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Myeloperoxidase, malondialdehyde and serum lipids in type 2 diabetes mellitus

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ABSTRACT

Introduction: In diabetics, oxidative stress is increased in all stages of cardiovascular disease; from lipoprotein modification to plaque rupture. Oxidative stress increases the risk of development of cardiovascular complications. Atherogenic index of plasma (AIP) is related directly to the atherosclerotic risk. Oxidative stress markers have value in long-term cardiovascular risk prediction. In view of this, the present study was taken to compare and correlate the levels of oxidative stress markers and AIP in type 2 diabetic patients. **Methods:** A total of 140 individuals were included in the study. Of which 70 are type 2 diabetics and 70 are age and sex matched controls. Levels of serum myeloperoxidase (MPO), malandialdehyde (MDA), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and AIP is measured in these study population. **Results and Conclusion:** Statistically significant increase in body mass index (27.2 ±1.2 kg/m^2), waist-hip ratio (1.02 ± 0.04), serum MPO (211.71 ± 2.24 μmol/min) and MDA (549 ± 45 nmole/100 ml), TC (274 ± 24 mg/dl), TG (189 ± 2.5 mg/dl), LDL-C (207 ± 45 mg/dl) and AIP (0.47 ± 0.02) was seen in type 2 diabetic patients when compared to controls. The levels of these parameters are higher in diabetic patients having diabetes ≥10 years and serum cholesterol ≥200 mg/dl. AIP was positively correlated with the levels of serum MPO, MDA, TC, TG, LDL-C and negatively correlated with serum HDL-C that was statistically significant. By measuring serum MPO, MDA, lipid profile and AIP helps in long-term cardiovascular risk assessment in patients with diabetes and by measuring these parameters help to detect these complications as early as possible and early interventions can be done to prevent future development of cardiovascular complications.

KEY WORDS: Atherogenic index of plasma, malandialdehyde, myeloperoxidase, oxidative stress, type-2 diabetes mellitus

INTRODUCTION

Diabetes is a metabolic disorder with hyperglycemia either due to an absolute or relative deficiency of insulin secretion or reduction in the biologic effectiveness of insulin or both [1]. The abnormalities in diabetics' mellitus contribute to cellular events that cause atherosclerosis and subsequently increase the risk of cardiovascular events [2]. The oxidative stress biomarker myeloperoxidase (MPO) enhances cardiovascular risk prediction [3]. Serum malandialdehyde (MDA) level was significantly higher in patients with diabetes for more than 5 years and in the presence of diabetic complications (both microvascular and macrovascular) [4].

Risk of cardiovascular disease (CVD) increased significantly with increasing total cholesterol (TC) and low-density lipoproteins cholesterol (LDL-C) [5]. Type 2 diabetic patients are more prone to develop cardiovascular complications. Atherogenic index of plasma (AIP) is the new marker of atherogenicity, since the AIP is related directly to the atherosclerotic risk.

The levels of MPO, MDA and AIP in these patients help to detect cardiovascular complications, and early interventions can be done to prevent future development of cardiovascular complications.

MPO (EC 1.11.1.7) a member of the heme peroxidase superfamily is a tetrameric hemoprotein (MW 144,000 Da) consisting of a pair of heavy (57 kDa) and light (15 kDa) chains. It is a stored in azurophilic granules of polymorph nuclear neutrophils and monocyte macrophages and when released (typically with inflammation), catalyzes the conversion of chloride anion and hydrogen peroxide to hypochlorite, a metal ion-independent chlorinating oxidant that possesses potent microbicidal activity. Hence, it has a role in host defense against pathogens. MPO activity leads to oxidation of LDL-C. Oxidation of high-density lipoprotein cholesterol (HDL-C)

reduce its capacity to transport cholesterol from tissues to the liver, and it consumes endothelial derived nitric oxide, which can lead to plaque formation and endothelial dysfunction [6].

MDA is generated by both lipid oxidation and as a by-product of prostaglandin and thromboxane synthesis. Its plasma concentration is increased in diabetes mellitus, and it is found in the atherosclerotic plaque, which is promoted by diabetes. The clinical relevance of the reaction between MDA and proteins is highlighted in atherosclerosis, in which the arterial intima becomes infiltrated with foam cells, resulting in thickened, non-resilient arteries with reduced blood flow. This is a major cause of CHD and strokes [7].

Diabetes mellitus and atherosclerosis are two main causes of cardiovascular morbidity and mortality. Epidemiological studies have shown that several factors contribute to the onset and progression of atherosclerosis. Factors such as age, gender and genetic predisposition are non-changeable parameters. Increased levels of serum cholesterol are the main risk factor for atherosclerosis, especially LDL-C fraction, and other risk factors for atherosclerosis: smoking, decreased glucose tolerance, obesity and sedentary lifestyle.

Prevalence of metabolic syndrome in developing countries is rising due to an increase in the incidence of obesity. Central obesity plays a key role in the pathogenesis of the metabolic syndrome: promotes inflammation, hypertension, dyslipidemia and it leads to the development of type 2 diabetes and atherosclerosis. Correcting and controlling these means less risk of increase or progression of atherosclerosis [8].

Objectives

1. To compare the serum levels of MPO, MDA, AIP, LDL-C, HDL-C, triglyceride (TG), TC body mass index (BMI) and waist hip ratio (WHR)
   a. In type 2 diabetics and age and sex matched healthy controls,
   b. In type 2 diabetics with duration of diabetes less than 10 years and ≥10 years.
   c. In type 2 diabetics with serum TC levels less than 200 mg/dl and ≥ 200 mg/dl.
2. To correlate AIP with serum levels of MPO, MDA, LDL-C, HDL-C, TG, TC, BMI and WHR in type 2 diabetic individuals.

MATERIALS AND METHODS

Study group included 140 individuals of which 70 patients were suffering from type 2 diabetes mellitus (newly diagnosed and diabetics on treatment) and 70 healthy controls. Subjects with the previous history of febrile illness, renal failure, chronic diseases, benign and malignant disorder were excluded. Study was approved by Institution Ethical Committee.

Height, weight, waist circumference, and hip circumference were measured using standard methods. BMI and WHR were calculated [9]. After obtaining the informed consent fasting blood sample (5 ml) was drawn from all the study population by venipuncture taking aseptic precaution in a plane tube and samples were centrifuged at 3000 rpm for 15 min and serum was obtained.

Analysis of TC [10], TG [11] and HDL-C [12] was done. LDL-C was calculated by using Friedwald's formula [12]. Serum MPO levels were estimated by spectrophotometric method using O-Dianisidine dihydrochloride as a substrate [13]. MDA is estimated as thiobarbituric acid reactive substances [14]. AIP was calculated by taking the log of (TGL/HDL) [15]. Data was analyzed using analysis of variance and Karl Pearson's correlation. All the analysis was done using the windows based SPSS statistical package (Version 10.0; SPSS Inc.; Chicago, IL, USA) and $P < 0.05$ were taken as the level of significance.

RESULTS

In comparison with non-diabetic individuals, type 2 diabetic individuals had statistically highly significant increase in BMI, WHR, serum levels of MPO, MDA, TC, LDL-C and AIP (P<001) and statistically significant decrease in serum HDL cholesterol as shown in Table 1.

When BMI, WHR, serum levels MPO, MDA,TC, LDL-C, TG and AIP were compared between type 2 diabetics with duration of diabetes <10 years and ≥10 years, individuals having diabetes for ≥10 years had statistically significant increase in levels of BMI, WHR, serum levels MPO, MDA,TC, LDL-C, TG and AIP ($P < 0.05$) [Table 2].

In comparison with type 2 diabetics with serum TC <200 mg/dl, the type 2 diabetic individuals with serum TC ≥200 mg/dl had statistically highly significant increase in BMI, WHR, serum levels of MPO, MDA, TG and AIP ($P < 001$) [Table 3].

Pearson's correlation analysis of AIP with other risk variables in cases had shown that in type 2 diabetic patients AIP had shown a positively correlated with BMI, WHR, MPO, MDA, TC, LDL-C and TG, which was statistically significant. However, a statistically significant negative correlation was found between AIP and serum HDL-C ($P < 001$) [Table 4 and Figure 1].

Table 1: Comparison of BMI, WHR, oxidative stress markers, lipid profile and AIP in study population

Parameters	Controls	Cases	*P* value
BMI (kg/m²)	19.2	27.2	<0.001
WHR	0.73	1.02	<0.001
MPO (μmol/min)	21.14	211.71	<0.001
MDA (nanomole/100 ml)	280	549	<0.001
TC (mg/dl)	163	274	<0.001
LDL-C (mg/dl)	85	207	<0.001
HDL-C (mg/dl)	52	28	<0.001
TG (mg/dl)	126	189	<0.001
AIP	0.016	0.47	<0.001

$P<0.05$ significant. BMI: Body mass index, WHR: Waist hip ratio, MPO: Myeloperoxidase, MDA: Malandialdehyde, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride, AIP: Atherogenic index of plasma

DISCUSSION

In the present study, we observed that diabetic subjects have higher BMI and WHR. Various studies have found that most adults with diagnosed diabetes were overweight or obese, Eric and John (2006) studies shows that prevalence of obesity was 85.2% and NHANES (2005) report indicates the prevalence of obesity was 54.8% [1].

Table 2: Comparison of BMI, WHR, Oxidative stress markers, lipid profile and AIP in type 2 diabetics with duration of diabetes <10 years and ≥10 years

Parameters	Type 2 diabetics with duration of diabetes <10 years	Type 2 diabetics with duration of diabetes ≥10 years	*P* value
BMI (kg/m²)	26.08	27.28	<0.05
WHR	0.9	1.04	<0.05
MPO (μmol/min)	196	217	<0.05
MDA (nanomole/100 ml)	506	566	<0.05
TC (mg/dl)	265	277	<0.05
LDL-C (mg/dl)	180	210	<0.05
HDL-C (mg/dl)	29	24	<0.05
TG (mg/dl)	185	191	<0.05
AIP	0.40	0.50	<0.05

P<0.05 significant. BMI: Body mass index, WHR: Waist hip ratio, MPO: Myeloperoxidase, MDA: Malandialdehyde, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride, AIP: Atherogenic index of plasma

Table 3: Comparison of BMI, WHR, oxidative stress markers, TG and AIP in Type 2 diabetics with serum TC levels <200 mg/dl and ≥200 mg/dl

Parameters	Type 2 diabetics with serum TC levels <200 mg/dl	Type 2 diabetics with serum TC levels ≥200 mg/dl	*P* value
BMI (kg/m²)	22.9	27.8	<0.001
WHR	0.8	1.05	<0.001
MPO (μmol/min)	125	224	<0.001
MDA (nanomoles/100 ml)	364	576	<0.001
TG (mg/dl)	167	192	<0.001
AIP	0.39	0.48	<0.001

P<0.05 significant. BMI: Body mass index, WHR: Waist hip ratio, MPO: Myeloperoxidase, MDA: Malandialdehyde, TG: Triglyceride, AIP: Atherogenic index of plasma

Table 4: Correlation between AIP and other risk variables in cases

Parameters	*r*	*P* value
AIP		
BMI (kg/m²)	0.973	<0.001
WHR	0.940	<0.001
MPO (μmol/min)	0.907	<0.001
MDA (nanomole/100 ml)	0.990	<0.001
TC (mg/dl)	0.962	<0.001
LDL-C (mg/dl)	0.974	<0.001
HDL-C (mg/dl)	−0.989	<0.001
TG (mg/dl)	0.987	<0.001

P<0.05 significant. BMI: Body mass index, WHR: Waist hip ratio, MPO: Myeloperoxidase, MDA: Malandialdehyde, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride, AIP: Atherogenic index of plasma

We observed significant increase of MPO in subjects with type 2 diabetes mellitus. These findings were in accordance to the study of Shetty *et al*. [16]. Low grade chronic inflammation and endothelial dysfunction are contributing factors in the initiation and progression of CVD.

The MDA levels were elevated in diabetic subjects compared with non-diabetic subjects. These findings were in accordance to the study of Slatter *et al*. [7]. The inter-molecular cross-linking of collagen through MDA is important in the late complications of diabetes mellitus because it contributes to the stiffening of the cardiovascular tissue.

Type 2 diabetes mellitus is associated with dyslipidemia, which is due to insulin deficiency, insulin resistance and hyperglycemia. The present study showed that serum TC were found to be increased in type 2 diabetics when compared with controls. Suryawanshi *et al*. [17] found a statistically significant increase in TC, TG and LDL cholesterol. Increase in TC may be due to decreasing muscular exercise or inhibition of cholesterol catabolism.

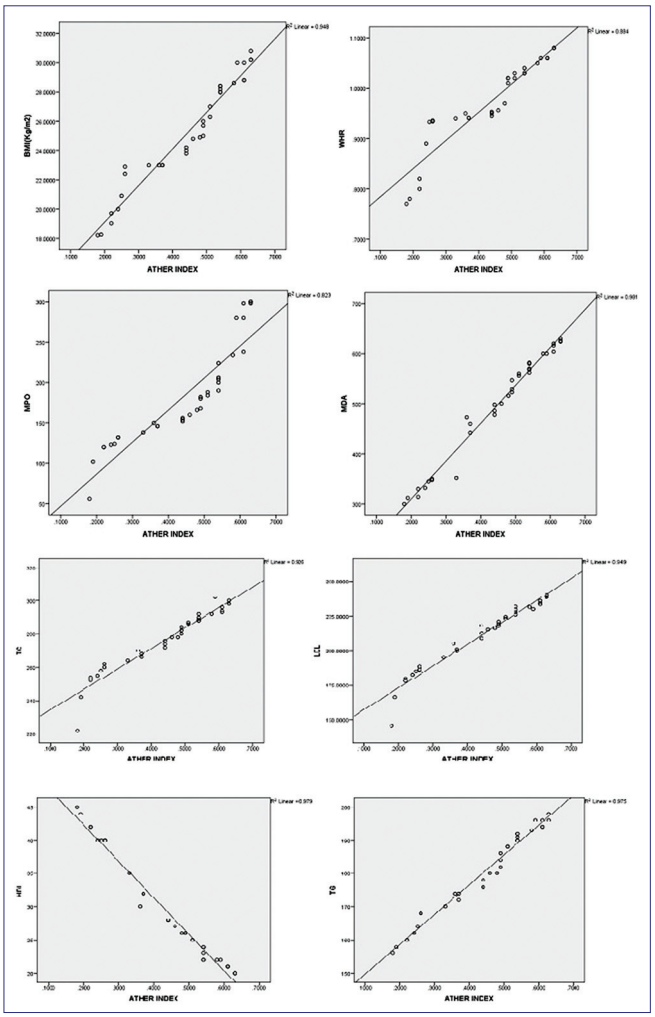

Figure 1: Correlation between atherogenic index and body mass index, waist hip ratio, myeloperoxidase, malandialdehyde, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride

Serum TG levels were found to be increased in type 2 diabetics when compared to controls. Sabzwari *et al.* [18] observed that in patients with type 2 diabetes mellitus, TG levels are elevated compared to normal subjects.

Serum LDL-C levels were found to be increased in type 2 diabetics when compared to controls. Nayak *et al*. found that chronic insulin increases the number of LDL receptor and hence chronic insulin deficiency might be associated with a diminished level of LDL receptor, causing an increase in LDL-C [19].

The serum HDL-C levels were found to be decreased in type 2 diabetics without hypertension and type 2 diabetics with hypertension when compared to controls. These findings were in accordance to the study of Abdel-Aal *et al*. [20].

AIP was found to be increased in type 2 diabetics when compared to controls, which was statistically significant. AIP values of less than (0.1) are associated with low risk, (0.1-0.24) with medium risk and above 0.24 with high CV risk [8].

Type 2 diabetic patients with duration of diabetes ≥10 years had higher mean levels MPO, MDA, TC, LDL-C, TG, BMI and WHR when compared to type 2 diabetics with diabetic duration <10 years. A greater number of atherosclerotic lesions may exist in those with longer duration of diabetes. It is possible that a longer duration of diabetes might be associated with autonomic neuropathy and reduced heart rate variability, increasing the risk of cardiovascular death and long-term exposure to increased amounts of oxidative stress explain for the increased risk of CHD death among diabetic patients [21].

Type 2 diabetic patients with serum TC ≥200 mg/dl had higher mean levels MPO, MDA,TC, LDL-C, TG, BMI and WHR when compared to type 2 diabetics with serum TC <200 mg/dl. The increase in LDL-C and TG seems to depend on a decrease in the oxidative capacity of the fatty acids, which is reflected in a reduction of their catabolism.

In type 2 diabetic patients AIP has shown a positive correlation with BMI, WHR, MPO, MDA, TC, LDL-C and TG and a negative correlation was found between AIP and serum HDL cholesterol. The logarithmically transformed ratio of plasma TG to HDL-C closely correlated with the LDL-C particle size and could serve as an indicator of the atherogenic lipoprotein phenotype [15].

Recent studies have indicates that TG/HDL-C ratio transformed logarithmically can estimate the atherogenic risk better than all others. TG and HDL-C perfectly reflect the balance between atherogenic lipoproteins and protective lipoproteins. Clinical studies revealed that AIP can estimate the cardiovascular risk [8].

SUMMARY

The MPO is marker of inflammation and a strong predictor of endothelial dysfunction.

Elevated serum levels of MDA indicate increase in the production of oxygen based free radicals, suggesting their possible role in atherogenesis.

AIP can be easily calculated from standard lipid profile. As a marker of lipoprotein particle size it adds predictive value beyond that of the individual lipids, and/or TC/HDL-C ratio By comparing each important parameter from our study, involved directly or indirectly in the initiation or maintenance of atherogenesis and CVD in type 2 diabetic patients, we were able to highlight the importance of MPO, MDA and AIP for the clinician in medical practice and it can be a useful and economical tool for diagnosis, treatment and prevention of chronic CVD and major cardiac events, especially myocardial infarct and stroke.

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