IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 3 Ver. VIII (March. 2017), PP 30-34
www.iosrjournals.org

Myeloperoxidase levels predicts the vascular dysfunction in patients with Type 2 Diabetes Mellitus

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Abstract
Background: *Myeloperoxidase (MPO) is a heme enzyme which is the major protein in neutrophils and, to a lesser extent, in monocytes. MPO uses H_2O_2 to generate HOCl that is a potent bactericidal agent, generating reactive oxygen species (ROS). MPO plays an essential part in the innate immune system by catalyzing the production of HOCl. However, MPO has also been implicated as a very harmful agent in an increasing number of inflammatory-mediated disorders*
Aim: *The aim of this study was to evaluate the relationship between MPO with albumin creatinine ratio and to explore possible correlation with HbA1c and Insulin resistance in type 2 diabetic patients.*
Materials and methods: *Sixty type 2 diabetic patients in the age group of 35 to 60 years were selected for this study and 30 age matched healthy individuals were selected as a control group. Serum MPO and Insulin levels were assessed by ELISA method and microalbumin by turbilatex method.*
Results: *The Serum MPO levels were significantly higher in the normoalbuminuric group with diabetes than in the control group, and significantly increased in microalbuminuric patients compared to normoalbuminuric patients. The serum MPO levels were positively correlated with the albumin creatinine ratio (ACR), glycated hemoglobin (HbA1C) and insulin resistance.*
Conclusion: *The serum MPO levels were associated with ACR, HbA1c and Insulin resistance in type 2 diabetic patients and increased levels in normoalbuminuric patients indicates that it could be useful to assess the vascular dysfunction in T2DM patients.*
Keywords: *Type 2 diabetes mellitus (T2DM), Myeloperoxidase (MPO), Albumin creatinine ratio (ACR)*

I. Introduction

Type 2 diabetes mellitus (T2DM) is a disease that affects more than 415 million people around the world. In 2040, there will be more than 642 million people with diabetes worldwide [1]. Micro vascular complications are common in type 2 diabetes includes retinopathy leading to various degrees of visual impairment including blindness and has become a major cause of blindness throughout the world [2-4]; neuropathy, leading to pain and numbness, chronic and recurrent infected ulcers in the extremities which can lead to amputation; and nephropathy characterized by proteinuria ultimately leading to end stage renal disease [5-6]. Microalbuminuria is the gold standard for detection and prediction of diabetic kidney disease in clinical practice. At the same time, microalbuminuria represents a marker of the generalized endothelial dysfunction present in DM, linking renal involvement with cardiovascular and cerebral impairment [7, 8]. However, many patients, who were normoalbuminuric and just recently had positive test for microalbuminuria, have advanced renal histopathological changes, decreased glomerular filtration rate (GFR) and progressive loss of kidney function [9,10].So, it is necessary to develop more sensitive markers rather than microalbumin for detecting the early stage of nephropathy and as well as cardiovascular complications in diabetic patients.

Hyperglycemia frequently induces endothelial dysfunction in diabetic patients. The severity of type 2 diabetic complications normally correlates with plasma glucose levels, and the inflammation provoked by their deregulation can contribute to insulin resistance [11]. It has been suggested that myeloperoxidase (MPO), a heme protein derived from leukocytes, plays a key role in leukocyte-mediated vascular injury responses in inflammatory cardiovascular disease (CVD). MPO is released from activated leukocytes at inflammatory sites, generating reactive oxygen species (ROS). However, the antimicrobial activity of MPO can also produce oxidative damage in the endothelium and vessel wall; thus, promoting CVD and clinical complications [12]. Therefore, present study was carried out to evaluate the relationship between MPO with albumin creatinine ratio and to explore possible correlation with HbA1c, homeostasis model assessment-estimated insulin resistance (HOMA-IR) in type 2 diabetic patients.

II. Materials and Methods

The study groups comprised of 60 type 2 diabetic patients of both sexes, aged between 35-60 years on oral hypoglycemic drugs, attending diabetic out-patient department of NIMRA institute of medical sciences, Jupudi, Andhra Pradesh, India, were selected for our study. We excluded the patients based on the following criteria: patients on insulin, hypertension, smokers, alcoholics, tobacco chewers, abnormal urinary sediment, urinary tract infection, history of other renal disease and active or chronic persistent infection or inflammatory disorders, neoplastic disorders, uncontrolled thyroid disorders, severe liver dysfunction, history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease. The included diabetic patients were categorized into two groups according to urinary albumin creatinine ratio (ACR). Groups were divided as follows: 30 patients with normoalbuminuria (< 30 mg/g creatinine), 30 patients with microalbuminuria (30–299 mg/g creatinine). Thirty healthy age, sex matched subjects were selected as a controls. The informed consent was obtained from all the study subjects and the study was approved by the Institutional Human Ethics Committee (IHEC). Experiments were done in accordance with Helsinki declaration of 1975.

Biochemical analysis:

A random spot urine and fasting blood samples were obtained from the subjects immediately after enrolment. Blood samples were centrifuged at 2000×g for 10 min. Samples were analyzed for fasting blood glucose, lipid Profile(Total Cholesterol, HDL, Triglycerides), by using Auto analyzer. HbA1C estimated by Ion exchange resin method and Myeloperoxidase, Insulin assessed by ELISA and the 2 hour post prandial venous blood sample collected for plasma glucose (PPG) analysis. Homeostasis model assessment for insulin resistance evaluation (HOMA-IR) was calculated using the equation: fasting plasma insulin × glucose/22.5 [13]. Urine samples were analyzed for microalbumin, creatinine by using auto analyzer.

Statistical analysis:

Statistical analyses were carried out with SPSS 20.0. Values were expressed as mean ± standard deviation, p value < 0.05 was considered statistically significant. Normally distributed data were analyzed by using one-way ANOVA. The Pearson correlation test was used for correlation analysis.

III. Results

Table 1: Baseline data of controls, normoalbuminuric and microalbuminuric type 2 diabetic patients

Parameters	Controls (n=30)	Normoalbuminuric T2DM (n=30)	Microalbuminuric T2DM (n=30)
Age	45.7±3.9	46.1±6.7	48.1±4.4
Body mass index (BMI)	25.3±1.9	26.9±3.6 [a#]	26.5±3.5 [a#]
Waist/Hip ratio	0.91±0.03	0.92±0.06	0.92±0.04
Systolic BP(mmHg)	112.9±6.8	124.5±13.5[a#]	129.6±12.5 [a*]
Diastolic BP (mm Hg)	74.1±5.4	78.7±7.5 [a#]	78.5±6.6 [a#]

a - Controls vs Normoalbuminuria T 2DM, Microalbuminuria T2DM
b -Normoalbuminuria T 2DM vs Microalbuminuria T 2 DM
* p value <0.001, # p value <0.05
Data are expressed as mean ±SD, p<0.05 was considered statistically significant.

Table 2: Clinical data of controls, normoalbuminuric and microalbuminuric type 2 diabetic patients

Parameters	Controls (n=30)	Normoalbuminuric T2DM (n=30)	Microalbuminuric T2DM (n=30)
Urine albumin Creatinine ratio (mg/gm. of creatinine)	19.4±2.1	23.8±3.4 [a*]	140.1±12.7 [a*,b*]
FPG(mg/dl)	82.7±5.1	123.9±19.5 [a*]	148.8±18.5 [a*, b*]
PPG(mg/dl)	107.4±10.3	168.7±20.9 [a*]	203.4±42.4 [a*, b#]
HbA1C	5.4±0.5	7.3±0.8 [a*]	8.2±0.9 [a*, b*]
Serum cholesterol (mg/dl)	168.4±8.6	186.7±25.6 [a#]	194.9±22.7 [a*]
Serum Triglycerides (mg/dl)	96.7±7.6	123.5±48.1 [a#]	140.2±35 [a#]
HDL cholesterol (mg/dl)	43.1±2.2	40.0±3.6 [a#]	38.5±2.4 [a*]
LDL cholesterol (mg/dl)	105.9±8.7	122.0±20.5 [a#]	128.3±22.2 [a*]
Insulin (µIU/mL)	6.6±0.6	10.3±3.6 [a*]	14.2±3.4 [a*, b*]
HOMA-IR	1.36±0.15	3.2±1.2 [a*]	4.9±1.5 [a*, b*]
Serum Myeloperoxidase (pg/ml)	154.5±13.6	237.1±21.4 [a*]	325.7±29.5 [a*, b*]

a - Controls vs Normoalbuminuria T 2DM, Microalbuminuria T2DM
b -Normoalbuminuria T 2DM vs Microalbuminuria T 2 DM
* p value <0.001, # p value <0.05
Data are expressed as mean ±SD, p<0.05 was considered statistically significant

Table 3: Correlation between Myeloperoxidase &measured parameters in type 2 diabetic patients

Parameters	Correlation Coefficient(r)
Albumin Creatinine Ratio	0.523**
FPG	0.486**
PPG	0.420**
HbA1C	0.487**
HOMA-IR	0.573**
Cholesterol	0.282*
TGL	0.342**
HDL	-0.298*
LDL	0.353**

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 4: Correlation between ACR &measured parameters in type 2 diabetic patients

Parameters	Correlation Coefficient(r)
FPG	0.282*
PPBS	0.345**
HbA1C	0.273*
HOMA-IR	0.573*
Cholesterol	0.285*
TGL	0.211
HDL	-0.293*
LDL	0.331**

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 5: Correlation between HbA1c &measured parameters in type 2 diabetic patients

Parameters	Correlation Coefficient(r)
FPG	0.298*
PPG	0.323**
Cholesterol	0.298*
TGL	0.198
HDL	-0.173
LDL	0.231

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 6: Correlation between HOMA- IR &measured parameters in type 2 diabetic patients

Parameters	Correlation Coefficient(r)
FPG	0.483**
PPG	0.321**
Cholesterol	0.289*
TGL	0.301*
HDL	-0.294*
LDL	0.368**

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

IV. Discussion

Type 2 diabetes mellitus is associated with oxidative stress and microvascular dysfunction. Although endothelial dysfunction and endothelial/leukocyte interactions are key features of diabetes and are thought to be a major cause of diabetes-associated vascular complications, the underlying molecular mechanisms remain unclear [14,15]. In the present study, we observed that serum myeloperoxidase levels were significantly increased in type 2 diabetic patients and also positively correlated with ACR indicating its relevance for diagnosis of vascular dysfunction. MPO levels were significantly increased in normoalbuminuric type 2 diabetics when compared with controls, even before the appearance of microalbuminuric state suggests that it could be useful as vascular dysfunction marker. MPO is most abundantly present in azurophilic granules of neutrophils, followed by monocytes and some macrophage subpopulations including resident tissue macrophages such as peritoneal macrophages, microglia, and Kupffer cells [16]. MPO catalyzes the reaction of hydrogen peroxide (H_2O_2), formed by dismutation of O2·−, and halide and pseudohalide ions (Cl−, Br−, and

SCN−) to the corresponding hypohalous acids (hypochlorous acid, HOCl; hypobromous acid, HOBr; and hypothiocyanous acid, HOSCN) [17] and formation of other reactive intermediates such as reactive nitrogen species and tyrosyl radicals. Oxidants produced by MPO are microbicidal factors and play an important role in innate immune response defending the body against bacteria, parasites, viruses, and other agents [18]. However, HOCl and other MPO-derived oxidants participate as mediators of oxidative damage to biomolecules, including proteins, nucleic acids, lipids, and carbohydrates, thus damaging the host tissue. In that way they may initiate and contribute to the development of atherosclerosis, endothelial dysfunction [19].

MPO levels showed strong positive correlation with HbA1C, HOMA-IR, FPG ,PPG, cholesterol , LDL and also negative correlation was observed between HDL cholesterol. Chronic high concentrations of glucose and dyslipidemias cause oxidative stress and along with proinflammatory mediators may impair insulin signaling, initiates insulin resistance [20-22]. MPO protein abundantly expressed in polymorphonuclear neutrophils (PMNs) and monocytes, is also a catalyst of 3-nitrotyrosine (NO_2Tyr) formation via nitrite oxidation to the potent nitrating species nitrogen dioxide ($\bullet NO_2$) [23-26]. In addition, MPO is considered a general index of inflammation, with increased tissue MPO activity thought to reflect neutrophil and monocyte extravasation. Importantly, there is often increased free MPO observed in the plasma of patients during inflammatory conditions [27, 28]. Thus, the intraluminal release of this highly cationic protein may facilitate electrostatic interactions with the negatively charged endothelial plasma membrane, thereby favoring neutrophil-independent binding of MPO with the vascular intima [29, 30]. Independently, the endothelium and the subendothelial space have been identified as a predominant site for NO2Tyr formation during tissue inflammatory reactions [31-33]. So, we are suggesting that MPO may catalyzes the oxidative protein modification and pathogenic constituent in diabetic endothelial dysfunction and it could be useful marker to assess the vascular dysfunction in T2DM patients. Further studies are needed to confirm it.

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