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Research Article

Association of nitrosative and oxidative stress in young type 2 diabetic patients

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ABSTRACT

Background: Nitric oxide levels play an important role in the pathogenesis of endothelial dysfunction in type 2 diabetes mellitus (DM). Aim of the study was to assess the association between serum nitric oxide levels (NOx), Oxidant load (FOX2) and total antioxidant status (FRAP) with fasting blood sugar (FBS) in type 2 diabetic patients.
Methods: Serum concentration NOx was measured by Griess method in seventy six type 2 diabetic patients with age & sex matched (aged 45-65yrs) controls. Serum total oxidant load was estimated by Ferrous oxidation product in xylenol orange version 2 (FOX2), total antioxidant status by ferric reducing capacity of serum (FRAP) .FBS was estimated by commercial kits adapted to autoanalyser.
Results: Significant difference was observed in serum NO level between patients and controls (78.6+ 8.6 vs. 37.59+ 4.19µmol/L: p<0.000). Significantly high FOX 2 levels (12.4+ 2.6µmol/L vs. 4.33+ 1.7 µmol/L) and lower FRAPS level was observed in patients as compared to controls (61.37+31.64 µmol/L vs. 226.46+ 15.23 µmol/L).
Conclusions: Hyperglycemia leads to nitrosative stress (increase NO level) and oxidative stress by increasing total oxidant load (FOX2), decreasing antioxidant capacity (FRAP).

Keywords: Diabetes mellitus, Nitric oxide, Total oxidant load, Antioxidant capacity

INTRODUCTION

Increased extracellular glucose concentration, a principal feature of diabetes mellitus induces deregulations of reactive oxygen & nitrogen generating pathways.[1] Reactive oxygen species (ROS) are leading cause of endothelial dysfunction & ensuing cardiovascular disease. Elevated glucose causes oxidative stress due to increased production of mitochondrial reactive oxygen species (ROS) and it up regulates the endothelial nitric oxide (NO) synthase gene and stimulate NO production.[2]

Reactive oxygen & nitrogen species trigger endothelial cell dysfunction through various mechanisms. Several mechanisms have been proposed to explain the role of glucose in the dysfunction of the vascular NO system in diabetes. One putative mechanism is that high glucose reduces the availability of NO by increasing oxidative destruction of the molecule. Hyperglycemia may activate the production of protein kinase C (PKC), which leads to production of advanced glycation end products (AGEs).[3]

This case control study aims to

a) Comparing blood sugar, serum nitric oxide metabolites (NO) in between young diabetic patients (age below 45yrs of age) & age and sex matched controls.
b) Correlation of serum NO, total oxidant load (FOX2) & antioxidant capacity (FRAP) in patients.

METHODS

Seventy six patients of type 2 diabetes mellitus of age group of 30-45 yrs. without any other infections, inflammations and other serious diseases were taken for this study. There are also same number of age & sex matched controls were taken. This prospective case control study was conducted in the department of Biochemistry, the patients attained the medicine OPD of MKCG medical college and hospital was selected for this study. Diagnosis of Diabetes was made using WHO Criteria for Diabetes Guideline. Fasting blood sugar (FBS) was estimated by commercial kits adapted to auto-analyzer Erba 360. Serum NO levels were estimated by Griess method. Serum total oxidant load was estimated by Ferrous oxidation product in xylenol orange version 2 (FOX2), total antioxidant status by ferric reducing capacity of serum (FRAP). Quantitative data were represented as mean ±SD. Data was analyzed by unpaired student's t test and Pearson's correlation. SPSS 19 was used for analysis.

RESULTS

Significant difference was observed in serum NO level between patients and controls (78.6+ 8.6 vs. 37.59+ 4.19µmol/L: p<0.000). Significantly high FOX 2 levels (12.4+ 2.6µmol/L vs. 4.33+ 1.7 µmol/L) and lower FRAPS level was observed in patients as compared to controls (61.37+31.64 µmol/L vs. 226.46+ 15.23 µmol/L). Positive correlation was observed between FBS, Serum NO and FOX2 level (FBS & NO r=0.964, p <0.000, NO & FOX2 r=0.952: p<0.000) and a negative correlation existed between serum NO and FRAP levels. (p<0.001 r-.943).

Table 1: Comparison of Clinical and Biochemical parameters in female type 2 Diabetic patients and controls.

Parameters	Controls	Diabetic patients	P value
FBS(mg/dl)	96.98±9.25	203.26± 46.56	0.000
Total cholesterol(mg/dl)	156.2± 16.53	259.0± 43.88	0.05
Triglycerides(mg/dl)	151.34± 32.19	245.46± 69.67	0.000
HDL-Cholesterol(mg/dl)	43.65± 6.0	38.62± 3.9	0.015
LDL Cholesterol(mg/dl)	86.07± 7.5	164.46± 43.03	0.005
Nitric oxide(µmol/L)	37.59± 4.19	78.6± 8.6	0.000
FRAP(µmol/L) equivalent of Ferrous Sulphate	226.46± 15.23	61.37± 4.71	0.000
FOX2(µmol/L) equivalent of H_2O_2	4.33± 1.7	12.4± 2.6	0.000

Data is represented as mean± SD. Statistical significance is derived by unpaired student's t test. A p value of <0.05was considered significant.

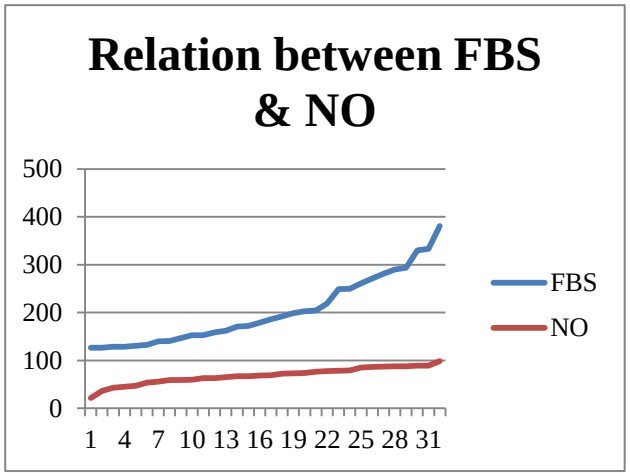


Figure 1: The relation between fasting blood sugar (FBS) & Serum nitric oxide (NO) in cases.

It shows when FBS level increases serum nitric oxide value rises correspondingly.

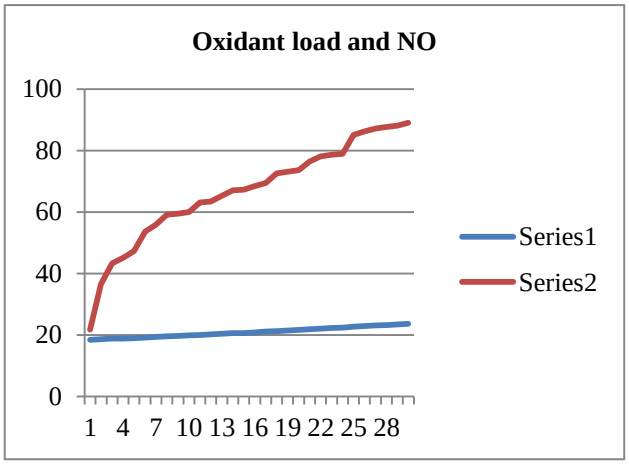
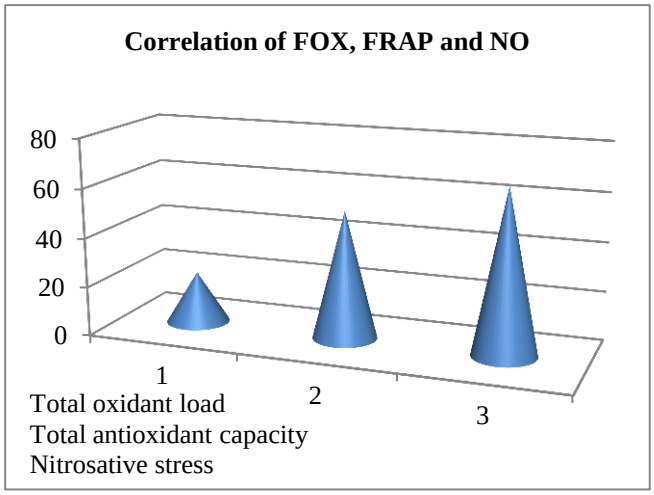


Figure 2: The relation between oxidant load and nitric oxide in cases.

It shows a significant positive relation between FOX2 and NO. Series 1 depicts serum nitric oxide levels and series 2 shows FOX2 levels.

Figure 3: The correlation of oxidant load (FOX2), antioxidant capacity (FRAP) and Nitric oxide (no) level in cases.



It shows comparison of mean FOX2, Total antioxidant capacity (FRAP) and NO.

Table 2: correlations between nitric oxide, oxidative stress and antioxidant load.

Nitric oxide(NO)	FOX2(µmol/L) equivalent of H_2O_2	FRAP(µmol/L) equivalent of Ferrous Sulphate
r value	0.952	-0.943
p value	.000	.000

Correlation is significant at the 0.01 level (2-tailed). Significant positive correlation was observed between NO and FOX2. Significant negative correlation was observed between NO and antioxidant load (FRAP).

DISCUSSION

The findings of the present study demonstrate a strong association between fasting blood sugar, nitrosative and oxidative stress shows that parameters. Assunta pandolfi et al found that elevated glucose concentrations can up regulate the endothelial NO synthase (eNOS) gene and stimulate NO production in both animal and human cells[5] Primary cultures of human umbilical vein endothelial cells(HUVEC) cultured in high glucose exhibit increased synthesis of NO.[6] Production of ROS and RNS occurs in response to extracellular and intracellular stimuli. Extracellular stimuli act through plasma membrane receptors and include TNFα, hormones, and growth factors, including platelet-derived growth factor, epithelial growth factor, and insulin. Intracellular stimuli include nutrients.[7] nicotinamide adenine dinucleotide phosphate (NADPH) oxidase,[8] NOS, and mitochondrial electron transfer.[9] These are the most relevant sources of reactive species (RS), including ROS and RNS. They can react with multiple cellular components (proteins, lipids, nucleic acids), and generate reversible or irreversible oxidative modifications. They also activate various signaling cascades, some of which are designated for sensing and responding to "stress" like the mitogen-activated protein (MAP) kinase family or c-Jun N-terminal kinase (JNK) There is reduced expression and activity of human equilibrative nucleoside transporter type 1(hENT1), the main mediator of adenosine uptake which down regulated by NO dependent reduced slc29A1 transcription leading to increased eNOS and hCAT-1 m RNA levels.[10]

In our study it was observed that hyperglycemia leads to oxidative stress there by increased FOX2level and decreased antioxidant load (FRAP). Similar findings were observed by Giugliano D, Tesfamariam B et al.[11]

Hyperglycemia and free fatty acid induced oxidative stress leads to the activation of stress sensitive signaling pathways. Stress activated signaling pathways include nuclear factor-kB (NF-kB), p38 MAPK, NH2-terminal Jun kinases/stress-activated protein kinases (JNK/SAPK) and protein kinase C (PKC).[12]

In our present study we observed a significant positive correlation between serum nitric oxide and total oxidant load (FOX2) p value <0.001. Several authors observed that with increasing oxidative stress serum nitric oxide increases. Vascular oxidative stress with an increased production of reactive oxygen species (ROS) contributes to mechanisms of vascular dysfunction. Oxidative stress is mainly caused by an imbalance between the activity of endogenous pro-oxidative enzymes (such as NADPH oxidase, xanthine oxidase, or the mitochondrial respiratory chain) and anti-oxidative enzymes (such as superoxide dismutase, glutathione peroxidase, heme oxygenase, thioredoxin peroxidase/peroxiredoxin, catalase, and paraoxonase). Also, small molecular weight antioxidants may play a role in the defense against oxidative stress. Increased ROS concentrations increase the amount of bioactive NO by chemical inactivation to form toxic peroxynitrite.[13,14]

CONCLUSION

From the present study it is concluded that hyperglycemia leads to nitrosative stress (increase NO level) and oxidative stress by increasing total oxidant load (FOX2), decreasing antioxidant capacity (FRAP).

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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