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OXIDANT, ANTIOXIDANTS AND SOME BIOCHEMICAL PARAMETERS IN PATIENTS WITH TYPE 2 DIABETIC NEPHROPATHY

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ABSTRACT

This work was conducted to study the role of oxidative stress and some biochemical parameters in type 2 diabetic nephropathy patients. Fifteen type 2 diabetic nephropathy and ten type 2 diabetic patients (at the age 57-68 years) attending to the abd- Almajid private hospital were recruited for this study. For the purpose of comparison, seven healthy controls subjects matched for age were also included. Random blood sugar, serum urea, serum creatinine, glycosylated hemoglobin, serum complements 3 and 4, serum malondialdehyde, serum glutathione and serum catalase activity were measured in blood. Microalbumin, creatinine and albumin / creatinine ratio were measured in urine. The results showed increase in random blood sugar in diabetic and diabetic nephropathy patients at the ages of 57-68 years. No change was observed in serum urea and creatinine concentrations in diabetic patients, whereas an increase was demonstrated in diabetic nephropathy patients. The percentages of glycosylated hemoglobin increased significantly in diabetic and diabetic nephropathy patients. No changes were seen in serum complements 3 and 4, malondialdehyde and glutathione concentrations in diabetic and diabetic nephropathy patients. A significant increase in serum catalase activity was demonstrated in diabetic and diabetic nephropathy patients. No change was observed in urine microalbumin concentration in diabetic patients, while a significant increase was seen in diabetic nephropathy patients. Urine creatinine concentrations in diabetic and diabetic nephropathy patients showed no change. Albumin / creatinine ratios showed increase in diabetic nephropathy patients. No change was observed in albumin / creatinine ratio in diabetic patients. It is concluded that

diabetic patients demonstrated hyperglycemia, an increase in the percentages of glycosylated hemoglobin and catalase activity with an associated no changes in serum complements 3, 4, MDA and GSH. Diabetic nephropathy patients showed more profound biochemical changes than diabetic patients as reflected by hyperglycemia, an increase in serum urea, creatinine, the percentages of glycosylated hemoglobin, catalase activity, urine creatinine and A/C ratio with an associated microalbuminuria at the different age groups. Serum complements 3 and 4, MDA and GSH remained unchanged.

KEYWORDS: Random blood sugar, serum urea, serum creatinine, MDA, GSH, Catalase, HbA1c.

INTRODUCTION

The term diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies characterized by disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. It is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to the development of nephropathy, neuropathy and retinopathy.[1] Three principal types of DM are recognized; Type 1 DM(T1DM), Type 2DM (T2DM) and gestational diabetes. T2DM is characterized differently and is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion.[2] Due to increasing obesity, sedentary life style and dietary habits in both Western and developing countries, the prevalence of T2DM is growing at an exponential rate. The increase in T2 DM is also seen in younger people and in developing countries and estimates in the Middle East and Africa revealed that the prevalence is high and set to increase dramatically during the next 18 years.[3] The etiology of T2DM is not well-understood, although associated health risk factors are recognized; for instance, a family history of diabetes age over 45 years, race or ethnic background, metabolic syndrome (also called insulin resistance syndrome), obesity, hypertension and history of vascular disease such as stroke, abnormal cholesterol levels and history of gestational diabetes.[2] Diabetic nephropathy is a major long-term complication of diabetes mellitus.[4,5] It develops in more than of 40% of patients in spite of glucose control.[6] Oxidative stress has been considered to be a pathogenic factor for diabetic nephropathy.[7] Hyperglycemia is believed to activate oxidative stress resulting in proteinuria.[8,9] It is suggested that increased oxidative stress through reduction of plasma antioxidants and increased lipid peroxidation could intensify mesangial cells susceptibility to free radical

injury.[9,10] The biological systems living in aerobic conditions are exposed to oxidants. Generally, these oxidants occur in two categories consisting of paramagnetic free radicals: reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS is a collective term used to describe the chemical species that are formed upon incomplete reduction of oxygen and includes superoxide anion (O2−), hydrogen peroxide (H2O2) and hydroxyl radical (HO•). All these species are able to initiate or mediate many enzyme- and gene-dependent reactions in both physiological and pathophysiological processes. Overproduction or deficiency of ROS and/or RNS may result in impaired homeostasis and associated pathology. Thus, it is widely believed that multiple pathogenic mechanisms involve disequilibrium in the redox balance as the final common pathway.[11]

The aim of the present work was to study the role of oxidative stress and some biochemical parameters in type 2 diabetic nephropathy patients.

MATERIALS AND METHODS

Type2 Diabetic and diabetic nephropathy patients

The study was conducted on 10 type 2 diabetic and 15 type 2 diabetic nephropathy patients, their mean ages (57-68 years). The diabetic and diabetic nephropathy patients having microalbuminuria or elevated serum creatinine were randomly selected from those attending the Al-fajer laboratory at Abdalmajid private hospital. The information about the age, the presence of hypertension and having treatment were documented from the patients.

Control subjects

For the purpose of comparisons, 7 healthy controls subjects comparable to diabetes mellitus and diabetic nephropathy patients in respect to age (57-68 years). The controls were selected among subjects who were apparently healthy in terms of being non-diabetic, with no other endocrine disorders or metabolic kidney diseases and were free of acute illness or infection at time of sampling.

Collection of samples

Collection of blood samples

Five ml of blood were obtained from patients and control subjects by venipuncture, using a 5 ml disposable syringe, 3 ml of blood and dispensed in a gel tubes and centrifuged at 3000 rpm for 10 minutes to collect serum. The serum was divided into aliquots in Eppendorf tubes, stored in deep freezer at (-20◦C) for measuring random blood sugar (RBS), blood urea, serum

creatinine, complements 3 and 4(C_3,C_4), catalase, malondialdehyde and glutathione. Other 2 ml were collected into EDTA tubes for measuring HbA1c.

Collection of urine samples

Urine was collected two times by spot urine collection. The urine was collected into sterile cups without any blood or urinary tract infection.

Determmination of biochemical parameters

Blood glucose, blood urea, serum creatinine and Glycosylated hemoglobin were determined using kits. (Roche- China).

Serum malondialdehyde, glutathione and catalase were determined using ELISA kits. (Bioassay Technology Laboratory- China).

Complements 3 and 4 was determined by radial immunodiffusion plate (LTA- China).

Creatinine, microalbumin and protein detected by urine test strip (Human- Germany).

RESULTS

Figure1.1 demonstrates that RBS increases significantly ($p<0.05$) in diabetic and diabetic nephropathy patients as compared with the healthy control subjects. No significant ($P>0.05$) change is observed in serum urea concentration in diabetic patients, while a significant ($P<0.05$) increase is noticed in serum urea concentration in diabetic nephropathy patients when compared with the healthy control subjects(Figure1.2). The results of serum creatinine concentrations in healthy control subjects, diabetic and diabetic nephropathy patients are shown in Figure 1.3. No change is observed in serum creatinine concentration in diabetic patients, whereas in diabetic nephropathy patients, serum creatinine concentration shows a significant ($p<0.05$) increase when compared with the healthy control subjects. The percentages of HbA1c increase significantly ($P<0.05$) in diabetic and diabetic nephropathy patients in comparison with the healthy control subjects (Figure 1.5). Figures 1.5 and 1.6 demonstrate a nonsignificant ($P>0.05$) change in the serum complements 3 and 4 in diabetic and diabetic nephropathy patients when compared with the healthy control subjects. Figure1.7 shows nonsignificant ($p>0.05$) changes in serum malondialdehyde concentration in diabetic and diabetic nephropathy patients as compared with the healthy control subjects. Serum glutathione (GSH) concentrations in healthy control subjects, diabetic and diabetic nephropathy patients are shown in Figure 1.8 no significant ($P>0.05$) changes are seen in

GSH concentration in diabetic and diabetic nephropathy patients as compared with the healthy control subjects. The Figure1.9 demonstrates a significant (P<0.05) increase in serum catalase activity in diabetic and diabetic nephropathy patients as compared with the healthy control subjects. No significant (P>0.05) change is observed in urine microalbumin concentration in diabetic patients, while a significant (P<0.05) increase is noticed in urine microalbumin concentration in diabetic nephropathy patients when compared with the healthy control subjects (Figure1.10). Urine creatinine concentration in healthy control subjects, diabetic and diabetic nephropathy patients is shown in Figure1.11 No significant (P>0.05) changes are seen in urine creatinine concentrations in diabetic and diabetic nephropathy patients as compared with the healthy control subjects. No change is observed in albumin/creatinine ratio in diabetic patients, whereas in diabetic nephropathy patients, albumin/creatinine ratio shows a significant (p<0.05) increase when compared with the healthy control subjects(Figure 1.12).

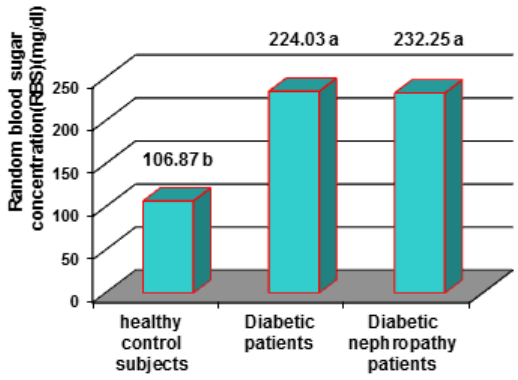

Figure 1.1 Random blood sugar concentrations in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). different letters mean significant differences at P< 0.05.

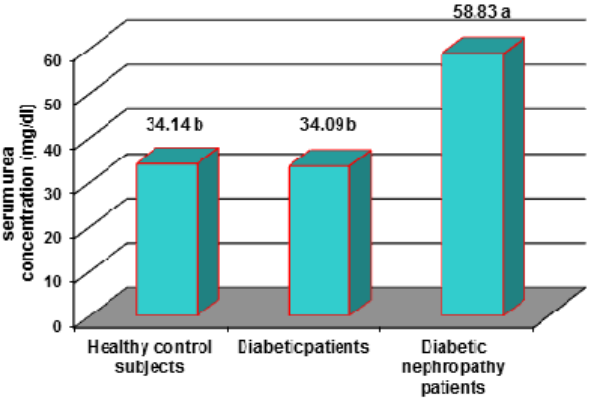

Figure 1.2 serum urea concentrations in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). different letters mean significant differences at P< 0.05.

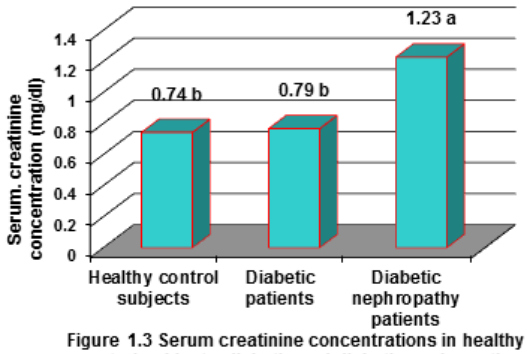

Figure 1.3 Serum creatinine concentrations in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). different letters mean significant differences at P< 0.05.

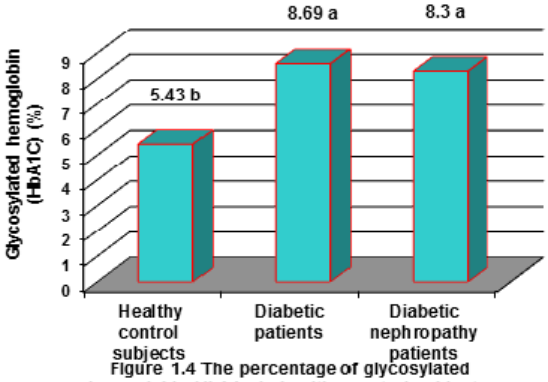

Figure 1.4 The percentage of glycosylated hemoglobin HbA1c in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). different letters mean significant differences at P< 0.05.

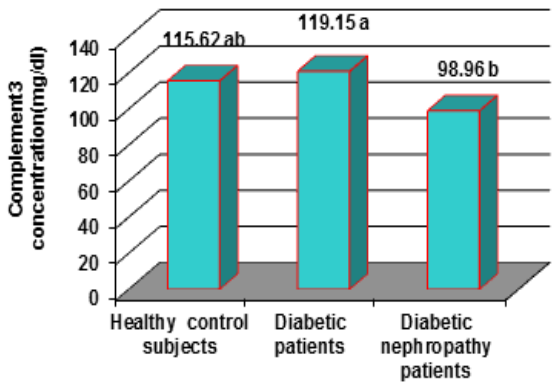

Figure 1.5 Serum complement3 concentrations in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). different letters mean significant differences at P< 0.05.

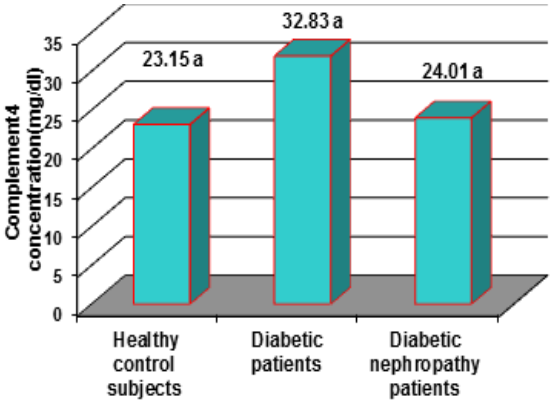

Figure 1.6 Serum complement 4 concentration in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). similar letters mean nonsignificant(P>0.05) differences.

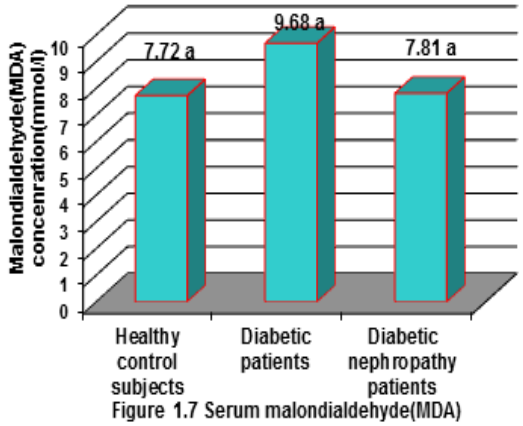

Figure 1.7 Serum malondialdehyde(MDA) concentration in healthy control subjects, diabetic and diabetic nephropathy patients(age 57-68 years). similar letters mean nonsignificant (P>0.05)differences.

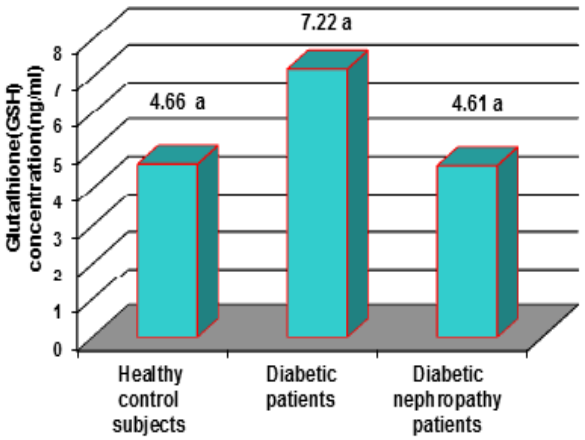

Figure 1.8 Serum glutathione(GSH) concentrations in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years).similar letters mean nonsignificant(P>0.05) differences.

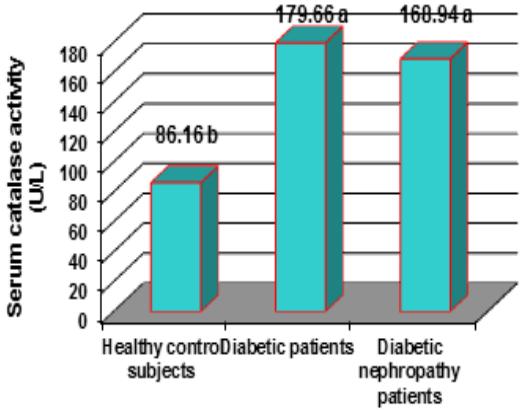

Figure 1.9 Serum catalase activity in healthy control subjects, diabetic and diabetic nephropathy patients (age 57- 68years). different letters mean significant differences at P< 0.05.

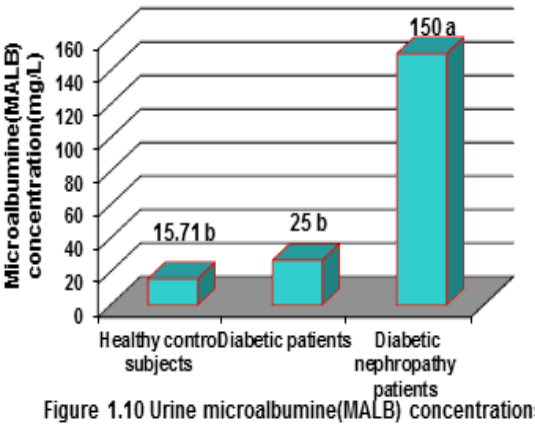

Figure 1.10 Urine microalbumine(MALB) concentrations in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). different letters mean significant differences at P< 0.05.

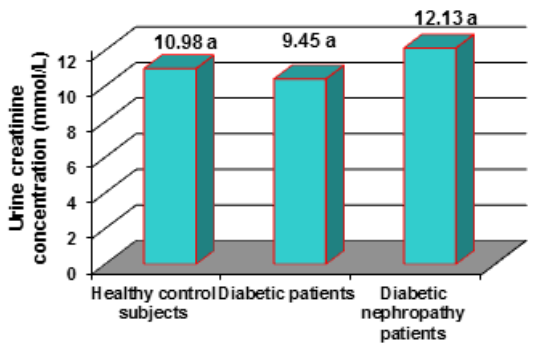

Figure 1.11 Urine creatinine concentrations in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). similar letters mean nonsignificant (P>0.05) differences.

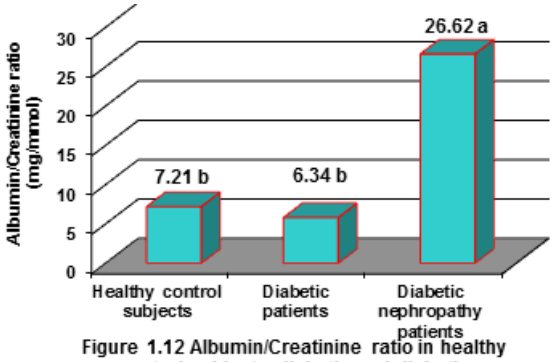

Figure 1.12 Albumin/Creatinine ratio in healthy control subjects, diabetic and diabetic nephropathy patients (age 57-68 years). different letters mean significant differences at P< 0.05.

DISCUSSION

Hyperglycemia results from defects in insulin secretion and insulin action or both. Type 2 diabetes mellitus is characterized by insulin resistance in peripheral tissue and a defect in insulin secretion from the beta cell of the pancreas.[12] The chronic hyperglycemia associated with diabetes mellitus is due to derangement in carbohydrate, fat and protein metabolism.[7] It can increase oxidative stress which has been considered to be a pathogenic factor of diabetic complications including nephropathy.[13] No change was observed in serum urea concentration in diabetic patients, whereas an increase was demonstrated in diabetic nephropathy patients at the ages of 45-80 years. Bamanikar *et al*. (2016) demonstrated that out of 100 diabetic samples, 18 had high urea level. They showed that poorly controlled blood sugar levels would cause increase in serum urea levels thus increasing the chances of the patient suffering from diabetic nephropathy. The duration and severity of diabetes strongly correlate with serum urea levels.[14] The increase in serum urea concentration in diabetic nephropathy patients in the current study is in accordance with other studies.[15,16,13] Bamanikar *et al.*(2016) reported that an increase in urea level is observed when there is damage to the kidney and the increase in blood urea level in the presence of hyperglycemia in diabetic patients indicates damage to the kidney. The findings of the current study indicated no change in serum creatinine concentrations in diabetic patients. Serum creatinine concentrations increased significantly in diabetic nephropathy patients. Creatinine is a waste product in the blood formed by the normal breakdown of muscles cells during activity and the healthy kidneys filters creatinine out of the blood into the urine.[17] When kidneys are not functioning well, creatinine levels build up in the blood, since creatinine is an indicator of normal functioning of the kidney and its increase in the blood indicates kidney dysfunction.[18] Anjaneyulu and Chopra (2004) reported that the increase in serum creatinine in diabetic rats indicated progressive renal damage. the percentages of glycosylated

hemoglobin increased in diabetic and diabetic nephropathy patients. No changes were seen in serum complements 3 and 4 concentrations in diabetic and diabetic nephropathy patients. Nikolova *et al*. (2004) found that serum complements 3 and 4 levels were within the normal range in diabetic nephropathy patients. On the contrary, Dezayee and Alnakshabandi (2011) demonstrated an increase in serum complement 3 in type 2 diabetic patients with a decrease in serum complement 4 as compared with the healthy subjects. On the other hand, There is evidence that complement may be involved in susceptibility to and progression of diabetic nephropathy.[19] No changes were observed in serum malondialdehyde concentrations in diabetic and diabetic nephropathy patients. Human body possesses natural antioxidants to control the level of reactive oxygen species by scavenging them both enzymatically and non-enzymetically.[20] In addition to the naturally occurring antioxidants, the antioxidant activity of drugs may play a role in ameliorating the oxidative stress induced renal damage during diabetes[21] and good glycemic control could be supportive and beneficial in reducing effects caused by oxidative stress.[22] Serum glutathione concentrations show nonsignificant changes in diabetic and diabetic nephropathy patients.). Glutathione can maintain SH groups of protein, detoxify foreign radicals and also act as coenzyme in several enzymatic reactions[23]. The results demonstrated an increase in serum catalase activity in diabetic and diabetic nephropathy patients. These results are in agreement with those obtained by others in type 2 diabetes mellitus with or without nephropathy.[7,22,24] Catalase acts as main regulator of hydrogen peroxide metabolism. It catalyzes the decomposition of hydrogen peroxide to water and oxygen.[22] The reason for increased catalase activity can be linked to a secondary compensatory activation of the enzyme.[24] The microalbuminuria levels were shown to be higher in complicated cases.[25] The development of vascular complications in diabetes correlates with the intensity of hyperglycemia and the high intracellular glucose concentration has been suggested to be a prerequisite for the development of structural and functional changes in the kidney.[7] The cause of microalbuminuria may be attributed to a defect in the glomerulare membrane filtration. The results of the present study showed no changes in urine creatinine concentrations in diabetic and diabetic nephropathy patients, It is assumed that creatinine excretion rates are fairly constant during the day, as long as the glomerular filtration rate (GFR) remains constant.[26] Diabetes affects the kidney in stages and at the onset of diabetes, the GFR becomes disturbed.[27] Diabetic nephropathy occurs in conjunction with hyperfiltrative period in which creatinine clearance and GFR are high.[28] followed by a gradual decrease in GFR that leads to kidney failure.[29] An increase in albumin / creatinine ratios was seen in diabetic nephropathy patients, Manjunatha Goud *et al*. (2010)

showed a significant increase in urine albumin / creatinine ratio in diabetic cataract patients without nephropathy and in diabetic cataract patients with nephropathy. The urine albumin / creatinine ratio is commonly used as an index of albuminuria.[30] The presence of microalbuminuria in type 2 diabetic patients is an important predictor of progressive renal failure.[31]

REFERENCES

1. World Health Organization 2005. EMRO strategies for diabetes control and prevention Reginal office for the Eastern Mediterranean. PO. Box 7608. Nasr City Cairo. 11371. Egypt.
2. American Diabetes Association 2012. Diagnosis and Classification of Diabetes mellitus. Diabetes Care; 35(suppl.1): S64-71.
3. Li, L., Yang, G., Shi, S., Yang, M., Liu, H. and Boden, G. 2009. The adipose triglyceride lipase, adiponectin and visfatin are down regulated byTNF- α in *vivo*. J. Cytokine, 45: 12-19.
4. Lu, H.J., Tzeng, T.F., Hsu, J.C., Kuo, S.H., Chang, C.H., Huang, S.Y., Chang, F.Y., Wu, M.C., Liu, I.M. 2013. An aqueous-ethanol extract of *liriope spicata var. prolifera* ameliorates diabetic nephropathy through suppression of renal inflammation. Evid Based Complement Alternat Med, 2013; 201643.
5. Ribeiro, M.C., Avila, D.S., Barbosa, N.B., Meinerz, D.F., Waczuk, E.P., Hassan, W., Rocha, J.B. 2013. Hydrochlorothiazide and high-fat diets reduce plasma magnesium levels and increase hepatic oxidative stress in rats. Magnes Res., 26(1): 32–40.
6. Gross, J. L., de Azevedo, M. J., Silveiro, S. P., Canani, L.H., Caramori, M.L., Zelmanovitz, T. 2005. Diabetic nephropathy: diagnosis, prevention and treatment. Diabetes Care, 28(1): 164–176.
7. Kumawat, M., Sharma, T.K., Singh, I., Singh, N., Ghalaut, V.S., Vardey, S.K., Shankar, V. 2013. Antioxidant enzymes and lipid peroxidation in type 2 diabetes mellitus patients with and without nephropathy. N Am J Med Sci., 2013; 5(3): 213–219.
8. Schena, F. P., Gesualdo, L. 2005. Pathogenetic mechanisms of diabetic nephropathy. J Am Soc Nephrol, 16(Suppl 1): S30–S33.
9. Hans, C.P., Chaudhary, D.P., Bansal, D.D. 2002. Magnesium deficiency increases oxidative stress in rats. Indian J Exp Biol, 40(11): 1275–1279.
10. Walti, M.K., Zimmermann, M.B., Spinas, G.A., Jacob, S., Hurrell, R.F. 2002. Dietary magnesium intake in type 2 diabetes. Eur J Clin Nutr, 56(5): 409–414.

11. D'Autreaux, B., Toledano, M. B. 2007. ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol. 8*: 813–824.
12. Garber, A. J. 2000. The importance of early insulin secretion and its impact on glycaemic regulation. Int. J. Obes. Relat. Metab. Disord., 24(suppl. 3): S32- S37.
13. Kanwar, G., Jain, N., sharma, N., shekhawat, M., Ahmed, J. and Kabra, R. 2015. Significance of serum urea and creatinine levels in type 2 diabetic patients. IQSR J. Dent. Med. Sci., 14(8): 65-67.
14. Bamanikar, S. A., Bamanikar, A. A. and Arora, A. 2016. Study of serum urea creatinine in diabetic and nondiabetic patients in a tertiary teaching hospital. J. Med. Res., 2(1): 12-15.
15. Ahmed, Sh. H., Abd- Ali, F. and Abdullah, M. R. 2010. Biochemical study on diabetic nephropathy patients. Ibn Al- Haitham J. Pure Appl. Sci., 23(3): 152-158.
16. Singh, P., Khan, S. and Mittal, R. K. 2014. Glycemic status and renal function among type 2 diabetics. Bangladesh J. Med. Sci., 13(4): 406-410.
17. Usman, A., Umar, R. A. Shehu, N. I., Wali, U. and Nasir, A. 2012. Markers of diabetic nephropathy in diabetic patients in gusau, Zamfara state, Nigeria. Nigerian J. Basic Appl. Sci., 20(2): 130-133.
18. Kamal., A. 2014. Impact of diabetes on renal function parameters. Indian J. Fundament. Appl. Life Sci., 4(3): 411-416.
19. Fearn, A. and Sheerin, N. S. 2015. Evaluation of glutathione dependent antioxidant enzymes in maternally inherited type 2 diabetes mellitus. J. Pharm. Sci. Res., 7(3): 137-140.
20. Matough, F. A. Budin, S. B., Hamid, Z. A., Alwahaibi, N. and Mohamed, J. 2012. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ. Med. J., 12: 5-18.
21. Agarwal, N., Sadhukhan, P., Saha S. and Sil, P.C. 2015. Therapeutic insights against oxidative stress induced diabetic nephropathy: A review. J. Autoimmune Disorders, 1(1:2): 1-16.
22. Jabeen, F., Rizvi, H. A., Aziz, F. and Akhtar, Y. 2013. Effect of glycemic control on lipid profile, platelet indices and antioxidant enzymes (catalase and superoxide dismutase) activities in type 2 diabetes. Int. J. Aes., 1(7): 207-215.
23. Tsai, C. J., Tung, S. C., Kuo, M. C. and shen, F. C. 2012. Acute blood glucose fluctuations can decrease blood glutathione and adiponectin levels in patients with type 2 diabetes. Diabetes Res. Clin. Pract., 98(2): 257-263.

24. Goicheva, P., Iliev, R. and Gadjeva, V. 2014. Comparative analysis of some markers of oxidativestreaa in patients with diabetes nephropathy on chronic hemodialysis _ four years study. Trakia J. Sci., 12(suppl. 1): 184-190.
25. Rani, K., Gavel, P. and Bharti, S. 2016. MDA, oxidative stress marker- role in diabetic nephropathy with special reference to type Π diabetes mellitus. Indian J. Appl. Res., 6(5): 128-130.
26. Price, C. P. Newall, R. G. and Boyd, J. C. 2005. Use of protein: creatinine ratio measurements on random urine samples for prediction of significant proteinuria: A systematic review. Clin. Chem., 51: 1577-1586.
27. Dabla, P. R. 2010. Renal function in diabetic nephropathy. World J. Diabetes, 1(2): 48-56.
28. United States Renal Data System USRDS. 2001. Annual report: Atlas of end stage renal diseases in the United State. Am. J. Kidney Diseases, 41: 57-254.
29. Gal, M. A. Houganard, P., Borch- Johnsen, K. and parving, H. H. 1997. Risk factors for development of incipient and overt diabetic nephropathy in patients with type Π diabetes mellitus. Brit. Med. J., 314: 783-788.
30. Wang, Z., Hoy, W. E. and Wang, Z. 2013. The correlates of urinary albumin to creatinine ratio (ACR) in a high risk Australian aboriginal community. BMC Nephrology, 14: 176-182.
31. Manjunatha Goud, B. K. Deepa, K., Sarsina Devi, O., Poornima, A. M., Nandini, M. and Asha, K. 2010. Comparison of blood sorbitol level and microalbuminuria in early detection of diabetic nephropathy in type 2 diabetes. Int. J. Pharma. Bio Sci., 1(4): B-335-B339.