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ESTIMATION OF URINARY ENZYMES FOR DIAGNOSIS OF DIABETIC NEPHROPATHY AT EARLY STAGE IN PATIENTS OF TYPE 2 DIABETES MELLITUS

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Abstract

Background: Diabetes mellitus (DM) is a metabolic disorder characterized by a rise in blood glucose levels. Uncontrolled diabetes may be associated with various complications, including both macrovascular and microvascular. Among these complications, diabetic nephropathy (DN) is particularly noteworthy, which eventually results in the onset of chronic kidney failure. **Aim:** to assess urinary enzymes as diagnostic markers for diabetic nephropathy at an early stage in type 2 diabetes mellitus.

Materials and methods: A total of 90 diabetic patients, 90 patients with diabetic nephropathy, and 90 control subjects were recruited. Following this, blood and urine samples were collected and sent to the laboratory for estimation of blood glucose levels (FBS mg/dl and PPBS mg/dl) and serum creatinine, urine albumin creatinine ratio (mg/g), urine alkaline phosphate (U/l), and urine gamma-glutamyl transpeptidase (U/l). The data was analyzed by ANOVA using SPSS software version 26.0.

Results: An increase in mean blood glucose and serum creatinine, urine albumin creatinine ratio, urine alkaline phosphate, and urine gamma glutamyl transpeptidase levels were noticed in both patients with DN and DM. Additionally, the DN group showed an even higher rise in blood glucose, serum creatinine, urine albumin creatinine ratio, urine ALP, and urine GGT levels than the diabetic group.

Conclusion: Based on the findings of the present study, it is concluded that estimation of serum creatinine, urine albumin creatinine ratio, and urinary enzymes are indicators for early diagnosis of diabetic nephropathy in DM patients, as all these parameters were elevated in patients with diabetic nephropathy and subjects with diabetes mellitus.

Keywords: Diabetic nephropathy (DN), Diabetes mellitus (DM), Urine alkaline phosphatase (ALP), Gamma Glutamyl transpeptidase (GGT).

INTRODUCTION

Diabetes mellitus is a metabolic and multifactorial condition characterized by high levels of glucose in the blood, referred to as hyperglycemia, due to deficiencies in insulin secretion, function, or both. This condition leads to a persistent metabolic imbalance that increases the risk of long-term complications, including both macrovascular and microvascular problems. Among these complications, diabetic nephropathy is particularly noteworthy. Type 2 diabetes mellitus is a prevalent and growing health issue globally. Presently, there are 415 million individuals worldwide living with diabetes, and this figure is expected to exceed 642 million by 2040. In India, the prevalence of diabetes mellitus is 8.7%, affecting around 69.2 million people, and it is expected to rise to 109 million by 2035. [1, 2]

Diabetic nephropathy is a major complication of diabetes that eventually results in the onset of chronic kidney failure.[3, 4] Individuals suffering from diabetes mellitus (DM) are at a much greater risk of developing end-stage kidney failure, with a tenfold higher chance. The International Diabetes Federation (IDF) reports that around 40% of people with diabetes could eventually experience final-stage renal failure. Furthermore, the onset of end-stage kidney failure is often linked to diabetes and hypertension, either together or separately, which account for roughly 80% of cases. [5] Microalbuminuria is an early sign of DN. About 20% of people with microalbuminuria develop nephropathy within ten years, and a similar percentage progress to end-stage kidney disease. On the other hand, T1DM patients have a 20% risk of developing end-stage kidney failure within ten years and 75% within less than two decades. Conversely, individuals with T2DM show signs of microalbuminuria and nephropathy shortly after being diagnosed with diabetes.[6, 7]

The first indication of diabetic nephropathy is the elevated release of albumin in the urine, coupled with the growth of the glomerular and renal components, hyperfiltration, and proliferation of the mesangial cells caused by the buildup of extracellular matrix proteins such as fibronectin, laminin, and collagen. [8] There exist two categories of risk factors linked to DN, specifically modifiable and non-modifiable factors. Modifiable factors include hypertension, glycemic level control, and dyslipidemia. Additionally, smoking has been recognized as an extra-modifiable risk factor. [9, 10] Non-modifiable factors include race, age, pregnancy, genetic makeup, and gender. Individuals with a familial background of DN have been found to be more prone to developing the disorder.[11]

DN undergoes three distinct phases. The initial phase begins with thickening of the glomerular basement membrane (GBM). During this phase, normal glomerular filtration rate (GFR), absence of albuminuria, and hypertension are commonly observed for a period of five years following the onset of GBM thickening. The subsequent phase involves the progression of mild to severe mesangial expansion. Even after two years from the initiation of GBM thickening and mesangial proliferation, normal GFR levels persist, and no other clinically significant symptoms are documented. [12] The final phase is characterized by glomerular damage and increased microalbuminuria, ranging from 30 to 300 mg per day. A recent study revealed that 38% of patients developed microalbuminuria, and 29% saw a decrease in GFR after being followed up for 15 years. The research also indicated a progression rate of 2.8% from microalbuminuria to end-stage renal disease (ESRD) and 2.3% from GFR to ESRD. [13]

MATERIALS AND METHODS

A prospective open-label comparative study was conducted in the department of biochemistry in collaboration with department of general medicine atPt. J.N.M. Medical College and DR. B.R.A.M. Hospital, Raipur, C.G., from June 2019 to August 2019. The study protocol was approved by the institutional ethical committee, and every participant had given their written informed consent before the commencement of the study. Participants were recruited as per the specified inclusion criteria.

Inclusion criteria:

- Type 2 diabetic patients of either sex for Group 1
- Patients of either sex with early diabetic nephropathy as diagnosed by the presence of microalbuminuria (30–300 mg/g of creatinine) for group 2
- Age range between 30-70 years
- Patients who were willing to give their written, informed concern

Exclusion Criteria

- Patients with type 1 diabetes mellitus
- Pregnancy and lactation
- Patients taking nephrotoxic drugs
- History of chronic illnesses like liver cirrhosis, coronary heart disease, malignancy, and psychiatric problems.
- Patients with symptoms of urinary tract infection.

Based on the above-mentioned specific inclusion and exclusion criteria and after explaining the purpose of the current study, a total of 270 participants were recruited. Out of 270, 90 were diagnosed with type 2 DM, 90 had diabetic nephropathy, and the remaining 90 were healthy individuals. At the beginning of the study, sociodemographic data along with the past medical history of all the participants were taken. Following this, blood and urine samples were collected and sent to the laboratory for estimation of basic blood glucose parameters such as HbA1c, FBS, and PPBS, as well as serum creatinine and urine albumin creatinine ratio (mg/g), urine alkaline phosphate (u/l), and urine gamma glutamyl transpeptidase (u/l), respectively. Data was collected and analyzed by ANOVA using SPSS software version 26.0. Results were expressed as mean ± standard deviation, and a p value >0.05 was considered to be statistically significant.

RESULTS

The current study included a total of 270 participants: 90 diagnosed with diabetic nephropathy, 90 with diabetes mellitus, and the remaining 90 being healthy individuals. Out of the 270 participants, 160 were male, and the remaining 110 were female. Additionally, the majority of participants (52%) were in the 41–50 age group, 26% were in the 31–40 age group, 18% were in the 51–60 age group, and the remaining 4% were in the 61–70 age group. **Figure-1**

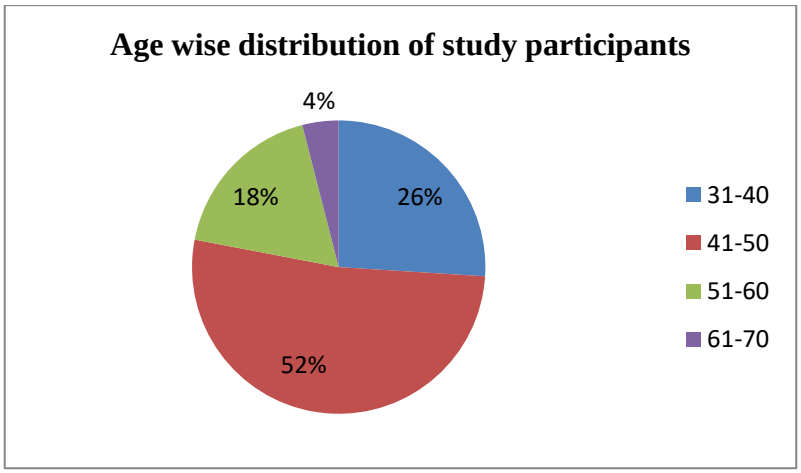

Figure-1: Showing age wise distribution of study participants

Upon comparison of the groups, a significant difference in mean FBS (mg/dl), PPBS (mg/dl), and BMI (kg/m2) was observed, which was statistically significant (P<0.05). Patients with diabetic

nephropathy and diabetes mellitus exhibited higher blood glucose levels compared to healthy individuals. Furthermore, patients with diabetic nephropathy showed an even greater increase in blood glucose levels than diabetic patients. **Table 1 and Figure 2.**

When comparison was done between the groups, a notable difference was observed in the mean levels of serum creatinine (mg/dl), urine albumin creatinine ratio (mg/g), urine alkaline phosphate (u/l), and urine gamma glutamyl transpeptidase (u/l), which was statistically significant (P<0.05). Patients with diabetic nephropathy and diabetes mellitus exhibited higher levels of these parameters compared to healthy individuals. Furthermore, patients with diabetic nephropathy displayed an even greater increase in the mentioned parameters than diabetic patients. **Table 2 and figures**

Table-1: Level of Blood glucose (FBS&PPBS) and BMI in study and healthy participants

Variables	Group	n	Mean± SD	p value (ANOVA)
FBS(mg/dl)	Diabetic Nephropathy	90	191.9±52.8	<0.05
	Diabetic Mellitus	90	151.6±23.7	
	Healthy Controls	90	83.71±9.21	
PPBS(mg/dl)	Diabetic Nephropathy	90	271.35±73.34	<0.05
	Diabetic Mellitus	90	211.75±51.8	
	Healthy Controls	90	158.3±15.4	
BMI(Kg/M^2)	Diabetic Nephropathy	90	28.6 ± 3.5	<0.05
	Diabetic Mellitus	90	24.5±3.5	
	Healthy Controls	90	22.0 ±2.14	

P<0.05 statistically significant**

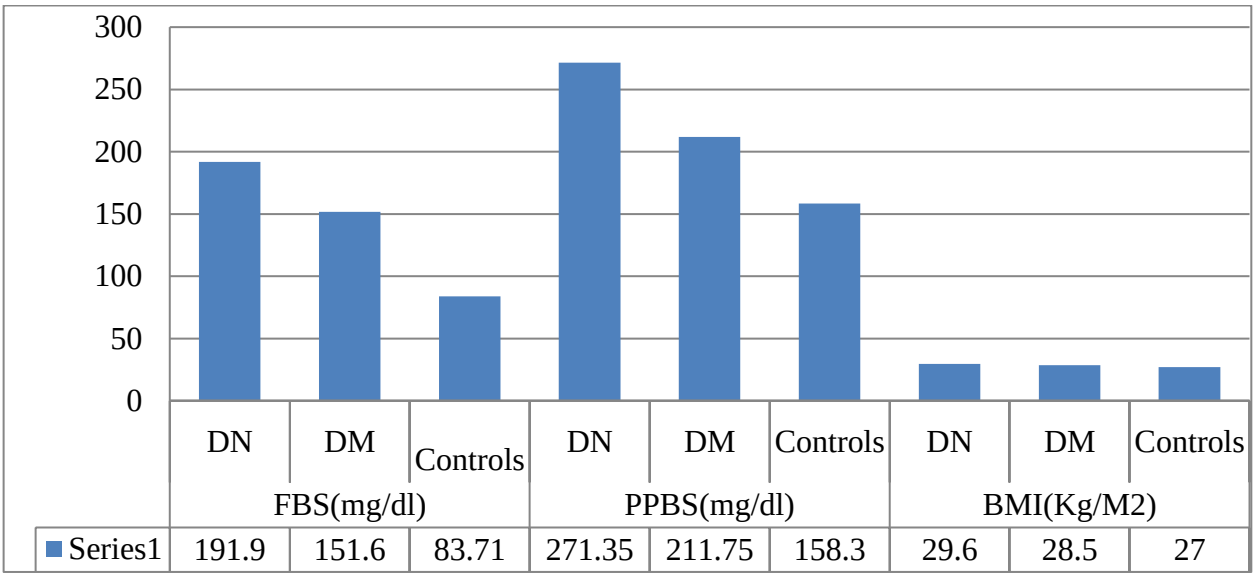

Figure-2: Histogram showing the mean value of blood glucose and BMI in study group and healthy participants

Table-2: Level of serum creatinine& urinary enzymes in study and healthy participants

Variables	Group	n	Mean± SD	p value (ANOVA)
Serum Creatinine (mg/dl)	Diabetic Nephropathy	90	1.60 ± 0.28	<0.05
	Diabetic Mellitus	90	0.78 ± 0.14	
	Healthy Controls	90	0.71 ± 0.14	

Urine Albumin Creatinine Ratio (mg/g)	Diabetic Nephropathy	90	268.86 ±37.0	<0.05
	Diabetic Mellitus	90	30.56±1.64	
	Healthy Controls	90	22.90±4.98	
Urine Alkaline phosphate (u/l)	Diabetic Nephropathy	90	16.7±3.87	<0.05
	Diabetic Mellitus	90	8.88± 1.85	
	Healthy Controls	90	4.35± 1.36	
Urine Gamma Glutamyl transpeptidase (U/L)	Diabetic Nephropathy	90	45.93 ±8.69	<0.05
	Diabetic Mellitus	90	12.25 ± 1.75	
	Healthy Controls	90	6.2 ± 1.48	

P<0.05 statistically significant**

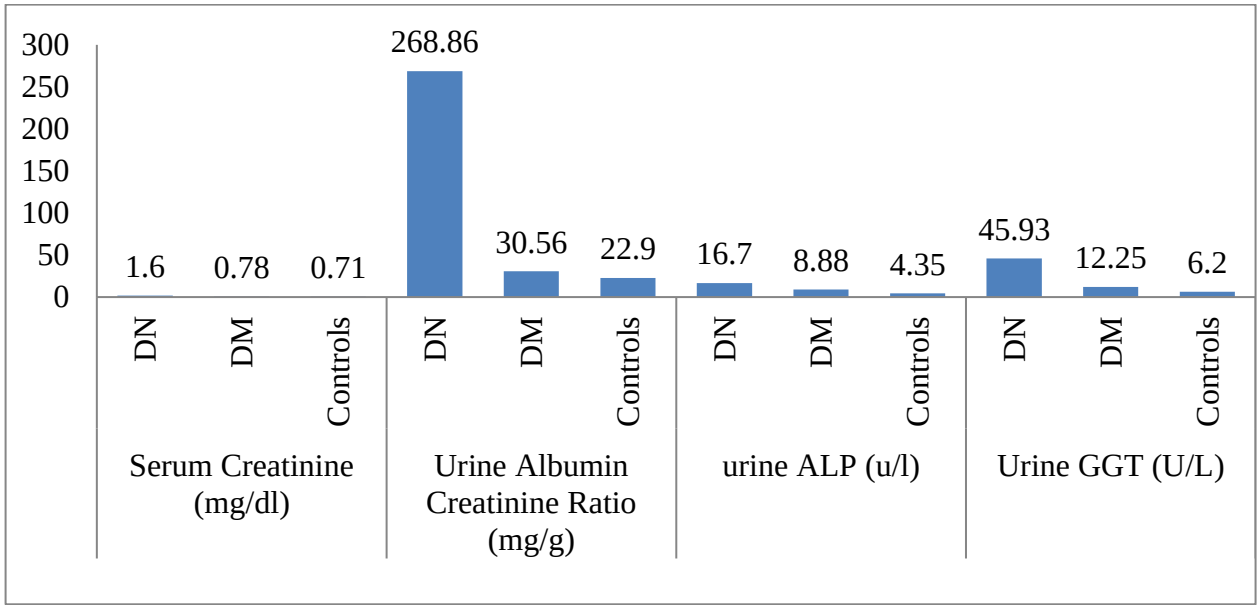

Figure-3: Histogram showing the mean value of serum creatinine& urinary enzymes in study and healthy participants

DISCUSSION

The primary objective of this study was to assess the urinary enzymes as potential early markers for diabetic nephropathy in individuals with diabetes. A total of 270 participants took part in this research, with 160 males and 110 females. The majority of participants (52%) fell within the 41–50 age group, while 26% were in the 31–40 age group, 18% in the 51–60 age group, and the remaining 4% were aged between 61 and 70 years. **Figure -1.** There were notable differences in BMI kg/M^2 among participants from the test and control groups, with DN: 28.6 ±3.5; DM: 24.5 ±3.5; and control group: 22.0 ±2.14, consistent with previous findings. [14] Significant variations in mean FBS (mg/dl) and PPBS (mg/dl) were observed between the groups, with diabetic nephropathy patients displaying even higher levels. **Figure-2** A similar study also reported a significant increase in mean PPBS levels in both diabetes mellitus and DN patients. [15]

Upon comparison of all three groups, a notable difference in average serum creatinine (mg/dl), urine albumin creatinine ratio (mg/g), urine alkaline phosphate (u/l), and urine gamma glutamyl transpeptidase (u/l) levels was observed among the groups, demonstrating statistical significance ($p<0.05$). Patients with diabetic nephropathy exhibited even higher levels of urinary enzymes and serum creatinine. **Figure-3** Furthermore, a significant increase in mean serum creatinine levels was reported in a previous study (16), although the diabetic group was not specifically compared with patients suffering from diabetic nephropathy. Another study also highlighted a significant elevation

in mean urine alkaline phosphate levels in diabetic nephropathy patients compared to those without diabetic nephropathy.[17] Previous studies have also shown an increase in mean urinary ALP in diabetic patients. [18, 19] Additionally, several previous studies have confirmed an increase in urine GGT levels in diabetic patients and also in diabetic nephropathy when compared to control subjects. [20, 21, 22, and 19]

Urinary enzymes such as alkaline phosphatase and gamma glutamyl transferase are indicators of tubular injury. ALP and GGT are located in the luminal brush border of the epithelial cell membrane within the proximal tubule lumen. [23, 24] Since tubular damage occurs before glomerular damage, the levels of these enzymes can rise, making them valuable early diagnostic markers for diabetic nephropathy. Microalbuminuria, or diabetic nephropathy associated with significant glomerular damage, is regarded as an early marker of kidney dysfunction in individuals with type 2 diabetes mellitus. Diabetic nephropathy is the primary cause of end-stage renal disease, and high blood sugar levels are a major factor in its progression.

CONCLUSION

The urinary enzymes alkaline phosphatase and gamma glutamyl transferase are markers of tubular damage. Based on the findings of the present study, it is concluded that estimation of serum creatinine, urine albumin creatinine ratio, and urinary enzymes are indicators for early diagnosis of diabetic nephropathy in DM patients, as all these parameters were elevated in patients with diabetic nephropathy and in subjects with diabetes mellitus.

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