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Abstract

The study was designed to find out the relation between lipid peroxidation, lipoprotein levels to severity and complication of diabetes mellitus. Degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) along with antioxidants, lipid profile and blood glucose in diabetes mellitus. Total 100 human subjects, out of which 50 healthy individuals of age group (50-70years) were taken as control & 50 elderly diabetic subjects of age group (50-70years) were taken as cases. There was significant increase in the lipid profile except HDL cholesterol, which is decreased, Also significant decrease in antioxidant enzymes such as Reduced glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase except catalase was seen as compared to the control subjects. Other findings observed was that the level of lipid peroxide (MDA) increased as per the increase in concentration of blood glucose. Our findings indicate that the increase in the lipid peroxidation product MDA and decline in glutathione-dependent antioxidant defences may appear early in non insulin dependent type 2diabetes mellitus patients.

Introduction

Diabetes mellitus is characterised by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation [1]. Diabetes mellitus is considered to be one of a rank of free radical diseases which propagates complications with increased free radical formation [2, 3]. Oxidative stress is increased in diabetes mellitus owing to the increase in the production of oxygen free radicals and a deficiency in antioxidant defence mechanisms [4, 5]. Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of diabetes mellitus [6]. Hyperlipidaemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes mellitus [7, 8].

The study aims to evaluate serum lipid peroxidation marker, malondialdehyde (an oxidant), Reduced glutathione (an antioxidant), glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase, plasma glucose and lipid profile in non-insulin-dependent diabetic patients.

Methods

The study was conducted on 100 human subjects, out of which 50 elderly diabetic subjects (Females-23 & males-27) of age group (50-70years) were taken as cases, attending the OPD and indoor of Department of Medicine, GR Medical College, Gwalior. These Type 2 diabetes mellitus patients were diagnosed on the basis of history, physical examination and biochemical investigations and according to the biochemical criteria laid down by the National Diabetes Data Group (NDDG) of the National Institute of health in 1980/WHO criteria [9]. The diagnosis of NIDDM was based on the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2000) [10]. Also, the subjects giving history of smoking/ alcoholism/ any chronic disease were excluded from the study. The NIDDM patients were not taking any medicines other than oral anti-diabetic pills for the past four years.

50 age & sex matched apparently healthy individuals with normal plasma glucose and with no symptoms suggestive of diabetes mellitus were taken as controls (Control group).

The written consent of all subjects was taken before starting the study. All ethical measures were taken prior and during the study.

Collection of blood samples

Under all aseptic conditions, 6ml of venous blood sample was collected from each subject. This sample was distributed in following vials:

- 0.4ml in heparinized vial for estimation of reduced glutathione (GSH)
- 1.6ml in citrated vial for estimation of catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) & malondialdehyde (MDA).
- 4ml in plain vial for estimation of superoxide dismutase (SOD) & lipid profile.

Both cases and controls were subjected to estimation

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of biochemical parameters like Fasting plasma Glucose (FPG), Post prandial plasma Glucose (PPPG)[11], Total cholesterol (TC), Triglyceride (Tg), HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol [12,13].

The lipid peroxidation product MDA formed a characteristic chromogenic adduct with TBA which was measured spectrophotometrically after butanol extraction [14] other antioxidant enzymes and reduced glutathione were also analyzed [15,16,17,18,19].

- The results were analyzed using students 't' test.

Results

In our study we observed a significant decrease ($p < 0.001$) in high density lipoprotein cholesterol (HDL-C), reduced Glutathione (GSH), Glutathione peroxidase (GPx), Glutathione reductase (GR) and Superoxide dismutase (SOD) as compared to the control subjects. However the fasting blood sugar (FBS), post prandial blood sugar (PPBS), total cholesterol (TC), Triglyceride (Tg), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and malondialdehyde (MDA) levels were found significantly increased ($p < 0.001$) as compared to the control subjects.

Discussion

Type 2 diabetes mellitus is associated with multiple metabolic derangements that result in the excessive production of reactive oxygen species and oxidative stress. Oxidative stress and resultant tissue damage are hallmarks of chronic disease and cell death. There is increasing evidence that, in certain pathologic states, the increased production and/or ineffective scavenging of such reactive oxygen species may play a crucial role in determining tissue injury. Endothelial dysfunction is considered an intrinsic element in the pathogenesis of diabetic angiopathies. A variety of potential mechanisms for the initiation of endothelial dysfunction in type 2 diabetes have been described including the effects of hyperglycemia, advanced glycation end products (AGE) and dyslipidaemia [20,21]. In addition, hyperglycemia has been shown to induce free radical release and reduce anti-oxidant defenses [22], both of which are associated with endothelial dysfunction.

Abnormal lipid metabolism often presents in patients with NIDDM [23]. Hypertriglyceridaemia usually accompanies decreased HDL-C, which is also a prominent feature of plasma lipid abnormalities seen in diabetic subjects [24, 25, 26]. The low level of HDL-C, which exerts anti-atherogenic and antioxidative effects when present in sufficient amounts, is a key feature of NIDDM (also known as type 2 diabetes mellitus). The reduced HDL-C levels are often accompanied by elevations in plasma TG levels, [27] a process mediated by cholesterol ester transfer protein (CETP), [28]. Resistance to insulin likely underlies the changes that occur in lipid parameters of NIDDM, and usually it is associated with higher concentrations of TC and TG, and lower concentrations of HDL-C [29]. The mechanism responsible for hypertriglyceridaemia may be an increased hepatic secretion of VLDL and a delayed clearance of TG-rich lipoproteins, which might mainly be due to increased levels of substrates for TG production, free fatty acids, and glucose. The latter could be secondary to decreased activity of lipoprotein lipase (LPL), a key enzyme for lipoprotein-TG [30]

Oxidative stress may be associated with the pathogenesis of NIDDM [31]. Increase lipid peroxide may be due to the increased glycation of proteins in diabetes mellitus. The glycated protein might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which may be also thought to play a role in increased lipid peroxidation in diabetes mellitus. In the present study, the increased levels of MDA clearly show that diabetic patients, were exposed to an increased oxidative stress via lipid peroxidation, [6, 7, 8, 32, 33]

The reports about the status of antioxidants and antioxidant enzymes in diabetic patients are very contradictory, both increase and decrease of antioxidant activity have been reported [33,34] The reports about the SOD activity in diabetes mellitus are controversial, with some authors reporting no change in SOD activity [35,36] while others reported increased [37,38] and decreased SOD activity [39]. In the present study, decreased SOD activity in diabetic patients was reported. Products of membrane lipid peroxidation and other oxidants like H_2O_2 may react with superoxide dismutase resulting in oxidative modification thereby causing loss of enzyme activity [39]. Also, diabetic hyperglycemia leads to glycation and inactivation of superoxide dismutase thus attributing to its decrease.

GSH is a ubiquitous tripeptide that presents in red cells and participates in GPx reaction. When H_2O_2 is detoxified by GPx, the GSH is simultaneously converted to the oxidized form (GSSG). In the present study, the authors found that GSH levels in type 2 DM

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patients were significantly lower than that in their same age-matched control subjects. These results are in good agreement with other studies [40-42]. As already mentioned GSH serves as an essential cofactor for the enzyme GPx and formed oxidized glutathione (GSSG) during the enzyme processes. Thus, increasing in GPx activities imply higher consumption of GSH. Other mechanisms that may explain the GSH reduction in diabetes are that the GSH is regenerated by the enzyme glutathione reductase, using reducing equivalents from NADPH. The NADPH is generated in red blood cells through the pentose phosphate pathway, which is stimulated by insulin [43]. NADPH production in DM may be sluggish, probably resulting in lowered glutathione reductase activity and reduced GSH recycle. The enzyme glutathione reductase was found to be decreased in type 2 diabetic patients as reported by Dincer et al [44]. Moreover in diabetes mellitus, the increased sorbitol synthesis via the polyol pathway occurred. This elevated sorbitol production caused the NADPH depletion that was required by aldose reductase enzyme in this pathway. This deficiency will also limit the GSH recycle [44].

There is still a controversial view regarding alteration in the activity of catalases in diabetic subjects. According to some scientist increase in level of catalase is compensatory for the removal of free radical, in the absence of glutathione peroxidase in type II diabetes mellitus [45]. We found in our study increased catalases level which is in agreement of other reports.

Conclusion(s)

In conclusion, the present study supported the hypothesis that hyperglycemia activates cellular and tissue damage by oxidative stress. However, there were compensatory mechanisms for defense against the ROS. Normalization of oxidative stress was not achieved in the diabetic patients.The results suggest that the increase in lipid peroxidation and the decline in antioxidant defences may appear early in NIDDM patients, before the development of secondary complications, and might play an important role in the initiation and progression of diabetic complications. Our results also suggest that there seems to be an imbalance between plasma oxidant and antioxidant systems in patients with NIDDM. Thus, any means that can reduce the oxidative damage may be beneficial for treatment of diabetic patients in the future.

Abbreviation(s)

FPG, Fasting plasma glucose; PPPG, Postprandial plasma glucose; TC, Total cholesterol; TG, Triglyceride; HDL-C, High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; VLDL-C, Very low density lipoprotein cholesterol; CAT, Catalase; MDA, Malondialdehyde; GSH, Reduced glutathione; GR, Glutathione reductase; GPx, Glutathione peroxidase; SOD, Superoxide dismutase

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Authors Contribution(s)

All authors contributed to the skillful editing of the manuscript and interpretation of results.

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Illustrations

Illustration 1

Table 1

Showing the status of FBS, PPBS and lipid profile in Type 2 diabetes Mellitus

GROUPS N =50	FBS mmol/l	PPBS mmol/l	TC mmol/l	TG mmol/l	HDL-C mmol/l	LDL-C mmol/l	VLDL-C mmol/l
CONTROL GROUP	4.62±0.75	6.31±0.59	5.39±0.52	1.39±0.25	1.37±0.26	3.42±0.53	0.64±0.12
TYPE 2 D M	9.91±1.56	15.65±2.7	6.42±0.9	2.33±0.67	0.95±0.26	4.31±0.97	1.07±0.3

($p<0.001$) Highly significant

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Illustration 2

Table 2

Showing the status of antioxidant enzymes (AOEs) and malondialdehyde (MDA) in Type 2 Diabetes Mellitus.

GROUPS N=50	GSH Mg%	GPx U/gmHb	GR U/gP	CAT U/gmP/ml	SOD UmgP/ml	MDA nmol/ml
CONTROL GROUP	14.53 ±2.30	8.82 ±1.16	16.84 ±0.56	6.00 ±0.66	6.65 ±1.18	4.79 ±0.72
TYPE 2D M	10.14 ±1.27	8.36 ±1.18	15.09 ±0.81	8.16 ±1.90	3.85 ±1.07	5.64 ±0.42

($p<0.001$) Highly significant

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