Urinary Aspartate Transaminase in Childhood

YUTAKA HASEGAWA, MASUHIDE MIYAO, YOSHIIO KITAMURA, TAKEO MATSUZAWA* AND NOBUHIKO KATUNUMA*1

Department of Pediatrics, School of Medicine, Tokushima University and *Department of Enzyme Chemistry, Institute for Enzyme Research, School of Medicine, Tokushima University, Tokushima

(Received May 30, 1967).

Urinary aspartate transaminase in normal children and children with various renal diseases including orthostatic proteinuria were determined. The mean values of urinary aspartate transaminase activities in healthy, nephrotic, acute nephritic and orthostatic proteinuric children were found to be 12.9 ± 7.2, 53.0 ± 38.4, 115.8 ± 66.6 and 100.3 ± 63.4 μmoles oxaloacetate formed per hour in 24-hour urine, respectively.

In orthostatic proteinuria, the enzyme level in urine together with urinary protein excretion was almost equal to the values obtained from nephritic children. On the basis of these observations, a possible pathogenesis of orthostatic proteinuria was discussed.

Determination of urinary aspartate transaminase activity was found to be of use for the differential diagnosis of renal diseases. An isoenzyme nature of urinary transaminase was also studied.

In recent years, the measurement of serum transaminase level has been increasingly used for an aid to the diagnosis of the heart and liver diseases. However, only a few papers have been reported in which the measurement of the urinary enzymes was proved to be of aid to the diagnosis of renal diseases (1-5). Rosalki and Wilkinson stated that the urinary lactic dehydrogenase and transaminase levels elevated significantly in nephritis (1). Katunuma also found that urinary aspartate transaminase increased more markedly in nephritis than nephrosis and assumed that the urinary enzyme in some renal diseases was derived from kidney cells, since the increased release of aspartate transaminase into urine was found in nephritic patients with no other apparent complications (5).

Orthostatic proteinuria which occurs most frequently during childhood and puberty, is known as a disease of benign nature, and usually not a result of renal
dysfunction and occurs temporarily when a child with extreme lumbar lordosis is in a state of standing up. But the pathogenesis of this disease has not yet been clarified.

The purposes of this investigation were to study an enzymatic aspect of orthostatic proteinuria and at the same time to examine urinary aspartate transaminase activity in various renal diseases. The results obtained suggested strongly that the measurement of urinary aspartate transaminase could be used as a reliable aid for the diagnosis as well as for the prediction of prognosis of various renal disorders.

**EXPERIMENTAL**

1. **Test Material**

   The urine was obtained from the children with nephrosis or acute nephritis who were admitted to the pediatric clinic of Tokushima University Hospital. Children showing orthostatic proteinuria were found during the physical examination in schools or routine urinary tests of patients in our clinic.

2. **Preparation of Samples**

   For the enzyme assay, urine was first treated as follows. Fifty ml of fresh urine was cooled and centrifuged at 3,000 rpm for 10 minutes. The resultant clear supernatant was fractionated by the addition of ammonium sulfate to 50% saturation, and then centrifuged. The precipitate was washed repeatedly with 50% ammonium sulfate solution by means of centrifugation. Then, the precipitate was dissolved in a small amount of distilled water and 100 μg of pyridoxal phosphate was added. This solution was further centrifuged and the resultant supernatant was used as the enzyme source (5).

3. **Determination of Aspartate Transaminase**

   Aspartate transaminase activity was measured using the diazonium salt as reported by Katunuma and Nishii (6). The reaction mixtures contained 100 μg pyridoxal phosphate; 0.1 ml sample (the urinary enzyme solution); 5 μmoles 2-oxoglutaric acid; 20 μmoles aspartic acid; 25 μmoles Tris-HCl buffer (pH 8.5). The reaction mixtures were incubated for 20 minutes at 37° in round-bottom centrifuge tubes in a final volume of 2.0 ml. The reaction was started by the addition of enzyme solution. The reaction was stopped by adding 4 ml of diazonium-ethanol solution containing 5 μmoles of the diazomium salt and centrifuged. The resultant supernatant was allowed to stand for 20 minutes at room temperature. After the completion of color development, 1 ml of 2 N HCl was added. Color intensity was measured at 520 mμ. One unit of aspartate transaminase activity expressed throughout this paper represents the amount of enzyme forming 1 μmole oxaloacetate per hour at 37°.

   The specific activity was expressed as μmoles oxaloacetate formed per hour per mg protein at 37°. Protein was determined by the method of Folin and Ciocalteau.
RESULTS

1. Urinary Aspartate Transaminase Activity in Various Renal Diseases

Table 1 shows the results obtained in this study. Aspartate transaminase activities per 100 ml urine were found to be from 0.2 to 2.5 (1.2 ± 0.8) in normal, from 0.7 to 16.0 (7.3 ± 6.3) in nephrosis, from 4.3 to 26.8 (14.7 ± 7.5) in acute nephritis and from 4.5 to 17.3 (8.7 ± 3.2) in orthostatic proteinuria, respectively. These results were plotted in Fig. 1. In two cases of nephrotic children the urinary aspartate transaminase activity were shown to be within the normal value. But in acute nephritis and orthostatic proteinuria, the figures were apparently higher than those of normal and nephrotic children.

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Aspartate transaminase activity</th>
<th>Protein</th>
<th>Aspartate transaminase activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units per 200 ml urine</td>
<td>mg per 100 ml urine</td>
<td>Units per 24 hr urine</td>
<td>pmoles per hr per mg protein</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
<td>1.2 ± 0.8 (0.2-2.5)</td>
<td>5.2 (1.0-9.6)</td>
<td>12.9 ± 7.2 (1.7-29.3)</td>
<td></td>
</tr>
<tr>
<td>Acute nephritis</td>
<td>32</td>
<td>14.7 ± 7.5 (4.3-28.8)</td>
<td>133.1 (40.6-606)</td>
<td>115.8 ± 66.6 (21.5-256.3)</td>
<td>0.17 (0.03-0.65)</td>
</tr>
<tr>
<td>Nephrosis</td>
<td>7</td>
<td>7.3 ± 6.3 (0.7-16.0)</td>
<td>216.9 (75.6-380)</td>
<td>53.0 ± 38.4 (5.6-108.5)</td>
<td>0.08 (0.01-0.04)</td>
</tr>
<tr>
<td>Orthostatic</td>
<td>22</td>
<td>8.7 ± 3.2 (4.5-17.3)</td>
<td>83.5 (40.3-313)</td>
<td>100.3 ± 63.4 (20.5-230.4)</td>
<td>0.13 (0.02-0.25)</td>
</tr>
</tbody>
</table>

Fig. 1. Aspartate Transaminase Activity per 100 ml Urine in Various Renal Diseases.

Fig. 2. Aspartate Transaminase Activity Excreted in the Urine during 24 Hours in Various Renal Disorders.
Enzyme activities per 24-hour urine were calculated from the enzyme activity per 100ml urine and the volume of 24-hour urine. Fig. 2 shows the plotting of each value of the enzyme activity per 24-hour urine in various renal disorders. This figure shows an almost similar pattern to Fig. 1. Normal children excreted 0–30 units of aspartate transaminase in the urine during 24 hours. In nephrosis the amount of enzyme excreted during 24 hours was moderately increased. However, acute nephritic, and orthostatic proteinuric children excreted a large amount of enzyme (about 20–250 units) during a day. Specific activity of urinary aspartate transaminase (enzyme unit per mg protein) was shown in Table 1. The specific activity in nephrosis was much lower than those of acute nephritis and orthostatic proteinuria.

2. Change of Urinary Aspartate Transaminase Activity during the Course of Acute Nephritis

The mean urinary aspartate transaminase levels in each week during four weeks after the onset of acute nephritis were found to be 124, 83, 64 and 45 units, respectively, as given in Fig. 3. The urinary transaminase level decreased to normal value with the lapse of time in this disease. Fig. 4 shows the course of clinical features of a nephritic boy who was nine years old and admitted to the clinic complaining of edema and hematuria. At the beginning of the disease, the serum nonprotein nitrogen level was 58 mg per 100 ml serum and gradually decreased up to 27 mg per 100 ml serum about one month later. Though urinary aspartate transaminase activity and protein excretion were high at the beginning, they fluctuated and gradually returned to the normal value after about one month. This result indicates that urinary aspartate transaminase activity fluctuated with other clinical features such as albuminuria and nonproteinous nitrogen in urine.
3. Isoenzyme Nature of Urinary Aspartate Transaminase

Aspartate transaminase associated with mitochondria has been found more labile than its cytoplasmic counterpart. Hence it is considered that the mitochondrial transaminase released into the serum in hepatitis or myocardial infarction tends to be inactivated in the earliest stage of disease.

However, aspartate transaminase in urine was expected to contain the mitochondrial type transaminase, since it was excreted directly in the urine from the kidney cells. The sample prepared as described above was analyzed for the isozymes by means of a starch zone electrophoresis. Fig. 5 shows the electrophoretic pattern of urinary aspartate transaminase obtained from a nephritic child. As evident, there were two fractions showing aspartate transaminase activity, one remaining on the original line and the other migrating fast to cathode. The former corresponded to the supernatant transaminase and the latter was identified as the mitochondrial enzyme, respectively. In nephrosis almost the same pattern was obtained.

**DISCUSSION**

Several investigators reported that many enzymes found in urine originated from the kidney or urinary tract tissue and considered that the estimation of certain urinary enzymes served to establish a diagnosis of kidney and urinary tract diseases. However, studies on this field have not been extensively explored and remain open. Kidney tissue possesses aspartate transaminase activity and this enzyme appears only in a small amount in normal urine.

However, urinary aspartate transaminase activities are considerably increased in acute nephritis and nephrosis, and the amount excreted was higher in the former
than the latter.

In the present study, urinary aspartate transaminase was shown to be very low in normal children and was increased in acute nephritis, orthostatic proteinuria and nephrosis. The mean value of urinary transaminase activity in orthostatic proteinuria was apparently higher than that of nephrotic children and lower than that of nephritic children. The value of aspartate transaminase in diurnal urine of orthostatic proteinuria was very close to that of acute nephritis.

Also a markedly high level of urinary aspartate transaminase was found in some cases of orthostatic proteinuria. The specific activity of this transaminase in nephrosis, acute nephritis and orthostatic proteinuria were 0.03, 0.14 and 0.17, respectively. This indicates that the specific activity in orthostatic proteinuria was definitely higher than that of nephrosis and close to that of acute nephritis. Two cases of orthostatic proteinuria showed very high specific activity and in this case the presence of a latent nephritis was suspected.

In acute nephritis urinary aspartate transaminase level was decreased during the course of disease and this was paralleled with other symptomatic improvement. This observation suggests that the determination of urinary aspartate transaminase may be clinically useful in differentiating acute nephritis and orthostatic proteinuria from nephrosis, and in evaluating the improvement of disease during the therapy. Aspartate transaminase was shown to be contained abundantly in renal tubular cells, by a histochemical investigation using the diazonium salt. Amelung et al. (7) stated that a small amount of aspartate transaminase appeared in normal urine and ascribed its origin to the destruction and renewal process of tubular cells.

As shown by the electrophoretic analyses, urinary aspartate transaminase contained two forms of isozymes, one derived from the supernatant and the other from mitochondria.

The pathogenesis of orthostatic proteinuria has been explained in several ways, but has not yet been clearly understood. Studies in a number of cases of orthostatic proteinuria showed an increase of urinary aspartate transaminase; in such cases almost equaled to that of acute nephritis. These observations suggest that some of the cases complaining orthostatic proteinuria may be complicated by latent nephritis or a certain condition is created in which the destruction of tubular cells occur and subsequently induce the leakage of intracellular enzymes into the urinary canals.

References