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Original Article: Clinical Investigation

Serum gamma glutamyl-transferase is a sensitive but unspecific marker of metastatic renal cell carcinoma

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Objective: To address the role of serum γ-glutamyl transferase (GGT) as a marker of metastases in patients with renal cell carcinoma.
Methods: Serum alkaline phosphatase and GGT were determined in 156 patients with localized renal cell carcinoma and 60 patients with metastases as proven by echosonography, computerized tomography and bone scan. The control group consisted of 50 healthy subjects matched for sex and age. Sensitivity and specificity of both enzymes as markers of metastatic disease were compared. In metastatic patients, enzyme activities were analyzed according to the site of metastases.
Results: Both alkaline phosphatase and GGT activities were normal in majority of patients with localized renal cell carcinoma and increased in most of the patients with metastatic disease (80% and 70%, respectively). GGT did not significantly differ from alkaline phosphatase in terms of sensitivity (70% vs 80%) and specificity (89% vs 92%). Concerning the site of metastases, high frequencies of increased GGT and alkaline phosphatase were found in patients with liver-only metastases (80% and 90%, respectively). All of the patients with both liver and bone metastases exhibited increased activity of both enzymes. Despite the fact that bone cells do not express GGT, increased activity was found in patients with bone metastases-only (45%), suggesting that enzymes might be released from tumor cells.
Conclusions: Our data provided evidence that GGT is a sensitive marker of metastatic renal cell carcinoma. However, findings of abnormal GGT activity cannot specify the site of involvement.

Key words: alkaline phosphatase, γ-glutamyl transferase, metastases, renal cell carcinoma.

Introduction

According to the College of American Pathologists,[1] serum alkaline phosphatase (AP) is a category I prognostic factor in the preoperative metastatic evaluation of patients with renal cell carcinoma (RCC).[2] Although elevated enzyme activity usually prompts a search for bone and/or liver metastases, the analysis lacks specificity. To differentiate whether AP activity elevation in RCC patients is a consequence of liver or bone involvement, an AP isoenzyme profile is determined or serum activity of another liver-specific enzyme is measured. Among liver enzymes that are released in plasma, γ-glutamyl transferase (GGT) has gained particular interest, since it is not expressed in bone.[3] Accumulating clinical and laboratory evidence suggest that serum GGT activity is widely used as a highly sensitive biochemical marker in the majority of malignant and non-malignant liver diseases.[4,5] Enhanced liver GGT expression has been observed during adult hepatocarcinogenesis and in the presence of secondary liver tumors.[6]

In addition to liver cells, GGT is also expressed by proximal convoluted tubules of the kidney, the presumed site of origin of RCC.[7,8] GGT has also been shown to be present in most RCC cells by monoclonal antibody stains.[9,10] Interestingly, serum GGT is not elevated in low-stage RCC, and therefore it is not useful as a diagnostic marker of RCC.[7] On the other hand, Sandock *et al*.[11] in a retrospective study, have shown elevated serum GGT activity in a large percent of patients with metastatic RCC. Based on these findings, determination of GGT in addition to AP has been suggested to be introduced in preoperative evaluation of RCC patients. However, analysis of GGT as a marker of metastatic disease, including determination of sensitivity and specificity of a GGT test, has not been performed as yet. Still, the question of whether increased GGT activity in serum of patients with metastatic RCC is a consequence of secretion/leakage of GGT from either liver or metastatic RCC cells, and the ability of the test to specify liver involvement, remain unanswered. For such a purpose, parallel assessment of both serum GGT and AP activities, and how complementary these findings are, in patients with metastases at different sites would be of great importance.

To address the ability of GGT testing to differentiate between the liver and other organ involvement in metastatic RCC, in this study we

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determined serum AP and GGT activities in patients with localized and metastatic RCC. Data on enzyme activities in RCC patients with metastases were analyzed with respect to site of metastasis.

Methods

Study participants

Serum AP and GGT were determined at the time of diagnosis in 156 patients with localized RCC and 60 patients with metastases as proven by echosonography, computerized tomography and bone scan. All patients were treated at the Institute of Urology and Nephrology, Clinical Center of Serbia between 2002 and 2005. Patients with metastatic RCC were further separated into groups based on the site of metastases, including liver-only ($n = 20$), bone-only ($n = 22$), liver and bone ($n = 12$) as well as lung and/or brain (metastatic disease but not in liver or bone, $n = 6$). None of the patients had a history of chemotherapy treatment. Exclusion criteria were alterations in liver function secondary to liver disease and cholecystitis. The control group consisted of 50 healthy subjects matched for sex and age. Both patients and controls gave informed consent to enter the study. The protocol for the research project was approved by a local Ethics Committee and it conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

Biochemical assays

Serum was separated from 5 mL of peripheral venous blood obtained from patients and controls. Biochemical tests for the assessment of liver and kidney function included urea, creatinine, uric acid, protein, bilirubin, aspartate transaminase and alanine transaminase determination. These parameters were measured spectrophotometrically.

Serum AP and GGT activity measurements were performed at 37°C using a Ultrospec K spectrophotometer. GGT activity was determined at 405 nm, using γ-L-glutamyl-4-nitroanilide as the substrate and glycil-glycine as an acceptor forming 4-nitroaniline.[12] One unit (U) of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of 4-nitroaniline per minute. Enzyme activity is expressed as U per liter of serum. Serum GGT activity exceeding 60 U/L was interpreted as an abnormal result. AP activity was determined at 405 nm based on enzymatic hydrolysis of the substrate p-nitrophenylphosphate which forms p-nitrophenyl. Aminometilpropanol was used as a buffer and acceptor of phosphate ions.[13] The diagnostic sensitivity of each test was calculated as the percentage of patients with the metastatic RCC whose test was abnormal (true positives). The diagnostic specificity of an each test was calculated as the percentage of patients with localized RCC who had normal enzyme activities within reference values (true negatives).

Statistical analysis was performed using the Mann–Whitney and χ^2 tests. Correlations between AP and GGT activities in each patient were analyzed using Pearson's method. $P < 0.05$ was considered statistically significant.

Results

Clinical and biochemical characteristics of the subjects are presented in Table 1. No differences in parameters tested were found between RCC patients with localized tumor and controls. However, patients with metastatic RCC exhibited moderate increase in both urea and creatinine levels (Table 1).

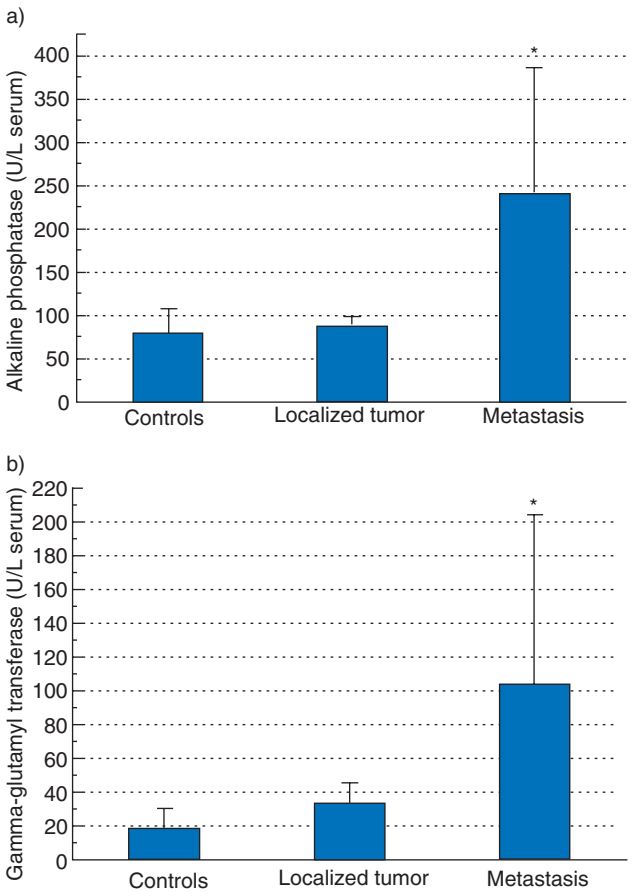

Fig. 1 Activities of (a) alkaline phosphatase (AP) and (b) γ-glutamyl transferase (GGT) in healthy controls, patients with localized and metastatic renal cell carcinoma. Enzyme activity expressed as mean ± SD. (*) mean AP and GGT activities in patients with metastatic disease were significantly increased in comparison to both patients with localized tumors ($P < 0.01$) and controls ($P < 0.01$) as evaluated by Mann–Whitney test.

Mean AP and GGT activities in controls, patients with localized tumor and metastatic disease are shown in Figure 1(a,b). As shown, mean activities of both AP and GGT in patients with localized tumor did not significantly differ from healthy controls. However, mean AP and GGT activities in patients with metastatic disease were significantly increased in comparison to both patients with localized tumors ($P < 0.01$) and controls ($P < 0.01$).

γ-Glutamyl transferase as a marker for RCC was 70% sensitive and had a specificity of 89%. Compared to AP (sensitivity and specificity were 80% and 92%, respectively) GGT exhibited lower sensitivity and similar specificity as AP.

Results obtained in patients with metastatic carcinoma were further stratified into different groups according to the site of metastases. Mean values and frequency of abnormal results for groups of patients with metastases at different sites are presented in Table 2. In patients with liver-only and liver and bone metastases, mean AP activity was markedly higher than values obtained in the group with localized tumors. A less pronounced but significant increase in AP activity was also found in patients with bone metastases, while mean AP activity was not significantly increased in patients with metastases at other sites in comparison to those with localized tumors. Regarding the frequency of increased AP activities, the rate of increased AP levels was much higher

Table 1 Clinical and biochemical characteristics of patients with renal cell carcinoma

	Controls	Patients with renal cell carcinoma	
		Localized tumor	Metastasis
Number of patients	$n = 50$	$n = 156$	$n = 60$
Age (year)	62.4 ± 3.7	62.2 ± 9.4	61.2 ± 8.1
Gender (M/F)	18/32	96/60	48/12
Urea (mmol/L)	5.48 ± 1.26	5.72 ± 2.24	11.2 ± 6.54*
Creatinine (μmol/L)	81.6 ± 14.8	108 ± 37.3	179 ± 126*
Urea N : Cr ratio	16.7 ± 4.94	13.3 ± 4.74	15.5 ± 6.52
Uric acid (μmol/L)	254 ± 86.1	305 ± 117	329 ± 129
Plasma proteins (g/L)	70.1 ± 3.70	67.5 ± 6.50	67.8 ± 6.10
Bilirubin (μmol/L)	13.4 ± 3.20	13.9 ± 4.10	10.7 ± 5.60
AST (U/L)	23.0 ± 4.00	22.0 ± 12.0	29.0 ± 31.0
ALT (U/L)	16.0 ± 6.00	12.0 ± 11.0	24.0 ± 31.0

*$P < 0.05$ compared to healthy controls. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; N, Nitrogen.

Table 2 Serum alkaline phosphatase and γ-glutamyl transferase activities in patients with renal cell carcinoma

Groups	n	Serum AP levels (U/L serum)		Serum GGT levels (U/L serum)	
		Mean ± SD†	>120 U/L‡	Mean ± SD†	>60 U/L‡
Localized RCC	156	89.2 ± 9.2	12 (8)	38.0 ± 10.9	18 (12)
Metastasis	60	263 ± 65.0**	48 (80)***	131 ± 64.0**	42 (70)***
Liver	20	360 ± 134**	18 (90)	202 ± 99.0**	16 (80)
Liver and bone	12	377 ± 9.00**	12 (100)	196 ± 82.0**	12 (100)
Bone	22	188 ± 65.0**	18 (82)	64.0 ± 45.0*	10 (45)
Other	6	107 ± 51.0	0 (0)	63.0 ± 30.0*	4 (67)

*$P < 0.05$ compared to localized tumor (Mann–Whitney test); **$P < 0.01$ compared to localized tumor (Mann–Whitney test); ***$P < 0.01$ compared to localized tumor (χ^2 test). †Data presented as mean ± SD. ‡Data presented as number of patients (%) in which increased enzyme activity were detected. Units of alkaline phosphatase and GGT activity defined in Material and Methods section. AP, alkaline phosphatase; GGT, γ-glutamyh transferase; RCC, renal cell carcinoma.

in patients with metastatic disease than that in patients with localized tumors (80% vs 8%, $P < 0.01$). It is important to note that the frequency of increased AP values was the highest in patients with both liver and bone metastases (100%, Table 2). Mean GGT activity levels in patients with metastatic disease were significantly increased in comparison to those with localized tumors in all patient groups tested independently of the site of metastases (Table 2). However, the overall frequency of increased GGT activities in the metastatic group (70%) was somewhat lower than that observed for AP (80%, Table 2). Among patient groups with metastases at different sites, the highest rate of increased GGT levels was found for patients with both liver and bone metastases (100%) and the lowest in patients with bone metastases (45%, Table 2).

We also studied the correlation between GGT and AP in each patient (Fig. 2) and whether metastases at a particular site were associated with increased levels in AP-only, GGT-only or both AP and GGT activities (Table 3). We found a statistically significant correlation between two enzymes ($r = 0.808$; $P < 0.001$; Fig. 2). The majority of patients with metastatic RCC exhibited both increased AP and GGT levels (Table 3). An isolated increase in AP level was observed in patients with liver-only and bone metastasis (20% of all patients with metastases). An isolated increase in GGT level was found in patients with liver-only and

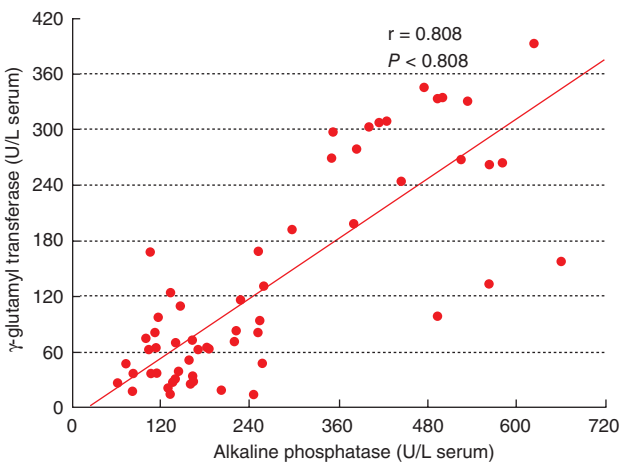

Fig. 2 Correlation between AP and GGT activities in patients with metastatic renal cell carcinoma.

Table 3 Frequency of increased alkaline phosphatase and/or γ-glutamyl transferase activities in serum of patients with metastatic renal cell carcinoma

Site of metastasis	Normal AP and GGT *n* (%)	Increased			Total *n* (%)
		AP *n* (%)	GGT *n* (%)	AP and GGT *n* (%)	
Liver	ND	4 (20)	2 (10)	14 (70)	20 (33)
Liver and bone	ND	ND	ND	12 (100)	12 (20)
Bone	4 (18)	8 (36)	ND	10 (46)	22 (37)
Other	2 (33)	ND	4 (67)	ND	6 (10)
Total	6 (10)	12 (20)	6 (10)	36 (60)	60 (100)

AP, alkaline phosphatase; GGT, γ-glutamyl transferase; ND, not detected; RCC, renal cell carcinoma.

patients with metastases at sites other than liver and bone (10% of all patients with metastases). However, normal AP and GGT levels were still observed in a small fraction of patients with metastases (10%, Table 3).

Discussion

In this study, we evaluated serum GGT as a marker of metastatic disease in patients with RCC and compared it with serum AP, a category I prognostic factor for metastatic RCC. Our data provided evidence that GGT is associated with RCC in a manner similar to that of AP; namely, both AP and GGT activities were normal in the majority of patients with localized RCC and increased in most of the patients with metastatic RCC. Findings of abnormal serum GGT activity could not specify the site of involvement, because increased GGT activities have been observed in patients with metastases at different sites. Sensitivity and specificity as markers of metastatic RCC did not significantly differ between AP and GGT tests.

Because alteration of kidney function might have an important impact on bone metabolism, which consequently could affect AP activity in serum, we carefully analyzed serum urea and creatinine concentrations as markers of renal function. In this context, it is important to note that none of our patients had bilateral tumors that would cause significant reduction in functional renal mass. In order to further discriminate between pre-renal and post-renal uremia, we calculated the urea nitrogen : creatinine ratio because high ratios with elevated creatinine concentrations might denote post-renal obstruction by tumor.[14] Although the mean urea nitrogen : creatinine ratio in patients with metastatic tumors was within normal reference intervals, we cannot exclude the possibility that some of the patients had mild obstruction due to the tumor. Serum urea concentration might also be elevated due to pre-renal reasons, such as increased protein catabolism, muscle wasting and some cases of liver disease.[14] Among these, we speculate that a hyper-catabolic state with overall increased protein catabolism might be a reason for increased urea levels in patients with metastatic RCC. The latter cause (chronic liver disease), could be reflected in alterations of serum AP and GGT activity. To be sure that serum AP and GGT in this study were reliable markers of metastatic disease, but not other chronic liver diseases, several biochemical markers of liver function including aspartate aminotransferase (AST), alanine transferase (ALT), bilirubin and plasma protein levels were tested and abnormal values served as exclusion criteria.

Our findings on normal GGT levels in patients with localized RCC are in agreement with those of Speights *et al*.[7] and Sandock *et al.* [11] It seems that early in the progression of RCC, GGT is not released from tumor cells.[7] Such lack of sustainable enzyme release might be partially explained by quantitative changes in GGT expression and changes in its cellular morphology. Namely, GGT expression in RCC is lower than that in corresponding renal parenchymal cells.[15] Besides, in normal kidneys GGT are located in the luminal brush-border membrane of proximal tubule cells, whereas in renal carcinomas GGT is found surrounding the whole tumor cells.[10] In our study, a small portion of RCC patients with localized tumors had abnormal serum GGT activities. It is interesting that these patients had large tumors (data not shown). Thus, it is possible that either a large tumor mass and/or necrotic changes within the tumor contributed to the increase in serum GGT activity in patients with localized RCC.

In the current study, we found elevated serum GGT activity in the majority of patients with metastatic RCC. The portion of patients with abnormal GGT levels in our study is almost the same as reported by Sandock *et al*.[11] in the only study that investigated GGT as a marker of metastatic RCC. In contrast to such consistent findings on GGT, the frequency of abnormal AP findings in metastatic RCC patients in our study was significantly higher than in their study. Moreover, we found significant correlation between the activities of both enzymes in each patient. In order to further evaluate the role of AP and GGT as markers of metastatic disease we analyzed their activities with respect to the site of metastases. Based on our data, it seems that liver involvement will result in increase of either AP or GGT, or both AP and GGT. Thus, complementary determination of both enzymes enabled detection of all patients with liver metastases. Elevations of both AP and GGT were observed in patients with liver and bone metastases. Moreover, almost half of the patients with bone-only metastases also exhibited increased GGT activity associated with abnormal AP levels. However, GGT exhibited lower sensitivity and similar specificity compared to AP. Besides, isolated increase in GGT was found in fewer patients with metastatic RCC. These results, together with a significant correlation between GGT and AP activities in each patient, suggest the potential use of GGT only as an additional biochemical marker of metastatic disease.

Because GGT is not expressed by bone cells,[3] elevated GGT activity in sera of patients with bone metastases could presumably originate from RCC cells. Based on these findings, we conclude that abnormal serum GGT cannot specify the site of metastases and therefore cannot differentiate between liver and bone involvement. Therefore, to specify the site of metastatic disease either another liver-specific or bone-specific enzyme should be tested. Thus, Bichler *et al*.[16] showed elevated serum ostase in sera of patients with RCC with bone metastases. Still, the question of why GGT was released from the majority of metastatic RCC and not from localized RCC needs to be addressed. As a possible explanation, Sandock *et al*.[11] suggested that metastatic RCC cells might

have been altered in a way to secrete or leak more GGT in the circulation. In addition to changes in malignant phenotype, it can be speculated that a large tumor mass in metastatic patients, which includes both primary and metastatic RCC cells, might also contribute to increased serum GGT levels.

Regarding the longing of urologists for a metastatic tumor marker that detects individuals with early signs of disease or recurrence rather than metastatic sites, or predict tumor biology or response to therapy, GGT is not likely to be the tumor marker that we are hoping for. Still, some patients with metastatic RCC evaluated in our and other studies[11] had increased serum GGT activity as the only biochemical marker of metastatic disease.

Acknowledgments

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