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RESEARCH ARTICLE

The relationship between Matrix Metalloproteinase-10 (MMP-10) and atherosclerosis in Patients with chronic kidney disease

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Abstract

Background: Cardiovascular diseases (CVD) are the leading cause of death in patients with chronic kidney disease (CKD).

Aim: As matrix metalloproteinases have a major role in atherosclerosis, we investigated the relationship between matrix metalloproteinase (MMP-10) and the severity of atherosclerosis in patients with chronic kidney disease. **Methods:** This study was evaluated in a cross-sectional study of 40 patients of CKD subdivided into 20 patients with different stages of kidney disease and 20patients on dialysis and 20 healthy controls. The severity of atherosclerosis was estimated with carotid intima media thickness (CIMT) by carotid ultrasound. Serum level of (MMP-10) was measured by ELISA.

Results: MMP-10 level in the control group was ranging between 346 and 776 pg/dl with a mean of (601 $\pm$ 132.12) and in the earlier stages of CKD group was ranging between 989 and 2569 pg/dl with a mean of (1857.45$\pm$ 387. 1). Also MMP-10 in patients on dialysis group (HDx) group was ranging between 1790 and 2986 pg/dl with a mean of (2306.45 $\pm$ 335.247). The difference between all groups was statistically significant with P value <0.001. The carotid intima media thickness (CIMT) between all groups was statistically significant with P- value <0.001 between the HDx group and other groups and P value= 0.045 between the control and the CKD group. Also there was statistically significant positive correlation between MMP-10 and CIMT in all groups.

Conclusion: MMP-10 was significantly increased in patients with kidney disease compared with the healthy controls, and was higher in patients on dialysis than in earlier stages of CKD. The severity of the atherosclerosis was also more prevalent in the dialysis group, in which serum levels of MMP-10 was significantly higher. Thus, patients with CKD exhibit elevated levels of circulating MMP-10, and this was independently associated with the severity of atherosclerosis and may represent a new biomarker of atherosclerotic diseases.

Introduction

Cardiovascular diseases (CVD) are the leading cause of death in patients with chronic kidney disease (CKD), and cardiovascular risk is higher in such patients than in the general population even at early stages of CKD [1].

Established cardiovascular risk factors (CVRFs) are associated with the development of new-onset kidney disease. Therefore, it is important to assess conventional CVRFs in patients with kidney disease to allow early intervention [2].

The lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described as an inflammatory disease. Their underlying pathogenesis involves an endothelial dysfunction [3**].**

The presence and severity of atherosclerosis is higher in patients with CKD compared with control subjects with normal kidney function [4].

The clinical use of the assessment of potential biomarkers of atherosclerosis is restricted due to lack of end-point data, with the exception of CRP [5].

Turnover of extracellular matrix proteins, crucial for atherosclerotic plaque development and rupture, is largely achieved through the balance between the action of matrix metalloproteinases (MMPs), which represent a major class of matrix-degrading proteinases and their specific tissue inhibitors (tissue inhibitor of metalloproteinases, TIMPs). MMPs are considered key factors in atherosclerosis being implicated in intimal thickening and in the subsequent plaque rupture [6].

The proteinase activities exerted by MMPs have been implicated in some of the biological processes associated with atherosclerosis and its ischemic clinical manifestations, such as myocardial infarction and stroke, and circulating MMP levels have been associated with subclinical atherosclerosis and increased cardiovascular risk [7].

However, the roles of MMPs and TIMPs in the pathogenesis of atherosclerosis in CKD are poorly understood. It was reported that MMP-10 is expressed in atherosclerotic plaques, being almost undetectable in healthy arteries, and that endothelial MMP-10 expression can be induced by inflammatory stimuli [8].

Therefore, we investigated the relationship between MMP-10 and the severity of atherosclerosis in patients with chronic kidney disease.

Subjects and methods:

Study population:

This study was performed from April 2013 to January 2014 included 60 subjects. Forty patients with chronic kidney disease subdivided into 20 patients in different stages of chronic kidney disease and 20 patients on dialysis who had attended the internal medicine and nephrology outpatient clinic and dialysis unit of Al Kasr al Ainy Hospital and twenty healthy age matched controls.

Exclusion crieteria:

1. Smokers
2. Previous cardiovascular accident.

Ethical aspects:

Research protocols were approved by the medical ethics committee of Al Kasr al Ainy medical school, Cairo University. All participants provided a written informed consent after the research protocols were carefully explained to them. Informed consent was obtained from all the study participants and their approval taken by signature

Procedures:

All subjects were subjected to full History, clinical examination, serum urea, creatinine, fasting plasma glucose (FPG), serum cholesterol, LDL, HDL, Triglycerides, calcium, phosphorus and uric acid. Measurements were done with the Beckman Coulter Synchron LX20 Clinical System auto analyser. Serum level of MMP-10 was assessed by specific enzyme linked immunoassay, double antibody sandwich ELISA **(Human MMP-10 Kit, Sunred bio, UK, 2013)** according to the manufacturer's instructions with a serum dilution of 1:50. The interassay coefficients of

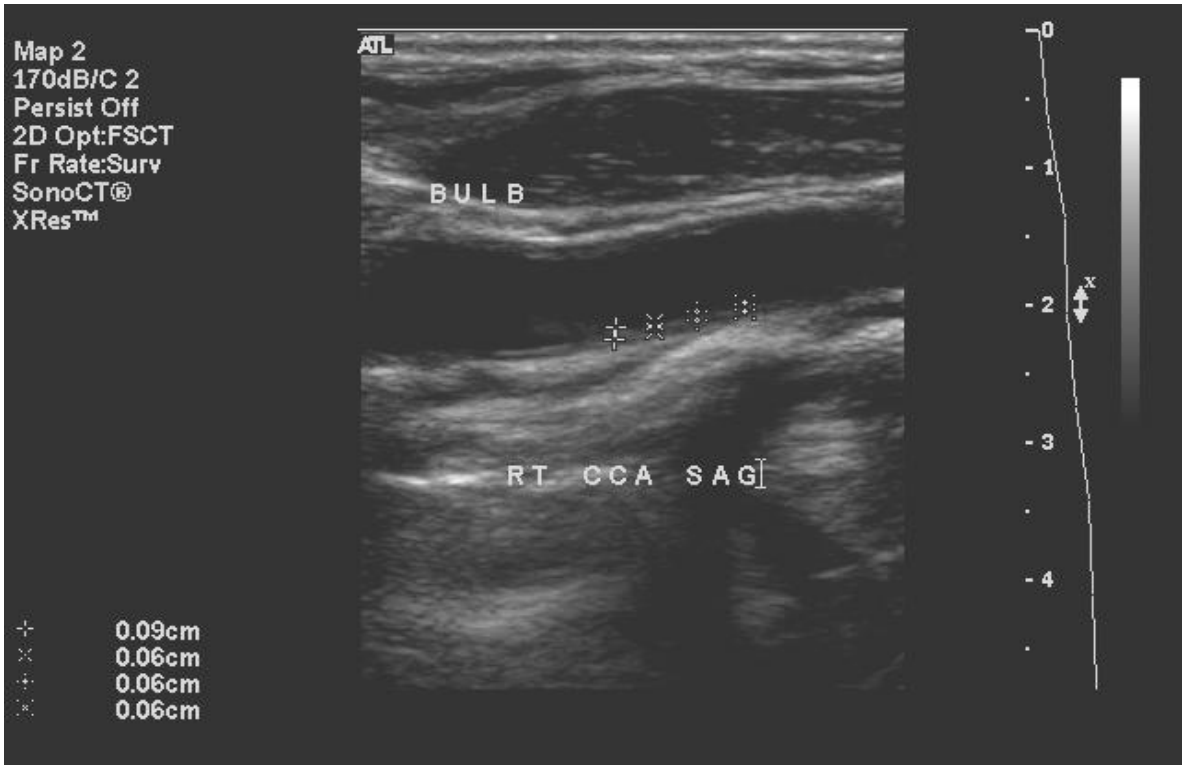
variation were 8% and detection limit for the assays were 78.1pg/ml. Carotid duplex was done to measure the carotid intima- media thickness and identify carotid plaques.

Measurement of intima media thickness (IMT):

Ultrasound examinations were performed by using the HDI- 5000 at a transducer frequency of 5 - 10 MHz. Computer-assisted acquisition, processing, storage of B-mode images, and calculation of IMT was performed with the software Intima Scope. Both the near and far walls of the common carotid arteries, the carotid bifurcations, and the origins (first 2 cm) of the internal carotid arteries were scanned longitudinally and transversely to assess the occurrence of plaques.

The definition and measurement of IMT were performed according to the method reported by Pignoli [9]**.** We adopted the scans of the far wall common carotid arteries, since several reviews on methodological considerations of ultrasound investigation of IMT have revealed that the IMT can only be measured accurately in the far-wall position and good-quality multiple scans may be achieved in nearly every case from the common carotid arteries, while the percentage of missing images is high from the internal carotid arteries [10].

The IMT was measured by using an automated edge-detection algorithm based on significant changes in density of a section between the lumen and subadventitial structures perpendicular to the vessel wall. The software estimated lines for the lumen-intima interface and the media-adventitia interface was designed to achieve increased accuracy and reproducibility with reduced variability for the measurements of IMT. Two measurements on longitudinal views of both the right and left common carotid arteries were made at the 20 mm segment distal to the carotid bulbs. The greatest value of IMT was used as the representative value for each individual.



Measuring common carotid IMT.

The normal values (mm) of maximum IMT measured by the above described method obtained from healthy subjects were 0.45-0.63 (for individuals aged 30 to 50 years) and 0.61-0.71 (for who aged 50 to 70 years) [11].

Statistical analysis

Data were statistically described in terms of range, mean, standard deviation (SD), frequencies (number of cases) and relative frequencies (percentages). Results are given as mean ± SD otherwise stated.

Comparison between different groups in the present study was done using Student *t* test for comparing continuous data when normally distributed and Mann Whitney *U* test when not normally distributed. For comparing categorical data, Chi square (x^2) test was performed. Yates correction was used instead when the frequency is less than 10. Linear Correlation coefficient was used for detection of correlation between two quantitative variables. A probability value (P value) less than 0.05 was considered significant and if less than 0.01 was considered highly significant.

All statistical analyses were performed with the statistical software package program SPSS V18 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA).

Results

The age of the Control group was ranging between 40 and 63 years old with a mean of (49 ±6.505) and in the CKD group was ranging between 38 and 61 years old with a mean of (49.45 ±6.58). In the HDx group the age was ranging between 38 and 62 years old with a mean of (52 ±6.67). The difference between groups were not significant as P value between control group and CKD group was 0.414, between control and HDx groups was 0.115 and between CKD and HDx groups was 0.079 as shown in table 1 and figure 1.

Table 1: Comparison of age in all groups

Groups	Age		P-value		
	Range	Mean ± SD	Control / CKD	Control / HDx	CKD / HDx
Controls	40 - 63	49 ± 6.505	0.414	0.079	0.115
CKD Patients	38 - 61	49.45 ± 6.581			
HDx	38 - 62	52 ± 6.672			

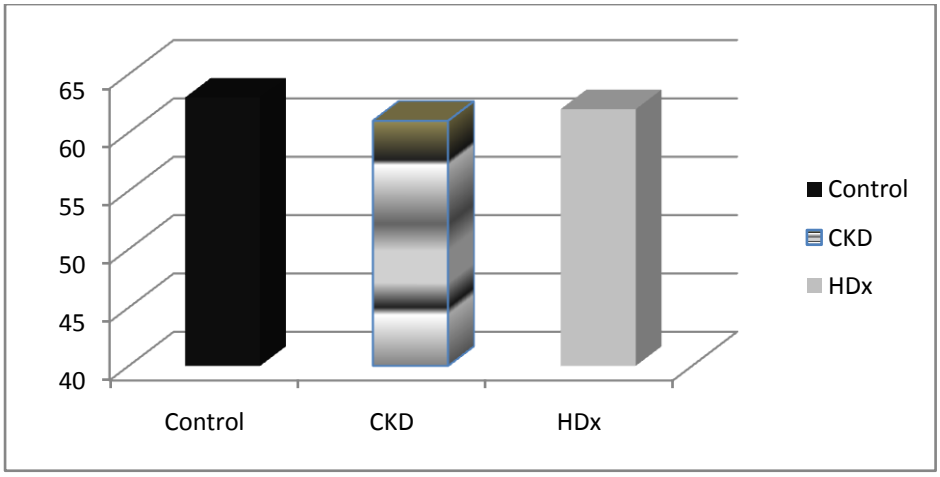


Figure (1): Comparison of age in all groups

Thirty-three females of total 60 persons (55%) were included in our study and there was no statistically significant difference in sex distribution in the three groups as shown in table 2 and figure2.

Table 2: Comparison of sex between all groups

Sex	Groups						Total		Chi-Square, P-value		
	Controls		CKD		HDx						
	No	%	No	%	No	%	No	%	Control / CKD	CKD/ HDx	Control / HDx
Female	12	60%	11	55%	10	50%	33	55%			
Male	8	40%	9	45%	10	50%	27	45%	0.75	0.74	0.52
Total	20	100%	20	100%	20	100%	60	100%			

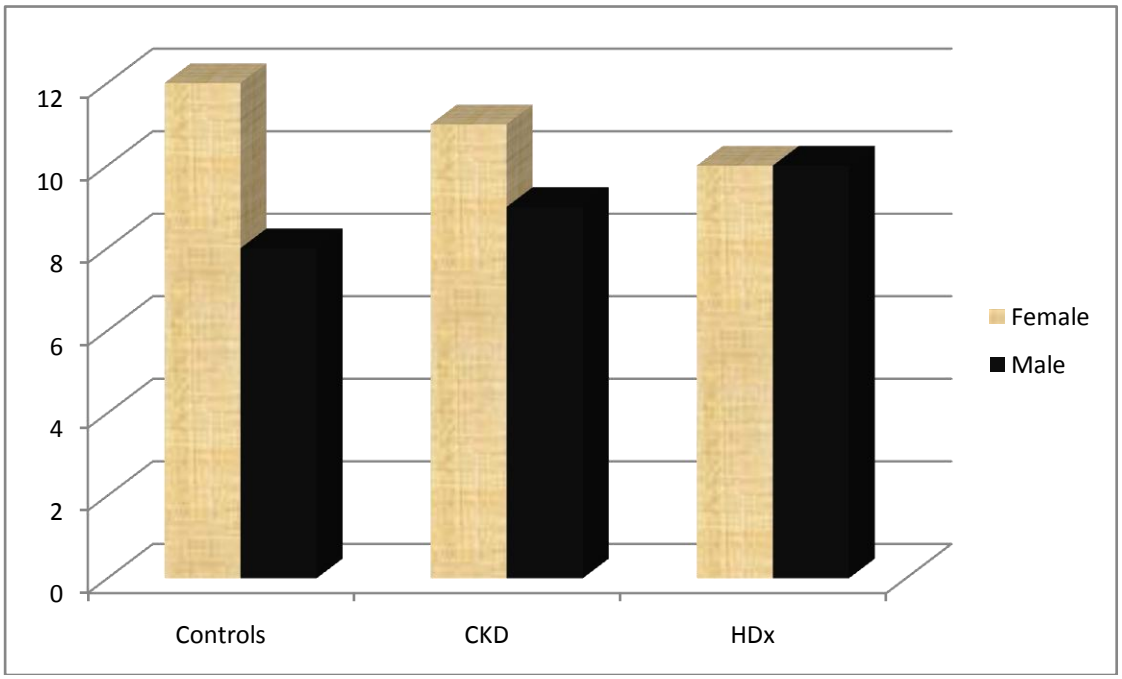


Figure (2): Comparison of sex in all groups

The difference of systolic blood pressure (SBP) between groups was not significant except between control and CKD groups as P value was 0.034, between control and HDx groups was 0.298 and between CKD and HDx groups was 0.079. (Table 3 and figure 3)

Table (3): Comparison of systolic blood pressure between all groups

Groups	SBP		P-value		
	Range	Mean ± SD	Control / CKD	Control / HDx	CKD / HDx

Controls	110 - 135	121.5 ± 7.626	0.034	0.298	0.079
CKD Patients	110 - 145	126.5 ± 9.191			
HDx	110 - 130	122.75 ± 7.158			

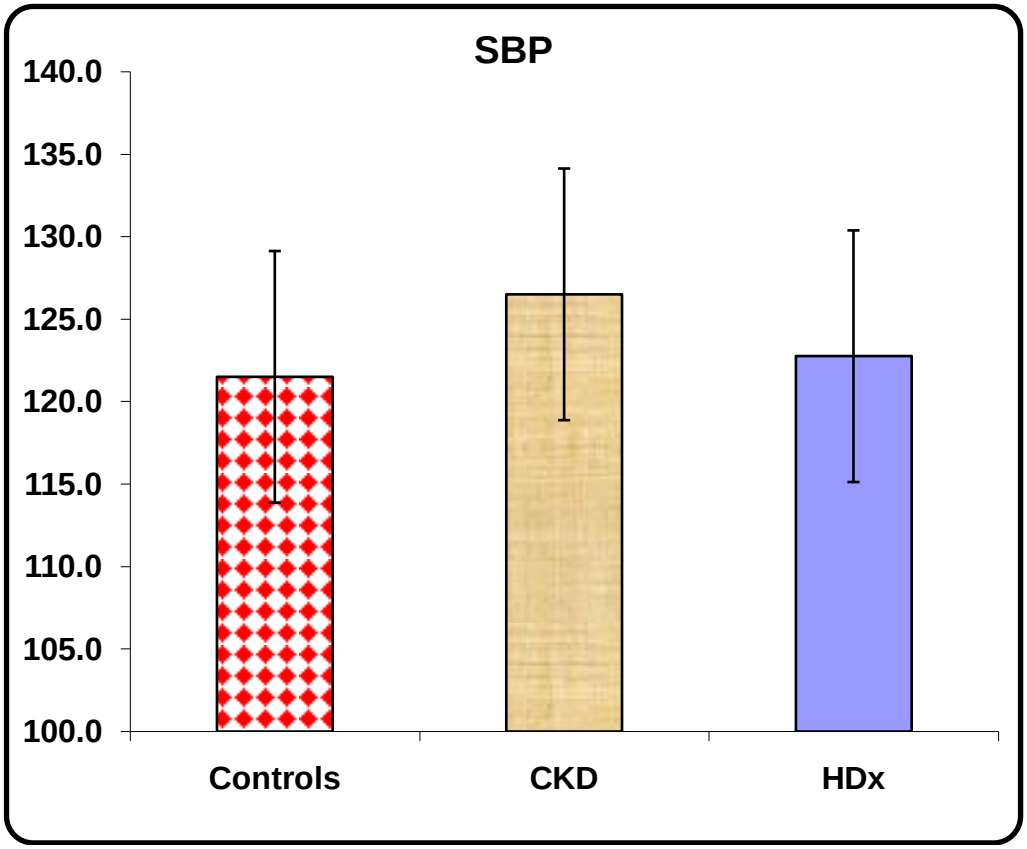


Figure (3): Comparison of systolic Blood Pressure between all groups

The difference of diastolic blood pressure (DBP) between groups wasn't significant as P value between the control and CKD group was 0. 359, between the control and HDx group was 0.339 and between CKD and HDx group was 0.5. (Table 4 and figure 4)

Table (4): Comparison of diastolic blood pressure in all groups

Groups	**DBP**		**P-value**		
	Range	**Mean ± SD**	**Control / CKD**	**Control / HDx**	**CKD / HDx**
Controls	60 - 90	78 ± 6.45716	0.359	0.339	0.5
CKD Patients	60 - 90	77.75 ± 8.025453			
HDx	70 - 85	77.75 ± 6.584471			

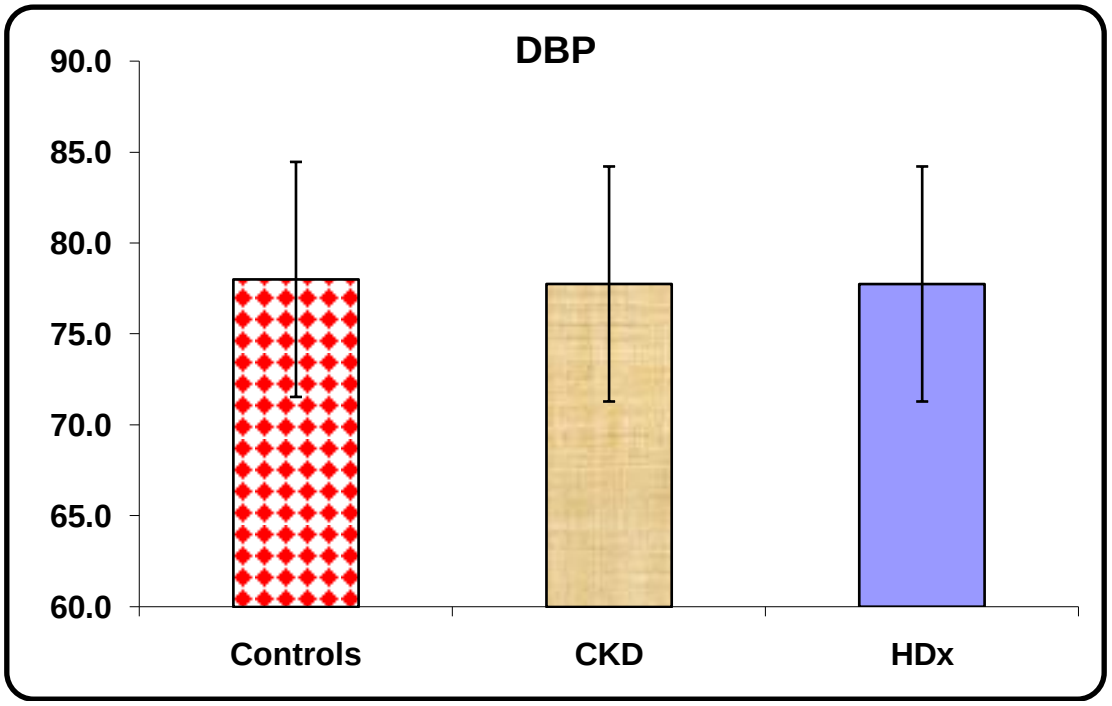


Figure (4): Comparison of diastolic blood pressure in all groups

The difference of triglyceride level between groups wasn't significant as P value between the control and CKD group was 0.21, between the control and HDx group was 0.39 and between CKD and HDx group was 0.097. (Table 5 and figure 5)

Table (5): Comparison of triglyceride levels in all groups

Groups	TG			P-value		
	Range		Mean ± SD	Control / CKD	Control / HDx	CKD / HDx
Controls	62 - 260		151.5 ± 52.18338	0.21	0.39	0.097
CKD Patients	95 - 190		140.95 ± 25.71601			
HDx	72 - 250		155.65 ± 42.35101			

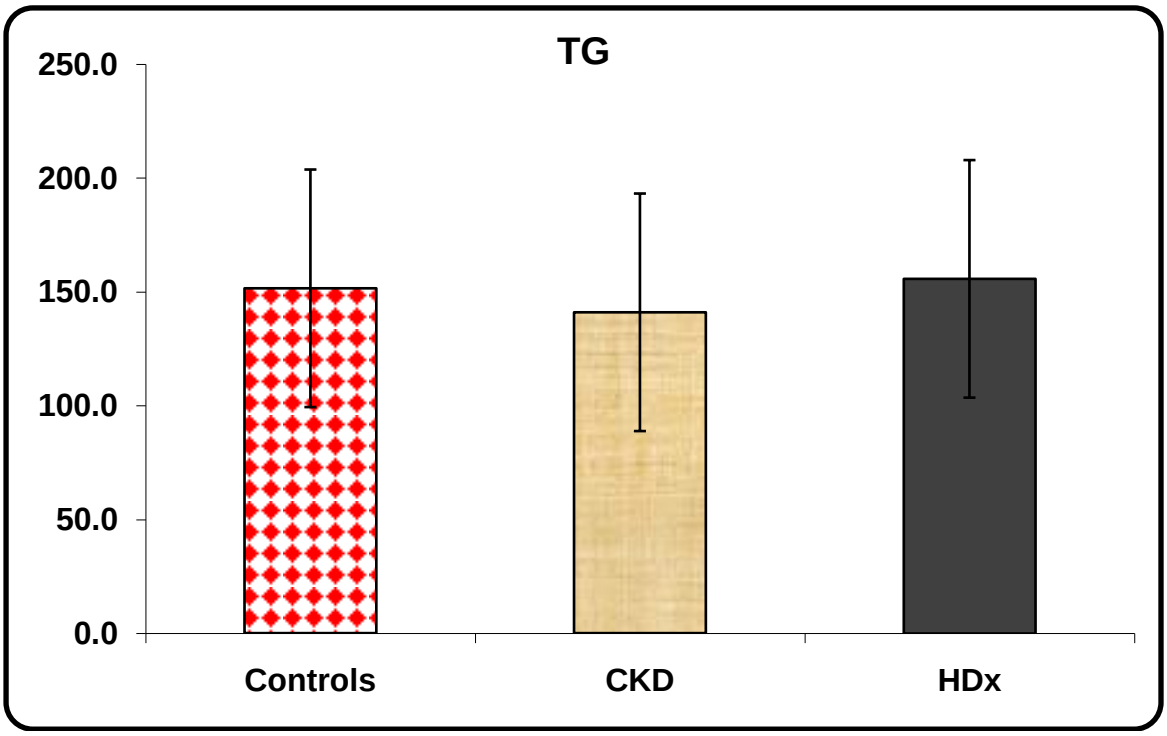


Figure (5): Comparison of triglyceride levels in all groups

The difference of cholesterol level between groups wasn't significant as P-value between control and CKD group was 0.208, between control and HDx group was 0.092 and between CKD and HDx group was 0.267. (Table 6 and figure 6)

Table 6: Comparison of cholesterol levels in all groups

Groups	Cholesterol		P-value		
	Range	Mean ± SD	Control / CKD	Control / HDx	CKD / HDx
Controls	109 - 240	184.2 ± 36.33557	0.2	0.09	0.27
CKD Patients	125 - 230	191.4 ± 26.09174			
HDx	127 - 238	199.5 ± 26.68629			

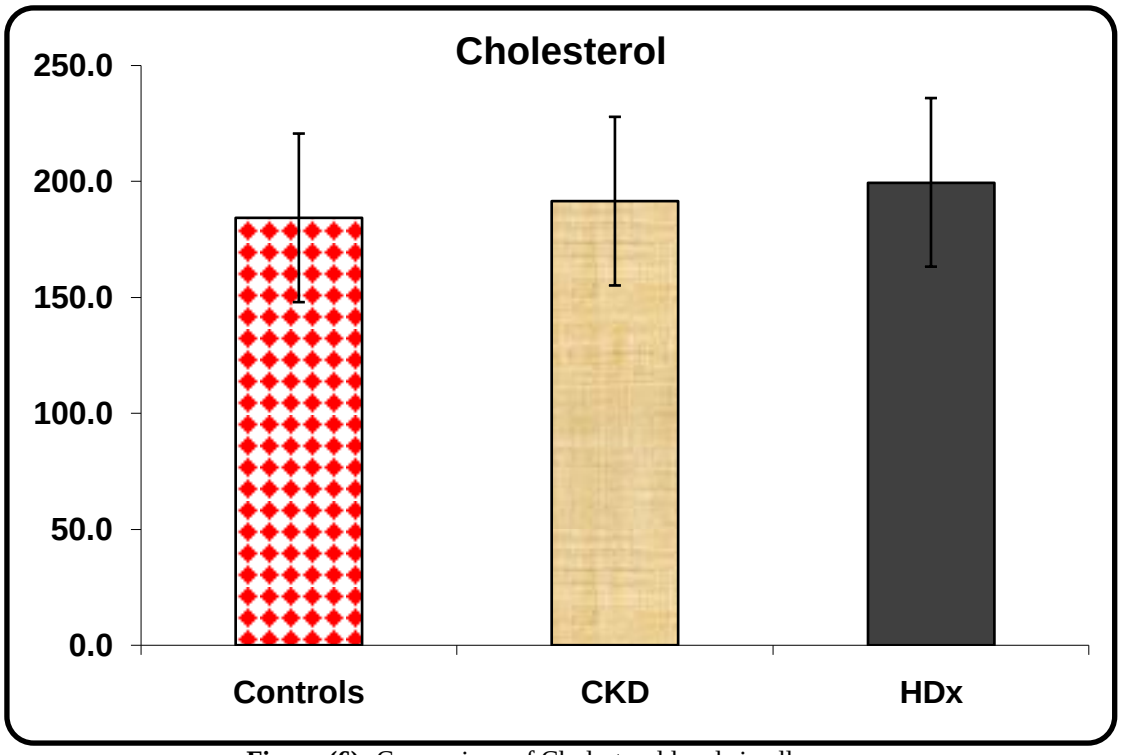


Figure (6): Comparison of Cholesterol levels in all groups

The difference of LDL level between groups wasn't significant as P value between the control and CKD groups was 0.437, between the control and HDx groups was 0. 2 and between CKD and HDx groups was 0. 131. (Table 7 and figure 7)

Table (7): Comparison of LDL levels in all groups

Groups	LDL		P-value		
	Range	Mean ± SD	Control / CKD	Control / HDx	CKD / HDx
Controls	70 - 160	117.6 ± 28.1563	0.437	0.2	0.131
CKD Patients	80 - 150	116.35 ± 21.6534			
HDx	80 160	124.7 ± 24.6536			

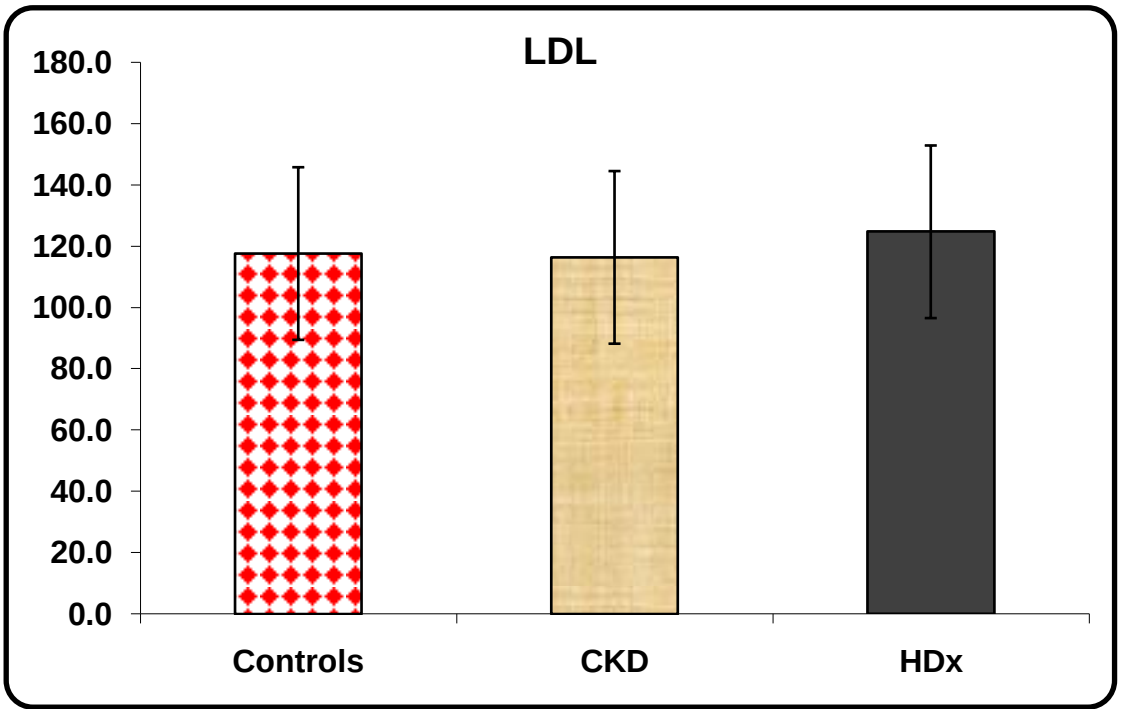


Figure (7): Comparison of LDL levels between all groups

The difference of HDL level between HDx group and other groups was statistically significant. (Table 8 and figure 8). No significant difference between the control and CKD group, P value was 0.35.

Table (8): Comparison between HDL levels in all groups

Groups	HDL			P-value		
	Range	Mean ± SD		Control / CKD	Control / HDx	CKD / HDx
Controls	39 - 80	52.2 ± 14.2961		0.35	0.05*	0.02*
CKD Patients	38 - 75	51.1 ± 10.9971				
HDx	38 - 86	60.05 ± 15.5511				

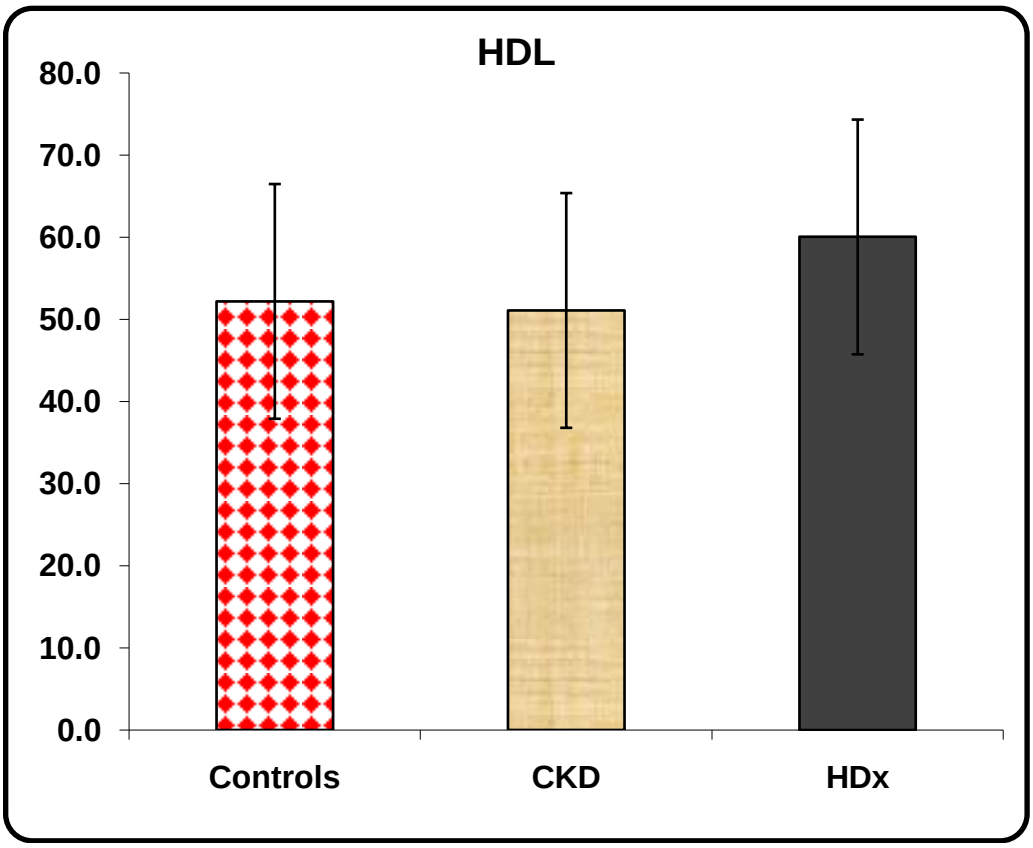


Figure (8): Comparison of HDL levels in all groups

The difference of creatinine level between all groups was statistically significant with P- value was <0.001. (Table 9 and figure 9)

Table (9): Comparison of creatinine levels in all groups

Groups	Creatinine			P-value		
	Range		Mean ± SD	Control / CKD	Control / HDx	CKD / HDx
Controls	0.5 - 1.4		0.835 ± 0.275824	<0.001*	<0.001*	<0.001*
CKD Patients	2.4 - 5		3.68 ± 0.800395			
HDx	6.9	12.3	9.13 ± 1.210089			

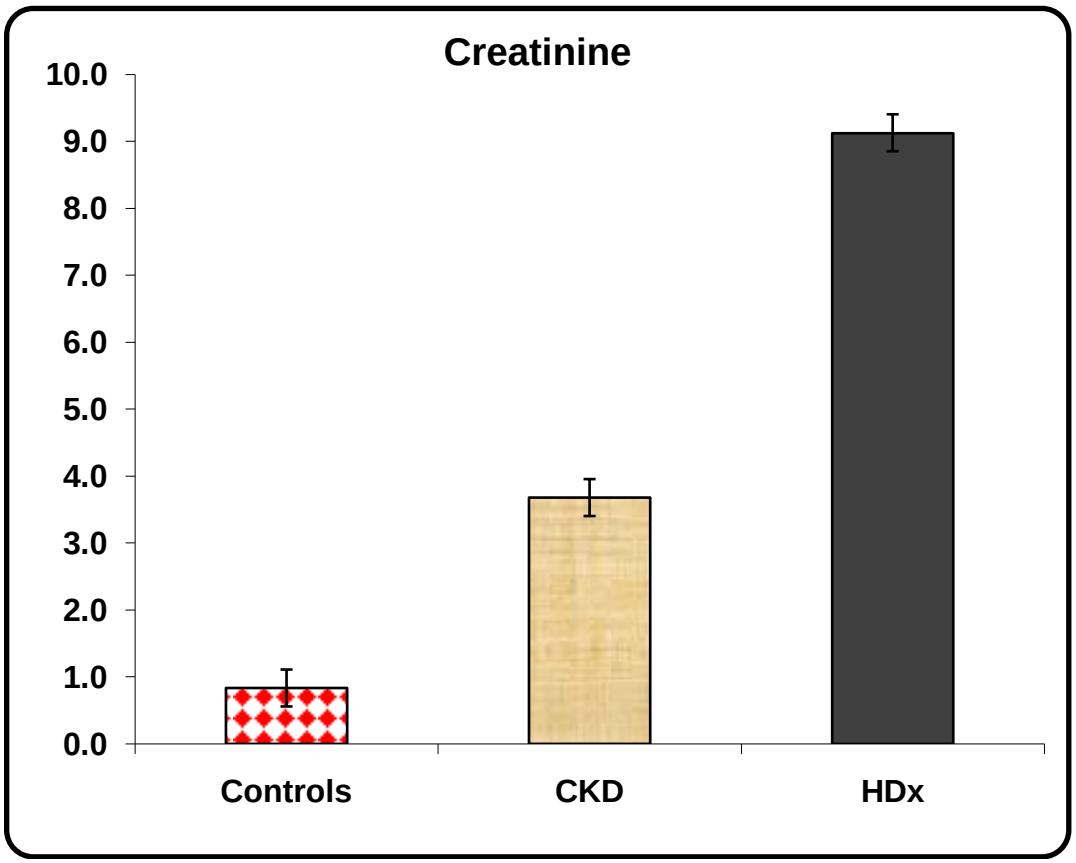


Figure (9): Comparison of creatinine levels in all groups

Measuring carotid intima media thickness (CIMT) in the control group was ranging between 0.48 and 1 mm with a mean of (0.647 ± 0.132) and in the CKD group was ranging between 0.5 and 1.2 mm with a mean of (0. 741± 0. 202). Also CIMT in the HDx group was ranging between 0.74 and 1.2 mg/dl with a mean of (0.921± 0.104). The difference between all groups was statistically significant with p- value <0.001 between the HDx group and other groups and 0.045 between the control and the CKD group. (Table 10 and figure 10).

Table (10): Comparison of CIMT between all groups

Groups	CIMT		P-value		
	Range	Mean ± SD	Control / CKD	Control / HDx	CKD / HDx
Controls	0.48 - 1	0.647 ± 0.132112	0.045*	<0.001*	<0.001*
CKD Patients	0.5 - 1.2	0.741 ± 0.20217			
HDx	0.74 - 1.2	0.9215 ± 0.104895			

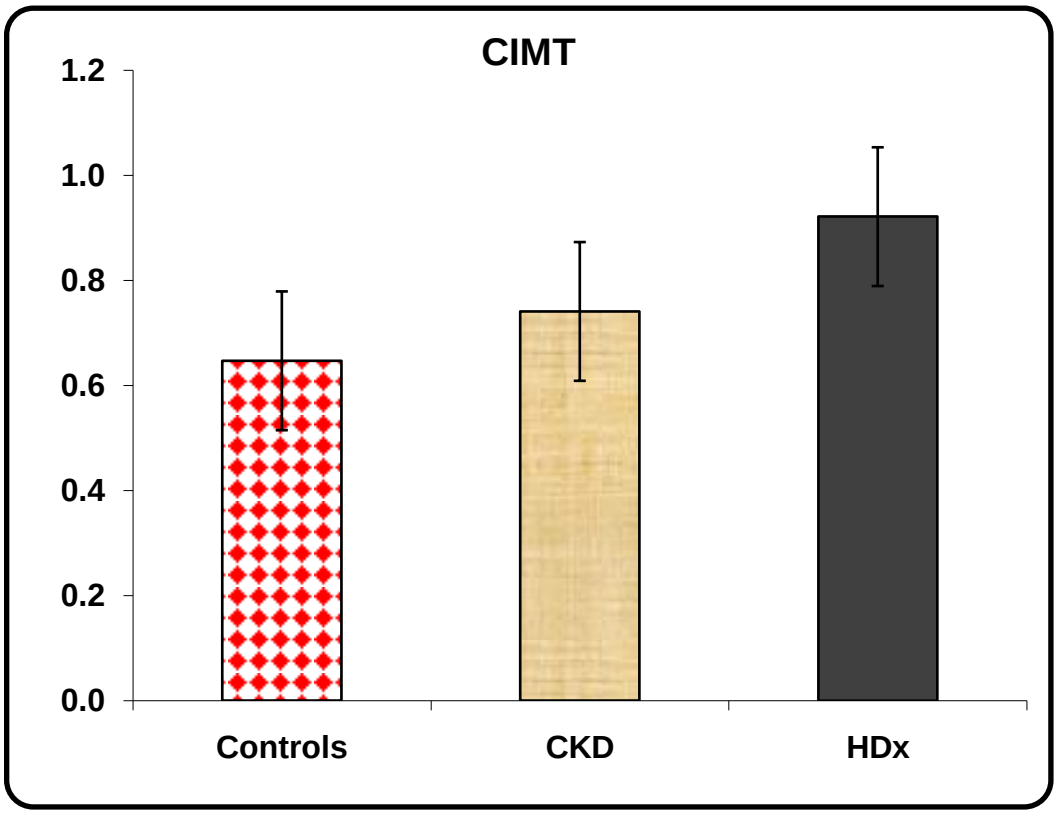


Figure (10): Comparison of CIMT between all groups

Matrix metalloprotienase10(MMP-10) level in the control group was ranging between 346 and 776 pg/dl with a mean of (601 ± 132.12) and in the CKD group was ranging between 989 and 2569 pg/dl with a mean of (1857.45± 387. 1). Also MMP-10 in the HDx group was ranging between 1790 and 2986 pg/dl with a mean of (2306.45 ± 335.247). The difference between all groups was statistically significant with P- value <0.001. (Table 11 and figure 11)

Table (11): Comparison of MMP- 10 between all groups

Groups	MMP 10		P-value		
	Range	Mean ± SD	Control / CKD	Control / HDx	CKD / HDx
Controls	346 - 776	601.4 ± 132.1285	<0.001*	<0.001*	<0.001*
CKD Patients	989 - 2569	1857.45 ± 387.1013			
HDx	1790 - 2986	2306.45 ± 335.2474			

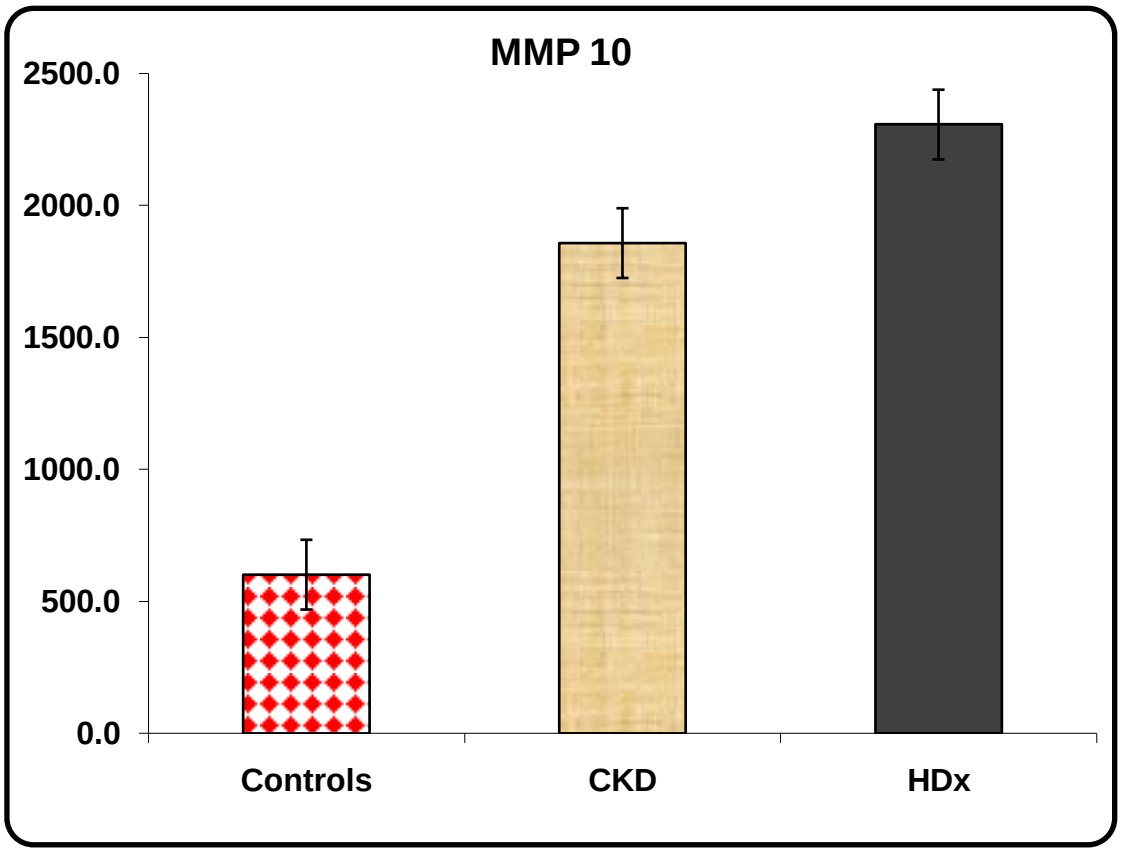


Figure (11): Comparison of MMP-10 between all groups

MMP- 10 level in diabetic patients was ranging between 408 and 2768 pg/dl with a mean of (1578± 868) and in non diabetics was ranging between 346 and 2986 pg/dl with a mean of (1592 ± 763). The differences between groups were not significant as p- value was 0.452. (Table 12 and figure 12)

Table (12): Comparison of MMP-10 between diabetics and non-diabetics

Groups	MMP 10		P-value
	Range	Mean ± SD	
Diabetics	408 - 2768	1578.118 ± 868.18	0.452
Non diabetic	346 - 2986	1592.512 ± 763.68	

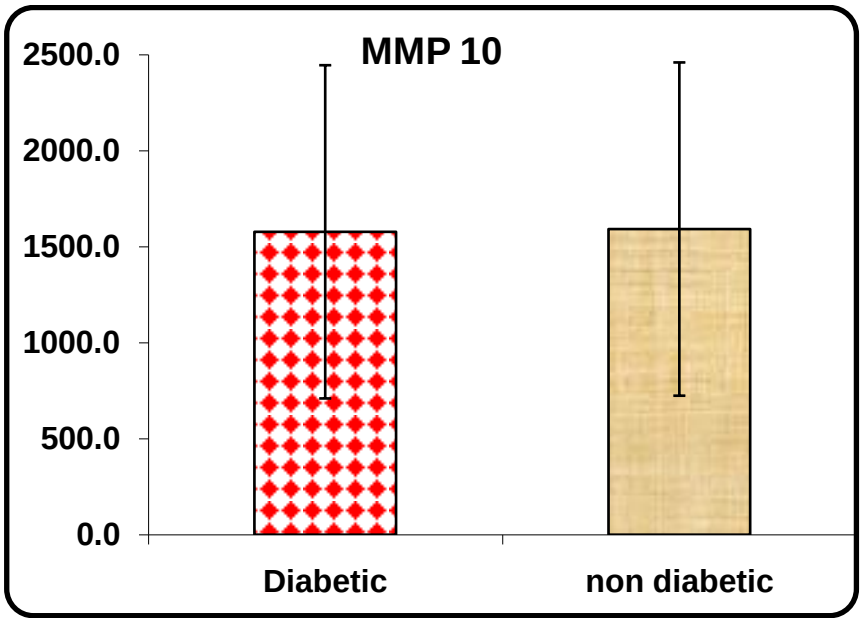
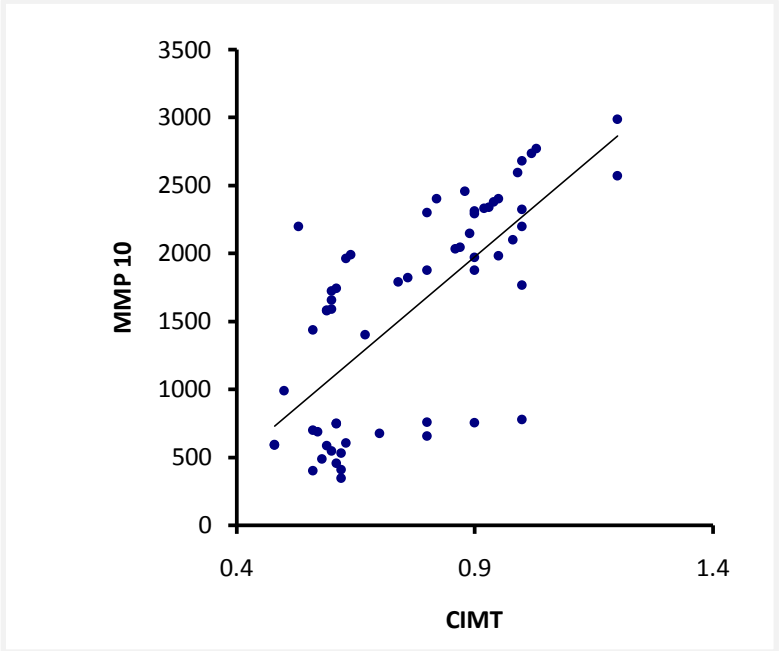


Figure (12): Comparison of MMP-10 between diabetics and non-diabetics

Correlations:

We found significant positive correlation between level of MMP- 10 with both serum creatinine and CIMT. Also we found a positive significant correlation between serum creatinine and CIMT in all groups.

Pair	R	95% CI		P-value
CIMT, MMP 10	0.709	0.555	to 0.816	<0.0001
CIMT, Creat	0.634	0.454	to 0.765	<0.0001
MMP 10, Creat	0.840	0.746	to 0.902	<0.0001



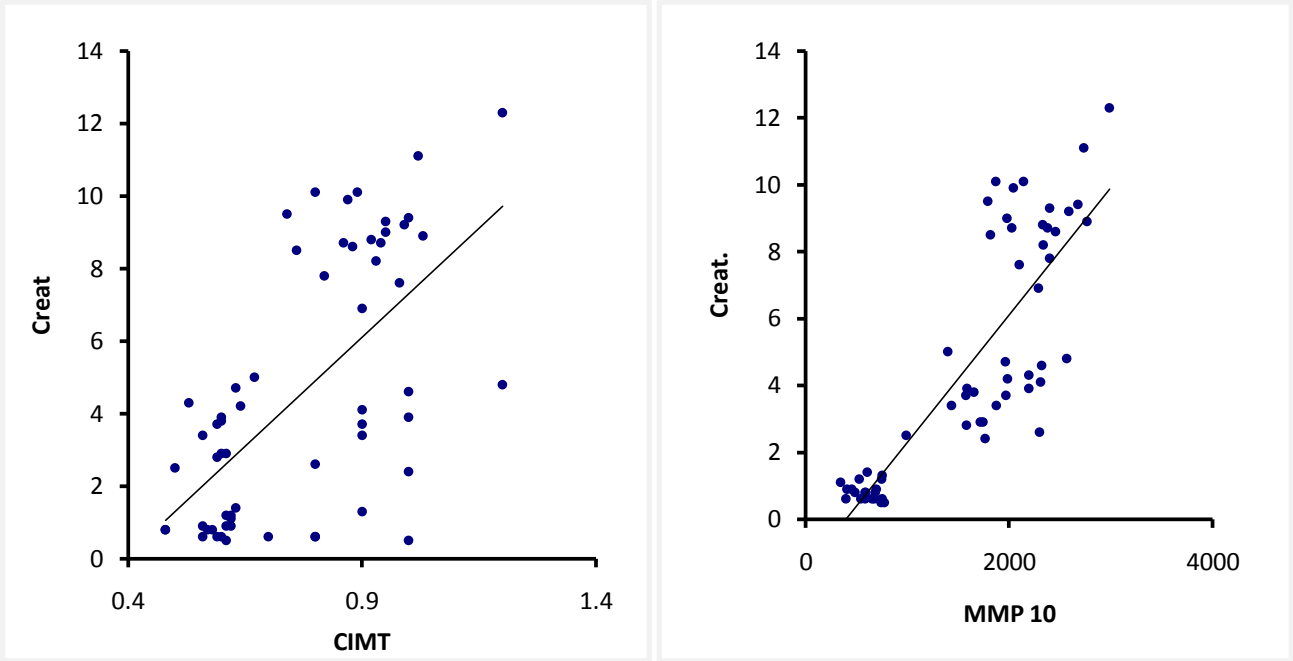
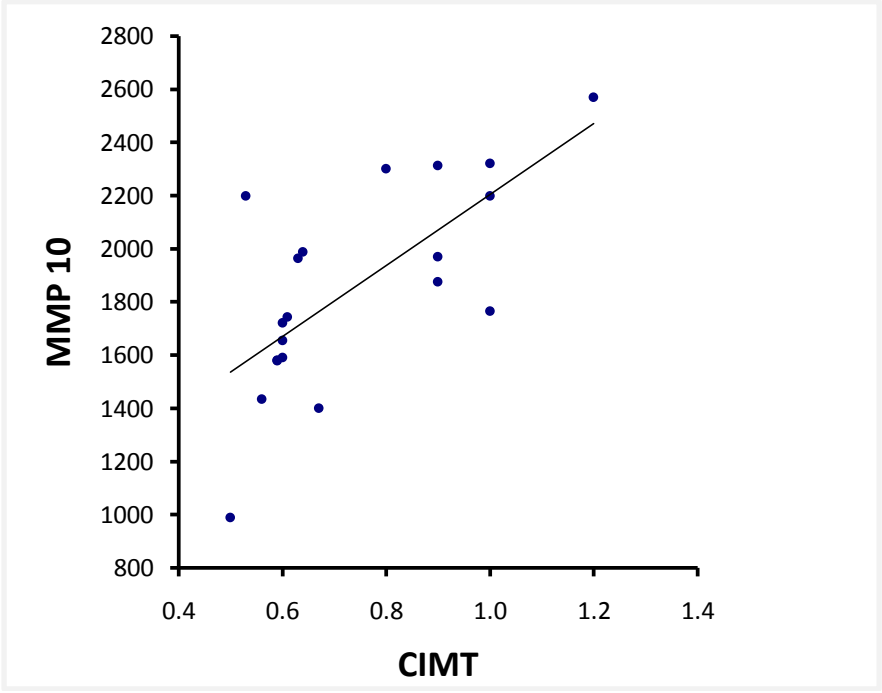


Fig (13): correlations between all groups

Analysis inside each group:

In the **CKD group** we found a positive significant correlation between MMP -10 level and CIMT with non-significant correlation between serum creatinine and CIMT and between serum creatinine and MMP- 10 levels.

Pair	r	95% CI		p-value
CIMT, MMP 10	0.697	0.369	to 0.871	0.0006*
CIMT, creatinine	0.221	-0.246	to 0.604	0.3499
MMP 10, creatinine	0.408	-0.042	to 0.720	0.0741



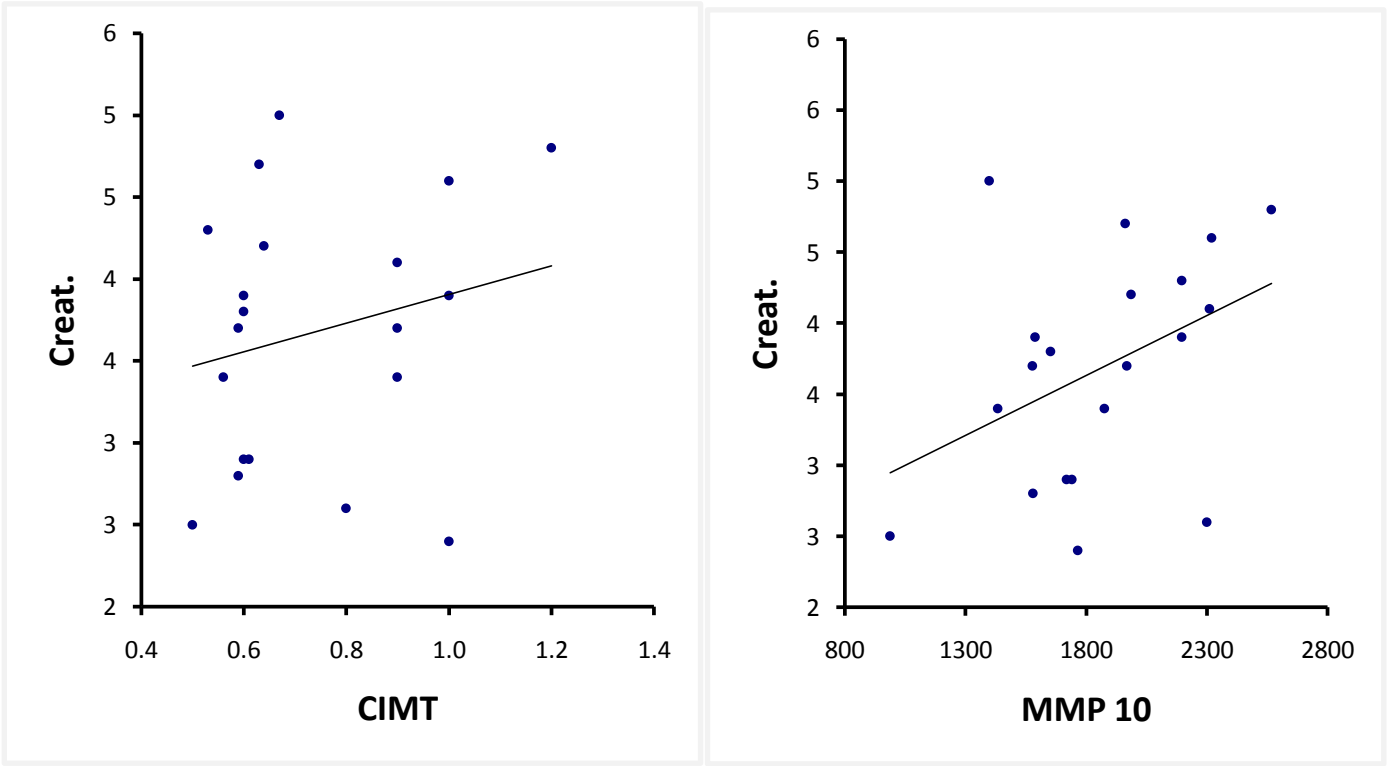


Fig (14): Correlations inside the CKD group

In the **HDx group:** also we found a positive significant correlation between MMP- 10 level and CIMT with non-significant correlation between serum creatinine and CIMT and between serum creatinine and MMP- 10 levels.

Pair	r	95% CI		p-value
CIMT, MMP 10	0.836	0.624	to 0.933	<0.0001*
CIMT, Creat	0.436	-0.008	to 0.737	0.0545
MMP 10, Creat	0.338	-0.123	to 0.679	0.14

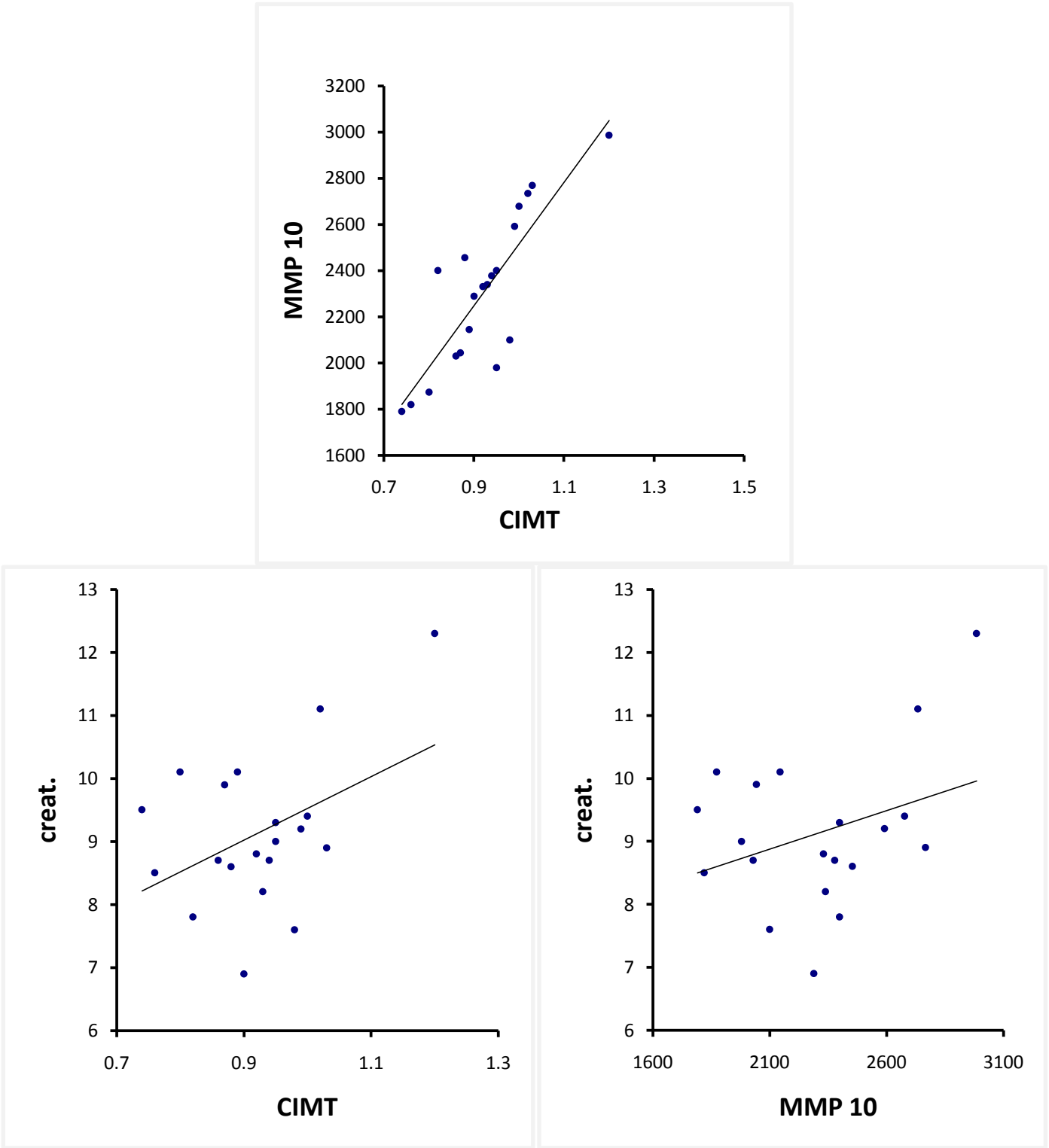
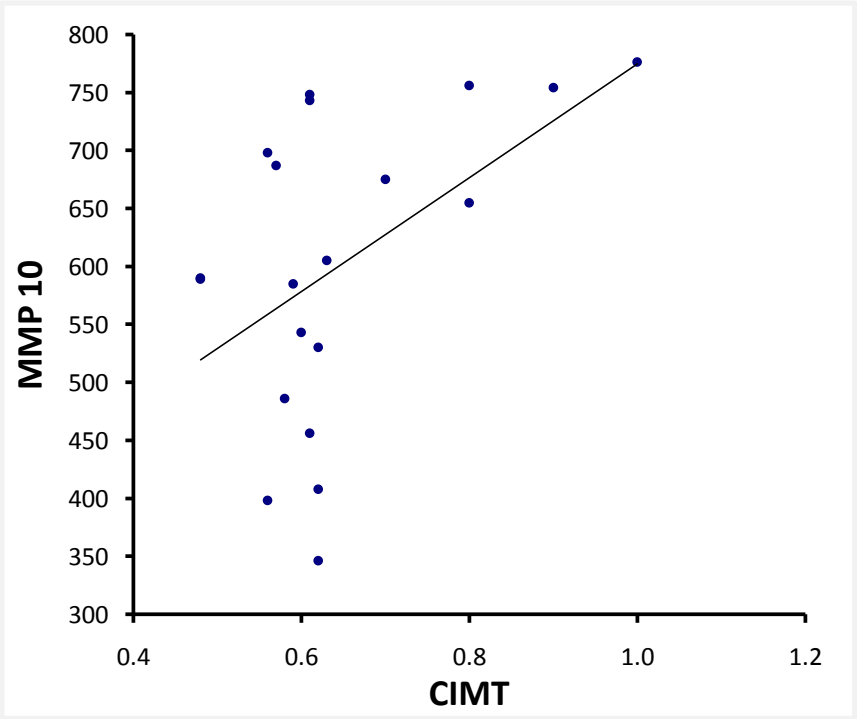
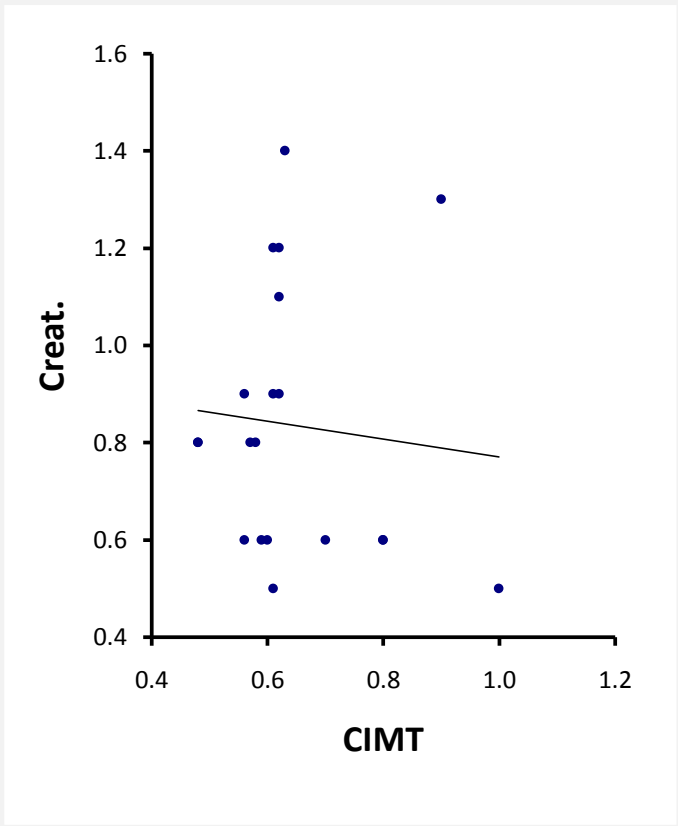


Fig (15): Correlations inside the HDx group

In the Control group: still there was significant positive correlation between MMP- 10 and CIMT and no significant correlation between creatinine with both MMP- 10 and CIMT

Pair	r	95% CI		p-value
MMP 10, CIMT	0.491	0.062	to 0.767	0.028*
MMP 10, Creat.	-0.127	-0.539	to 0.334	0.59
CIMT, Creat.	-0.088	-0.511	to 0.369	0.71





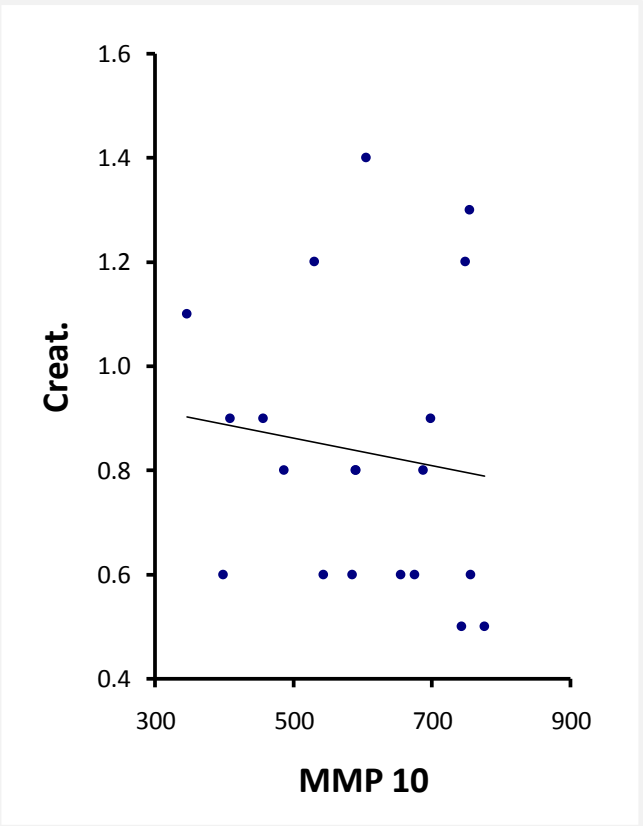


Fig (16): Correlations inside the control group

Discussion

The objective of this study is to investigate the relationship between MMP-10 and severity of atherosclerosis in patients with chronic kidney disease.

In our work we measured carotid IMT as this correlates well with the pathological findings of atherosclerosis. It has gained acceptance and validity as a noninvasive, inexpensive and reproducible method to assess the occurrence and extent of atherosclerosis [12]**.**

Smokers were not included in our study as MMP-10 levels are higher in smokers and there is an

independent association between smoking and the MMP-10 concentration in asymptomatic individuals. Also smoking is the single most important risk factor for CVD other than advanced age [13].

Regarding the relation between MMP- 10 and hypertension, in our study we didn't find any correlation between Blood Pressure (systolic and diastolic) and MMP- 10.This may be due to our Patients have been already treated for hypertension, and hyperlipidemia. Thus the relationship between the obtained values of blood pressure and lipid profiles with IMT and MMP- 10 were likely confounded by the treatment. But In a study done by Friese **et al., 2009** [14] found that MMP-10 did not differ between the normotensive and hypertensive groups, but they found that MMP-10 was elevated in ESRD compared to both the normotensive and hypertensive groups. This suggests that MMP-10 does not play a major role in essential hypertension but instead may be involved in the development of renal injury once essential hypertension has been established.

Also we didn't find any significant relation between the age of our patients and MPP-10 but the data presented by **Komosinska-Vassev et al., 2011**[15] showed negative correlation between age and MMP-10 (-0.53; p

= 0.000). This may be due to the wide range of age taken by Komosinska-Vassev et al, (individuals aged 6-62 years) and also may be due to the effect of the diseases that alter MMP-10 in our study.

We didn't find significant difference in MMP-10 level between diabetic patients and non diabetics. **Toni M et al., 2013**[16] found elevated MMP-10 in patients with microvascular complications in type 1 diabetic patients, but they didn't compare diabetics with non diabetics. No other studies compared MMP-10 levels in diabetics and non diabetics.

In our study we found that HDL is significantly higher in hemodialysis group than both control and CKD groups. This may be explained that plasma HDL cholesterol levels do not always accurately predict HDL function including reverse cholesterol transport and modulation of inflammation. In healthy individuals HDL is anti inflammatory in the absence of systemic oxidative stress and inflammation. In those with chronic illnesses such as renal failure HDL may become dysfunctional and actually promote inflammation [17].

We found that MMP-10 is significantly higher in patients on dialysis compared with those on other stages CKD group and the control group. It was also significantly higher when comparing CKD group and controls. We found a significant positive correlation between MMP-10 and creatinine in all groups. This is in agreement with **Coll et al., 2010** [4] as they observed that serum MMP-10 is abnormally increased in CKD patients especially patients on dialysis.

Also we found that CIMT as a marker of atherosclerosis is significantly increased in patients on dialysis compared with those on other stages of CKD and also significantly higher in CKD group not on dialysis in comparison with the controls. We found significant positive correlation between CIMT and creatinine in the study population. These findings are in agreement with other studies [18, 19] that had demonstrated the strong and independent associations between CVD and CKD people with end stage renal failure.

In our study we found a significant positive correlation between CIMT and MMP-10. This result was in agreement with **ORBE et al., 2007** [8] as they found an independent association of matrix metalloproteinase-10, cardiovascular risk factors and subclinical atherosclerosis. As raised serum MMP-10 levels which are associated both with increased carotid IMT as well as with the presence of carotid plaques, Circulating MMP-10 may be useful to identify subclinical atherosclerosis in subjects free from cardiovascular disease.

This means that MMP-10 still correlates with atherosclerotic burden even in renal impairment patients. This may indicate that MMP-10 may represent a new biomarker of atherosclerosis in this clinical setting.

Conclusion: Atherosclerosis is more common in patients with chronic kidney disease than in patients with normal kidney function, and more common in dialysis population. MMP-10 can be used as a marker of atherosclerosis in patient with different stages of chronic kidney. This warrants further studies of MMP-10 targeted therapy in high risk patients for atherosclerosis.

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