Original Article

Assessment of serum nitric oxide level and its correlation with anthropometric parameters and lipid profile in diabetic patients: A hospital-based study from Tripura

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Abstract

Background: Endothelial dysfunction is a well-known finding in hyper-cholesterolaemic patients. Multiple factors including increased inactivation of nitric oxide by radicals and inhibition of nitric oxide formation by different mechanisms contribute to this. **Objectives:** (i) To estimate serum nitric oxide (NO) levels among diabetic and non-diabetic subjects attending a tertiary care hospital of Tripura and (ii) to determine the correlation of serum nitric oxide with different anthropometric parameters and lipid profile among the study subjects. **Methods:** This cross-sectional study was conducted during June 2019 to May 2020 among 227 subjects. Anthropometric measurements like weight, body mass index (BMI), body fat percentage, visceral fat percentage were measured by using OMRON Body Composition Monitor (HBF 701). Serum NO levels were measured using standard NO colorimetric assay kit and HbA_1C and lipid profile were analyzed by using a Biochemical Autoanalyser. Statistical analysis was performed by using SPSS software version 25. **Result:** One hundred fifteen (115) diabetics were considered as test group whereas One hundred twelve (112) non-diabetic subjects were included as control. The mean serum level of NO in the diabetic group was 86.91 ± 14.13 μmoles/L whereas in the non-diabetic group it was 33.23 ± 12.90 μmoles/L which is statistically significant. Significant correlation is also found between serum NO level and different anthropometric parameters, namely, age, BMI and visceral fat percentage. **Conclusion:** In this study, positive correlation is found between serum NO, BMI, and body visceral fat. As NO is considered as a potential biomarker for diabetic patients developing hypertension, BMI, and body visceral fat may be considered as a good prognostic parameter in future development of diabetic complications. While dealing with diabetic patients the family physicians should be aware of these two parameters and besides treating them, physicians should convince the diabetic patients to maintain ideal BMI and body visceral fat by following proper life style.

Keywords: Anthropometric measurements, lipid profile, serum nitric oxide, type 2 diabetes

Introduction

The reports of International Diabetes Federation (IDF), 2020 reveal that, 463 million people have diabetes in the world and out of that 77 million belong to India.[1] According to this report, the prevalence of diabetes among the Indian population is 8.9%. The rapid urbanization, sedentary lifestyle, high-calorie diet, visceral adiposity, and high genetic predisposition have been identified as the major factors that elevate the risk of type 2 diabetes mellitus (T2DM) among Indians even at a much younger age than the Western population.[2] Many population-based studies also reported that the average onset of T2DM among Indians is gradually increasing in the age groups below 40 years of age.[2,3] The major factors inT2DM are chronic

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hyperglycemia, dyslipidemia, and increased insulin resistance, which induce a series of metabolic and molecular alterations leading to the development of diabetes-associated vascular complications.[4] Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of diabetic vascular complications and shown to originate from hyperglycaemia. Hyperglycaemia and its biochemical effects either alter endothelial function directly or influence endothelial cell functioning indirectly by affecting the pathways of growth factors.[5] Endothelial dysfunction is also a well-known finding in hypercholesterolaemic patients.[6] It is reported that multiple factors contribute to this including inactivation of NO by radicals and inhibition of NO formation by different mechanisms. From previous studies it has been observed that Hyperlipidemia, that is, oxidized LDL brings endothelial dysfunction by uncoupling the eNOS (endothelial nitric oxide synthase) resulting in increase in superoxide anions (O_2^-) production. This superoxide spontaneously reacts with NO to form peroxynitrite anion ($ONOO^-$) which is highly reactive, cytotoxic, and induces lipid peroxidation causing to endothelial dysfunction.[7] The role of serum NO in the management of diabetes and its co-existing complications may be associated with the various parameters of lipid profile such as Cholesterol, Triglyceride, Very Low Density Lipoprotein (VLDL), High Density Lipoprotein (HDL), and Low Density Lipoprotein (LDL). Information regarding correlation of serum NO level with anthropometric parameters among diabetic and non-diabetic subjects from this north eastern part of India is very scarce. So, this study was designed (i) to estimate serum levels of NO in diabetics and non-diabetics subjects and (ii) to determine the correlation between serum levels of NO and anthropometric parameters, lipid profile in the study subjects.

Materials and Methods

This hospital based cross-sectional study was conducted during June 2019 to May 2020 among 227 subjects. They were allocated into two study groups which is as follows:

- Group 1: Subjects without diabetes as Control (CT) (n = 112)
- Group 2: Subjects having Type 2 diabetes mellitus (T2DM) (n = 115) at least from last one year as test group.

Simple random sampling technique was used for determining sample size. The participants having co-morbidities like hormonal disorders, benign or malignant disorders, diabetic ketoacidosis, infection, chronic renal and cardiac disease, inflammatory diseases, transplant rejection, central nervous system disorders, and other chronic diseases were excluded from the study. Other than these, subjects taking anti-cancer, anti-TB drugs and pregnant women and those who were on antioxidant supplementation or lipid lowering drugs were also excluded. But the diabetic patients were allowed to continue their regular lifestyles. Out of 227 patients, 115 patients came under test group having history of T2DM and 112 accompanying person of the patients who were non-diabetic, were considered as control group as per selection criteria. Written informed consent was obtained from each subject before including them in this study. Selected subjects were interviewed using questionnaire. Fasting glucose ≥126 mg/dl and HbA1c level ≥6.5% were considered for diagnosis of diabetes as per ICMR (Indian Council of Medical Research) 2018 guidelines.[8] All anthropometric measurements like weight, BMI, body fat percentage, visceral fat percentage were measured by using OMRON Body Composition Monitor (HBF-701). After completing the interview and anthropometry measurement, 5 ml venous blood sample was collected from each of the respondents following standard guidelines[9] for performing different biochemical tests. Random blood glucose, HbA_1C and lipid profile was analyzed by using a Biochemical Autoanalyser [XL-640]. Serum NO level was estimated by using standard NO colorimetric assay kit (Make: Invitrogen).

Statistical analysis

Data were analyzed by using 'Statistical Package for the Social Sciences' (SPSS) software for Windows version 25.0. Student t-test was used for testing significance of difference between two variables expressed in Mean ± SD. Probability value < 0.05 was considered as statistically significant.

Ethical clearance

Before conducting this study, ethical clearance was obtained from Institutional Ethics Committee vide order no. F.No. (6-9)/AGMC/Academic/IEC Committee/2015/8962, Dated, 25.04.2018.

Result

This research study was conducted among 227 participants. Out of them, 115 patients having history of diabetes came under test group whereas 112 subjects were non-diabetic. Out of total participants, 53% were female and 47% were male. Table 1 shows correlation status of serum NO level with anthropometric parameters of all the study subjects. In this present study, significant correlation was found between serum NO level with systolic blood pressure (P = 0.001), diastolic blood pressure (P = 0.001), age (P = 0.001), BMI (P = 0.002) and V-Fat (P = 0.023) percentage among all the participants. Among all the study subjects, majority, that is, 42.04% comes under 47–57 age group [Figure 1]. According to this study, significant correlation has also been observed between serum NO level and different parameters namely BMI, visceral fat%, SBP, DBP, FBS, and HbA1c among two groups [Table 2]. Mean values of lipid parameters were also well comparable among two groups [Table 3]. The significant difference (P < 0.001) in serum NO levels were also observed between the groups which imply the role of NO in pathogenesis of type-2 diabetes with dyslipidemia.

Discussion

Some studies showed lower serum NO level in diabetic patients comparing to their non-diabetic counterparts[10]

but in other studies[11,12] it is claimed that hyperglycaemia enhances NO production. It is now clear that oxidative stress plays an important role in progression and development of diabetes mellitus and its complications. Studies on influence of anthropometric parameters and its correlation with NO production and lipid profile are very scarce and inconclusive. Hence this study was undertaken to determine correlation of NO level with various anthropometric parameters and lipid profile among the population of Tripura.

In the present study, there was a significant difference in many of the independent and dependant variables in terms of FBS and HbA1c with serum NO level, anthropometric parameters, and different lipid parameters.

Table 1: Correlation between serum nitric oxide level and anthropometric parameters

Name of the parameter	Mean±SD (*n*=227)	Pearson Correlation (*r*)	*P*
Serum nitric oxide level (60.42±30.10 µmoles/L)			
SBP (mm Hg)	127.02±17.25	0.696	0.001**
DBP (mm Hg)	82.03±8.06	0.551	0.001**
Age (Years)	51.05±9.35	0.227	0.001**
BMI (kg/cm^2)	24.15±3.57	0.206	0.002**
Weight (kg)	58.31±10.98	0.127	0.056
Height (cm)	154.88±9.11	-0.033	0.624
Visceral fat (%)	10.82±5.74	0.151	0.023**
Body fat (%)	30.96±7.67	0.172	0.101

Table 2: Correlation of serum NO level with different parameters between two groups

Co-relation of NO with	Control (*n*=112)		Test (*n*=115)	
	r	*P*	*r*	*P*
SBP (mm Hg)	0.379	0.000	0.273	0.000
DBP (mm Hg)	0.298	0.000	0.194	0.000
BMI (kg/cm^2)	0.679	<0.001	0.589	<0.001
Weight (kg)	0.792	<0.001	0.562	<0.001
Height (cm)	-0.052	0.414	-0.046	0.321
Visceral fat (%)	-0.526	0.001	-0.712	0.001
Body fat (%)	0.037	0.522	0.043	0.517
FBS	0.643	0.000	0.702	0.000
PP	0.123	0.058	0.224	0.067
HbA1c (%)	-0.823	0.000	-0.811	0.000

In a study conducted by Adela *et al.*,[11] it was noticed that elevated levels of glucose may enhance NO production through increased expression of eNOS and iNOS gene and protein levels. Increased levels of NO in in-vivo might have both beneficial as well as detrimental effect depending upon the NO concentration. On one hand, NO can cause relaxation of blood vessels and reduce hypertension and on the other hand, NO may interact with superoxide radical ($O2^-$) leading to inactivation of NO. The interaction of O2 − with NO is rapid and leads to the formation of potent oxidant radical, namely, peroxynitrite ($ONOO^-$). This may contribute to impaired endothelial function by stimulating arachidonic acid metabolism, lipid peroxidation, and prostanoid production.[13] Our results clearly indicated that NO levels were increased in T2DM patients in comparison to normal subjects. Adera *et al.*[11] showed that serum NO levels were also increased in type 2 DM patients in comparison to control group (without diabetes) in South Indian individuals which supports our present finding.

In this present study, diabetic patients were grouped depending on the duration of diabetes. Patients having five years or less duration have higher serum NO levels (64.53 ± 29.37 µmoles/L) in comparison to the group of patients having more than five years of duration (59.48 ± 32.05 µmoles/L) which is in accordance with study of Adera *et al.*[11] This reduction in serum NO level indicates some protective role of serum NO in relation to the advancement of diabetes.

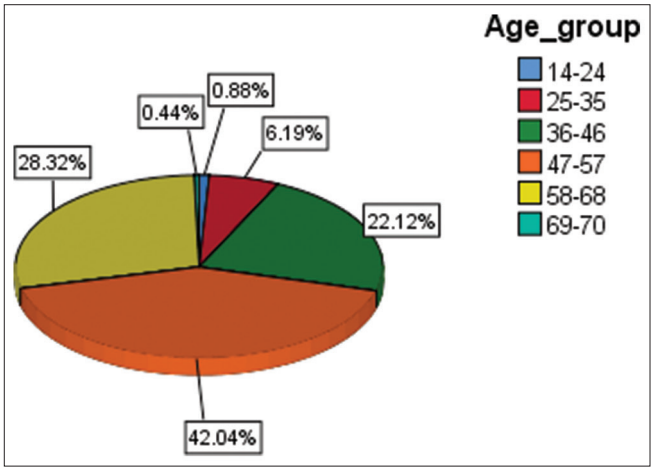

Figure 1: Age group distribution

Table 3: Comparison of two groups based on serum NO level and Lipid parameters

Name of the Parameter	Control (*n*=112)			Test (*n*=115)		
	Serum Nitric Oxide (33.23±12.90) µmoles/L			Serum Nitric Oxide (86.91±14.13) µmoles/L		
	Mean±SD	Pearson Correlation (*r*)	*P*	Mean±SD	Pearson Correlation (*r*)	*P*
Cholesterol (mg/dl)	172.66±35.75	0.088	0.358	192.80±51.53	0.274	0.003**
Triglyceride (mg/dl)	199.40±92.06	-0.008	0.934	192.01±98.61	0.198	0.034*
HDL (mg/dl)	52.89±13.89	-0.044	0.641	48.64±13.02	-0.019	0.841
LDL (mg/dl)	111.98±31.55	0.111	0.242	126.32±36.01	0.214	0.023*
VLDL (mg/dl)	41.38±18.59	-0.001	0.989	43.01±23.14	0.090	0.340

Hoshiyama *et al.*[14] showed that high glucose exposure increases NOS protein expression, but decreases release of NO in human glomerular endothelial cells. Decreased NO bioavailability was associated with over production of superoxide and L-arginine deficiency. The increased glucose levels in blood may enhance the NO levels in blood. However, it could be possible that other factors like geographical location, genetic background, and anthropometric measurements of the population may also influence the change.

Earlier, it was shown that endothelial NO pathway abnormalities are present in human with atherosclerosis.[15] This evidence also suggests that several key steps may be inhibited by NO in the atherosclerotic process and an alteration of NO production within the vascular endothelium could contribute to the pathogenesis of atherosclerosis.[16]

In this study, statistically significant positive correlation was found between serum NO level and lipid parameters like cholesterol, triglyceride, and LDL among test group whereas no significant correlation was present among the non-diabetic subjects. Significant correlation with cholesterol, triglyceride, and LDL among diabetic patients indicates that increased levels of serum NO may lead to the over production of cholesterol, triglyceride, and LDL and eventually lead to hypercholesterolemia and atherosclerotic complications in diabetic patients.

In addition to this, significant negative correlation of serum NO levels with HDL was also observed (P = 0.023) among all the study subjects of this present study. In recent studies, Tomas Vaisar *et al*. demonstrated that diabetes is associated with endothelial dysfunction and HDL can protect endothelium.[17] In our study, negative or inverse correlation of serum Nitric Oxide levels with HDL indicates that increased serum NO level in diabetic patient leads to endothelial dysfunction and reduction in HDL level which may lead to atherosclerotic complication in diabetic patients.

In this present study, significant correlation has been observed between serum NO level, BMI, and increased blood pressure. Increased serum NO levels in overweight subjects might be a reflection of increased NO production. Previous studies also found that obese and overweight subjects had relatively higher serum NO levels as compared to normal weight individual.[18,19] In a study done by Vitale *et al.*,[20] there was a positive correlation between BMI and salivary NO concentrations in overweight and obese subjects. To the best of our knowledge, this is the first report of increased serum NO level and higher BMI in relation to onset of diabetes from this north eastern part of India.

High visceral fat is an indicator of obesity. Along with BMI, visceral fat contributes to the progression of obesity which in turn leads to diabetes. In our study, significant correlation of BMI and visceral fat with serum NO level is found which may contribute to the increase in SBP and DBP. So, in future, serum NO might be used by family physicians as a prognostic biomarker for treating the diabetic patients. However, with advancement of diabetes, the decreased serum NO levels may have some beneficial effect which needs further study.

Conclusion

In this study, positive correlation is found between serum NO, BMI, and body visceral fat. As NO is considered as a potential biomarker for diabetic patients developing hypertension, BMI, and body visceral fat may be considered as a good prognostic parameter in future development of diabetic complications. While dealing with diabetic patients the family physicians should be aware of these two parameters and besides treating them, physicians should convince the diabetic patients to maintain ideal BMI and body visceral fat by maintaining proper life style.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Ethics approval

Ethics approval was obtained from institutional ethics Committee of Agartala Govt. Medical College before conducting this study.

Abbreviation: T2DM – Type 2 Diabetes Mellitus, NO – Nitric Oxide, ICMR – Indian Council of Medical Research

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Conflicts of interest

There is no conflict of interest.

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