Study of the role of endothelial progenitor cells and oxidative stress in myocardial dysfunction in patients with type 2 diabetes mellitus

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Abstract: Background: Type 2 Diabetes mellitus (T2DM) is a major cause of significant morbidity, associated with microangiopathy and myocardial dysfunction. The pathology of myocardial dysfunction in these patients is likely multifactorial and includes increased oxidative stress, dyslipidemia and altered myocardial metabolism specially in patients with poor glycemic control for long duration. Oxidative stress, represented by an over production of reactive species, plays a role in all stages of diabetic heart disease ranging from cardiac hypertrophy to myocardial fibrosis and dysfunction. T2DM is characterized by a widespread endothelial dysfunction and a bi- to tri-fold increased risk of developing cardiovascular diseases. **Objective:** Studying the role of endothelial progenitor cells and oxidative stress in myocardial dysfunction in T2 DM patients. **Patients and Methods:** After departmental ethics committee approval and patient consents were obtained, Sixty patients included in this work with T2DM (31 females and 29 males) having variable duration of DM and thirty healthy subjects [15 females and 15 males]. All patients were subjected to history taking and clinical examination, complete blood count (CBC), liver function tests, renal function tests, lipid profile, fasting plasma glucose (FPG) and post-prandial plasma glucose (PPPG), lycatedhaemoglobin (Hb-A1c), Quantitative determination of serum serum super oxide dismutase (SOD) and Quantification of circulating endothelial progenitor cells (EPCs). In addition, echocardiography was done. **Results:** T2DM having variable duration of DM (group I) has significant increase in HbA1c, FPG and 2h-PPPG, SBP, DBP, TC, TG, LDL, ALT, diastolic dysfunction and its severity, significant decrease in EPCs and SOD compared with healthy control group (group II). Also, (group I) has significant negative correlations between SOD and EPCs and severity of myocardial diastolic dysfunction but not in control group suggesting that the decrease in the circulating EPCs numbers might contribute to diastolic dysfunction which had taken place in T2DM and its cardiovascular complications. **Conclusion:** T2DM is associated with increase oxidative stress and reduced EPCs numbers that might have a role in myocardial diastolic dysfunction and its severity, independent of traditional cardiovascular risk factors. EPCs numbers might be used as a laboratory test for identifying myocardial diastolic dysfunction in patients with T2DM.

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1. Introduction

The incidence of T2DM is increasing at an alarming rate both nationally and worldwide. Many observations linking OS to T2DM, currently, there is evidence supporting that OS is an important to regulate some pathways related to T2DM and its complications **(Miki et al., 2012).**

Diastolic myocardial dysfunction predicts cardiovascular events and represents with vascular endothelial dysfunction an underlying event for vascular abnormalities observed in T2DM patients and both of them early markers of atherosclerosis seen in T2DM **(Yuen-Fung et al., 2011).**

T2DM is characterized by a widespread endothelial dysfunction and a bi- to tri-fold increased risk of developing cardiovascular diseases **(Nichols et al., 2004).** Although the pathology of myocardial dysfunction in these patients is unclear, it is likely multifactorial and includes increased oxidative stress **(Aksakal et al., 2011).**

2. Patients and Methods

Patients:

This study was conducted on 90 subjects. They were divided into 2 groups:

- Group I (diabetics): 60 Diabetic patients having variable duration of DM,
- Group II (controls): 30 Normal healthy subjects (age and sex matched).

Diabetic patients were classified into subgroups according to myocardial diastolic function (preserved and impaired diastolic function subgroups).

Patients were presented to Emergency Department of Sayed Galal Hospital, Al-Azhar University, and the outpatient clinic, and admitted to the Internal Medicine Department.

The study was carried out during the period from March 2013 - March 2018.

Exclusion criteria:

- ❖ Poorly controlled DM (HbA1c ≥ 11%).
- ❖ Dilated cardiomyopathy.
- ❖ Significant valvular heart disease.
- ❖ Chronic atrial fibrillation.
- ❖ NYHA class III/IV heart failure.
- ❖ History of coronary heart disease.
- ❖ Cerebrovascular stroke.
- ❖ Acute heart failure within the past 6 months.

Methods:

❖ Thorough history taking and full clinical examination were done for all patients including BMI [body weight (in kg) divided on height (in m2)].

❖ Laboratory investigations for selected patients included liver function tests evaluation including alanine transaminase (ALT), aspartate transaminase (AST), serum albumin, serum bilirubin, and prothrombin time (PT) using (Hitachi, 911 automatic analyzer), renal function tests assessment including blood urea and serum creatinine using (Hitachi, 911 automatic analyzer), CBC including hemoglobin (Hb), white blood cells (WBCs) and platelets (PLT), lipid profile assessment including TC, TG, LDL and high density lipo-protein (HDL), fasting plasma glucose (FPG), 2hour post-prandial plasma glucose (2h-PPPG), glycatedhaemoglobin (Hb-A1c), quantitative determination of serum Super Oxide Dismutase (SOD) by ELISA technique and peripheral blood EPCs were analyzed by flow Cytometry.

❖ Transthoracic echocardiography for assessment of diastolic dysfunction.

❖ Informed consents were taken from all patients.

❖ Statistical analysis of data by IBM computer using statistical program for social science (SPSS) version 20: Chi-square test to compare qualitative variables between groups, unpaired (Independent) t-test to compare quantitative variables between groups, and one way analysis of variance (ANOVA) followed by post hoc analysis (LSD test) to compare between more than two groups regarding quantitative data with parametric distribution.

3. Results

CD34CD133 count (marker of endothelial dysfunction) was significant positive correlations with SOD level (marker of oxidative stress) among diabetics, i.e the greater the degree of EPCs count, the greater the degree of SOD level and vice versa. The lesser SOD means more oxidative stress. CD34 CD133 count and SOD was significant negative correlations and age, BMI, SBP, DBP, TC, TG, LDL, DM duration, FPG, 2h-PPPG, HbA1c, (markers of glycemic control), i.e. the greater the degree of age, BMI, DM duration, FPG, 2h-PPPG and HbA1c, SBP, DBP, TC, TG, LDL, the lesser the degree of SOD (more oxidative stress) and vice versa (Table 1).

Table (1): Correlations of EPCs and SOD and other studied parameters among diabetics

Parameters	CD34 + CD133		SOD (U/ml)	
	R	P	R	P
Age (yrs)	-0.411	0.001	-0.580**	0.000*
BMI (Kg/m^2)	-0.334	0.009	-0.506**	0.000
SBP (mm Hg)	-0.411	0.001	-0.640**	0.000
DBP (mm Hg)	-0.364	0.004	-0.514**	0.000
DM duration (yrs)	-0.382	0.003	-0.617**	0.000*
FPG (mg/dL)	-0.492	0.000	-0.609**	0.000*
2h-PPPG (mg/dL)	-0.480	0.000	-0.600**	0.000*
HbA1c (%)	-0.295	0.022	-0.638**	0.000*
Creatinine (mg/dL)	-0.026	0.843	-0.097	0.461
ALT (IU/L)	0.000	0.999	0.024	0.855
T. Bilirubin (mg/dL)	0.125	0.342	0.168	0.199
TG (mg/dL)	-0.510	0.000	-0.641**	0.000
TC (mg/dL)	-0.480	0.000	-0.587**	0.000
HDL (mg/dL)	-0.059	0.655	-0.056	0.670
LDL (mg/dL)	-0.370	0.004	-0.619**	0.000
EF%	-0.287	0.026	-0.036	0.786
LVEDD (cm)	0.014	0.917	-0.096	0.467
LVESD (cm)	-0.016	0.905	0.190	0.146
SOD (U/ml)	0.478	0.000	-	-

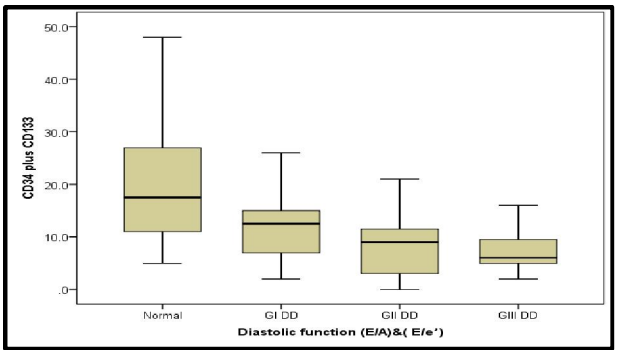

Figure (1): Diagrams of EPCs and diastolic function (DF) among diabetics. The lesser the degree of EPCs count, the greater the degree of DD among diabetics.

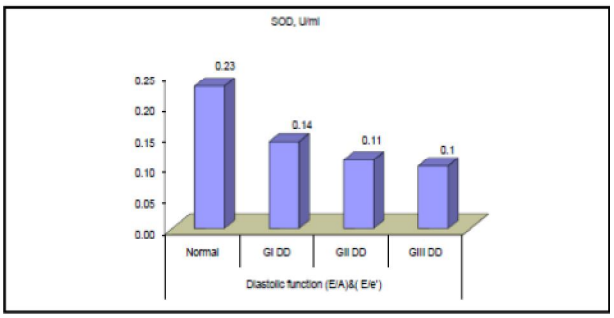

Figure (2): Histograms of SOD among diabetics with normal and different grades of DD. The decrease of SOD level (more oxidative stress) is associated with increase DD grading among diabetics

4. Discussion

The obtained results in the current study showed that60 diabetics have a significantly lower SOD level (0.15 ± 0.07ng/dl) compared to that of 30 controls (0.28 ± 0.05 ng/dl) (P < 0.001). In the current study; diabetics showed that, there were significant negative correlation between SOD and markers of glycemic control as HbA1c, FPG and 2h-PPPG (P < 0.000, r -0.609,- 0.600,- 0.638),i.e., diabetics with poor glycemic control have less antioxidant activity than diabetics with good glycemic control implying that glycemic control may affect OS in diabetics.

These results were in agreement with those reported by **Manisha et al. (2016)** who found that, malondialdehyde (MDA) levels in diabetics with poor glycemic control were increased compared with diabetics with good glycemic control and the differences were statistically highly significant (P < 0.0001).

In the same way in the current work, there were significant negative correlation between SOD and both of age of diabetics and duration of DM (P < 0.000), i.e., age advancement in diabetics and more duration of diabetics are associated with depletion of antioxidant and more OS which may take place in the development of diabetic complications over time.

Also, the current results were in agreement with **Kuldipand Gurpreet (2017)** who found that the activity of SOD was significantly reduced by 46.01% (P < 0.01) in diabetics without and with microalbuminuria in compared with healthy controls suggesting that OS is increased in diabetics.

In the current study, diabetics had significant decrease in EPCs counts (CD34 CD133). The counts was; 14.08 ± 11.82 cells compared with those of controls 35.3 ± 14.1 cells in lymphocyte gate per 10000 WBCs, EPCs were found to have negative correlations with markers of glycemic control as: HbA1c, FPG and 2h-PPPG, i.e., diabetics with good glycemic control had more EPCs counts than diabetics with poor glycemic control implying that glycemic control may affect EPCs counts in T2DM patients. In another way; diabetics with impaired diastolic function subgroup have reduced EPCs counts, less glycemic control and more HbA1c, FPG and 2-h-PPPG than diabetics with preserved diastolic function subgroup. Also, it is noted that there were significant negative correlations between EPCs counts and duration of DM. Patients with long duration of DM tend to have reduced EPCs count. These findings suggested that the duration of DM may have deleterious effect on EPCs counts.

These results were in agreement with findings of **António et al, (2014)** who reported that diabetics had circulating numbers of CD34 cells reduced by 63%, CD34 CD133 EPCs and reduced by 50% when compared with non diabetics. They also found significant negative correlations between circulating EPCs and both HbA1c and FPG.

The current study demonstrated that, depletion of antioxidant system in T2DM patients was associated with decrease in circulating CD34 CD133 EPCs count and both of them appears to have possible negative impact on myocardial diastolic function. However, the current work did not found causal relationship between decrease SOD level and circulating EPCs in T2DM patient's inspite of positive correlation between SOD and EPCs.

In another way, the obtained results showed that there were positive correlation between SOD level and the EPCs counts in the diabetics but not in the controls, i.e, in the diabetics, the better SOD level (less oxidative stress), the greater EPCs counts. Diabetics with impaired diastolic function subgroup had significant decrease in EPCs counts (CD34 CD133: 8.7 ± 7.5 cells) compared with, those with preserved diastolic function (CD34 CD133: 12.1 ± 7.3 cells). Diabetics with preserved diastolic function subgroup had significant increase in EPCs counts but still less than those of controls.

Our results were in agreement with the study made by **Chun and ting (2012)** who found that patients with an impaired circumferential strain had a lower number of CD34 EPCs and SOD level (0.13 ± 0.06 U/ml vs. 0.20 ± 0.08 U/ml, $P < 0.01$), suggested that myocardial dysfunction in patients with T2DM is related to depletion of EPCs and increased oxidative stress.

Also, another in vitro study done by **Peng and Jin (2016)** who found that hyperglycemia caused oxidative stress in EPCs, resulting in the dysfunction of EPCs, which in turn attenuated repair in the ischemic heart. The mechanism underlying this process may be due to decreased SOD mRNA and protein expression levels, leading to a reduction in EPC resistance to oxidative stress.

According to the results of the current study and the previous studies, alterations in the circulating EPC number can be suggested to have an important role in the development and progression of myocardial dysfunction in DM. Also, it may be related to OS. Both EPC number and OS could be considered as a target of therapeutic interventions for diabetic cardiovascular protection.

The current study showed that, the metabolic parameters e.g. BMI, TG, TC and LDL being higher in diabetics with impaired diastolic function than those with normal DF. In the same manner, it was found that age advancement and more duration of diabetes where associated with increase severity of diastolic dysfunction. These results were in agreement with findings of **Hisashi et al, (2014)** showed that the duration of DM had significant inverse associations with CD34 cell number in T2DM patients.

All these factors were associated with more or less similar reduction of EPCs, more or less similar reduction of SOD. So in diabetic's poor glycemic control, more dyslipidemia, high BP and increase BMI may be responsible for increase oxidative stress and depletion EPCs which may be responsible for development of diastolic dysfunction observed in T2DM.

Conclusion:

T2DM is associated with increase oxidative stress and reduced EPCs numbers that might have a role in myocardial diastolic dysfunction and its severity, independent of traditional cardiovascular risk factors. EPCs numbers might be used as a laboratory test for identifying myocardial diastolic dysfunction in patients with T2DM.

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