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Variants of MMP-9 and TIMP-1 Levels Could be a Predictor of an Early Development of Cardiovascular Diseases in Type 2 Diabetes among Iraqi Patients

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Abstract

Dysregulation of matrix metalloproteinases-9 (MMP-9) and tissue inhibitors of matrix metalloproteinases-1 (TIMP-1) may contribute to the development of cardiovascular diseases in type 2 diabetes mellitus (T2DM) patients. The aim of this study was to determine the effects of chronic hyperglycemia on serum concentrations of MMP-9 and TIMP-1of T2DM patients without dyslipidemia (one of atherosclerosis risk factors) and with duration less than 5 years in comparison with T2DM patients with dyslipidemia and with duration more than 10 years and controls. Also to investigate if serum levels of MMP-9 and TIMP-1 could be potential markers for early detection of the development of cardiovascular complications in T2DM patients without dyslipidemia. This study consisted of 24 T2DM patients without dyslipidemia, 30 T2DM patients with dyslipidemia, and 26 healthy subjects. A variety of inflammatory markers including: MMP-9, TIMP-1, IL18 and hs-CRP were compared among the three groups. The BMI was similar among the three groups. A significant increase of WHR, WHtR, FPG, TC, TG, LDL-C, VLDL-C, AIP, atherogenic ratio-1, atherogenic ratio-2, hs-CRP, MMP-9, TIMP-1 and IL-18 with a significant decrease of HDL-C, β Cell% and S% among the three groups. MMP-9 of T2DM patients without dyslipidemia and with duration less than 5 years showed a significant positive correlation with FPG and a significant negative correlation with TC. MMP-9 of T2DM patients with dyslipidemia and with duration more than 10 years showed significant negative correlation with LDL-C. TIMP-1 of T2DM patients with dyslipidemia and with duration more than 10 years showed a significant negative correlation with TC, TG, VLDL-C and atherogenic ratio-1. The significant increased levels of both MMP-9 and TIMP-1 in T2DM patients without dyslipidemia and with duration less than 5 years compared to controls showed that those patients have risk factor for cardiovascular complications. This study suggests that MMP-9 and TIMP-1 may be potentially useful as markers in T2DM patients at risk of progression of cardiovascular diseases.

Keywords: MMP-9; TIMP-1; T2DM; dyslipidemia; cardiovascular disease.

التغيرات في مستويات MMP-9 و TIMP-1 الالتهابية ترتبط مع مخاطر امراض القلب الوعائية في السكري من النوع الثاني بين المرضى العراقيين

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الخلاصة

الخلل في تنظيم matrix metalloproteinases-9 (MMP-9) و tissue inhibitors of matrix metalloproteinases-1 (TIMP-1) قد تسهم في تطور امراض القلب الوعائية في مرضى السكري من النوع الثاني.الهدف من هذه الدراسة هو تحديد تأثيرات ارتفاع السكر المزمن في الدم على التراكيز المصلية ل MMP-9 و TIMP-1 لمرضى السكري من النوع الثاني بدون اضطراب دهون الدم (واحد من عوامل الخطر للاصابة بتصلب الشرايين) وكانت مدة المرض اقل من ٥ سنوات بالمقارنة مع مرضى السكري من النوع الثاني مع اضطراب دهون الدم وكانت مدة المرض اكثر من ١٠ سنوات ومقارنتها مع مجموعة الاصحاء. أيضا للتحقق فيما اذا المستويات المصلية ل MMP-9 و TIMP-1 قد تكون علامات محتملة للكشف المبكر عن تطور مضاعفات القلب الوعائية لمرضى السكري من النوع الثاني بدون اضطراب دهون الدم. هذه الدراسة تضمنت (٢٤مريض) سكري من النوع الثاني بدون اضطراب دهون الدم، (٣٠مريض) سكري من النوع الثاني مع اضطراب دهون الدم، و(٢٦) اشخاص اصحاء. مجموعة متنوعة من العلامات الالتهابية تتضمن: IL18 MMP-9,TIMP-1,وhs-CRP تمت مقارنتها بين المجاميع الثلاثة. قيم الBMI كانت متشابهة بين المجاميع الثلاثة. هذه الدراسة اضهرت زيادة معنوية لكل من WHR، WHtR، FPG, TC, TG, LDL-C,VLDL-C، AIP، atherogenic ratio-1، atherogenic ratio-٢، hs-CRP، MMP-9, TIMP-1و IL-18 واظهرت نقصان معنوي في HDL-C، β Cell% و S% ضمن المجاميع الثلاثة. MMP-9لمرضى السكري من النوع الثاني وبدون اضطراب دهون الدم ومع مدة مرض اقل من ٥ سنوات اضهرت ارتباط معنوي موجب مع FPG وارتباط معنوي سالب مع TC. MMP-9 لمرضى السكري من النوع الثاني ومع اضطراب دهون الدم ومع مدة مرض اكثر من ١٠ سنوات اضهرت ارتباط معنوي سالب مع LDL-C. TIMP-1لمرضى السكري من النوع الثاني ومع اضطراب دهون الدم ومع مدة مرض اكثر من ١٠ سنوات اضهرت ارتباط معنوي سالب مع TC, TG، VLDL-C، atherogenic ratio-1. الزيادة المعنوية في المستويات المصلية لكل من TIMP1,MMP-9 لمرضى السكري من النوع الثاني بدون اضطراب دهون الدم ومع مدة المرض اقل من ٥ سنوات بالمقارنة مع الاصحاء اظهرت ان هؤلاء المرضى لديهم عامل الخطر لمضاعفات القلب الوعائية. اقترحت هذه الدراسة ان قياس مستويات MMP-9 و TIMP-1 قد تكون مفيدة كدلالات في مرضى السكري من النوع الثاني المعرضين لخطر تطور أمراض القلب الوعائية.

Introduction

Cardiovascular complications are the leading cause of increasing premature mortality in diabetic patients despite the progress in the diagnosis and the treatment of this disease [1]. Matrix metalloproteinases (MMPs) are members of a family of Zn^{+2} and Ca^{+2} dependent endopeptidases, which are essential for cellular migration and tissue remodeling in both physiological and pathological conditions [2]. MMPs are secreted by many types of cells as pro-enzymes. On activation by proteolytic cleavage, activated enzymes are capable of degrading many extracellular matrix (ECM) components [3]. It has been proven that MMPs play an important role in atherosclerosis and the rebuilding of vascular wall [4]. The relationship between MMPs and their inhibitors (tissue inhibitors of matrix metalloproteinases – TIMPs) and diabetic angiopathy is less well defined .Hyperglycemia directly or indirectly (e.g., via oxidative stress or advanced glycation products) might increase MMP expression and activity in large vessels [5]. Among MMPs, MMP-9 (92 KDa) is capable of degrading gelatins, fragments of collagens degraded by collagenases, and type IV collagen, which forms basement membranes [6]. The role of MMP-9 in cardiovascular disease is supported by genetic study that showed that variations in the MMP-9 gene were related to the presence and severity of atherosclerosis [7]. Circulating MMP-9 levels are increased in type 2 diabetes mellitus (T2DM) patients with coronary artery disease [8]. Within atherosclerotic plaques an imbalance between MMP-9 and TIMP-1 may induce matrix degradation, resulting in an increased risk of plaque rupture [9]. Hyperglycemia associated with T2DM can lead to inflammation [10]. Inflammation is the major pathogenetic mechanism associated with the vascular complications of T2DM [11,12]. Interleukin-18 (IL-18), as a pro-inflammatory cytokine, is involved in the development and progression of both

atherosclerosis and T2DM [13]. IL-18 induces the production of tumor necrosis factor (TNF)-α, which in turn promotes the synthesis and release of IL-6 and C-reactive protein (CRP) [14].
Low high density lipoprotein (HDL-C) and high triglyceride (TG) concentrations have been separately discussed as possible independent predictors of cardiovascular disease, the combination of these 2 conditions, also called atherogenic dyslipidemia may be even more deleterious. Such atherogenic dyslipidemia profile is particularly prevalent in patients with T2DM, obesity, metabolic syndrome, and/or established cardiovascular disease—a number of conditions associated with a high vascular risk [15, 16].

The aim of this study is to determine the effects of chronic hyperglycemia in T2DM patients (without dyslipidemia and with duration less than 5 years) on serum MMP-9 and TIMP-1 concentrations compared with T2DM patients with dyslipidemia and duration more than 10 years and control group, also to investigate if serum MMP-9 and TIMP-1 levels could be used as potential markers for early detection of the development of cardiovascular complications in T2DM patients.

Materials and methods

StudyPopulation

Patients enrolled in the present study were subdivided to with or without dyslipidemia, which is one of the risk factors for atherosclerosis (HDL-C $\leq$40 mg/dL and TG $\geq$150 mg/dL) [16]. Iraqi T2DM patients (24) without dyslipidemia (group P1) with diabetes duration 2.47±1.85 years and (30) T2DM patients with dyslipidemia (group P2) with diabetes duration 11.40±1.81 years were selected from National Diabetes Center for Treatment and Research, Al-Mustansiriya University, Baghdad, Iraq from October 2013 to March 2014 with the ages ranging from 40 to 63 years. All T2DM patients were diagnosed based on their medical history according to the American Diabetes Association criteria [17]. Healthy subjects (26) with the ages ranging from 42 to 62 years were used as control (group C). All participants gave written informed consent and the exclusion criteria include: Cushing's disease, acromegaly , chronic pancreatitis, pancreatactomy , pregnancy, history or even manifestation of nephropathy, chronic renal failure, malignancies and chronic or acute inflammatory disease, also patients who were taking aspirin, lipid lowering therapy and insulin therapy, history of smoking or alcohol drinking were excluded.

The systolic (SBP) and diastolic (DBP) blood pressure (mmHg) were measured by mercury sphygmomanometer with adult cuff in sitting position. Venous blood samples were collected after overnight fasting between 08.00 and 11.00 a.m. The blood sample was divided into two aliquots; 2 and 8 ml. The first aliquot was dispensed for tube containing ethylene diamine tetra acetic acid and used for estimation of fasting plasma glucose (FPG), insulin level and lipid profile. The second aliquot was dispensed in a plain tube and left to clot at room temperature. Then, it was centrifuged at 3000 rpm to collect serum. The serum was divided into aliquots (250µl) in Eppendorff tubes and stored at (-20ºC) until used. Anthropometrical indexes: these include: body mass index (BMI), waist to hip ratio (WHR), and waist to height ratio (WHtR) were calculated.

Laboratory Methods

FPG and lipid profile (total cholesterol TC and TG) were measured by enzymatic colorimetric methods with commercially available kits from SPINREACT Company, Spain.
(HDL-C) was measured using kit from Randox Company, U.K. Fasting plasma Insulin (FPI) was measured by enzyme linked immunosorbent assay (ELISA) [DRG Company (Germany)]. HOMA2 parameters [Insulin resistance (HOMA2-IR), beta cell% (β cell%) and sensitivity% (S %)] were calculated using HOMA2 calculator [18]. MMP-9, TIMP-1, IL-18, and hs- CRP (Intra- and inter-assay coefficients of variation were CV<10% and CV<12% respectively), were measured by (ELISA) kits from RayBiotech. Company, USA. Atherogenic Index of Plasma (AIP) was determined using the equation of Dobiášová [19]: AIP =Log (Tg/HDL-C), while Atherogenic ratios were determined using the equation of Georgiadis [20]: Atherogenic ratio-1= (TC/HDL-C) and Atherogenic ratio-2= (LDL-C/HDL-C).

All experimental procedures involving human participants were conducted with due attention to the guidelines approved by the research ethical committee at National Diabetes Center for Treatment and Research at Al-Mustansiriya University.

Statistical analysis

Statistical Package for the Social Sciences (SPSS), version 16.0 for windows software (SPSS Inc., Chicago, Ill, USA) was used for statistical analysis. The data were normally distributed and were

expressed as mean ± standard deviation (SD). Statistical tests were carried out by one-way analysis of variance (ANOVA) followed by post hoc test. A difference among groups was defined to be statistically significant if the corresponding p-value was less than 0.05. Correlations between variables were determined by Pearson correlation coefficients (*r*-values).

To assess the diagnostic accuracy of the MMP-9, TIMP-1, IL-18, and hs- CRP for predicting the risk of atherosclerosis in diabetic patients, receiver operating characteristic (ROC) plots [21] were performed. The area under the ROC curve (AUC) and 95% confidence interval were calculated for each plot.

Results

Anthropometric indexes& lipid profile

The summary of the data of the groups (C), (P1) and (P2) are shown in Table-1. The BMI was similar among the three groups. The WHR and WHtR showed a significant difference among the three groups. The lipid profile showed a significant difference in the TC, TG, HDL-C, LDL-C, VLDL-C, AIP, atherogenic ratio-1 and atherogenic ratio-2 among the three groups.

Comparison of inflammatory markers among the three groups

As shown in Table (1), The inflammatory markers such as MMP-9, TIMP-1, IL-18 and hsCRP increased significantly in group (P1) and group (P2) in comparison with group (C) Figure-1. TIMP-1of group (P2) increased significantly in comparison with group (P1). MMP-9/TIMP-1 ratio increased in group (P1) as compared to group (C) but this difference was not statistically significant. While this ratio in group (P2) as the same as of group (C).

Table 1- The differences of various parameters among the three groups (C), (P1) and (P2).

Parameter	C (n=26) Mean (±SD)	(P1)(n=24) Mean (±SD)	(P2)(n=30) Mean (±SD)	P value
Gender(male/female)	17/9	16/8	20/10	0.832
age(year)	51.54±75	53.33±6.81	55.20±5.27	0.501
Diabetes duration(year)	-----	2.47±1.85^{***a}	11.40±1.81^{***b,c}	0.000
BMI(Kg/m^2)	26.38±1.68	26.83±1.79	26.88±1.83	0.661
WHR	0.93±0.05	0.98±0.05^{***a}	0.99±0.03^{***b}	0.034
WHtR	0.56±0.04	0.59±0.04	0.60±0.06^{**b}	0.047
SBP mmHg	119.96±2.86	120.42±2.10	120.67±3.65	0.723
DBP mmHg	79.96±0.34	80.00±0.42	80.50±2.08	0.543
Drugs				
Aspirin	٠	٠	٠	
Statin	٠	٠	٠	
Diet alone	-	5	٠	
Metformin	-	9	8	
metformin and sulfonyl urea	-	10	22	
Antihypertensive medication	-	٠	٣٠	
FPI (µlU/ml)	7.24±5.90	15.53±10.84^{*a}	11.75±7.94	0.017
FPG (mg/dl)	87.46±7.96	158.71±47.43^{***a}	224.27±72.83^{***b,c}	0.000
β Cell%	95.57±50.77	64.65±44.85^{*a}	23.10±11.20$^{***b, **c}$	0.000
%S	148.94±67.33	63.79±50.76^{***a}	53.40±21.00^{***b}	0.000
HOMA2-IR	0.93±0.74	2.27±1.40^{*a}	1.90±0.90^{*b}	0.011
TC (mg/dl)	148.85±18.86	157.25±22.94	225.80±33.29^{***b,c}	0.000

TG (mg/dl)	92.50±17.24	118.79±26.29	218.67±134.10^{***b,c}	0.000
HDL-C(mg/dl)	54.04±8.43	51.75±7.09	38.50±1.44^{***b,c}	0.000
LDL-C(mg/dl)	77.12±17.70	82.38±20.91	139.57±22.49^{***b,c}	0.000
VLDL-C(mg/dl)	18.96±4.48	23.79±5.18	43.73±26.84^{***b,c}	0.000
AIP	0.23±0.10	0.36±0.13	0.66±0.30^{***b,c}	0.000
atherogenic ratio-1	2.77±0.52	3.09±0.58	5.51±1.34^{***b,c}	0.000
atherogenic ratio-2	1.46±0.41	1.63±0.47	3.37±0.66^{***b,c}	0.000
hs-CRP (mg/l)	1.51±0.78	2.75±1.53^{**a}	3.27±1.55^{***b}	0.000
MMP-9(ng/ml)	1203.41±357.83	1455.73±281.61^{*a}	1476.75±260.81^{**b}	0.002
TIMP-1 (ng/ml)	669.65±95.72	739.08±112.36^{*a}	817.13±69.44$^{***b, **c}$	0.000
MMP-9/TIMP-1 ratio	1.82±0.64	2.03±0.59	1.82±0.36	0.267
IL-18(pg/ml)	175.97±44.36	238.70±50.11^{**a}	274.83±90.02^{***b}	0.000

Data were expressed as mean ±SD.
BMI: body mass index; WHR: waist to hip ratio ;WHtR: waist to height ratio; FPG: fasting plasma glucose; FPI: fasting plasma insulin; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglycerides ;LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein-cholesterol; VLDL-C: very low density lipoprotein-cholesterol; HOMA: homeostasis model assessment ; Atherogenic Index of Plasma (AIP):[Log(TG/HDL-C)]; Atherogenic ratio-1: (TC/HDL-C) ; Atherogenic ratio-2: (LDL-C/HDL-C); hs-CRP: high sensitivity C-reactive protein ; MMP-9: matrix metalloproteinase-9; TIMP-1: tissue inhibitors of metalloproteinase-1.
Statistical tests were carried out by one-way analysis of variance (ANOVA) followed by post hoc test.
* p<0.05; **p<0.01; ***p<0.001; no asterisk: P>0.05.
[a] indicate significant difference between groups (C) and (P1) based on multiple comparison.
[b] indicate significant difference between groups (C) and (P2) based on multiple comparison.
[c] indicate significant difference between groups (P1) and (P2) based on multiple comparison.

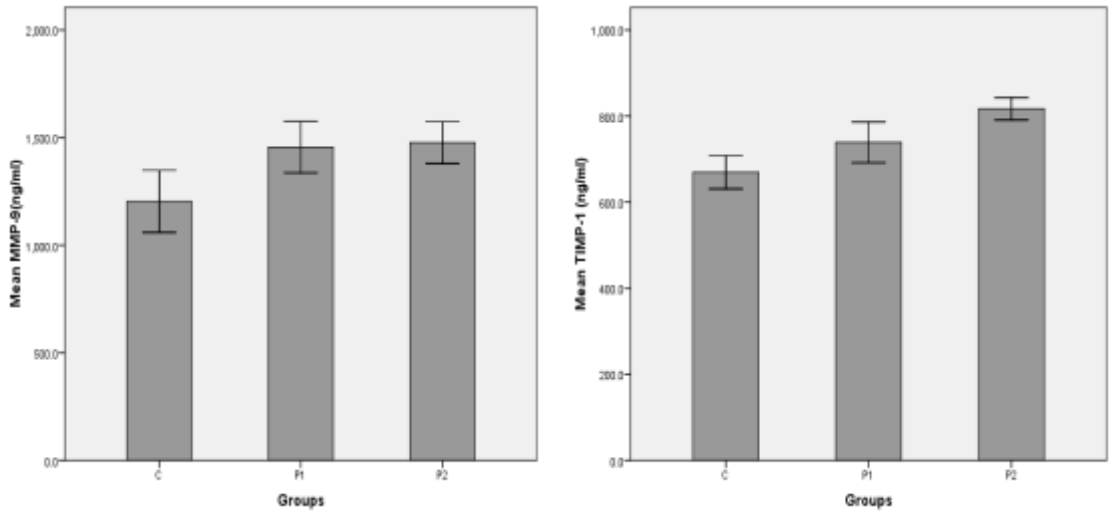

Figure 1-Comparison of inflammatory markers MMP-9 and TIMP-1 among the three groups (C), (P1) and (P2).

Correlations of inflammatory markers

MMP-9 of group (P1) showed a significant positive correlation with FPG and a significant negative correlation with TC, as shown in Table-2 and Figure-2. hs-CRP showed a significant positive correlation with weight and a significant negative correlation with DBP. MMP-9 of group (P2), as shown in Table-3 and Figure-3, showed a significant positive correlation with weight, height, DBP and a significant negative correlation with LDL-C. TIMP-1 showed a significant negative correlation

with TC, TG, VLDL-C and atherogenic ratio-1 Figure-4. IL-18 showed a significant positive correlation with BMI, waist, hip, WHtR, TG, VLDL-C, HOMA2-IR, AIP, atherogenic ratio-1 and showed a significant negative correlation with HDL-C.

Table 2-The Pearson correlation analysis of group (P1).

Parameter	hs-CRP (mg/L)		MMP-9(ng/ml)		TIMP-1 (ng/ml)		IL-18(pg/ml)	
	r	p	r	p	r	p	r	P
weight(Kg)	0.522**	0.009	-0.089	0.679	0.040	0.851	0.208	0.331
Height(cm)	-0.138	0.521	0.101	0.638	-0.054	0.802	0.334	0.111
BMI(Kg/m2)	0.212	0.319	-0.218	0.307	0.055	0.800	0.067	0.756
waist(cm)	0.090	0.677	0.102	0.637	-0.067	0.756	0.175	0.413
Hip(cm)	0.156	0.467	0.218	0.306	-0.318	0.130	-0.038	0.862
WHR	-0.040	0.854	-0.113	0.600	0.302	0.151	0.288	0.173
WHtR	0.114	0.596	0.043	0.841	-0.053	0.805	-0.003	0.987
SBP mmHg	0.034	0.875	-0.012	0.956	-0.356	0.088	-0.269	0.203
DBP mmHg	-0.491*	0.015	-0.162	0.449	0.082	0.703	-0.055	0.797
FPI(µlU/ml)	0.186	0.385	0.113	0.599	0.213	0.317	0.188	0.378
FPG(mg/dl)	0.176	0.412	0.554**	0.005	-0.265	0.211	0.231	0.277
β Cell%	-0.095	0.658	-0.144	0.503	0.188	0.379	0.026	0.903
%S	-0.353	0.091	-0.208	0.329	0.146	0.497	-0.114	0.594
HOMA2-IR	0.219	0.305	0.279	0.186	0.140	0.515	0.306	0.146
TC(mg/dl)	-0.082	0.703	-0.418*	0.042	-0.174	0.417	-0.197	0.356
TG (mg/dl)	-0.026	0.903	-0.180	0.400	0.107	0.619	0.047	0.826
HDL-C(mg/dl)	0.192	0.369	-0.215	0.313	0.114	0.597	-0.160	0.456
LDL-C(mg/dl)	-0.195	0.362	-0.364	0.08	-0.236	0.266	-0.177	0.407
VLDL-C(mg/dl)	-0.021	0.924	-0.175	0.413	0.107	0.618	0.061	0.779
AIP	-0.095	0.659	-0.044	0.839	0.020	0.925	0.102	0.635
atherogenic ratio-1	-0.208	0.329	-0.135	0.529	-0.207	0.333	-0.037	0.864
atherogenic ratio-2	-0.254	0.232	-0.178	0.406	-0.241	0.256	-0.079	0.714
hs-CRP (mg/L)	1	—	0.025	0.908	0.112	0.603	-0.091	0.672
MMP-9(ng/ml)	0.025	0.908	1	—	-0.229	0.281	0.347	0.097
TIMP-1 (ng/ml)	0.112	0.603	-0.229	0.281	1	—	0.142	0.508
MMP-9/TIMP-1 ratio	-0.079	0.715	0.795***	0.000	-0.749***	0.000	0.117	0.586
IL-18(pg/ml)	-0.091	0.672	0.347	0.097	0.142	0.508	1	—

* p<0.05; ** p<0.01; *** p<0.001; no asterisk: P>0.05.

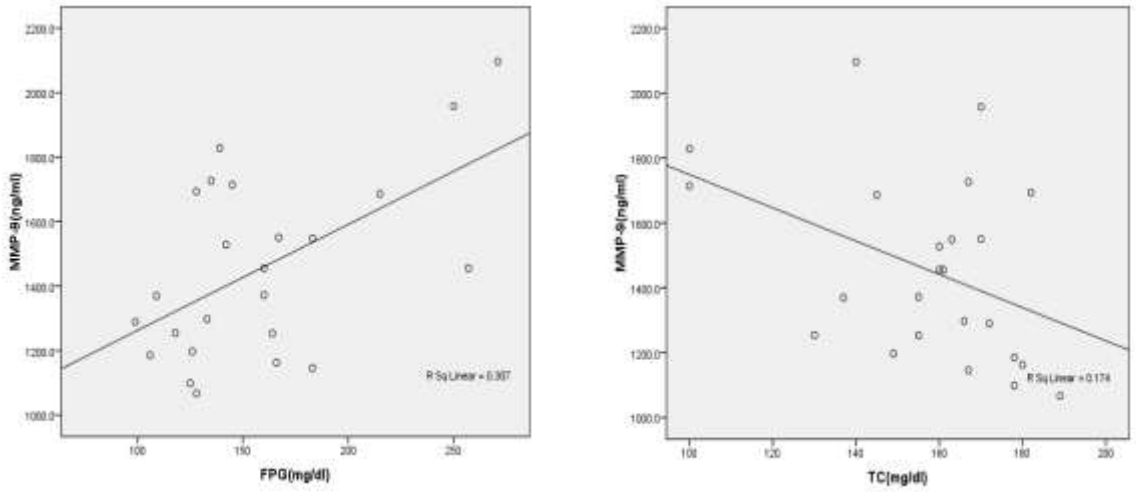

Figure 2-The significant correlation of serum MMP-9 of group (P1) with (a) FPG and (b) TC.

Table 3-The Pearson correlation analysis of group (P2).

Parameter	hs-CRP (mg/L)		MMP-9(ng/ml)		TIMP-1 (ng/ml)		IL-18(pg/ml)	
	r	p	r	p	r	p	r	P
weight(Kg)	-0.059	0.756	0.389*	0.034	0.180	0.340	0.294	0.115
Height(cm)	-0.010	0.959	0.518**	0.003	0.164	0.386	-0.006	0.974
BMI(Kg/m2)	-0.040	0.834	-0.106	0.579	-0.060	0.753	0.442*	0.014
waist(cm)	0.190	0.314	-0.015	0.938	0.071	0.708	0.468**	0.009
Hip(cm)	0.266	0.155	-0.057	0.765	0.136	0.473	0.437*	0.016
WHR	-0.255	0.173	0.127	0.502	-0.171	0.366	0.055	0.772
WHtR	0.190	0.314	-0.257	0.171	0.005	0.979	0.448*	0.013
SBP mmHg	0.090	0.635	0.050	0.791	-0.053	0.781	0.164	0.386
DBP mmHg	-0.155	0.413	0.423*	0.020	0.247	0.188	-0.300	0.107
FPI(µlU/ml)	0.168	0.375	0.267	0.153	-0.047	0.803	0.340	0.066
FPG(mg/dl)	-0.006	0.977	0.177	0.349	-0.051	0.788	0.074	0.696
β Cell%	0.200	0.289	0.014	0.940	-0.172	0.365	0.238	0.205
%S	-0.207	0.272	-0.257	0.171	0.216	0.251	-0.353	0.056
HOMA2-IR	0.126	0.508	0.280	0.134	-0.141	0.457	0.373*	0.042
TC(mg/dl)	-0.241	0.199	-0.270	0.149	-0.440*	0.015	0.059	0.758
TG (mg/dl)	-0.273	0.145	-0.028	0.885	-0.373*	0.042	0.372*	0.043
HDL-C(mg/dl)	0.187	0.321	0.035	0.856	0.076	0.691	-0.451*	0.012
LDL-C(mg/dl)	-0.102	0.592	-0.379*	0.039	-0.241	0.200	-0.187	0.322
VLDL-C(mg/dl)	-0.273	0.144	-0.028	0.882	-0.368*	0.045	0.371*	0.044
AIP	-0.332	0.073	0.015	0.937	-0.253	0.177	0.429*	0.018
atherogenic ratio-1	-0.281	0.132	-0.147	0.439	-0.369*	0.045	0.387*	0.035
atherogenic ratio-2	-0.234	0.213	-0.300	0.107	-0.333	0.073	0.264	0.158
hs-CRP (mg/L)	1	—	-0.247	0.188	-0.046	0.808	0.352	0.057
MMP-9(ng/ml)	-0.247	0.188	1	—	-0.072	0.707	-0.207	0.273
TIMP-1 (ng/ml)	-0.046	0.808	-0.072	0.707	1	—	-0.265	0.157
MMP-9/TIMP-1 ratio	-0.175	0.356	0.892***	0.000	-0.500**	0.005	-0.100	0.600
IL-18(pg/ml)	0.352	0.057	-0.207	0.273	-0.265	0.157	1	—

*p<0.05; **p<0.01; ***p<0.001; no asterisk: P>0.05.

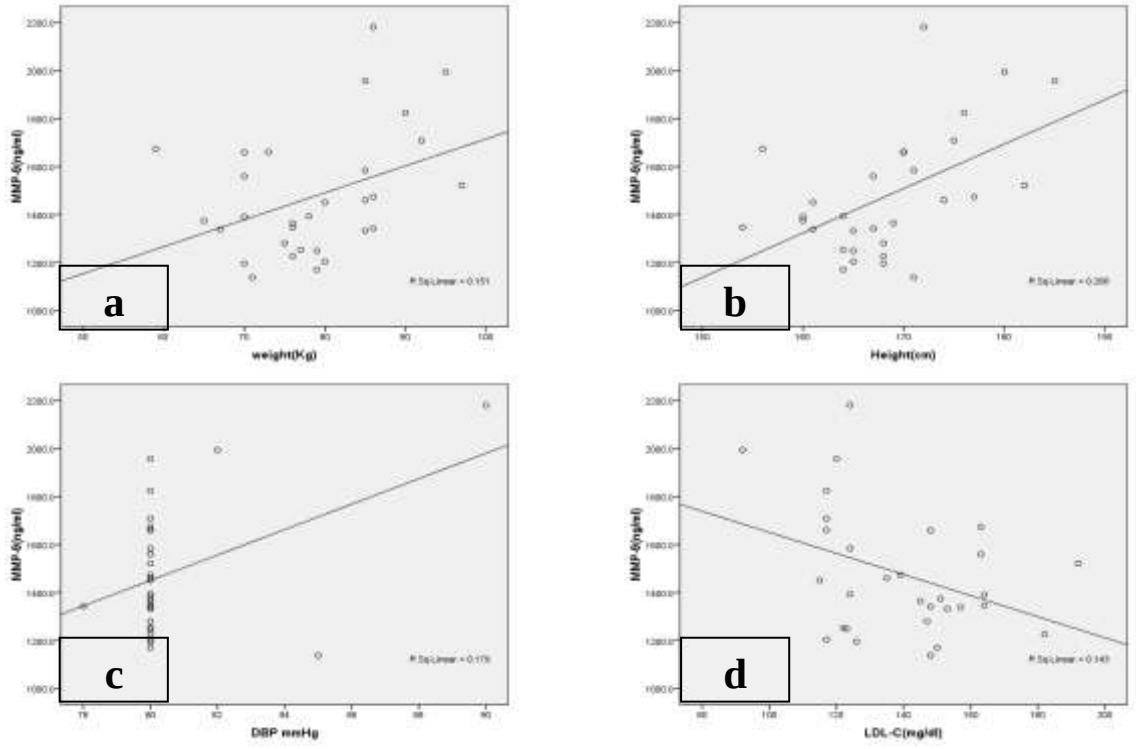

Figure 3-The significant correlation of serum MMP-9 of group (P2) with (a) weight, (b) height, (c) DBP and (d) LDL-C.

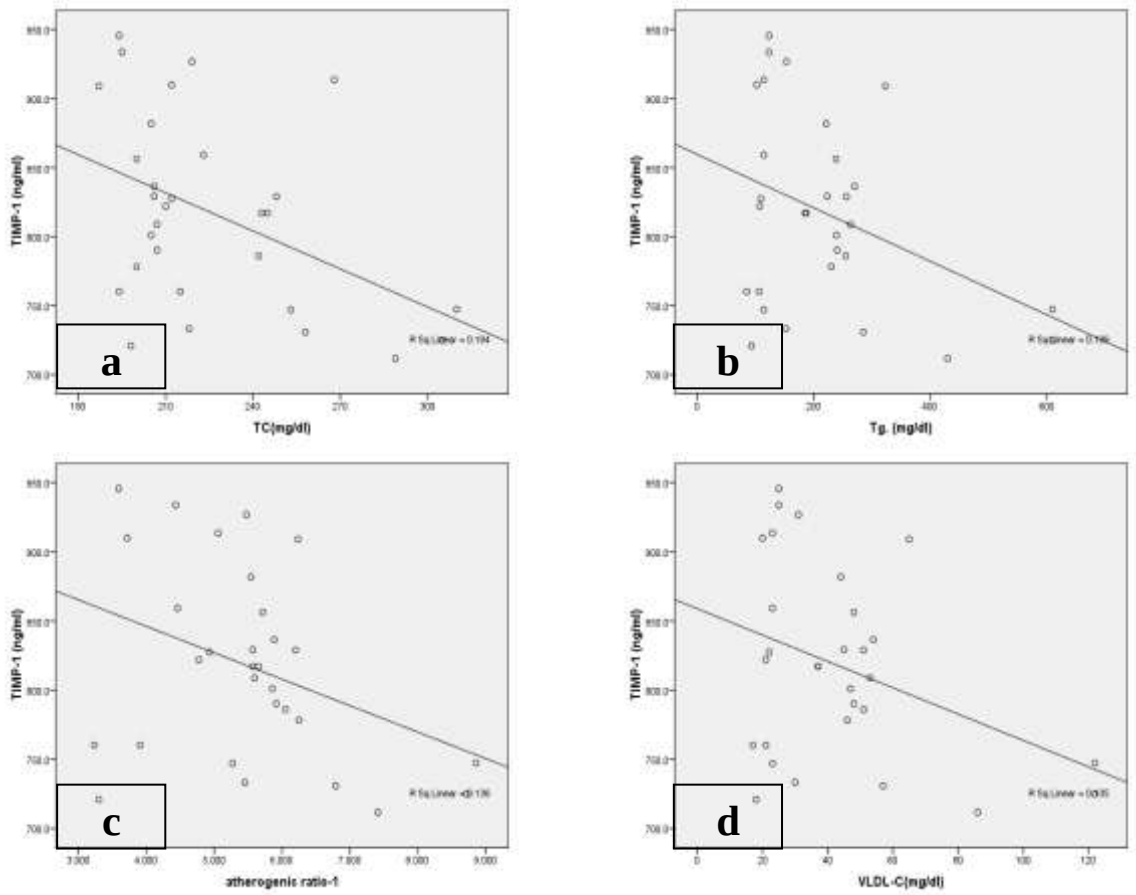

Figure 4-The significant correlation of serum TIMP-1 of group (P2) with (a) TC, (b) TG, (c) atherogenic ratio-1 and (d) VLDL-C.

ROC analyses

Comparison of AUC showed that TIMP-1 had the highest diagnostic values for detection between groups (C) & (P1), (C) & (P2) and (P1) & (P2) and the MMP-9 had the lowest values as shown in Table-4.

Table 4-The Areas under the ROC curves (AUC).

Parameter	groups (C) & (P1)	groups (C) & (P2)	groups (P1) & (P2)
MMP-9(ng/ml)	0.697*	0.753**	0.524
TIMP-1 (ng/ml)	0.703*	0.922***	0.702*

Values are areas under the ROC curves (AUC). * $p<0.05$; ** $p<0.01$; *** $p<0.001$; no asterisk: $P>0.05$.

Discussion

High glucose significantly amplifies MMPs expression and activity, leading to an upset in the balance between ECM synthesis and degradation [22]. Rupture prone atherosclerotic lesions are characterized by the accumulation of activated mononuclear inflammatory cells (MNCs) and their increased expression of MMPs [23]. In MNCs, MMP-9 is strongly inducible by a number of inflammatory mediators, including TNF-α and oxidized LDL [24]. Studies suggest that interaction of primary monocytes with ox-LDL and proinflammatory cytokines may contribute to vascular remodeling and plaque rupture [25]. MMP-9 appears to participate in several stages of atherosclerosis. Matrix degradation is a prerequisite for monocyte recruitment because cell transmigration across the endothelium requires disruption of the basement membrane [26].As shown in Table-1, serum levels of MMP-9, TIMP-1, IL-18 and hs-CRP increased in groups (P1) and (P2) in comparison with group (C) that may confirm the inflammatory state in these groups. Although there was no significant difference between groups (P1) and (P2) (except TIMP-1), but an increase in their levels as the duration of the disease increased was found.

TIMP-1 showed significant increase in group (P2) in comparison with groups (P1) and (C). This may be explained that this increase is to inhibit the increased levels of MMP-9.

In this study, serum levels of MMP-9 were higher in patients than in control groups. These findings were in line with previous study, who they concluded that the expression and the gelatinolitic activity of MMP-9 were induced by high glucose exposure [27]. Another study observed that MMP-9 activity was enhanced in diabetic rats [22]. Our finding was also in agreement with previous studies [28a-30]. In this study we demonstrated that serum TIMP-1 concentration is significantly higher in diabetic patients as compared to control group. These data can confirm what has already been observed in the previous studies [30, 31], [28a], who they found a significant increased in TIMP-1 levels in patients with T2DM in comparison with controls.

It was suggested that MMP-9 is released from blood vessels into the bloodstream in T2DM to a greater extent than in healthy individuals, which may contribute to increased chronic local inflammation of blood vessels among T2DM. Additionally, the endothelium in blood vessels from diabetics may activate MMP-producing cells in the circulation [32].

Another issue that warrants discussion is the possible relationship between MMP-9 and insulin. Our data do not provide convincing support for a significant direct effect of either acute or chronic hyperinsulinemia on serum MMP-9. These findings were in agreement with previous study on MMP-9 in women with polycystic ovary syndrome [33]. Our results were also in line with another study, who they reported either lack of a direct correlation between MMP-9 and HOMA-IR [28b], or just a relatively weak correlation between plasma MMP-9 and fasting insulin ($r2 = 0.16$, $p = 0.04$) [34]. There were, however, studies that demonstrated an increase in free MMP-9 (zymographic method) in aortic tissue of male rats during euglycaemic hyperinsulinaemic clamp [35], or in monocytes of hyperinsulinaemic and obese mice [36].

As shown in Table-2, serum MMP-9 levels of group (P1) showed a significant positive correlation with FPG ; a finding that shares the demonstration of investigator who reported that a hyperglycemic environment could up-regulate the mRNA expression of MMP-9 in tendon cells [37].

These findings may explain, in part, the molecular mechanisms underlying diabetes associated CVD. Serum TIMP-1and MMP-9 levels were higher in group (P2) as compared to group (P1). The MMP-9/TIMP-1 ratio is an independent predictor of the stability of atherosclerotic plaque and the severity of coronary atherosclerosis [38]. In this study, the MMP-9/TIMP-1 ratio increased in group (P1) in comparison to groups (C) and (P2) which may be due to increased levels of TIMP-1 to counter the increased levels of MMP-9. Our finding was in agreement with previous study [30]. It was suggested that circulating IL-18 level is a useful biomarker for atherosclerosis prone patients with metabolic syndrome [39]. Another study showed that IL-18 induces smooth muscle cell (SMC) migration through the induction of activator protein-1(AP-1) and necrosis factor (NF-κB) mediated MMP9 expression [40].

The present study showed significant increase in serum IL-18 and hs-CRP levels in group (P1) as compared to group (C) and this may reflect the chronic inflammation in group (P1) and propagation of atherosclerosis. This occur in accompanied with significant increase in serum MMP-9 and TIMP-1 levels in group (P1) as compared to group (C) and as shown in Table-2, there is association between hyperglycemia and MMP-9 in group (P1).

5. Conclusion

The results showed that serum levels of MMP-9, TIMP-1, IL-18 and hs-CRP increased in diabetic patients without dyslipidemia and with duration less than 5 years group (P1) as compared to healthy subjects and this may reflect abnormal ECM metabolism. This lead to suggest that serum levels of MMP-9 and TIMP-1 may be potential predictor of early development of cardiovascular complications in diabetic patients.

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