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Assessment of the serum levels of nitric oxide among diabetic patients and its correlation with lipid profile as well as oxidative stress

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Abstract

Background and Aim: Endothelial dysfunction appears to be a consistent finding in all diabetic patients. Indeed, there is a general agreement that chronic hyperglycemia and DM lead to impairment in nitric oxide (NO) production and activity. The present study was conducted to assess the serum levels of NitricOxide (NO) among diabetic patients and its correlation with lipid profile as well as oxidative stress.

Material and Methods: The present study was conducted among 99 patients of diabetes of both genders. The patients were divided into three groups: Group I were type 2 diabetics with dyslipidemia and hyperuricaemia, group II was type 2 diabetics with dyslipidemia and normouricaemia and group III was type 2 diabetics with normolipidemic and normouricaemia. Lipid profile and nitric oxide were determined.

Results: There was a non-significant difference in HbA1c, TC, TG, HDL and LDL among different differences ($P > 0.05$). There was poor correlation of antioxidants with NO levels ($P > 0.05$)

Conclusion: There was a role of Nitric Oxide (NO) in the pathogenesis of type -2 diabetes mellitus with dyslipidemia and hyperuricaemia. The oxidative stress parameters had poor correlation with NO level in all the groups.

Keywords: Diabetics, dyslipidemia nitric oxide, oxidative stress

Introduction

Diabetes mellitus is a disease with a rapidly increasing prevalence needing continue research for novel methods to both prevent and treat this disorder. Now it is obvious that obesity and decreased physical activity are the well known major risk factor for the development of diabetes [1]. Recently the emphasis is focused on oxidative stress in pathogenesis of type two diabetes mellitus and its complication [2].

Diabetes mellitus is a disease with a rapidly increasing prevalence needing continue research for novel methods to both prevent and treat this disorder. Now it is obvious that obesity and decreased physical activity are the well known major risk factor for the development of diabetes [3].

Depending on the intensity and duration of exposure to hyperglycemia, structural damage may occur in vascular endothelium and nervous tissue, leading to the dysfunction, and even failure of different organs and tissues, characterizing the diabetic chronic complications. These complications are divided into macrovascular- (coronary artery disease, peripheral vascular disease and stroke) and microvascular- complications (diabetic kidney disease, diabetic retinopathy, and neuropathy), and are associated with high morbidity and mortality rates among diabetic patients [4].

Endothelial dysfunction appears to be a consistent finding in all diabetic patients. Indeed, there is a general agreement that chronic hyperglycemia and DM lead to impairment in nitric oxide (NO) production and activity [5, 6]. NO is a short-lived gaseous free radical secreted by endothelium. Modifications in its bioavailability have been found to cause endothelial dysfunction, increasing susceptibility to hypertension, progression of atherosclerosis, hypercholesterolemia, thrombosis, stroke, DM and its chronic complications [7-9]. NO is synthesized as a byproduct of the conversion of its physiological precursor L-arginine to L-citrulline by a family of NO synthases (NOS). These enzymes comprise three distinct isoforms, encoded by three different genes: neuronal (nNOS codified by NOS-1), inducible (iNOS/NOS-2), and endothelial (eNOS/NOS-3) forms [10].

Although, at baseline, the main source of plasma NO is related to eNOS, during several clinical conditions, such as inflammation, iNOS is activated [11]. NO acts as pleiotropic intracellular messenger, exerting a variety of biological actions under both physiological and pathological conditions. While low levels of NO are beneficial for several physiological and cellular functions, keeping vascular tonus, coagulation and inflammation well balanced; high levels of NO may cause detrimental effects [12, 13].

Elevated concentration of superoxide dismutase causes impairment of endothelial isoform of nitric oxide synthase (eNOS) by triggering advanced glycation end products and poly (ADP-ribose) polymerase. NO is synthesized as a byproduct of the conversion of its physiological precursor l-argininetol-citrulline. This reaction is catalyzed by a family of enzymes known as NO synthases (NOS). Malondialdehyde (MDA) is known to be a by-product of reactive oxygen species metabolism [14].

The present study was conducted to assess the serum levels of NitricOxide(NO) among diabetic patients and its correlation with lipid profile as well as oxidative stress.

Material and Methods

The present study was conducted among 99 patients of diabetes of both genders. All patients were informed regarding the study and their consent was obtained. The demographic profile of patients was recorded. Patients were defined as diabetes mellitus using the following criteria: those with symptoms of diabetes with random blood glucose level >200 mg/dl or fasting plasma glucose >126 mg/dl or HbA1C>6.5% or impaired oral glucose tolerance test with two-hour postprandial plasma glucose level > 200mg/ dl. The patients were divided into three groups: Group I were type 2 diabetics with dyslipidemia and hyperuricaemia, group II was type 2 diabetics with dyslipidemia and

normouricaemia and group III was type 2 diabetics with normolipidemic and normouricaemia. A thorough clinical assessment was performed. FBS and PPBS were measured using a glucose oxidase (GOD/POD) method. Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), High-Density Lipoprotein (HDL), and serum creatinine levels were estimated and nitric oxide was determined by a colorimetric kit.

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.

Results

Table 1: Demographic data of study participants

| Variables | Group 1 | Group 2 | Group 3 | P value |
|------------------|---------|---------|---------|---------|
| Age Group | | | | |
| 20-40 | 9 | 13 | 8 | 0.01* |
| 40-60 | 12 | 11 | 17 | |
| >60 | 12 | 9 | 8 | |
| Gender | | | | |
| M:F | 22:11 | 18:15 | 17:16 | 0.06 |
| BMI | | | | |
| 18.5-24.9 | 1 | 2 | 4 | 0.02* |
| 25-29.9 | 12 | 10 | 19 | |
| 30-34.9 | 6 | 16 | 9 | |
| >35 | 14 | 5 | 1 | |

*indicates statistically significance at p<0.05

Table 2: Correlation of NO levels with antioxidant

| Anti-oxidant | Group 1 | | Group 2 | | Group 3 | |
|--------------|---------|------|---------|------|---------|------|
| | r | p | r | p | r | p |
| MDA (nmol/l) | 0.11 | 0.8 | -0.10 | 0.3 | 0.03 | 0.6 |
| CAT (Umg/ml) | 0.07 | 0.5 | 0.2 | 0.14 | 0.2 | 0.01 |
| SOD (Umg/ml) | -0.10 | 0.20 | 0.04 | 0.69 | -0.05 | 0.7 |
| GR (Umg/p) | 0.09 | 0.23 | -0.06 | 0.6 | -0.09 | 0.4 |
| GPx (UmgHb) | 0.16 | 0.1 | -0.04 | 0.7 | -0.2 | 0.3 |

Age group 20-40 years had 9 in group I, 13 in group II and 8 in group III, 40-60 years had 12 in group I, 11 in group II and 17 in group III and age group >60 years had 12 in group I, 9 in group II and 8 in group III. There were 22 males and 11 females in group I, 18 males and 15 females in group II and 17 males and 16 females in group II. BMI (Kg/m²) was 18.5- 24.9 seen 1 in group I, 2 in group II and 4 in group III, 25- 29.9 seen 12 in group I, 10 in group II and 19 in group III, 30-34.9 seen 6 in group I, 16 in group II and 9 in group III and >35 seen 14 in group I, 5 in group II and 1 in group III. The difference was significant (P< 0.05). (Table 1) There was a non-significant difference in HbA1c, TC, TG, HDL and LDL among different differences (P> 0.05). Table 2 shows that there was poor correlation of antioxidants with NO levels (P> 0.05)

Discussion

Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of diabetic vascular

complications and is being shown to originate from hyperglycaemia. Hyperglycaemia and its biochemical sequelae either alter endothelial function directly or influence endothelial cell functioning indirectly by affecting the pathways of growth factors [15]. Endothelial dysfunction is a well-known finding in hypercholesterolaemic patients and it is reported that multiple factors contribute to this, including increased inactivation of nitric oxide by radicals and inhibition of nitric oxide formation by different mechanisms [16]. It was also observed that the peroxidation of lipids in lipoproteins in the vascular wall leads to local production of reactive carbonyl species that mediate recruitment of macrophages, cellular activation and proliferation [17]. In the present study, age group 20-40 years had 10 in group I, 15 in group II and 10 in group III, 40-60 years had 15 in group I, 13 in group II and 20 in group III and age group >60 years had 15 in group I, 12 in group II and 10 in group III. There were 25 males and 15 females in group I, 22 males and 18 females in group II and 20 males and 20

females in group II. BMI (Kg/m^2) was 18.5- 24.9 seen 2 in group I, 4 in group II and 6 in group III, 25- 29.9 seen 14 in group I, 12 in group II and 22 in group III, 30-34.9 seen 8 in group I, 20 in group II and 10 in group III and >35 seen 16 in group I, 4 in group II and 2 in group III. Ghosh *et al.* [18] assessed serum nitric oxide level among type 2 diabetic patients along with other biochemical parameters. There was a significant difference when age- and sex-matched cases and controls were compared in regard to waist circumference and body mass index. The values of fasting and postprandial serum glucose, and lipid profiles between the study group and the control group differed significantly. The mean serum level of NO in the study and control group was $43.83 \pm 11.3 \mu\text{moles/L}$ and $58.85 \pm 12.8 \mu\text{moles/L}$ respectively, and this difference was statistically significant. with non-diabetic controls. The present results are in agreement with previous experimental studies showing that hyperglycemia may enhance NO production [19-20] or decrease its bioactivity leading to increased superoxide formation [21]. Yang *et al.* [19] demonstrated that hyperglycemia induced iNOS (NOS-2) expression and nitrosative stress in mouse embryonic tissues, and pharmacological inhibition of the proapoptotic JNK1/2 was able to blockade this effect. Accordingly, Zhang *et al.* [20] showed that high glucose treatment increased NO generation and iNOS expression in vascular endothelial cells co-cultured with retinal pigment epithelium cells. In a Japanese study, when nitric oxide levels were measured by high performance liquid chromatography method, it was significantly high in diabetics than in control ones [22]. Prospective studies showed that a decrease in bioavailability of nitric oxide which is an anti-atherosclerotic endogenous molecule, leads to dyslipidemia. Dysfunction in L-arginine-nitric oxide pathway causes a decreased synthesis of NO which results in various cardiovascular risk factors including hypercholesterolaemia and dysfunction of vascular wall [23, 24]. In a Turkey study, the serum levels of NO were significantly higher among the patients with type 2 diabetes than the non-diabetics [25]. Another study from Turkish on micro- and normoalbuminuric type 2 diabetics and healthy controls found that serum NO levels was higher in both micro-albuminurics and normo-albuminurics than controls in early diabetes [26].

We found that there was a non-significant difference in HbA1c, TC, TG, HDL and LDL among different difference ($P > 0.05$). There was poor correlation of antioxidants with antioxidants ($P > 0.05$). Kumar *et al.* [27] included subjects suffering from type 2 diabetes for more than 1 year and age between 30 to 50 years with hyperuricaemia. The patients were divided into three groups: Group I- Type 2 diabetics with dyslipidemia and hyperuricaemia, Group II- Type 2 diabetics with dyslipidemia and normouricaemia and Group III- Type 2 diabetics with normolipidemic and normouricaemia. The nitric oxide level was significantly lower in Group I and Group II than Group III. The oxidative stress parameters had poor correlation with NO level in all the groups. A study in Karachi showed that serum NO level was significantly low in diabetic normotensive and diabetic hypertensive patients as compared to controls whereas HbA1c levels and FBG were significantly high. These data imply that there is definitive correlation between glycosylated hemoglobin (HbA1c) and Nitric Oxide in diabetics and hypertensive subjects. A negative correlation was observed between serum nitric oxide and serum glucose

and HbA1c among subjects with diabetes and hypertension, suggesting that HbA1c can modulate the NO metabolism and vice versa [28].

Conclusion

There was a role of Nitric Oxide (NO) in the pathogenesis of type -2 diabetes mellitus with dyslipidemia and hyperuricaemia. The oxidative stress parameters had poor correlation with NO level in all the groups. Our data suggests the role of Nitric Oxide (NO) in pathogenesis of type-2 diabetes with dyslipidemia and hyperuricaemia.

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