

## OXIDATIVE STRESS WITH HOMOCYSTEINE, LIPOPROTEIN (A) AND LIPID PROFILE IN DIABETIC NEPHROPATHY

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**ABSTRACT:** Diabetic nephropathy is the single most common disorder leading to renal diseases. Reactive oxygen species (ROS) play a major role in the development of diabetic nephropathy. Nephrotic syndrome is often manifesting in progression of diabetic nephropathy. Therefore, this study was carried out to investigate oxidant and antioxidant status in diabetic nephropathy patients. The blood samples were analyzed for quantitation of malondialdehyde as index of lipid peroxide, vitamin C, total antioxidant capacity, homocysteine, lipoprotein (a) and lipid profile. Significantly increased levels of serum total cholesterol, triglycerides, low density lipoprotein, malondialdehyde as index of lipid peroxide, lipoprotein (a), homocysteine ( $p < 0.001$ ) and decreased levels of serum total antioxidant capacity, total protein, albumin, high density lipoprotein & plasma vitamin C ( $p < 0.001$ ) were noticed in the patients with diabetic nephropathy as compared to control subjects.

**KEYWORDS:** Malondialdehyde (MDA), Total antioxidant capacity (TAC), vitamin C (vit C), Diabetic nephropathy (DN), Lipoprotein (a), Homocysteine, Reactive oxygen species.

### INTRODUCTION

DN is characterized by excessive accumulation of extracellular matrix in the kidney, reactive oxygen species (ROS) play a central role in the extracellular matrix synthesis and degradation in the glomeruli and tubulointerstitium leading to renal diseases (Ha H, et.al., 2005 a). Oxidative stress has been known to play an important role in the development and progression of diabetic nephropathy (Ha H, et.al., 2001 b). Diabetic nephropathy is a leading cause of end stage renal failure, DN has several pathways for development such as glomerular hyperfiltration, upregulation of protein kinase C, advanced glycation end products, activation of polyol pathway, increased oxidative stress and upregulation of growth factors (Ohgas, et.al., 2004). There is considerable evidence that hyperglycemia represents the main cause of complications of diabetes mellitus (DM) and oxidative stress resulting from increased generation of reactive oxygen species plays a crucial role in their pathogenesis (Davi G, et.al., 2005). Classical factors contributing to the pathology of diabetic nephropathy e.g., hypertension, hyperglycemia, hypoinsulinemia, and hyperlipidemia (Miyata T, et.al., 2009). Recent studies, mainly perform new markers such as hypoxia, advanced glycation, oxidative stress, and other bioactive molecules in the pathogenesis of DN (Miyata T, et.al., 2009). Diabetic nephropathy has several distinct phases of development and multiple mechanisms contribute to the development of the disease and its outcomes (Dronavalli S, et.al., 2008). HCY is a link between DN and both chronic inflammation and hypercoagulability increasing cardiovascular risk (Wotherspoon F, et.al., 2006, Zdemir G, et.al., 2005, Aso Y, et.al., 2004). Lp (a) is an independent risk factor for the progression of diabetic nephropathy in type 2 diabetic patients with overt proteinuria (Kramer GA, et.al., 1996).

The objective of this study was to investigate possible associations between oxidative stress and the severity of diabetic nephropathy in nephrotic syndrome patients with the estimation of the serum HCY, Lp(a), TAC, MDA, plasma ascorbic acid (vit C), interrelationship of all biochemical parameters and correlate with severity of DN.

## MATERIALS AND METHODS

### Location

Patients included in the present study were all admitted to the intensive care unit (ICU) or attending the OPD of medicine of M.Y. Hospital attached to M.G.M. Medical College, Indore, Madhya Pradesh, India.

**The study group:**-The present study was case control study conducted on 2 groups.

**Group I:-** Comprised with control (135).

**Group II:-** Comprised with adult DN patients (65).

Age of the patients group I & II ranged from 30 to 80 years, patients were from same geographical area and none was taking a special diet, untreated DN patients newly diagnosed by biopsies evidences of nephritis. Fasting blood glucose levels  $\geq 126.0$  mg/dl, BMI  $> 24.0$  kg/m<sup>2</sup>, HTN – SBP  $\geq 140$  mm Hg and DBP  $\geq 90$  mm Hg. Group I<sup>st</sup> was judged to be free of any illness by clinical examination, DN patients were not with any other active complication medical condition or with systemic diseases. Excluded the subjects or patients taking vitamins tablet from prolonged time, alcohol abusers, smokers, acute and chronic renal failure and hemodialysis patients, other systemic diseases such as amyloid nephropathy, hepatic impairment, lupus nephritis, cardiovascular nephropathy, sickle cell anemia, amyloidosis, sacroidosis, leukemia, lymphoma, cancer of breast, colon and stomach, reaction to drugs, allergic reactions. Fasting venous blood were drawn from all.

Total antioxidant capacity (TAC) in serum was estimated by using spectrophotometric method described by D-Koracevic et al (Koracevic D, et.al., 2001). MDA one of the aldehydic by product of lipid peroxidation in serum was estimated by its thiobarbituric acid reactivity, spectrophotometric method described by Hunter et al (Hunter MI, et.al., 1985). Plasma ascorbic acid (vit C) was measured by colorimetric method described by Roe and Kuether et al (Roe JH, et.al., 1943). Lp(a) was estimated by 'Turbidimetric method' a commercially available kit from "human diagnostic kit". HCY was estimated by a commercially available kit from a "Keragen diagnostic kit method". Lipid profile, total protein and albumin were estimated by a commercially available kit from "AGAPPE" in auto analyzer. LDLC and VLDLC were calculated using friedwalds formula.

Present work was approved by institutional research and ethical committee. The mean and standard deviation were determined for each variable in all groups. All the results were expressed as mean  $\pm$  SD. Student "t" test was used to assess statistical significance of the results.

## RESULTS

All results of group II were compared with group I. The level of all biochemical parameters were significantly changed between groups I and II. Descriptive statics of diagnostic parameters in group I & group II presented in Table I & Table II. There was a statistically significant decreased level of the serum HDLC, total protein, albumin, TAC, plasma vit C level and increased serum Tchol, TGs, LDLC, MDA, HCY, Lp(a) level in group II when compared to group I.

Table III- Description about correlation coefficient and significance with diagnosed parameters in the study group II. There were positive correlation between Lp(a) & MDA, HCY was positively correlated to the serum MDA & Lp(a) where HCY supported to oxidative stress in study group II. HCY was negatively correlated to the serum TAC, TP & Alb it was related to the decreased defense system of antioxidant protection of the body, which is related to increased oxidative stress in study group II & proteinuria and albuminuria was not related to the HHCY in study group II. Total antioxidant capacity was negative correlated to serum Lp(a), supported for decreased antioxidant defense and oxidant/antioxidant imbalance in the study group II. Total protein was negative correlated to MDA, where decreased concentration of total protein supported to increased lipid peroxidation in the patients group II.

## DISCUSSION

In the present study DN patients had more severe oxidative stress than normal persons where oxidative stress plays an important intermediary role in the pathogenesis of diabetes complications. Diabetic nephropathy seemed to occur as a result of an interaction between metabolic and hemodynamic factors, which activate common pathways that lead to renal damage (Yamagishi S, et.al., 2007).

**Table-I: Comparison of routine diagnosed parameters-lipid profile, serum proteins in group I & group II**

Parameters	Group I	Group II
n	135	65
TGs (mg/dL)	112.09±10.16	213.7±8.9*
Tchol (mg/dL)	173.71±15.44	358.06±20.5*
VLDLC (mg/dL)	22.40 ± 1.98	42.74±2.7*
HDLC (mg/dL)	49.15 ± 7.4	26.71±6.7*
LDLC (mg/dL)	103.68± 8.24	288.78± 21.2*
TP(g/dL)	6.90 ± 1.6	3.53± 0.45*
Alb (g/dL)	4.34 ± 0.37	1.82±0.23*

n=No. of subjects and patients \*group I compare to group II

\* p<0.001; Highly Significant

All results expressed in mean and standard deviation (SD).

**Table II: Comparison of diagnosed biochemical parameters between control (group I) and patients (group II) with DN**

Parameters	Group I	Group II
n	135	65
Lp(a) (mg/dL)	18.15 ± 9.7	40.55±6.2**
HCY (umol/L)	10.75 ± 3.1	26.89±7.5**
TAC(mmol/L)	2.37 ± 0.87	1.16± 0.34**
MDA(nmol/mL)	1.56 ± 0.96	7.54±0.31**
Vit C(mg/dL)	1.48 ± 0.65	0.47±0.25**
p value	-----	**group I compare to group II ** p<0.001

n=No. of subjects and patients; \*\*group I compare to group II; p<0.001; Highly Significant

All results expressed in mean and standard deviation (SD).

**Table III: - Correlation coefficient and significance in the patients group II**

Parameters	Correlation coefficient(r)	Significance
Lp(a) and MDA	+0.93	p<0.001*a
HCY and MDA	+0.85	p<0.001*a
LDL and Lp(a)	+0.88	p<0.001*a
Alb and HCY	-0.55	p<0.001*a
TP and HCY	-0.61	p<0.001*a
Lp(a) and HCY	+0.80	p<0.001*a
HCY and TAC	-0.42	p<0.01*b
Lp(a) and TAC	-0.33	P<0.0001*c
TP and MDA	-0.65	P<0.001*a

\*a-Highly significant,\*b & \*c-Significant

The oxidative stress was increased in patients with DN compared to diabetic patients without nephropathy and this increase seems to be related to the severity of micro albuminuria levels (Pan HZ, et.al., 2009, Aslan M, et.al., 2007). An oxidative stress was increased in diabetes and the overproduction of ROS in diabetes was a direct consequence of hyperglycemia. Various types of vascular cells including renal cells were able to produce ROS under hyperglycemic condition. Both NADPH oxidase and mitochondrial electron gradient play roles in hyperglycemia induced ROS generation. ROS mediate hyperglycemia induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes in the kidney (Dave GS, et.al., 2007, S P Wolff, et.al., 1993). Conventional and catalytic antioxidants have been shown to prevent or delay the onset of DN, Renal lesions were associated with increased oxidative stress and decreased renal nitric oxide availability ((Dave GS, et.al., 2007, S P Wolff, et.al., 1993, Prabhakar S, et.al., 2007). Oxidative stress occurs as a result of the imbalance between ROS production and antioxidant defenses. Sources of ROS included the mitochondria, auto-oxidation of glucose, and enzymatic pathways including nicotinamide adenine dinucleotide phosphate reduce oxidase (Tan AL, et.al., 2007, Fukami K, et.al., 2008).

Oxidative stress was increased in diabetes and the overproduction of ROS in diabetes was a direct consequence of hyperglycemia. Various types of vascular cells including renal cells were able to produce ROS under hyperglycemic condition. Both NADPH oxidase and mitochondrial electron gradient play roles in hyperglycemia-induced ROS generation. In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules. ROS mediate hyperglycemia-induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes in the kidney. Furthermore, ROS-activated signaling molecules generate and signal through ROS and thus ROS act as a signal amplifier. Intensive glycemic control and inhibition of angiotensin II delay the onset and progression of diabetic nephropathy, in part, through prevention of overproduction of ROS. Conventional and catalytic antioxidants have been shown to prevent or delay the onset of diabetic nephropathy (Ha H, et.al., 2008 c).

Oxidative stress has been known to play an important role in the development and progression of diabetic nephropathy, but the intracellular signal transduction pathways regulated by reactive oxygen species (ROS) have not been clearly defined. High glucose (HG) induces intracellular ROS directly via glucose metabolism and auto-oxidation and indirectly through the formation of advanced glycation end products and their receptor binding. ROS mimic the stimulatory effects of HG and up regulated transforming growth factor-beta 1, plasminogen activator inhibitor-1, and extracellular matrix (ECM) proteins by glomerular mesangial cells, thus leading to mesangial expansion. ROS activated other signaling molecules, such as protein kinase C and mitogen-activated protein kinases and transcription factors, such as nuclear factor-kappa B, activator protein-1, and specificity protein 1 leading to transcription of genes encoding cytokines, growth factors, and ECM proteins. Finally, various antioxidants inhibit mesangial cell activation by HG and ameliorate features of diabetic nephropathy. These findings qualify ROS as intracellular messengers and as integral glucose-signaling molecules in glomerular mesangial cells in diabetic nephropathy (Maryam S, et.al., 2005). With this new concept; ROS assume a greater importance in the pathogenesis of diabetic nephropathy. Lower Se and GPx levels in diabetic patients may be implicated in diabetic nephropathy (Kromhauser C, et.al., 2008).

Serum MDA concentration was significantly higher value with diabetic nephropathy ( $p < 0.001$ ) than control, catalase & SOD activity in group of diabetic nephropathy being significantly lower than group without diabetic nephropathy (Bhatia S, et.al. 2003). Erythrocyte GSH contents was significantly lower in group of diabetic nephropathy as compared to controls (Bhatia S, et.al., 2003). Results of present study indicate the oxidative stress was increased and oxidant-antioxidant defense was imbalance with DN. These dearrangements were of higher magnitude in patients of type 2 diabetes mellitus with nephropathy (Bhatia S, et.al., 2003). No independent correlation between proteinuria (or albuminuria) and HCY levels, this study improves the external of previous negative finding (Friedman AN, et.al., 2002 a). Therefore it is unlikely that the observed positive association between proteinuria and CVD was directly related to HHCY (Buyschaert M, et.al., 2001, L Martinez CA, et.al., 2002). In diabetic nephropathy, oxidant injury and renal tubular damage accompany and may even precede microalbuminuria. The presence of these abnormalities in the absence of glomerular proteinuria favours the hypothesis that alterations first occur in the peritubular microcirculation, which by causing oxidant injury and tubular damage, may initiate diabetic nephropathy (M. Yaqoob, et.al., 1994). There were evidences for increased oxidative stress due to hypoproteinemia in DN patients.

Elevated blood levels of homocyst(e)ine represent a known independent risk factor for macrovascular disease; the link between hyperhomocyst (e) inemia and diabetic microvascular complications, hyperhomocysteinemia related to endothelial dysfunction and supported to oxidative stress (Hofmann MA, et.al., 1997).

Total serum homocysteine has been shown to predict de novo and recurrent cardiovascular events in many studies. However, results in diabetic populations with minimal nephropathy are mixed. The independent relationship between tHCY and arteriosclerotic outcomes and congestive heart failure (CHF) events in a population with high cardiovascular risk and diabetic nephropathy (Friedman AN, et.al., 2005 b). Patients with both types of diabetes and nephropathy had higher plasma homocysteine concentrations than those without nephropathy. Increases of homocysteine in plasma were related to increases in the severity of the nephropathy, increases in fasting homocysteine in diabetic patients were associated with increased albumin excretion rate, especially in those with NIDDM, thus providing a potential new link between microalbuminuria, diabetic nephropathy and cardiovascular disease (A Chico, et.al., 1998). Increased plasma homocysteine concentrations may contribute to increased morbidity and death from cardiovascular disease in adolescents and young adults with diabetic retinopathy and nephropathy (Chiarelli F, et.al., 2000).

The underlying pathogenic mechanism that links diabetic nephropathy (DN) to a high risk for CVD (Cardiovascular disease) remains unclear. In addition to traditional risk factors, including hypertension, hyperglycemia and dyslipidemia, hyperlipoproteinemia, hyperhomocysteinemia identification of novel modifiable risk factors was important in preventing CVD in people with diabetes & DN (L Martinez CA, et.al., 2002, Iwasaki T, et.al., 2008). Inflammation/oxidative stress were known to be associated with an increased risk for CVD in patients with DN (Iwasaki T, et.al., 2008). Moreover homocysteine advanced glycation end products asymmetric dimethylarginine and anemia may play a role in the development and progression of atherosclerosis in patients with DN (Iwasaki T, et.al., 2008, Aso Y, et.al., 2008).

## CONCLUSION

We conclude that oxidative stress is enhanced in DN patients due to hyperhomocysteinemia, hyperlipoproteinemia & hypoproteinemia which may contribute to the development of DN related complication with more frequency such as cardiovascular diseases and end stage renal diseases and many other complications.

Several evidences suggest that patients with DN had imbalance oxidant/antioxidant status and increased subsequent oxidative stress is due to oxidation of LDL and lipoprotein, low intake of antioxidants in diet, HHCY, hyperlipoproteinemia & hypoproteinemia. We can only hypothesize that in patients at the acute phase of the disease, decreased total antioxidant capacity may lead to abnormal lipid peroxidation, resulting in a high rate of glomerular injury. On the other hand prolonged lipid oxidation may lead to diminished antioxidant activity. Long term follow up in a large number of patients would be necessary to confirm these results. Antioxidant supplements for oxidative stress can achieve excellent long term results in the treatment of DN.

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## ABBREVIATIONS

CHD - Cardiac heart diseases, tHCY - Total homocysteine,  
HHCY - Hyperhomocyst (e) inemia, DM - Diabetes mellitus,  
CVD - Cardiovascular diseases, Tchol - Total cholesterol,  
TGs - Triglycerides, BMI - Body mass index  
VLDLC - Very low density lipoprotein cholesterol  
HDLC - High density lipoprotein cholesterol  
LDLC - Low density lipoprotein cholesterol  
TP - Total protein, Alb - Albumin, SOD - Superoxide dismutase,  
HTN - SBP - Hypertension systolic blood pressure  
DBP - Diastolic blood pressure, HG - High glucose,  
NADP - Nicotinamide adenine dinucleotide phosphate,  
ECM - Extracellular matrix, GPx - Glutathione peroxidase,  
GSH - Glutathione, Se - Selenium, CHF - Congestive heart failure,  
NIDDM - Non insulin dependent diabetes mellitus

## REFERENCES

- A Chico, A Perez, A Cordoba et al (1998) Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? [Diabetologia 41\(6\)](#): 684-693.
- Aslan M, Sabuncu T, Kocyigit A et al (2007) Relationship between total oxidant status and severity of diabetic nephropathy in type 2 diabetic patients. [Nutr Meta Cardiovasc Dis 17\(10\)](#): 734-740.
- Aso Y (2008) Cardiovascular disease in patients with diabetic nephropathy. [Curr Mol Med 8\(6\)](#): 533-543.
- Aso Y, Yashida N, Okumura K et al (2004) Coagulation and inflammation in overt diabetic nephropathy: association with hyperhomocysteinemia. [Clin Chem Acta 348\(1-2\)](#): 139-145.
- Bhatia S, Shukla R, Venkata MS et al (2003) Antioxidant status lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. [Clin Biochem 36 \(7\)](#): 557-562.
- Buysschaert M, Dramais AS, Wallemaeg PE, Hermans MP (2001) Hyperhomocysteinemia in type 2 diabetes: relationship to macroangiopathy, nephropathy and insulin resistant. [Diabetes Care 24\(6\)](#): 1123-1125.
- [Chiarelli F](#), [Pomilio M](#), [Mohn A](#) et al (2000) Homocysteine levels during fasting and after methionine loading in adolescents with diabetic retinopathy and nephropathy. [J Pediatr 137\(3\)](#): 386-392.
- Dave GS, Kalia K (2007) Hyperglycemia induced oxidative stress in Type-1 and Type-2 diabetic patients with and without nephropathy. [Cell Mol Biol 53\(5\)](#): 68-78.
- Davi G, Falco A, Patrono C (2005) Lipid peroxidation in diabetes mellitus. [Antioxid Redox Signal 7\(1-2\)](#): 256-268.
- [Dronavalli S](#), [Duka I](#), [Bakris GL](#) (2008) The pathogenesis of diabetic nephropathy. [Nat Clin Pract Endocrinol Metab 4\(8\)](#): 444-452.
- Friedman AN, Hunsicker LG, Selhub J, Bostom AG (2002) Collaborative study group proteinuria as a predictor of total plasma HCY levels in type 2 diabetes mellitus. [Diabetes Care 25\(11\)](#): 2037-2041.
- [Friedman AN](#), [Hunsicker LG](#), [Selhub J](#), [Bostom AG](#) (2005) Total plasma homocysteine and arteriosclerotic outcomes in type 2 diabetes with nephropathy. [J Am Soc Nephrol 16\(11\)](#): 3397-3402.
- Fukami K, Yamagishi S, Veda S, O Kudas (2008) Role of AGEs in diabetic nephropathy. [Curr Pharm Des 14\(10\)](#): 946-952.
- Ha H, Lee HB (2005) Reactive oxygen species amplify glucose signaling in renal cells cultured under high glucose and in diabetic kidney. [Nephrology 10 Suppl](#): 57-60.
- [Ha H](#), [Lee HB](#) (2001) Oxidative stress in diabetic nephropathy: basic and clinical information. [Curr Diab Rep 1\(3\)](#): 282-287.
- [Ha H](#), [Hwang IA](#), [Park JH](#), [Lee HB](#) (2008) Role of reactive oxygen species in the pathogenesis of diabetic nephropathy. [Diabetes Res Clin Pract 13](#); 82 Suppl 1: S42-5.
- [Hofmann MA](#), [Kohl B](#), [Zumbach MS](#) et al (1997) Hyperhomocyst(e)inemia and endothelial dysfunction in IDDM. [Diabetes Care 20\(12\)](#): 1880-1886.
- Hunter MI, Nlemadin BC, Davidson DL (1985) Lipid peroxidation product and antioxidant activity protein in plasma and cerebrospinal fluid from multiple sclerosis patients. [Neurochem Res 10](#): 1645-1652.
- Iwasaki T, Togeshi Y, Tenanchi Y (2008) Significant association of serum albumin with severity of retinopathy and neuropathy in addition to that of nephropathy in Japanese type 2 diabetes mellitus patients. [Endocr J 55\(2\)](#): 311-316.
- Koracevic D, Koracevic G, Jordjevic VD et al (2001) Method for the measurement of antioxidant activity in human fluids. [J Clin Pathol 54](#): 356-361.
- [Kramer GA](#), [Quaschnig T](#), [Greiber S](#), [Wanner C](#) (1996) Potential role of lipids in the progression of diabetic nephropathy. [Clin Nephrol 46\(4\)](#): 262-265.
- Kromhauser C, Garcia Ramirez JR, Wrobel K et al (2008) Serum Se and GPx concentration in type 2 diabetes mellitus patients. [Prim Care Diabetes 2\(2\)](#): 81-85.
- L Martinez CA, Ramos R, Gonzalez MT, Castineiras MJ (2002) Dyslipidemia and cardiovascular risk in type: 2 diabetes mellitus patients with associated diabetes nephropathy. [Nephrologia 22 Suppl 1](#): 51-58.
- [Maryam S](#), [Farvid](#), [Mahmood J](#), [Fereydown S](#), [Mostafa H](#) (2005) Comparison of the effects of vitamins and/or mineral supplementation on glomerular and tubular dysfunction in type 2 diabetes. [Diabetes Care October 28\(10\)](#): 2458-2464.
- [Miyata T](#) (2009) Novel mechanisms and therapeutic options in diabetic nephropathy. [Pol Arch Med Wewn 119\(4\)](#): 261-264.
- M Yaqoob, P McClelland, AW Patrick et al (1994) Evidence of oxidant injury and tubular damage in early diabetic nephropathy. [Q J Med 87](#): 601-607.

- Ohgas, Shikata K, Makinoh (2004) Nephrotic syndrome due to metabolic disease - special reference to diabetic nephropathy. *Nippon Rinsho* 62(10): 1907-1913.
- Pan HZ, Zhang L, Guomy et al (2009) The oxidative stress status in diabetes mellitus and diabetic nephropathy. *Acta Diabetol* 28.
- Prabhakar S, Starnes J, Shi S, Lonis B, Tran R (2007) Diabetic nephropathy is associated with oxidative stress and decreased renal nitric oxide production. *J Am Soc Nephrol* 18(11): 2945-2952.
- Roe JH, Kuether CA (1943) The determination of ascorbic acid in the whole blood and urine through the 2, 4 dinitrophenylhydrazine derivative of dehydroascorbic acid. *J Biol Chem* 147: 399-407.
- SP Wolff (1993) Free radicals, transition metals and oxidative stress in the etiology of diabetes mellitus and complications. *British Medical Bulletin* 49: 642-652.
- Tan AL, Forbes TM, Cooper ME (2007) AGE, RAGE and ROS in diabetic nephropathy. *Semin Nephrol* 27(2): 1301-1343.
- Wotherspoon F, Laight DM, Browne DL et al (2006) Plasma homocysteine oxidative stress and endothelial function in patients with type 1 diabetes mellitus & microalbuminuria. *Diabet Med* 23(12): 1350-1356.
- Yamagishi S, Fukami K, Ueda S, Okuda S (2007) Molecular mechanisms of diabetic nephropathy and its therapeutic intervention. *Curr Drug Targets* 8(8): 952-959.
- Zdemir G, Ozden M, Maral H, Kushay S, Centihalp P, Tarkun I (2005) Malondialdehyde, glutathione peroxidase and homocysteine level in type 2 diabetic patients with or without microalbuminuria. *Ann Clin Biochem* 42(8): 99-104.

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