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Influence of cytokine status on insulin resistance and circulating endothelial progenitor cells in type 2 diabetes mellitus

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM), a chronic metabolic disorder caused by insulin resistance (IR) and elevated blood glucose level, may lead to endothelial dysfunction. This can result in the development of various vascular complications, even in clinically controlled glycemic state.

Aim: It has been experimentally proven that cytokine influences both IR and endothelial progenitor cell (EPC) dysfunction in T2DM patients. The present study evaluated the effect of clinical and metabolic risk factors and cytokine levels on IR and EPC, which are used as critical early biomarkers for estimating the risks associated with T2DM.

Methods: The study involved 58 T2DM patients. They were further subdivided into three groups based on IR score: 32 (55.17%) with normal, 11 (18.97%) with mild-moderate and 15 (25.86%) with severe IR. The relationship of clinical, metabolic and immune mediators with IR and EPCs was verified.

Results: HbA1c% was significantly elevated in severe ($P = 0.022$) and mild-moderate IR groups ($P = 0.012$) than the normal group. The IR normal group had significantly elevated TNF levels compared to mild-moderate and severe groups. The regression analysis indicated that patients with increased body mass index (BMI) were 19.5% more likely to be significantly associated with severe IR. Association studies demonstrated that IL6 and IL10 values correlated with EPCs.

Conclusion: IL6 and IL10 were associated with circulating EPCs than IR and other clinical characteristics including glycemic control (glycated hemoglobin). TNF-α was associated with IR, but had no relationship with EPCs. The effect of cytokine status on IR and circulating EPCs in T2DM may indicate the risk of vascular complications.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by the development of micro and macrovascular complications. Inadequate control of blood sugar and inflammatory parameters has been demonstrated to positively influence the development of these complications. Many studies postulate local and systemic inflammation as one of the underlying pathophysiological mechanisms in cardiovascular diseases [1,2]. Pro-inflammatory (IL6, TNF-α) and anti-inflammatory cytokines (IL10) are found to be significantly associated with metabolic abnormalities [3]. Plasma biomarkers produced by adipose tissue including TNF are elevated in patients with insulin resistance (IR) [4]. Therefore, these cytokine may serve as biomarkers, if evaluated periodically, to estimate the cardiovascular risks associated in DM.

The circulating endothelial progenitor cells (EPC) play an essential role in regulating the vascular tone and structure of the blood vessels [4]. EPCs are regarded as a significant biomarker as they depict the patient's cardiovascular stress level. Classical cardiovascular risk factors such as elevated BMI, smoking, hypertension, dyslipidemia, and diabetes induce adverse inflammatory microenvironment, which modulates EPC survival and function [5]. Circulating EPCs mediate endogenous endothelial repair. Reduced levels of circulating EPCs has been reported in patients with increased risk of cardiovascular diseases [6].

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Knowledge on circulating EPCs, and its relationship with traditional risk factors and cytokine levels may help in understanding the pathophysiology of vascular complications in T2DM. Studies have correlated conventional risk factors of vascular diseases with changes in EPC levels [7–9]. Non-classical risk factors such as CRP and vitamin D are also known to be involved in vascular damage, and have an impact on circulatory EPC levels [5,6]. CRP attenuates endothelial nitric oxide synthase mRNA expression in EPCs, thereby inhibiting their differentiation, survival, and function [10]. Literature evidence suggests positive association between vitamin D levels and EPCs [11]. A study conducted in human umbilical vein cord endothelial cells (HUVEC) has reported that calcitriol treatment have an inhibitory effect on the pro-inflammatory parameters such as adhesion molecules, receptor of advanced glycation end product (RAGE), and IL-6. The same study demonstrated that anti-inflammatory properties of calcitriol are mediated through modulation of endothelial pro-inflammatory transcription factor nuclear factor κ B (NFkB) and phosphorylated-p38 mitogen-activated protein kinase (MAPK) activities [12]. Additionally, cytokines and therapy could modulate inflammatory processes and mobilization of peripheral circulating EPCs [9]. Circulating cytokines are involved in recruiting EPCs to the site of vascular injury from the bone marrow [13].

Improved clinical applicability of EPCs and insulin resistance by identifying risk factors influencing them can lead to therapeutic interventions in T2DM patients to reduce cardiovascular risk. The purpose of this study was to evaluate the effect of clinical and metabolic risk factors and cytokine status on IR and circulating endothelial cells in type 2 diabetes mellitus.

2. Methodology

2.1. Study design

2.1.1. Patient recruitment

The study was conducted as a part of the main study organized to estimate the impact of vitamin D3 supplementation on cardiovascular risk in patients with T2DM. This sub-study was approved by the institutional ethics committee and informed consent was obtained from all the participants. The patients with T2DM complying with the American Diabetes Association (ADA) 2015 criteria were recruited in 2015 for the main study. The inclusion criteria considered were: patients within the age range 25–65 years, T2DM with HbA1c < 9%, vitamin D levels < 30 ng/ml, and those with dyslipidemia and on adequate doses of statins based on the ADA guidelines. Patients with comorbidities that can influence the cytokine status were excluded from the study.

The patient's demographic data and clinical characteristics such as height, weight, body mass index, and blood pressure were documented at screening visit. Biochemical and serological tests performed included: fasting blood glucose and postprandial blood glucose, HbA1c, triglyceride, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C), total cholesterol to high density lipoprotein cholesterol (TC/HDL-C) ratio, blood urea levels, serum creatinine, high-sensitivity c-reactive protein (hs-CRP), and vitamin D at fasting insulin levels. Assessment of cytokines was also done using the collected blood samples.

The Homeostatic Model assessment (HOMA) was used to calculate IR score for each patient and was graded into three categories as follows: < 3: normal, 3 to 5: mild-moderate, and > 5: severe IR.[14]

The cardiovascular risk index was assessed using atherogenic index of plasma (AIP), which is logarithmically transformed value of triglyceride to high density lipoprotein ratio. The concentrations were taken in mg/dl.[15,16,17,18]

2.1.2. Quantification of cytokines by ELISA

Serum levels of the cytokines IL-6, IL-10 and TNF-α were measured by enzyme linked immunosorbent assay (ELISA) following the manufacturer's instructions (BD Biosciences, USA). The assay detection limits of the cytokine detection system were 2.2 pg/ml for IL-6, 2 pg/ml for IL-10, and 2 pg/ml for TNF-α. IL6 and IL10 levels were categorized as below detection limit (BDL) and above detection limit (ADL) values: ≤2.2 pg/ml and > 2.2 pg/ml for IL-6, and ≤2 pg/ml and > 2 pg/ml for IL-10. The TNF values in all the subjects were above assay detection limit values. Hence it could not be classified into BDL and ADL values, and continuous values of TNF were considered for analysis.

2.1.3. Quantification of peripheral blood EPCs by flow cytometry

The EPCs were analyzed by flow cytometry (FC500; Beckman Coulter, MarselleCedex 9, France) using mouse anti-human CD34 phycoerythrin (PE) (555822; BD Biosciences, San Jose, CA, USA), mouse anti-human CD45 PE (555484; BD Biosciences, San Jose, CA, USA) and mouse anti-human CD133VioBright Fluorescein isothiocyanate (130-105-225/226; MiltenyiBiotec GmbH, BergischGladbach, Germany). 100 μl of whole blood was incubated with 10 μl each of CD34, CD45, and CD133 antibodies after gentle mixing in dark for 15 min at room temperature. Subsequently, 500 μl of optilyse (Beckman Coulter, France) was added for red blood cell lysis, vortexed and incubated in dark for 15 min at room temperature. The sample was evaluated after adding 1 ml of sheath fluid (Beckman Coulter, France), followed by 100 μl of flow count beads (Beckman coulter, France) and vortexing. After appropriate gating, the peripheral blood cells positive for the stained antibody reagents were determined by two-dimensional side scatter fluorescence for $CD45^+$. $CD34^+$ cells were gated followed by the examination of dual positive CD34+ and CD133+ population of circulating cells. Circulating EPCs considered were $CD45^+34^+133^+$ cell population. A total of 2×10^4 events were acquired per tube and scored. Internal calibration was performed at each analysis of EPCs for exactness and stability of cell count by using CAL 992 (Beckman Coulter, Marselle Cedex 9, France).

2.2. Statistics

Data were reported as mean ± standard deviation or median (range) for continuous and counts for categorical variables. The IR groups were compared for differences in demographic, clinical, biochemical and immune parameters by ANOVA and Kruskal-Wallis test for continuous variables. Post-hoc analysis by Turkey's honest significant difference (HSD) and Games-Howell was done for ANOVA, and Dunn-Bonferroni method for Kruskal-Wallis test. Chi-square or Fisher's exact test were performed for categorical variables. The relationship between endothelial progenitor cells and independent variables was examined by Mann-Whitney *U* test or Kruskal-Wallis test for categorical variables and Spearman's correlation test for continuous variables. Statistical significance was assessed at $P < 0.05$. The association of the independent variables with IR was verified by univariate and multivariate ordinal regression. The variables were entered by simultaneous entry method. Association of independent variables with endothelial progenitor cells was tested by univariate and multiple linear regressions. For univariate analysis, $P \leq 0.2$ was considered as cut-off for inclusion of variables in multivariate regression. Statistical significance was considered at < 0.05 level for multivariate regression analysis.

3. Results

A total of 65 consecutive patients who met the inclusion criteria were recruited for the study. Seven patients were excluded due to incomplete data, consent withdrawal, loss to follow-up or extreme values of fasting insulin levels (> 50 mlU/L). The study finally considered a sample size of 58 patients. Their mean ± sd age was 53.09 ± 7.89 with F:M ratio of 0.93:1 (28 women and 30 men). The patients had 101.34 ± 69.58 (mean ± sd) months of duration of diabetes. The

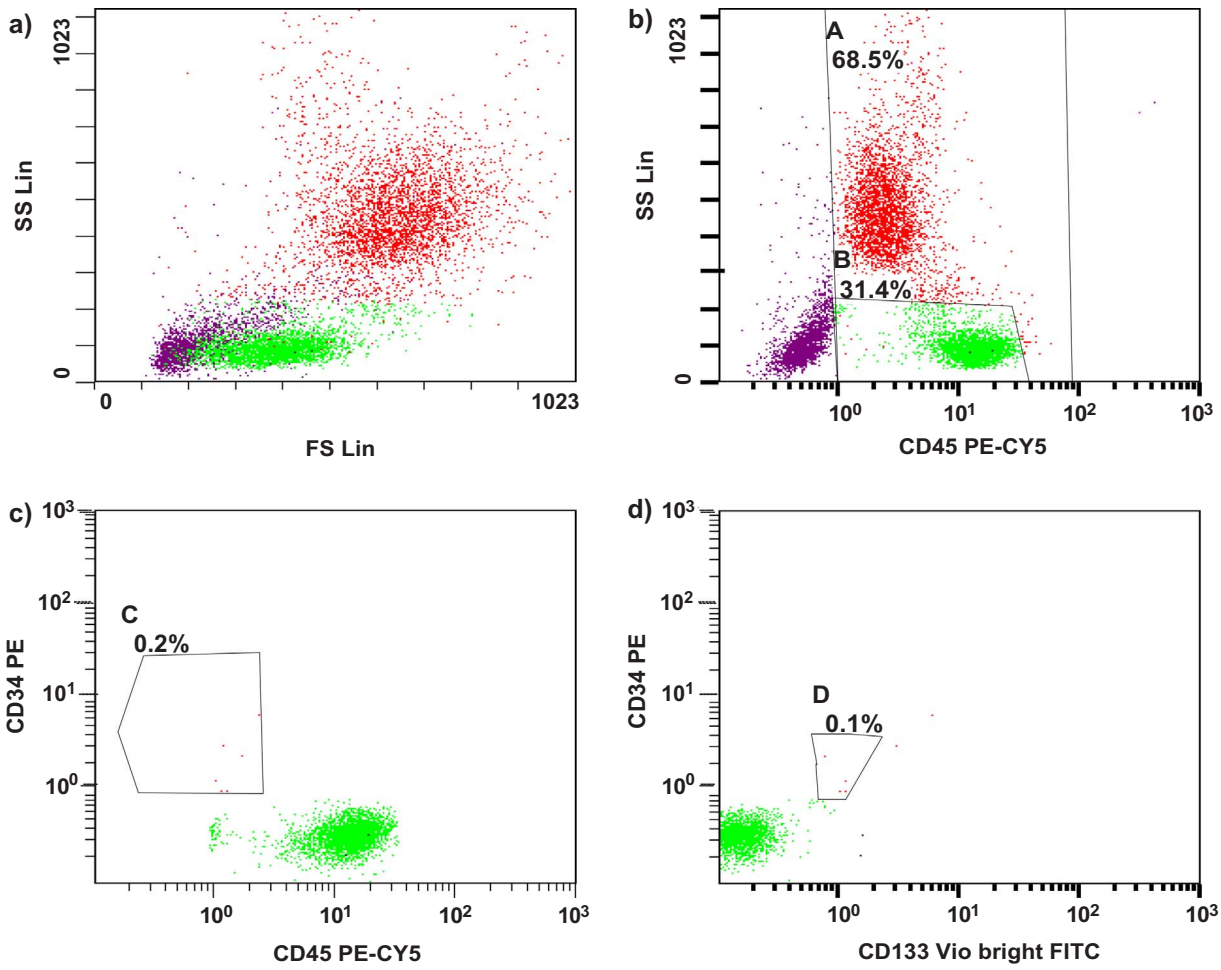

Fig. 1. Representative flow cytometric dot plots for identification of circulating epithelial progenitor cells in patients with type 2 diabetes mellitus. (a) Forward vs. side scatter plots of lymphocytes, monocytes and granulocytes (b) CD45 vs. side scatter plot of cells gated (B) to include CD45+ cells. CD34+/CD133+ progenitor cells express CD45 with lower staining intensity when compared to lymphocytes (c) CD45 vs. CD34 plot indicates the expression of CD45+CD34+ cells (d) CD34 vs. CD133 plot shows the CD45+CD34+CD133+ circulating endothelial progenitor cells.

patients' IR score was used to classify them into 3 groups. Thirty-two (55.17%) patients had normal score, 11 (18.97%) had mild-moderate score and 15 (25.86%) had severe score. The median (range) of EPCs in patients were 4 (1–12) cells/μl. The graph representing the CD34+/CD133+ circulating endothelial progenitor cell population is given in Fig. 1. Cardiovascular risk assessed by AIP showed that 4 (6.9%) patients had scores for medium risk (Normal IR = 3 & Mild-moderate IR = 1) and 54 (93.1%) patients had high risk (Normal IR = 29, Mild-moderate IR = 10 and severe IR = 15). The group-wise comparison of demographic, clinical, metabolic and immune parameters of the study subjects are listed in Table 1.

The comparison of IR groups showed HbA1c% was significantly elevated in severe (P = 0.022) and mild-moderate groups (P = 0.012) than the normal group. The IR normal group had significantly elevated TNF levels compared to mild-moderate and severe groups. Multiple comparisons showed that the differences were significant between mild-moderate and normal groups (P = 0.005). The remaining variables did not show differences among the groups at $P < 0.05$ level. However, duration of diabetes was smaller in normal IR group compared to the other groups. The average log (TG/HDL) ratio was > 0.24 in all the three groups (P = 0.051). The number of patients with BDL values of IL6 (82.76%) and IL10 (94.83%) were higher than those with ADL values. Hs-CRP, vitamin D, HbA1c and EPCs showed uniform distribution among the IR groups.

The univariate ordinal regression demonstrated duration of diabetes, HbA1c%, and log(TG/HDL) had significantly increased odds of severe IR. BMI and TNF were within $P \leq 0.2$ cut-off values (Table 2). HbA1c% and log(TG/HDL), the widely known factors influencing IR outcome, were excluded from multivariate regression, as these could also serve as end-points in diabetes disease models. The multivariate ordinal regression was fitted by including duration of diabetes, BMI and TNF as variables and IR as outcome. The result indicated that patients with increased BMI were 19.5% more likely to be associated with severe IR. BMI and duration of diabetes were risk factors, while TNF was a protective factor. The model fitted the parallel line assumption of the ordinal regression.

Association analysis demonstrated that IL-6 was associated with EPCs (P = 0.045) and IL-10 was weakly associated with EPCs (P = 0.056) (Table 3). Spearman's rank correlation for continuous variables showed that none of the variables were significantly associated with EPCs (Table 3). Hs-CRP (P = 0.08) was weakly associated with increase in EPCs. Linear regression of independent variables with EPCs as outcome for univariate association revealed an inverse association of EPCs with age and a significant direct association with IL10 (Table 4). IL6, hs-CRP, IR and type of treatment were within the $P \leq 0.2$. IR and hs-CRP were excluded from multivariate model. IR was separately modeled for independent variables and hs-CRP correlated with IL6. Two multiple linear regression models were fitted with age, IL6, IL10 and type of treatment as variables. The full model (Model I) with all the four variables showed that increase in EPCs was moderately associated with increase in IL10 levels and weakly related to IL6 and type of treatment. To get a more parsimonious model, we checked the

Table 1
Characteristics of diabetes patients according to insulin resistance at screening visit.

Variables	Insulin resistance			P value[#]
	Normal n = 32	Mild-moderate n = 11	Severe n = 15	
Age (years)	52.69 ± 8.49	53.45 ± 5.75	53.67 ± 8.32	0.914
Gender, n (female/male)	17/15	6/5	5/10	0.403
Duration of diabetes (months)	48 (1–252)	132 (6–228)	132 (12–264)	0.082
Body mass index (kg/m^2)	26.18 ± 3.5	26.62 ± 3.28	28.5 ± 4.48	0.144
Type of treatment				
Metformin	8	3	2	0.69
Metformin + other oral drugs	19	6	12	
Metformin + other oral drugs + insulin	5	2	1	
Hypertension, n (absent/present)	15/17	4/7	5/10	0.716
Hs-CRP (mg/l)	1.42 (0.34–8.44)	1.39 (0.22–22)	1.3 (0.16–4.66)	0.967
Vitamin D (ng/ml)	14.27 ± 6.05	13.52 ± 4.77	13.67 ± 6.59	0.914
HbA1c (%)	6.9 (5.4–8.3)	8.3 (6.1–8.8)	7.5 (6.8–8.9)	0.002
log(TG/HDL)	0.42 (0.19–1.1)	0.56 (0.22–0.96)	0.56 (0.38–1.19)	0.051
IL6, n (BDL/ADL)[*]	26/6	9/2	13/2	1
IL10, n (BDL/ADL)[*]	29/3	11/0	15/0	0.411
TNF (pg/ml)	9.21 (3.82–40.55)	7.66 (5.37–9.51)	8.69 (6.96–10.57)	0.007
Endothelial progenitor cells (cells/μl)	4.5 ± 2.97	4.09 ± 2.17	6 ± 3.44	0.193

[*] BDL = below detection limit, ADL = above detection limit.
[#] P value of anova for normal variables, Kruskal-Wallis test for non-normal data and chi-square or Fischer exact for categorical data.

reduced model by excluding age (Model II). The reduced model demonstrated that increase in all the three variables were significantly correlated with increase in EPCs. The R^2 change diagnostic of full model vs. reduced model showed that the exclusion of age reduced the R^2 by 0.03 and the change was not significant (Table 5). Hence the reduced model was a better model for evaluating the EPCs. Multicollinearity and homoscedasticity diagnostics were performed and showed no violation of assumptions and residuals were normally distributed.

Table 2
Univariate and multivariate ordinal regression analyses between patient characteristics and insulin resistance groups.

Variables[*]	Odds ratio	95% Confidence interval	P value
Univariate ordinal regression			
Age (years)	1.015	0.952–1.083	0.643
Gender			
Female	0.573	0.210–1.565	0.277
Male	1		
Duration of diabetes (months)	1.008	1.001–1.016	0.036
Body mass index (kg/m^2)	1.14	0.994–1.309	0.062
Type of treatment			
Metformin	1.073	0.178–6.473	0.939
Metformin + other oral drugs	1.891	0.403–8.884	0.42
Metformin + other orals drugs + insulin	1		
Hypertension			
Absent	0.609	0.218–1.699	0.344
Present	1		
Hs-CRP (mg/l)	1.021	0.870–1.197	0.801
Vitamin D (ng/ml)	0.982	0.901–1.070	0.674
HbA1c (%)	2.532	1.264–5.069	0.009
log (TG/HDL)	11.33	1.055–121.59	0.045
IL6			
Below detection limit	1.324	0.345–5.088	0.683
Above detection limit	1		
TNF (pg/ml)	0.78	0.565–1.075	0.129
Multivariate ordinal regression model[#]			
Duration of diabetes (months)	1.008	1–1.061	0.052
Body mass index, (kg/m^2)	1.195	1.030–1.386	0.019
TNF (pg/ml)	0.71	0.498–1.011	0.058

Nagelkerke R^2 = 0.234, Goodness of fit: P = 0.400, test of parallel lines: P = 0.846.
[*] Last group is the reference group for categorical variable estimates.
[#] Model-fit: -2LL = 102.15; chi^2 model = 13.06, df = 3, p = 0.005.

Based on the above findings we examined the levels of both IL6 and IL10 in study subjects. Ten patients had IL6 ADL and IL10 BDL, and 3 patients had IL6 BDL and IL10 ADL. A total of 45 patients were found to have both IL6 as well as IL10 BDL. None had ADL values for both the cytokines.

Table 3
Assessment of relationship between clinical, immune and metabolic markers and endothelial progenitor cells in diabetes patients.

Variables	Groups	N	Correlation co-efficient[#]	P value
Age (years)			−0.192	0.150
Duration of diabetes (months)			0.102	0.447
Body mass index, (kg/m^2)			0.066	0.622
Hs-CRP (mg/l)			0.232	0.080
Vitamin D (ng/ml)			0.111	0.405
TNF (pg/ml)			0.100	0.457
log (TG/HDL)			0.065	0.630
HbA1C (%)			−0.08	0.549
			Median (range)[*]	P value
Gender	Female	28	4(1–11)	0.832
	Male	30	4(1–12)	
Type of treatment	Metformin	13	3(1–9)	0.141
	Metformin + oral drugs	37	5(1–12)	
	Metformin + orals + insulin	8	3.5(2–9)	
HTN	Absent	24	4(2–12)	0.534
	Present	34	4(1–12)	
IL6	Below detection limit	48	3.5(1–12)	0.045
	Above detection limit	10	7 (2–9)	
IL10	Below detection limit	55	4(1–12)	0.056
	Above detection limit	3	11(4–12)	
Insulin resistance	Normal	32	3.5(1–12)	0.265
	Mild-Moderate	11	4(1–8)	
	Severe	15	6(1 −1 2)	

[#] Spearman's correlation co-efficient for non-parametric and Pearson correlation co-efficient for parametric continuous data.
[*] median (range) of EPCs are given for each variable and assessed by Mann-Whitney *U* test for 2 group and Kruskal-Wallis test for 3 group.

Table 4
Linear regression between clinical, immune and metabolic markers and endothelial progenitor cells in diabetes patients.

Variables	Regression co-efficient[#]	95% CI (Lower, Upper)	P value
Univariate regression			
Age (years)	−0.278	−0.204, −0.008	0.035
Gender	−0.015	−1.69, 1.509	0.910
Duration of diabetes (months)	0.111	−0.007, 0.016	0.405
Body mass index (kg/m^2)	0.066	−0.159, 0.264	0.622
Type of treatment	0.175	−0.444, 2.199	0.189
Hypertension	−0.100	−2.222, 1.007	0.454
Hs-CRP (mg/l)	0.195	−0.066, 0.449	0.143
Vitamin D (ng/ml)	0.129	−0.07, 0.202	0.335
IL6	0.228	−0.260, 3.860	0.086
IL10	0.328	1.009, 7.827	0.012
TNF, pg/ml	−0.060	−0.217, 0.138	0.657
Insulin resistance	0.188	−0.261, 1.583	0.157
Log(TG/HDL)	0.134	−1.802, 5.463	0.317
HbA1c (%)	−0.080	−1.292, 0.694	0.549
Multiple linear regression			
Model 1			
(Constant)		−0.146, 11.240	0.056
Age (years)	−0.177	−0.162, 0.027	0.157
IL6	0.248	0.020, 3.908	0.048
IL10	0.349	1.410, 7.986	0.006
Type of treatment	0.236	−0.015, 2.382	0.053
Model 2			
(Constant)		−0.627, 4.375	0.139
IL6	0.289	0.376, 4.194	0.020
IL10	0.382	1.884, 8.403	0.003
Type of treatment	0.237	−0.020, 2.399	0.054

Model 1- F statistic 4.686, P value = 0.003, R^2 = 0.261, Model 2- F statistic 5.451, P value = 0.002, R^2 = 0.232.
[#] Standardized regression co-efficient.

Table 5
Comparison of Model 1 Vs Model 2 multivariate linear regression R^2 change statistics.

Model	R^2 change	F change	df1	df2	F change P value	Durbin-Watson
Model 1	0.261	4.686	4	53	0.003	
Model 2	−0.029	2.065	1	53	0.157	1.645

4. Discussion

The present study found that clinical and biochemical characteristics of duration of diabetes, pro-inflammatory cytokines (TNF-α) and BMI had significant impact on IR, IL6 and IL10 as metabolic markers of circulating EPCs.

From the literature review, it is evident that IR impacts HbA1c, as blood glucose gets irreversibly attached to the hemoglobin inside the cell [19]. Previous research reveals that elevated TNF-α level are usually associated with the pathophysiology of IR in T2DM. It has been found that TNF-α gene polymorphism is strongly associated with T2DM (though some studies are contradicting these findings) [20]. The relation between insulin sensitivity and TNF- α has been demonstrated even in non-diabetic subjects [21]. It is evident from several *in vitro* studies that the elevated levels of TNF- α is directly proportional to the rate of circulating EPCs and their apoptosis could further negatively impact the heart's vasculature [1]. Hence, elevated TNF-α in T2DM patient implies significant risk associated with future cardiovascular diseases. However, in the current study, reduced level of TNF-α was associated with severe IR (non-significantly) and did not show association with EPCs. Elevated TNF-α production in the adipose tissue of obese subjects has been reported, however there are conflicting reports on circulating levels of TNF and its association with IR [22,23,24]. Native Canadian subjects with abnormal glucose tolerance and overt diabetes showed association of elevated levels of TNF-α with increase in severity of IR [25]. Bluher et al. concluded that TNF-α is not elevated at early stage of diabetes development in patients with IR. They explained that TNF- α, produced in adipose tissue, is not released into the circulation at early stages, hence the changes in TNF-alpha levels are not detectable in the peripheral blood [26]. In contrast, Miyazaki et al. reported that levels of TNF-α was increased before the onset of diabetes and no further increase was observed with IR. They further explained that pathogenic factors such as hyperglycemia, glucose toxicity, hyperinsulinemia, elevated plasma free fatty acid concentration and worsening obesity could play an important role in the development of IR. These factors mask the correlation between serum TNF-α concentration and IR observed in non-diabetic individuals, but not in diabetic patients at the time of overt diabetes manifestation [24]. This could be one of the reasons for reduced TNF noted in the present study. Further, treatment could have influenced the TNF levels of study subjects, as metformin and insulin therapy suppress markers of inflammation and upregulates anti-inflammatory markers [27–30].

An elevated degree of IR is found to be directly proportional to high BMI. The current study corroborated these findings. Studies suggest that, BMI possess inverse association with β cell function, which results in developing insulin resistant conditions [31]. Therefore, BMI could be useful for the early detection of risk associated with the disease.

Evidence from research claims a strong connection of IL6, obesity, and inflammation with disease pathogenesis of T2DM. IL6 was associated with EPCs and did not show association with the IR groups in the present study. The study did not have the power to detect the effect of IL6 on IR groups due to the smaller sample size. It has also been proposed that IL6 plays an important role in IR as well as EPC proliferation and migration. CRP is found to be responsible for inhibition of differentiation, survival and function of EPC. IL6 is also responsible for the production of CRP in liver [1,4]. Therefore, significantly elevated values of IL6 can directly and/or indirectly impact the EPC function and number, which could further lead to increased risk of cardiovascular diseases [1]. Therefore, IL6 can be regarded as a candidate biomarker for risk assessment of cardiovascular diseases in T2DM [20,32].

It is evident from the literature that there is a proximal connection between IL10 and impaired EPC survival and function. It was reported that IL10 has been proven to be a protective parameter in various atherosclerotic diseases [33]. IL10 promotes EPC survival and function by activation of *STAT3/VEGF* signaling cascades and modulation of other downstream signaling target like *NFkB* and *HO-1* in the myocardium [34]. *In vitro* findings suggest that IL10 inhibits adhesion of monocytes to endothelial cells by depressing CD18 and CD 62-L adhesion on immunocompetent cells [35]. Therefore IL10 can be considered as an ideal biomarker for the risk assessment of the T2DM − mediated cardiovascular disease even during initial stages. The current study substantiates these findings as IL10 levels correlated with EPCs.

Findings from the current study indicate that, despite elevated level of IL10 in 3 patients, there was no significant reduction in IL6 level (as 10 patients were found to possess elevated IL6 level), which could probably be due to the inflammatory nature of the disease pathogenesis. Reduced mobilization, cell death and functional impairment of EPCs have been associated with prolonged inflammation [34]. No significant difference in EPC levels was noted for different IR groups in the current study. Studies have shown that patients with impaired glucose tolerance have reduced number of EPCs when compared to those with normalized glucose levels. Factors attributed to defective EPC mobilization with insulin resistance include abnormalities in NO bioavailability and *PI3K/Akt* signaling [36]. António et al. have noted a progressive decrease in EPCs levels in patients with acute myocardial infarction, from pre-diabetes to DM. The study further suggested that the glycemic control may serve as a determinant for circulating EPCs levels [37]. The average age of the subjects (> 50 years), co-occurrence of comorbidities and treatment could have probably influenced the result obtained in the present study. Some of the studies have found that both EPC counts and IL10 are inversely proportional to the age

[38,39]. Similarly, the present study also showed negative correlation of age with EPCs.

The limitations of the present study were lesser number of patients and not considering other predicting factors for analysis. The patients were on statins and studies have shown that lipid-lowering therapy can increase EPCs in the circulation [40]. The study, being a preliminary one, needs future studies with large sample size and diverse age groups to corroborate the findings.

5. Conclusion

Clinical, metabolic and immune factors are associated with IR and EPCs in a distinctively different way in T2DM. Current study indicates that the levels of IL6 and IL10 are associated with circulating EPCs than IR and other clinical characteristics including glycemic control (glycated Hb). Though TNF-α, was associated with IR, it had no relationship with EPCs. Hence, the cytokines IL6 and IL10 can serve as biomarkers in predicting the micro and macrovascular complications in patients with established T2DM.

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Author contribution

BS and CS conceptualized and monitored the study, BS and AS patient recruitment, RP monitoring laboratory work-up and data, AKR conducted cytokine ELISA assay and statistical analysis and interpretation of the data. All the authors had access to anonymous data. All the authors contributed for preparation of the manuscript and approved the finalized manuscript.

Disclosure

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