

Evaluation of the Serum Levels of Nitric Oxide among Diabetic Patients and its Correlation with Lipid Profile as well as Oxidative Stress in North Indian Setting

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ABSTRACT

Introduction: Diabetes mellitus is a disease with a rapidly increasing prevalence, needs continue research for novel methods to both prevent and treat this disorder. Obesity and decreased physical activity are the major risk factor for the development of diabetes. Recently the emphasis is focused on oxidative stress in pathogenesis of this disease.

Aim: To assess the serum levels of Nitric Oxide (NO) among diabetic patients and its correlation with lipid profile as well as oxidative stress in north Indian setting.

Materials and Methods: This was a cross-sectional study. Subjects suffering from type 2 diabetes for more than 1 year and

age between 30 to 50 years with hyperuricaemia were included in the study. 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 normolipidemia and normouricaemia.

Results: 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.

Conclusion: Our data suggests that there is definite role of Nitric Oxide (NO) in pathogenesis of type -2 diabetes mellitus with dyslipidemia and hyperuricaemia.

Keywords: Dyslipidemia, Free radical, HbA1c, Hyperuricaemia, Middle aged

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]. Basic principal behind the management of type -2 diabetes mellitus is to control the hyperglycaemia because persistent hyperglycaemia leads to development of oxidative stress [3]. It has been reported that the oxidative stress ameliorate the endothelial dysfunction vasculopathy and neuropathy within erectile tissue, in conditions like persistent hyperglycaemia [4]. Malondialdehyde (MDA) is known to be a by-product of reactive oxygen species metabolism [5]. Griesmacher et al., reported a significant rise in MDA levels among the patients of diabetes [6]. With increased peroxidation and reduced antioxidant levels, the oxidative stress may play an important role in the pathogenesis of diabetic vascular complications [5].

AIM

Study was conducted with the aim to assess the serum level of nitric oxide among the diabetic patients and its correlation with lipid profile as well as oxidative stress in north Indian subjects.

MATERIALS AND METHODS

This was a cross-sectional study, conducted at the Department of Medicine, LLRM Medical College, Meerut, Uttar Pradesh, India. A total number of 223 patients suffering from type 2 diabetes for more than 1 year, aged between 30 to 50 years with hyperuricaemia were included in the study. Patients with coronary artery disease due to other causes diagnosed for hypothyroidism, chronic alcoholics, tobacco users, taking antioxidant medication, using lipid lowering

drugs and with chronic diseases were excluded from the study. The study was approved by the ethical committee of the college. An informed consent was taken from each participant before enrolling in the study. The demographic profile, anthropometric parameters, habit of tobacco, alcohol intake and basic clinical parameters of the patients were noted.

Criteria for Diabetes Mellitus: 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 (n=73)- Type 2 diabetics with dyslipidemia and hyperuricaemia, Group II (n=76)- Type 2 diabetics with dyslipidemia and normouricaemia and Group III (n=74)- Type 2 diabetics with normolipidemia and normouricaemia.

Estimation of biochemical parameters: After a minimum of 12 hours of fasting, venous blood sample was collected in plain vial for nitric oxide estimation. Blood was collected from the antecubital vein, following precautions. The sample was then allowed to clot in the aliquot at room temperature for about 30 minutes and was then centrifuged at 3000 rpm for 10 minutes to separate the serum. FBS and PPBS were measured using a glucose oxidase (GOD/POD) method available as a kit manufactured by Transasia. Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), High Density Lipoprotein (HDL), and serum creatinine levels were estimated by commercially available Ecoline kits (Merck, Germany) [6]. Nitric Oxide was assayed by a colorimetric kit that used a convenient measure of stable decomposition product total nitrate/nitrite in serum by a simple two-step process. The first step was to convert nitrate to nitrite utilizing nitrate reductase. The second step was to convert nitrite to a deep purple azo compound. The amount of the

azochromophore accurately reflected the amount of nitric oxide in the samples.

STATISTICAL ANALYSIS

Data obtained were summarized as mean±SD and percentages. The statistical analysis was done using Graph Pad 3 and the comparison among the groups was done by one way Analysis of Variance (Anova) followed by Tukey's post-hoc multiple comparison tests and Chi-square test. Pearson correlation was used to find the correlation between NO and oxidative stress parameters. The p-value<0.05 was taken as statistically significant.

RESULTS

The anthropometric parameters, age and habits were well comparable among all the three groups. The significant difference (p=0.001) in the BMI was observed among the groups. The presence of metabolic syndrome was observed to be significantly higher among the patients of Group I (90.4%) compared with Group II (64.5%) and Group III (18.9%) [Table/Fig-1].

The analysis of variance revealed that there was significant (p=0.0001) difference in the NO level among the groups in the patients whose lipid levels and HbA1c were either increased

or decreased [Table/Fig-2]. The post-hoc multiple comparison tests revealed that the NO level was significantly (p=0.001) lower in Group I than Group II. It was also observed that the NO was significantly (p=0.001) lower among the patients whom HbA1c, FBG, TC and TC/HDL were decreased. However, a significant decrease level of NO was also observed in Group II than Group III among the patients whom TG was increased. Within group analysis revealed that there was no significant (p>0.05) difference in NO level between increased and decreased level of HbA1c and lipid profiles.

The post-hoc comparison tests revealed that there was significant difference in the level of NO among the three groups according to demographic profile, anthropometric parameters and clinical profile of the patients [Table/Fig-3]. However, NO was found to be significantly (p=0.001) lower in Group II than Group III among age>80 years, females, smokers and BMI with 25-29.9 kg/m². A poor correlation was observed between NO levels and antioxidants in all the three groups [Table/Fig-4].

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 [7]. 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 [8]. 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 [9]. The present study investigated the correlation of NO with lipid levels, HbA1c and oxidative stress among the three groups of diabetic patients. We did not find significant difference in the demographic profile, tobacco & alcohol intake habits, anthropometric parameters and past & current history of hypertension among the three groups. We found the significant difference in the NO levels among the groups which was similar to the findings of Samant et al., [10]. We found significant difference in the level of NO between increased or decreased level of HbA1c, FBG and lipid levels among the groups. We also found increased level of NO in Group III compared with Group I and II. There was not much difference in the level of NO according to demographic profile, addiction habits and clinical profile within the groups in this study.

In a Japanese study, when nitric oxide levels were measured by high performance liquid chromatography method (Griess methods), it was significantly high in diabetics than in control ones [11].

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 [12,13].

In a Turkey study, the serum levels of NO were significantly higher among the patients with type 2 diabetes than the non-diabetics [14]. 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 [15].

In an Iranian cross-sectional study, NO was measured in 3505 subjects, aged 20–94 years, using the Griess reaction. It was observed that NO level was significantly higher in subjects with type -2 diabetes mellitus hence these data support the fact

Basic characteristics	Group I (n=73)		Group II (n=76)		Group III (n=74)		p-value ¹
	No.	%	No.	%	No.	%	
Age in years							
41-60	20	27.4	28	36.8	27	36.5	0.41
61-80	49	67.1	42	55.3	45	60.8	
>80	4	5.5	6	7.9	2	2.7	
Gender							
Male	43	58.9	45	59.2	38	51.4	0.55
Female	30	41.1	31	40.8	36	48.6	
Smoking habit							
Non-smoker	53	72.6	60	78.9	58	78.4	0.60
Smoker	20	27.4	16	21.1	16	21.6	
Habit of tobacco							
Tobacco non-users	45	61.6	50	65.8	45	60.8	0.80
Tobacco users	28	38.4	26	34.2	29	39.2	
Alcohol use							
Alcohol non-users	52	71.2	60	78.9	58	78.4	0.47
Alcohol users	21	28.8	16	21.1	16	21.6	
BMI							
18.5-24.9	5	6.8	6	7.9	10	13.5	0.001*
25.0-29.9	27	37.0	25	32.9	42	56.8	
30.0-34.9	14	19.2	37	48.7	18	24.3	
>35	27	37.0	8	10.5	4	5.4	
Waist Circumference							
Normal WC	5	6.8	26	34.2	31	41.9	0.001*
Raised WC	68	93.2	50	65.8	43	58.1	
Past history of hypertension							
Normotensive	39	53.4	46	60.5	36	48.6	0.34
Hypertensive	34	46.6	30	39.5	38	51.4	
Current hypertensive							
Normotensive	12	16.4	9	11.8	8	10.8	0.55
Hypertensive	61	83.6	67	88.2	66	89.2	
Metabolic Syndrome							
Absent	7	9.6	27	35.5	60	81.1	0.001*
Present	66	90.4	49	64.5	14	18.9	

[Table/Fig-1]: Basic characteristics of the patients.
1: Probability with 95% confidence

Biochemical parameters		Group I (n=73)		Group II (n=76)		Group III (n=74)		p-value ¹
		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	
HbA1c (%)	<7	25	43.57±13.55 ^a	36	57.60±14.71 ^b	33	70.86±13.39 ^{a, b}	0.001*
	>7	48	46.76±15.03 ^a	40	60.26±13.02	41	74.83±12.64 ^a	0.001*
FBG (mg/dl)	<100	51	46.54±15.20 ^a	60	58.51±13.86 ^b	51	73.17±13.02 ^{a, b}	0.001*
	>100	22	44.31±13.44 ^a	16	62.81±13.00	23	73.22±13.33 ^a	0.001*
TC (mg/dl)	<200	11	32.30±7.74 ^a	17	57.48±15.11 ^b	62	73.18±13.01 ^{a, b}	0.001*
	>200	62	47.13±14.46 ^a	59	59.43±13.58	12	72.04±12.24 ^a	0.001*
TG (mg/dl)	<150	7	43.15±3.46 ^a	7	72.30±0.00	63	73.18±13.01 ^a	0.001*
	>150	66	45.90±14.80 ^a	69	58.93±13.74 ^b	11	74.23±13.15 ^{a, b}	0.001*
HDL (mg/dl)	>40	39	47.47±14.60 ^a	59	58.63±13.86	60	72.18±11.01 ^a	0.001*
	<40	34	43.60±14.52 ^a	17	61.78±13.31	14	71.34±13.24 ^a	0.001*
LDL (mg/dl)	<100	13	42.45 ±13.24 ^a	7	60.40±0.00	61	73.18±13.01 ^a	0.001*
	>100	60	45.82±14.59 ^a	69	59.10±13.83	13	72.12±13.29 ^a	0.001*
TC/HDL (mg/dl)	<3.5	9	43.24±14.34 ^a	8	57.57±12.25 ^b	57	73.67±12.06 ^{a, b}	0.001*
	>3.5	64	45.82±14.59 ^a	68	59.12±13.73	17	71.03±16.97 ^a	0.001*

[Table/Fig-2]: Comparison of nitric oxide (µmol/L) levels among the groups according to biochemical parameters.
¹ANOVA tests (among the groups), *Significant, ^a, ^bp=0.001 (Post-hoc comparison tests)

Basic characteristics		Group I (n=73)		Group II (n=76)		Group III (n=74)		p-value ¹
		No.	Mean±SD	No.	Mean±SD	No.	Mean±SD	
Age in years	41-60	20	49.8±14.5 ^a	28	57.0±16.8 ^b	27	72.3±12.6 ^{a, b}	0.001*
	61-80	49	44.1±14.9 ^a	42	60.5±11.6	45	73.3±13.4 ^a	0.001*
	>80	4	45.4±9.10 ^a	6	60.7±10.10 ^b	2	82.5±11.3 ^{a, b}	0.001*
Gender	Male	43	47.27±14.30 ^a	45	59.90±12.93	38	74.10±13.27 ^a	0.001*
	Female	30	43.75±15.01 ^a	31	57.95±15.02 ^b	36	72.21±12.85 ^{a, b}	0.001*
Smoking habit	Non-smoker	53	46.32±15.63 ^a	60	60.17±13.65	58	73.82±12.97 ^a	0.001*
	Smoker	20	44.19±10.75 ^a	16	54.05±13.52 ^b	16	70.11±13.34 ^{a, b}	0.001*
Habit of tobacco	Tobacco non-users	45	45.91±15.34 ^a	50	58.49±14.19	45	74.08±12.58 ^a	0.001*
	Tobacco users	28	45.53±12.24 ^a	26	62.91±10.39	29	68.87±14.73 ^a	0.001*
Alcohol use	Alcohol non-users	52	49.36±14.76 ^a	60	58.91±13.57	58	73.89±13.18 ^a	0.001*
	Alcohol users	21	38.42±11.28 ^a	16	59.55±14.35	16	71.91±12.86 ^a	0.001*
BMI (kg/m ²)	18.5-24.9	5	42.30±14.56 ^a	6	58.67±12.34	10	69.05±14.59 ^a	0.001*
	25.0-29.9	27	47.31±15.45 ^a	25	56.98±12.85 ^b	42	73.33±12.93 ^{a, b}	0.001*
	30.0-34.9	14	43.91±15.02 ^a	37	59.62±14.11	18	75.13±12.53 ^a	0.001*
	>35	27	45.31±13.86 ^a	8	63.51±15.13	4	76.56±12.25 ^a	0.001*
Waist Circumference	Normal WC	5	43.45±13.26 ^a	26	59.26±13.59	31	73.45±15.17 ^a	0.001*
	Raised WC	68	45.82±14.59 ^a	50	59.07±13.92	43	73.01±11.64 ^a	0.001*
Past history of hypertension	Normotensive	39	46.57±16.00 ^a	46	58.79±13.16	36	74.06±15.13 ^a	0.001*
	Hypertensive	34	44.98±13.01 ^a	30	59.68±14.90	38	72.40±10.94 ^a	0.001*
Current hypertensive	Normotensive	12	43.60±19.35 ^a	9	64.93±18.81	8	73.90±11.89 ^a	0.001*
	Hypertensive	61	46.07±14.12 ^a	67	58.86±13.59	66	73.14±13.16 ^a	0.001*
Metabolic Syndrome	Absent	7	56.85±30.19 ^a	27	60.50±14.44	60	73.16±13.07 ^a	0.001*
	Present	66	45.48±14.19 ^a	49	58.53±13.53	14	73.32±13.33 ^a	0.001*

[Table/Fig-3]: Comparison of nitric oxide levels among the groups according to basic characteristics of the patients.
¹ANOVA tests (among the groups), *Significant, ^a, ^bp=0.001 (Post-hoc comparison tests)

that overproduction of NO affects metabolic actions of insulin hormone [16].

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 [17].

Evaluation of oxidant-antioxidant status in diabetic patients with

or without complications has been a regular area of research. Verma et al., conducted a study to compare antioxidant levels between diabetics and age matched, healthy controls and they found impaired antioxidant status in diabetic patients which is a definite sign of ongoing oxidative stress as has been noted in our study [18]. Serum NO was observed significantly low in diabetic participants as compared to control, along with difference in other biochemical parameters [19]. But in another studies [20,21] it was claimed that Hyperglycaemia enhances NO production in diabetes. Now it became clear that oxidative stress plays an important role in progression and development of diabetes and its complications [22,23]. We found poor correlation between NO

Correlation of NO with	Group I (n=73)		Group II (n=76)		Group III (n=74)	
	"r"	"p"	"r"	"p"	"r"	"p"
MDA (nmol/l)	0.129	0.295	-0.117	0.335	0.047	0.699
SOD (Umg/ml)	0.090	0.467	0.159	0.189	0.247	0.039
CAT (Umg/ml)	-0.131	0.288	0.038	0.755	-0.040	0.743
GR (Umg/p)	0.074	0.550	-0.017	0.755	-0.091	0.454
GPx (UmgHb)	0.162	0.187	-0.043	0.724	-0.125	0.302

[Table/Fig-4]: Correlation of NO levels with Antioxidants.
MDA- Malondialdehyde, SOD- Superoxide dismutase, CAT- Catalase, GR- Glutathione reductase, GPx- Glutathione peroxidase

levels and oxidative stress parameters in all the three groups. This should be further investigated in a larger sample size.

CONCLUSION

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. Our data suggests the role of Nitric Oxide (NO) in pathogenesis of type-2 diabetes with dyslipidemia and hyperuricaemia. Our study was an attempt to analyses nitric oxide in a background of redox stress. It should be studied on a much larger scale.

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