**Original article:**

**A study of urinary enzymes as a marker of early renal damage in patients suffering from diabetes mellitus**

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Abstract:

**Introduction:** Diabetic nephropathy is a serious major microvascular complication leading to the death of patients suffering from diabetes mellitus. Therefore, this study was designed to determine whether the activities of these tubular enzymes could be used as screening markers for renal dysfunction in diabetic patients.

**Materials and methods:** The study consisted of 90 diabetic patients (subdivided into normoproteinuria, microproteinuria and renal failure) admitted in Gauhati Medical College and Hospital and 30 healthy controls. Proximal tubular structural integrity was studied by determining the activities of the enzymes U.NAG, U.ALP, U.GGT, and U.LDH.

**Observations and results:** A statistically significant increase in diabetic patients was found in urinary NAG, ALP, LDH, and GGT excretion compared with control groups (p < 0.0001, p < 0.01, p < 0.01, and p < 0.0001, respectively). The values of serum urea and serum creatinine were the same for the control, diabetic patients with normoproteinuria and with microproteinuria (p>0.05) but different between the diabetic patients with microproteinuria and with renal failure (p<0.05).

**Conclusion:** The results suggest that site-specific urinary biochemical markers provide valuable information about early renal proximal tubular damage that ultimately may precede glomerular permeability in Diabetes.

**Key words:** Diabetic nephropathy, Tubular integrity, Urinary tubular enzymes,microalbuminuria,renal failure

**Introduction**

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Diabetes mellitus has recently assumed epidemic proportions and affects more than 285 million individual worldwide. Global estimates for the year 2030 predict a further growth of almost 50%, with the greatest increases in the developing countries of Africa, South America and Asia. [2]

Currently India leads the world with the largest number of diabetic subjects and this is expected to further rise in the coming years. Given the high prevalence of diabetes in Indians with over 50 million diabetics already and the numbers expected to increase to 87 million by the year 2030, this could place considerable burden on the health budgets of this country. The medical and socioeconomic burden of the disease is caused by the associated complications, which impose enormous strains on health-care system. Diabetic nephropathy...
is major microvascular complication of diabetes, a leading cause of end-stage renal disease and is associated with increased cardiovascular mortality [2].

Diabetic nephropathy is characterised structurally both by glomerular lesions and changes in the tubulointerstitial compartment of the kidney, and functionally by increasing severity of microproteinuria and altered Glomerular Filtration Rate (GFR), the later usually being assessed in the laboratory by measurements of serum or plasma creatinine concentrations. Furthermore, End-Stage Renal Disease (ESRD) in diabetics is increasing and now accounts for approximately 40% of treated ESRD by either transplantation or dialysis [3].

The routinely available clinical parameters of kidney disease - blood urea nitrogen, serum creatinine and urinary microprotein are usually not sensitive since these parameters could be within normal ranges despite considerable impairment of the renal function because of the great reserve capacity of the renal function.

It is well established that the detection of microalbuminuria in a patient with diabetes mellitus indicates the presence of glomerular involvement in early renal damage. It would be better prognostically, if diabetic nephropathy can be detected at an even earlier stage, before the appearance of microalbuminuria, so that intervention could reverse the process, or even prevent the onset of nephropathy altogether. Recent studies have demonstrated that, there is a tubular component in renal complications of diabetes as shown by the detection of renal tubular enzymes and low molecular weight proteins in urine. In fact, tubular involvement may precede glomerular involvement as several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine [4].

Alkaline phosphatase a phosphohydrolase enzyme attached to the cell wall by glycosyl phosphatidylinositol anchors, and lactate dehydrogenase, a key enzyme in energy metabolism located in the cell cytoplasm, are more prominent even before the appearance of microalbuminuria [5]. Gamma glutamyl transferase in urine originates from the surface of brushy border of epithelial cell membrane in the proximal tubules lumen. Gamma GT is a specific and sensitive indicator of these cells damages [6].

This study aimed to investigate whether urinary activities of NAG, ALKP, GGT, and LDH can be used as a marker of early renal damage in diabetic patients.

**Aims and objectives of the present study**

1. To determine the blood urea and serum creatinine in the study groups.
2. To determine the urinary levels of N-acetyl-β-D-glucosaminidase, Gamma glutamyl transferase, Alkaline phosphatase and lactate dehydrogenase enzymes in the study groups.
3. To compare the levels of all four urinary enzymes in all the study groups.
4. To utilize the data for a new clinical approach for early diagnosis of patients suffering from diabetes mellitus.

**Methods**

**Study population:** The study was carried out in the department of biochemistry between the months May 2013 – June 2014. The study was approved by the Institutional Ethics Committee, Gauhati Medical College and Hospital and informed, written consent was obtained from all the participants in the study. The study subjects were recruited from the department of medicine, department of endocrinology, and department of nephrology of Gauhati medical college and hospital, Guwahati, while the control group with
age and sex matched were selected from the hospital who came as attendants in the different departments. The study consisted of a total of 120 participants. The participants were divided into four groups as follows:

1. Healthy control group – This group contained 30 sex-matched controls drawn from healthy population. Age range was 25-65 years, with 42.96 ± 10.59 years as mean.

2. Test groups was further divided into the following groups:
   A) Diabetic patients with normoalbuminuria (test group 1) – This group contained 30 diabetic patients without significant albuminuria (<30 mg/day). Age range was 26-72 years, with a mean of 49.5 ± 11.11 years.
   B) Diabetic patients with microalbuminuria (test group 2) – This group contained 30 diabetic patients with significant albuminuria (30-300 mg/day). Age range was 27-75 years, with a mean of 54.43 ± 11.66 years.
   C) Diabetic patients with macroalbuminuria or renal failure (test group 3) – This group contained 30 diabetic patients with kidney failure (>300 mg/day). Age range was 29-80 years, with a mean of 59.23 ± 17 years.

Exclusion criteria: All patients in this study had no medical history of, urinary tract diseases, ischemic heart disease, use of nephrotoxic drugs, smoking, renal parenchymal disease, and infections over the previous months.

Sample collection: 24 hours urine specimen in a closed container was collected from each participant. The urine was centrifuged at 3000 rpm for 5 min. The supernatant was distributed in vials of 1.5ml each and biochemical analysis was done within four hours. The remaining sample was kept frozen at -20°C. Five ml of blood was drawn from the participants under aseptic conditions from the median cubital vein. It was collected in properly labelled vacutainers and then centrifuged at 3000 rpm for 15 minutes. The serum thus obtained was subjected to biochemical analysis within 8 hours of collection of blood.

Sample analysis: Estimation of urinary enzymes was done using MERCK microlab 300 semiautoanalyser. Urinary NAG activity was measured without freezing, using a commercially available colorimetric kit assay based on MNPGlcNAc substrate [7]. The developed color was measured using MERCK microlab 300 semiautoanalyser. Urinary GGT [8], ALKP [9], and LDH [10] were measured using commercially available assay kit based on kinetic photometric test. Serum creatinine and Blood urea were measured by using commercially available assay kits by Modified Jaffe’s Kinetic Method [11] and Modified Berthelot Method [12] respectively.

All data were expressed as Mean ± SD. One way ANOVA was performed to evaluate whether the difference in the mean of all four urinary enzymes of the four respective groups was significant or not. P-value of less than 0.05 was considered to indicate statistical significance. Statistical analysis was performed using IBM SPSS Statistics Program Version 20.0.0.
Results

Biochemical parameters in diabetic patients: A statistically significant increase in diabetic patients was found in urinary NAG, ALKP, LDH, and GGT excretion compared with control groups (p < 0.0001, p < 0.01, p < 0.01, and p < 0.0001, respectively). The values of serum urea and serum creatinine were the same for the control, test group 1 and test group 2 (p>0.05) but different between the test group 2 and test group 3 (p<0.05). (Graph 1.2, 1.3, 1.4 & 1.5)

Correlations in the three test groups under study: There were significant positive correlations between urinary NAG, ALKP, GGT, and LDH in all the three test groups (p<0.05). The study also demonstrated significant positive correlations between serum urea and NAG, ALKP, GGT, and LDH in diabetic patients with renal failure (p<0.05). The same trend was observed between serum creatinine and all the other urinary renal markers in the same study group.

[Table/Fig1]: Levels of urinary and serum biomarkers of renal injury in normal controls and in various stages of diabetic subjects/patients

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>HEALTHY CONTROL</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>U.NAG (IU/L)</td>
<td>8.94±5.22</td>
<td>13.84±8.28</td>
<td>18.50±8.47</td>
<td>30.32±21.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>U.GGT (U/L)</td>
<td>5.02±0.76</td>
<td>8.01±4.67</td>
<td>11.83±8.20</td>
<td>15.86±9.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>U.ALKP (U/L)</td>
<td>2.44±1.02</td>
<td>2.65±1.36</td>
<td>3.21±1.45</td>
<td>3.72±1.56</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>U.LDH (U/L)</td>
<td>4.24±2.59</td>
<td>5.30±2.77</td>
<td>7.85±5.03</td>
<td>8.11±7.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>S.UREA (mg/dl)</td>
<td>30.40±10.75</td>
<td>32.82±11.34</td>
<td>34.38±14.30</td>
<td>180.30±73.38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S.CREAT (mg/dl)</td>
<td>1.05±0.52</td>
<td>1.17±0.71</td>
<td>1.35±0.78</td>
<td>8.67±3.79</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

[Table/Fig2]: Pearson correlation coefficient of urinary biomarkers in the various study groups

**: p-value<0.01; *: p value<0.05; r = Pearson Correlation Coefficient

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PARAMETER</th>
<th>NORMOALBUMINURIC SUBJECTS</th>
<th>MICROALBUMINURIC SUBJECTS</th>
<th>RENAL FAILURE SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)U. NAG</td>
<td>U.GGT</td>
<td>r = 0.43*</td>
<td>r = 0.41*</td>
<td>r = 0.53**</td>
</tr>
<tr>
<td></td>
<td>U.ALKP</td>
<td>r = 0.41*</td>
<td>r = 0.50**</td>
<td>r = 0.50**</td>
</tr>
<tr>
<td></td>
<td>U.LDH</td>
<td>r = 0.44*</td>
<td>r = 0.52**</td>
<td>r = 0.54**</td>
</tr>
<tr>
<td>2)U.ALKP</td>
<td>U.GGT</td>
<td>r = 0.39*</td>
<td>r = 0.45*</td>
<td>r = 0.40*</td>
</tr>
<tr>
<td></td>
<td>U.LDH</td>
<td>r = 0.51*</td>
<td>r = 0.40*</td>
<td>r = 0.38*</td>
</tr>
<tr>
<td>3)U.GGT</td>
<td>U.LDH</td>
<td>r = 0.70**</td>
<td>r = 0.41*</td>
<td>r = 0.36*</td>
</tr>
</tbody>
</table>
Graph 1.2: Histogram Showing the mean value of Urinary NAG in the control and test groups.

Graph 1.3: Histogram Showing the mean value of Urinary GGT in the control and test groups.

Graph 1.4: Histogram Showing the mean value of Urinary ALKP in the control and test groups.
Graph 1.5: Histogram Showing the mean value of Urinary LDH in the control and test groups.

Discussion

Diabetes mellitus is a global disorder and complications resulting from the disease are the third leading cause of death in the world [3]. Kidneys are one of the important organs that are involved in diabetes. Since with untreated diabetic nephropathy, a significant decrease in life expectancy of patients happens; therefore, prevention of this debilitating condition and if there is, early diagnosis and treatment is important [13]. In clinical trials, 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, reliable, specific, as there is a time delay between renal injury and detection [3] and when nephropathy is diagnosed by these classical methods, little can be done to prevent the progressive downhill course of renal failure [4]. So, interest has focused on the use of novel urinary biomarkers for the early diagnosis of diabetic nephropathy. They show greater sensitivity and specificity than conventional biomarkers.

It was found that the level of urinary NAG in the test group 1, test group 2, and test group 3 were significantly higher than in the control group with p value of <0.0001, indicated a progressive increase of NAG with increase renal involvement. These findings corroborate with the findings of Gatua et al [3] who observed in their study that diabetic patients with normoalbuminuria also excreted high level of U.NAG compared with healthy individuals and patients with kidney impairment excreted high levels of the U.NAG compared to diabetic patients without kidney failure. Similar observations have also been reported by various authors across the globe. [5], [14], [15], [16]. Assal et al [17] also observed in their study, a highly significant increase in level of U.NAG in all patient groups than in control group.

The enzyme NAG is a high-molecular weight hydrolytic lysosomal enzyme. Principally, it originates in proximal tubules and normally cannot pass through the glomerular filtration [18]. The increased activity of this enzyme would be regarded as reflecting lysosomal enzyme activation in tissues, occurring in response to the metabolic need to degrade either various constituents of cells themselves; in a situation involving increased tissue catabolism, or mucopolysaccharides and
glycoproteins that have accumulated in tissues - as is the case in diabetics with vasculopathies [19]. Increased urinary NAG excretion supposedly indicates tubular dysfunction. Detection is useful for assessing the preclinical stage of diabetic nephropathy. Oxidative stress has been considered a common pathogenic factor in diabetes mellitus and its complications, including nephropathy. Hyperglycemia leads to enhanced reactive oxygen species production, and as a result tubular cell damage and abnormal urinary enzyme excretion develop [14]. Shokeir A.A [20] has demonstrated that NAG is the most widely assayed urinary enzyme for the detection of renal damage and the diagnosis of renal disease. This is due to its stability in urine, its relatively large molecular mass (130KDa) which precludes filtration by the glomerulus and its presence in high activity in the tubular lysosomes. Therefore, elevation of NAG activity in urine provides a marker for renal tubular damage or, more precisely, loss of lysosomal integrity.

In the present study, it was found that the level of urinary ALKP in the test group 1, test group 2, and test group 3 were significantly higher than in the control group with p value of <0.01, indicated a progressive increase from the control group with renal involvement. These findings corroborate with the findings of Gatua et al [3]. Mohammadi-Karakani et al [5] and Jung et al [15] have found that urinary ALKP were excreted higher in diabetic patients compared with control groups. Uslu et al [14] have also demonstrated that urinary ALKP were higher in diabetics than in controls with a p value of <0.001. The level of urinary GGT in the test group 1, test group 2, and test group 3 were also significantly higher than in the control group with p value of <0.0001, indicated a progressive increase from the control through the renal group. These findings corroborate with the findings of Gatua et al [3] and Nikolov et al [18].

In this study the level of urinary LDH in the test group 1, test group 2, and test group 3 were significantly higher than in the control group with p value of <0.01, indicated a progressive increase from the control with the test group with renal involvement. These findings corroborate with the findings of Gatua et al [3], Uslu et al [14], and Mohammadi-Karakani et al [5]. Increase in urinary LDH reflects tubular injury in patients with diabetic nephropathy.

In our study, we observed a significant positive correlation between all the urinary enzymes with one another in patients with normoalbuminuria, microalbuminuria, and with renal failure. These findings corroborate with the findings of Gatua et al [3]. This can be explained by the fact that all the studied urinary enzymes are proximal tubular enzymes whose excretion is influenced by the same pathological conditions such as diabetes mellitus [3].

There were no significant correlation between serum creatinine and blood urea with U.NAG, U.GGT, U.ALP and U.LDH in the diabetic patients with normoalbuminuria and diabetic patients with microalbuminuria. However, there were significant positive correlations between blood urea and serum creatinine with all the measured urinary renal markers in the diabetic patients with renal failure. These findings corroborate with the findings of Gatua et al [3]. This can be explained by the fact that serum creatinine and blood urea are poor early renal markers and are usually elevated when too much pathological damage has already occurred [3].

**Conclusion**

Diagnosis of diabetic nephropathy in the early stages is very important since there are no clinical signs or symptoms and this disease should be
prevented rather than reversed. Traditional markers of renal function like blood urea, serum creatinine and microalbuminuria are insensitive and fail to identify early renal damage. Therefore there is great interest in identifying early reliable biomarkers. In the present study, we reviewed four biomarkers of tubular injury implicated in diabetic kidney disease. Urinary concentrations of these damaged markers are elevated in patients with diabetes when compared with nondiabetic control subjects. Our results suggest a relationship between changes in urinary enzymes and development of renal disease. A more elaborate study would have been desirable to precisely establish the role of urinary enzymes in diagnosing early renal damage in diabetics but, due to paucity of time, resource and due to conduction of the study in a sole institution it was not implementable. However, we made a modest effort to fulfil the same through whatever resources available. And the results of this study tally with most of the studies conducted in foreign land as well as with the few conducted in India. Hence, it is hoped that the present study will encourage further studies on the present topic in a bigger way. A humble beginning has been made, which if followed would prove useful to the diabetic nephropathy patients.

References


