International Journal of Biotechnology and Biochemistry
ISSN 0973-2691 Volume 15, Number 1 (2019) pp. 17-25

http://www.ripublication.com

Study of Serum Sialic Acid, Microalbuminuria, Oxidative Stress and Antioxidant Status in Diabetic Nephropathy

A Rajeshwari[1], D A Divija[2*], G N Somshekhar [3]

[1]Associate Professor, [3]Assistant Professor
Department of Biochemistry, AIMS, Mandya District, Karnataka, India.

[2]Assistant Professor, Department of Biochemistry, KoIMS, Kodagu District, Karnataka, India.*
[*]Corresponding author

Abstract

Diabetes mellitus is the most common endocrine disorder characterized by metabolic abnormalities and long term complications such as retinopathy, neuropathy and nephropathy. Diabetic nephropathy is a major cause of morbidity and mortality in both type 1 and type 2 diabetes mellitus patients. Serum sialic acid, a marker of acute phase response is related to the presence of diabetic micro and macrovascular complications. Diabetes results in increased oxidative stress which plays an important role in the pathogenesis of diabetic complications such as neuropathy, nephropathy and retinopathy. Microalbuminuria is the most important screening tool for identifying early nephropathy. Hence objective of this study was to estimate serum sialic acid, glycated hemoglobin, microalbuminuria and to evaluate oxidative stress by malondialdehyde (MDA) levels and antioxidant status by measuring vitamin A, vitamin C and vitamin E levels in diabetic nephropathy patients. In this Study 100 participants were involved of which 50 were age and sex matched healthy controls and 50 were diagnosed to have diabetic nephropathy. Detailed medical history was taken. Blood samples were drawn and analyzed for fasting blood sugar (FBS), postprandial blood sugar (PPBS), blood urea, serum creatinine, glycated hemoglobin, seum sialic acid levels, vitamin A, vitamin C, vitamin E and malondialdehyde (MDA) levels and an early morning urine sample was collected for microalbuminuria levels in both diabetic nephropathy cases and controls. Statistical analysis of the data was done and results were represented as mean ±SD. Pearson correlation was done to establish the relation between the study variables. Statistical significance and difference from control and test values were evaluated by student t test.

Keywords: Antioxidant, Diabetic nephropathy, Malondialdehyde, Microalbuminuria, Reactive oxygen species, Sialic acid.

INTRODUCTION

Diabetes mellitus is the most common metabolic disorder affecting the people all over the world. Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting from an absolute or relative deficiency of insulin and / or insulin resistance. An estimated 346 million people have diabetes worldwide. The International Diabetes foundation estimated that this number is expected to increase by 522 million by 2030[1]. Diabetic nephropathy is a major microvascular complication of long standing diabetes mellitus. Diabetic nephropathy is characterized by proteinuria, increased blood pressure and a late decline in glomerular filtration rate (GFR) resulting in end stage renal disease[2]. In diabetes mellitus due to chronic hyperglycemia production of reducing equivalents is more which saturates the electron transport chain leading to the production of more superoxide radical beyond its threshold resulting in increased reactive oxygen species (ROS) production[3]. These reactive oxygen species can activate a number of other superoxide production pathways such as formation of advanced glycation end products, sorbitol pathways, activation of protein kinase C and hexosamine pathway[3]. The oxidative stress causes damage to proteins, lipids and DNA which occurs when the production of reactive oxygen species has exceeded the body or cell's capacity to protect itself and effectively repair oxidative damage. Normally in body there is an abundant supply of naturally occurring antioxidants such as vitamin A, vitamin E, vitamin C, superoxide dismutase, glutathione peroxidase and catalase etc., which delay or inhibit oxidation and neutralize oxygen free radicals[4]. Malondialdehyde (MDA) is a highly toxic product formed by lipid peroxidation by free radicals. The concentration of MDA is high in diabetes mellitus which correlates well with poor glycemic control. The membrane lipids are more liable to attack by free radicals and produce damage to the integrity of the membrane[2]. Sialic acid is a constituent of acute phase reactants which have been associated with microvascular sequelae in diabetes. Sialic acid contributes to the maintenance of the negative charge of renal glomerular basement membrane, one of the regulators of membrane permeability. Due to increased vascular permeability there is shedding of vascular endothelial sialic acid leading to increased levels in circulation[5]. Microalbuminuria is a predictor of progressive renal damage. Damage to the basement membrane allows more protein to leak through the glomeruli which can be reabsorbed and results in complications of diabetes. Screening for the earliest stages of renal damage can have a significant impact on prevention and progression of diabetic nephropathy. Hence the current study was undertaken to estimate sialic acid, glycated haemoglobin and microalbuminuria levels and to establish the effect of reactive oxygen species in diabetic nephropathy patients, measurement of oxidative stress and antioxidant levels is undertaken.

MATERIALS AND METHODS

This was a case control study comparing the sialic acid, microalbumin, oxidative stress and antioxidant vitamins. The study included 100 participants. Clinically diagnosed 50 diabetic nephropathy cases attending medicine outpatient department

and admitted in wards in Adichunchanagiri Hospital and research centre, B G Nagara, Mandya were included in the study. Medical records were screened by specialist physicians. Age and sex matched 50 healthy individuals were taken as control group. Patients with pre-existing chronic kidney disease, chronic glomerulonephritis, nephrotic syndrome, metabolic conditions like ketoacidosis, cerebrovascular accidents, preeclamptic patients, smoking, alcohol intake, history of cardiac diseases were excluded from the study. Study was approved by institutional ethical and research committee. None of the subjects were on antioxidant supplementation. After obtaining the informed consent selected subjects were asked to fast overnight and with all aseptic precautions around 10 ml blood sample was drawn from the study subjects and divided into 3 test tubes. 2ml of blood in both fasting and postprandial state with an anticoagulant was used for the estimation of blood glucose (Glucose Oxidase method). 2 ml of blood sample was collected in heparin coated tube for the estimation of glycated hemoglobin (Affinity chromatography), 6 ml of blood collected in plain tube was processed to obtain serum and serum was used for the measurement of sialic acid (Modified thiobarbituric acid method of Warren), blood urea (Glutamate dehydrogenase (GLDH) urease method), serum creatinine (Jaffe's method), Vitamin A (Spectrophotometric Method), Vitamin C (2,4,dinitrophenyl hydrazine method DNPH), Vitamin E (Baker and Frank method) and serum malondialdehyde (Thiobarbituric acid method). Urine sample was collected in a clean, dry and sterile container for the estimation of urinary microalbumin (Immunoturbidimetry). Statistical analysis of the data was done using statistical software SPSS 15.0. Results were represented as mean ±SD. Pearson correlation was performed to establish the relation between the study variables. Statistical significance and difference from control and test values were evaluated by student t test.

OBSERVATION AND RESULTS

In the present study 50 clinically diagnosed diabetic nephropathy cases and 50 healthy controls were included to evaluate FBS, PPBS, blood urea, serum creatinine, glycated hemoglobin, sialic acid, vitamin A, vitamin C, vitamin E, malondialdehyde (MDA) levels and urinary microalbumin levels. There was a statistically significant increase ($p<0.001$) in FBS, PPBS, blood urea, serum creatinine, glycated hemoglobin, sialic acid and urinary microalbumin levels in cases compared to controls as shown in the table 1. There was a statistically significant increase in malondialdehyde (MDA) levels and decrease in vitamin A, vitamin C and vitamin E in diabetic nephropathy cases compared to controls as shown in the table 2. Table 3 shows the correlation of serum sialic acid and microalbumin with MDA levels and various vitamins. Sialic acid showed positive correlation with MDA and negative correlation with vitamins A, E and C indicating the relationship between variables is weak. Microalbumin showed positive correlation with MDA and negative correlation with vitamins A, E and C indicating the relationship between variables is weak.

Table 1: A Comparison of study variables between two group

Study variables	**Cases (n=50)**	**Controls (n=50)**	**P value**
FBS (mg/dl)	187.60±58.14	90.86±13.29	<0.001**
PPBS (mg/dl)	295.22±81.87	119.72±10.57	<0.001**
Blood Urea (mg/dl)	61.63±12.37	23.79±4.44	<0.001**
Serum creatinine(mg/dl)	2.83±0.68	1.06±0.20	<0.001**
HbA1c (%)	11.17±1.63	5.50±0.45	<0.001**
Serum sialic acid (mmol/l)	3.06±0.35	1.90±0.30	<0.001**
Microalbumin (mg/l)	153.38±69.64	10.35±2.77	<0.001**

FBS – Fasting blood sugar, PPBS - Postprandial blood sugar, HbA1c - Glycated haemoglobin.
(** highly significant)

Table 2: Comparison of serum MDA levels and vitamin A, C and E between two groups

	Controls Mean **(n=50)**	SD	Cases Mean **(n=50)**	SD	p Value
MDA nmol/ml	4.29	±8.01	7.36	±1.71	0.01**
Vitamin A µg/dl	53.33	±12.71	27.03	±8.87	0.0001**
Vitamin C mg/l	12.63	±2.89	4.72	±1.65	0.0001**
Vitamin E mg/l	14.03	±2.79	7.46	±2.80	0.0001**

MDA - Malondialdehyde (** highly significant)

Table 3: Pearson correlation of serum sialic acid and microalbumin and other study variables in cases

Pair	Cases	
	r value	P value
Serum sialic acid (mmol/l) MDA(nmol/ml)	0.117	0.416
Serum sialic acid (mmol/l) vs Vitamin A (µg/dL)	-0.118	0.414
Serum sialic acid (mmol/l) vs VItamin C (mg/l)	-0.117	0.418
Serum sialic acid (mmol/l) vs Vitamin E(mg/l)	-0.116	0.249
Microalbumin (mg/l) vs MDA (nmol/ml)	0.194	0.177
Microalbumin (mg/l) vs Vitamin A (µg/dL)	-0.235	0.108
Microalbumin (mg/l) vs Vitamin C (mg/l)	-0.103	0.476
Microalbumin (mg/l) vs Vitamin E(mg/l)	-0.135	0.349

DISCUSSION

Diabetic nephropathy is a progressive and irreversible renal disease characterized by the accumulation of extra cellular matrix in glomerular mesangium and kidney interstitial tissue that eventually leads to renal failure. In diabetes mellitus, oxidative stress is caused by an imbalance between increase in production and or impaired removal of reactive oxygen species (ROS) and sharp reduction in antioxidant defence and altered cellular redox status[2]. Reactive oxygen species generated within the nephrons are counter balanced by a large number of antioxidant enzymes and free radical scavenging systems like vitamin A, vitamin E, vitamin C, glutathione peroxidase, superoxide dismutase and catalase[6]. Hyperglycemia induced ROS generation induces activation of signal transduction cascade and transcription factors and over expression of genes and proteins in glomerular mesangial and tubular epithelial cells results in extracellular matrix (ECM) accumulation in diabetic kidney. This endogenous oxidative stress causes toxic effects through the production of peroxides and free radicals that can cause oxidation of proteins, lipid peroxidation and damage to DNA, which is an important etiological factor in the pathophysiology of complications of diabetes mellitus[6,7]. In our study the mean values of FBS and PPBS were 90.86±13.29 and 119.72±10.57 in controls and 187.60±58.14 and 295.22±81.87 in diabetic nephropathy patients which is statistically highly significant with $p<0.001$. Measurement of blood urea is used to evaluate renal function. The mean blood urea values were 23.79 ±4.44 in controls and 61.63±12.37 in diabetic nephropathy cases which is statistically highly significant with $p<0.001$. Measurement of serum creatinine is done to determine kidney function. The mean serum creatinine values were 1.06±0.20 in controls and 2.83±0.68 in cases respectively which is statistically highly significant with $p<0.001$. Several studies have reported increased serum creatinine levels in diabetic nephropathy cases compared to controls[8,9]. Glycated hemoglobin is an index of long term control of blood glucose level and as a measure of risk for the development of complications in patients with diabetes mellitus. The mean values of HbA1c were 5.50±0.45 and 11.17±1.63 in controls and in cases which is statistically highly significant with $p<0.001$. Our study is in accordance with other studies who found that HbA1c levels were increased in diabetic patients with and without diabetic nephropathy compared to controls[8,9,10]. Serum sialic acid is a marker of micro and macrovascular complications in diabetic patients. Sialic acid regulates vascular permeability. Diabetic vascular complications stimulate local cytokine secretions from macrophages and endothelium which induces an acute phase response and involves the release of acute phase glycoproteins with sialic acid from the liver into the general circulation leading to increased serum sialic acid concentration[11]. The vascular endothelium carries a high concentration of sialic acid hence extensive microvascular damage associated with diabetes results in its shedding into the circulation. This leads to an increase in vascular permeability and increased serum sialic acid concentration[11]. The mean values of serum sialic acid were 1.90±0.30 and 3.06±0.35 in controls and in diabetic nephropathy cases respectively which is statistically highly significant with $p<0.001$. Our study is in agreement with several study done by Shahid SM and Mahaboob T[10], Prajna k etal[12], Krishnamurthy U, Halyal SS[13], Shivananda Nayak B and Geetha Bhaktha[14], who have demonstrated the

increased sialic acid levels in diabetic patients with the progression of complications such as nephropathy[10,12-14]. Microalbuminuria is defined as urinary excretion of 30-300 mg/day of albumin. Microalbuminuria is a clinically important indicator of deteriorating renal function in diabetic patients. Altered glomerular endothelial permeability in the kidneys due to the disruption of the integrity of the endothelial barriers allows increased amount of albumin to escape into the glomerular ultrafiltrate. The tubular reabsorptive mechanism for albumin from the ultrafiltrate is exceeded beyond its threshold capacity, leading to increased excretion of albumin in the urine[15]. The mean values of urinary microalbumin were 10.35±2.77 in controls and 153.38±69.64 in diabetic nephropathy cases which is statistically highly significant with $p<0.001$. These findings were in accordance to the study of Melidonis A, Tournis S[9], Shivananda Nayak B and Geetha Bhaktha[14] who demonstrated that urinary microalbumin levels were significantly increased in diabetic nephropathy patients compared to healthy controls[9,14].

In the present study serum MDA levels were measured as a marker of oxidative stress. Malondialdehyde is a toxic product formed by lipid peroxidation by free radicals. The mean values of MDA were 4.29 ±8.01 in controls and 7.36±1.71 in diabetic nephropathy cases which is statistically highly significant with $p<0.01$. Several authors Kumawat M[16], Bhutia Y[17], Vivian Samuel T[18] demonstrated higher levels of MDA in diabetic nephropathy cases as compared to those without nephropathy and healthy controls which suggested permanent structural membrane alterations in diabetes and also increased production of reactive oxygen species in the circulation[16-18]. Vitamin A is a fat soluble vitamin which plays an important role in vision, reproduction and maintenance of epithelial tissues. The mean values of vitamin A were 53.33±12.71 and 27.03±8.87 in controls and in cases which is statistically highly significant with $p<0.0001$. Present study is in accordance with several studies who found decreased levels of vitamin A in diabetic nephropathy cases[18,19]. Vitamin C is one of the important component of antioxidant and can scavenge physiologically important reactive oxygen species. In diabetes mellitus there is increased oxidation of ascorbic acid to dehydro ascorbic acid which promotes the oxidative damage. Both ascorbate and ascorbyl radical have low redox potential and prevents oxidative damage to biological macromolecules including DNA, lipids and protein[20]. The mean values of vitamin C were 12.63±2.89 in controls and 4.72±1.65 in cases which is statistically highly significant with $p<0.0001$.These findings were in accordance with other study done by Vivian Samuel who have shown decreased levels of vitamin C in diabetic nephropathy patients compared to controls[18]. It has been shown that Vitamin C supplementation improves glucose tolerance, reduces sorbitol levels and nonenzymatic glycosylation of proteins. Vitamin E is the most important lipophilic membrane antioxidant which protects red cell membrane by preventing lipid peroxidation and maintains structural and functional integrity of all cell membranes[20]. The deficiency of vitamin E results in damage to internal structures by enhancing free radical production. Vitamin E by improving the activity of insulin improves glucose tolerance within the body. The mean values vitamin E were 14.03±2.79 in controls and 7.46±2.80 in diabetic nephropathy cases respectively which is statistically highly significant with $p<0.0001$. These findings were in

accordance with other study where decreased levels of vitamin E is noted in diabetic nephropathy patients compared to controls[18,19]. It has been shown that vitamin E supplementation has a beneficial role in preventing the development of diabetic complications associated with oxidative stress. Correlation study revealed that sialic acid is positively correlated with MDA and negatively correlated with vitamins A, E and C. Microalbumin is positively correlated with MDA and negatively correlated with vitamins A, E and C indicating the relationship between variables is weak. The decrease in mean values of vitamin A, C and E in cases compared to controls is statistically highly significant with $p<0.0001$[18-20]. The increase in mean values of MDA in cases is statistically highly significant with $p<0.01$. So it is suggested that, there is a high correlation between oxidative stress in diabetes and the development of complications including diabetic nephropathy.

SUMMARY AND CONCLUSION

In diabetes hyperglycemia leads to an increased generation of free radicals via multiple mechanisms. Patients prone to acute and chronic oxidative stress enhances the development of late diabetic complications. Antioxidant therapy is suggested as one of the most important treatment strategies for diabetic patients for the prevention and slowing of diabetic nephropathy before reaching to end stage renal disease (ESRD). Hence the study was designed to estimate sialic acid, glycated hemoglobin and microalbuminuria levels and to evaluate oxidative stress and antioxidant status in diabetic nephropathy patients. Correlation study was done between serum sialic acid and microalbumin with MDA levels and vitamins A, C and E. There was a statistically significant increase in serum sialic acid, glycated hemoglobin, malondialdehyde (MDA) levels and urinary microalbumin levels and statistically significant decrease in vitamin A, vitamin C and vitamin E in diabetic nephropathy cases as compared to control. Many studies reported that Vitamin E and Vitamin C antioxidant supplementation significantly improved renal function in Type 2 diabetes. The above discussion has highlighted that elevated serum sialic acid and microalbumin concentrations and the presence of oxidative stress were strongly related to the presence of microvascular complications like diabetic nephropathy. In conclusion determining the markers of oxidative stress, in addition to antioxidant status in diabetes, enable the formulation of specific therapeutic strategies for an early intervention and better management of the disease and its complications.

REFERENCES

[1] Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo et al, editors: Harrison's principles of internal medicine. 19th ed. McGraw-Hill: Medical publishing division; 2015.

[2] Jakus V "The role of free radicals, oxidative stress and antioxidant system in diabetic vascular diseases". Bratesl Lek Listy 2000 ; 101(10): 541-551.

[3] Kronenberg HM, Melmed S, Kenneth S, Polonsky, Larsen PR, editors:

Williams textbook of endocrinology. The complications of diabetes mellitus. 11th ed. Saunders Elsevier publishing Division; 2008: 1417-1482.

[4] Lipid peroxidation and antioxidant status in patients with diabetic retinopathy." Indian Journal of physiology pharmacology 2005; 49(2): 187-192.

[5] Mohammad JS, Muhammad TM, Ahmad M, Riaz M, Umair M. Serum Sialic acid concentration and type 2 DM. Professional Med J Dec 2006;13(4):508-510.

[6] Renu A. Kowrluru and Pooi-see chan; Review article "Oxidative stress and diabetic Retinopathy." Experimental diabetes research Hindawi publishing corporation volume 2007, article ID 43603.

[7] Arya A, Aagarwal S, Yadav HN. Pathogenesis of diabetic nephropathy. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(4): 24-29.

[8] Chen JW, Gall MA, Yokoyama H, Jensen JS, Deckert M, Parving HH: Raised serum sialic acid concentration in NIDDM patients with and without nephropathy. Diabetes Care 1996 February; 19(2):130-134.

[9] Melidonis A, Tournis S, Hraklioti S, Hraklianou S: Serum sialic acid concentration and diabetic nephropathy in type 2 diabetes mellitus. Diabetologia Croatica 1998 Decemb; 29;27(4).

[10] Shahid SM, Mahaboob T: Clinical correlation between frequent risk factors of diabetic nephropathy and serum sialic acid. Int J Diabetes and Metabolism 2006; 14:138-142.

[11] Nayak BS, Roberts L**.** Relationship between inflammatory markers, metabolic and anthropometric variables in the Caribbean type 2 diabetic patients with and without microvascular complications. Journal of Inflammation 2006, 3:17 doi:10.1186/1476-9255-3-17.

[12] Prajna K, Kumar AJ, Raj S, Shetty SK, Raj T, Shrinidhi , Begum M, Shashikala MD. Predictive Value Of Serum Sialic Acid in Type-2Diabetes Mellitus and its Complication (Nephropathy).2013 (11):2435-2437.

[13] Krishnamurthy U, Halyal SS, Jayaprakash Murthy DS: Serum sialic acid and microalbuminuria in non insulin dependent diabetes mellitus. Biomedical Research 2011; 22(1):31-34.

[14] Shivananda Nayak B, Geetha Bhaktha: Relationship between sialic acid and metabolic variables in Indian type 2 diabetic patients. Lipids in Health and Disease. 2005 Aug; 10; 4:15.

[15] Basu S, Chaudhuri S, Bhattacharyya M, Chatterjee TK, Todi S, Majumdar A. Microalbuminuria ; An inexpensive, non invasive bedside tool to predict outcome in critically ill patients. Indian journal of clinical Biochemistry 2010 April; 25(2):146-152.

[16] Kumawat M, Sharma TK, Singh I, Singh N, Ghalaut VS, Vardey SK, Shankar V: Antioxidant enzymes and lipid peroxidation in Type 2 diabetes mellitus patients with and without nephropathy. North Am J Med Sci.2013; 5:213-9.

[17] Bhutia Y, Ghosh A, Sherpa M L, Pal R, Mohanta P K: Serum malondialdehyde level: Surrogate stress marker in the Sikkimese diabetics. J Nat Sci Biol Med. 2011 Jan-Jun; 2(1): 107–112.

[18] Vivian Samuel T, Smilee Johncy S, Jayaprakash Murthy D.S, Rekha M, Poornima. R T: Potential Role of oxidative Stress and Antioxidant Deficiency in pathogenesis of Diabetic Nephropathy Vivian Samuel et al /J. Pharm. Sci. & Res. Vol.3(2), 2011,1046-1051.

[19] Kesavulu MM, Giri R, Rao KR et al. Lipid peroxides and antioxidant enzyme levels in type 2 diabetics with microvascular complications. *Diabetes and Metab* 2000; 26(3): 387– 92.

[20] Vasudevan D.M and Sreekumari S "Text Book of Bio-chemistry for Medical Students". 8th Ed. Jaypee brother's medical publishers (P) Ltd; 2016.