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Original Research Article

Estimation of plasma glutathione levels in NIDDM patients with and without cardiovascular disease

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Abstract

Diabetes mellitus is a group of metabolic derangements characterised by hyperglycemia resulting from defective insulin secretion or action or both. Persistent hyperglycemia generates reactive oxygen species causing oxidative damage to cells and various biomolecules. In this study the levels of reduced glutathione antioxidant were estimated in NIDDM patients with cardiovascular disease (n=50) by HPLC method and compared to healthy controls (n=50). The results indicated that plasma glutathione levels are significantly decreased showing that antioxidant defence is lowered in diabetic patients with cardiovascular complications.

Keywords: NIDDM, cardiovascular diseases.

1.Introduction

Diabetes mainly Non insulin dependent diabetes mellitus (NIDDM) affects nearly 150 million people worldwide and 30% rise is predicted by 2025 due to increased rate of obesity and the ageing population living in industrial countries [1,2]. Diabetes Mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several types of Diabetes mellitus exist caused by a complex interaction of genetic, environmental factors and lifestyle choices. The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual and on the health care system [3].

Glutathione (GSH), a tripeptide consisting of L-γ-glutamyl-L-cysteinylglycine, is the most abundant intracellular thiol compound present in virtually all mammalian cells. Functions of GSH in reductive processes are essential for the synthesis and degradation of proteins. It also affords protection to cells against Reactive oxygen species (ROS) and free radicals generated by metabolic processes. GSH participates in reactions that destroy H_2O_2, organic peroxides, free radicals and certain foreign compounds. It protects thiol groups in proteins from oxidation, functions as an intracellular redox buffer and serves as a reservoir of cysteine[4].

In physiological conditions, the metabolism of oxygen generates highly reactive compounds called reactive oxygen species (ROS) or free radicals including superoxide, hydroxyl radicals, nitric oxide and hydrogen peroxide. However uncontrolled production of ROS is deleterious. Sustained hyperglycemia is linked to increased oxidative stress and an increased risk of diabetic micro and macrovascular complications[5]. Oxidative stress mediates insulin resistance and its progression to glucose intolerance and diabetes mellitus, subsequently favoring development of secondary complications like atherosclerosis and cardiovascular disorders [6]. Due to oxidative stress the metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of blood vessels and myocardium [7]. The antioxidant defense system is divided into two groups: endogenous enzymes like superoxide dismutase, catalase and glutathione peroxidases and exogenous free radical scavengers.

Three mechanisms have been proposed to explain the reduced levels of GSH in type 2 DM- a) glucose affects GSH synthesis by decreasing the

activity of γglutamylcysteine ligase[8], b)cofactor for GSH regeneration from GSSG, NADPH gets consumed when excess glucose is converted to sorbitol via polyol pathway [9]**,** and c)AGE induced formation of superoxide and hydrogen peroxide which deplete GSH[10].

2. Materials and Methods

This one year cross sectional study was conducted in the Department of Biochemistry, Goa Medical College & Hospital, Bambolim, Goa after ethical clearance was obtained from The Hospital Ethical Committee. The study population comprised of 120 patients of NIDDM, in the age group of 40 to 70 years, attending the medicine and cardiac out-patient departments of Goa Medical College & Hospital.

They were grouped into two groups. Group 1 (n=60 men=38, women=22) patients of NIDDM without complications, group 2 (n=60: men=30, women=30) patients of NIDDM with cardiovascular complications. Sixty age and sex matched healthy controls were included in the study. Patients with history of smoking, chronic alcoholism, rheumatoid arthritis, hepatic, renal, neurological, gastrointestinal disorders, tuberculosis and neoplasia were excluded from the study. Written informed consent was obtained from the participants of this study.

Under aseptic precautions, fasting plasma samples from the study groups were obtained from whole blood collected into EDTA Vacutainer tubes and stored for a maximum of 3 months at -70 °C before assay.

HPLC was carried out by an isocratic system with fluorescence detection (SFM 25 spectrofluorometer), autosampler (SA 360), and HPLC pump supplied by SHIMADZU. The method has been adapted from that of Ubbink *et al*[11] on the basis of the chemical description provided by Araki and Sako [12-14]. Homocysteine calibrators (DL form) were prepared in borate buffer (0.1 mol/L, pH 9.5, with and without 2 mmol/L EDTA). Internal standard was added to the plasma or calibrator to achieve a final concentration of 10.0 µmol/L (30 µL of 50.0 µmol/L cysteamine + 120-µL sample).The calibration slopes were calculated with homocysteine/ cysteamine peak area ratios. The data obtained was tabulated and statistical analysis was done using student unpaired't' test and comparison between the groups was done by analysis of variance.

Ethical approval: obtained from hospital ethical committee.

3. Results

The Coefficient of variation (CV) with internal standard for low control plasma (6.2 µmol/L) was 3.2% (n = 10), and for high control plasma (22.8 µmol/L) was 2.8% (n = 10).In group I, majority (50%) of the patients were in the age group of 51 to 60 yrs and in group II 62 % of the patients were in the 50 to 60 yrs age group. No significant difference was observed in distribution of cases among males and females.

Compared with non diabetic subjects, subjects with diabetes had lower plasma reduced glutathione concentrations (3.42+0.18 vs. 2.62 $\pm$ 0.38µmol/L). NIDDM patients with CVD also showed low values of glutathione (2.85+0.33µmol/L). The p values of both group I and group II are <0.003 which indicates that the result is significant.

Table I: Age incidence of the patients in the control and study groups

Age (yrs)	Controls		Group I		Group II	
	(n=60)	%	n=60)	%	(n=60)	%
41- 50	36	60	25	42	20	33
51- 60	22	47	30	50	37	62
>60	2	3	5	8	3	5

Table II: Values of plasma gluthione

Group	No. of Patients	Mean (µmol/L)	S.D.±	t Value	Remarks at p
Controls	60	3.42	0.18		
NIDDM Patients	60	2.62	0.38	13.27	<0.003
NIDDM+CVD events	60	2.85	0.33	15.07	<0.003

4. Discussions

The hypothesized physiologic processes by which hyperglycemia contributes to cardiovascular disease are probably gradual and cumulative, occurring during decades of exposure to chronically elevated blood glucose levels. The results of this study demonstrate that plasma concentrations of GSH are decreased in diabetic patients compared to healthy adults indicating an imbalance between oxidative stress and antioxidant defence system of the body.

Baynes in 1991, then Ramesh *et al* in 2012 [15,16] reported that lipid peroxidation in diabetes

induced secondary chronic complications like atherosclerosis and neural disorders. Several studies has pointed out that advanced oxidative protein products and oxidative stress markers increase in adult subjects with type 2 DM with and without micro/macrovascular complications [17,18].

GSH is present in all mammalian tissues at 1-10 mM concentrations (highest concentrations in liver) as the most abundant nonprotein thiol that defends against oxidative stress [19]. Decreased GSH levels may be one of the factors in oxidatine DNA in type II DM [20]. Significantly higher values of thiobarbituric acid-reactive substances in RBCs and decreased RBC antioxidant enzymes have been reported in diabetic condition [21,22]. In most tissues, H_2O_2 and organic hydroperoxides, decomposition is obtained by Glutathione peroxidase which are very specific for glutathione as the reducuing substrate [23].

Deficiency of S containing aminoacids[5], or protein content in the diets of healthy humans has shown to result in suppression of GSH turnover in vivo which indicates that GSH deficiency in DM is due to decreased availability of precursor aminoacids cysteine and glycine [24]. Mechanisms implicated in hyperglymia driven tissue damage include abnormal signaling through protein kinase C, elevated advanced glycation end products and aldose reductase pathways[9]. Since ROS stimulates these pathways, reducing levels of mitochondrial ROS and thereby oxidative stress, successfully prevents induction of these mechanisms [25].

A study shows that depletion of GSH in mice leads to increased susceptibility to infections, reduced production of IL-12p70 and poorer disease outcomes [26].

In the present study Glutathione was measured by HPLC method along with homocysteine, cysteine, cysteinylglycine and other thiols, which is simple and less time consuming. The lower detection limit by HPLC was found to be 50fmol of glutathione following derivatization by N-(1-pyrenyl) maleimide. Various new and other methods have been described for glutathione estimation [27].

5. Conclusions

Type II diabetes is an endocrine disorder affecting many organ systems. The resulting hyperglycemia causes generation of toxic free radicals which oxidize cellular membranes and cellular biomolecules. Free radicals are counteracted by the body's antioxidant defence systems like superoxide dismutase, catalases and glutathione. Glutathione (L-γ-glutamyl-L-cysteinylglycine) acts as a redox buffer. This study concludes that diabetes coexists with oxidative stress and antioxidant glutathione levels are diminished as measured by HPLC.

Conflict of interest: None declared

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