Diabetes & Metabolic Syndrome: Clinical Research & Reviews 10S (2016) S123–S126

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

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

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ARTICLE INFO

Keywords:
Interleukin-6 (IL-6)
Myeloperoxidase (MPO)
Microalbuminuria (MA)
Prediabetes

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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http://dx.doi.org/10.1016/j.dsx.2016.03.009
1871-4021/

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.