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Nitric Oxide and Paraoxonase 1 in Type 2 Diabetes Mellitus

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ABSTRACT

Diabetes mellitus is a progressive and complex metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion and /or insulin action, the lack of effective insulin leads to disturbances in carbohydrate, lipid and protein metabolism. Type-2 diabetes mellitus is on track to become one of the major global public health challenges of the 21st century. Patients with type-2 diabetes may have complications like cardiovascular disease, nephropathy, retinopathy and polyneuropathy. The present study was planned to measure the serum levels of nitric oxide (NO) and paraoxonase 1 (PON1) in patients with type 2 diabetes mellitus (D M) and to compare with healthy controls. Study includes 100 patients with type 2 DM and 100 healthy controls. Nitric oxide level was estimated by Cortas NK and Wakid NW. Whereas Paraoxonase 1 activity was estimated by spectrophotometric method described by Therry FD and Gean D. The data were evaluated statistically. The mean nitric oxide level in type 2 DM patients was significantly ($p<0.001$) increased than in the controls. Whereas mean PON1 activity in type 2 DM patients was significantly ($p<0.001$) decreased than in the controls. Increased nitric oxide and decreased PON1 activity may contribute to the increased chances for the development of cardiovascular disease (CVD) in type 2 DM patients.

KEY WORDS

Cardio vascular disease, Diabetes mellitus, Nitric oxide, Paraoxonase 1.

INTRODUCTION

Diabetes mellitus is a progressive and complex metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion and /or insulin action, the lack of effective insulin leads to disturbances in carbohydrate, lipid and protein metabolism [1].

endothelium derived vasodilator that participates in the general homeostasis of the vasculature. NO is a diffusible, liposoluble radical gas produced from L-arginine by a family of enzymes known as the nitric oxide synthase (NOS) (7). NO is highly reactive FR and induces adverse alterations in the structure of proteins, carbohydrates, nucleotides and lipids. It also has a role in cell and tissue destruction and formation of adhesions [3]. Generation of NO may be related to development of diabetic complications.

Paraoxonase-1 (PON1) is a calcium dependent enzyme. PON1 has lactonase and esterase activity and thus is able to catalyze the hydrolysis of lipid peroxides and organophosphate pesticides. Its physiologic function has not been fully elucidated. The PON1 enzyme attaches to high-density lipoprotein (HDL) particles in serum, and inhibits low-density lipoprotein (LDL) oxidation, and plays a preventive role in atherogenesis. Increased susceptibility for oxidation of LDL -

cholesterol has been reported in diabetes mellitus and diabetic nephropathy. Accelerated atherosclerosis and altered lipoprotein metabolism is responsible for increased cardiovascular events and cardiac death in these conditions [4].

With this background in mind present study was planned to estimate serum nitric oxide and Paraoxonase 1levels in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

The present study was carried out in Department of Biochemistry, Govt. Medical College, Miraj. Study protocol was approved by ethics committee of Government Medical College, Miraj.

Sample size:

Study cases: The study group includes a total of 200 subjects. This includes patients as well as control.

Patients: Total 100 patients with confirmed diagnosis of type 2 D.M. done by physician on the basis of W.H.O. criteria were included in this study. The patients in the study group were those attending Medicine OPD of Government medical college and hospital, Miraj.

Control: 100 healthy control were taken who attended the OPD of the Government Medical College and Hospital, Miraj during the same period.

Study and control subjects having history of smoking, alcoholism and other diseases which induce oxidative stress such as Pulmonary diseases, Respiratory diseases, Liver diseases etc were excluded from the study.

Collection of blood samples:

Informed consent was obtained from the participants. Venous blood was collected in plain bulb from the subjects under aseptic condition by venipuncture using sterile disposable syringe and needle. Blood samples were centrifuged and clear serum was separated and used for estimation of Nitric oxide and Paraoxonase 1 activity. Nitric oxide level was estimated by method described by and Cortas NK and Wakid NW [5]. This method uses cadmium granules for reduction of NO to nitrate, followed by colorimetric determination of nitrate. Whereas Paraoxonase 1 activity was estimated by spectrophotometric method described by Therry FD and Gean D. [6]. The data was analyzed by student's 't' test. P values <0.001 were considered significant.

RESULTS AND DISCUSSION

We found significantly increased ($p<0.001$) nitric oxide level in type 2 D.M. patients as compared to control. This may indicate increased production of nitric oxide by activating inducible isoforms of nitric oxide synthase (NOS). In type 2 diabetes, there is increased production of free radicals and hyperglycemia [3]. The elevated glucose binds to proteins, forming advanced glycation end products (AGEs) and many free radicals. Advanced glycation end products are associated with tissue damage and aging. Once formed these bind to their cell surface receptors termed RAGE to endothelial cells and macrophages, resulting in the activation of post-receptor signaling, generation of intracellular reactive oxygen species (ROS) and the activation of gene expression. Exposure of endothelial cells to AGEs activates tumor necrosis factor alpha, increases adhesion molecule (including vascular cell adhesion molecule -1) production and iNOS activity [3].

Inducible NOS, is induced by inflammatory cytokines such as interleukin-1 or tumor necrosis factor alpha in macrophages and many other cell types. NO overproduction through inducible NOS induction in inflammatory conditions appears to inhibit insulin metabolic

actions which lead to insulin resistance [3]. Sarita A et al also found increased nitric oxide levels in type 2 D.M. [7].

The mean value of serum PON1 was significantly decreased ($p<0.001$) in type 2 DM patients as compared to the control. PON1 is an esterase associated with HDL it acts like an antioxidant, prevents lipid peroxidation of LDL. Decreased levels of PON1 in type2 DM may be due to increased glycation of various plasma enzymes due to increased glucose concentration which further reduce the capacity of PON1 to prevent lipid peroxidation leading to increased tendency for lipid peroxidation and producing complications like diabetic retinopathy, neuropathy and atherosclerosis [8].

Utilization of Paraoxonase for balancing the oxidative stress in diabetes mellitus can explain a decrease of PON1 activity. A study from Paris supports our study and reports that PON1 activity decreased even in early stages of Diabetes Mellitus [9].

Several mechanisms have been postulated for this decrease in Activity [9]: According to the study of Mira Rosenblat et al [10] this is due to the dissociation of PON1 from HDL as a free, unstable PON1. Another study gives an explanation at the secretory level. It appears that large sized HDL complexes favor PON1 secretion. In clinical syndromes like Diabetes the HDL size tends to diminish. Further the fact that free cholesterol addition to reconstituted HDL influences its capacity to promote PON1 release may also have clinical implications. Unesterified cholesterol partitions into the outer lipid layer in native lipoprotein complexes, and it can adversely affect the lipoprotein function if present in elevated concentrations. Pathological changes due to unesterified cholesterol have been reported for diabetic patients. These factors together set the stage for the progression of associated complications of Diabetes Mellitus [9].

We could not find any significant correlation between the two biochemical parameters that is NO and PON1.The reason may be that each parameter is altered in diabetes mellitus involving an independent mechanism.

To conclude, the present study showed that NO level is associated with diabetes mellitus and PON1 activity is negatively associated with type 2 DM. These two biochemical parameters may be involved in the development of complications in diabetic patients.

Table No 1: Serum NO and PON1 in healthy control and type 2 DM

Parameters	Healthy control (n=100)	Type 2 DM (n=100)	Z and p value	Significance
NO (µmol/l)	17.56 ± 6.7	51.49 ± 6.13	Z = 33.93 P < 0.001	Highly significant
PON1 (U/ml)	82.72 ± 3.21	78.28± 6.54	Z = 6.094 P < 0.001	Highly significant

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