A STUDY OF URINARY AND SERUM NON-ENZYMATIC GLYCOSYLATED PROTEINS IN DIABE1'ES MELLITUS AND RENAL DISORDERS

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Summary

Glycosylated Haemoglobin (GHB), glycosylated serum proteins (GSP) and glycosylated urinary proteins (GUP) were studied in 45 individuals with diabetes mellitus, 22 with nondiabetic renal diseases and 19 age and sex matched normal individuals. The GUP levels in normoproteinuric diabetics and in microproteinuric diabetics were significantly higher ($p < 0.001$) than macroproteinuric diabetics and those of control group. GUP/GSP ratio was significantly higher in normoproteinuric diabetics (3.12±0.35) than the microproteinuric (1.82±0.35), macroproteinuric diabetics (1.17±0.12) and controls (2.56±0.23). GUP/GSP ratio in patients with nephrotic syndrome and that of chronic renal failure is 0.98±0.04 and 0.96±0.06 respectively that is close to unity indicating that in major renal involvement the proteinuria is non selective. Thus measurement of GUP/GSP ratio could be one of the parameters in monitoring the progress of diabetic nephropathy.

Introduction

There is increasing evidence that chronic hyperglycemia is one of the major determinants of diabetic complications. The mechanism by which this is brought about is presently unknown, but it has been postulated that there is glucose dependent chemical alteration of body protein structures and functions leading to various complications in diabetes mellitus[1]. One of the processes by which these changes are brought about is nonenzymatic glycosylation of body proteins. Some authors have reported an increase in nonenzymatic glycosylation of diabetic rat glomerular basement membrane and suggest that this phenomenon might change the selective permeability of the membrane itself[2]. These authors have demonstrated an altered state of renal handling of glycosylated proteins with different grades of proteinuria in diabetes mellitus. The available literature point toward an increase in excretion of glycosylated proteins in urine in patients with early nephropathy[2].

The present study has been undertaken for finding the possible relationship of glycosylated urinary proteins with different grades of nephropathy, glycosylated serum proteins and hemoglobin as well as for finding any difference between diabetic renal disease.

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Material and Methods

Eighty six cases were studied of which 67 were patients of diabetes mellitus and renal diseases. Age, sex, weight and socioeconomic status-matched nondiabetic healthy persons served as the control group. Diabetes was excluded in the control group by standard oral glucose tolerance test. Subjects with normal renal functions, as judged by normal urine examination on two different occasions, with normal blood urea, serum creatinine were included in the control group. All the healthy controls were free from obvious illness and were not taking any drug.

Modified criteria as laid down by National Diabetes Data Group USA (1979)[3] for blood sugars were used for the diagnosis of diabetes mellitus.

Patients of CRF, diabetic or nondiabetic, included in the study group satisfied the following criteria.

1. Blood urea more than 50 mg/ 100 ml
2. Serum creatinine > 2 mg/100 m)
3. Symptoms suggestive of uraemia for more than 3 months

Patients of nephrotic syndrome had a proteinuria of > 3.5 g/day per 1.73 sq. m of body surface area.

1. Haemogram
2. Urine examination for proteins, sugars, ketones and microscopic elements.
3. Blood sugars-fasting, pp and OGTT whenever indicated.
4. Blood urea; Serum creatinine
5. Other investigations-glycosylated haemoglobin, glycosylated serum proteins, glycosylated urinary proteins

Measurement of glycosylated haemoglobin and serum/urinary proteins were done according to the colorimetric method of Fluckiger (1978) with minor modifications. Results were expressed as nanomole of 5-HMF/mg Hb and/mg protein.

Results

A total of 86 persons were studied :
GROUP I-CONTROLS-19
GROUP II-Diabetic with proteinuria-45
A-Normoproteinuria <150 mg/24 hrs.-15

B-Microproteinuria 150-500 mg/24 hrs-19
C-Macroproteinuria > 500 mg/24 hrs-11
GROUP III-Non-Diabetic with renal disease-22
A-Nephrotic Syndrome-14
B-CRF-8

Table 1

GHB, GSP, GUP in Diabetics having proteinuria vs controls

Group	No. of Patients	GHB Mean±SD	GSP Mean±SD	GUP Mean±SD	Proteinuria Mean±SD
IIA	15	8.62±2.98	8.89±2.39	24.59±2.36	126.2±13.71
IIB	19	9.15±3.70	9.10±2.88	15.48±2.10	323.0±73.27
IIC	11	9.54±3.68	9.46±2.90	11.41±1.77	1028.7±357.30
I	19	3.95±0.40	4.72±0.27	11.98±0.67	132.6±8.73

Table 2

GUP/GSP ratio in the study group I & II

Group	No. of Patients	Proteinuria mg/24 hrs Mean ± SD	GUP/GSP Mean ± SD
Group I			
Controls	19	132.58 ± 8.73	2.56 ±0.23
Group II			
Diabetics			
IIA	15	126.20± 13.71	3.12±0.57
IIB	19	323.25±73.27	1.82±0.35
IIC	11	1028.28±357.3	1.17±0.12

Statistical significance

P value between	
IIA Vs IIB	P <0.001
IIB Vs IIC	P <0.001
IIA Vs IIC	P <0.001

Table 3

GHB, GSP, & GUP levels in patients with renal disease Group I & Group III

Group	No. of Patients	Proteinuria mg/24 hrs Mean±SD	Serum Creatinine mg% Mean±SD	GHB Mean±SD	GSP Mean±SD	GUP Mean±SD	GUP/GSP Mean±SD
IIIA							
Nephrotic syndrome	14	6536.0±80	1.10±0.28	4.10±0.37	4.01±0.22	4.88±0.17	0.98±0.04
IIIB							
CRF	8	1054.0±296	2.12±3.90	5.99±0.20	5.20±0.16	5.26±0.11	0.96±0.06
I							
Controls	19	132.6±9	0.90±0.15	3.95±0.40	4.72±0.27	11.98±0.67	2.56±0.23

Statistical significance

P values between

IIIA Vs I	NS	NS.	Sig
IIIB Vs I	Sig.	Sig.	Sig
IIIA Vs IIIB	Sig.	NS	NS

Discussion

The GHB and GSP values have been found to be raised in the diabetic patients, the increase being approximately three times the normal ($p<0.001$)[6,7].

The mean GHB and GSP were similar in all the diabetic subgroups (according to degree of proteinuria $p>0.1$). There was no significant difference in GUP levels among males and females or with increasing age.

The GUP levels were significantly higher ($p<0.001$) in normoproteinuric diabetics (24.59±2.36 nm HMF/mg protein) than of controls. The GUP levels in microproteinuric diabetics (15.48 ±2.1 nm HMF/mg protein) were higher than those of the control group. However, the levels of GUP were similar ($p>0.1$) in macroproteinuric diabetics and of control group. Thus we see that as proteinuria increases the GUP level decreases. Thus inspite of similar GHB and GSP levels, there were differing levels of GUP in the above different diabetic subgroups.

To have a correct idea of glycosylated protein filtration kinetics a ratio of GUP/GSP was suggested. Thus considering this GUP/GSP ratio it can be seen that with similar GSP levels the GUP/GSP ratio varies in different subgroups.

The ratio being lowest in macroproteinuric diabetics (1.17 ± 0.12) and highest (3.12±0.96) in normoproteinuric while in microproteinuric diabetics this was in between (1.82±0.37)[2].

Candino et al (1983) suggested that nonenzymatic glycosylation of serum albumin alters the charge characteristics of the originally anionic aibumin molecule i.e., glycosylated and nonglycosylated albumin molecules are electrostatically different. Similarly nonenzymatic glycosylation of glomerular basement membrane has been shown to produce loss of its fixed negative charge[5]. An interaction between these altered electrostatic forces subsequent to glycosylation of structural proteins can be expected to explain the variability.

The proteinuria in mg/24 hrs and GUP have a negative correlation between each other in each of the groups of diabetics ($r = 0.402$) in normoproteinuric diabetics and ($r = 0.248$) in microproteinuric while there is no correlation between GUP and proteinuria per 24 hrs in the macroproteinuric diabetics. Thus whenever small amounts of proteins are filtered through a diabetic kidney it consists of higher amounts of the glycosylated fraction and as the level of proteinuria increases the degree of glycosylation decreases.

This suggests that with higher degree of proteinuria we get somewhat unselective loss of proteins through nephrons while when small amount of proteins is passing in the urine most of it is glycosylated and is preferentially filtered.

GUP in nephrotic syndrome and in renal failure were similar to GSP levels in these patients respectively. Thus we see that the whole of glycosylated proteins is excreted.

Hence the ratio of GUP/GSP in both nephrotic syndrome (0.98±0.04) and in CRF (0.96±0.04) respectively, is close to unity suggesting thereby that there is non selective proteinuria in nondiabetic renal disease.

Thus by analogy one can say that by the time the diabetics reach macro proteinuric stage they also develop non selective proteinuria. Thus estimation of levels of GUP may roughly denote the various stages of diabetic nephropathy viz higher levels of GUP and higher GUP/GSP ratio indicating normoproteinuria and microproteinuria suggesting incipient nephropathy and lower levels of these indicating overt nephropathy.

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