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Original Research Article

Oxidative Stress and Antioxidant Defences in Type- I Diabetic Cases of Southern Rajasthan

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Abstract

Oxidative stress is the outcome of an imbalance between the production and neutralization of reactive oxygen and nitrogen species (RONS) such that the antioxidant capacity of cell is overwhelmed. The present review briefly summarized the underlying role of overwhelming levels of RONS in the pathophysiology of diabetes mellitus (DM). The primary causative factor of oxidative stress in DM is hyperglycemia, which operates via several mechanisms. However, the individual contribution of other intermediary factors to hyperoxidative stress remains undefined, in terms of the dose response relationship between hyperglycemia and overall oxidative stress in DM. Intuitively, the inhibition and/or scavenging of intracellular free radical formation provide a therapeutic strategy to prevent oxidative stress and ensuing pathologic conditions.

Keywords: Diabetes Mellitus Type-I, Oxidative stress, Lipid peroxide, Superoxie dismutase, Catalase, Reduced Glutathione.

INTRODUCTION

Diabetes mellitus is considered to be one of the most common chronic diseases worldwide, and recognized as one of the leading causes of morbidity and mortality [1]. It has been reported that the prevalence of diabetes mellitus will increase from 6% to over 10% in the next decade [2].

According to the World Health Organization in 2000, a total of 171 million people in all age groups worldwide (2.8% of the global population) have been affected by diabetes mellitus, and the number of persons is expected to increase to 366 million (4.4% of the global population) by 2030 [3].

Type 1 diabetes mellitus accounts for 5-10% of all diagnosed cases of diabetes mellitus, and exhibits hyperglycemia as its hallmark. It is caused by pancreatic β-islet cell failure with resulting insulin deficiency mortality and risk factors may be autoimmune, genetic, or environmental [4]. Type 1 diabetes mellitus is an autoimmune disorder involving immune-mediated recognition of islet β-cells by auto-reactive T cells. This subsequently leads to the liberation of pro-inflammatory cytokines and reactive oxygen species. There is destruction of pancreatic β-cells in the islets of Langerhans and loss of insulin secretion [5]. The Jun kinase pathway is also activated by the pro-inflammatory cytokines, and there is evidence that oxidative stress is involved in β-cell destruction [6]. The loss of β-cell mass consequential to the activation of pro-apoptotic signaling events is increasingly recognized as a causal and committed stage in the development of type 1 diabetes mellitus [7].

Moreover, pancreatic β-cells are sensitive to cytotoxic damage caused by reactive oxygen species as gene expression and activity of antioxidant enzymes such as glutathione peroxidise activity is decreased in these cells [8].

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a central role in the onset of diabetes mellitus as well as in the development of vascular and neurologic complications of the disease [9]. Studies advancing the role of oxidative stress in vascular endothelial cells proposed that oxidative stress mediate the diversion of glycolytic intermediates into pathological pathways [10, 11]. Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals and a deficiency in antioxidant defense mechanisms. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the

subsequent oxidative degradation of glycated proteins [12]. 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 [13].

MATERIAL AND METHODS

The present case control study was carried out in the Department of Biochemistry, RNT Medical College, Udaipur (Rajasthan) Patients and controls were selected from Endocrinology and Medicine wards, as well as outdoor patients of MB Govt. Hospital, RNT Medical College, Udaipur. Case history in detail was recorded on a proforma. Cases as well as controls were analyzed for oxidative stress, antioxidant stress, antioxidant activity, ascorbic acid, α- Tocopherol, β-Carotene, Retinol, SOD, Catalase, GSH, Uric acid, fasting blood sugar (FBS) and HbA1c (Glycosylated hemoglobin).

Control Group: (n=130)

Age matched healthy controls, without Type- I Diabetes Mellitus were included. The selected control subjects were healthy family members, staff members and attendants of patients visiting M.B. Govt. Hospital and RNT Medical College, Udaipur.

Case group: (n=180)

- Known cases of Type- I Diabetes Mellitus were included in this category.
- Written consent was obtained from all the participants on whom the study was conducted.

Exclusion Criteria

- Non willingness for participation.
- Hemolysed samples.

Inclusion Criteria

- Known cases of Type- I Diabetes Mellitus.
- Smokers, alcoholics and tobacco users.
- Diabetic cases with acute and chronic complications.

Collection of Blood Samples of Patients and Control

10 ml of blood from the control, as well as the study group was drawn from antecubital vein and was collected in plain, EDTA and fluoride vials (According to the order of draw). Serum was separated by centrifugation of blood samples. TBARS, total antioxidant activity, ascorbic acid, α- tocopherol, β-carotene, Retinol, Uric acid. Fasting blood sugar (FBS) was estimated from the fluoride vial. The EDTA blood was centrifuged at 2500 rpm for 20 minutes. Compact RBC were washed twice with equal volume of normal saline and centrifuged again at 2500 rpm for 15 minutes. Supernatant layer was removed and hemolysate was prepared by adding double amount of distilled water to compact RBC. SOD, catalase and Glutathione were estimated in hemolysate.

Analysis of blood for various analytical parameters:

- Thiobarbituric Acid Reactive Substance (TBARS) [14].
- Total Antioxidant Activity [15] (Frap Assay).
- Retinol and β- Carotene [16].
- Ascorbic Acid [16].
- α Tocopherol [17].
- Superoxide Dismutase [18] (Ransod kit method).
- Reduced Glutathione [19].
- Catalase [20].
- Glycosylated Hemoglobin (HbA1C).

A hemolysed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation- exchange resin. During this time non glycosylated hemoglobin binds to the resin. After the mixing period, a filter is used to separate the supernatant containing the glycohemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbances gives the percent hemoglobin.

Reference Range:- (As per the recommendation endorsed by ADA)
4.6% – 6.0% (Normal)
6.0% - 7.0% (Good control)
7.0% - 9.0% (Fair control)
9.0% - 14% (Poor control)

Blood Glucose [21]. Estimated by enzymatic glucose kit (GOD/POD method).

RESULT AND DISCUSSION

Table-1: Oxidative stress, Antioxidant and Glycemic status in cases and control

Parameters	Cases (n=61)		Controls (n=130)		t-test / Value	P-value
	Mean	±SD	Mean	±SD		
MDA (nmol/ ml) (TBARS)	4.76	1.34	1.65	0.49	306.3	0.001
Total Antioxidants (µmol/L)	720.84	181.14	1305.54	302.87	0.58	NS
Nutrient Antioxidants						
Ascorbic Acid (mg/ dl)	0.52	0.29	1.01	0.15	882.27	0.001
α- Tocopherol (mg / dl)	1.10	0.50	1.37	0.39	122.67	0.001

β - carotene (mg / dl)	57.72	10.36	158.83	17.12	37.68	0.001
Retinol (mg / dl)	26.30	7.98	56.15	10.33	25.65	0.001
Endogenous Antioxidants						
SOD (U/ml)	223.21	97.45	329.64	145.78	0.84	NS
catalase (U/ mg Protein)	2.54	1.59	1.9	1.28	41.31	0.001
GSH (mg/ dl)	25.90	6.92	16.98	5.23	26.52	0.001
Uric Acid (mg / dl)	5.40	2.20	3.27	0.66	155.42	0.001
Glycemic Index						
FBS (mg/ dl)	340.57	119.13	76.54	7.64	3.57	NS
HbA1c (%)	11.33	2.05	4.76	0.12	282.86	0.001

P Value < 0.05 was considered as significant.

When type- I diabetic cases were compared with control, they reflected high significance for TBARS ($p < 0.001$), lower ascorbic acid, α- tocopherol, β- carotene and Vitamin A ($p < 0.001$), higher levels of catalase, GSH, Uric Acid and HbA1c ($p < 0.001$).

In the patients with type- I diabetes, oxidative stress seems to be caused by both increased production of plasma ROS and reduction of antioxidant defences as reported by Giugliano *et al.*, [22], Ramakrishna and Jailkhani [23].

Cunningham *et al.*, [24] reported 33% reduction of vitamin C in type 1 patients, even though their intake of dietary vitamin C was adequate. Will *et al*., [25] has also reported a significant negative correlation coefficient between blood glucose and vitamin C.

Martin- Gallan *et al.*, [26] reported low levels of vitamin E to be associated with increased evidence of type 1 diabetes and stated that enhanced lipid peroxidation increased the need for lipid soluble antioxidants as α- tocopherol, vitamin A and β-carotene which is reflected by our findings too.

No difference in SOD activity between diabetic groups were found, which is supported by findings of Ruiz *et al*., [27], while Diamon *et al.*, [28] has reported a reduction of SOD activity in type 1 cases.

An increase in catalase and GSH in type 1 diabetic cases was observed when compared to control, which is supported by findings of Fridovich [29] stating that an increased activity of catalase and GSH scavenges H_2O_2 thereby conserving SOD. A decrease in catalase activity has been reported by Ramakrishna and Jailkhani [23]. An increase in GP_X activity is reported by Ndahimana *et al.*, [30] and Djordjevic *et al.*, [31] while Dominguez *et al.*, [32] reports a decrease in GPx activity related to low GSH content of the diabetic patients.

Hyperuricemia indicates body's defence against deleterious effects of free radicals as reported by Baynes [33] and reflected by our observations.

The poor glycemic control of type 1 cases Vs control group (11.33 ± 2.05 Vs 4.76 ± 0.12) is supported by the study of Ramakrishna and Jialkhani [23] (11.5 ± 3.2 Vs 6.12 ± 0.3) while Szaleczky *et al.*, [34] reports a GHb % of 6.06 ± 0.48 in well controlled type 1 diabetic cases, reflecting that blood glucose when strictly controlled by intensive insulin therapy is not accompanied by changes in pro- oxidant-antioxidant balance.

Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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