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Systemic Inflammation and Nitric Oxide Levels in the Patients with Type 2 Diabetes Mellitus

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Abstract: Presence of systemic inflammation is a hallmark of variety of pathophysiological such as hypertension, atherosclerosis, myocardial infarction and inflammatory joint diseases. Although previous evidences provided extensive literature about the low grade inflammation in Type 2 diabetes mellitus (T2DM), there is a paucity of satisfactory explanation regarding the alteration in the level of nitric oxide along with marker of systemic inflammation and lipid peroxidation in Type 2 DM patients. Therefore, the objective of present study was to estimate the level of C-reactive protein (CRP), malondialdehyde (MDA) and nitric oxide (NO) in T2DM patients and to determine its relation with future risk of atherosclerosis. 40 patients of either sex (35-50 years age group) suffering from T2DM and 40 normal healthy individuals served as control; were included in the study. Above mentioned parameters were estimated using standard methods and data from patients and controls were compared by using Student's t-test and Pearson co-relation coefficient. In addition to hyperglycemia, erythrocyte MDA and plasma CRP levels were significantly high in T2DM subjects ($P<0.001$ & $P<0.05$) whereas plasma NO level was found to be significantly low ($P<0.001$) as compared to healthy controls and negative correlated with MDA and CRP levels ($p<0.05$). These findings suggest that collective effect of inflammation and oxidative stress on endothelial dysfunction plays a crucial role in the development of future atherosclerosis in T2DM, characterized by low nitric oxide levels and enhanced CRP and MDA levels. Thus, these markers should be monitored regularly in T2DM patients for early detection and adoption of preventive strategy against cardiac complications.

Keywords: Oxidative stress, endothelial dysfunction, lipid peroxidation, C-reactive protein.

INTRODUCTION

Diabetes mellitus (DM) is a major health concern of the developing nations and accounts for the 5% deaths around the world annually [1]. DM is considered as a coronary heart disease (CHD)- risk equivalent and frequently associated with various other cardiovascular (CV) risk factors including hypertension. With T2DM, there is an increase in hypertension risk which is mostly attributable to vascular endothelial dysfunction [2,3].

Moreover, risk factors for developing diabetes, such as obesity, physical inactivity, smoking, dietary habits, psychological stress and infections, are considered to be activators of the innate immune system that induce a state of inflammation[4]. C-reactive protein (CRP), a marker of systemic inflammation and synthesized in liver, has been received considering attention in hyperglycemia and various aging associated disorders including diabetes mellitus. Previous studies have demonstrated an association between diabetes progression and inflammation as measured by plasma C-reactive protein [5,6].

Nitric oxide (NO) is a powerful endothelium derived vasodilator, produced from the precursor L-arginine in all human body cells. It takes part in blood pressure control, inhibits mast cells degranulation, possesses anti-aggregant properties, and regulates vascular tone. It also inhibits both proliferation of smooth muscle cells and adhesion of leukocytes and platelets [7,8]. Reduction in NO levels plays a crucial role in the development of cardiac complications.

Oxidative stress has been considered to be a pathogenic factor of diabetic complications including CHD. The intensification of free-radical reactions in patients with type 2 diabetes was a factor responsible for the development of vascular changes [9,10]. Amongst various free radicals mediated destructive

events, lipid peroxidation is a free radical mediated process in which the polyunsaturated fatty acids contained in the LDL or present in the cell membrane are degraded to variety of aldehydes mainly malondialdehyde (MDA). These aldehydes may alter endothelial function and inhibit NO synthase activity leading to the development of hypertension [11].

Although limited information is available on systemic inflammation in the patients with T2DM, alteration in serum CRP levels along with marker of endothelial dysfunction and oxidative stress in T2DM patients at a single platform is still not documented. Therefore, the objectives of the present study were to estimate serum CRP, and nitric oxide levels along with erythrocyte MDA in patients with T2DM and to determine its relation in prediction of hypertension risk.

MATERIALS & METHODS

In the present study 40 patients of either sex with diabetes mellitus belonged to age group 35-50 years and 40 age matched healthy individuals, served as control, were taken. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed in all the subjects after taking their informed consent and approval of protocol by ethics committee of college.

Inclusion criteria

The inclusion criteria adopted were: age 35-50 years, newly diagnosed and untreated cases of T2DM. Diabetes mellitus was diagnosed by following the American Diabetes Association criteria 2015 [12].

Exclusion criteria

Patients suffering from cardiovascular disease, hepatic disease, tuberculosis, renal disease and taking drugs like steroid, amiodarone, lithium, antioxidant vitamin supplement or non-steroidal anti-inflammatory drugs, antihypertensive drugs and other medications were excluded from the study.

Fasting blood samples were collected in fluoride and EDTA vials from the anticubital vein of the study group subjects and processed immediately. Fasting and postprandial blood glucose levels were estimated by hexokinase method (Beckman coulter, USA). Plasma CRP levels were measured using commercially available ELISA kits (R&D Systems, USA), according to manufacturer's instructions.

The measurement of plasma NO is difficult because this radical is poorly soluble in water and has a short half-life in tissue (10-60 s), but its half-life may be as long as 4 minutes in the presence of oxygen. For these reasons, the end products of the phenomenon, nitrate and nitrite, are preferentially used in clinical biochemistry. Plasma total nitrate and nitrite levels were measured with the use of Griess reagent as described earlier [13].

Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate. The heat induced reaction of malondialdehyde (MDA) with thio barbituric acid (TBA) in the acid solution forms a trimethine coloured substance, which is measured spectrophotometrically at 532 nm [14].

STATISTICAL ANALYSIS

The data from both the study group subjects and controls were expressed as Mean ± SD and compared by using Student's t-test and Pearson correlation coefficient test (r).

RESULTS

In the present study, abnormalities in biochemical markers such as glycemic profile, erythrocyte MDA, plasma CRP and nitric oxide levels in study group subjects are represented in Table 1. Fasting and postprandial blood glucose levels were significantly high ($P< 0.001$; 79.43 and 105.32% high) in patient group as compared to healthy controls. Similarly, erythrocyte MDA and plasma CRP levels were significantly high ($P< 0.001$, 38.15% and 28.5% high) in T2DM patients as compared to healthy controls whereas plasma nitric oxide level was significantly low ($p<0.05$; 26.8% low) in T2DM patients which authenticated the fact that assessment of oxidative stress and systemic inflammation along with marker of endothelial dysfunction may be an important determinant for future cardiac complication in T2DM patients. In addition, after performing correlation coefficient analysis of plasma NO levels with erythrocyte MDA, plasma CRP and fasting blood glucose levels, it was observed that endothelial dysfunction was inversely correlated with oxidative stress, systemic inflammation and hyperglycemia ($p<0.05$; $r = -0.532$; -0.605 and -0.498) in diabetic patients as presented in Table 2.

Table-1: Glycemic profile and markers of oxidative stress, systemic inflammation and endothelial dysfunction in study group subjects (Mean ± SD)

Sl.No.	Particulars	Control Group (n=40)	Patient group (n=40)	Level of Significance
1.	Fasting Blood Glucose (mg/dl)	85.16± 7.82	152.80± 40.35***	p < 0.001
2.	Post Prandial Blood Glucose (mg/dl)	128.2± 10.8	263.22± 48.5***	p < 0.001
3.	CRP (mg/L)	3.27± 0.11	4.20± 0.14***	P < 0.001
4.	Malondialdehyde (µmol MDA/ml)	2.76± 0.12	3.81± 0.16***	*P < 0.001*
5.	NO level (µ mol/L)	8.34± 2.1	6.11± 1.8**	P < 0.05

Where,

* p<0.1: Non-significant

** p<0.05: Significant

*** p<0.001: Highly significant

Table-2: Correlation coefficient (r) between Nitric oxide (NO) and various biomarkers in T2DM patients

Particulars	MDA	CRP	FBS
Nitric oxide	- 0.532 **	+ 0.605**	+ 0.498**

where,

*p<0.1: Non-significant

** p<0.05: Significant

DISCUSSIONS

Despite significant progress in diagnosis, treatment and prevention, diabetes particularly continues to remain the leading cause of cardiovascular complications and represents global socioeconomic burden. It has now been proved that oxidative stress is increased in patients with diabetes owing to increase in free radical production [15]. Involvement of free radicals in membrane damage via lipid peroxidation and its resultant products such as lipid radicals (L◦), lipid peroxides (LOO◦), lipid hydroperoxides (LOOH) and highly reactive aldehydes plays a crucial role in the development and progression of inflammatory and age related disorders including diabetes[16,17]. Free radicals via lipid peroxidation play an etiopathogenic role in the development of T2DM and its related cardiac complication as obvious in the findings of present study [18]. Marked elevated levels of malondialdehyde (i.e. marker of lipid peroxidation) were observed in patient group (P<0.001) as compared to healthy control along with its negative correlation (p<0.05) with nitric oxide levels which reflect the role of oxidative stress in making diabetic patients more susceptible to develop endothelial dysfunction followed by cardiac complications. Our findings were in concordance with the findings of Nandkeoliar *et al*. According to them, lipid peroxides are toxic to the cellular components and lipid peroxidation may be responsible for vascular disorder in T2DM patients [19].

It has been reported that vascular cell apoptosis and inflammatory necrosis are caused by increased oxidative stress through ischemia-reperfusion in diabetes. Moreover, inflammation medicated free radicals and their intermediates plays a crucial role in inducing inflammatory disorders by promoting various culprit events such as activation of proteolytic enzymes, reduction of antioxidant reserves in body fluid, induction of lipid peroxidation and inhibition of glycolytic enzymes[20,21]. In the present study, plasma CRP levels were found to be significantly high (p<0.001) in T2DM patient which clarify the combined role of inflammation and oxidative stress not only in inducing endothelial dysfunction but also in the etiopathogenesis of T2DM and its related cardiac complications. Similar findings have been documented in T2DM patients suggesting that inflammation not only plays a crucial role in the development of cardiac complications but also considered as its risk factor as well [22-24].

CONCLUSION

In conclusion our study shows that plasma CRP and MDA levels are inversely related to endothelial dysfunction which plays an etiopathological role in the development of cardiac complication in diabetic patients. The present study also authenticates that malondialdehyde and CRP along with NO levels may be an excellent marker of oxidative stress and inflammation mediated endothelial dysfunction in diabetic patients. Furthermore, our study suggests that the routine blood sugar monitoring and its management along with consumption of diet rich in antioxidants are essential for diabetic patients in order to combat the deleterious effect of hyperglycemia mediated oxidative stress and its associated future cardiac complications.

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REFERENCES

1. Vij AS, Bali K, Marwaha TS, Sareen S. Insulin Acceptance among Type 2 Diabetes Mellitus Patients. Sch. J. App. Med. Sci. 2017; 5(11B):4459-4462.
2. Mithal A, Majhi D, Shunmugavelu M, Talwarkar PG, Vasnawala H, Raza AS. Prevalence of dyslipidemia in adult Indian diabetic patients: A cross sectional study (SOLID). Indian J Endocr Metab. 2014;18:642-647.
3. Saydah SH, Fradkin J, Cowie CC. Poor control of risk factors for vascular disease among adults with previously diagnosed diabetes. JAMA. 2004; 291:335-42.
4. Pitsavos C, Tampourlou M, Panagiotakos DB, Skoumas Y, Chrysohoou C, Nomikos T, Stefanadis, C. Association between Low-Grade Systemic Inflammation and Type 2 Diabetes Mellitus Among Men and Women from the ATTICA Study.The review of diabetic studies : RDS. 2007;4(2):98-104.
5. Colhoun HM, Schalkwijk C, Rubens MB, Stehouwer CD. C-reactive protein in type 1 diabetes and its relationship to coronary artery calcification. Diabetes Care. 2002; 25(10):1813-1817.
6. Saxena R, Suneja S, Saxena R, Sharma D, Lal AM .Cumulative effect of systemic inflammation and oxidative stress in 40 known cases of active rheumatoid arthritis. Int J Res Ortho. 2015; 1(1): 7-10.
7. Suneja S, Saxena R, Saxena R, Sharma D and Lal AM. Association between serum paraoxonase and plasma nitric oxide in pre-eclampsia. Int J Adv Med. 2014; 1(1):19-23.
8. Saxena R, Jaiswal G. Vitamin E, markers of oxidative stress and nitric oxide levels in senescence. J. Indian. Acad. Geriat. 2010; 6: 71-77.
9. Sinclair AJ. Free radical mechanisms and vascular complications of diabetes mellitus. Diabetes Rev. 1993; 2:7–10.
10. Saxena R, Agarwal A, Sharma D, Lall AM. Plasma total antioxidant activity and its relation with serum uric acid in type 2 diabetes: a clinical approach. Sch J App Med Sci. 2016; 4(12A): 4193-4196.
11. Saxena R, Jaiswal G. Lipid peroxidation mediated electrolyte imbalance in patients with different stages of Essential Hypertension. S Asian J Prev Cardiology. 2006; 10(3): 143-147.
12. ADA Standards in Medical care in Diabetes-2013. Diabetes Care. 2013; 36: S66 – S74.
13. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 1995; 41:892-6.
14. Sinnhuber RO, Yu TC, Yu TC. Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative rancidity. Food Res 1958; 23: 626-630.
15. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003; 17 (1), 24–38.
16. Saxena R, Lal A M. Effect of Aging on antioxidant enzyme status and lipid peroxidation. J. Indian Acad Geriat. 2006; 2(2): 53-56.
17. Bhattacharya I, Saxena R, Gupta V. Efficacy of vitamin E in knee osteoarthritis management of North Indian Geriatric population. Therap Adv Musculo Dis. 2012; 4(1):11-19.
18. Shrestha S, Saxena R, Srivastava S, Thakur RK. Evaluation of cardio metabolic profile, endothelial dysfunction and oxidative stress in Metabolic Syndrome: A comparative perspective. Medical Science. 2016; 4(3): 334-340.
19. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-Reactive Protein, Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. *JAMA.* 2001;286(3):327-334.
20. Nandkeoliar MK, Saxena R, Lall AM Association of systemic inflammation and oxidative stress in adolescent diabetes mellitus patients: A clinical approach. SJAMS. 2017; 5(3A): 723-726.
21. Siddiqui M H, Saxena R, Verma S, Sharma GD. 25(OH) vitamin D level in Type 2 Diabetics and Non Diabetics: A comparative study. Int J Pharma and Clin Res. 2016; 8(4): 284-288.
22. Nandkeoliar MK, Goyal R, Saxena R. Type 2 diabetes in children and adolescents- A Study of C-peptide and insulin levels. SJAMS. 2017; 5(3E): 1038-1042.
23. Prasad RS, Nandkeoliar MK, Rai G, Srivastava S, Saxena R. Assessment of insulin resistance in diabetic hypothyroidism patients – A clinical insight. SJAMS. 2017; 5(2C): 531-535.
24. Saxena R, Suneja S, Saxena R, Sharma D, Lal AM. Systemic inflammation, oxidative stress and apolipoprotein B/A1 ratio in Active Psoriasis: bridging an apparent paradox. Int J Res Dermato. 2015; 1(1): 10-13.