ANTIOXIDANT STATUS IN DIABETIC NEPHROPATHY

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ABSTRACT

BACKGROUND
Hyperglycemia and dislipidemia in DM induce increased lipid peroxdation and free radical formation. This is an important mechanism of microangiopathy.

AIM
To measure the antioxidant status in type 2 DM with nephropathy and compared with nondiabetic control group.

MATERIALS AND METHODS
50 type 2 DM patients aged between 50 to 70 years according to national diabetes data group criteria with nephropathy diagnosed on the basis of history, physical examination and biochemical parameters were included. 50 age and sex matched apparently healthy individuals with normal plasma glucose, normal renal parameters and with no symptoms suggestive of DM were taken as controls.

RESULTS
Antioxidant status was significantly less in patients with diabetic nephropathy.

CONCLUSION
Data suggests that alteration in antioxidant status may help predict the risk of diabetic nephropathy.

KEYWORDS
Antioxidant, Diabetes, Nephropathy.

HOW TO CITE THIS ARTICLE: K. V. Giriraja, Pavitra Chandrashekar, Bindumathi P. L. "Antioxidant Status in Diabetic Nephropathy". Journal of Evidence based Medicine and Healthcare; Volume 2, Issue 53, December 03, 2015; Page: 8716-8719, DOI: 10.18410/jebmh/2015/1212

INTRODUCTION: Diabetic Nephropathy is a major cause of morbidity and mortality is patients with type 2 diabetes mellitus in India. Diabetic nephropathy is a clinical syndrome characterised by the following:

- Persistent albuminuria (>300 mg/d or >200ug/min) that is confirmed on at least 2 occasions 3-6 months apart.
- Progressive decline in the glomerular filtration rate (GFR).
- Elevated arterial blood pressure.

Proteinuria was first recognised in DM in late 18th century in 1930s, Kimmelstiel and Wilson described the classic lesions of nodular glomerulosclerosis in diabetes associated with proteinuria and hypertension. In 1950s kidney disease was clearly recognised as a common complication of diabetes with as many as 50 % of patients with DM of more than 20 years having this complication.[1,2]

Submission 22-11-2015, Peer Review 23-11-2015
Acceptance 28-11-2015, Published 01-12-2015.
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DOI: 10.18410/jebmh/2015/1212

Diabetic Nephropathy is considered after a routine urine analysis and screening for microalbuminuria in the setting of Diabetes. Patients will have physical findings associated with long standing DM. Good evidence suggests that early treatment delays or prevents the onset of diabetic nephropathy or diabetic kidney disease. Regular outpatient follow up is key in managing diabetic nephropathy successfully. The exact cause of diabetic nephropathy is unknown with various postulated mechanisms are hypergylcemia causing hyper filtration and renal injury, advanced glycation products and activation of cytokines. Glycemic control reflects the balance between the dietary intake and gluconeogenesis and tissue uptake are utilisation through storage as glycogen or fat and oxidation.[1,2,3]

The high blood glucose levels and high levels of saturated fatty acids create an inflammatory medium resulting in activation of the nuclear transcription factor-kappa B, and release of inflammatory mediators, including, interleukin -1B and tumour necrosis factor-a, promoting systemic insulin resistance and B-cell damage as a result of autoimmune insulitis. Hyperglycemia and high serum levels of free fatty acids and interleukin -1 lead to glycotoxcity and lipotoxcity IL1 toxicity resulting in apoptic B cell death.[3]

Hyperglycemia also increase the expression of transforming growth factor B in the glomeruli and of matrix proteins specifically stimulated by this cytokin. transforming growth factor B and vascular endothelial growth factor may contribute to cellular hypertrophy and enhanced collagen sysnthesis sand may induce the vascular changes observed in person with diabetic nephropathy. Hyperglycemia also may activate protein kinase C which may contribute to renal disease and other vascular complications of diabetes. Some evidence has accrued for a polymorphism in the gene for angiotensin-convering enzyme in either predisposing to nephropathy or accelerating its course. However, definitive genetic markers have yet to be identified. More recently, the role of epigenetic modification in the pathogenesis of diabetic nephropathy has been highlighted.[4,5,6]

In recent years free radicals have assumed an overwhelming importance for the etiopathogenesis of diabetic nephropathy. The most common damaging affect produced by free radicals being lipid peroxidation. The hyper glycemia and dislipidemia in diabetic nephropathy produces a number of reactive hydroperoxides and aldehyes leading to microangipathy in diabetes. Under normal conditions free radicals are formed in minute quantities and are rapidly scavenged by natural cellular defence mechanisms comprising enzymes like superoxide dismutase, glutathione peroxidise, glutathione reductase, catalase, etc. An increased production of malondialdehyde a marker for lipid peroxidation has been found in erythrocyte membrane of diabetic patients together with depressed erythrocyte membrane of diabetic patients together with depressed erythrocyte content ie, anti-oxidant enzymes and reduced glutathione (GSH).[3,5,6]

In view of the above considerations the present study was aimed to evaluate the degree of oxidative stress in diabetic nephropathy patients.

Material and Methods:

Ethics: Study protocol was approved by the institutional ethical committee

Inclusion Criteria: 50 type 2 DM patients aged between 50 to 70 years according to national diabetes data group criteria with nephropathy diagnosed on the basis of history, physical examination and biochemical parameters were included.

50 age and sex matched apparently healthy individuals with normal plasma glucose, normal renal parameters and with no symptoms suggestive of DM were taken as controls.

Exclusion Criteria: Acute and chronic inflammatory conditions like Diabetic ketoacidosis, cerebro vascular accidents, smokers, alcoholics, patients on anti oxidant drugs.

Sample Collection: Under all aseptic conditions, 6ml of venous blood sample was collected from each subject, by disposable syringe. This sample was distributed in the following vials.

- 0.4ml in heparinised vial for estimation of reduced GSH.
- 1.6ml in citrated vial for estimation of plasma MDA, CAT, GR and GPx.
- 4ml in plain for estimation of SOD and lipid profile.

RESULTS: In the present study the levels of GSH, GPx, GR, SOD significantly reduced in patients with diabetic nephropathy compared normal healthy adults. There is significant increase in the level of CAT in diabetic nephropathy patients compared normal healthy controls. The level of MDA significantly increased compared to normal healthy controls.

DISCUSSION: In diabetic nephropathy patients elevated blood glucose levels increased NADPH production resulting in GSH reduction. The decrease in GSH levels in diabetic nephropathy patients is due to inactivation GR which is responsible for the regeneration of GSH. Hyperglycemia causes glycation of GPx which is responsible for the decreased affinity of this enzyme. In our study levels of SOD are reduced, products of lipid peroxidation and H_2O_2 react with superoxide dismutase resulting in modification of this enzyme causing loss of enzyme activity[6,7,8]. In diabetic nephropathy hyper glycemia lead to glycation of the SOD thus reducing its activity. In our study there is increase in the level of CAT in diabetic nephropathy patients compared to normal healthy individuals. Increase level of CAT is explained by the fact that, CAT is compensatory for the removal of free radical. We found in our study increase catalyse level which is in agreement with other studies.[8,9]

IN DM patients there is formation of excessive lipid peroxide MDA in the cell. In our study there is significant rise in serum lipid peroxidise in the study group. As the duration of the diseased increases the level of MDA also increases. Thus it is evident that Diabetic nephropathy patients are susceptible to oxidative damage by lipid peroxidation.[10,11,12]

Hyperglycemia increases the production of free radicals in our body, damages tissue like kidney. Free radicals produce lipid peroxidises which are toxic to microvascular cell. The disorders of lipid metabolism and disorders of lipid peroxidation may be one of the factor to develop diabetic nephropathy.[6,7] Also in our study we observed significantly high levels of TC, Tg, LDL-C,VLDL-C and significantly low levels of HDL-C in diabetic nephropathy subjects as compared to age and sex matched control group.

Many biochemical abnormalities have been identified in kidney in diabetes, including elevated oxidative stress, activation of protein kinase C, non enzymatic glycation, polyolpathway.[13,14,15]

Both dislipidemia and lipid peroxidation contributes to oxidative stress. Metabolically active free fatty acids results in oxidative stress. Advanced glycation products bind to renal endothelial cells, initiating a chain of cellular events leading to nephropathy. Apart from hyperglycemia and dislipidimia weakness of antioxidants with defence system

may be the biochemical background for the pathogenesis of the endothelial dysfunction, associated with DM and lead to microvasular damage resulting in diabetic nephropathy.[15,16,17]

The decrease in the levels of antioxidants produces 3 major histological changes in glomeruli of person with diabetic nephropathy. First mesangial expansion is directly induced by hyperglycemia perhaps via increased matrix production or glycation of matrix proteins due to reduction in anti oxidant status. Second the thickening of glomeruli basement membrane. Third glomerular sclerosis caused by intra glomerular hypertension produced by ischemic injury due to narrowing of the in the antioxidant levels. The key change of diabetic glomerulopathy is augmentation of extra cellular matrix. The earliest morphological abnormality in diabetic nephropathy is the thickening of glomerular basement membrane and explanation of mesangium due to accumulation of extra cellular matrix, due to reduction in antioxidant levels. Light microscopy shows increase in solid spaces of the tufts frequently observed as chores branching of solid material. Large acellualr accumulations also may be observed with in these areas, these are known as Kimmelstiel-Wilson lesions. The reason for KW lesions is decrease in antioxidant level.[18,19,20,21,22]

Immunoflourensec microscopy revel disposition of albumin immnoglobins fibrin and other plasma proteins, along GBM in a linear pattern, most likely as a result of exudation from the blood vessels. Renal vasculature typically displays evidence of artiosclerosis usually due to concomitant hyperlipidimia and athrosclerosis. The above changes are due to decrease in the level of antioxidant status.[15,17,18]

Electron microscopy provides a more detailed definition of the structure involved in advanced diseases the mesangial regions occupy a large portion of the tuft with prominent matrix content. Further the basement membrane in the capillary wall is thicker than normal. The above changes are due to reduction in the antioxidant levels. The severity of diabetic glomerulopathy is estimated by the thickness of the peripheral basement membrane and mesangium and matrix, expressed as a fraction of appropriate spaces. The more severe the lesion the less is the antioxidant status in diabetic nephropathy.[10,11,12]

CONCLUSION: In our study it has been established that antioxidant such as GSH, GPx, GR, SOD were significantly reduced in Diabetic Nephropahty patients compared to normal healthy controls. Levels of CAT, MDA were elevated in Diabetic Nephropathy patients compared to healthy adults. Hence level of antioxidative status can predict the future onset of diabetic nephropathy.

Showing the Status of Anti-oxidant Enzymes and Lipid Peroxides in Diabetic Nephropathy Patients

Subjects	GSH mg%	GPx U/gHb	GR U/gP	CAT U/gP/ml	SOD UmgP/ml	MDA Nmol/ml
Normal Healthy Control (n=50)	14.53±2.30	8.82±1.16	16.84±0.56	6.00±0.66	6.65±1.18	4.79±0.72
Diabetic Nephropathy (n=50)	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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