Estimation of Renal Tubular Markers for Predicting Early Stage Diabetic Nephropathy in Egyptian Children with Type I Diabetes Mellitus

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Abstract: Background: Early detection of renal dysfunction is important since this is the first step in the progressive loss of renal functions and ultimately progression to renal failure. Urinary modern technology, minute quantities of LMWP and urinary enzymes can be measured. Excretory patterns that are characteristic for site and mechanism of renal injury often can be found. Objectives: This study was carried out to evaluate the correlation between the duration of diabetes, metabolic control (glycemic control, HbAlc) and the urinary tubular marker: B2-microglobulin, Retinol binding protein as (LMWP) and lysosomal enzyme N.acetyl B-D-glucosaminidase for early detection of subclinical nephropathy. Methods: Study population consisted of 20 randomly selected patients with type 1 diabetes mellitus that diagnosed before the age of 16 years and with a normal serum creatinine (<1.3mg/dl) and no signs of clinical nephropathy. 20 age and sex matched healthy controls were randomly selected from outpatient clinic of general pediatrics. Hemoglobin Alc (HbAlc), serum and urinary creatinine, creatinine clearance, urinary excretion of low molecular weight proteins and urinary N-B-D glucosaminidase were measured in all cases and controls. Results: The study showed a significant increase in B2-microglobulin (P<0.0001), RBP (P<0.0001), NAG (P<0.0001) in diabetic children compared to control. There was a positive correlation between the duration of diabetes and B2-microglobulin (P<0.0001), RBP (P<0.0001), NAG (P<0.05), HbAlc (P<0.0001). There was a significant positive correlation between glycemic control (HbAlc) and B2-microglobulin, RBP and N-acetyl B-D-glucosaminidase (P<0.001, P<0.0001, P<0.03 respectively. In the same time, there was no significant difference between the patients and controls in respect of serum creatinine, protein/24h in urine, creatinine in urine and creatinine clearance. Conclusion: tubular markers appear to be useful in early detection of diabetic nephropathy with positive correlation with the duration of type 1diabetes and glycemic control (HbAlc).

Key words: Diabetic nephropathy - renal tubular markers.

INTRODUCTION

Diabetes mellitus is a world health problem, affecting all age groups. Renal damage is a serious major micro-vascular diabetic complication in patient with types I diabetes mellitus leading to the death of diabetic patients[13]. Approximately, 30-40% of those patients develop end-stage renal disease and require either dialysis or renal transplantation for survival[22]. Thus diagnosis of diabetic nephropathy in an earlier stage would be critical and would help to reduce morbidity and mortality. The routine classical evaluation of diabetic nephropathy includes: appearance of micro albuminuria, decrease in creatinine clearance and increase in serum creatinine[13].

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 the urine. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine[6,30]. One of the most important frequently evaluated urinary enzymes is N-acetyl-beta-D-glucosaminidase (NAG), which is a hydrolytic lysosomal enzyme with high molecular weight (150,000 Da) and very low physiological activity[27]. It originates principally in proximal tubules and normally cannot pass through the glomerular filtration. NAG has been reported to be a very sensitive and reliable marker of renal failure[28].

Therefore, estimation of this biomarker is being done in various conditions involved with renal injury or dysfunction. Some of the uses of urinary NAG...
includes: nephritic syndrome, nephrotoxic drugs, urinary tract infection, heavy metal poisoning, kidney transplants, vesico-ureteral reflux and in diabetic children and adolescents. Tubular low molecular weight proteins (B2-microglobulin), retinol binding protein (RBP), ??? microglobulin and urine protein have been studied in the urine of diabetic patients and their roles as early indicators of diabetic nephropathy have been evaluated. Retinol binding protein (RBP), a low molecular weight protein, is filtrated by the glomeruli and then reabsorbed and catalyzed by the proximal tubules. Increased urinary RBP excretion occurs with altered tubular function and when tubular reabsorptive capacity is exceeded. The excretion of B2-microglobulin was found to be correlated with RBP.

Therefore, this study was conducted to assess renal tubular function in type I diabetic patients by measuring urinary excretion of B-NAG, B2-microglobulin and RBP and correlating values to the duration of diabetes, glycemic control using glycosylated hemoglobin level (HbAlc), creatinine clearance and protein 24h/urine, as markers of tubular dysfunction for early detection of diabetic nephropathy (pre-clinical organ dysfunction).

MATERIALS AND METHODS

Study population consisted of 20 randomly selected patient with type I diabetes mellitus attended the diabetic outpatient pediatric and diabetic clinic of research institute of ophthalmology, with mean age 12.6±3.1 years, with disease duration more than 5 years. All patients included in the study had diabetes onset before the age of 16 years. They were receiving human insulin from the time of diagnosis, had a normal serum creatinine (<1-3mg/dl) and had no signs of clinical nephropathy. Twenty age and sex matched healthy controls were randomly selected from outpatient clinic of general pediatrics of research institute of ophthalmology (Coming with minor complaints, with no history of either diabetes or kidney disease).

Procedure: Glycemic control of all cases and controls was assessed by measuring the hemoglobin Alc (HbAlc) by Glycohemoglobin in HbAl test kit from STANBIO laboratory, San Antonio, Taxes, USA according to the manufacturer instruction. Serum and urinary creatinine were estimated in all cases and controls by quantitative calorimetric kit from STANBIO laboratory, San Antonio, Taxes and USA according to the manufacturer instruction. Serum creatinine was used as marker for glomerular filtration rat (GFR). 24-hour urine was collected from cases and controls for measuring urinary creatinine, creatinine clearance and proteins.

Spot urine samples were used to measure urinary excretion of low molecular weight proteins: B2-microglobulin and Retinol Binding Protein (RBP) by using Radial immunodiffusion kits from binding site England, according to the manufacturer instruction. Urinary N-B-D – glucosaminidase was measured by enzyme assay using P-nitrophenol linked substrate from Sigma according to the method of Yang et al.

Statistical Analysis: Results were expressed as the mean ± standard deviation (S.D.). Data were analyzed using an IBM personal computer. Data were compared by Paired t-test for independent variables. Values of P<0.05 were considered significant. Linear correlations between different variable and duration of diabetes and glycemic control (HbAlc) were done.

RESULTS AND DISCUSSION

Results: Table (1) shows the demographic data of studied groups as regard age and sex. There was no significant difference between diabetic patients and controls as regard age & sex. Tables (2 and 3) show the descriptive data of studied variables expressed as mean and standard deviation and shows the comparison between both groups as regard the studied variables. There was no significant difference between both groups as regards to protein per 24 hours urine (p<0.124), serum creatinine (p<0.32), urinary creatinine (p<0.108), and creatinine clearance (p<0.702).

As regards tubular injury markers, urinary B2microglobulin and urinary RBP were significantly higher in patients than controls (p<0.0001); urinary NAG was significantly higher in patient compared to control (p<0.01). Metabolic control shown by HbAlc was significantly higher in patients compared to control (p<0.0001).

Table (4) shows significant positive correlation between diabetes duration and other variables including HbAlc, urinary B2microglobulin (p<0.0001), urinary RBP (p<0.0001), and urinary NAG (p<0.05) as well as the correlation between HbAlc and other variables. It demonstrates highly positive correlation between HbAlc and B2microglobulin (p<0.0001) RBP (p<0.0001) and NAG (p<0.03).

Discussion: In our work we have studied the excretion of two markers of low molecular weight protein (LMW) B2 microglobulin and Retinol binding protein and a lysosomal enzyme: urinary N-acetyl B-D-glucosaminidase. Hong and Chia stated that the detection of renal tubular proteins and enzymes may precede glomerular involvement, as several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria.
Table 1: The demographic data of studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients (N=18)</th>
<th>Controls (N=18)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>12.6 ± 3.1</td>
<td>13.6 ± 1.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>34.6%</td>
<td>51.35%</td>
<td>0.41</td>
</tr>
<tr>
<td>females</td>
<td>65.4%</td>
<td>48.64%</td>
<td></td>
</tr>
</tbody>
</table>

P value is not significant P >.05

Table 2: Comparison between renal functions of patients and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Creatinine(mg/dl)</td>
<td>0.630 ± 0.101</td>
<td>0.722 ± 0.109</td>
<td>0.32</td>
</tr>
<tr>
<td>Urine Creatinine(mg/kg/24h)</td>
<td>13.180 ± 0.429</td>
<td>14.490 ± 1.01</td>
<td>0.108</td>
</tr>
<tr>
<td>Creatinine clearance(mg/24h)</td>
<td>95.50 ± 2.715</td>
<td>98.00 ± 6.95</td>
<td>0.702</td>
</tr>
<tr>
<td>Protein/24h urine gm/24h</td>
<td>0.210 ± 0.04</td>
<td>0.256 ± 0.01</td>
<td>0.124</td>
</tr>
</tbody>
</table>

Significance P<0.05

Table 3: Comparison between patients and controls considering glycemic control and tubular injury makers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBALC</td>
<td>4.81 ± 0.12</td>
<td>9.87 ± 3.41</td>
<td>0.0001</td>
</tr>
<tr>
<td>B2-microglob. µg/L</td>
<td>1.256 ± 350</td>
<td>2.394 ± 792</td>
<td>0.0001</td>
</tr>
<tr>
<td>Urinary RBPµg/L</td>
<td>0.689 ± 0.030</td>
<td>1.443 ± 0.363</td>
<td>0.0001</td>
</tr>
<tr>
<td>Urinary NAG ( µ/gCreatinine)</td>
<td>1.986 ± 0.397</td>
<td>3.406 ± 2.608</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Significance P<0.05

Table 4: Correlation between duration of DM and HbA1c with tubular markers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Duration</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>0.75</td>
<td>0.000*</td>
</tr>
<tr>
<td>Urinary B2 microglobulin</td>
<td>0.67</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>0.0001*</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Urinary RBP</td>
<td>0.64</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>0.0001*</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Urinary NAG</td>
<td>0.39</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>0.05*</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

*Correlation is significant at the level 0.05

Jung et al. [14,13] stated that, urinary excretion of renal tubular enzymes and low molecular weight proteins have been recommended as useful markers for detection of minor changes in proximal tubular function long before elevation in other markers as proteinuria and rise in serum creatinine.

Our study showed that urinary excretion of B2 microglobulin (LMWP) was significantly higher in diabetic children (p<0.0001) than controls with positive correlation with the duration of diabetes (p<0.001), which indicates an increase in glomerular permeability and/or decrease re-absorption of proximal tubules. Musialik et al. [19] stated that urinary B2 microglobulin is a sensitive marker of increased glomerular filtration and proximal renal tubular function. Chiaramonte et al. [17] stated that elevated urinary microprotein (B2 microglobulin) might be a useful marker of renal injury in children.

On the other hand, Mojiminiyi and Abdella[16] showed that there is no significant difference between diabetic and controls as regard B2 microglobulin, but Mortada et al.[17], determined urinary excretion of B2 microglobulin, as a marker of tubular damage. Aksun et al.[2], stated that increased B2 microglobulin in diabetes may be early indicator of diabetic nephropathy. Also, in our study we found a significantly higher increase in retinol binding protein excretion (p<0.0001) in diabetic children compared to control with a positive correlation with the duration of diabetes (p<0.0001).

This co-inside with the results of Matti et al.[20] who stated that retinol binding protein (RBP) and B2 microglobulin were higher in diabetic patients than in controls. Pontuch et al.[24] and Galanti et al. [10] suggested that increased urinary RBP in diabetic patients showed impaired proximal renal tubular function in early stage of diabetic nephropathy, this is also correlated with several previous studies of Dubrey et al.[13] where they showed that urinary RBP was significantly increased in diabetic patients compared to controls. In a follow up study, Sarasua et al.[26] confirmed the utility of RBP as a kidney biomarker of preclinical organ dysfunction in diabetic patients. Lysosomal enzymes which are high molecular weight protein (HMWP) may appear in the urine, but those usually derived from damaged tubular cells directly rather than from the circulation. Urinary NAG is known to be distributed more widely among the nephron and is released as a result of tubular damage [22].
Our study showed that, the urinary excretion of NAG was significantly higher in diabetic group compared to controls (p<0.01), with positive correlation with the duration of diabetes (p<0.05), which was contradict to the result of Mungan et al. who found no correlation between the duration of diabetes and elevation of NAG. Our data agrees with who found that urinary excretion of NAG was significantly higher in IDDM subjects compared to control subject (p<0.001). Also, our data agrees with who stated that excretion of NAG and RBP indicate proximal tubular dysfunction and may identify early diabetic nephropathy. At the same time who stated that, urinary NAG excretion was significantly greater in patients with diabetes Mellitus in respect to healthy controls. Regression analysis showed that, urinary NAG excretion was significantly associated with duration of D.M.

As regards glycemic control, we demonstrated a significant positive correlation between it and each of $B_2$ microglobulin, RBP and NAG. This corresponds with the finding of who reported a significant correlation between urinary $B_2$ microglobulin, NAG, RBP and HbA1c (p<0.001), and who found high correlating between urinary RBP and HbA1c and with as they found that urinary NAG excretion is associated with glycemic control (p<0.01) and they suggested that increased urinary excretion in hyperglycemic subjects may be due to the adverse effect of plasma proteins highly filtered through the glomerular capillary on tubular cells.

However, Mungan et al. found that, urinary NAG activity was not associated with levels of HbA1c. Holm et al. found no correlation between HbA1c urinary RBP excretion. In addition, we reported a significant positive correlation between urinary $B_2$ microglobulin globulin, urinary RBP and urinary NAG excretion. This agrees with the findings of who found positive correlation between RBP and NAG urinary excretion rate in diabetics. It also corresponds with findings of who found a significant correlation between urinary excretion of $B_2$ microglobulin and RBP and the excretion of $B_2$ microglobulin was higher than RBP.

In conclusion, this study suggests that, tubular dysfunction is an important component of diabetic renal disease. For early detection of diabetic nephropathy, it would be necessary to include some markers of tubular dysfunction. Of these, RBP, $B_2$ microglobulin and NAG appear to be useful. These documented tubular dysfunctions appear to be correlated with duration of type I diabetes and glycemic control (HbA1c).

REFERENCES


