Original Article

Impairment of Renal Function in Non-proteinuric Diabetic Patients

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Objective: To evaluate renal function in non-proteinuric diabetic patients.

Material and Methods: It was a descriptive analytical study, conducted in University of Health Science Lahore, from February 2010 to January 2011, with a sample size of 195 diabetic subjects. They were divided equally among normoalbuminuric, microalbuminuric and macroalbuminuric groups, according to their daily urinary albumin excretion rate (AER), with 65 patients in each group. Their renal function status and GFR was evaluated by conducting tests on serum and urine samples.

Kruskal-Wallis test and Mann-Whitney U test were used to observe differences of medians in different groups. p value less than 0.05 was taken statistically significant.

Results: There was predominance of males in microalbuminuric and macroalbuminuric groups while females were more in number in normoalbuminuric group. Majority of normoalbuminuric individuals were unmarried, while married individuals were prevalent in microalbuminuric and macroalbuminuric group. Significant differences were found in serum urea concentration, serum creatinine concentration, serum uric acid concentration, glomerular filtration rate, urinary creatinine concentration, urine flow rate, daily albumin excretion rate and urinary albumin concentration among the three groups. Urinary creatinine concentration and glomerular filtration rate were in the highest ranges in normoalbuminuric group and in the lowest ranges in macroalbuminuric group. While rest of the parameters (ie. age, duration of diabetes, serum urea concentration, serum creatinine concentration, urine flow rate, daily AER , urinary albumin concentration and serum uric acid concentration) were in the lowest ranges in normoalbuminuric group and in the highest ranges in macroalbuminuric group. There was significant renal function impairment in microalbuminuric stage of diabetic nephropathy. $p < 0.05$ was taken statistically significant.

Conclusion: It is concluded that impairment in renal function occurs even in microalbuminuric stages of diabetic nephropathy. Renal functions are impaired even in the diabetic patients who have not yet developed frank proteinuria.

Keywords: Diabetic nephropathy, Proteinuria, Renal function, Glomerular filtration rate.

Introduction

Diabetic nephropathy (DN) accounts for about 40% of new cases of end-stage renal disease (ESRD) in the United States (ADA, 2004)[1] and it is the leading cause of diabetes related morbidity and mortality (Powers, 2008)[2]. Diabetic nephropathy (DN) is staged on the basis of degree of urinary albumin excretion rate (AER) per day. According to American Diabetic Association (ADA), DN is classified in terms of microalbuminuria (or incipient nephropathy) (urinary AER: 30-299 mg/day); and macroalbuminuria (or overt nephropathy) (urinary AER: ≥300 mg/day) (ADA, 2004)[1]. A more extensive classification has been proposed by Mogensen (1997)[3] and is now generally accepted for both research and clinical purposes **(Table-1).** The complex pathogenesis for the development of DN is not fully clarified (Parving et al, 2004)[4].

Stages of Diabetic Nephropathy:

Stage -1: Glomerular hypertension and hypertrophy

Stage-2: Silent stage with normoalbuminuria (AER: < 30 mg/24 hours)

Stage-3: Incipient diabetic nephropathy or microalbuminuria (AER: 30-300 mg/24 hours)

Stage-4: Overt diabetic nephropathy or macroalbuminuria (AER: > 300 mg/24 hours)

Stage -5: End stage renal disease (Mogensen, 1997)[3]

But like other microvascular complications, the pathogenesis of DN is related to chronic hyperglycemia. The mechanisms by which chronic hyperglycemia leads to ESRD, though incompletely defined, involve the effects of soluble factors, hemodynamic alterations in the renal microcirculation and structural changes in the glomerulus (Powers, 2008)[2]. Hyperglycemia can lead to the activation of oxidative stress and increased

Production of reactive oxygen species (Ruggenenti *et al.*, 2010).[5] The earliest functional abnormality in diabetic kidney is renal hypertrophy associated with raised glomerular filtration rate (GFR). This appears soon after diagnosis and is related to poor glycaemic control. As the kidney becomes damaged by DM, the afferent arteriole becomes vasodilated to a greater extent than the efferent arteriole. This increases the intraglomerular filtration pressure, further damaging the glomerular capillaries. This increased intraglomerular pressure also leads to increased shearing forces locally which are thought to contribute to mesangial cell hypertrophy and increased secretion of extracellular mesangial matrix material. This process eventually leads to glomerular sclerosis. The initial structural lesion in the glomerulus is thickening of the basement membrane. Associated changes result in disruption of the protein cross-linkages which normally make the membrane an effective filter. In consequence, there is a progressive leak of large molecules, particularly proteins, into the urine (Gale and Anderson, 2009)[6]. The earliest evidence of this is 'microalbuminuria' in which the amount of urinary albumin is so small as to be undetectable by standard dipstick. At the later stage of glomerulosclerosis, the glomerulus is replaced by hyaline material. A rise in plasma creatinine is a late feature that progresses inevitably to renal failure, although the rate of progression may vary widely between individuals (Gale and Anderson, 2009)[6]. After 5-10 years of type 1 DM, 40% of individuals begin to excrete small amounts of albumin in the urine. Microalbuminuria is defined as 30-300 mg/day in a 24 hour collection or 30-300 µg/mg creatinine in a spot collection. Although the appearance of microalbuminuria in type 1 DM is an important risk factor for progression to overt proteinuria (>300 mg/day), only 50% of individuals progress to macroalbuminuria over the next 10 years **(Figure 1).** The relationship of time from onset of diabetes, the glomerular filtration rate, and serum creatinine are shown. (Powers, 2008)[2]

Once macroalbuminuria is present, there is a steady decline in GFR, and 50% of individuals reach ESRD in 7-10 years. Blood pressure rises slightly and the pathologic changes are likely irreversible. Some individuals with type 1 or type 2 DM have a decline in GFR in the absence of micro- or macroalbuminuria and this is the basis for assessing the GFR on an annual basis (Powers, 2008)[2]

Renal function changes in DN conventionally have been linked to progression of urinary AER. More contemporaneous research findings have challenged this paradigm. Rather, the process of renal function loss appears to begin prior to the onset of proteinuria. The lower limit of normal GFR is considered as 90 mL/min/1.73 m2. GFR decreases with advancing kidney disease (NKF, 2002)7 and may be impaired even before macroalbuminuria starts (Rosolowsky et al, 2008)8. Detection of renal function decline before the development of macroalbuminuria may be helpful for initiating preventive measures so as to delay the advanced kidney disease.

Materials and Methods

It was a descriptive, analytical study, conducted in the Department of Physiology and Cell Biology, University of Health Sciences, Lahore. The study span was one year.

A target population of 195 diabetic subjects was selected according to inclusion and exclusion criteria, and was categorized into 3 groups, as follows:

Group A: 65 macroalbuminuric diabetics.
Group B: 65 microalbuminuric diabetics.
Group C: 65 normoalbuminuric diabetics.

Convenient sampling was done among the diagnosed registered cases of type 1 and type 2 diabetes mellitus from SIMS, Lahore.

The subjects selected were:

Diagnosed patients of diabetes mellitus. Both male and female patients irrespective of age. The subjects with following conditions were excluded:

Current use of diuretics or uricosuric drugs.
Urinary tract infection.
Serum creatinine > 2 mg/dL.

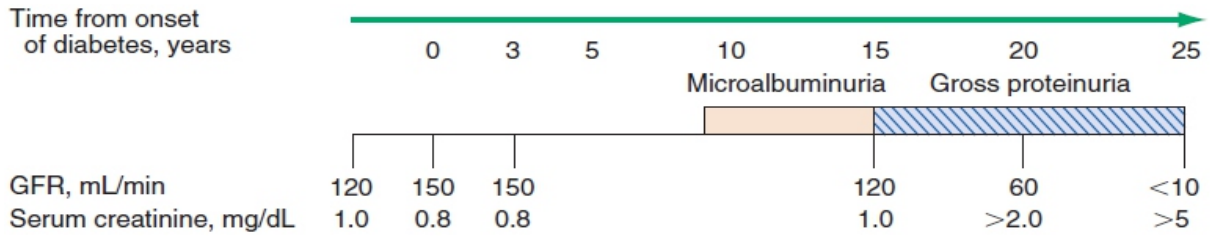

Figure-1: Time course of development of diabetic nephropathy.

Overt kidney disease, other than diabetic nephropathy.
Gout.
Diabetic patients were selected from the medical wards and diabetic clinics of the tertiary care hospitals of Lahore and medical record of every patient was evaluated for any concomitant medical condition (gout) or any overt kidney disease other than diabetic nephropathy. After getting written informed consent, the demographic data of all the subjects was collected and every individual was assessed by taking history and performing physical examination, using specially designed questionnaire. Blood and urine samples were taken. Patient's urine was assessed for proteinuria on the bedside, with the help of Urinalysis Reagent Strips. Proteinuric state determined that the patient was having macroalbuminuria (frank proteinuria), while non-proteinuric state determined that the patient was either having microalbuminuria or normoalbuminuria. So for the quantitative, accurate and final determination of albumin levels in the urine, more sensitive technique of radioim- munoassay was used on 24 hour urine sample. GFR was calculated using the formula: GFR = U.V/P.
The data was entered into and analyzed by SPSS (Statistical Package for Social Sciences) version 17.0. Kruskal-Wallis test and Mann-Whitney U test were used to observe differences of medians in different groups. P value less than 0.05 was taken statistically significant.

Results

All three groups were compared for demographic data as well as renal functions parameters. Following results were obtained:

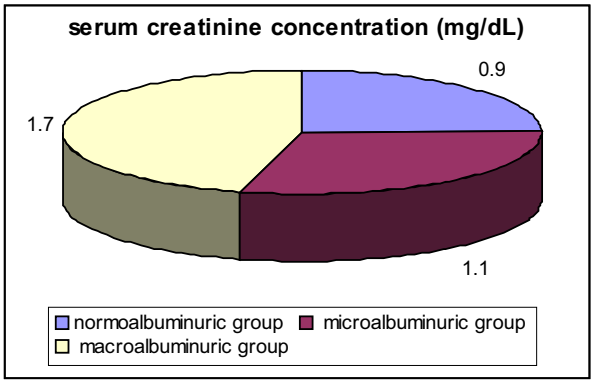

Figure-1: Comparison of serum urea concentration among normoalbuminuric, microalbuminuric and macroalbuminuric group. p <0.001.

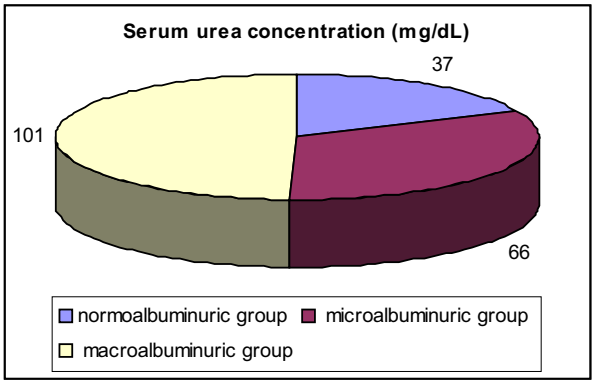

Figure-2: Comparison of serum creatinine concentration among normoalbuminuric, microalbuminuric and macroalbuminuric group. p <0.001.

Discussion

The results of the study showed remarkable differences in various parameters among the three groups. Overall, macroalbuminuric group was the worst in renal function parameters among the three

Table-1: Comparison of demographic data of three groups.

Variables	Normoalbuminuric Group (n=65)	Microalbuminuric Group (n=65)	Macroalbuminuric Group (n=65)	P-value
Gender	Male: 26 (40%)	Male: 51 (78.5%)	Male: 44 (67.7%)	<0.001
	Female: 39 (60%)	Female: 14 (21.5%)	Female: 21 (32.3%)	<0.001
Marital status	Married: 16 (24.6%)	Married: 61(93.8%)	Married: 65 (11%)	<0.001
	Unmarried: 49 (75.4%)	Unmarried: 04 (6.2%)	Unmarried: 0 (0%)	<0.001
Median Age (Years)	21	36	55	<0.001
	(18-24.5)*	(33.41)*	(49.57)*	
Median duration of DM (year)	04	11	20	<0.001
	(2-5)	(9-12)*	(15-24.5)*	

Table-2: Comparison of renal function tests of three groups.

Variables	Normoalbuminuric Group (n=65)	Microalbuminuric Group (n=65)	Macroalbuminuric Group (n=65)	P-value
Median Serum urea concentration (mg/dL)	37	66	101	<0.001
	(28.5 - 46.5)*	(58 - 73)*	(87.5 - 50.5)*	
Median serum creatinine concentration (mg/dL)	0.9	1.1	1.7	<0.001
	(0.8 - 0.95)*	(1.1 - 1.1)*	(1.6 -1.9)*	

Groups. However the differences existed in every parameter of study. Although the number of females was greater in normoalbuminuric group, where they were constituting 3/5th of the subjects, the males clearly outnumbered females in rest of the two groups. In microalbuminuric group, males were nearly 4/5th and in macroalbuminuric group, they constituted slightly more than 2/3rd of the subjects in the group. The higher percentage of males in the groups which were having advanced stage of DN may reflect that the prevalence of DN may be greater in male individuals of the society, and therefore they presented in higher numbers in tertiary care hospitals and had greater chances of being selected as study subjects. Hovind et al. in 2009 worked on patients with DN and majority of the subjects in all three groups in their study were males, with 56% in normoalbuminuric, 67% in microalbuminuric and 70% in macroalbuminuric group[9]. The marital status also exhibited a peculiar trend in the present study. The married subjects in normoalbuminuric group were only about one fourth of the total diabetics in the group but they exceeded 90% of the cases in microalbuminuric group and there was even no unmarried subject in macroalbuminuric group. This is also consistent with the fact that the unmarried subjects were younger and had less advanced disease and vice versa. Although marital status does not directly alter the course of DN but it is suggestive of the age of the individual which is directly correlated with the stage of DN. In the present study, the median age of the normoalbuminuric group was only 21 years as compared to 36 years in microalbuminuric group and 55 years in macroalbuminuric group. This also suggests advanced disease in older age group and mild changes in younger age group. The median duration since the diagnosis of DM in the subjects was less (only 4 years) in normoalbuminuric group as compared to microalbuminuric group (11 years) and macroalbuminuric group (20 years). The AER showed a wide range of values among the three groups. This was the parameter on which all the subjects were divided into three different groups. There was significant difference of renal function status among the three groups. Also significant negative correlation was found between AER and GFR in normoalbuminuric and macroalbuminuric groups. However, there was no significant correlation between the two in microalbuminuric group. The renal function status was assessed by estimating the GFR, serum urea and creatinine levels. The GFR significantly differed among the three groups which is consistent with the previous work (Rigalleau et al., 2007)[10]. Also the GFR was inversely correlated with the albuminuric state in the normoalbuminuric and macroalbuminuric groups. Rigalleau et al. in 2007 observed that GFR has a declining trend from normoalbuminuric to macroalbuminuric stage, in the start of their study, as well as at the end of it. They calculated GFR from MDRD equation, which is an estimated GFR (eGFR) rather than real values obtained by measuring urinary creatinine concentration in 24 hour urine. But for the current discussion, their values give sufficient evidence of renal function decline over the stages of DN. So their findings are consistent with our results in this regard. But for better and more descriptive results, a follow up study is required in our set up. Also Rosolowsky et al. in 2008 determined GFR by cystatin c method in normoalbuminuric and microalbuminuric subjects in their study of 675 subjects and reported that although mean GFR was not abnormally low in the microalbuminuric group, but it did decrease to a statistically lower value than that of normoalbuminuric subjects. GFR depends on various factors: urine flow rate, daily water intake, dietary protein and even the temperature of the environment. These factors may be different in different countries, and even different regions in the same country. So although the reference range of GFR is taken to be 80-130 mL/min/1.73 m^2 (Granerus and Aurell, 1981)[11], the normal values must be described for every region and ethnic group of the world, especially for our country, in order to have a clear picture of the normal and abnormal values.

With the advancing stage of DN, GFR decline occurs at the rate of 10 mL/min/decade (Granerus and Aurell, 1981)[11]. In our study this correlation was also found in normoalbuminuric and macroalbuminuric group but not in microalbuminuric group. This finding prompts us to further evaluate our population for improving our diagnosis and treatment of DN. ADA now recommends screening of chronic kidney disease (CKD) in diabetic patients, based both on daily AER and GFR (Kramer and Molitch, 2005).[12] Serum urea and creatinine are also parameters of renal function status. They are taken to be routine markers for renal function decline and therefore have been incorporated in various equations of GFR calculation (Bostom et al., 2002).[13] In our study, serum urea and serum creatinine gave expected results in relation to stages of DN. These parameters were significantly different among the three groups with the lowest values in normoalbuminuric group and highest values in the macroalbuminuric group. Serum urea and creatinine were increased even in microalbuminuric patients. Serum creatinine is of particular importance, because it is also used for indirect measurement of GFR and has been used by various researchers for this purpose. Creatinine clearance is a routine but not perfect marker of renal function status and GFR, because a small amount of it is secreted by the tubules, so that the amount of creatinine excreted slightly exceeds the amount filtered. On the other hand, there is normally a slight error in measuring plasma creatinine, that leads to an overestimation of the plasma creatinine concentration. These two errors tend to cancel each other, so that the creatinine clearance provides a reasonable estimate of GFR (Hall, 2011)[14]. The values of serum creatinine were given in μmol/L in majority of the research works, with the conversion method being to divide the value of μmol/L by 88.4 to obtain values in mg/dL. As daily AER increases, serum creatinine rises while there is concomitant decline in the GFR. These results are in consistent with the previous work done by Perkins et al. in 2007, in which one third of the microalbuminuric population in their study had early progressive renal function decline[15].

Conclusion

It is concluded that impairment in renal function occurs even in microalbuminuric stages of diabetic nephropathy (ie. before the development of frank proteinuria). Renal functions are impaired even in the diabetic patients who have not yet developed frank proteinuria.

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Answer Picture Quiz

Extremely extensive new necrosis is demonstrated involving the stomach, the entire small bowel, and right side of colon extending to the splenic flexure. There is no appreciable neural enhancement. Extensive portal vein, with extensive gas filling of branches of the left portal vein within the liver. The descending colon and sigmoid colon appear unremarkable, with normal enhancing walls. Part of the stomach mucosa appeared to enhance, PEG in situ.

The celiac trunk, superior mesenteric artery, and inferior mesenteric artery opacify normally, with no thromboembolism or occlusion evident. The abdomen appears distended, with compression of the IVC.

A 10 cm pelvic mass is present, which is smaller and hypodense compared to earlier CT scans from last year. Degenerative fibroid. The kidneys, liver, pancreas and spleen are normally in appearance.

Conclusion:

Very extensive necrosis involving the stomach, small bowel and right-sided colon. Portal venous gas.