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# Elevated levels of serum aldolase A in patients with renal cell carcinoma

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**Summary.** To clarify whether serum aldolase A is a useful biomarker for renal cell carcinoma (RCC), we determined serum levels of the aldolase A isozyme by an enzyme immunoassay in patients suffering from RCC, other urological tumors, and benign urological diseases. Forty-six of 126 patients with RCC (37%) had elevated serum aldolase A. The positive rates were 23% in stage I, 40% in stage II, 63% in stage III, and 46% in stage IV. In 10 (83%) of 12 patients whose serum levels had been elevated preoperatively, these were reduced to within the normal range after nephrectomy. Four of 7 patients (57%) with progressive disease had elevated levels of aldolase A. In contrast, the positive rates were only 9.9% in 71 patients with other urological tumors and 5.8% in 52 cases of benign urological diseases. High concentrations of aldolase A isozyme in RCC tissues might be reflected in elevated serum levels. The present findings indicate that serum aldolase A is a useful biomarker for monitoring the clinical course of patients with RCC.

**Key words:** Aldolase – Isozyme – Renal cell carcinoma – Biomarker

Fructose-1,6-bisphosphate aldolase (EC 4.1.2.13), a glycolytic enzyme, has a tetrameric form with three immunologically distinct subunits: A, B, and C [6, 8]. The A subunit (aldolase A), which is the dominant fetal form of aldolase, is present in large amounts of muscles, and in smaller amounts in the kidney. The B subunit (aldolase B) is found predominantly in the liver and kidney, whereas the C subunit (aldolase C) is localized mainly in the brain [6]. Aldolase A can be hybridized with aldolase C; thus aldolases A and C form a group of five members consisting of $A_4$, $A_3C$, $A_2C_2$, $AC_3$, and $C_4$ [8].

Aldolase A has attracted attention as a potential biomarker for malignant tumors including hepatocellular carcinomas [1]. In recent years we have purified monospecific polyclonal antibodies to human aldolases A, B, and C, and developed highly sensitive immunoassays for each isozyme [4, 5, 7]. In addition we localized aldolases A and C in renal tissues and those of renal cell carcinoma (RCC) and showed that both isozymes are enhanced during renal carcinogenesis [15, 18]. In the present study, in order to clarify whether serum aldolase A is a useful biomarker for RCC, we determined serum levels of aldolase A in RCC patients and compared them with values for cases of other urological tumors or benign urological diseases.

## Materials and methods

### *Serum samples*

Serum samples from 126 patients with RCC, 71 with other urological tumors, and 52 with benign urological diseases were preoperatively obtained by venipuncture. One hundred healthy subjects supplied sera for the controls. Serum samples for 41 of the 126 patients with RCC were also obtained between 2 and 4 weeks after nephrectomy. Serum samples were kept at either −80°C or −20°C until analysis.

According to Robson's staging system [10], of the 126 patients with RCC, 60 (47%) had stage I, 15 (12%) stage II, 16 (13%) stage III, and 35 (28%) stage IV tumors. The 71 patients with other urological tumors comprised 25 with testicular cancer, 14 with renal pelvis cancer, 12 with bladder cancer, 10 with prostatic cancer, 6 with renal angiomyolipoma, 3 with ureteral cancer, and 1 with an adult Wilms' tumor. The 52 patients with benign urological diseases were made up as follows: 15 with benign prostatic hypertrophy, 9 with ureteral stones, 8 with renal cysts, 4 with renal stones, 4 with polycystic kidneys, 4 with ureteral stenosis, 3 with acute prostatitis, 2 with acute epididymitis, 2 with acute pyelonephritis, and 1 with testicular injury.

### *Immunoassay method for aldolase A isozyme*

Concentrations of aldolase A in the sera were determined by the sandwich-type enzyme immunoassay system reported previously [7]. In brief, the serum samples were incubated with polystyrene balls bearing immobilized monospecific antibodies to the human aldolase A antigen, and then the balls were incubated with the same antibodies labeled with β-D-galactosidase from *Escherichia coli*. The galactosidase activity bound to the balls was assayed with 4-methylumbelliferil-β-D-galactoside as a substrate. The assay was

**Table 1.** Concentrations and positive rates of serum aldolase A in patients with renal cell carcinoma (RCC) and in healthy subjects

| | No. of samples | Concentration (ng/ml) | | Positive rate |
|---|---|---|---|---|
| | | Mean ± SD | (Range) | |
| Renal cell Ca: | 126 | 331 ± 283 | (64–1710) | 37% (46/126) |
| Stage I | (60) | 248 ± 150 | (64– 715) | 23% (14/60) |
| Stage II | (15) | 295 ± 205 | (70– 807) | 40% (6/15) |
| Stage III | (16) | 498 ± 366 | (140–1140) | 63% (10/16) |
| Stage IV | (35) | 413 ± 382 | (112–1710) | 46% (16/35) |
| Healthy subjects | 100 | 198 ± 51 | (85– 409) | – |

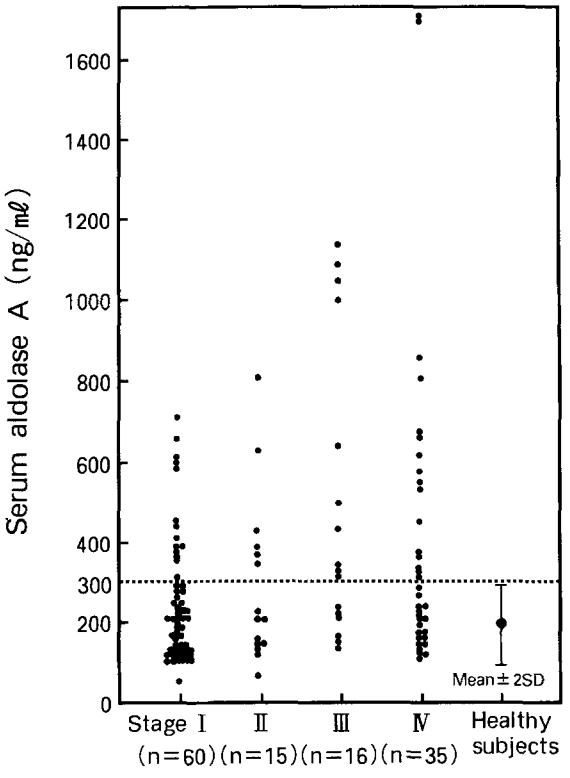


**Fig. 1.** Levels of serum aldolase A in patients with renal cell carcinoma. In healthy subjects, the average serum aldolase A value was 198 ± 51 ng/ml (mean ± SD). Upper limit of normal serum aldolase A (mean plus two SDs) was 300 ng/ml *(dotted line)*

confirmed to be highly sensitive, and the minimum detection limit of aldolase A was 10 pg/assay tube [7]. Purified human aldolase $A_4$ was used as a standard, and the results were expressed as nanograms of homomeric aldolase $A_4$ equivalent per milliliter of serum (ng/ml).

### *Statistical analysis*

Quantitative data were expressed as mean ± standard deviation (SD) values. The results were compared by Wilcoxon's rank-sum or signed-rank test. Serum levels of aldolase A of healthy adults ($n = 100$) were estimated to be 198 ± 51 ng/ml. Any value higher than 300 ng/ml, which was the mean plus 2 SDs, was tentatively defined as abnormal [7].

## Results

### *Serum levels of aldolase A in patients with RCC*

Serum levels of aldolase A in 126 patients with RCC ranged from 64 to 1710 ng/ml. The mean value, 331 ± 283 ng/ml, was significantly higher than that for healthy subjects (198 ± 51 ng/ml, $P<0.001$). Serum levels of aldolase A were elevated (more than 300 ng/ml) in 46 of the 126 patients (37% positive rate). Figure 1 shows the distribution of serum aldolase A levels in patients with state I to IV tumors. Table 1 summarizes concentration values and positive rates for each stage of RCC. The mean value in patients with high-stage tumors (III and IV: 440 ± 375 ng/ml) was significantly higher than that in patients with low-stage tumors (I and II: 257 ± 162 ng/ml, $P<0.01$). Patients with high-stage tumors had a higher positive rate than those with low-stage tumors (51% vs 27%, $P<0.01$).

### *Changes in levels of serum aldolase A during the clinical course*

In 41 RCC patients whose sera were obtained before and after operation, levels of serum aldolase A were significantly reduced after nephrectomy. Preoperative levels were 256 ± 148 ng/ml and postoperative levels were 192 ± 79 ng/ml ($P<0.01$). In 10 (83%) of 12 patients whose serum levels were elevated preoperatively, the levels were reduced to within the normal range after nephrectomy (Fig. 2).

Serum levels of aldolase A were measured serially in 7 patients during the clinical course (Fig. 3). All of them had undergone nephrectomy. In 4 (57%) of the 7 patients levels of serum aldolase A increased to more than 300 ng/ml at recurrence.

### *Serum levels of aldolase A in patients with other urological tumors or with benign urological diseases*

Table 2 summarizes the concentration values and positive rates of aldolase A in 71 patients with urological tumors other than RCC. Of 13 patients with testicular seminoma, 3 (23%) had elevated levels of serum adolase A. Positive

**Table 2.** Concentrations and positive rates of serum aldolase A in patients with urological tumors other than RCC

| | No. of samples | Concentration (ng/ml) | | Positive rate |
|---|---|---|---|---|
| | | Mean ± SD | (Range) | |
| Testicular Ca: | | | | |
| Seminoma | 13 | 248 ± 159 | (131–662) | 23% (3/13) |
| Nonseminoma | 12 | 222 ± 70 | (143–409) | 8% (1/12) |
| Renal pelvis Ca | 14 | 193 ± 64 | (109–345) | 7% (1/14) |
| Ureteral Ca | 3 | 173, 206, 211 | (173–211) | 0% (0/3) |
| Bladder Ca | 12 | 209 ± 77 | (105–353) | 17% (2/12) |
| Prostatic Ca | 10 | 140 ± 40 | (100–211) | 0% (0/10) |
| Renal AML[a] | 6 | 176 ± 37 | (114–228) | 0% (0/6) |
| Adult Wilms' tumor | 1 | 120 | (120) | 0% (0/1) |
| Total | 71 | 201 ± 91 | (100–622) | 9.9% (7/71) |

[a] Angiomyolipoma

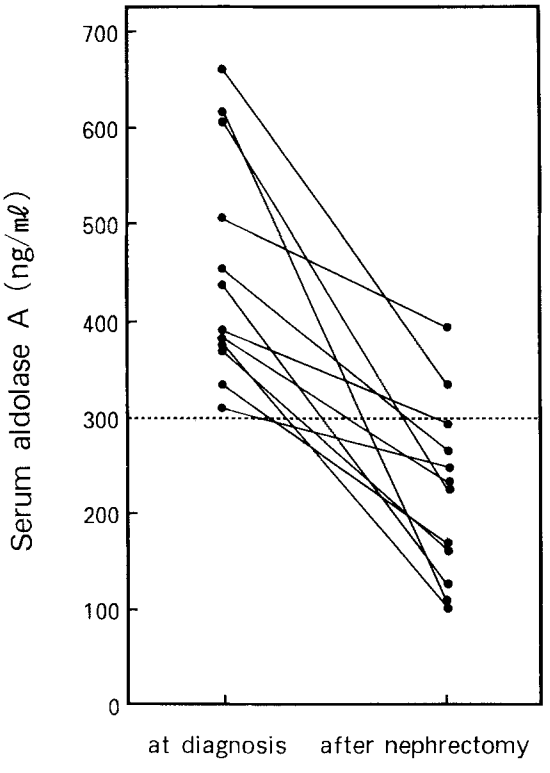


**Fig. 2.** Serum aldolase A values before and after nephrectomy in 12 patients whose preoperative levels were elevated (>300 ng/ml). In 10 (83%) of the 12 patients the levels were reduced to within the normal range after nephrectomy

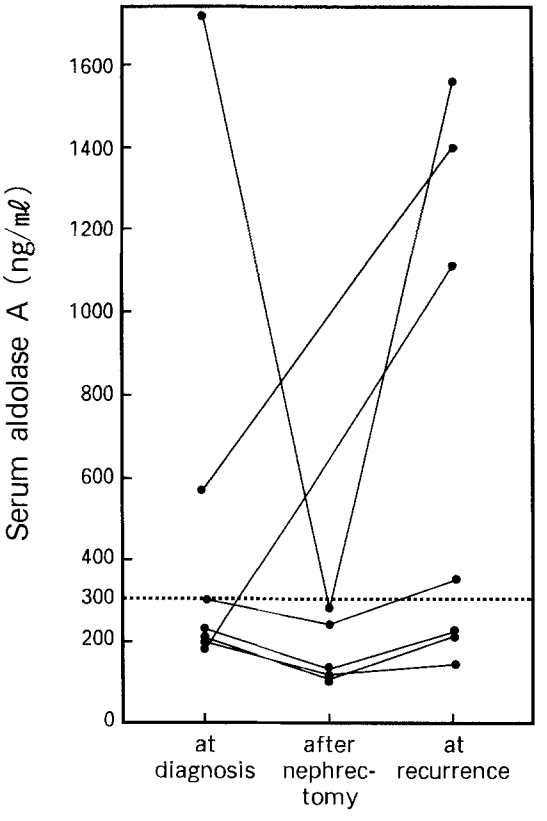


**Fig. 3.** Changes in levels of serum aldolase A during the clinical course in 7 patients with renal cell carcinoma who underwent nephrectomy. In 4 (57%) of the 7 patients levels of serum aldolase A became elevated (>300 ng/ml) at recurrence

values were found also for 1 case of nonseminomatous germ cell testis tumor, 1 of renal pelvis cancer, and 2 of bladder cancer. Overall, the positive rate was 9.9% (7/71) for urological tumors other than RCC.

Table 3 summarizes the concentration values and positive rates in 52 patients with benign urological diseases, 3 (5.8%) of whom (2 with renal cysts and 1 with renal stones) had elevated levels of serum aldolase A.

## Discussion

In normal renal tubules the three aldolase isozymes have distinctly different distributions. Immunohistochemical studies have localized aldolase A in distal tubules [9, 16], aldolase B in proximal tubules [5, 9, 16], and aldolase C in loops of Henle and collecting ducts [15]. Using the immunohistochemical approach, we have demonstrated

**Table 3.** Concentrations and positive rates of serum aldolase A in patients with benign urological diseases

| | No. of samples | Concentration (ng/ml) | | Positive rate |
|---|---|---|---|---|
| | | Mean ± SD | (Range) | |
| Renal cysts | 8 | 240 ± 85 | (148–360) | 25% (2/8) |
| Renal stones | 4 | 225 ± 69 | (159–319) | 25% (1/4) |
| Ureteral stones | 9 | 213 ± 46 | (131–297) | 0% (0/9) |
| Ureteral stenosis | 4 | 225 ± 48 | (167–276) | 0% (0/4) |
| Polycystic kidneys | 4 | 191 ± 53 | (119–248) | 0% (0/4) |
| BPH[a] | 15 | 192 ± 48 | (98–262) | 0% (0/15) |
| Acute prostatitis | 3 | 152, 234, 237 | (152–237) | 0% (0/3) |
| Acute epididymitis | 2 | 225, 274 | (225–274) | 0% (0/2) |
| Acute pyelonephritis | 2 | 193, 218 | (193–218) | 0% (0/2) |
| Testicular injury | 1 | 286 | (286) | 0% (0/1) |
| Total | 52 | 214 ± 56 | (98–360) | 5.8% (3/52) |

[a] Benign prostatic hypertrophy

aldolases A and C in RCC tissues and showed that their concentrations are significantly elevated in the tissues [18]. Previous electron microscopical and histochemical studies suggested that RCCs are derived from proximal renal tubules [2], which contain only aldolase B [5, 9, 16]. Thus, the above findings indicate that aldolases A and C expression is switched on during renal carcinogenesis. Schapira et al. [11–13] and Asaka et al. [1] demonstrated a similar change in aldolase isozymes from B to A and C in rat and human hepatocellular carcinomas as well as in human RCCs. High concentrations of aldolase A in RCC tissues, which we previously demonstrated [18], entail elevated serum levels of the isozyme.

It is interesting that the composition of aldolase isozymes in neoplastic tissues is similar to that in fetal tissues, where an anaerobic glycolysis is predominant. Anaerobic glycolysis is generally enhanced in neoplastic tissues [17], the activities of glycolytic enzymes in tumor cells being increased. Although the biological significance of the change in aldolase isozymes remains unknown, the expression of aldolases A and C might be associated with a specific metabolism of cancer cells, suitable for anaerobic glycolysis.

We have been seeking a useful serum biomarker for RCC because there are few valuable markers for the diagnosis and monitoring of RCC patients. Our previous studies revealed that RCC tissues contain high concentrations of γ-enolase and showed that serum γ-enolase is a useful biomarker for RCC [3, 14]. It is interesting that aldolase A is a glycolytic enzyme like γ-enolase. On the other hand we reported that, while the concentrations of aldolase C in RCC were about 6 times as high as those in the normal cortex, patients with RCC had no serum elevation of this enzyme. This may be ascribed to the low absolute concentrations of aldolase C (48.0 ± 8.0 ng/mg protein) compared with the concentrations of aldolase A (7300 ± 6300 ng/mg protein).

The present study showed that 37% of patients with RCC had elevated levels of aldolase A. Thus, the sensitivity of this marker was somewhat lower than that observed earlier for γ-enolase (51%). Further study is needed with simultaneous measurement of both serum aldolase A and γ-enolase, to determine whether serum aldolase A provides additional information to that gained from assessment of γ-enolase alone. Nevertheless, it is noteworthy that patients with other urological tumors and with benign urological diseases had only low positive rates of aldolase A (9.9% and 5.8%, respectively) because for other markers, including acute phase reactants, relatively high positive values have been found in cases of benign disease.

In conclusion, the present study revealed serum aldolase A to be a potentially useful biomarker for monitoring the clinical course of patients with RCC, but not a marker for staging.

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