Original Research Article

Oxidative Stress, Endothelial Dysfunction, Inflammation and Impaired Coagulopathy in Prediabetic Individuals

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ABSTRACT

Background and objectives: Prediabetic is the precursor state of the disease Diabetic Mellitus (DM).It is a metabolic diathesis with high blood sugar and dyslipidaemia. This increases the risk for the development of DM and cardiovascular diseases. The prediabetic state is classified into: - (1) the impaired fasting glycemia (IFG) and (2) impaired glucose tolerance (IGT). WHO has defined impaired fasting glucose level as 110 to 125mg% and impaired glucose tolerance as that 2hour plasma glucose to be within the range of 140 to 200 mg% after an oral glucose load of 75grams. Hence, this study was designed to(1) evaluate the role oxidative stress, endothelial dysfunction , inflammation (2) find diagnostic markers for early detection of changes in prediabetes to prevent DM.

Material and method: This case control study included 112prediabetic individuals and an equal number of matched healthy volunteers. The FBG, lipid profile, HbA1c and CRP were estimated. We used ferrous oxidation in xylenol orange version 2 (FOX2) assay to estimate the level of oxidative load and FRAP assay for total antioxidant capacity; ratio of FOX2 & FRAP assay provided the oxidative stress level. We estimated endothelial dysfunction by serum nitric oxide (NO) level, inflammation by CRP and coagulopathy by plasma Fibrinogen level.

Results: A positive correlation was seen between oxidative stress, glycated haemoglobin, inflammation and dyslipidemia and negative correlation with serum nitric acid.

Conclusion: Biomarkers of oxidative stress, inflammation, dyslipidemia, serum nitric oxide should be monitered along with, glycated haemoglobin in prediabetic patients so that DM can be prevented.

Keywords: endothelial dysfunction, inflammation, impaired coagulopathy, oxidative stress, prediabetes

INTRODUCTION

Prediabetic is the precursor state prior to the development of the disease Diabetes Mellitus (DM). In the prediabetic state the blood sugar is high but without the other signs & symptoms of DM. DM is a worldwide health problem affecting approximately more than 6% of the global population. [1] The prevalence of DM is predicted to increase to about 552 million in 2030. [1] These figures do not estimate the prediabetic individuals. Most of the prediabetic individuals are not aware of their disease status. Prediabetic is the precursor state prior to the development of the disease DM. [2] In the prediabetic state the blood sugar is high but without the other signs & symptoms of DM. As per the American Diabetes Association it is a metabolic diathesis with high blood sugar as the only sign and associated with obesity and dyslipidemia. This state increases the

high risk for the development of DM and cardiovascular diseases (CVD). [2]

The prediabetic state is again classified into two forms:- (1) the impaired fasting glycemia (IFG) and (2) impaired glucose tolerance (IGT). [3] The WHO criteria for IFG is that the fasting glucose level more than 110 mg% and less than or equal to 125 mg%. [4]

The IGT stage of prediabetes is associated with dysglycemia and insulin resistance. [4] The prediabetic individuals are usually unaware of their condition though they are at a risk of developing DM, CVD. [5,6] The huge socioeconomic burden for diagnosis and treatment of DM and its complications can be averted by early detection of prediabetic stage. This can be done by identifying the biomarkers of prediabetic state. The commonly used biomarkers are FBG level, HbA1c and Lipid profile. The other biomarkers associated with the disease progression are markers of inflammation, oxidative stress and endothelial dysfunction. Recent studies have associated oxidative stress and endothelial dysfunction with CVD in DM. Endothelial dysfunction and oxidative stress has a positive correlation with hyperglycemia. [5,6]

Hence, this study was designed to (1) evaluate the role oxidative stress, endothelial dysfunction, inflammation in disease progression in prediabetes (2) to find diagnostic markers for early detection of changes in prediabetes so the treatment can be ensured to prevent DM, CVD.

MATERIAL AND METHOD

This case control study included 112 prediabetic individuals and an equal number of matched healthy volunteers. The FBG, lipid profile, HbA1c and CRP were estimated by commercial kits adapted to the autoanalyser. We used ferrous oxidation in xylenol orange version 2 (FOX2) assay [7] to estimate the level of oxidative load and FRAP [8] assay to measure the total antioxidant capacity and the ratio of FOX2 & FRAP assay provided the oxidative stress level. [9] We estimated endothelial

dysfunction by serum nitric oxide (NO) level using Griess method, [10] inflammation by CRP and coagulopathy by plasma Fibrinogen level. [11] The study included prediabetic individuals attending the Regional diagnostic centre for availing health checkups. All the prediabetic individuals belonged to the age group of 38 - 55 yrs. The study included 120 males and 104 females. The screening process included measuring the BMI, FBG, Lipid profile, Atherogenic index of plasma (log TG/ HDLc). [12]

Sample preparation & Processing

Venous blood was collected after an overnight fast into serum separating tubes and sodium citrate tubes. The oxidant load, antioxidant capacity, nitric oxide & plasma fibrinogen levels were measured immediately.

The FBG, lipid profile, HbA1c, CRP and plasma fibrinogen were estimated by commercial kits adapted to the autoanalyser. We used FOX2 assay, ferrous ion oxidation xylenol orange assay [7] to estimate the level of oxidative load and FRAP, ferric reducing antioxidant [8] assay to measure the total antioxidant capacity and the ratio of FOX2 & FRAP assay provided the oxidative stress level. [9] The oxidant load was measured by ferrous ion oxidation xylenol orange, FOX 2 assay method. The principle of this method is based on oxidation of ferrous to ferric ion by hydrogen peroxide. The ferric ion reacts with xylenol orange to produce a coloured complex. The intensity of the colour is measured at 560nm. The FRAP assay measures the antioxidant status of the serum. The principle is ferric ion is reduced to ferrous ion at a low pH and reacts with tripyridyltriazine to form a coloured complex and readings are taken at 593nm. We estimated endothelial dysfunction by serum nitric oxide (NO) level by Griess method. [10] Serum nitric oxide is estimated by the Griess method, follows the principle of chemical diazotization reaction using sulphanilamide and N-1-napthylethyenamidediamine dihydrochoride

under the acidic conditions provided by phosphoric acid.

Statistical Analysis

All the data is represented as mean ± SD. The data was compared by unpaired student's 't' test and correlation was derived by Pearson correlation analysis. SPSS version 19 was used to derive all statistical analysis. A 'P' value <0.05 was considered significant.

RESULTS

We observed that the prediabetic had a significantly higher level of serum triglyceride, CRP and plasma fibrinogen level. The glycated HbA1c is also slightly higher though within the normal range. As shown in Table – II the oxidant load

(FOX2) is higher and the total antioxidant capacity (FRAP) is lower in the prediabetics. The nitric oxide level is also low in the prediabetics as compared to the controls.

Table – 1: Demography characteristics and Biochemical parameters of Control and Prediabetic individuals

Parameters	Control	Prediabetic
FBG(mg%)	98 ± 3.4	122 ± 4.4
Age (yrs)	55±8	57±6
Sex (M/F)	128/100	128/100
Family history of DM (%)	32	43
Medications		
Statin	2	4
Antihypertensive	6	8
BMI (kg/m^2)	24±6	26±4
HbA1c (%)	5.7±0.89	5.68±0.9*
Total Cholesterol (mg%)	156±1.7	158±2
Triglyceride(mg%)	172±3.6	188±2.8*
HDL-C (mg%)	50±8.46	38±9.2
LDL-C (mg%)	79 ± 2.22	100 ± 2.8
VLDL	34 ± 0.7	37 ± 0.5
CRP (mg/L)	3.08±1.06	6.4±1.5*
Plasma Fibrinogen (mg/dl)	206±14	218±17.3*
Atherogenic index	0.044	0.059

*p value < 0.05

Table – 2: Oxidation stress parameters and serum nitric oxide level in controls and prediabetics

Parameters	Control	Prediabetics
FOX2 (equivalent of H_2O_2)µmol/L	4.12±1.46	14.20±4.1*
FRAP (equivalent of ferrous sulphate) µmol/L	444.54±16.22	100.2±46.44*
Nitric Oxide level (µM/L)	52.68±9.4	36.99±3.1*

*P value < 0.05

DISCUSSION

Prediabetes is the asymptomatic state of DM. In this study we tried to evaluate the existing biomarkers for diagnosis of prediabetes and also tried to find new markers which can indicate disease progression. In our study we observed that the prediabetics had slightly higher HbA1c, FBS. The inflammatory marker CRP, coagulopathy marker plasma fibrinogen and FOX2 oxidant load is also high in prediabetics. The protective mechanisms such as the total antioxidant capacity, FRAP and marker of endothelial dysfunction, nitric oxide levels are low. We observed the changes levels of the biomarkers begin in the prediabetic stage. The prediabetics had a significantly higher oxidant load and lower antioxidant status as compared to controls. This shows that oxidative stress exists in prediabetic stage and is associated with disease progression. This observation is in concurrence with previous studies. [13]

We observed a higher plasma fibrinogen level. Plasma fibrinogen is an important molecule in the coagulation cascade and is also an acute plasma protein. [14] The increased level of plasma fibrinogen in prediabetics show an increase in inflammatory and atherothromboembolic activity, this predisposing to vascular complications of diabetes. [15]

Studies have suggested that increased oxidative stress prevent fibrinogen degradation and increase its synthesis leading to the hypercoagulable state in diabetes. [16] Similar changes also commence in the prediabetic stage.

We observed a lower serum nitric oxide level in prediabetic as compared to controls. Studies have observed a similar trend in the DM patients. [17] Nitric oxide, is a marker of endothelial dysfunction and is synthesised by nitric oxide synthase using L- Arginine and molecular oxygen as raw materials. Nitric oxide is required to mantain the blood pressure; relax the

vascular smooth muscles, release of growth hormone and Insulin. [18,19] Oxidative stress impairs the function of nitric oxide synthase and thus lowers the nitric oxide level in the serum. Thereby, impairing the endothelial function and predisposes to CVD and PVD. [20] These changes begin during prediabetic stage.

This implies that an increased oxidative stress level is found in the Prediabetic state. In normal physiological conditions the blood glucose molecules enter the glycolytic pathway and undergo oxidative phosphorylation. In the hyperglycemic conditions as seen in prediabetes and type 2 DM can overwhelm the glycolytic pathway and stall glyceraldehyde catabolism, leading to shunting of glucose, fructose-1-6 bisphosphate, glyceraldehyde-3-phospate to othe pathways such as enol and ketoaldehyde formation, protein kinase activation, dicarbonyl formation, glycation, sorbitol metabolism, hexosamine formation and an increase in oxidative phosphorylation. All these pathways affect the NADPH system and increase production of reactive oxygen production and oxidative stress. Oxidative stress causes inflammation and leads to a prohypercoagulable state, which has been implicated by various previous studies and our study also has similar observations. [21]

Prediabetics, both IGT & IFG are the asymptomatic stages of type 2 DM. Hence the prediabetic patients are ideal for starting early therapeutic interventions so that the disease progression to frank DM & its complications CVD & PVD can be prevented.

The observations of our study show that oxidative stress, endothelial dysfunction, increased inflammation and hypercoagulable state commence in the prediabetic stage. Hence, these biomarkers could be used to screen the prediabetic individuals and appropriate therapeutic and preventive measures should be taken to prevent the disease progression to frank DM.

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