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Study of serum malondialdehyde and vitamin c status in type 2 diabetes mellitus

K. K. Kshitiz[1]*, Sanjay Kumar Varun[2], Amar Ranjan[3] and J.R. Kesari[4]

[1]Shridhar Sharma Centre of investigation medicine, Central Institute of Psychiatry, Kanke, Ranchi-834006.
[2]Department of Medicine, Jeevan Jyoti Hospital, Forbesganj, Araria, Bihar -854318
[3]Laboratory Oncology Unit, BRA Institute-Rotary Cancer Hospital, All India Institute of Medical Science, New- Delhi -110029
[4]Department of Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna, Bihar 800014
Corresponding author

KEYWORDS

Diabetes mellitus , Oxidative stress, Malondialdehyde, vitamin C

A B S T R A C T

Diabetes mellitus (DM) is fast gaining the status of a potential epidemic in India and it is associated with high incidence of morbidity and mortality. The pathogenesis of diabetes complications remains illusive, however oxidative stress seems to be most favorable linkage amongst factors suggested. The present study was carried out to evaluate oxidative stress (MDA, VIT C) and to correlate these parameters with the disease process along with its complications. A total number of 80 subjects comprising of 30 healthy controls and 50 cases of diabetes mellitus studied. Out of 50 diabetes cases, 30 were without any complications and 20 were with complications like atherosclerosis, retinopathy and nephropathy etc. In all the subjects, serum levels of malondialdehyde (MDA) and serum vitamin C were estimated. Serum MDA was significantly increased in complicated diabetes in comparison to uncomplicated type 2 DM and control. The antioxidant serum vitamin C was significantly decreased in complicated diabetes when compared to non complicated diabetes and controls. MDA and vitamin c levels correlated with plasma fasting glucose and 2 hrs post prandial plasma glucose and also with lipid profile explaining the contributory role of hyperglycemia and dyslipidemia for the disease process. MDA and serum vitamin C level showed a negative correlation. The presence of increased systemic oxidative stress in diabetes seems to be associated with severity of complication.

Introduction

Increased free radical production and attenuation of antioxidant system is currently receiving the highest attention when discussing pathogenesis of diabetic mellitus and its complications. Hyperglycaemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in diabetes mellitus (Ramachandran *et al.,* 2010). Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production. Weak defense system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress (Halliwell *et al.,* 2007).

Malondialdehyde is a organic compound with the formula $CH_2(CHO)_2$. This reactive species occurs naturally and is a marker for oxidative stress. Reactive oxygen species degrade polyunsaturated lipids present on cell membrane forming malondialdehyde. This aldehyde product is used as a biomarker to measure the level of oxidative stress in an organism (Wikipedia, 2015).

Antioxidants depletion or deficiency may contribute to oxidative stress. Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of oxidative stress related diseases.

Vitamin C is a water soluble free radical scavenger, can directly scavenge O_2 and OH- radicals and help to neutralize physiological oxidant burden created by both exogenous and endogenous sources (Rai *et al.,* 2006).

Vitamin C is an important antioxidant in human, capable of scavenging oxygen-derived free radicals. Several studies showed decreased basal vitamin C level in diabetic patients (Cunningham *et al.,* 1991) and also it is suggested that oxidative stress is increased in diabetes (Ting *et al.,* 1996).

The present study was conducted with an objective to evaluate the oxidative status and serum vitamin antioxidant levels in diabetics & to correlate them with the disease process and its complications.

Materials and Method

The present study was conducted on 80 subjects of age groups 40–70 years comprising of 30 healthy age and sex matched healthy control and 50 cases of diabetes mellitus. Out of 50 diabetes cases, 30 were without complications and 20 were with complications like nephropathy, cardiovascular diseases, retinopathy etc. Controls and cases were selected from a tertiary care hospital in Bihar after obtaining informed consent and this study was approved by the ethical committee.

Type 2 DM patients were diagnosed and selected on the basis of their history, physical examination and laboratory investigations as per WHO criteria (WHO, 2006).

Patients with diseases like jaundice, renal disease, hypertension, thyroid, acute metabolic complications, cardiovascular accidents, acute infections were discarded. Smokers and alcoholics were also excluded from the study.

The following tests were done in each sample during the study: (1) Blood urea (2) serum creatinine (3) Serum total Cholesterol (4) Serum HDL & LDL Cholesterol (5) Serum Triglyceride (6) Plasma glucose - Fasting & 2½ hrs after 75 gm glucose (7) MDA (8) seum vitamin C (9) Complete blood count.

Estimation of serum malondialdehyde (Satoh, 1978)

Serum malondialdehyde estimated by Kei Satoh Method. It is based on the principle of auto-oxidation of unsaturated fatty acids, involves the formation of semistable peroxides, which then undergo a series of reactions to form malondialdehyde (MDA). MDA reacts with thiobarbituric acid (TBA) to form pink colored chromogen. The resulting chromogen is extracted with 4.0ml of n-butyl alcohol and the absorbance of which is measured at 530 nm.

Estimation of serum vitamin C (Gunter *et al.,* 1985)

Serum vitamin C was estimated by 2, 4 – dinitrophenyl hydrazine method. This method is based on the principle that ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2,4-dinitrophenyl hydrazine (DNPH) to form the derivative bis-2,4-dinitrophenyl hydrazone. This compound in strong sulfuric acid, undergoes rearrangement to form a colored product which is measured at 520nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogen.

Results and Discussion

The rise in glucose level with dyslipidemia of the study group were contributed by the impaired insulin secretion (Table 2). However the mechanism of insulin resistance is not very clear. Hyperglycemia work through different mechanisms such as activation of protein kinase C, polyol and hexosamine pathways and advanced glycation end products production. All of these pathways, in association to hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote reactive oxygen species (ROS) accumulation that, in turn, promote cellular damage and contribute to the diabetic complications development and progression (Fiorentino *et al.,* 2013).

MDA level were significantly raised in both the group of uncomplicated and complicated type of type 2 DM cases. This explains the generation of free radicals during disease process. Vitamin C level also significantly increased in both the groups of uncomplicated and complicated DM type 2 patients. MDA and glucose level showed a significant positive correlation thus showing contributory role of hyperglycemia towards generation of oxidative stress. This generation of ROS has been potentiated by the marked dyslipidemia and increased lipid peroxidation of the disease process (West, 2000).

The negative correlation between MDA and vitamin C reveals about negative impact of ROS on the bioavilability on vitamin C. Oxidative stress may be a common pathway linking diverse mechanism for the pathogenesis of diseases as well as complications in DM. ROS can directly damage lipids, proteins or DNA and modulate intracellular signaling pathways, such as mitogen activated protein kinases and redox sensitive transcription factors causing changes in protein expression and, therefore, irreversible oxidative modifications (Fiorentino *et al.,* 2013).

Fig.1 Correlation between plasma MDA and vitamin C in study group

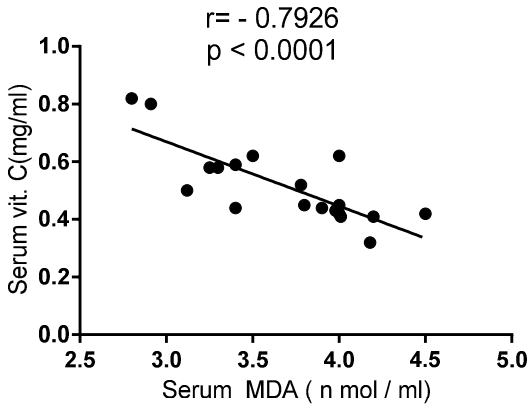

Table.1 Clinical data of control and diabetics with and without complications (Mean±SD)

Parameters	Control	Diabetes without complications	Diabetes with complications
Age (yrs)	56.84 ±7.45	55.30±6.49	63.04±4.83
Weight (kg)	60.21±4.42	61.80±6.99	60.24±4.71
Body mass Index (kg/m2)	23.31±2.82	24.47±2.81	24.40±2.57
Duration of DM (yrs)	Nil	6.37±1.78	11.44±1.77

Table.2 Biochemical parameters in the study group (Mean+SD)

Parameters	Control	Diabetes without complications	Diabetes with complications
FPG (mg/dl	82.67±10.11	146±30.26 **	240±34.34**
2 hr PPPG (mg/dl)	110.56±10.87	241.34±67.10**	300.34±35.32**
Total cholesterol (mg/dl)	160.49±10.32	200.78±18.14**	220.78±24**
TAG (mg/dl)	100.78±12.34	128.89±9.67**	156.45±9.90**
HDLc (mg/dl)	45.89±8.40	35.49±16.64*	30.29±13.27*
LDLc (mg/dl)	95.78±6.85	139.53±12.34**	157.47±37**

*P<0.05 **P<0.001

Table.3 Plasma MDA and Vitamin-C in study groups

Parameters	Control	Diabetes without complications	Diabetes with complications
Plasma MDA(n mol/ml)	2.02±01.23	3.98±12.00*	5.56±10.26**
Plasma Vit. C (mg/ml)	1.12±0.08	0.70±0.04**	0.43±0.003**

*P<0.05 **P<0.001

Table.4 Correlation of plasma MDA with biochemical parameters in study groups

Parameters	Plasma MDA (r- value)	Serum vit. C (r – value)
FPG (mg/dl)	0.74**	- 0.68**
2 hr PPPG (mg/dl)	0.68**	- 0.65**
Total cholesterol (mg/dl)	0.35**	- 0.38**
TAG (mg/dl)	0.20*	- 0.21*
HDLc (mg/dl)	-0.50**	0.48**
LDLc (mg/dl)	0.41**	- 0.43**

Oxidative stress induces several phenotypic alterations also in vascular smooth-muscle cell (VSMC). ROS is one of the factors that can promote both VSMC proliferation/migration in atherosclerotic lesions and VSMC apoptosis, which is potentially involved in atherosclerotic plaque instability and rupture. Currently, there are contrasting clinical evidences on the benefits of antioxidant therapies in the prevention/treatment of diabetic cardiovascular complications. Appropriate glycemic control, in which both hypoglycemic and hyperglycemic episodes are reduced, in association to the treatment of dyslipidemia, hypertension, kidney dysfunction and obesity, conditions which are also associated to ROS overproduction, can counteract oxidative stress and, therefore, both microvascular and macrovascular complications of diabetes mellitus (Fiorentino *et al.*, 2013).

Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (West, 2000). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus.

The significant hyperglycemia and dyslipidemia along with raised plasma MDA level and lowered vitamin C level in both diabetes groups explain their contribution towards free radical production as well as generation of oxidative stress. The extent of ROS formation might be determinant of bioavailability of vitamin C.

Though several mechanism represent a common pathway in the disease process of diabetes mellitus and its complications, yet future result may be carried out to get in-depth knowledge pertaining to the fundamental informations.

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