Role of Hypertension and Metabolic Abnormalities in the Development of Diabetic Nephropathy among Egyptian Patients with Type 2 Diabetes

[1*]Azza M. El-Wakf; [2]Tarek M. Abbas ; [3]Rizk A. El-Baz and [1]Wafaa A. Mohammed.

[1]Zoology Department, Faculty of Science, Mansoura University, Egypt.
[2]Urology & Nephrology Center, Faculty of Medicine, Mansoura University, Egypt.
[3]Genetics Unit, Childern Hospital, Faculty of Medicine, Mansoura University, Egypt.
[*] dr_azzaelwakf@yahoo.com

Abstract: Diabetic nephropathy is one of the most severe diabetic micro-angiopathies. Various factors are involved in the pathogenesis of diabetic nephropathy including, interactions between metabolic and haemodynamic factors, as well as genetic susceptibility to develop nephropathy. This study aims to evaluate the role of metabolic abnormalities and hypertension in the development of chronic renal failure among type 2 diabetic cases. The measurement of fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), total cholesterol, triglycerids (TGs), high density lipoprotein cholesterol (HDL-C), creatinine (Cr) and urinary albumin was done. This study included 90 type 2 diabetic patients (mean age: 57.47 ± 0.77 year, male/female: 30/60) and 30 non diabetic healthy volunteers served as control (mean age: 51.17 ± 1.07 year, male/female: 8/22). Diabetic patients were further divided into 2 main groups: patients without nephropathy, (n =30) and those with nephropathy, (n = 60) who were subdivided into: micro-albuminuric and macro-albuminuric patients. Diabetic patients with nephropathy were older, more obese and had higher systolic and diastolic blood pressure (SBP and DSP). Diabetic patients also exhibited higher blood glucose, HbA1c and lipid profile, as well as increased serum creatinine and urinary albumin concentrations, which in all tended to be markedly pronounced in diabetic patients with macro-albuminuria (overt renal failure). The study concluded that obesity, hypertension and metabolic abnormalities are risk factors related to prevalence of nephropathy among diabetic subjects and for individual differences in its onset and severity. It is therefore possible to prevent the increasing prevalence of diabetic nephropathy by improving these factors.
[Azza M. El-Wakf; Tarek M. Abbas ;Rizk A. El-Baz and Wafaa A. Mohammed. **Role of Hypertension and Metabolic Abnormalities in the Development of Diabetic Nephropathy among Egyptian Patients with Type 2 Diabetes** Nature and Science 2011; 7(9): 220-228].(ISSN: 1545-1003). http://www.sciencepub.net/nature

Keywords: Diabetic nephropathy, type 2 diabetes, blood pressure, metabolic abnormalities.

1. Introduction:

Diabetes mellitus (DM) is a disease in which the hallmark feature is elevated blood glucose concentrations due to loss of insulin-producing pancreatic β-cells (type 1 diabetes) or through loss of insulin responsiveness in its target tissues (type 2 diabetes). Type 1 diabetes usually begins to manifest in childhood and early adulthood, but type 2 diabetes is typically a disease for which increased age is a risk factor *(Schwarz et al., 2009)*. Different studies have described diabetes as one of the main threat to human health in the 21st century *(Zimmet et al., 2001)*.

Diabetes mellitus is associated with microvascular, macrovascular and non-vascular complications *(Gispen & Biessels, 2000; Montilla et al., 2005)*.

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes *(Giunti et al., 2006)* defined as rise in urinary albumin excretion rate, often associated with an increase in blood pressure, but without evidence of other causes of renal disease *(Viberti et al., 1982; Gnudi et al., 2007)*. Specifically, it represents a major cause of morbidity and mortality in diabetic subjects *(Giunti et al., 2006)*. It is a leading cause of end-stage renal disease (ESRD), and its prevalence is progressively increasing worldwide *(Nakai et al., 2004; Foley & Colins, 2007)*.

DN develops in both type 1 and type 2 diabetes and is observed in about 5-10% of patients who suffer from non insulin dependent diabetes (NIDD) *(Gambaro et al., 1992)*. Despite this relatively low prevalence in type 2 diabetes, those patients have been widely studied, since type 2 is far more common than type 1 *(Parving, 1998)*. Classically the development of DN depends mostly on the duration of diabetes *(Pirart, 1977)* however modern theory assumed that the pathogenesis of DN is multi-factorial. In addition to chronic hyperglycemia, the major cause which activates factors and mediators of diabetic renal disease, hypertension *(Bakris et al., 2003)*, dislipidemia *(Thomas et al., 2006)* and obesity *(Price et al., 2002; Ejerblad et al., 2006)* have suggested to be risk factors and to be related for differences in onset and severity of renal disease among diabetic patients.

The present study was mainly conducted to further understand the role of those risk factors in the diabetic nephropathy, which may provide new strategies for monitoring diabetic patients before the onset of this disease.

2. Subjects and Methods

This study included 90 type 2 diabetic patients (mean age: 57.47 ± 0.77 year, male/female: 30/60) and 30 non diabetic healthy volunteers served as controls (mean age: 51.17 ± 1.07 year, male/female: 8/22). Diabetic patients

were further divided into 2 main groups: patients without nephropathy, recruited from the Internal Medicine Specialized Hospital, Mansoura University, Egypt **[**n=30 & Albumin Excretion Rate (AER)< 30 mg/24-hrs**]** and those with nephropathy, (n = 60) recruited from Urology & Nephrology Center, Mansoura University, Egypt, who were subdivided into: micro-albuminuria patients **[**n=30 & Albumin Excretion Rate (AER)= 30-300 mg/24-hrs**]** and macro-albuminuria patients [n=30, Albumin Excretion Rate (AER)> 300 mg/24-hrs & renal impairment with serum creatinine (S-Cr) levels> 1.3 mg/dl]. Type 2 DM was diagnosed according to WHO criteria (*World Health Organization, 2006*). Nephropathy was assessed based on the presence of persistent albuminuria> 30 mg/24-hrs in at least two of three consecutive measurements on 24-hrs collected sterile urine samples. Urinary albumin concentration in 24-hrs urine samples was measure by using quantitative determination of total urinary albumin. This Study Was performed after obtaining informed consent from all participating subjects.

For evaluation of nephropathy, the study subjects were matched regarding the following: Age (yr), sex (M/F), weight (Kg) duration of diabetes (yr), drugs used, clinical investigations done and family history.

Clinical investigation:
Cardiovascular examination: was performed thorough investigating the patient's cardiovascular system for evidence of heart failure, ischemic heart disease (IHD), valvular lesions, etc. Blood pressure was measured as recommended by the American Association (*Kirkendall et al.,1980)*, using mercury sphygmomanometer. A detailed Chest and abdominal examination were done for any abnormalities. Lower limbs were examined for evidence of oedema and peripheral vascular disease. Fundus examination for evidence and grade of retinopathy and neurological examination for evidence of peripheral neuropathy and hemiplegia, were also performed.

Blood collection:
Venous blood samples were collected from each examined subject, after over night (12 hrs) fasting in two separated fractions. The first blood fraction (3ml) was collected in ethylene diamine tetracetic acid (EDTA) containing tube for glycosylated hemoglobin (HbA1c) measurement. The second blood fraction (5ml) of peripheral blood sample was collected in clean centrifuge tube without anticoagultant to separate serum for biochemical analysis (fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C) and creatinine (Cr)).

Urine collection:
Subjects were instructed to collect 24-hrs urine samples for measurement of urinary albumin.

Biochemical investigations:
Blood biochemical investigations to all studied subjects were carried out by RA-50 chemistry analyzer (Bayer) using ready made chemicals (kits) supplied by Spinreact Co. Spain, according to the following methods. FBS was evaluated using quantitative method *(Kaplan,*

1984). HbA1c was evaluated by an exchange quantitative colorimetric method *(Trivelli et al., 1971)*. S-Cr was evaluated using Jaffe`. Colorimetric-Kinetic method *(Murray, 1984)*. TC was measured by enzymatic - colorimetric method *(Allain et al., 1974)*, while TGs was measured by enzymatic- colorimetric method *(Fossati & Prencipe, 1982)*. HDL-C was determined by precipitation method *(Warnick & Wood, 1995)*. Low-density lipoprotein cholesterol (LDL-C) was calculated according to formula applied by *Friedewald et al., (1972).*

Urine analysis:
Urinary albumin level was evaluated in random urine samples using dipstick test *(Duncan et al., 1994)*. Urinary albumin level / 24-hrs was evaluated by quantitative determination of total urinary albumin *(Orsonneau et al., 1989)*.

Statistical analysis*:*
All data were collected and tabulated and statistically analyzed using SPSS statistical computer package version 10 software *(SPSS 10, 1999)*. Quantitative variables were expressed as mean ± SE, while the qualitative variables were presented as numbers and percentages. Comparison of qualitative data was done using chi-square test (χ^2) or Fisher's Exact test. Quantitative data were compared using Independent- Samples T test. Statistical significance was set at $p < 0.05$.

3. Results
Analysis of clinical characteristics of the study subjects (Table 1) generally indicated that all diabetic patients were older than control subjects, however no significant variations were detected between the age of different groups. The study groups also showed no significant variations according to gender distribution. On the other hand, all diabetic groups exhibited significantly higher body weights when compared to control subjects. Similar body weight increase was also recorded on comparing diabetics with macro (D-macro) to those with micro-albuminuria (D-micro). So, it is of importance to consider the impact of increased body weight (or obesity) on development and progression of renal disease in diabetic patients. For factor of duration, significant differences were observed on comparing each of D-micro & D-macro with D-normo, but no significant variation was detected when the two albuminuric groups were compared. Meanwhile, all diabetic patients were observed to have elevated blood pressure (DBP & SBP) which tended to be significant in all diabetic subjects when compared to control subjects.

Additionally, the two albuminuric (D-micro & D-macro) groups showed highly significant blood pressure (DBP & SBP) when compared to D-normo. It was noticed that diabetic patients with macro-albuminuria are characterized by higher blood pressure (DBP & SBP) relative to other diabetic groups, suggesting that susceptibility to develop overt renal failure might be influenced by predisposition to develop higher blood pressure.

Besides, other diabetic complications (retinopathy, neuropathy, ischemic heart disease) have been recorded only in the two albuminuric diabetic group, in particular

those exhibiting macro-albuminuria (or overt renal failure), indicating the association between incidence of these complications and the severity of renal disease in diabetic patients.

In table 2 serum fasting blood sugar (FBS) was significantly increased in all diabetic subjects when compared to control. At the same time, all diabetic subjects showed significantly increased levels of glycosylated hemoglobin (HbA1c) when compared to control subjects. For the two studied variables FBS and HbA1c, statistically significant increases were detected on comparing each of diabetic groups with micro- and macro-albuminuria relative to normo- diabetic patients. All diabetic subjects exhibited marked increases in serum TC, TGs and LDL-C levels, which were significant when compared to control and normo-diabetic patients. Additionally, significant increases were also observed for all tested parameters by comparing diabetic with macro relative to diabetic with micro groups. On the other hand, HDL-C showed reverse behaviour, where decreased values were observed in all diabetic patients that tended to be significant only in the two diabetic groups with micro and macro-albuminuria when compared to control and normo-diabetic subjects. All diabetic subjects showed significantly increased serum creatinine levels when compared to control subjects. Similarly, further statistical analysis exhibited significant elevations of serum creatinine levels in diabetic patients with macro-albuminuria if compared to diabetic patients with normo and those with micro-albuminuria. All diabetic patients exhibited increased urinary albumin that tended to be markedly significant in the two diabetic groups with moderate and overt renal failure. Similarly, urinary albumin

values showed significant increase in the two diabetic groups with micro and macro-albuminuria if compared to those with normo-albuminuria, as well as on comparing diabetics patients with macro to patients with micro-albuminuria. Thus, confirming the relation between urinary albuminuria concentration and the severity of renal failure.

4. Discussion

Patients with diabetes mellitus (DM) have higher susceptibility to develop a variety of chronic complications *(Rahman et al., 2007; American Diabetes Association, 2008).* Of these the most common is nephropathy, which is the major risk factor for end stage renal disease (ESRD) *(Khan et al., 2005).* As

agreement, the present study indicated that diabetic subjects tended to develop nephropathy, which seemed to be associated with a number of complications (retinopathy, neuropathy and ischemic heart disease) indicating the association between incidence of these complications and the development of diabetic renal disease *(Sasso et al., 2006; Chandy et al., 2008; Pradeepa et al., 2010).*

By comparing the present subjects with respect to clinical characteristics, all diabetic patients were older than control subjects, however no significant variations were detected between the age of different groups. The study groups also showed no significant variations regarding the gender distribution. These results agree with findings of *Prasad et al., (2006)* and *Rahimi et al., (2011)* who showed that age and sex exhibited no significant variations among diabetic patients with micro and macro-albuminuria compared to those patients with normo-albuminuria.

Table 1. Clinical characteristics of the study subjects

Groups	Control n=30	All diabetic n=90	Diabetic n=90		
			DM with normo-albuminuria n=30	DM with micro-albuminuria n=30	DM with macro-albuminuria n=30
Age (yrs).	51.17±1.07	57.47±0.77	54.07±1.48	56.83±0.98	61.50±1.17
Sex					
Male n(%)	8(26.7%)	30(33.3%)	10(33.3%)	9(30%)	11(36.7%)
Female n(%)	22(73.3%)	60(66.7%)	20(66.7%)	21(70%)	19(63.3%)
Weight(Kg)	81.07±0.48	94.53±1.45[a]	92.47±3.38[a]	94.49±2.03[b†]	96.63±1.89[c††]
Duration (yrs).	-	12.63±0.72	9.57±1.62	12.83±0.71[†]	15.50±0.98[††]
SBP (mmHg).	122.33±1.71	155.78±2.96[a]	145.00±3.18[a]	152.67±5.69[b†]	169.67±5.20[c††]
DBP (mmHg).	76.00±1.13	88.72±1.95[a]	83.17±2.18[a]	85.33±3.66[b†]	97.67 ±3.57[c††]
Retinopathy(%)	-	(43.3%)	-	(56.7%)	(73.3%)
Neuropathy(%)	-	(60%)	-	(86.7%)	(93.3%)
Ischemic heart disease(%).	-	(41.1%)	-	(56.7%)	(66.7%)

Data are presented as mean± SE.   n: number of cases, (%)= percentage of cases. DM=diabetes mellitus, SBP= Systolic blood pressure, DBP=Diastolic blood pressure. p<0.05 (significant). a=significant difference if compared DM with normo-albuminuria and control. b=significant difference if compared DM with micro-albuminuria and control. c=significant difference if compared DM with macro-albuminuria and control.† =significant difference if compared DM with micro-albuminuria and DM normo-albuminuria. † † =significant difference if compared DM with macro-albuminuria and normo-albuminuria.

Table 2. Biochemical parameters in diabetic and control subjects

Groups	Control n=30	Diabetic n=90		
		DM with normo albuminuria n=30	DM with micro-albuminuria n=30	DM with macro- albuminuria n=30
FBS(mg/dl)	88.30±1.47	160.27±3.71[a]	14.02 [b†]± 166.63	208.53±12.29[c††]
HbA1c(%)	4.28±0.11	6.09 ±0.09[a]	7.34 ±0.27[b†]	9.01±0.29[c††]
T-Cholesterol (mg/dl)	182.10±0.54	202.03±6.95[a]	204.93±9.27[b†]	240.20±19.05[cd††]
TG(mg/dl)	83.83±0.71	139.60± 3.91[a]	147.60±16.18[b]	177.27±20.88[cd††]
HDL-C(mg/dl)	49.83±0.68	42.50±3.92[a]	39.79±2.20[b†]	36.27±2.79[c††]
LDL-C(mg/dl)	115.50 ±0.82	131.61 ±4.27[a]	135.62±7.37[b†]	168.48±18.64[cd††]
S-creatinine(mg/dl)	0.80±0.02	0.89±0.03[a]	1.06±0.04[b]	4.24±0.46[cd††]
Urinary albumin (mg/24-hr)	-	9.01±0.16	184.50±6.82[†]	606.97±28.65[d††]

Data are presented as mean± SE. n: number of cases. p <0.05(significant)
DM=diabetes mellitus, FBS= fasting blood sugar, HbA1c= Glycosylated hemoglobin. T-cholesterol=total cholesterol, TG=Triglyceride, HDL-C=High density lipoprotein-cholestrol, LDL-C=Low density lipoprotein-cholestrol. S-creatinine= serum creatinine. a=significant difference if compared DM with normo-albuminuria and control. b=significant difference if compared DM with micro-albuminuria and control. c=significant difference if compared DM with macro-albuminuria and control, d=significant difference if compared DM with micro-albuminuria and DM with macro-albuminuria. † = significant difference if compared DM with micro-albuminuria and DM with normo-albuminuria. † † = significant difference if compared DM with macro-albuminuria and DM with normo-albuminuria.

For factor of duration, the present study demonstrated significant differences on comparing each of diabetic micro and macro with diabetic normo- albuminuria, but no significant variation was detected when the two albuminuric groups were compared. In agreement, *Unnikrishnan et al., (2007)* reported that duration of diabetes was significantly higher in subjects with micro- and macro- albuminuria as compared to those in normo-albuminuric group. Thus, indicating that progression of diabetic renal disease occurs in association with increase in duration of diabetes.

According to other studies, progression of diabetic renal disease was characterized by increased serum creatinine levels *(Anjaneyulu &* chopra*, 2004)* which occurs in association with a fall in glomerular filtration rate (GFR), indicating the increasein serum creatinine as a well accepted marker for impaired renal function *(Adler et al., 2003)*. Similar findings were also detected in the present study regarding the association between the progression of diabetic renal disease and the increase in the levels of serum creatinine.

Other workers have presented albuminuria as a powerful predictor of progress to nephropathy in patients with type 2 diabetes *(Keane et al., 2003)*. Albuminuria (or elevated urinary albumin excretion) may reflect underlying renal expression of vascular damage, hypertension, endothelial dysfunction *(Stehouwer et al., 2004)*, and inflammation *(Ritz, 2003)*. Therefore, it has become clear that albuminuria is not only indicator for diabetic renal disease, but also for progress to more advanced stages of the disease *(Murussi et al., 2009)*. If diabetic renal disease progresses, GFR slowly decreased due to the reduced filtration surface *(Osterby et al., 1988)*.With decreased GFR, hypertension will develop, which in turn accelerates the filtration process *(Hovind et al., 2001)* and thereby

facilitates albumin leakage from glomerular capillaries into the urine *(Tryggvason & Pettersson, 2003)*. At first, there will be relatively small amounts of urinary albumin, (also called microalbuminuria which is defined as levels of albumin ranging from 30 to 300 in a 24-hrs urine collection). As the kidney damage progresses the amount of albumin increases and the patient develops macroalbuminuria (also called overt albuminuria or proteinuria) which is defined as levels > 300 mg/24-hrs *(Molitch et al., 2004)*.

In the present study, all diabetic patients exhibited increased urinary albumin concentration that tended to be markedly significant in the two diabetic groups with micro and macro-albuminuria. Similary, urinary albumin values showed significant increase in the two diabetic group with micro and macro-albuminuria if compared to those with normo-albuminuria, as well as on comparing diabetic patients with macro to patients with micro-albuminuria. Thus, confirming the relation between urinary albumin concentration and the severity of renal disease.

Obesity is a strong risk factor for renal failure, especially in patients with diabetes and hypertension *(Hsu et al., 2006)*. Recent studies have further highlighted the role of obesity in the renal damage observed not only in patients with obesity-related glomerulopathy but also in over weight subjects with type 2 diabetes *(Locatelli et al., 2006)*. Increased body weight may have independent effect on increasing proteinuria in type 2 DM patients and therefore body weight remains an important target of control in overweight DM patients *(Rossi et al., 2010)*. Weight loss is noted to be significantly decrease urinary protein excretion in diabetic patients with nephropathy *(Saiki et al., 2005)*.The etiology may be ascribed to the fact that adipose tissue is a source of hormones, including angiotensinogen, renin and leptin that may influence renal function and blood pressure (BP) *(Gross & Amann, 2004)*. Numerous inflammatory mediators such as TNFα, IL-6,

resistin and others *(Wisse, 2004)* are also secreted from adipose tissue. These in all may contribute to chronic inflammation, general atherosclerosis and probably insulin resistance associated with kidney disease. In the present study, all diabetic groups exhibited significantly higher body weights when compared to control subjects. Similar body weight increase was also recorded on comparing diabetics with macro to those with micro-albuminuria. So, it is of importance to consider the impact of increased body weight (or obesity) on development and progression of renal disease in diabetic patients.

Renal function and blood pressure are tightly linked. Physiologically, kidney provides a key mechanism for autoregulation of glomerular blood pressure *(Guyton, 1991)*, whereas elevated blood pressure affects renal function via pressure natriuresis mechanism *(Romero & Knox, 1988; Firth et al., 1990)*. Pathophysiologically, long standing hypertension attenuates pressure natriuresis *(Roman & Cowley, 1985)* and can cause or at least contribute to renal damage *(Griffin & Bidani, 2004)*. Therefore, hypertension is one of the imperative contributing factors associated with both causation and progression of renal failure *(Levey et al., 2002)*. Several reports have indicated the reno-protective effect of blood pressure reduction *(Lewis et al., 1999; Bakris et al., 2000)*, thus providing a more pronounced recommendation for blood pressure control in diabetics, aiming for a target of blood pressure less than 130/80 *(Chobanian et al., 2003)*. In the current study, all diabetic patients were observed to have significantly elevated blood pressure (DBP & SBP) as compared to control subjects, except for diabetics with normo-albuminuria, where non significantly increased DBP was observed. Additionally, it was noticed that diabetic patients with macro-albuminuria are characterized by the higher blood pressure (DBP & SBP) relative to other diabetic groups, suggesting that susceptibility to develop overt renal failure might be influenced by predisposition to develop higher blood pressure *(Hsu et al., 2005)*.

Higher blood pressure can adversely affect kidney function through various mechanisms. One of them is that the increased blood pressure within the glomerulus (glomerular hypertension) enhances GLUT-1 expression with concomitant increase in intracellular glucose accumulation, amplifying the deleterious effects of glucose and its metabolites within the kidney *(Gnudi et al., 2003)*. Increased blood glucose level induces injury in the kidney through various pathways, including increases in the formation of reactive oxygen species (ROS), as well as advanced glycation end products, (AGEs) which play a critical role in the development of diabetic nephropathy *(Chen et al., 2001; Twigg et al., 2002)*. AGEs are heterogeneous groups of macromolecules that are normally formed non-enzymatically by the interaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids, but their formation increases under high glucose ambience *(Brownlee, 2005; Huebschmann et al., 2006; Ahmed & Thornalley, 2007)*. Engagement of AGEs to their receptors (RAGE) has been shown to play a critical role in diabetic complications, including DN *(Yan et al., 2008)*. This is because activation of RAGE induces production of a variety of cytokines, including tumor necrosis factor β (TNFβ), which mediates an inhibition of

metalloproteinase and increases production of mesangial matrix, leading to glomerulosclerosis *(Yan et al., 2007)* and thus diabetic nephropathy.

In other way, a major role of AGEs in the development of diabetic nephropathy is through enhancing vascular permeability *(Pugliese et al., 1997)* thereby accelerating the vasculopathy of end stage diabetic renal disease *(Sugiyama et al., 1996; Fishbane et al., 1997; oldfield et al., 2001)*.

Among the most investigated AGEs is glycated hemoglobin (HbA1c) which is the product of a slow and largely irreversible reaction that occurs through non enzymatic glycation of hemoglobin *(John, 1997)*. Chronic hyperglycemia, as measured by HbA1c, is an established risk factor for diabetes associated microvascular diseases *(The Diabetes Control and Complications Trial Research Group, 1993)*. A reduction of 1% in HbA1c is associated with a 37% decrease in microvascular endpoints *(Stratton et al., 2000)*. In other studies, fasting plasma glucose concentration and HbA1c, each predict elevated albuminuria, deined as an albumin / creatinine ratio (ACR) $\geq$ 30 mg/g, after adjusting for age, sex, and duration of diabetes *(Nelson et al., 1995)*. The risk of hyperglycemia (HbA1c) amplifies the risk of micro-albuminuria conferred by increased systolic blood pressure *(Tapp et al., 2004)*. In support, *Nikzamir et al., (2009)* found that more severe albuminuria was significantly associated with higher levels of HbA1c among diabetic groups with (normo-, micro-, and macroalbuminuria). In this regard, the present study showed that, serum fasting blood sugar (FBS) was significantly increased in all diabetic subjects when compared to control. At the same time, all diabetic subjects showed significantly increased levels of HbA1c when compared to control subjects. For the two studied variables [(FBS) and (HbA1c)], statistically significant increases were detected on comparing each of diabetic groups with micro- and macro-albuminuria relative to normo- diabetic patients. Thus, indicating the importance of both glucose and HbA1c levels as predictors for developing nephropathy status (micro- and macro- albuminuria) among diabetic patients.

Apart from the role of glucose metabolic changes , it is worth stating that, plasma lipid levels have emerged as potentially important predictors of DN risk *(Hovind et al., 2004)*. In diabetes, multiple lipid abnormalities are already present at an early stage of diabetic nephropathy *(Tolonen et al., 2008)*. In other studies, increased circulating lipids and enhanced glomerular lipid synthesis have been clearly implicated in diabetic glomerulosclerosis *(Leiter, 2005)*. In addition, several recent studies, have documented enhanced kidney synthesis of triglycerides and cholesterol in diabetes *(Proctor et al., 2006; Jiang et al., 2007)*. This increased local lipid synthesis appears to be stimulated in diabetes due to a number of factors, including increased renal expression of the transcription factor, sterol regulatory element–binding protein-1, which, when over expressed in mice, causes lipid accumulation and induces expression of transforming growth factor β (TGF-β), plasminogen activator inhibitor-1, and vascular endothelial growth factor (VEGF). This endogenous kidney lipid synthesis pathways appear to directly result in enhanced accumulation of extra-cellular matrix (ECM) proteins, mesangial expansion, and glomerulosclerosis, suggesting that diabetes induces renal

glomerular synthesis of triglycerides and cholesterol, which then promotes glomerulosclerosis. In the present study, all diabetic subjects exhibited marked lipid abnormalities characterized by increased serum T-cholesterol, triglycerides and LDL-C levels, which were significant only in the two diabetic groups with micro- and macro-albuminuria when compared to control and normo-diabetic patients. Additionally, significant increases were also observed for all tested parameters by comparing diabetic with macro relative to diabetic with micro- albuminuria group. On the other hand, HDL-C showed reverse behaviour, where decreased values were observed in all diabetic patients that tended to be significant only in the two diabetic groups with micro and macro-albuminuria when compared to control and normo-diabetic subjects. Therefore, providing a mechanism whereby lipid abnormalities facilitate progression of diabetic nephropathy.

Conclusions

Diabetic patients with nephropathy were older, more obese and had higher systolic and diastolic blood pressure (SBP and DSP), blood glucose & lipid profile in both sexes (male and female). These facts underscore the necessity for correcting BP, body weight increase (obesity) and metabolic abnormalities for retarding development and progression of nephropathy among type 2 diabetic patients.

Corresponding author

Azza M. El-Wakf
Zoology Department, Faculty of Science, Mansoura University
dr_azzaelwakf@yahoo.com

References

Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR and UKPDS GROUP.,2003.Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int.; 63:225-32.

Ahmed N. and Thornalley PJ., 2007. Advanced glycation endproducts: what is their relevance to diabetic complications? Diabetes Obes Metab.;9:233-45.

Allain CC, Poon LS, Chan CS, Richmond W. and Fu PC., 1974. Enzymatic determination of total serum cholesterol. Clin Chem.;20:470-75.

American Diabetes Association, 2008. Standards of medical care in diabetes--2008. Diabetes Care.;31:12-54.

Anjaneyulu M. and Chopra K., 2004. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol.;31:244-48.

Bakris GL, Weir MR, Shanifar S, Zhang Z, Douglas J, van Dijk DJ, Brenner BM and RENAAL Study Group,2003. Effects of blood pressure level on progression of diabetic nephropathy: results from the RENAAL study. Arch Intern Med.; 163: 1555-65.

Bakris GL, Williams M, Dworkin L, Elliott WJ, Epstein M, Toto R, Tuttle K, Douglas J, Hsueh W. and Sowers J., 2000. Preserving renal function in adults with hypertension and diabetes: a consensus approach. National Kidney Foundation Hypertension and Diabetes Executive Committees Working Group. Am J Kidney Dis.;36:646-61.

Brownlee M., 2005.The pathobiology of diabetic complications: a unifying mechanism, Diabetes.;54:1615-25.

Chandy A, Pawar B, John M. and Isaac R., 2008. Association between diabetic nephropathy and other diabetic microvascular and macrovascular complications. Saudi J Kidney Dis Transpl.;19:924-28.

Chen S, Cohen MP, Lautenslager GT, Shearman CW and Ziyadeh FN., 2001. Glycated albumin stimulates TGF-beta 1 production and protein kinase C activity in glomerular endothelial cells. Kidney Int.;59:673-81.

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; 2003. National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating

Committee. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA.;289:2560-72.

Duncan JR, Prasse KW and Mahaffey EA., 1994. Veterinary Laboratory Medicine: Clinical Pathology.3rd ed. Iowa State University Press,Ames.; 162-183.

Ejerblad E, Fored CM, Lindblad P, Fryzek J, McLaughlin JK, Nyrén O., 2006. Obesity and risk for chronic renal failure. J Am Soc Nephrol.;17:1695-702.

Firth JD, Raine AE and Ledingham JG., 1990.The mechanism of pressure natriuresis. J Hypertens.;8:97-103.

Fishbane S, Bucala R, Pereira BJ, Founds H. and Vlassara H., 1997. Reduction of plasma apolipoprotein-B by effective removal of circulating glycation derivatives in uremia. Kidney Int.;52:1645-50.

Foley RN and Collins AJ., 2007. End-stage renal disease in the United States: an update from the United States Renal Data System. J Am Soc Nephrol.;18:2644-48.

Fossati P. and Prencipe L.,1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem.;28:2077-80.

Friedewald WT, Levy RI and Fredrickson DS., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem.;18:499-502.

Gambaro G, Cavazzana AO, Luzi P, Piccoli A, Borsatti A, Crepaldi G, Marchi E, Venturini AP and Baggio B., 1992. Glycosaminoglycans prevent morphological renal alterations and albuminuria in diabetic rats. Kidney Int.;42:285-91.

Gispen WH. and Biessels GJ., 2000. Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci.;23:542-49.

Giunti S, Barit D. and Cooper ME., 2006. Diabetic nephropathy: from mechanisms to rational therapies. Minerva Med.;97:241-62.

Gnudi L, Thomas SM. and Viberti G., 2007. Mechanical forces in diabetic kidney disease: a trigger for impaired glucose metabolism. J Am Soc Nephrol.;18:2226-32.

Gnudi L, Viberti G, Raij L, Rodriguez V, Burt D, Cortes P, Hartley B, Thomas S, Maestrini S. and Gruden G., 2003. GLUT-1 overexpression: Link between hemodynamic and metabolic factors in glomerular injury? Hypertension.;42:19-24.

Griffin KA. and Bidani AK., 2004. Hypertensive renal damage: insights from animal models and clinical relevance. Curr Hypertens Rep.;6:145-53.

Gross ML. and Amann K., 2004. Progression of renal disease: new insights into risk factors and pathomechanisms. Curr Opin Nephrol Hypertens.;13:307-12.

Guyton AC., 1991. Blood pressure control--special role of the kidneys and body fluids. Science; 252:1813-16.

Hovind P, Rossing P, Tarnow L, Smidt UM. and Parving HH., 2001. Progression of diabetic nephropathy. Kidney Int.;59:702-09.

Hovind P, Tarnow L, Rossing P, Jensen BR, Graae M, Torp I, Binder C. and Parving HH., 2004. Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study. BMJ.;328(7448):1105.

Hsu CY, McCulloch CE, Darbinian J, Go AS. and Iribarren C., 2005. Elevated blood pressure and risk of end-stage renal disease in subjects without baseline kidney disease. Arch Intern Med.;165:923-28.

Hsu CY, McCulloch CE, Iribarren C, Darbinian J, Go AS., 2006. Body mass index and risk for end-stage renal disease. Ann Intern Med.;144:21-28.

Huebschmann AG, Regensteiner JG, Vlassara H. and Reusch JE., 2006. Diabetes and advanced glycoxidation end products. Diabetes Care.;29:1420-32.

Jiang T, Liebman SE, Lucia MS, Phillips CL. and Levi M., 2005. Calorie restriction modulates renal expression of sterol regulatory element binding proteins, lipid accumulation, and age-related renal disease. Am Soc Nephrol.;16:2385-94.

John WG., 1997. Glycated haemoglobin analysis. Ann Clin Biochem.;34 :17-31.

Kaplan L.A., 1984. Glucose. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton;1032-1036.

Keane WF, Brenner BM, de Zeeuw D, Grunfeld JP, McGill J, Mitch WE, Ribeiro AB, Shahinfar S, Simpson RL, Snapinn SM. and Toto R., 2003. RENAAL Study Investigators. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. Kidney Int.;63:1499-507.

Khan.SA., Muhammad H. and Rehman G., 2005. Studies on Diabetic Nephropathy and Secondary Diseases in Type 2 Diabetes. Int. J. Diab. Dev. Countries ; 25: 25-29.

Kirkendall WM, Feinleib M, Freis ED. and Mark AL., 1980. Recommendations for human blood pressure determination by sphygmomanometers. Subcommittee of the AHA Postgraduate Education Committee. Circulation.;62:1146-55.

Leiter LA., 2005. The prevention of diabetic microvascular complications of diabetes: is there a role for lipid lowering? abetes Res Clin Pract.;68:3-14.

Levey AS., 2002. Clinical practice. Nondiabetic kidney disease. N Engl J Med.;347:1505-11.

Lewis JB, Berl T, Bain RP, Rohde RD. and Lewis EJ., 1999. Effect of intensive blood pressure control on the course of type 1 diabetic nephropathy. Collaborative Study Group. Am J Kidney Dis.;34(5):809-17.

Locatelli F, Pozzoni P. and Del Vecchio L., 2006. Renal manifestations in the metabolic syndrome. J Am Soc Nephrol.; 17:81-85.

Marre M., 1996. Diabetic renal disease: genetic pattern and risk factors. Reducing The Burden Of Diabetes Journal ISSN.;5-8.

Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, Steffes MW. and American Diabetes Association., 2004. Nephropathy in diabetes. Diabetes Care.;27 :79-83.

Montilla P, Barcos M, Munoz MC, Bujalance I, Munoz-Castaneda JR. and Tunez I., 2005. Red wine prevents brain oxidative stress and nephropathy in streptozotocin-induced diabetic rats. J Biochem Mol Biol.;38:539-44.

Murray RL., 1984. Creatinine In: Clinical Chemistry; Theory, Analysis and Correlation, Kaplan, L.A. and A.J. Pesce (Eds.). CV Mosby Co., St. Louis, pp: 1247-1253.

Murussi M, Murussi N, Campagnolo N, Silveiro SP., 2008. Early detection of diabetic nephropathy. Arq Bras Endocrinol Metabol.;52:442-51.

Nakai S, Shinzato T, Nagura Y, Masakane I, Kitaoka T, Shinoda T, Yamazaki C, Sakai R, Ohmori H, Morita O, Iseki K, Kikuchi K, Kubo K, Suzuki K, Tabei K, Fushimi K, Miwa N, Wada A, Yanai M, Akiba T; Patient Registration Committee, Japanese Society for Dialysis Therapy, Tokyo, 2004. An overview of regular dialysis treatment in Japan (as of 31 December 2001). Ther Apher Dial.;8:3-32.

Nelson RG, Knowler WC, Pettitt DJ, Hanson RL. and Bennett PH., 1995. Incidence and determinants of elevated urinary albumin excretion in Pima Indians with NIDDM. Diabetes Care.;18:182-87.

Nikzamir A, Esteghamati A, Feghhi M, Nakhjavani M, Rashidi A, Reza JZ., 2009. The insertion/deletion polymorphism of the angiotensin-converting enzyme gene is associated with progression, but not development, of albuminuria in Iranian patients with type 2 diabetes. J Renin Angiotensin Aldosterone Syst.;10:109-14.

Oldfield MD, Bach LA, Forbes JM, Nikolic-Paterson D, McRobert A, Thallas V, Atkins RC, Osicka T, Jerums G. and Cooper ME., 2001. Advanced glycation end products cause epithelial-myofibroblast transdifferentiation via the receptor for advanced glycation end products (RAGE). J Clin Invest.;108:1853-63.

Orsonneau JL, Douet P, Massoubre C, Lustenberger P. and Bernard S., 1989. An improved pyrogallol red-molybdate method for determining total urinary protein. Clin Chem.;35:2233-36.

Osterby R, Parving HH, Nyberg G, Hommel E, Jørgensen HE., Løkkegaard H. and Svalander C., 1988. A strong correlation between glomerular filtration rate and filtration surface in diabetic nephropathy. Diabetologia.;31:265-70.

Parving HH., 1998. Renoprotection in diabetes: genetic and non-genetic risk factors and treatment. Diabetologia.; 41:745-59.

Pirart J., 1977. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabetes Metab.;3:245-56.

Pradeepa R, Anjana RM, Unnikrishnan R, Ganesan A, Mohan V, Rema M., 2010. Risk factors for microvascular complications of diabetes among South Indian subjects with type 2 diabetes--the Chennai Urban Rural Epidemiology Study (CURES) Eye Study-5. Diabetes Technol Ther.;12:755-61.

Prasad P, Tiwari AK, Kumar KM, Ammini AC, Gupta A, Gupta R, Sharma AK, Rao AR, Nagendra R, Chandra TS, Tiwari SC, Rastogi P, Gupta BL. and Thelma BK., 2006. Chronic renal insufficiency among Asian Indians with type 2 diabetes: I. Role of RAAS gene polymorphisms. BMC Med Genet.;7:42.

Price DA, Lansang MC, Osei SY, Fisher ND, Laffel LM. and Hollenberg NK., 2002. Type 2 diabetes, obesity, and the renal response to blocking the renin system with irbesartan. Diabet Med.;19:858-61.

Proctor G, Jiang T, Iwahashi M, Wang Z, Li J. and Levi M., 2006. Regulation of renal fatty acid and cholesterol metabolism, inflammation, and fibrosis in Akita and OVE26 mice with type 1 diabetes. Diabetes.;55:2502-09.

Pugliese G, Pricci F, Romeo G, Pugliese F, Mené P, Giannini S, Cresci B, Galli G, Rotella CM, Vlassara H. and Di Mario U., 1997. Upregulation of mesangial growth factor and extracellular matrix synthesis by advanced glycation end products via a receptor-mediated mechanism. Diabetes.; 46:1881-87.

Rahimi Z, Felehgari V, Rahimi M, Mozafari H, Yari K, Vaisi-Raygani A, Rezaei M, Malek-Khosravi S. and Khazaie H., 2011. The frequency of factor V Leiden mutation, ACE gene polymorphism, serum ACE activity and response to ACE inhibitor and angiotensin II receptor antagonist drugs in Iranians type II diabetic patients with microalbuminuria. Mol Biol Rep.; 38:2117-23.

Rahman S, Rahman T, Ismail AA. and Rashid AR., 2007. Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. Diabetes Obes Metab.;9:767-80.

Ritz E., 2003. Minor renal dysfunction: an emerging independent cardiovascular risk factor. Heart.;89:963-64.

Roman RJ and Cowley AW Jr., 1985. Abnormal pressure-diuresis-natriuresis response in spontaneously hypertensive rats. Am J Physiol.;248:199-205.

Romero JC. and Knox FG., 1988. Mechanisms underlying pressure-related natriuresis: the role of the renin-angiotensin and prostaglandin systems. State of the art lecture. Hypertension.;11:724-38.

Rossi MC, Nicolucci A, Pellegrini F, Comaschi M, Ceriello A, Cucinotta D, Giorda C, Pomili B, Valentini U, Vespasiani G. and De Cosmo S., 2010. Obesity and changes in urine albumin/creatinine ratio in patients with type 2 diabetes: the DEMAND study. Nutr Metab Cardiovasc Dis.;20:110-16.

Saiki A, Nagayama D, Ohhira M, Endoh K, Ohtsuka M, Koide N, Oyama T, Miyashita Y. and Shirai K., 2005. Effect of weight loss using formula diet on renal function in obese patients with diabetic nephropathy. Int J Obes (Lond).;29:1115-20.

Sasso FC, De Nicola L, Carbonara O, Nasti R, Minutolo R, Salvatore T, Conte G. and Torella R., 2006. Cardiovascular risk factors and disease management in type 2 diabetic patients with diabetic nephropathy. Diabetes Care.;29:498-503.

Schwarz PE, Li J, Lindstrom J. and Tuomilehto J., 2009. Tools for predicting the risk of type 2 diabetes in daily practice. Horm Metab Res.;41:86-97

SPSS 10., 1999. "SPSS for windows release 10.0.1 27 Oct 1999" Standard version, Copyright SPSS Inc.

Stehouwer CD, Henry RM, Dekker JM, Nijpels G, Heine RJ. and Bouter LM., 2004. Microalbuminuria is associated with impaired brachial artery, flow-mediated vasodilation in elderly individuals without and with diabetes: further evidence for a link between microalbuminuria and endothelial dysfunction--the Hoorn Study. Kidney Int Suppl.;(92):42-44.

Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC. and Holman RR., 2000. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ.;321(7258):405-12.

Sugiyama S, Miyata T, Horie K, Iida Y, Tsuyuki M, Tanaka H. and Maeda K., 1996. Advanced glycation end-products in diabetic nephropathy. Nephrol Dial Transplant.;11:91-94.

Tapp RJ, Shaw JE, Zimmet PZ, Balkau B, Chadban SJ, Tonkin AM, Welborn TA. and Atkins RC., 2004. Albuminuria is evident in the early stages of diabetes onset: results from the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). Am J Kidney Dis.;44:792-98.

The Diabetes Control and Complications Trial Research Group, 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med.;329:977-86.

Thomas MC, Rosengård-Bärlund M, Mills V, Rönnback M, Thomas S, Forsblom C, Cooper ME, Taskinen MR, Viberti G. and Groop PH., 2006. Serum lipids and the progression of nephropathy in type 1 diabetes. Diabetes Care.;29:317-22.

Tolonen N, Forsblom C, Thorn L, Wadén J, Rosengård-Bärlund M, Saraheimo M, Heikkilä O, Pettersson-Fernholm K, Taskinen MR, Groop PH. and FinnDiane Study Group., 2008. Relationship between lipid profiles and kidney function in patients with type 1 diabetes. Diabetologia.;51:12-20.

Trivelli LA, Ranney HM, Lai HT., 1971. Hemoglobin components in patients with diabetes mellitus. N Engl J Med.; 284: 353-57.

Tryggvason K. and Pettersson E., 2003. Causes and consequences of proteinuria: the kidney filtration barrier and progressive renal failure. J Intern Med.;254(3):216-24.

Twigg SM, Joly AH, Chen MM, Tsubaki J, Kim HS, Hwa V, Oh Y. and Rosenfeld RG., 2002. Connective tissue growth factor/IGF-binding protein-related protein-2 is a mediator in the induction of fibronectin by advanced glycosylation end-products in human dermal fibroblasts. Endocrinology; 143:1260-69.

Unnikrishnan RI, Rema M, Pradeepa R, Deepa M, Shanthirani CS, Deepa R. and Mohan V., 2007. Prevalence and risk factors of diabetic nephropathy in an urban South Indian population: the Chennai Urban Rural Epidemiology Study (CURES 45). Diabetes Care.;30:2019-24.

Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U. and Keen H., 1982. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet.;1:1430-32.

Warnick GR. and Wood PD., 1995. National Cholesterol Education Program recommendations for measurement of high-density lipoprotein cholesterol: executive summary. The National Cholesterol Education Program Working Group on Lipoprotein Measurement. Clin Chem.; 41:1427-33.

Wisse BE., 2004. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. J Am Soc Nephrol.;15:2792-800.

World Health Organization, 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Report of a WHO consultation World Health Org, Geneva

Yan HD, Li XZ, Xie JM. and Li M,. 2007. Effects of advanced glycation end products on renal fibrosis and oxidative stress in cultured NRK-49F cells. Chin Med J (Engl).;120:787-93.

Yan SF, Ramasamy R, Schmidt AM., 2008. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. Nat Clin Pract Endocrinol Metab.;4:285-93.

Zimmet P, Alberti KG. and Shaw J., 2001.Global and social implications of the diabetes epidemic. Nature.;414 (6865): 782-