Original Research Article

Evaluation of Proteinuria in Normotensive Diabetics in a Tertiary Care Hospital

Authors

Mukherjee Brijesh1*, Mishra Prafulla Kumar2

1Associate Professor, Dept of Biochemistry, Hi-tech medical College and Hospital, Rourkela, Odisha, India
2Professor & Head, Dept of Biochemistry, Hi-tech medical College and Hospital, Rourkela, Odisha, India

*Corresponding Author

Dr Brijesh Mukherjee
Associate Professor, Hi-tech medical College and Hospital, Rourkela, Odisha, India
Mobile: 919437115479, Email: taararia@gmail.com

Abstract

Background: Proteinuria has been generally regarded as a marker for the degree of glomerular damage in diabetes. The levels of proteinuria correlate well with the progression for renal functions and interventions that retard the progression of diabetes renal disease also reduce proteinuria. The aim of this study is to collate information on the incidence of proteinuria among normotensive diabetic patients attending Hi-tech Medical College and Hospital, Rourkela, Odisha

Materials and Methods: The study involved 100 diabetics and 50 healthy controls. Proteinuria was estimated using biuret method, while fasting blood glucose using glucose oxidase method.

Result: The study shows that an overall 22% of the patients have diabetes related proteinuria with 17% males and 5% females. The duration of the disease < 5 years (42.0%) and > 5 years (58.0%) have 14.5% and 31.1% diabetes related proteinuria respectively. An average age of < 30 years (70%) and > 30 years (30%) have diabetes related proteinuria of (17.1%) and (24.6%) respectively. FBG differed significantly (p<0.05) between patients (11.01±1.03Mmol/L) and controls (4.38±0.07Mmol/L). Urinary protein excretion was significantly higher in diabetics (143.7±5.78) than in controls (90.43±5.78). Increased urinary protein excretion was observed (p<0.05) with duration of diagnosis <5 years (228±5.4mg/24hrs) and >5 years (264±9.1mg/24hrs). Statistically significant increase in proteinuria (p<0.05) was also observed in males (254±10.0mg/24hrs) than in females (194±29mg/24hrs).

Conclusion: Given the large number of individuals with diabetes is increasing; the number of diabetic nephropathy is undoubtedly enormous. This could produce major constraints on health care budgets in the future. This urgently calls for not only good control of diabetes to prevent nephropathy but also to address the larger issue of primary prevention of diabetes, that is, reduction in the prevalence of diabetes itself by aggressive life style modifications.

Keywords: Proteinuria, diabetes mellitus, normotensive, fasting blood glucose.

Introduction

Proteinuria has been generally regarded as a marker for the degree of glomerular damage in diabetes [1]. The levels of proteinuria correlate well with the progression for renal functions and interventions that retard the progression of
diabetes renal disease also reduce proteinuria. However, it has not yet been known whether the flux of proteins across the glomerular basement membrane is causally implicated in the evolution of diabetes renal disease or simply reflects glomerular damage \[1\].

The association of proteinuria with diabetes mellitus was first recognized in the eighteenth century and later Kimmelstied and Wilson in 1936 who defined the condition by describing the lesions of nodular glomerulosclerosis and the association with proteinuria and hypertension in type 2 diabetes \[2\]. These features represent a late stage in the progression of the condition \[2\]. Complication associated with proteinuria in diabetes include: increased risk of CVD, arterial and venous thrombosis, including renal vein thrombosis, pulmonary oedema due to fluid overload, acute renal failure due to intravascular depletion and increased risk of bacterial infection including spontaneous bacteria peritonitis \[3\].

According to the centers for Disease Control and Prevention in 2008, approximately 44% of new cases of kidney were caused by diabetes. About 48,374 diabetics already began treatment to ESRD, and about 202,290 diabetics with ESRD have been on long-term dialysis or had a renal transplant \[4\]. In 2010 United State Renal Data System Reported 29.1% of individuals with self reported diabetes had stage 2 or 3 chronic kidney diseases \[4\]. Diabetes renal disease typically manifests after 10 years’ duration in type 1, whereas approximately 3% of newly diagnosed type 2 have overt nephropathy \[5\].

Hypertension is strongly associated with insulin resistance, even in the absence of diabetes \[6\]. About 40-70% and only about 25% of type 2 diabetes and type 1 diabetes respectively have been described to be hypertensive \[7\]. Hypertension has been considered to be an independent risk factor for development of proteinuria \[8\]. Diabetes renal disease characterised by nephritic syndrome and diffuse scarring of the glomeruli is due to long-standing diabetes and a prime reason of dialysis in many developed countries. It is also identified as a small blood vessel complication of diabetes \[3\]. During its early course, diabetes nephropathy often has no symptoms and can take 5-10 years after kidney damage begins \[9\]. These late symptoms include severe tiredness, headaches, a general feeling of illness, nausea, vomiting, frequent voiding, lack of appetite, itching skin and leg swelling \[9\].

The aim of this study is to collate information on the incidence of proteinuria among normotensive diabetic patients attending Hi-tech Medical College, Rourkela.

**Material and Methods**

All chemicals and reagents for this study are of analytical grade. Kits for proteinuria and blood glucose estimation were purchased from Randox company ltd.

**Ethical Consideration and Clearance**

An ethical clearance for this study was sought and obtained from ethical committee of the hospital prior to the commencement of this study.

**Analytical Design**

One hundred (100) diabetic patients and fifty (50) apparently healthy individuals (normoglycemic) as control was recruited for this study. Biuret method was employed for urinary protein estimation and blood glucose using glucose oxidase method. Systolic and diastolic blood pressure of more than 140/90 mmHg for both controls and the patients were excluded for this study.

**Sample Collection**

First morning void and 24 hours urine samples, and venous blood sample from both controls and patients was collected into their appropriate containers. Boric acid was used as preservative for urine samples and EDTA as anticoagulant in blood samples.

**Statistical/Data Analysis**

The data obtained was analyzed using Microsoft Excel for Windows VII version, SPSS (Statistical Package for Social Sciences) and GraphPad Prism 6.0 version. The values obtained were compared using students’ t-test. P-value less than or equal to
0.05 (≤0.05) was considered statistically significant.

Results
A total of one hundred and fifty (150) subjects of both sexes were recruited for this study. They consisted of seventy (70) type 2 diabetics, thirty (30) type 1 diabetics and fifty (50) controls (Table 1). The demographic and clinical characteristics of the study subjects are presented in Table 1. Thirty percent (30%), (15/50) of the control subjects, 33.3% (10/30) of type 1 diabetes, 28.5% (20/70) of type 2 diabetes and 36% (45/150) of the total subjects were females. There was no significant difference (p>0.05) between males and females within the group as regards to age, duration of disease and glycemic status.

The Means±SEM of 24 hours proteinuria and first morning void in Type 1 diabetics and controls is shown in table 2. The means proteinuria (both 24 hours and first morning void) in the type 1 diabetics were significantly higher (P<0.05) than the corresponding values in controls.

Tables 3 shows the Mean±SEM for proteinuria in type 2 diabetics and controls. Both 24 hours proteinuria and first morning void was shown to be significantly higher (P<0.05) in type 2 diabetics than in control.

The incidence of proteinuria according to sex, age and duration of disease is shown in Table 4. Males, age and duration of disease greater than five years have greater influence on the incidence of proteinuria.

Predictors of proteinuria are presented in Table 5. Statistically significant difference (p<0.05) was observed between the duration of diagnosis <5 years (228±5.4) and >5 years of diagnosis (264±9.1). There is also statistically significant difference (p<0.05) between males (254±10.0) and females (194±29.0). However, there is no statistically significant difference (p>0.05) between patients with <30 years of age (273±7.4) and >30 years of age (271±10.0).

Table 1: Demographic and clinical characteristics (Mean±SEM) of the study subjects

| Subject         | N  | Mean age | Mean DOD/Yrs | Mean FBG (Mmol/L) |
|-----------------|----|----------|--------------|-------------------|
| Control         | 50 | 47.9±1.68 |              | 4.38±0.07        |
| Males           | 35 | 49.3±2.0  |              | 4.25±0.07        |
| Females         | 15 | 46.7±1.85 |              | 4.67±0.16        |
| Type 1 DM       | 30 | 37.0±2.84 | 3.28±0.58    | 11.44±0.35       |
| Males           | 20 | 37.5±2.29 | 3.01±0.46    | 11.37±0.59       |
| Females         | 10 | 36.1±3.7  | 3.54±0.65    | 11.56±1.03       |
| Type 2 DM       | 70 | 50.2±3.12 | 7.18±0.91    | 10.69±1.01       |
| Males           | 50 | 47.5±2.8  | 6.98±0.84    | 10.59±0.84       |
| Females         | 20 | 52.5±4.3  | 8.15±0.93    | 10.95±0.73       |
| p-value         |    | >0.05     | >0.05        | <0.05             |

N=number of population group, SEM=standard error of mean, DOD =duration of disease, FBG= fasting blood glucose, Mmol/L= millimole per liter, Yrs=years, p-value is within the group.

Table 2: Proteinuria (Mean±SEM) in type 1 diabetics and controls subjects

| Parameters                  | Males      | Females    | Pooled     |
|-----------------------------|------------|------------|------------|
| Prot(mg/24 hrs)- Control    | 90.4±5.78  | 104±6.06   | 96.2±4.48  |
| Prot(mg/24 hrs)- Type 1 Diabetics | 140.7±25.18 | 112.5±27.29 | 131.3±23.81 |
| Prot(mg/dl)(1’st mv)- Control | 5.9±1.16   | 11.9±0.75  | 6.9±0.98   |
| Prot(mg/dl)(1’st mv)- Type 1 Diabetics | 8.8±2.86   | 5.4±2.03   | 8.2±2.46   |
| p-values                    | <0.05      | <0.05      | <0.05      |

SEM=standard error of mean, Prot= proteinuria, 1st mv= first morning void, Mg/24hrs= milligram/24hours, p-value is between the groups.
Table 3: Proteinuria (Mean±SEM) in type 2 diabetics and controls subjects

| Parameters                          | Males       | Females     | Pooled     |
|-------------------------------------|-------------|-------------|------------|
| Prot(mg/24 hrs)- Control            | 90.43±5.78  | 104±6.06    | 96.24±4.48 |
| Prot(mg/24 hrs)- Type 2 Diabetics   | 143.7±26.20 | 127.5±22.6  | 136.86±27.24 |
| Prot(mg/dl)(1st mv)- Control        | 7.9±1.16    | 11.93±10.75 | 6.91±0.98  |
| Prot(mg/dl)(1st mv)- Type 2 Diabetics| 10.94±0.73  | 9.94±3.1    | 10.15±2.02q|
| p-values                            | <0.05       | <0.05       | <0.05      |

SEM=standard error of mean, Prot= proteinuria, 1st mv= first morning void, Mg/24hrs= milligram/24hours, p-value is between the groups.

Table 4: Prevalence of proteinuria according to demographic, incidence of variables and duration disease

| Subject                        | N  | With proteinuria | Percentage (%) |
|--------------------------------|----|------------------|----------------|
| Males                          | 70 | 17               | 24.3           |
| Females                        | 30 | 05               | 16.6           |
| <30 years of age               | 30 | 06               | 17.1           |
| >30 years of age               | 70 | 16               | 24.6           |
| <5 years of DOD                | 42 | 08               | 14.5           |
| >5 years of DOD                | 58 | 14               | 31.1           |

DOD= duration of diagnosis, Yrs= years, %= percentage, N= Number of population group.

Tables 5: Predictors of proteinuria (Mean±SEM) in the diabetes subjects

| Subject variables          | Prot (mg/24hrs) | p-value |
|----------------------------|-----------------|---------|
| DOD <5 years               | 228±5.4         | <0.05   |
| DOD >5 years               | 264±9.1         | <0.05   |
| <30 years of age           | 273±7.4         | <0.05   |
| >30 years of age           | 271±7.0         | >0.05   |
| Males                      | 254±100         | <0.05   |
| Females                    | 194±29          | <0.05   |

Prot= proteinuria, DOD= duration of diagnosis, mg/24hrs= milligram/24hours, p-value is between the groups.

Discussion
The present study shows the overall 22% of the patients have diabetes related proteinuria, with 17% males and 5% females. After 5 years' duration, the frequency of diabetes related proteinuria increases to 31%. Stephenson et al reported that type 1 and type 2 diabetic patients had similar prevalence rates of proteinuria [10]. In type 1 diabetes, proteinuria is associated with nephropathy and renal failure [11], while in type 2 diabetic patients proteinuria is widely associated with cardiovascular rather than renal disease [12]. Among Europeans, diabetic nephropathy is reported to develop in 35% of patients with type 1 and 3%–15% of patients with type 2 diabetes [13]. Prevalence rates would of course vary widely depending on the methodology and definitions used for proteinuria. Fabre et al reported a prevalence of 48% with abnormal protein excretion (>150 mg in 24 hours) among 510 type 2 diabetic patients [14].

Studies from the UK have shown that among migrant Asian Indians, the prevalence of both diabetic and “nondiabetic” renal disease is higher compared with Europeans [15]. John et al, in a report from Vellore in southern India found that 8.4% of patients had persistent proteinuria (over 500 mg in 24 hours) [16]. Vijay et al, working at another diabetes centre at Chennai, found a much higher prevalence of proteinuria (18.7%) [17]. However the later study was confined to inpatients, that is, patients admitted to hospital. This would undoubtedly introduce an additional bias of more severely ill patients being included in the study and this could explain the difference between the two studies.

The result of our study suggests that duration of diabetes is associated with proteinuria. The duration of diabetes has been shown to be a risk factor for nephropathy by almost all earlier studies [18]. The recent Diabetes Control and Complications Trial Research Group [18] and UK
Prospective Diabetes Group studies have shown the impact of blood glucose control on reducing risk of retinopathy and nephropathy \[^{19}\].

**Conclusion**

In conclusion, given the large number of individual with diabetes is increasing, the number of diabetic nephropathy is undoubtedly enormous. This could produce major constraints on health care budgets in the future. This urgently calls for not only good control of diabetes to prevent nephropathy but also to address the larger issue of primary prevention of diabetes, that is, reduction in the prevalence of diabetes itself by aggressive life style modifications.

**References**

1. Remuzzi, G., Bertani, T. Is glomerulosclerosis consequence of altered glomerular permeability to macromolecules? (Edictorial). Kidney Int. 1990; 38:384-394.
2. Kimmelstiel, P., Wilson, C. Intracapillary lesions in the glomeruli in the kidney. Am. J. Pathol. 1936; 12:83-97(PMC free article).
3. Kittell, F. Diabetes Management.” In Thomas LK, Othersen J.B. Nutrition therapy for chronic kidney disease, CRC. Press. 2012; 198.
4. Centers for Disease Control and Prevention. National Diabetes Sheet. Atlanta, Ga. US. department of health and human services. 2012. http:www.edu.gov/diabetes /pubs.
5. Mogensen, CE. Microacy, blood pressure and diabetics renal disease: origin and development of ideas. Diabetologia. 1999; 42: 263-285
6. Hypertension in Diabetes Study (HDS). 1. prevalence of hypertension in newly presenting type 2 diabetes and the association with risk factors for cardiovascular and diabetic complication J. Hypertens, 1993; 11:309-317.
7. Hasslacher, C., Stech, w., Wahl, P., Ritz, E. Blood pressure and metabolic control as risk factors for nephropathy in type 1 DM. Diabetologia. 1985; 28:6-11.
8. Rossing, P., Hommel, E., Smidt, UM., Parving, HH. Impact of arterial blood pressure and albumin on the progression of DN in type 1 diabetic patients. Diabetes. 1993. 42:715719.
9. Medical Encyclopedia (Medline plus). Diabetes and kidney disease. 2015. www.nlm.nih.gov.
10. Stephenson, JM., Kenny, S., Stevens, LK. Proteinuria and mortality in diabetes: the WHO multinational study of vascular disease in diabetes. Diabet Med. 1995; 12:149–155.
11. Krolewski, AS., Warram, JH., Christlieb, AR. The changing natural history of nephropathy in type 1 diabetes. Am J Med. 1985; 78:785–794.
12. Mogensen, CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity onset diabetes. N Engl J Med. 1984; 310:356–360.
13. Tung, P., Levin, SR. Nephropathy in non insuiln dependent diabetes mellitus. Am. J. Med. 1988; 85:131–136.
14. Fabre, J., Balant, LP., Dayer, PG. The kidney in maturity onset diabetes mellitus: a clinical study of 510 patients. Kidney Int. 1982; 2:730–738.
15. Burden, AC., McNally, PG., Feehally, J. Increased incidence of end-stage renal failure secondary to diabetes mellitus in Asian ethnic groups in the United Kingdom. Diabet. Med. 1992; 9:641–645.
16. John, L., Sunda, PS., Kanagasabapathy, SS. Prevalence of diabetic nephropathy in non-insulin dependent diabetics. Indian J Med Res. 1991; 94:24–29.
17. Vijay, V., Snehalatha, C., Ramachandran, A. Prevalence of proteinuria in non insulin dependent diabetes. J Assoc Physicians India. 1994; 42:792–794.
18. Diabetes Control and Complications Trial Research Group. 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. 1993; 329:977–986.

19. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS). Lancet. 1998; 32:1-18.