

# Urinary Enzymes as Early Diagnostic Marker for Diabetic Nephropathy: A Comparison with Cystatin C

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## ABSTRACT

**Background:** Renal damage is a serious major microvascular diabetic complication leading to the death of diabetic patients. Thus, diagnosis of diabetic nephropathy in an earlier stage would be critical. Urinary enzymes which are markers of tubular damage viz. urinary GGT/ALP/LDH and their creatinine standardized ratio can serve as cost effective, feasible, widely available early diagnostic markers for diabetic nephropathy

**Materials and Methods:** Study enrolled 60 subjects for each group. Measurement of fasting blood sugar, random blood sugar, serum urea, serum creatinine, and serum cystatin c were done in serum. Measurement of ALP, LDH, GGT was done in urine.

**Results:** The urinary enzyme levels were significantly high in patients of diabetic nephropathy in comparison to other study subjects. Correlation analysis of Urine Albumin Creatinine Ratio and Urine ALP (r value- -0.1, p value-<0.0001), and Urine GGT (r value- -0.2, p value- <0.0001) Urine LDH (r value- -0.3, p value- <0.0001) of patients suffering from Diabetic Nephropathy was performed using Pearson's correlation analysis. Correlation analysis of Serum Cystatin C and Urine ALP (r value- 0.2, p value-<0.0001), Urine LDH (r value- 0.01, p value- <0.0001), and Urine GGT (r value- 0.14, p value-<0.0001) of patients suffering from Diabetic Nephropathy was performed using Pearson's correlation analysis.

**Conclusion:** Correlation studies of urinary enzyme levels with albumin-creatinine ratio (urine) and cystatin C (serum) levels didn't show any strong correlation (positive or negative) among the enzyme's levels with established markers of diabetic nephropathy. Given the fact that urinary enzyme levels are increased in case of tubular dysfunction of the

nephrons, the result of our study shows that, diabetic nephropathy primarily affects the glomerular function.

**Keywords:** Urinary enzymes, diagnostic markers, diabetic nephropathy, cystatin C.

## INTRODUCTION

Diabetes mellitus refers to group of metabolic disorders that share the common phenotype of hyperglycaemia. <sup>(1)</sup> Diabetes mellitus is one of the most common noncommunicable diseases in the world and also a leading cause of death. <sup>(2)</sup> India is leading in the world with largest number of people suffering from diabetes mellitus with prevalence of over 50 million which is expected to increase up to 87million by 2030. <sup>(3)</sup> As there is an increase in diabetic patients this also leads to increase in complications of diabetes. It has been observed that Asians has a higher prevalence of complications of diabetes compared to Europeans. <sup>(4)</sup> One of the most common complications of diabetes is diabetic nephropathy or diabetic kidney disease. <sup>(5)</sup>

Diabetic Nephropathy, also known as Diabetic kidney disease is one of the most common long-term microvascular complications of Diabetes Mellitus Type 1 and 2. It is one of the leading causes of End Stage Renal Disease (ESRD) in developing countries. <sup>(6)</sup> The routine classical evaluation of diabetic nephropathy includes: appearance of microalbuminuria. Urinary microalbumin is not prevalently available, difficult to standardize, relatively

costly and appears in urine only after significant glomerular damage have taken place, urine albumin level is also increased in urinary tract infection, following exercise, fever, posture, hyperglycaemia, marked hypertension, congestive cardiac failure, it may also be affected by patient's state of hydration and the method of sample collection. (7,8) It is well established that the detection of microalbumin in a patient having diabetes indicates the presence of glomerular involvement in early renal damage. Recent studies have shown that, tubular involvement may precede glomerular involvement as several of the tubular proteins and enzymes can be detected even before the appearance of microalbuminuria and rise in serum creatinine. Hence, it would be better prognostically, if we can detect Diabetic Nephropathy at an even earlier stage, before appearance of microalbuminuria.

Cystatin C is an endogenous low molecular weight cysteine proteinase inhibitor. (9) The main site of catabolism of Cystatin C is kidney- as >90% of Cystatin C is removed from circulation by glomerular filtration. Cystatin C has been suggested as useful early marker for glomerular damage as it produced at a constant rate and completely removed from circulation by glomerular filtration and proximal tubule reabsorption. (10) Cystatin C level detection test are not widely available (high cost of the immunoassay), and also not all assays have been universally calibrated. (11)

Urinary enzymes Alkaline phosphatase, Gamma glutamyl transferase and Lactate dehydrogenase are markers of tubular damage. ALP, GGT are present in luminal brush border of epithelial cell membrane in the proximal tubule lumen. (12,13) Lactate dehydrogenases is located in the cell cytoplasm and are more prominent even before the appearance of microalbuminuria. (14) As tubular damage precedes glomerular damage; thus, markers of tubular damage can serve as early diagnostic marker for diabetic nephropathy. Urinary enzymes ALP, GGT and LDH can

serve as early markers for Diabetic nephropathy, and tests to detect their level are widely available and cost effective to reduce the burden on patients of lower socioeconomic status.

## **MATERIALS AND METHODS**

### **STUDY POPULATION**

The study was conducted in the Department of Biochemistry, Pt. J.N.M. Medical College and DR. B.R.A.M. Hospital, Raipur, C.G. from June 2019 to August 2019 among patients who attended the Medicine OPD, Department of Medicine, DR. B.R.A.M. Hospital, Raipur (C.G.). Study was conducted after the approval of institutional scientific and ethical committee, and informed, written consent was obtained from all the participants in the study. The study consisted of a total of 180 participants. The participants were divided into three groups as follows:

1. 60 diabetic patients as diagnosed by WHO/ADA criteria
2. 60 subjects were patients with early Diabetic Nephropathy as diagnosed by presence of microalbuminuria,
3. 60 healthy controls age and sex matched.

### **EXCLUSION CRITERIA**

All patients in this study had no medical history of, urinary tract diseases, coronary heart disease, malignancy, pregnancy and lactation, and use of nephrotoxic drugs.

### **SAMPLE COLLECTION.**

Five ml of venous blood samples were obtained after overnight fasting with usual precautions in a plain vial. Early morning second urine sample – spot was collected in a sterile container. Serum was separated after allowing the samples to clot for 30 minutes at room temperature and then centrifuging the samples at 1500 rpm for 10 minutes. The serum thus obtained was utilized for determination of fasting blood sugar, post prandial blood sugar, serum urea, serum creatinine. Samples were stored in labelled aliquoted tubes and stored in a refrigerator at - 20<sup>0</sup>C for estimation of

serum cystatin C as per the manufacturer protocols. Urine samples obtained will be utilized for determination of urinary creatinine, microalbumin gamma glutamyl transpeptidase, lactate dehydrogenase, alkaline phosphatase.

### Statistical Analysis

Determination of microalbumin done using nephelometry. Determination of serum creatinine, urinary creatinine, urinary alkaline phosphatase gamma glutamyl transpeptidase and lactate dehydrogenase done by using automated biochemistry I-Lab 650 autoanalyzer using manufacturer's protocol. Determination of serum cystatin C done by double sandwich ELISA. Statistical analysis was performed using IBM SPSS Statistics Program and Microsoft excel. t-test, ANNOVA, and Pearson correlation, was applied.

### RESULTS

The study group consisted of total 180 subjects. Out of 180, 60 subjects were diagnosed with diabetes mellitus by WHO/ADA criteria, 60 subjects diagnosed with early Diabetic Nephropathy as diagnosed by presence of microalbuminuria, 60 subjects are healthy controls age and sex matched.

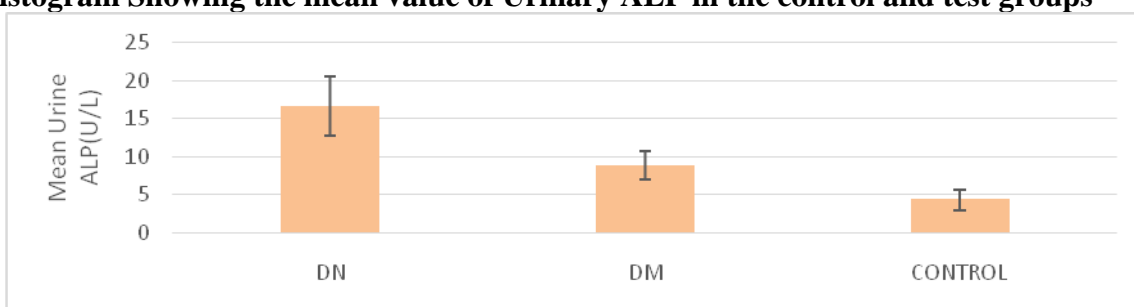
In this study out of 180 subjects 46 (25.56%) were between 30 to 40 yrs of age, 51 (28.33%) were between 41yrs to 50yrs of age, 42 (23.33% were between 51yrs to 60yrs of age, 31(17.22%) were between 61yrs to 70yrs of age, 9 (5.00%) were between 71yrs to 81yrs of age, 1 (0.56%) were between 81 to 90yrs of age.

Gender distribution in study subjected, Out of 180 subjects in total 83 (46.11%) were female, 97 (53.89%) were male.

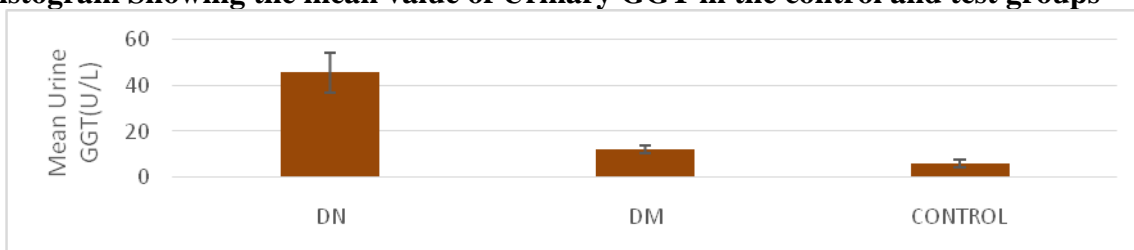
### Levels of urinary and serum biomarkers of renal injury in normal controls and in subjects suffering diabetes mellitus and diabetic nephropathy

Parameters	Diabetes mellitus (Mean SD)	Diabetic Nephropathy (Mean SD)	Healthy Control (Mean SD)	P Value
BMI	28.5 ±3.5	28.6 ±3.5	27.0 ±2.4	<0.01
FBS	145.6 ±23.7	182.9 ±52.8	83.71 ±9.21	<0.01
PPBS	207.75 ±51.8	265.35 ±73.34	112.98±14.11	<0.01
Serum Urea	23.95 ±10.81	51.46 ±13.45	21.18 ±8.13	<0.01
Serum Creatinine	0.73 ±0.14	1.50 ±0.28	0.71 ±0.14	<0.01
UACR	28.56 ±39.0	263.86 ±39.0	22.90 ±14.9	<0.01
Urine ALP	8.88 ±1.85	16.7 ±3.87	4.35 ±1.36	<0.01
Urine GGT	12.25 ±1.75	45.93 ±8.69	6.2 ±1.48	<0.01
Urine LDH	9.8 ±1.57	18.25 ±3.97	5.23 ±1.61	<0.01
Serum Cystatin C	1174.56 ±194.6	1894.9 ±369.8	741.6 ±132.6	<0.01

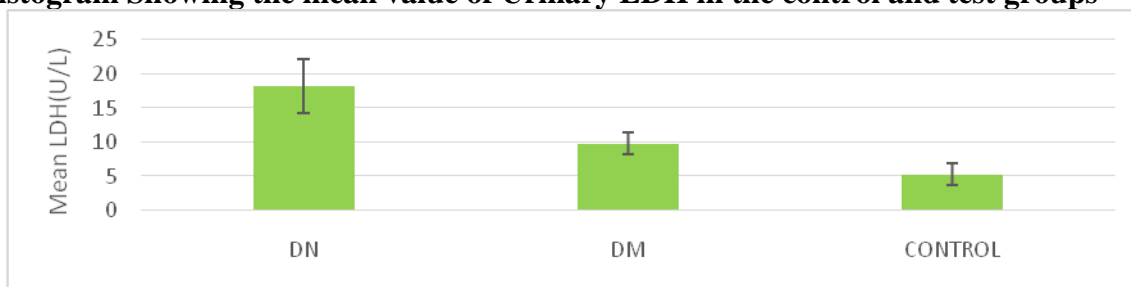
### Histogram Showing the mean value of Urinary ALP in the control and test groups



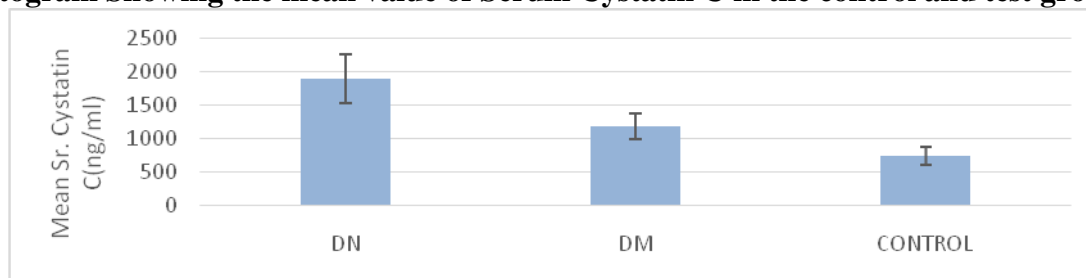
### Histogram Showing the mean value of Urinary GGT in the control and test groups



**Histogram Showing the mean value of Urinary LDH in the control and test groups**



**Histogram Showing the mean value of Serum Cystatin C in the control and test groups**



**Pearson correlation coefficient of urinary biomarkers in the subjects suffering Diabetes nephropathy**

Parameter	Parameter	Diabetic Nephropathy	p Value
1. serum Cystatin C	U.ALP	0.2	<0.0001
	U. GGT	0.14	<0.0001
	U. LDH	0.01	<0.0001
2.UACR	U. ALP	-0.1	<0.0001
	U. GGT	-0.2	<0.0001
	U.LDH	-0.3	<0.0001

**DISCUSSION**

Diabetes mellitus is a global disorder and resulting complications from diabetes are the third leading cause of death in the world. (14) Since with untreated diabetic nephropathy, there is a significant decrease in life expectancy of patients, therefore, prevention of this debilitating condition and early diagnosis and treatment is important. In routine, decrease in creatinine clearance, increase in serum creatinine, and especially appearances of microalbuminuria are used as key indicators of diabetic nephropathy. But these markers are not sensitive, specific, reliable, as there is a time delay between renal injury and detection and when nephropathy is diagnosed by these classical methods, little can be done to prevent the progressive downhill course of renal failure. (14)

This study was done to evaluate urinary enzymes as early diagnostic marker for diabetic nephropathy. Total 180 subjects were enrolled in the study, out of which, 120 subjects were patients with diabetes

mellitus, among diabetic subjects 60 subjects were diagnosed with diabetic nephropathy, and 60 healthy controls were enrolled in this study according to predetermined exclusion and inclusion criteria. All patients fasting and postprandial blood sugar were estimated, to determine the functional status of their kidney, serum urea, serum creatinine, and serum cystatin c estimation was done. Urinary alkaline phosphate, gamma glutamyl transpeptidase, and lactate dehydrogenase were measured in all subjects.

In present study it was found that urinary level of Alkaline phosphatase in subjects with DM and diabetes nephropathy were significantly higher than in control group (p value <0.01). These findings correlate with the findings of Mohammadi-Karakani et al, (15) Gatua et al (16) and Jung et al (17) have found that urinary ALP was excreted higher in diabetic patients compared with control groups. Uslu et al (18) have also demonstrated that urinary ALP

was higher in diabetics than in controls with a p value of <0.001.

It was found that urinary level of Gamma glutamyl transpeptidase in subjects with DM and diabetes nephropathy were significantly higher than in control group (p value <0.01). These findings correlate with the findings of Gatua et al <sup>(16)</sup> and Nikolov et al <sup>(19)</sup>

In present study it was found that urinary level of Lactate Dehydrogenase in subjects with DM and diabetes nephropathy were significantly higher than in control group (p value <0.01). These findings correlate with the findings of Gatua et al, <sup>(16)</sup> Uslu et al, <sup>(18)</sup> and Mohammadi-Karakani et al <sup>(15)</sup>

Correlation analysis of Urine Albumin Creatinine Ratio and Urine ALP (r value- -0.1, p value-<0.0001), and Urine GGT (r value- -0.2, p value- <0.0001) Urine LDH (r value- -0.3, p value- <0.0001) of patients suffering from Diabetic Nephropathy was performed using Pearson's correlation analysis. No Strong correlation was found between the urinary albumin creatinine ratio and urinary enzymes

Correlation analysis of Serum Cystatin C and Urine ALP (r value- 0.2, p value-<0.0001), Urine LDH (r value- 0.01, p value- <0.0001), and Urine GGT (r value- 0.14, p value-<0.0001) of patients suffering from Diabetic Nephropathy was performed using Pearson's correlation analysis. No Strong correlation was found between serum cystatin c and urinary enzymes.

## CONCLUSION

The study was conducted with an aim to find out the feasibility of urinary enzymes to act as early markers for diabetic nephropathy. The diagnosis was based on the levels of urinary albumin-creatinine ratio and serum cystatin C. The urinary enzymes, ALP, GGT and LDH were measured in all the study participants.

The urinary enzyme levels were significantly high in patients of diabetic nephropathy in comparison to other study

subjects. The levels of urinary enzymes were also found to be higher in diabetic patients without nephropathy than non-diabetic subjects, but the difference was not statistically significant. Correlation studies of urinary enzyme levels with albumin-creatinine ratio (urine) and cystatin C (serum) levels were also done. The results didn't show any strong correlation (positive or negative) among the enzymes levels with established markers of diabetic nephropathy (ACR and cystatin C).

Increased albumin/creatinine ratio in the urine and cystatin-C levels in the serum are established markers of glomerular injury. Given the fact that urinary enzyme levels are increased in case of tubular dysfunction of the nephrons, the result of our study shows that, diabetic nephropathy primarily affects the glomerular function.

Our study further strengthens the idea of glomerular damage being the primary pathology in diabetic nephropathy ruling out tubular dysfunction in early stages. The study also had a few limitations. Due to time constraints we could not follow up the patients to look for the changes in the study parameters with respect to time. We also couldn't assess the relation between severity and duration of diabetes with the urinary markers. Given the high and increasing prevalence of diabetes mellitus in the current global scenario, a larger sample size would have helped in getting a more reliable result. Future prospective cohort studies may be undertaken where similar parameters can be estimated which would help in improving our understanding the pathology of the commonest and most dreadful complication of diabetes, i.e diabetic nephropathy.

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