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Assessment of oxidative stress and inflammation in prediabetes—A hospital based cross-sectional study

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ABSTRACT

Background and aim: Prediabetes is associated with dysglycemia, obesity, inflammation and endothelial dysfunction, contributing towards the pathogenesis of cardiovascular diseases rendering them vulnerable for the same. The current study intended to explore the risk of cardiovascular disease (CVD) related with prediabetes by assessing oxidative stress and inflammation using serum interleukin-6 (IL-6), myeloperoxidase (MPO) and urine microalbumin (MA) and their correlation with fasting plasma glucose (FPG) and physical measurements.

Materials and methods: Based on FPG values, 80 subjects were grouped into prediabetes and healthy controls. IL-6 and MPO were estimated in serum sample whereas MA was estimated in random urine sample.

Results: Prediabetes group had significantly increased ($p < 0.05$) mean anthropometric measurements and IL-6, MPO and MA as compared to healthy controls. MPO had significant correlation with FPG (r-0.388) in the prediabetes group. IL-6 and MPO showed a positive correlation with body mass index (BMI (r-0.339, r-0.327)), waist circumference (WC (r-484, r-0.493)) and waist-to-hip ratio (WHR (r-0.430, r-0.493)) while MA did not correlate with FPG and anthropometric measurements.

Conclusion: This study suggests that prediabetes is associated with central adiposity, inflammation and oxidative stress predisposing them to an increased risk for CVD.

1. Introduction

Prediabetes is generally defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or both. It is associated with dyslipidemia, endothelial dysfunction, obesity, dysglycemia, pro-coagulant state, insulin resistance, hypertension and inflammation placing individuals with prediabetes at an increased risk of cardiovascular events [1].

Low grade inflammation is one of the major underlying pathophysiologic mechanisms responsible for development of cardiovascular disease (CVD). A major pro-inflammatory cytokine interleukin-6 (IL-6), contributes in the initiation and acceleration of chronic low grade inflammation resulting in endothelial dysfunction and atherosclerotic plaque formation in type 2 diabetes [2]. Myeloperoxidase (MPO) is an enzyme linked to both oxidative stress and inflammation and has been implicated in the pathogenesis of atherosclerosis and is associated with an increased CVD risk in diabetes population [3]. Microalbuminuria (MA), i.e., increased albumin excretion than normal in urine, is linked to CVD through oxidative stress and endothelial dysfunction. It is a prognosticator of cardiovascular mortality in the diabetic population [4].

IL-6, MPO and MA are associated with the development of CVD in diabetes patients but their role in prediabetes is still debatable. Therefore, estimation of IL-6, MPO and MA as indicators of CVD risk [3,5,6] in prediabetes and their correlation with fasting glucose and anthropometric measurements forms the basis of this study.

2. Materials and methods

A cross sectional study was conducted for study subjects who came with requisition for fasting plasma glucose (FPG) test in a tertiary care hospital, Mangalore. A total of 300 subjects of either gender aged 25–45 years were screened over a period of one year (December 2013–December 2014) and based on FPG values of 101–125 mg/dl or 70–100 mg/dl were categorized into prediabetes and healthy controls respectively. Eighty subjects were selected

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and 220 subjects were excluded on the basis of history of diabetes, endocrine disorders, kidney diseases, cardiac diseases, any infectious disease in the past two weeks and pregnancy. The study was conducted according to the guidelines of the Helsinki Declaration. This study was approved by the institutional ethics committee of Manipal University for medical research. All study procedures were explained to the participants and each provided written informed consent to participate in this study.

2.1. Anthropometric measurements

Anthropometric measurements were made for each participant. Body weight was measured using an adult balance and standing height was measured to the nearest centimeter using a wall-mounted stadiometer without shoes prior to eating in the morning. Body mass index (BMI) values were determined by weight (kg) divided by height (m) squared. Waist circumference (WC) was directly measured on the skin midway between the mean point of iliac peak and the inferior border of the last rib at the level of the umbilicus while in a standing position at the end of gentle expiration. Hip circumference was measured over the widest part of the gluteal region at the level of pubic tubercle in standing position. Waist to Hip ratio were determined by WC (cm) divided by hip circumference (cm).

2.2. Biochemical measurements

Blood sample was collected in plain vacutainer for IL-6 and MPO estimation and random urine sample was collected in sterile container for MA estimation. Serum samples were stored at −20 °C and urine samples were stored after addition of sodium azide at −20 °C until further analysis. All the serum and urine samples were brought to room temperature before analysis. Urine was centrifuged before testing.

IL-6 and MPO were analyzed using solid phase enzyme-linked immunosorbent assay (ELISA) based on sandwich principle in ELx 800 by BIO TEK® instruments, Inc. using commercially available kits. IL-6 kit was provided by RayBiotech, Inc., USA with detection range of 3–1000 pg/ml and sensitivity of 3 ng/ml. MPO kit was made available by Immunology Consultants Laboratory, Inc., USA with detection range of 1.875–120 ng/ml and sensitivity of 0.994 ng/ml. Urine MA was analyzed by the Latex-turbidimetric method in STAR 21 Plus semi-autoanalyser using commercially available kit provided by the Euro Diagnostic Systems Pvt. Ltd., India having a detection range of 2–1000 mg/l and Sensitivity of 3.8 mA mg/l.

2.3. Statistical analysis

Statistical package SPSS vers.16.0 was used for statistical analysis. Comparison between the groups was done by an independent sample '*t*' test and the Mann-Whitney '*U*' test for normal distribution and skewed data respectively. Possible associations between the FPG levels and other measurements were assessed using the Pearson correlation analysis and the Spearman's rank correlation coefficient. Normally distributed data are reported as means ± SD and skewed data are represented as median (interquartile range). Statistical inference is based on 95% confidence intervals (CIs) and the significance level was set at 0.05.

Table 1
Baseline characteristics comparison of the prediabetes and healthy group.

Variable	Prediabetes group ($n=40$)	Healthy controls ($n=40$)	*p*-Value
Age (years)	37.95 ± 6.08	36.05 ± 5.89	0.16
FPG (mg/dl)	109.18 ± 7.51	92.98 ± 4.23	0.000*
BMI (kg/m^2)	27.29 ± 1.38	22.81 ± 1.50	0.000*
WC (cm)	99.10 ± 4.74	87.22 ± 7.44	0.000*
HC (cm)	104.62 ± 3.45	102.53 ± 4.55	0.023*
WHR	0.94 ± 0.04	0.85 ± 0.05	0.000*

Results are shown as Mean ± SD, *n*—number of subjects, FPG—fasting plasma glucose, BMI—body mass index, WC—waist circumference, HC—hip circumference, WHR—waist-to-hip ratio.
* $p < 0.05$ was considered significant.

Table 2
Comparison of oxidative stress and inflammatory markers between the two groups.

Marker	Prediabetes group	Healthy controls	*p*-Value
IL-6 (pg/ml)	66.29 ± 15.39	12.59 ± 2.69	0.000
MPO (ng/ml)	67.46 ± 13.77	46.78 ± 9.93	0.000
MA (mg/l)*	19.07(14.75,28.96)	12.60(9.64, 15.81)	0.000

Results are shown as Mean ± SD.
* Median (interquartile range), IL-6—interleukin 6, MPO—myeloperoxidase, MA—microalbumin.
$p < 0.05$ was considered significant.

3. Results

Table 1 shows the baseline characteristics of the study participants in the two groups. The mean age of the participants in prediabetes group is 37.95 years and that in healthy controls is 36.05 years with no significant difference suggesting that the subjects of both the groups were age matched. Mean FPG differed significantly between the groups as per selection criteria. Participants with prediabetes had a significantly greater mean BMI, WC and waist-to-hip ratio (WHR). Study subjects with prediabetes had higher rate of general obesity (based on BMI) and central obesity (based on WC and WHR), respectively.

Table 2 shows the comparison of oxidative stress and inflammatory markers between the two groups. The mean serum IL-6, MPO and median urinary MA levels were found to be significantly increased in prediabetes group when compared with healthy controls.

Fig. 1 shows the association of IL-6, MPO and MA with FPG in prediabetes group. IL-6 has a correlation coefficient of 0.227, MPO has 0.388 and MA has 0.059 with FPG. Among all the three, only MPO correlation has a *p*-value <0.05.

Table 3 shows the correlation of IL-6 and MPO with BMI, WC, HC and WHR. IL-6 and MPO correlated significantly with BMI

Table 3
Correlation of IL-6 and MPO with anthropometric measurements.

Parameter	IL-6 (pg/ml)		MPO (ng/ml)	
	PD	*H*	PD	*H*
BMI (Kg/m^2)	0.339*	−0.044	0.327*	0.121
WC (cm)	0.484*	0.225	0.493*	−0.083
HC (cm)	0.141	0.240	0.074	0.175
WHR	0.430*	0.145	0.493*	−0.249

PD—prediabetes group, H—healthy controls, BMI—body mass index, WC—waist circumference, HC—hip circumference, WHR—waist-to-hip ratio, IL-6—interleukin-6, MPO—myeloperoxidase.
* $p < 0.05$ considered significant.

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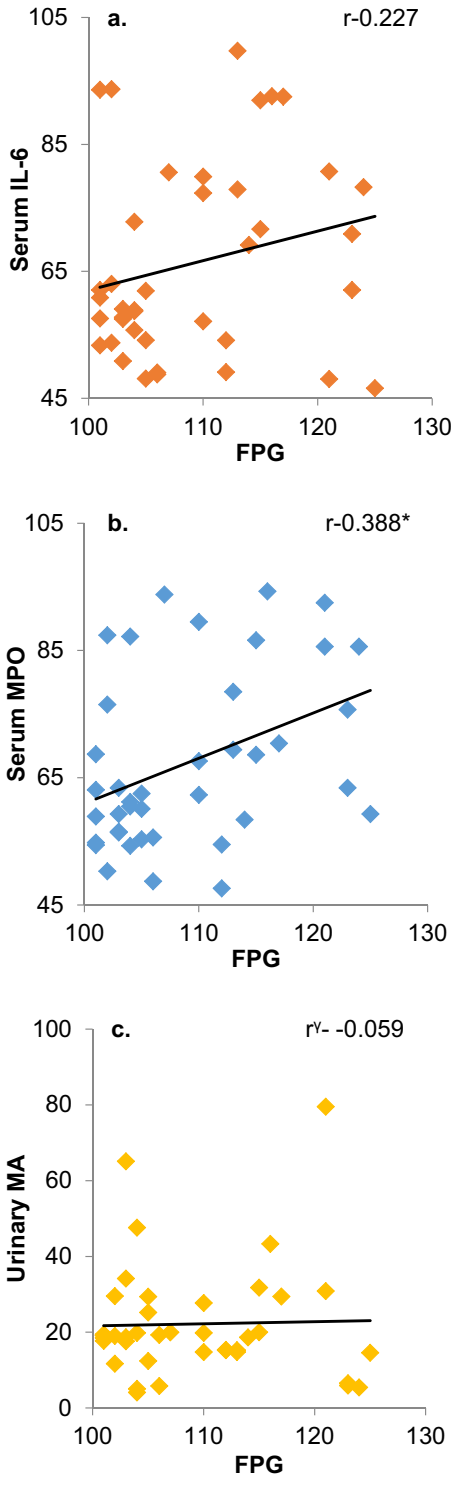

Fig. 1. Correlation of IL-6 (a), MPO (b) and MA (c) with FPG. FPG—fasting plasma glucose, IL-6—Interleukin-6, MPO—Myeloperoxidase, MA—microalbumin, *r*—Pearsons correlation, r^{γ}—Spearman correlation, $^{*}p < 0.05$ considered significant.

(*r*-0.339 and 0.327), WC (*r*-0.484 and 0.493) and WHR(*r*-0.430 and 0.493). MA had no correlation with anthropometric measurements.

4. Discussion

The current study was intended to explore the risk of CVD linked with prediabetes as compared to healthy population by assessing oxidative stress and inflammation using serum IL-6, MPO and urine MA. Besides having hyperglycemia, our data also suggests that prediabetes have higher rate of general obesity and central obesity. These finding in our study are consistent with the results of study conducted by Ferrannini [7] suggesting that prediabetes individuals, have mild hyperglycemia, a higher BMI, WC and higher WHR compared with normoglycemic subjects predisposing them to an increased risk of CVD.

Chronic inflammation has been suggested to play a causal role in endothelial dysfunction and atherosclerotic plaque formation contributing to the development of vascular complications in patients with diabetes [8]. Sommer et al. [9] found that hyperglycemia induces IL-6 production. This has been attributed to the formation of advanced glycation end products by persistent hyperglycemia, contributing to the development of chronic inflammation [10]. Elevated IL-6 in the prediabetes group indicates the presence of chronic ongoing inflammatory process in this group. But no correlation could be established between FPG and IL-6. Hossain et al. [11] reported IL-6 to correlate with glucose levels in prediabetes with IGT but not IFG.

IL-6 correlated with weight, BMI, WC and WHR in prediabetes subjects indicating that increased weight strongly contributes to the development of chronic inflammation. In vitro study [9] has demonstrated that adding the extract of adipocytes to human umbilical venous endothelial cells increases production of IL-6 by these cells. Previously documented findings [8,12] indicate that IL-6 is produced by adipose tissue macrophages, which may have an important role in the development of obesity and insulin resistance. The mechanism has been attributed to impaired insulin sensitivity and insulin resistance in target tissues with increased lipolysis and decreased glucose uptake in the adipose tissue [13,14] due to IL-6 induced reduction in tyrosine phosphorylation, and elevation of serine phosphorylation.

Recent advances in diabetes research have attributed hyperglycemia-mediated endothelial dysfunction, micro and macrovascular complications to reactive oxygen species (ROS). Oxidative stress marker MPO was elevated and correlated positively with FPG in prediabetes group indicating presence of endothelial dysfunction. Earlier studies have demonstrated a similar correlation in diabetes [15] and elevated levels of MPO with poor glycemic control [16]. There are two proposed mechanisms of endothelial dysfunction by MPO. Firstly, H_2O_2 mediated consumption of NO by MPO [17] and secondly production of $HOCl^{-}$ and its chlorinating species by reaction with high-glucose-stimulated H_2O_2 [18] resulting in reduced NO bioavailability. Anthropometric measures like BMI, WC and WHR correlated well with MPO. Increased BMI is related to inflammation and oxidative stress [19]. Similarly abdominal obesity as suggested by increased WC and WHR is also associated with systemic oxidative stress [20]. Thus it can be deduced that prediabetes group had signs of central obesity, inflammation and oxidative stress which predisposes them to dyslipidemia and CVDs.

Albuminuria is a robust and independent prognosticator of cardiovascular and all-cause mortality [21]. In the present study Urinary Albumin concentration was significantly increased in prediabetes group suggesting that hyperglycemia of prediabetes also leads to renal damage. A Korean study showed that subjects with MA had a higher FPG than those without [22]. Bahar et al. [23] reported a substantial correlation between FPG and urine albumin excretion (*r*-0.32, $p < 0.001$) in prediabetes patients, where the prevalence of MA was 18% in IFG group. In the current study, though 30% of prediabetes group were found to have MA, no correlation was observed between FPG and MA in this group. Current study enrolled people with only IFG. In the AusDiab study, [24] a significant relation was seen between prevalence of albuminuria and dysglycemia, predominantly postprandial

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glycemia [25]. Urinary Albumin concentration has been linked to obesity in earlier studies [26,27]. Though prediabetes group had higher BMI, WC, WHR and increased MA level, MA did not correlate with anthropometric measures.

The present study had several limitations. The principal limitation relevant to the interpretation of these results is the use of cross-sectional data, which limits inferences about causal pathways. Subjects with IFG were enrolled in prediabetes group, underestimating the overall risk of CVD in prediabetes which also includes IGT. A single fasting blood glucose measurement was the inclusion criteria which might have misclassified prediabetes.

In conclusion, MPO and IL-6 being markers of oxidative stress and inflammation, BMI and WHR being predictors of general and central obesity were found to be elevated in prediabetes group with IFG predisposing these people to increased risk of CVD. The study proposes an utmost need for preventive measures at the initial level of hyperglycemia and obesity to decrease the overall risk and development of CVD.

Conflicts of interest

The authors have none to declare.

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