞	0.6
MMP-9 (ng/mL)	0.72¶	0.56-0.83	≥350	36 %	100 %	100 %	27.3 %	∞	0.64
sE-selectin (ng/mL)	0.51*	0.32-0.69	≤53	36 %	100 %	100 %	27.3 %	∞	0.64
Predicting LVH									
Variables	**AUC**	**95 % CI**	**Cut-off values**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**LR+**	**LR-**
MMP-2 (ng/mL)	0.71¶	0.56-0.83	≥160	55.56 %	76.92 %	76.9 %	55.6 %	2.41	0.58
MMP-9 (ng/mL)	0.54*	0.35-0.72	≥450	16.67 %	100 %	100 %	46.4 %	∞	0.83
sE-selectin (ng/mL)	0.55*	0.36-0.73	≥70	38.89 %	92.31 %	87.5 %	52.2 %	5.06	0.66

C-IMT: Carotid intima media thickness; LVH: Left ventricular hypertrophy; AUC: Area under the ROC curve; PPV: Positive predictive value; NPV: Negative predictive value; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio; MMP-2: Matrix metalloproteinase-2; MMP-9: Matrix metalloproteinase-9. ¶ p value < 0.05, * p value > 0.05.

Discussion

In this study, we indicated the associations between MMP-2, MMP-9, and two endothelial dysfunction markers (sE-selectin, and FMD) with two cardiovascular abnormalities (atherosclerosis and LVH) in a population of children with ESRD. Although there are several studies in the literature including our previous work which have evaluated the interrelationships between mentioned MMPs and ED markers with known CVD risk factors in children with CKD (21-25), the associations between them and cardiovascular abnormalities had not been determined completely. Therefore, in this study, we attempted to clarify this issue. Furthermore, in the present study, we appraised traditional and uremia-related CVD risk factors with two major cardiovascular problems in children with ESRD.

In our study, the frequency of abnormal c-IMT and LVH in children with ESRD was 80.6 % and 54.8 %, respectively. The rate of abnormal c-IMT values is 71 % (35), 72 % (36), and 74 % (37) in different studies. These differences are mostly due to differences in ethnicity, age distribution, cut-off points for normal c-IMT, and duration of hemodialysis or peritoneal dialysis of the patients (35). Regarding LVH, our reported value was lower than similar studies that reported values of 40-75 % (36, 38). It seems that these differences between reported values are mostly due to differences in cut-

off points, duration of dialysis, and also distinct methods of LVMI measurement in the studies.

The results showed that MMP-2 and MMP- 9 had a positive correlation with c-IMT, which was consistent with studies performed in adults with CKD or ESRD (16-20). This correlation was stronger for MMP-2 than MMP-9 (16-20). c-IMT is an indicator of subclinical atherosclerosis (3); therefore, its association with these two MMPs may suggest their involvement in development of atherosclerotic plaques. In fact, MMPs contribute to tissue remodeling and eventually atherosclerosis plaque formation; this issue has been established in animal models and human atherosclerotic plaques (39). ROC curve analysis was performed to evaluate the predictive values of these two MMPs for the diagnosis of subclinical atherosclerosis, which showed acceptable predictive values for MMP-2 and MMP-9 in diagnosing an abnormal c-IMT with a high specificity.

LVH is a common cardiovascular abnormality in children undergoing dialysis (38). The causes of LVH have been investigated in several studies in this population, which include hypertension, salt and water retention, anemia, hyperparathyroidism, and oxidative stress (40). Moreover, the role of MMPs in the pathogenesis of LVH has been studied; MMPs contribute to the regulation of extracellular matrix and extracellular remodeling in the myocardium and eventually LVH (41). The results of the present study showed that LVMI had a positive correlation with MMP-2; moreover, this biomarker had an acceptable predictive value in differentiating patients with LVH from others. In a study conducted in adults for diagnosis of LVH, MMP-9 had an AUC of 0.62 (42). Martos et al studied 85 hypertensive adults and found that MMP-2 had an excellent predictive value in differentiating patients with diastolic heart failure from other patients (43). Altogether, it seems that these biomarkers could be useful in CVD diagnosis; however, large prospective studies are needed to establish the results.

Since ED has been introduced as a new CVD risk factor in patients with ESRD (44), we examined it in our patients and evaluated its possible relationship with c-IMT and LVMI. For this purpose, two ED indices were measured in the participants. The results demonstrated that the values of FMD and sE-selectin were significantly lower and higher in patients compared to controls respectively, indicating ED in children with ESRD. This finding was similar to previous studies (14, 21, 45, 46). Furthermore, this study found that c-IMT and LVMI had a negative correlation with FMD; in other words, this finding indicated the relationship between ED and these two cardiovascular indices. Similar to our results, two studies in children with CKD reported a negative correlation between c-IMT and FMD (36, 47). In contrast, regarding LVMI, Civilibal et al failed to find a significant correlation between LVMI and FMD (36). However, an association has been established between other ED markers such as endothelin-1 (48) and thrombomodulin (49) with LVMI.

The traditional and CKD-related CVD risk factors and their associations with subacute atherosclerosis and LVH were also investigated in the present study. The results showed that c-IMT correlated with DBP, hemoglobin, triglyceride, LDL, HDL, phosphorus, calcium, phosphorus × calcium, and PTH. In addition, multiple regression analysis introduced triglyceride, calcium, and phosphorus × calcium product as independent predictors of c-IMT. According to the literature, these variables are associated with c-IMT: cholesterol, LDL, triglyceride, phosphorus, phosphorus × calcium, and PTH (35, 36, 50). As for LVMI, the results showed that SBP, DBP, hemoglobin, triglyceride, HDL, phosphorus, phosphorus × calcium, and PTH had significant correlations with LVMI. The results of this study regarding the correlation of LVMI with other variables were similar to a study performed in ESRD children (36); however, in this study, SBP and hemoglobin were independent predictors of LVMI (36), while we found that HDL was an independent predictor. Overall, these correlations suggest that traditional and uremia-related CVD risk factors contribute to subclinical atherosclerosis and LVH.

Our study had some limitations including: (i) a relatively small sample size that could affect the results, (ii) the cross-sectional design of the study that prevented us from determining definite associations between measured variables and c-IMT and LVMI properly, (iii) not measuring MMP-2 and MMP-9 inhibitors, including tissue inhibitor matrix metalloproteinase-1 (TIMP-1) and TIMP-2 that could clarify the associations between metalloproteinases and CVD more effectively.

Conclusion

In conclusion, the results showed that MMP-2, MMP-9 and endothelial dysfunction markers were associated with two major CVD markers in children with ESRD. Furthermore, MMP-2 and MMP-9 may be useful as new biomarkers for diagnosis of subclinical atherosclerosis and MMP-2 may be used for diagnosis of LVH in children with ESRD. However, sE-selectin failed to discriminate patients with abnormal c-IMT and LVH values properly. Further studies with larger sample sizes and a longitudinal design are warranted to elucidate all aspects of these markers in association with CVD.

Conflict of Interest

The authors declare no conflicts of interest.

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