Original Research Article

ASSESSMENT OF CARDIOVASCULAR DISEASE RISK IN PREDIABETES

ABSTRACT

Introduction: Prediabetes is associated with dysglycemia, endothelial dysfunction, obesity and inflammation, placing them at an increased risk of cardiovascular events.
Aims: The present study aimed to investigate the risk of cardiovascular disease associated with prediabetes by estimation of serum interleukin-6, myeloperoxidase and urine microalbumin and their correlation with fasting plasma glucose and anthropometric measurements.
Study design: Cross sectional study.
Place and duration of study: Study was conducted at Department of Biochemistry, Kasturba Medical College Hospitals, Mangaluru between 2014 and 2015.
Methodology: Eighty subjects were categorised into prediabetes and healthy controls based on their fasting plasma glucose values. Anthropometric data (weight, body mass index, waist circumference, hip circumference and waist-to-hip ratio from all subjects were recorded. Interleukin-6 & myeloperoxidase were estimated in serum sample whereas microalbumin was estimated in random urine sample.
Results: The mean anthropometric measurements and cardiovascular disease risk markers (interleukin-6, myeloperoxidase and urine microalbumin) were found to be significantly higher ($p<0.05$) in prediabetes group. Myeloperoxidase had significant correlation with fasting plasma glucose (r-0.388) in the prediabetes group. Interleukin-6 and myeloperoxidase also showed a positive correlation with body mass index (r-0.339, r-0.327), waist circumference (r-484, r-0.493) and waist-to-hip ratio (r-0.430, r- 0.493) while urine microalbumin did not correlate with fasting plasma glucose and anthropometric measurements in prediabetes group.
Conclusion: This study suggests that prediabetes is associated with central adiposity and have an increased risk for cardiovascular disease.

Keywords:Interleukin-6(IL-6); Myeloperoxidase(MPO); Microalbuminuria(MA); Cardiovascular risk; Prediabetes.

1. INTRODUCTION

Pre-diabetes is generally defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both. It is associated with dyslipidaemia, 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 & 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 associated with oxidative stress and endothelial dysfunction. It is a predictor of cardiovascular mortality and an independent risk factor for the development of CVD in the diabetic population [4].

IL-6, MPO and microalbuminuria are associated with endothelial dysfunction, low grade inflammation and oxidative stress which are the mechanisms for the development of CVD in diabetes patients but their role in prediabetes associated CVD is still debatable. Therefore, estimation of IL-6, MPO and microalbuminuria 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

2.1 Study design

A cross sectional study was conducted for study subjects who came with requisition for fasting plasma glucose test in a tertiary care hospital, Mangalore. Around 300 patients were screened over a period of one year out of which 80 subjects aged 25-45 years who met the inclusion criteria i.e. FPG of 101-125mg/dl or 70-100mg/dl were selected and categorised into prediabetes and healthy controls respectively. Subjects with history of diabetes, endocrine disorders, kidney diseases, cardiac diseases, any infectious disease in the past two weeks and pregnant women were excluded.

This study was carried out with the approval of the institutional Ethics Committee. After obtaining informed consent, history of the subjects was taken through a structured interview and a thorough physical examination of the subjects was done which included measuring the BP, pulse rate, height, weight, BMI, hip circumference, waist circumference and waist-to-hip ratio followed by systemic examination.

2.2 Biochemical Measurements

Along with sample for fasting plasma glucose in fluoride vacutainer, additional blood sample was collected in plain vacutainer for IL-6 & MPO and random urine sample was collected in sterile container for MA estimation. Serum samples for IL-6, MPO and urine for MA were stored at -20°C until further analysis.

Serum IL-6 and MPO were analysed using solid phase enzyme-linked immunosorbent assay (ELISA) based on sandwich principle in ELx 800 by BIO TEK® instruments, Inc. using commercially available kits provided by RayBiotech, Inc. and Immunology Consultants Laboratory, Inc. respectively. Urine MA was analysed by Latex-turbudimetric method in STAR 21 Plus semiautoanalyser using commercially available kit provided by Euro Diagnostic Systems Pvt. Ltd.

2.3 Statistical Analysis

Statistical package SPSS vers.16.0 was used and $P < 0.05$ was considered significant. Comparison between the groups was done by independent sample 't' test for normal distribution data and for data with skewed distribution Mann-Whitney U test was used. Correlation was done by Pearson's correlation for normal distribution data and for data with skewed distribution Spearman's correlation was used.

3. RESULTS

3.1 Baseline Characteristics

Following the inclusion criteria a total of eighty patients were enrolled in the study. Table 1 shows the baseline characteristics of the patients from both the groups. The mean serum glucose, weight, BMI, WC, HC and WHR were found to be significantly increased in prediabetes group when compared with the healthy controls. The mean age and height were comparable between the groups.

Table 1: Baseline characteristics of the Prediabetes and Healthy group.

Variable	Prediabetes group (n=40)	Healthy controls (n=40)	*P* -value
Age (years)	37.95 ± 6.08(0.96)	36.05 ± 5.89(0.93)	>0.05
FPG (mg/dl)	109.18 ± 7.51(1.18)	92.98 ± 4.23(0.66)	<0.05*
Height (cm)	158.22 ± 5.80(0.91)	159.72 ± 8.09(1.28)	>0.05
Weight (kg)	68.55 ± 7.59(1.20)	58.33 ± 7.06(1.11)	<0.05*
BMI (kg/m^2)	27.29 ± 1.38(0.21)	22.81 ± 1.50(0.23)	<0.05*

WC (cm)	99.10 ± 4.74(0.75)	87.22 ± 7.44(1.17)	<0.05*
HC (cm)	104.62 ± 3.45(0.54)	102.53 ± 4.55(0.71)	<0.05*
WHR	0.94 ± 0.04(0.006)	0.85 ± 0.05(0.008)	<0.05*

*Results are shown as Mean ± SD(SE- standard error of mean), 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.*

3.2 CVD risk markers

Table 2 shows the central values of cardiovascular disease risk markers (IL-6, MPO and MA) in both the 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.

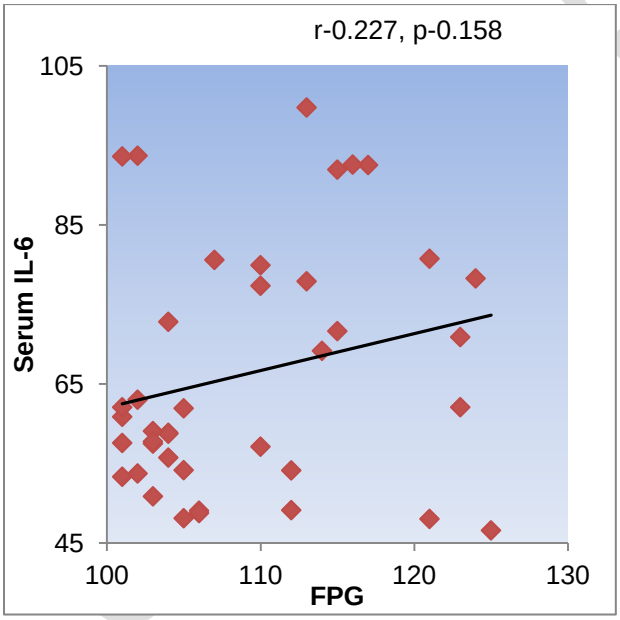
Table 2: Comparison of Cardiovascular Disease Risk Markers between the two groups.

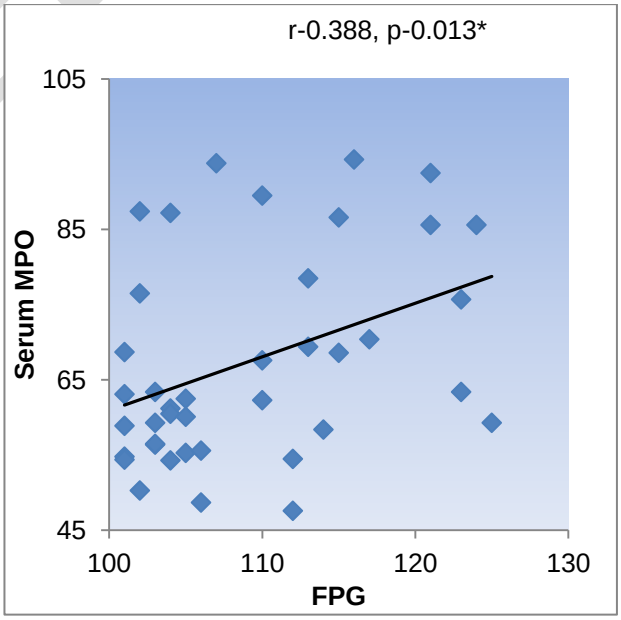
Marker	Prediabetes group	Healthy controls	*P* value
IL-6 (pg/ml)	66.29 ± 15.39(2.43)	12.59 ± 2.69(0.42)	<0.05
MPO (ng/ml)	67.46 ± 13.77(2.17)	46.78 ± 9.93(1.57)	<0.05
MA (mg/L)	19.07(14.75,28.96)*	12.60(9.64, 15.81)*	<0.05

Results are shown as Mean ± SD (SE-standard error of mean), median (interquartile range), IL-6 - Interleukin 6, MPO – Myeloperoxidase, MA-microalbumin, P <0.05 was considered significant*

3.3 FPG and CVD risk markers correlation

When Pearson's correlation was applied for FPG, versus serum IL-6, MPO and Spearman's correlation for urinary MA in both the groups as shown in Fig. 1, only MPO had a significant positive correlation (r- 0.388, *P*-0.013) with FPG in prediabetes group but had no correlation in the healthy controls. IL-6 and Urine MA had no correlation with FPG in both the groups.





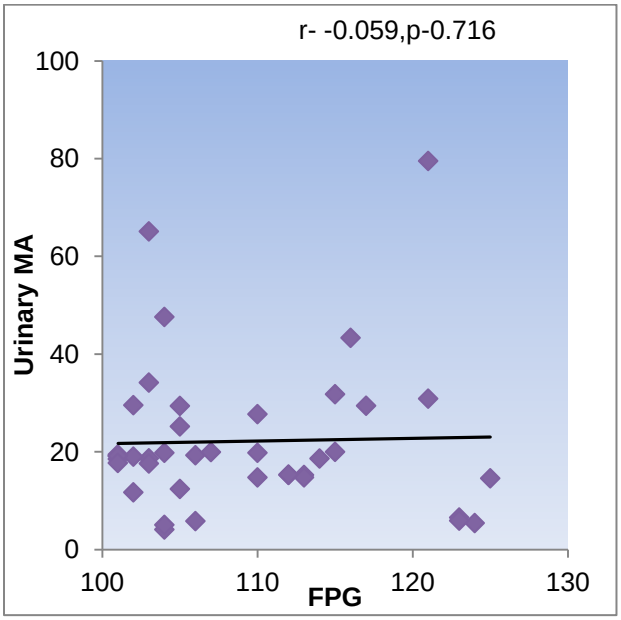


Fig. 1: Scatter plot showing correlation between serum IL-6, MPO and urinary MA with FPG in prediabetes group.
*FPG-Fasting Plasma Glucose, IL-6 - Interleukin 6, MPO –Myeloperoxidase, MA-microalbumin, r- correlation coefficient, * P <0.05 was considered significant*

3.4 Anthropometric measurements and CVD risk markers correlation

When Pearson's correlation was applied for serum IL-6 and MPO and Spearman's correlation for urinary MA versus anthropometric measurements i.e weight, BMI, WC, HC and WHR in both the groups as shown in Table 3, IL-6 were found to be positively correlated with weight, BMI, WC and WHR, MPO was found to be positively correlated with BMI, WC and WHR and Urine MA was not correlated with anthropometric measurements in prediabetes group.

Table 3: Correlation of Serum IL-6, MPO and Urinary MA with anthropometric measurements between the groups.

Parameter		IL6 (pg/ml) r value (p value)	MPO (ng/ml) r value (p value)	Urine MA (mg/L) # r value (p value)
Weight (Kg)	**PD**	0.341 (<0.05*)	0.274 (>0.05)	0.178# (>0.05)
	H	0.113 (>0.05)	0.033 (>0.05)	-0.217# (>0.05)
BMI (Kg/m^2)	**PD**	0.339 (<0.05*)	0.327 (<0.05*)	0.214# (>0.05)
	H	-0.044 (>0.05)	0.121 (>0.05)	-0.048# (>0.05)
WC (cm)	**PD**	0.484 (<0.05*)	0.493 (<0.05*)	-0.116# (>0.05)
	H	0.225 (>0.05)	-0.083 (>0.05)	0.161# (>0.05)
HC (cm)	**PD**	0.141 (>0.05)	0.074 (>0.05)	-0.110# (>0.05)
	H	0.240 (>0.05)	0.175 (>0.05)	0.147# (>0.05)
WHR	**PD**	0.430 (<0.05*)	0.493 (<0.05*)	-0.076# (>0.05)
	H	0.145 (>0.05)	-0.249 (>0.05)	0.100# (>0.05)

*BMI – Body Mass Index, WC – waist circumference, HC – hip circumference,WHR – waist-to-hip ratio, PD – Prediabetes group, H – Healthy controls, IL-6 – Interleukin-6, MPO- Myeloperoxidase, MA- microalbumin, r value – Pearson's correlation,# - Spearman's correlation, *P<0.05 considered significant.*

4. DISCUSSION

Prediabetes is associated with microangiopathy and also with more advanced atherosclerotic vascular damage than normoglycemia. Contribution of both glycemic and non-glycemic factors in the development of CVD during prediabetes is supported by the different pathophysiologic pathways leading to vasculopathy. [1] The risk of cardiovascular disease in prediabetes as compared to healthy population was evaluated using serum interleukin-6, myeloperoxidase and urine microalbumin in the current study. At baseline, it was observed that the subjects in prediabetes group had increased weight, BMI, waist circumference, hip circumference and waist-to-hip ratio in contrast to healthy group (Table 1). We found increased levels of IL-6, MPO and urine MA in prediabetes group as compared to healthy group. Further, we found a correlation of MPO with fasting glucose in prediabetes. We also found correlation of IL-6 and MPO, but not urine MA with anthropometric measurements in prediabetes.

The increase in anthropometric measurements found in prediabetes subjects in the present study is supported by the study conducted by Ferrannini which suggested that prediabetes individuals, aside from having mild hyperglycemia, have a higher BMI as well as more central fat distribution and higher waist-to-hip ratio compared with normoglycemic subjects [7].

Chronic inflammation could be one of the reasons of endothelial dysfunction and atherosclerotic plaque formation, processes which contribute to the development of vascular complications in patients with diabetes [8]. Elevated serum IL-6 concentration in the prediabetes group(Table 2) indicates the presence of chronic ongoing inflammatory process in this group,[9] which has been confirmed by the results of a study conducted by Sommer et al., where it has been found that hyperglycemia induces IL-6 production[10]. This can be attributed to the formation of advanced glycation end products by persistent hyperglycemia, contributing in the development of chronic inflammation[11]. No correlation could be established between IFG and IL-6 concentration in the present study (Table 2). Hossain et al [12] reported correlation of IL-6 with IGT but not with IFG. In present study only IFG subjects were enrolled which could be a reason for this result.

A corrrelation between IL-6 concentration with weight and BMI in prediabetes subjects indicates that increased weight strongly contributes to the development of chronic inflammation(Table 3)[13]. The results of an in vitro study has demonstrated that after adding the extract of adipocytes to human umbilical venous endothelial cells, there is increased production of IL-6 by these cells[14]. A strong corrrelation between IL-6 concentration and abdominal obesity (suggested by waist circumference (WC) and waist-hip ratio (WHR) was observed in our study. Previously documented findings [8,15] indicate that IL-6 is produced by adipose tissue macrophages, which may have an important role in the development of obesity and insulin resistance. IL-6 reduces tyrosine phosphorylation, impairing insulin sensitivity and increases serine phosphorylation leading to insulin resistance in target tissues, increasing lipolysis and decreasing glucose uptake in the adipose tissue [16].

Recent advances in diabetic research suggest that hyperglycemia-mediated endothelial dysfunction and micro and macrovascular complications can be attributed to reactive oxygen species (ROS). Positive correlation was observed between MPO with FPG only in the prediabetes group (Fig. 1). Earlier studies have demonstrated a similar correlation in diabetes[17] and higher levels of MPO with poor glycemic control[18]. There are two proposed mechanisms that may be involved in endothelial dysfunction by MPO. Firstly, H_2O_2 mediated consumption of NO by MPO [19] and secondly production of $HOCl^-$ and its chlorinating species by reaction with high-glucose–stimulated H_2O_2 [20] resulting in reduced NO bioavailability. Elevated serum MPO concentration in the prediabetes group (Table 2) indicate the presence of chronic inflammation and endothelial dysfunction in them[19]. MPO levels also correlates with anthropometric measures like BMI, WC and WHR in prediabetes group (Table 3) Increased BMI is related to inflammation and oxidative stress[21]. Similarly abdominal obesity as suggested by increased WC and WHR is also associated with systemic oxidative stress[22]. Thus it can be deduced that prediabetes group had raised levels of MPO as a marker of oxidative stress and signs of central obesity seen in this group predisposes them to dyslipidemia and cardiovascular diseases.

Proteinuria is a sign of more advanced renal disease and is a precursor to renal failure. Importantly, albuminuria is a strong and independent predictor of cardiovascular and all cause mortality [23]. In the present study Urinary Albumin concentration was significantly increased in prediabetes group (Table 2) suggesting that hyperglycemia of prediabetes also leads to renal damage [24]. A recent study of the Korean general population also showed that subjects with urine albumin in microalbuminuria range had a higher fasting plasma glucose than subjects without microalbuminuria [25]. Bahar et al reported a significant 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 (Fig. 1). In the AusDiab Study, [26] the prevalence of albuminuria increased significantly with increasing glycaemia, particularly postprandial glycaemia [27]. Current study had enrolled people with IFG only, hence there was no correlation observed between FPG and MA.

Urinary Albumin concentration has been linked to obesity in earlier studies [28,29]. The prediabetes group had higher BMI, WC, WHR and increased MA level. But MA did not correlate with anthropometric measures of this group because of low prevalence in prediabetes subjects.

Therefore, our data suggest that prediabetes may play a role in the development and progression of cardiovascular disease. This is important because, screening for diabetes in prediabetes stage may have an importance for CVD in the future.

5. CONCLUSION

In view of MPO and IL-6 being markers of oxidative stress and CVD risk their elevation in IFG predisposes these people to increased CVD and mandates preventive measures to be taken at the initial level of hyperglycemia.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication as per international and university standards.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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ABBREVIATIONS

CVD	Cardiovascular Disease
IL-6	Interleukin-6
MPO	Myeloperoxidase
MA	Microalbumin
FPG	Fasting Plasma Glucose
BMI	Body Mass Index
WC	Waist Circumference
HC	Hip Circumference
WHR	Waist-To-Hip Ratio
R	Pearson's Correlation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
BP	Blood Pressure
ELISA	Enzyme-Linked Immune Sorbent Assay
SPSS	Statistical Package For Social Science
PD	Prediabetes Group
H	Healthy Controls
ROS	Reactive Oxygen Species
H_2O_2	Hydrogen Peroxide
NO	Nitric Oxide
$HOCL^-$	Hypochlorus Acid
RSSDI	Research Society For Study Of Diabetes In India
MU	Manipal University