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Evaluation of Serum Levels of Tissue Inhibitor Metalloproteinase-1and Matrix Metalloproteinase-1 in Egyptian Patients with Diabetic Nephropathy

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We aimed to evaluate the levels of serum tissue inhibitor metalloproteinase-1 (TIMP-1) and matrix metalloproteinase-1 (MMP-1) in patients with type 2 diabetes (T2D)alone and in diabetic nephropathy(DN) and assess their relation with other clinical features. This study was conducted on 30 patients withT2D, 30 with diabetic nephropathy (DN) and 50 age- corresponded healthy individuals. Serum MMP-1 and TIMP-1 were measured with ELISA in patients and controls. Elevated serum concetrations of TIMP-1 and MMP-1 were recognized in DN group compared to patients with T2D alone as well as controls. Positive correlation was also observed between MMP-1, TIMP-1, FBG and HbA1c in DN group. In multiple linear regression analysis HBLA1c, TG and low HDL were selected as components significantly related to MMP-1 and TIMP-1 in DN patients. in conclusion, serum MMP-1 and TIMP-1were significantly increased in DN. Further, in DM type 2 both markers were not significantly increased than controls. These blood biomarkers are associated with DN and might be salutary in clinical discrimination of DN in patients with type 2 diabetes.

Keywords: DN; HbA1c; MMP-1; TIMP-1.

Matrix metalloproteinases (MMPs) are a family of zinc and calcium dependent proteolytic enzymes that mediate the degradation of extracellular matrix [12]. The balance between metalloproteinase-1 (MMP-1) and tissue inhibitor metalloproteinase-1 (TIMP-1) is an imperative controller point in tissue remodeling and an imbalance could encourage destruction of tissue. Evidence regarding altered MMP and TIMP activity in diabetes mellitus remains ambiguous. Moreover, although MMPs and TIMPs were investigated in the search for a possible role in diabetic nephropathy, they were not studied previously in the particular Egyptian clinical setting of type 2 diabetes mellitus. In the predominance of diabetic patients, albuminuria is one of the most common and earliest indicators of kidney illness[3]. Albuminuria regression is more common

with ameliorations in triglycerides,HbA1c and blood pressure, as well as by using inhibitors of renin angiotensin aldosterone (RAAS). Another demographic predictors of regression versus progression have been discussed, comprising sex alterations, which has been debatable[4].Data are limited regarding rates of albuminuria progression and regression. Since not all diabetic persons develop all the conceivable complications of the situation, systematic screening for pertinent complains has become a chief portion of diabetes care nowadays. Early detection of complexity permits definite treatment to delay progression of a complication or extra focused preventive treatment in its early phases.

This work was designed for assessment of serum levels of MMP and TIMP in cases with type 2 diabetes with and without diabetic nephropathy and to assess the effects of diabetes on MMP-1 and TIMP-1 levels and to analyze these biomarkers to unmask early diabetic complications. Our hypothesis that high plasma levels of MMP-9 could be associated to chronic microvascular complications in persons with type 2 diabetes, as diabetes nephropathy.

SUBJECTS AND METHODS

A total of 60 patents with DM2 were recruited from outpatient clinics of National institute of diabetes and endocrinology; between December 2017 and November 2019. Their median age was 43.5 years (range, 34-50 years). There were 50 healthy volunteer controls without family history of DM2. The individual variability of these assays in a given patient did not exceed 10%.

Blood samples were collected from all fasting participants in this study, Lipemic and hemolyzed samples were excluded.

Assessment of Lipid profile

Total cholesterol (TC) and triglycerides (TG) in serum were measured by colourimetric enzymatic method[5] . Also, high-density lipoprotein cholesterol (HDL cholesterol) was measured[6] , and low-density lipoprotein cholesterol (LDL cholesterol) was evaluated by Friedewald formula [7]

Quantification of MMP-1

Serum concentrations of MMP-1determined with enzyme linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN,USA).

Quantification of TIMP-1

Serum concentrations of TIMP-1 determined with enzyme linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN,USA).

Assessment of HbA1c

HbA1c levels were evaluated by high-performance liquid chromatography (BioRad). HbA1c by cation - exchange resin method[8] .

Statistical analyses

Statistical analysis was performed using the statistical package for the social sciences, version 13 (SPSS Inc., Chicago, Illinois, USA). Data are presented as mean ± SD fornormal distribution samples, whereas the mean differences between two groups were compared by Student's *t* test, and one-way analysis of variance was applied to three groups. Pearson's correlation (correlation coefficient) test was used for testingcorrelations between variables.A *P* value less than 0.05 was considered statistically significant multiple linear regression analysis was performed to find significant determinants of both markers, including BMI, TG, HDL-C, SBP, and DBP with as independent variables. The descriptive statistics were presented as mean ± SD. Differences between groups were assessed by ANOVA for normally distributed measurement data, Wilcoxon's test for non-normally distributed measurement data. The distribution of the variables was examined using Kolmogorov–Smirnov test of normality to verify whether it is followed a Gaussian pattern.

RESULTS

Table 1 shows that serum concentration of MMP-1 in diabetic nephropathy *was considerably elevated when compared with healthy group and TIMP-1 was significantly increased when compared with control and DM2 groups.*

Table 2 displays patients and controls clinical characteristics. DN patients showed significant higher levels of BMI, HBLA1c, TG, LDL and low HDL than controls as well as compared to DM2 patients.

Table 3 shows significant positive correlations between serum levels of MMP-1, TIMP-1, FBG and HbA1c in DN group.

Table 1. Serum MMP-1andTIMP- 1levels in controls and patients

Variables	Control Median (range)	T2Dwithout DN Median (range)	DN Median (range)
MMP-1	131.78 (102-361)	141.52(201-307)	153.60 (311-767) †
TIMP-1(ng/mL)	540.84(398-497)	671.76 (599-960)	1209.26 (798-1699)†

p<0.05, vs. ControlandT2D without DN

Table 2. Patient characteristics and other clinical findings

Variables	Group	Mean	SD	P value
Age (years)	Control	45.80	1.98	0.67
	T2D without DN	46.40	2.01	
	DN	48.90	1.52	
BMI (kg/m2)	Control	20.04	3.4715	<0.001
	T2D without DN	23.66	3.83	
	DN	28.04	2.17	
HbA1c	Control	3.99	0.50	<0.001
	T2Dwithout DN	4.45	0.75	
	DN	7.60	.65	
TC (mg/dL)	Control	136.29	26.17	0.69
	T2Dwithout DN	144.84	27.89	
	DN	134.45	27.85	
TG(mg/dL)	Control	77.87	28.28	<0.03
	T2Dwithout DN	92.42	22.12	
	DN	157.48	30.54	
HDL(mg/dL)	Control	43.73	5.4718	<0.005
	T2Dwithout DN	41.53	6.89	
	DN	32.21	6.96	
LDL(mg/dL)	Control	76.76	22.16	<0.036
	T2Dwithout DN	79.68	24.36	
	DN	192.55	25.702	

Table 3. Univariate correlation analysis between serum levels of MMP-1, TIMP-1 and hemoglobin A1c and fasting blood glucose (FBG) in DN

b	MMP-1	TIMP-1
TIMP-1	0.490**	-
FBG	0.459**	0.441*
HbA1c	0.557**	0.520**

** Correlation is significant at the 0.01 level (2-tailed).

Table 4. Multiple linear regression analysis forTIMP-1 and MMP-1 in Diabetic nephropathy patients

	β coefficient	t	p
TIMP-1			
HbA1c	0.55	4.12	<0.001
LDL	0.25	6.095	<0.005
HDL	-0.030	2.86	<0.014
TG	0.01	2.515	<0.001
MMP-1			
HbA1c	0.62	3.14	<0.001
LDL	0.357	5.07	<0.004
TG	0.021	2.66	<0.013
HDL	-0.020	2.51	<0.012

In table 4, the outcome of stepwise multiple linear regression analysis including all the studied parameters including age, BMI, HpA1c, TC, TG, HDL and LDL as independent variables to find significant determinants for TIMP-1 and MMP-1 among DN patients. HbA1c, LDL, HDL and TG were selected for both TIMP-1 and MMP-1.

DISCUSSION

In this study, we examined TIMP-1 and MMP-1 levels' in T2Dwith and without DN and their comparison with a control group. Also we inspected their relations with clinical features. The most applicable finding from our study is that diabetic nephropathy is related to a profound increase in serum concentrations of TIMP-1 and MMP-1.MMPs might play a role as "predictive" biomarkers, as their valuation could identify a population of patients at risk.

Serum TIMP-1 and MMP-9 concentrations in patients with type 2 DM have been described to be more than the control group in numerous studies[9,10]. Li and partners notified an augmented MMP-9 production during the motivation of diverse cytokines in the kidneys of diabetic rats in their study[11]. augmented concentrations of MMP-9 have been stated to participate in DN development via changing the ECM composition as well as the function and structure of podocytes,.

Opposing to the above explanations, various researchers revealed that MMP-9 levels were higher in the controls than in type 2 diabetic patients. Variances in the MMPs concentrations in the serum or tissue of diabetic individuals when compared to healthy ones. As revealed by the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS), hyperglycemia is a significant factor in the diabetic complications development [12]. Alterations in the MMPs concentrations in the serum or tissue of diabetic patients when compared with healthy controls have been authenticated in numerous researches, leading scientists to hypothesize that the levels are in agreement with the stages of diabetes and the severity of complications[13].

Our results of the positive relation between T2D and TIMP1harmonize with results from numerous case-control and cross-sectional Korean studies (cases n=80, controls n=80),[14] Iraq (cases n=54, controls n=26)[15], the UK (cases n=86,[10] controls n=63) and the USA (n=1069).[16]Whilein Greece, a case-control study (cases n=60, controls n=60) has detected diminished concentrations of TIMP1 in T2D patients in comparison to controls, the diversified outcomes might be clarified by the altered features of patients involved in the study[17]. The varied results might be elucidated by the dissimilar features of patients comprised in the study. In comparison to all other researches, the Greek study patients were at more progressive phase of T2D and might have received additional intensified treatment. The concentrated diabetes remedy have revealed to diminish levels of TIMP1 significantly, that might elucidate for the lower levels of TIMP1 among diabetic patients in the Greek research. The alternation of MMP activity in diabetic nephropathy is likely modified by genetic factors, as an association among diabetic nephropathy and the dinucleotide polymorphism of the MMP-9 gene has been identified[18]. DN, as one of the mainly widespread complications in type 2 DM, encompasses the progressive gathering of ECM in a number of constituents of the kidney[19]. Matrix metalloproteinases are the proteolytic enzymes that are responsible for protein turnover /degradation in the ECM, therefore abnormalities in MMP activity or expression are significant components in the progression and development of DN[20]. Genetic divergence mediating expression of MMP-2 might result in individual alterations in susceptibility to certain diseases. Recent study investigated the probable link of definite MMP-2 gene variants with the susceptibility of type 2 diabetes (T2D) in a Tunisian population, demonstrated a consistent relationship of the rs243866 and rs243865 genotype with a protection for T2D[21]. Moreover, previous study identified essential genes and pathways in DN using bioinformatics analysis, exploring the DN intrinsic mechanisms and discriminate new possible therapeutic and diagnostic markers of DN indicating that BTK, FOS, IRF4, JUN, PRKCB,MDFI,LCK,EGR1, NR4A1 and ALB might be the prospective novel biomarkers for diagnosis and the promising therapeutic target of DN [22].

CONCLUSION

Data from the present study suggest that both MMP-1 and TIMP-1 biomarkers are risk factors for DN among Egyptian patients.

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