ORIGINAL ARTICLE

Excretion of Urinary IgG as a Diagnostic Factor in Overt Diabetic Nephropathy using Oral Hypoglycemic Drugs

M. MUSTAFA ALI SIDDIQUI[1], MUHAMMAD IRFAN QADIR KHAN[2], MOHAMMAD MOHSIN KHAN[3], *RUKHSHAN KHURSHID[4], M. ZAMIR AHMAD[5], ZAFAR ALI CH[6]*

ABSTRACT

Aim: To attempt to conclude the urinary IgG as the marker for early stages of diabetic nephropathy in patients with type II diabetes using oral hypoglycemic drug.
Study design: 42 female patients and 27 male patients, age range 40-60 yrs with longstanding diabetes, were included in the study.
Methods: The patients using oral hypoglycemic drug were taken from the In and Out patient department of Sir Ganga Ram Hospital. The electrophoretic patterns of 24 hr urinary proteins in diabetic patients were studied on 10% SDS gradient polyacrylamide gel electrophoresis. 24 hr urinary protein of patients was estimated.
Results: Electrophoretic profile showed a condition of proteinurea (both high molecular weight and low molecular weight). A significantly increased raw volume (concentration) and density of IgG was observed in urine of patient.
Conclusion: Urinary IgG excretion is an important prognostic factor in idiopathic membranous nephropathy. It is therefore recommended that early recognition of level of IgG is indicative of the renal changes that may increase the chance of preventing the development of diabetic nephropathy.
Keywords: Diabetes Type-II, IgG, SDS electrophoresis

INTRODUCTION

Glucose toxicity is a major cause of glomerular injury in patients with diabetic nephropathy. Prolonged increase in blood glucose levels result in the formation of glycation end products which interfere with normal collagen turnover and promote vessel permeability, matrix accumulation, and the formation of adhesion molecules. Extended inappropriate increases in angio-tensin-II lead to decreases in renal blood flow and glomerular filtration rate and the release of cyto-kines and growth factors. An important glomerular result of these multiple cytokine activations is destruction of the podocytes, which (like neurons) are difficult to replace when lost. (Townsend, R., 2005)

Changes in the renal tubules are significant for the development of progressive diabetic kidney disease. Tubular hypertrophy,decreased organic ion transport, and other tubular changes usually develop before the onset of diabetic proteinuria. Furthermore, elevated tubulo-glomerular feedback and defective uptake may independently contribute to hyperfiltration and urinary protein loss.(Townsend, R.,2002)

Current studies have focused on the possibility that albumin and other proteins that accumulate in the lumen of proximal tubular cells as a consequence of glomerular permeability dysfunction are a direct reason of tubular cell injury. Certain proteins that have been shown to be cytotoxic are transferrin/iron, lipoproteins and complement components, all of which appear in the urine in proteinuric states. Reabsorption of high molecular weight proteins may stimulate proximal tubular cells to produce matrix proteins, cytokines, chemoattractants and vasoactive mediators that may arouse interstitial inflammation and scarring.(Townsend R.,1997)

The excess of tubular cells with filtered proteins may play an important role in the progression of diabetic nephropathy by translating glomerular protein leakage into cellular signals of interstitial inflammation (Townsend R.1998)

Nephelometry classifies diabetic patients in different pattern of nephropathy: non nephropathic, normoalbuminuric with hyperfiltration, with incipient (microalbuminuric) and overt nephropathy (macroalbuminuric). Urinary excretion of IgG was significantly increased only in macroalbuminuric

[1]*Assistant Professor Radiology Department CPEIC Multan*
[2]*Associate Professor Anatomy Department Amna Inayat Medical College, Lahore*
[3] *Associate Professor/HOD Community Medicine, Amna Inayat Medical College, Lahore &*
[4]*Assistant Professor Fatima Jinnah Medical College, Lahore Pakistan*
[5]*Professor Biochemistry Amna Aniyat Medical College & Research Institute Sheikhupura, Lahore*
[6]*Professor/Principal Amna Aniyat Medical College & Research Institute Sheikhupura, Lahore*
Correspondence to Dr. M. Mustafa Ali Siddiqui

diabetic patients. This marker might therefore characterize the stage of overt nephropathy.(Solerte SB, Severgnini S, Locateli M,1997)

IgG antibodies were composed of two types of polypeptides (heavy and light chains) held together by disulfide bonds and noncovalent bonds**.** Two heavy (H) and light (L) chains by partial reduction of IgG showed that the H and L chains in each IgG molecule by taking into account their molecular weights are ~50kD for H and ~25kD for L. However the molecular weight of intact IgG is ~150kD.(Khurshid R.,2000)

MATERIAL AND METHODS:

Forty two female patients and 27 male patients, age range 40-60 yrs with longstanding diabetes, were included in the study. All the patients had old diabetes, impaired renal function, and overt proteinuria. The patients were taken from the therapeutic ward of Sir Ganga Ram Hospital. The electrophoretic patterns of 24 hr urinary proteins in diabetic patients and their controls were scrutinized on 10 % SDS gradient polyacrylamide gel electrophoresis**.(** Laemmli UK.,1970). Densitometric measurements were used to differentiate protein excretion patterns. 24 hr urinary protein of patients was estimated with standard kit method.

RESULTS

Mean age and 24 hr urinary protein and blood sugar of male and female patients with chronic diabetes was tabulated (Table 1). It is observed that the mean age of male patient with chronic diabetes was 49.06 years while of female patients was 52.89 years. The levels of urinary protein in male and female patients were 0.84 and 0.81 gm/24 hour urine sample. Where as the level of urinary protein of normal male and female subjects were 0.16 and 0.17 gm/24 hour urine sample respectively. When the levels of urinary proteins of male and female patients were compared with normal male and female (with no history) of diabetes, it showed a highly significant difference ($P<0.001$).

The levels of blood sugar in male and female patients were 259 mg/dl 259mg/dl. Where as the level of blood sugar of normal male and female subjects were 140.0 and 150.0 mg/dl respectively. When the levels of blood sugar of male and female patients were compared with normal male and female (with no history) of diabetes, it showed a highly significant difference ($P<0.001$).

Electrophoretic profile of patients of both sexes showed proteins with a molecular weight range from 32.00 KDa to 80 KDa with a density range of 0.00525 to 0.01771. Whereas in normal subject, the protein with a molecular weight range of 41.77 to 71.99 KDa with a density range of 0.109 to 0.585 (data not shown). Raw volume (concentration) and density of IgG in male, female and normal subjects was noted. It is observed that raw volume (concentration) and density of IgG in female was non significantly decreased than male patients. However the level of IgG in female was increased than normal subject and showed a highly significant difference ($P<0.001$). On the other hand, raw volume (concentration) and density of IgG in male was greater than the concentration and density of this protein of normal subjects and this shows a highly significant difference ($P<0.001$).

Fig. Urine protein profile of diabetic patients (from left to right) as separated on 10% resolving gel. Molecular markers are on extreme left range from 15 - 200 Kda. Where C is control. RD1-7 is for renal dysfunction of diabetics and M is for molecular markers

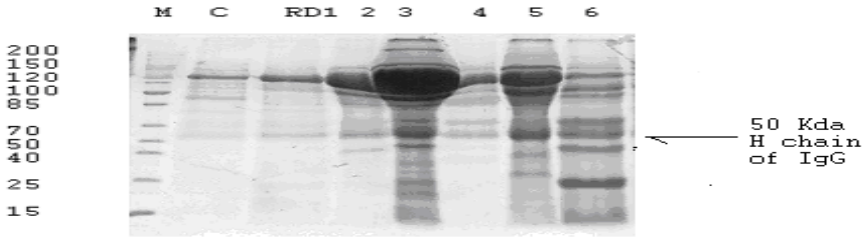

Table1: Mean age and 24 hr urinary protein of male and female patients with chronic diabetes Values expressed as mean±SD.

Parameter	Male (29)	Female (42)	Normal Male (10)	Normal Female (10)
Age (yr)	49.06±11.83	52.89±9.04	44.90±9.10	50.28±10.8
Urinary Protein (gm/24 hr urine)	0.84±0.50**	0.81±0.65**	0.16±0.12	0.17±0.56
Blood sugar (mg/dl)	249.00±36.42**	259.00±40.42**	140.00±28.2	145.00±25.2

**P>0.001= Highly significant difference

Table 2:Raw volume (concentration) and density of IgG in patients of both sexes.

Sample	Raw volume	Density
Male	6896.23±985**	0.00740±0.002**
Female	6784.68±1011**	0.00664±0.001**
Normal	182.83±110	0.43308±0.002

**P<0.001= highly significant difference

DISCUSSION

Urinary protein patterns were found to be useful to predict the high risk group for diabetic nephropathy in the preclinical stage. The protein pattern also discriminate nephropathic types of glomerular or tubular origin. These are also useful for clinicians to know the risk stage and prognosis for diabetic nephropathy (Hiratsuka N, Shiba K,1997).

Mean age of male patient with chronic diabetes was 49.06 years while of female patients was 52.89 years. A study instituted that glomerular alterations present in early diabetes, similar to those occurring with age, strengthen the concept that diabetes is an accelerated form of aging (Acevedo LM, Londono I, Oubaha M, 2008).

Level of 24 hr urinary protein was estimated in patients of both sexes with chronic diabetes. It was observed that the levels of urinary proteins of male and female patients were compared with normal male and female (with no history) of diabetes, it shows a highly significant difference (P<0.001). A study observed the additional proteins in urine samples of diabetes patients. Study instituted that these proteins can be used as markers for specific and accurate clinical analysis of Diabetic nephropathy (Jain S, Rajput A, Kumar Y, Uppuluri N,2005). It is reported that the pattern of proteinuria in group of diabetic patients may reflect hyperfiltration as well as tubular injury (Woo KT,1997).

Present study used the technique of sodium dodecyl sulphate gel electrophoresis (SDS PAGE) to analyze the protein pattern of urinary sample of chronic diabetics of normal subjects and patients of both sexes. The profile showed that in chronic diabetes there is a condition of proteinurea (both high molecular weight and low molecular weight), which may cause to excretes the protein which normally not appear in normal protein. The study is in accord with the studies (Koliakos G, Papachristou F,2001) who used the technique of SDS-PAGE to study the pathological changes in the protein pattern of urine. Their results prop up the view that early stages of diabetic nephropathy may involve both glomerular and tubular dysfunction. However the exact clinical and prognostic significance of the information provided by SDS PAGE analysis remains to be elucidated. On the other, the findings of a group of workers (Raicevic S, Trnacevic S,1991) demonstrate the low-molecular-weight proteinuria characteristic for the early stages of nephrology.

Raw volume (concentration) and density of IgG in male, female and normal subjects was noted. It is observed that raw volume (concentration) and density of IgG in female was lesser than male patients but the level of IgG was significantly increased in female when compared these levels with normal subjects. On the other hand, raw volume (concentration) and density of IgG in male was greater than the concentration and density of this protein of female patients and normal subjects and this shows a highly significant difference (P<0.001). Our study is in accord to number of studies who observed a significantly increased level of IgG (Calzada-Garcia JA, 1996). It is postulated that increased excretion of high molecular weight protein showed a mixed glomerular pattern (Garcia). Which showed that the proximal tubules and the membranes of renal glomeruli are damaged as early as during the period of subclinical diabetic nephropathy[15]. Another study established that high molecular weight protein IgG accurately predicted renal outcome in patients with idiopathic membranous nephropathy (Reichert LJ, Koene RA,1997). Recently it is reported that IgG nephropathy is one of the most described glomerulopathies in patients with renal problem (Lim BJ, Hong SW, 2009).

CONCLUSION

It is concluded that urinary IgG excretion is an important prognostic factor in idiopathic membranous nephropathy. It is therefore recommended that early recognition of level of IgG is indicative of the renal

changes that may increase the chance of preventing the development of diabetic nephropathy.

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