Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 31, Issue 3, 2020 DOI: http://doi.org/10.23851/mjs.v31i3.848

Research Article Open Access

Increasing Vascular Complications Depends on The Duration Disease of Having Been Diabetic Type 2

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Article Info

Received
08/02/2020

Accepted
07/06/2020

Published
20/08/2020

ABSTRACT

Angiopathy is a major complication in (T2-DM), Endothelin-1 (ET-1) is considered the main vasoconstrictor (a mitogenic endothelium-derived peptide is mostly, produced by endothelial cells, as well by vascular smooth muscle cells, macrophages, and other cells). Previous studies suggested a link between T2-DM and ET-1. This paper aims to study the association between Endothelin-1 (ET-1) as a marker of endothelial dysfunction and the duration disease among patients with T2-DM, and to identifying the effects of obesity and insulin resistance on elevated Endothelin level. The study includes 96 patients with T2-DM, aged between (45-70) years, (56.73 ± 9.14 years) is an average duration of having T2-DM (14.541± 11.462) years, and 96 healthy control subjects, aged (45-70) years, (56.42 ± 8.74 years). We show that the excess of ET-1 level was clearly linked with the duration of T2-DM, where the plasma Endothelin level was significantly changed among the studied groups (according to their duration) (11.607 ±0.783), (13.641± 0.729), (17.736 ± 3.409), (33.816 ± 12.902), (81.165 ± 35.404), and (156.783 ± 12.671) pg/mL respectively. Plasma ET-1 levels significant is positively correlated with T2-DM ($R^2 = 0.9711$, $p \leq 0.01$), with obesity, insulin resistance, age, HOMO-IR, hypertension and with HDL level ($p \leq 0.05$). As a conclusion**,** the plasma ET-1 level was significantly elevated as long as having been T2-DM.

KEYWORDS: Endothelin (ET-1); diabetes type II (T2-DM); Insulin resistance (IR); Lipid profile.

INTRODUCTION

Chronic T2-DM is prevalent in worldwide, and it most commonly affects elder people. It is an endocrine disorder with elevated level of blood glucose and insufficient secretion or dysfunction of insulin, which is produced by Beta cells in the islets of Langerhans. Body's resistance to insulin is frequently presented in obesity, cardiovascular disease (CVD), and the core defect of metabolic abnormalities and highly contributed to the pathogenesis of T2-DM [1,2]. Etiology of diabetic vascular diseases, insulin resistance (IR), hypertension, oxidative stress, and abnormal levels of fats are consequently lead to endothelial inflammation [3]. Endothelial dysfunction is the first-stage of T2-DM complication, characterized by an overproduction of the vasoconstriction and reduction of the vasodilatation nitric oxide (NO). Complication, including micro-and macroangiopathy, are a major cause of impaired life expectancy [4,5]. Prolong poorly controlled hyperglycemia lead to atherosclerosis and increased risk of CVD, therefore, controlled level of glucose in blood and reduce body weight can play major roles in reducing the risk of developing diabetes complication [7,8]. The vasoconstrictor ET-1 is a hormone, close to 80% of it is produced by vascular endothelial cells [9]. ET-1 is the most potent known endothelial vasoconstrictor, appears to play a central pathophysiological role in endothelial dysfunction [10, 11]. In this paper, we tested the hypothesis of the relationship between ET-1 and the duration of being diabetic T2.

MATERIALS AND METHODS

Ethical clearance

This study was approved by the Mustansiriyah university ethics committee. All volunteers were informed about the study and agreed to collect their data from medical records.

Sampling

This study was carried out in the Ghazi Hariri hospital-Baghdad through February 2018 to June 2019. The study included 192 volunteers their information and history of T2-DM were collected by a questionnaire form. Volunteers were divided into two groups, healthy subjects as controls group A (n=96, 48 males and 48 females) and patients with T2-DM (group B) who had fasting blood glucose (FBG) ≈ 200 mg/dl or above. Patients with diabetes complication, (e.g. nephropathy, retinopathy, and CVD) were excluded age ranged between (45-70) years and the average duration of having T2-DM was (5-30) years. Fasting blood samples (10 ml) were collected at morning, left for thirty min at room temperature and centrifuged at 3000 rpm for 20 min. Hemolysed samples were discarded, 10 µL of serum was used to measure FBG, Lipids, Malondialdehyde (MDA).

Fasting blood glucose was determined according to the enzymatic colorimetric assay method [12] Insulin and Endothelin were determined using sandwich enzyme immunoassay (ELISA) [13] Endothelin [14].

Lipid Profile Measurement

Cholesterol was determination using enzymatic method [15,16]. HDL was determined using precipitation method [17]. VLDL and LDL were mathematically calculated [LDL-C (mg/dl)] = TC mg/dl -[HDL-C mg/dl] + TG/5 (mg/dl). MDA was measured [18], by a MDA reaction with thiobarbituric acid in an acidic condition to produce a pink color chromophore, which spectrophotometrically detected at 535 nm.

Statistical Analysis

Student t-test was used to compare the significance of the differences in between control and cohort groups. High significance: p-value was ≤ 0.01 (HS), Correlation coefficient was used to indicate the relationship between ET-1 and other parameters by using origin 2018 SRI Build 9.5.1.195 version. ANOVA analysis was used to indicate the significance of changes observed in the progressive disease status. All values were expressed by mean ± SD.

RESULTS

Table 1 shows the description of the studied groups where a significant increase in FBG mg/dl, ET-1(pg/mL), HOMO-IR, MDA, TG (mmol/L), cholesterol (mmol/L), LDL (mmol/L) and VLDL. While, there was a significant decrease in HDL in patientscompared with control group. Table 2 shows a high significant positive correlation between serum ET-1 and duration of T2-DM ($r = 0.764$, $p < 0.0001$ HS), Figure 1. Additionally, there was a non-significant positive correlation between ET-1 and FBS ($r = 0.189$, $p = 0.156$), Figure 2, but a significant positive correlation with HOMA-IR levels in T2-DM ($r = 0.581$, $p < 0.0001$ HS), Figure 3. Results also shows that a non-significant positive correlation between ET-1 and LDL, VLDL, TC, TG serum levels ($p > 0.05$), but a significant negative correlation between Endothelin and HDL ($r = -0.329$, $p = 0.012$S), Figure 4. In addition, there was non-significant negative correlation between ET-1 and serum levels of MDA ($r = -0.006$, $p = 0.962$), Figure 5. ET-1 levels were studied according to the duration of disease, Figure 6 and Table 3.

Table 1. Clinical characteristics of diabetic patients and control subjects.

Variable	Control		Patients		P-value	
	Mean	SD	Mean	SD		
Age	56.42	8.74	56.73	9.14	P > 0.05	NS
Height	166.57	6.56	164.20	9.10	0.155	NS
Weight	85.83	8.18	87.17	14.55	0.651	NS
BMI	25.12	2.83	30.74	4.66	P < 0.05	S
Endothelin	6.91	3.40	81.94	54.36	P < 0.0001	HS
Insulin	18.59	15.47	23.65	16.94	0.265	NS
FBS	91.87	28.29	211.53	62.79	P < 0.0001	HS
HOMO-IR	3.56	2.43	12.44	8.09	P < 0.0001	HS
MDA	5.17	2.60	11.48	0.94	P < 0.0001	HS
T.G.	165.23	50.91	242.63	100.13	0.001	HS
Cholesterol	156.43	32.36	240.41	42.90	P < 0.0001	HS
HDL	54.00	6.90	45.47	6.64	P < 0.0001	HS
LDL	69.68	32.16	151.82	45.32	P < 0.0001	HS
VLDL	33.05	10.18	58.66	56.36	0.028*	S

Data are expressed as mean ± SD * Significance: P-Value ≤ 0.05 ** High significance: P-Value ≤ 0.01.

ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 31, Issue 3, 2020 DOI: http://doi.org/10.23851/mjs.v31i3.848

Table 2. Correlation coefficient (r) between serum Endothelin (ET-1) and other parameters.

Variables		*Group of T2-DM (n=96)*	
Age (years)	***r***	0.317	
	P	0.015	S
Duration of disease (years)	***r***	0.764	
	P	0.0001	HS
FBG (mg/dL)	***r***	0.189	
	P	0.156	NS
Insulin (uIU/ml)	***r***	0.475	
	P	0.0001	HS
HOMO-IR	***r***	0.581	
	P	0.0001	HS
TC (mg/dL)	***r***	0.025	
	P	0.851	NS
TG (mg/dL)	***r***	0.003	
	P	0.982	NS
HDL-C (mg/dL)	***r***	-0.329	
	P	0.012	S
VLDL (mg/dL)	***r***	0.026	
	p	0.846	NS
LDL-C (mg/dL)	***r***	0.053	
	P	0.691	NS
MDA (µmol/L)	***r***	-0.006	
	P	0.962	NS

*Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

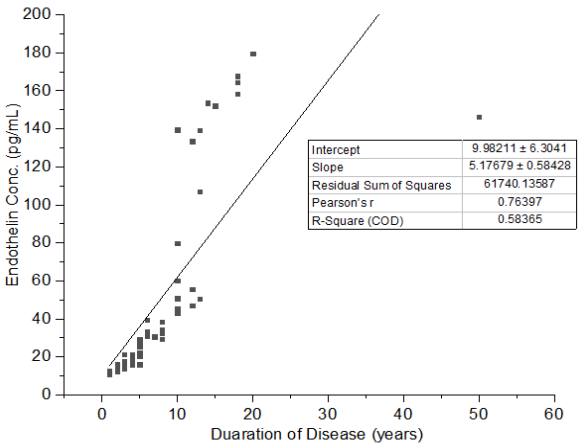

Figure 1. Correlation between Endothelin concentration and duration of disease.

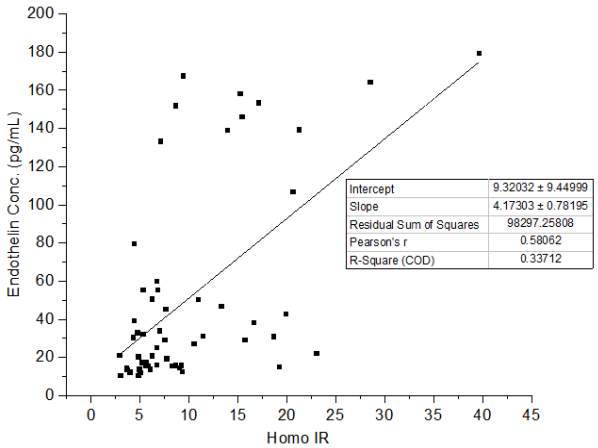

Figure 3. Correlation between Endothelin concentration and Homo IR.

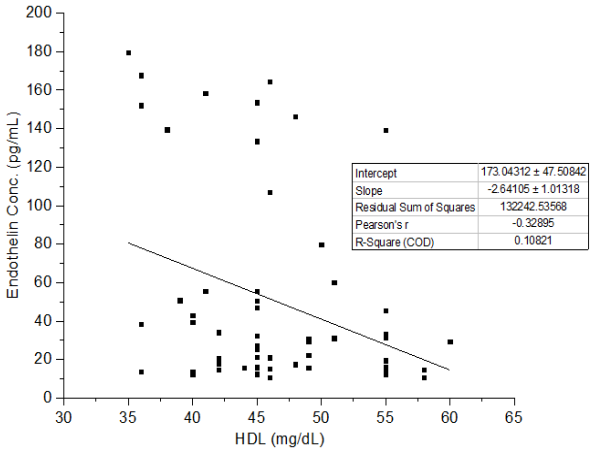

Figure 4. Correlation between Endothelin concentration and HDL concentration

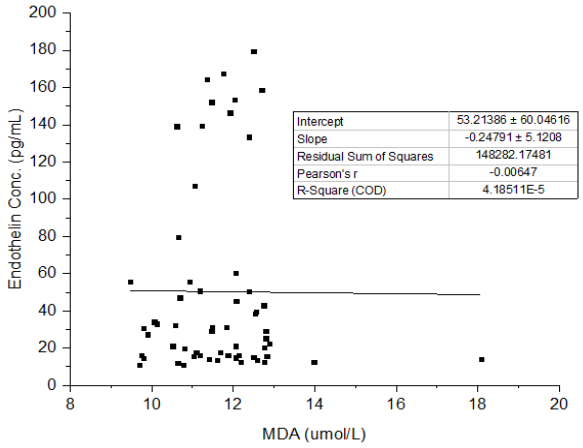

Figure 5. Correlation between Endothelin concentration and MDA concentration.

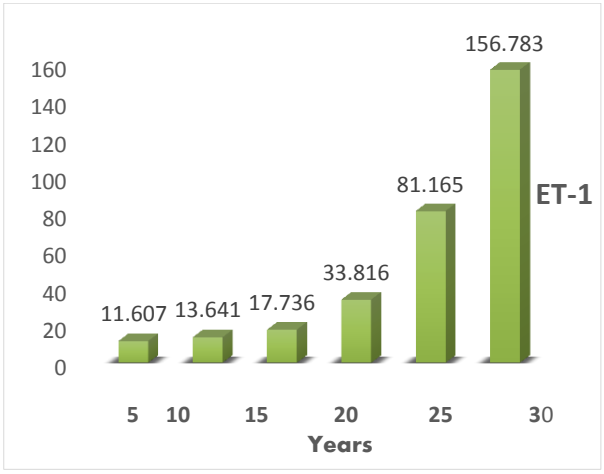

Figure 6. The effect of duration of diabetes (T2-DM) on plasma ET-1

Table 3. Correlation between ET-1 levels and duration of being T2-diabetic.

Group	Duration of disease	Mean	SD
1	1-5	11.6075	0.78393
2	5-10	13.6419	0.72960
3	10-15	17.7369	3.40955
4	15-20	33.8169	12.90266
5	20-25	81.1650	35.40472
6	25-30	156.7838	12.67172
ANOVA	p<0.0001 (Sig.)		

DISCUSSION

Results of this study showed that (ET-1) levels were higher in diabetic patients with that of control ($p \leq 0.01$). ET-1 levels were 11-folds higher in diabetic patients compared with healthy control. These results agreed with a previous result [19], which showed that ET-1 was 3-folds higher than healthy controls. Several studies suggested that endothelial dysfunction played an important role in the pathogenesis of vascular disease related with T2-DM. As a response to insulin mechanism, T2-DM produces an imbalance between productions of Nitric oxide and ET-1, which causes up regular release of ET-1. On the other hand ET-1 level was higher in women than men ($p \leq 0.01$). Additionally, and positively correlated with the age of patients. In our study, we found that Endothelin expression levels were significantly affected by the diseases duration where a positive correlation have detected ($p < 0.00001$). Elevated ET-1 levels in diabetics and a positive correlation between ET-1 and the disease duration of microvascular complications, and metabolic control have been previously reported [20]. Results also were in agreement with Gursel *et al* [21], who found that ET-1 levels elevated in patients had been diabetic for more than 10 years compared with patients had the disease for less than 10 years ($p= 0.02$). These results were disagreed other results [22], where no correlation between ET-1 levels and the duration of diabetes mellitus ($r = 0.108$, $p > 0.1$) was indicated and the age was not significantly correlated with plasma ET-1 levels in T2-DM ($p > 0.05$). On other studies, plasma ET-1 level did not change in diabetic subjects compared with healthy control, and no correlation between ET-1 levels and diabetic microangiopathy, duration of diabetes, and metabolic control [23-26]. This probably, due to not exhibit positive correlations between ET-1 levels and BP or difference in the number of patients, gender, genetic factors, BMI, different Immunological

methods, and environmental factors might also have an influence on plasma ET-1 levels. Level of Endothelin was raised in patients had a history of T2-DM for more than 20 years. However, we suggest that the duration of the disease is one of many causes of elevated Endothelin in these patients Results showed that serum FBG, insulin and HOMO-IR levels were highly substantial increased in T2-DM as compared with control groups ($p \leq 0.01$), especially in 55 years old and above. HOMO-IR was higher 4-folds in patients with T2-DM as compared with healthy control. There was a non-significant positive correlation between ET-1 and FBS ($p > 0.05$), in agreement with others, who found a non-significant correlations between plasma immunoreactive - Endothelin concentrations and FBS ($r=0.086$, $p> 0.1$) [27]. In our study we found a highly significant positive correlation between plasma Endothelin and IR ($p \leq 0.01$), which is in consistent with another study, where insulin appeared to be a direct stimulus to Endothelin release [28,29]. There were multiple mechanisms to explain the contribution of ET-1 to IR, including impairment of insulin signaling [30]. There was a highly significant increase in serum TC, TG and HDL, LDL in T2-DM patients ($p \leq 0.01$) as compared with controls. Dyslipidemia was related to high risk of CVD in T2-DM, which means that all patients had dyslipidemia. Results here indicated a non-significant positive correlation between ET-1 with LDL, VLDL, TC and TG serum levels ($p > 0.05$). Some reports described a significant increase of ET-1 in patients with T2-DM and dyslipidemia ($p < 0.0001$) [33]. In addition, there was a significant decreased HDL in diabetic patients compared with healthy group. As HDL removes the excess cholesterol from tissue membranes, including from macrophages of the vascular atherosclerotic lesions, its low level HDL in diabetic patients could have harmful effects on cholesterol content in vascular walls [34]. There was a non-significant negative correlation between ET-1 and serum levels of MDA ($p > 0.05$). However, many studies showed a direct relation between T2-DM, oxidative stress and oxidized LDL. By vascular transport the lipoproteins molecules can cross the endothelial cell layer [35,36]. Oxidative-LDL accumulates on the walls of the blood vessels, and poisons the endothelial cells. Oxidative-LDL decreases production of vasodilator (NO), and enhances the release of vasoconstrictor (ET-1) [37]. A high significant increase of FBG in T2-DM group as compared with control group, hyperglycemia promote formation reactive oxygen species (ROS), which contribute to vascular inflammation in T2-DM and oxidative stress. Indeed, we found high significant increase in MDA (as a marker for oxidative stress) in T2-DM compared to healthy group, oxidative stress also play a major role in the pathogenesis of atherosclerosis in T2-DM.

CONCLUSIONS

Endothelin levels rises with increases the duration disease with T2-DM, due to that in patients with T2-DM, insulin signal is impaired and consequently, it causes an imbalance in the releasing of nitric oxide (NO) and ET-1 (ET-1 release is up-regulated). SO, promote levels of Endothelin (ET-1) may participate to development of endothelial dysfunction by inhibiting NO secretion and increased ROS, ROS play essential role in the development vascular inflammation, and pathogenesis of T2-DM complication, but we suggested that the duration of disease not the only effective agent to elevated Endothelin levels in T2-DM patients. So, you can control of early T2-DM complication by reducing other factors such as hypertension, BMI, Low levels of HDL. In addition, different levels of endothelin in both genders, plasma ET-1 levels were higher in women in patients with T2-DM.

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ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 31, Issue 3, 2020 DOI: http://doi.org/10.23851/mjs.v31i3.848

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