



A Clinical Comparative Study of Glycosylated Haemoglobin, Urinary Protein Creatinine Ratio, 24-Hour Urinary Protein Concentration in Diabetic Subjects and Controls

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ABSTRACT

Objectives: Diabetes is the most common endocrine disorder. Long-term complications of diabetes mellitus cause morbidity and premature mortality. The present study aims to compare the glycosylated haemoglobin, urinary protein creatinine ratio, 24-hour urinary protein concentration in diabetic subjects and controls.

Materials and methods: The present study was carried out at Kurnool Medical College and General Hospital Kurnool. One hundred subjects (50 diabetic and 50 non-diabetic) were taken from OPD, following biomedical parameters like fasting blood sugar, glycosylated haemoglobin, Serum creatinine, Urine creatinine, urine protein, protein and creatinine ratio, and 24hr urinary protein.

Results: The levels of FBS, Serum creatinine, HbA1c, 24 hr urinary protein, and protein: creatinine ratio in random urine samples are increased in the diabetic subjects compared to controls; the increase is highly significant ($p < 0.0001$). The protein creatinine ratio in random urine samples is found to have a highly significant ($p < 0.001$) correlation with Serum creatinine, 24 hr urinary protein in random urine samples.

Conclusion: The present study suggests that estimating glycosylated hemoglobin as an indicator of glycemic control and protein: creatinine ratio in random urine samples for renal involvement in diabetic subjects provides a convenient early diagnosis and intervention method.

Keywords: Diabetes, HbA1C, Serum creatinine, 24-hour urinary protein, protein: creatinine ratio.

INTRODUCTION

Diabetes mellitus is a significant health problem affecting people all over the world. Long-term glycemic control is assessed by measuring the glycosylated hemoglobin level in people with diabetes mellitus. Renal involvement in diabetes is a leading cause of morbidity and mortality and the most common cause of end-stage renal disease¹⁻². The degree of proteinuria is a valuable marker for renal involvement and response to treatment. The protein-to-creatinine ratio in a random urine sample was found to correlate with 24-hour urinary protein concentration, and this method could be considered helpful for screening purposes and as an outpatient procedure³⁻⁴. In the present study, we evaluated glycosylated hemoglobin as a marker for glycemic control. We also tried to find a correlation between 24-hour urinary protein concentration and the protein-to-creatinine ratio in random urine samples in patients with diabetes mellitus.

MATERIALS AND METHODS

After obtaining institutional ethical committee approval and written informed consent from diabetic subjects and controls who voluntarily participated in the study, this prospective clinical comparative study of glycosylated hemoglobin and urinary protein-to-creatinine ratio in diabetic subjects and controls was conducted at Kurnool Medical College, Government General Hospital, Kurnool,

from May 2023 to May 2024. One hundred subjects participated in the study, out of which 50 were diabetic (both IDDM and NIDDM) and 50 were non-diabetic controls.

Exclusion criteria

Patients with chronic renal failure, glomerulonephritis due to other systemic conditions, and hypertensive patients were excluded from the study.

METHODOLOGY

Three milliliters of venous blood were collected from the antecubital vein under aseptic precautions. This was divided into two parts: 2 mL was collected in a plain vial, and 1 mL with EDTA. Twenty-four-hour urine was collected by instructing subjects to begin collection immediately after completing the first voiding in the morning and to collect all urine into the same container containing 4mL of 10% thymol in isopropanol as a preservative for 24 hours, including the final void after the 24 hours. This was thoroughly mixed, and a sample of 2 mL was taken to evaluate proteins. The total volume was noted, and the calculation was done for 24 hours. A random urine sample of 5 mL was collected the next day after the completion of the 24-hour collection. The following parameters were taken for the present study: following biomedical parameters like fasting blood sugar, glycosylated hemoglobin, Serum creatinine, Urine creatinine, urine protein, protein and creatinine ratio, and



24-hour urinary protein. Fasting blood sugar was estimated using the glucose oxidase and peroxide method⁵. The glycosylated hemoglobin was assessed using the cation exchange resin method⁶. The serum creatinine was estimated by modifying Jaffe's method⁷. Estimation of urine creatinine by modified Jaffe's method⁷. Estimation of urinary protein by turbidimetry method⁸.

RESULTS

It is evident from Table 1 (Figure 1) that the mean levels of FBS, serum creatinine, HbA1c, 24-hour urinary protein, and protein-to-creatinine ratio in random urine samples are increased in the diabetic subjects compared to the controls. The increase is highly significant (p<0.0001). Table 2 (Figure 2) shows that the HbA1c value in diabetic subjects with a protein-to-creatinine ratio of <0.2 was 7.42±0.56. In diabetic patients with a protein-to-creatinine ratio of >0.2, the HbA1c value was 8.07±1.62, which was statistically significant (p<0.001). Table 3 shows an increase in the mean values of 24-hour urinary protein concentration and the protein-to-creatinine ratio in random urine samples of patients with diabetes for

more than 5 years compared to those with diabetes for less than five years. This increase was statistically significant (p<0.05). The protein creatinine ratio in random urine samples is found to have a highly significant (p<0.001) correlation with Serum creatinine, 24 hr urinary protein in random urine samples (Figure 4).

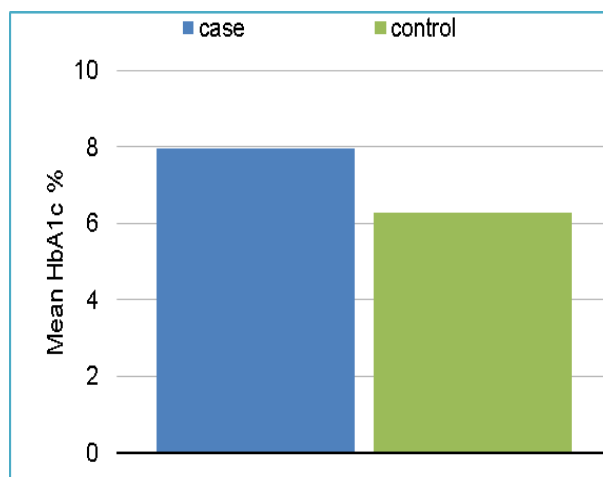


Figure 1: Comparison of HbA1C between cases and control

Table 1: Comparison of FBS, Serum creatinine, HbA1C, 24hr urinary protein, protein: creatinine ratio in random urine samples in controls and diabetic subjects

Study population	FBS (mg/dl) Mean±SD	Serum creatinine (mg/dl) Mean±SD	HbA1C (%) Mean±SD	24hr urinary protein (g/day) Mean±SD	Protein: creatinine ratio in a random urine sample Mean±SD
Control (n=50)	91.2±11.8	0.84±0.26	6.26±0.74	0.10±0.07	0.09±0.08
Cases (n=50)	198.2±92.4	1.18±0.62	7.96±1.52	1.08±1.76	1.19±1.92
Cases vs. control 't' value	8.12	3.576	7.11	3.934	4.048
'p' value (<0.05 significant)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 2: Comparison of FBS, Serum creatinine, HbA1C, 24 hr urinary protein, and protein creatine Ratio in diabetic subjects with protein creatine ratio 0.2 as a cut-off value

Study population	Number of cases	FBS (mg/dl) Mean±SD	Serum creatinine (mg/dl) Mean±SD	HbA1c (%) Mean±SD	24-hour urinary protein (g/dl) Mean±SD	Protein creatinine ratio in random sample Mean±SD
Diabetic subjects with Protein: creatinine ratio in a random urine sample >0.2	29	201.7±101.4	1.34±0.7	8.07±1.62	1.62±1.94	1.71±1.82
Diabetic subjects with Protein: creatinine ratio in random urine sample <0.2	21	182.7±64.2	0.79±0.17	7.42±0.56	0.09±0.06	0.07±0.06
'p' value		>0.05	<0.001	>0.05	<0.001	<0.001

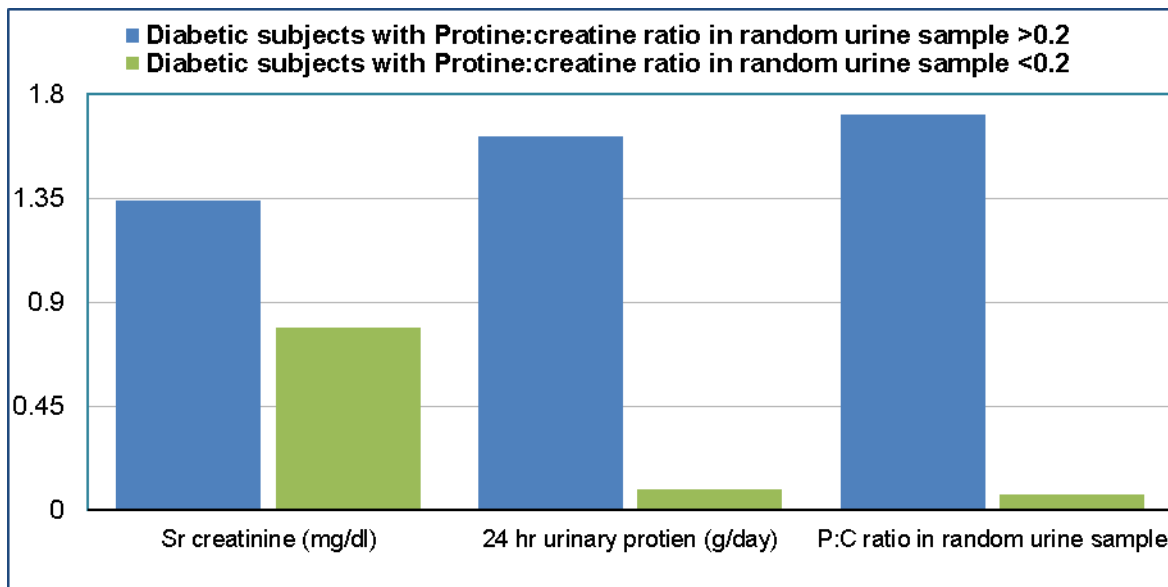


Figure 2: Comparison of FBS, Serum creatinine, HbA1C, 24 hr urinary protein, and protein creatine Ratio in diabetic subjects with protein creatine ratio 0.2 as a cut-off value

Table 3: Comparison of 24hr urinary protein concentration and protein: creatinine ratio in random urine sample level in diabetic subjects about Duration of diabetes

Diabetic subjects with Duration	Number of cases	24-hour urinary protein (g/day)	Protein: creatinine ratio in a random urine sample
< 5years	29	0.78±1.34	0.82±1.42
>5years	21	1.39±1.98	1.52±2.01
'p' value		<0.05	<0.05

Table 4: Pearson correlation coefficient (r)

Parameters	r value	'p' value
Serum creatinine and 24-hour urinary protein	0.293	<0.001
Serum creatinine and protein creatinine ratio in a random urine sample	0.291	<0.001
24hr urinary protein and protein: creatinine ratio in a random urine sample	0.328	<0.001

DISCUSSION

Diabetes is a joint endocrine disorder. Long-term complications of diabetes mellitus cause morbidity and premature mortality. Renal involvement is one of such complications, so screening and early diagnosis for renal participation are to be considered. In the present study, we conducted 100 subjects, out of which 50 were diabetic subjects and 50 were nondiabetic controls. Diabetic subjects are further divided depending on the Duration of diabetes as < 5 years and >5 years. We studied HbA1C, a marker of glycemic control, and protein: creatine ratio in random urine samples, which correlates with 24-hour urinary protein. HbA1C reflects an individual's degree of glycemic control better than FBS and PPBS levels. HbA1C is formed by post-transcriptional glycosylation of hemoglobin A in the amino-terminal of the Beta chain at valine, and it is a slow nonenzymatic reaction that occurs

throughout the lifespan of RBC(1). It is well documented that the level of HbA1C correlates with the degree of glycemic control (2,3). The mean value of HbA1C in cases was 7.96±1.52; in controls, it was 6.26±0.74, which was statistically highly significant (p<0.001). These findings were based on the study of Trivelli et al., Gonen and Nathan et al., Koenig et al. The HbA1c value in diabetic subjects with Protein: Creatinine <0.2 was 7.42±0.56, and its value in diabetic patients with protein: creatinine ratio >0.2 was 8.07±1.62. It was also observed that HbA1C value positively correlates with 24-hour urinary protein concentration and urine protein: creatinine ratio in random urine samples. This finding correlated well with Viberti et al. ¹⁰, the collaborative study group, and Krolewski et al. ⁹ The mean concentration of 24-hour urinary protein in cases and controls was 1.08±1.76, 0.10±0.07 respectively, which was statistically significant. This is in accordance with studies done by Nathan et al.



[10], Banerjee et al.¹¹, and Nelson et al.¹². Statistically significant ($p < 0.05$) in mean 24-hour urinary protein concentration and protein-creatinine ratio were observed in patients with diabetes duration >5 years compared to those with <5 years. This study was done by Croal et al.¹³ and Nelson et al.¹² The protein creatinine ratio in random urine samples is found to have a highly significant correlation with Serum creatinine, 24 hr urinary protein. This is to other studies by Thompson et al.¹⁴ and Durnwald et al.¹⁵

CONCLUSION

The present study suggests estimating glycosylated hemoglobin as an indicator of glycemic control and protein: creatinine ratio in random urine samples for renal involvement in diabetic subjects provides a convenient method for early diagnosis and intervention. The possibility to prevent, delay, or reverse the progression of diabetic nephropathy can be achieved only by perfect long-term metabolic control.

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