ORIGINAL RESEARCH PAPER

Clinical Biochemistry

ASSESSMENT OF REDOX BALANCE IN NEPHROPATHY ASSOCIATED WITH TYPE 2 DIABETES MELLITUS

KEY WORDS: Diabetic nephropathy, Glutathione, Superoxide dismutase, Catalase

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ABSTRACT

The study was assessing the role of oxidative stress in diabetic nephropathic group in comparison with diabetic group without any secondary complications. We observed that there was a significant imbalance in redox status in nephropathic diabetic group when compared to diabetic group without metabolic problems. This was evident from our result which showed that there was depletion in glutathione status in diabetic nephropathy along with reduction in activity of glutathione peroxidase as well as catalase. However, we also observed that the superoxide dismutase activity was elevated in diabetic nephropathic group in comparison with diabetic control. This could be attributed to increased generation of superoxide anion in nephropathy where SOD activity removes excess superoxide anion which is deleterious. The other finding was there was significant elevation of thiobarbituric acid reactive substances in diabetic nephropathy which was a compounding factor for severe oxidative stress in diabetic complications associated with nephropathy.

Introduction

Nephropathy associated with Type 2 diabetes mellitus (T2DM) is a significant cause of renal complications which ultimately resulted in fatal end stage renal disease (1). Diabetic nephropathy is manifested with microalbuminuria (2), a condition in which albumin is excereted in urine and loss of glomerular filtration rate associated with poor creatinine clearance. Diabetic nephropathy in common can affect haematological, immunological and metabolic functions. Because of the activation of inflammatory molecules associated with renal disease, there could be a great possibility of oxidative damage to cells in association with diabetic nephropathy (3 – 6). In the present study we focussed on assessing the antioxidant status in diabetic nephropathic cases in comparison with diabetic patients with no secondary complications and normal healthy age matched controls.

The major biochemical pathways linked with diabetic nephropathy are activation of renin – angiotensin system, hexosamine pathways, polyol advanced glycation end product pathway and activation of Protein kinase C (7 – 10). Accumulating evidence has demonstrated that overproduction of free radicals is responsible for altered metabolic pathways in the kidneys with disturbed hemodynamics. Renal oxidative stress is often a consequence of upregulation of pro-oxidant induced reative oxygen species production such as lipid peroxides, hydroxyl radicals and simultaneous depletion of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidise. Excessive Reactive oxygen species (ROS) formation causes significant tissue damage affecting renal functions. Hyperglycemia induced ROS production stimulates the recruitment of numerous inflammatory cells with concomitant increase in signalling pathways associated with NF-kB and TNF-alpha (11). In the biological system, the molecular oxygen undergoes a series of reductive biosynthetic steps forming several reactive oxygen intermediates which include superoxide, peroxyl and alkoxyl free radicals along with non radicals such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Under physiological conditions, both endogenous and exogenous antioxidants counteract on pro-oxidant molecules which is disturbed in pathological states such as diabetes mellitus according to severity spectrum (12- 14). For instance, superoxide free radical which is the primary reactive oxygen intermediate could be neutralised by superoxide dismutase (SOD) to hydrogen peroxide which is further acted upon by catalase and Glutathione peroxidise to O_2 and H_20. This mode of redox balancing is investigated in diabetic nephropathy cases in comparison with control in this study.

Methodology

Approximately 7 mL blood was collected in the participants in heparinised tubes and centrifuged. The plasma obtained was stored at -20°C till further biochemical analysis. The level of lipid peroxidation product, malondialdehyde, quantitated as Thiobarbituric acid reactive substances (TBARS) at 540 nm spectrophotometrically (15). The catalase activity was measured using colorimetric reaction using ammonium molybdate complexed with hydrogen peroxide (16). Superoxide dismutase activity in plasma was assayed based on the ability of the enzyme to inhibit the autooxidation of pyrogallol and represented as U/mL plasma (17). One unit of SOD activity is defined as amount of enzyme required to cause 50% inhibition of pyrogallol autooxidation. Estimation of glutathione in serum was done by colorimetric method using 5,5 dithionitro benzoic acid and absorbance measured spectrophotometrically at 450 nm (18). Glutathione peroxidise activity in plasma was determined from kinetic assay involving rate of NADPH to $NADP^+$ oxidation at 340 nm (19).

Group 1: Normal healthy control

Group 2: Type 2 Diabetes mellitus without secondary complications

Group 3: Type 2 diabetes mellitus with nephropathy

The grouping was classified based on standard reference interval for fasting glucose between 70 and 110 mg% as control, above 110 mg% as diabetic and the creatinine upto 1.2 mg% was grouped as diabetic internal control and above 1.2 mg% along with microalbuminuria along with fasting glucose above 180 mg% as diabetic nephropathic groups.

Statistical analysis:

The statistical analysis between groups was done using Microsoft excel. The difference between groups were analysed by student t test with $p < 0.01$ was considered as significant. The sample size was n = 22 in each group.

Results

The plasma obtained was subjected to biochemical analysis which included assay of Glutathione quantitation and activity of Glutathione peroxidase, Catalase and Superoxide dismutase along with Thiobarbituric acid reactive substances quantitation. All values were compiled in excel and results were represented in the form of graph with each bar representing Mean ± SD.

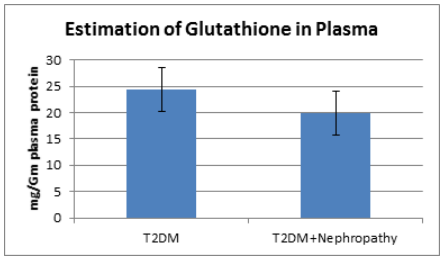

Figure -1

In figure 1 the glutathione (GSH) was estimated in plasma between the experimental groups and the values were represented as mg/Gm plasma protein. It was found that GSH was significantly decreased in Diabetic nephropathic group (Group 3) when compared to Type 2 diabetes mellitus group (Group 2) with P value < 0.001. However, there was no significant difference in GSH level between normal healthy control (Group 1) and group 2. This implied that GSH, the major antioxidant defence molecule was depleted in Type 2 diabetes with secondary complications such as diabetic nephropathy.

Estimation of Glutathione peroxidase activity

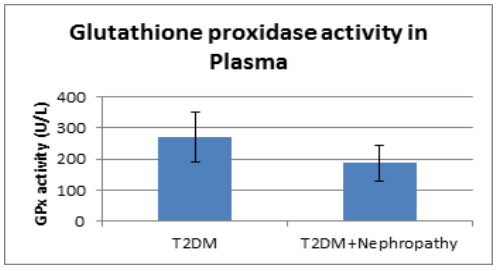

Figure – 2

In figure 2 the Glutathione peroxidase (GPx) was estimated in plasma between the experimental groups and the values were represented as U/L plasma. It was found that GPx activity was significantly decreased in Diabetic nephropathic group (Group 3) when compared to Type 2 diabetes mellitus group (Group 2) with P value < 0.001. However, there was no significant difference in GPx level between normal healthy control (Group 1) and group 2. This implied that GPx, the major antioxidant enzyme to detoxify H_2O_2 utilizing GSH as substrate was depleted in Type 2 diabetes with secondary complications such as diabetic nephropathy.

Estimation of Catalase activity

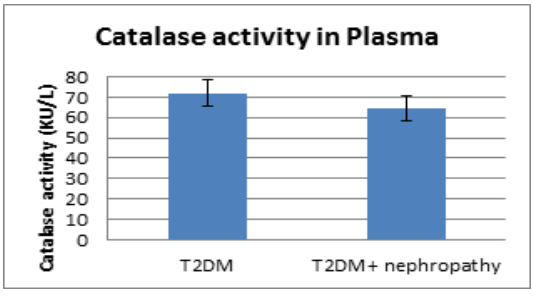

Figure – 3

In figure 3 the Catalase was estimated in plasma between the experimental groups and the values were represented as Katal U/L plasma. It was found that catalase activity was significantly decreased in Diabetic nephropathic group (Group 3) when compared to Type 2 diabetes mellitus group (Group 2) with P value < 0.001. However, there was no significant difference in catalase activity between normal healthy control (Group 1) and group 2. This implied that catalase, the other antioxidant defence enzyme to detoxify H_2O_2 was depleted in Type 2 diabetes with secondary complications such as diabetic nephropathy.

Estimation of Superoxide dismutase activity

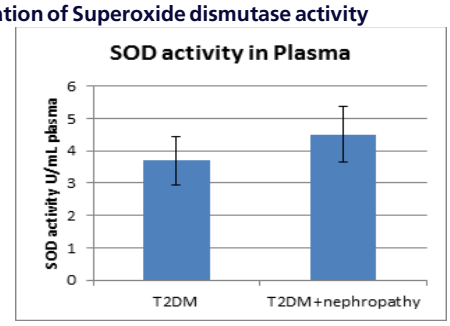

Figure -4

In figure 4 the Superoxide dismutase (SOD) activity was estimated in plasma between the experimental groups and the values were represented as % inhibition of autooxidation of pyrogallol. It was found that SODactivity was significantly increased in Diabetic nephropathic group (Group 3) when compared to Type 2 diabetes mellitus group (Group 2) with P value < 0.01. However, there was no significant difference in SOD activity between normal healthy control (Group 1) and group 2. This implied that SOD, the antioxidant defence enzyme to detoxify superoxide anion was elevated in Type 2 diabetes with secondary complications such as diabetic nephropathy to neutralise the increased generation of superoxide anion. This elevation is as a result of increased free radical generation due to severe oxidative stress.

Estimation of malondialdehyde

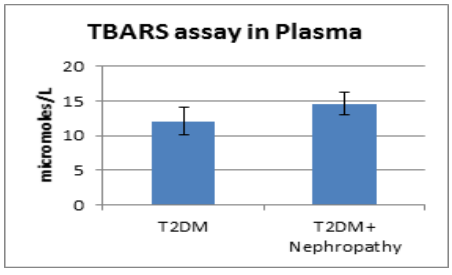

Figure – 5

In figure 5 the Thiobarbituric acid reactive substances (TBARS) was estimated in plasama between the experimental groups and the values were represented as micromoles/L. It was found that TBARS level was significantly increased in Diabetic nephropathic group (Group 3) when compared to Type 2 diabetes mellitus group (Group 2) with P value < 0.001. However, there was no significant difference in TBARS level between normal healthy control (Group 1) and group 2. This implied that TBARS, the indirect estimate of the extent of lipid peroxidation in serum was elevated in diabetic nephropathic group could be a reflection of increased oxidative stress.

Discussion

In the present study we assessed the level of antioxidant molecules and enzymes as well as pro oxidant molecules in the form of lipid peroxides in diabetic nephropathic blood samples in comparison with diabetic samples without any secondary complications such as nephropathy. We observed that the depletion of glutathione (GSH) in diabetic nephropathy revealed the fact that there was severe attenuation of antioxidant defence status in diabetic nephropathy. Consequently, the activity of glutathione peroxidase and catalase activity was decreased in diabetic nephropathy, as GSH is the major substrate for GPx. Hence, when the GSH as well as GPx and catalase activity came down there was little resistance to neutralise the pro oxidant molecules such as lipid peroxides. This was evident from our study that these depletions of antioxidant defence molecules were responsible for elevated presence of thiobarbituric acid reactive substances (TBARS) in blood samples of diabetic nephropathic patients. In addition to increased formation of lipid peroxides there are other free radical molecules such as hydroxyl and hydroperoxy free radicals as well as non free radical

pro oxidant molecules such as hydrogen peroxide in diabetic nephropathy (20). The increased superoxide dismutase (SOD) activity observed in diabetic nephropathy could be a mechanism to neutralise the excess superoxide anion generated in response to renal complications associated with diabetes. The same pattern of SOD activity was reported in a study of diabetic retinopathy where there was increased SOD activity reported in comparison with control (20). This implicated that apart from depletion of other antioxidant enzymes such as Glutathione peroxidase and catalase, SOD activity persisted and even increased in diabetes with renal complications to neutralise the increased superoxide anion.

Conclusion

In the present study we observed that diabetes mellitus *per se* would not disturb the redox balance. However, when diabetic mellitus is accompanied by chronic metabolic complications such as nephropathy there is severe imbalance between antioxidant and pro oxidant molecules. This resulted in increased oxidative damage to cells and tissues in diabetic nephropathy.

Acknowledgement:

The support provided by faculty and other staff members of JCMR is gratefully acknowledged. The support of technical staff members in central lab of the host institute is also acknowledged.

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