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Diagnostic value of high molecular weight alkaline phosphatase in detection of liver metastasis in gastrointestinal system malignancies

Gastrointestinal sistem kanserlerinde yüksek molekül ağırlıklı alkalen fosfatazın karaciğer metastazlarının saptanmasında tanısal değeri

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Background/aims: *This study was undertaken to evaluate the diagnostic value of high molecular weight alkaline phosphatase as determined by agarous gel electrophoresis.* ***Methods:*** *High molecular weight alkaline phosphotase levels were electrophoretically determined in fifty patients with different gastrointestinal system malignancies and 20 healthy volunteers were included.* ***Results:*** *The levels of high molecular weight alkaline phosphatage were high 13 of 25 patients with liver metastasis, in three of 25 patients in whom no metastasis was detected by biochemical tests or imaging techniques and in two of 20 healthy volunteers in the control group. When the pathological lower limit of 7.1 U/L was taken as a criteria, the sensitivity, specificity and accuracy were 54.2%, 88.5%, and 70% respectively.* ***Conclusion:*** *High mole-cular weight-ALP is a useful marker to determine liver metastasis in gastrointestinal system malignancies.*

Key words: Liver metastasis, gastrointestinal system malignancies, high molecular weight alkaline phosphatase.

Amaç: *Değişik Gastrointestinal sistem maligniteli 50 hasta ile sağlıklı kontrol grubunu oluşturan 20 olgunun serumlarında yüksek molekül ağırlıklı alkalen fosfatazın karaciğer metastazlarının saptanmasındaki tanısal değeri araştırıldı.* ***Yöntem:*** *Gastrointestinal sistem malignitesi olan elli hasta ve 20 sağlıklı kontrolde elektroforetik olarak yüksek molekül ağırlıklı alkale fosfataz düzeyleri ölçüldü.* ***Bulgular:*** *Yüksek molekül alkalen fosfatazın, karaciğer metastazı bulunan 25 hastanın 13'nde, biyokimyasal testler ve görüntüleme yöntemleriyle karaciğer metastazı saptanmayan 25 hastanın 3 'nde, ve kontrol grubundaki 20 olgunun 2' nde anormal değerlerde olduğu saptandı. Patolojik alt sınır olarak 7.1 U/L kriter alındığında bu testin duyarlılığı %54.2, özgüllüğü %88.5 ve doğruluğu %70 olarak bulundu.* ***Sonuç:*** *Yüksek molekül ağırlıklı alkalen fosfatazın karaciğer metastazlarının gösterilmesinde bir belirleyici olarak kullanılabileceğini gösterdi.*

Anahtar kelimeler: Karaciğer metastazı, gastrointestinal sistem maligniteleri, yüksek molekül ağırlıklı alkalen fosfataz.

INTRODUCTION

The liver is one of the most common sites of distant metastasis in patients with gastrointestinal system malignancies and early detection of hepatic metastasis is important in the management of diseases. Symptoms of liver metastasis appear late and radiological diagnostic procedures, such as x-rays, ultrasound and CAT scans are sensitive but costly, time-consuming and in the early stages of metastasis, can give false negative results. Simple laboratory techniques for the early detection of liver metastasis in oncology patients are needed (1).

Liver function tests have been used as supplemental diagnostic tools for the detection of hepatic metastasis in a variety of cancers (2). Alkaline phosphatase (ALP) in particular has been reported to be of value (3,4). ALP is a membrane-bound enzyme that can be resolved into tissue nonspecific ALP (liver-bone type), placental ALP and intestinal ALP (5). Hepatocellular and cholestatic diseases, such as acute and chronic hepatitis, cirrhosis, carcinoma of the liver, metastatic carcinoma of the liver and acute and chronic biliary obstruction are all associated with increased liver ALP activity and frequently, high molecular

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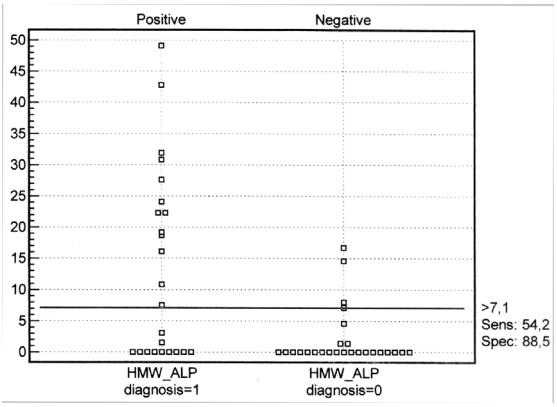

Figure 1. Individual levels of HMW-ALP
Diagnosis 1: Patients with hepatic metastasis, Diagnosis 0: Patients without hepatic metastasis.

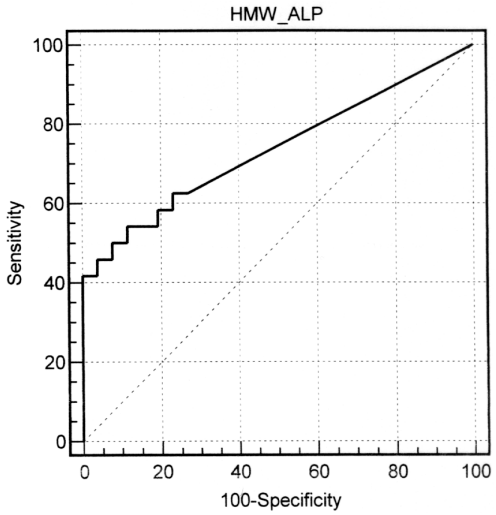

Figure 2. Receiver- operator characteristics curves (ROC) for HMW-ALP, in detection of liver metastasis

weight alkaline phosphatase (HMW-ALP). This enzyme is also known as fast-liver ALP, koinozyme and bile ALP (1).

HMW-ALP was first described in 1970 by using sefadex gel filtration and starch gel electrophoresis (6). It does not migrate during starch gel and polyacrylamide gel electrophoresis, a rapid movement is seen in agar gel or cellulase acetate (5,7-9)

In this study, an electrophoretic method on agarose gel was used for the separation and quantification of HMW-ALP and its diagnostic value in the detection of hepatic metastasis was investigated.

MATERIALS and METHODS

Patients

The study included 50 patients treated in the internal medicine and general surgery clinics, between January 1999 and June 1999 with different gastrointestinal system malignancies. A control group comprised 20 healthy volunteers with normal total ALP levels.

The primary cancers of these 50 patients were: stomach, 22; rectum, 12; colon, 10; pancreas, 2; esophagus, 2; liver, 2. The patients and controls were separated into three groups:

Group I: Twenty-five patients with liver metastasis: 11 had stomach carcinoma, 5 rectum carcinoma, 4 colon carcinoma, 2 pancreas carcinoma, 2 hepatocellular carcinoma (HCC) and one had esophagus carcinoma. Liver metastases were confirmed by clinical and biological tests, sonograms and scans. Ten patients with liver metastases and two patients with HCC had biopsies taken with guided USG or CT in order to determine histological diagnosis.

Group II: Twenty-five patients without liver metastasis: 11 had stomach carcinoma, 7 rectum carcinoma, 6 colon carcinoma and one esophagus carcinoma. The patients were apparently free of liver metastases with all biological liver function tests, scans and sonograms being completely normal. Evaluation for the existence of metastasis was undertaken during surgery.

Group III: Comprised 20 healthy volunteers with normal total ALP levels were admitted.

Procedures

Serum total ALP, γ-Glutamyltransferase (GGT), aspartate transaminase (AST) and alanine transaminase (ALT) levels were determined by autoanalyzer. Five ml of venous blood was taken in order to determine HMW-ALP. Serum was separated and stored at -20 °C. Agarose electrophoresis of the ALP isoenzymes was performed with the Isopal system (Sebia Lab.No:4022-4023, Moulineaux- France). Specimens were put on gel plaque and electrophoresis was performed at 100 volts for 45 minutes. Plaques were then incubated for 50 minutes by indolyl phosphate. After the incubation period, bands were analyzed by dansitometer at 570 nm. Absolute value and relative

Table 1. Patient characteristics

	Group I Met +	*Group II Met -*	*Group III Control*
No. Of patients	25	25	20
Mean age (years)	58 (31-75)	55 (32-78)	53 (23-68)
Sex, F/M	10/15	8/17	11/9
Primary cancer/n	Stomach - 11	Stomach - 11	
	Rectum - 5	Rectum - 7	
	Colon - 4	Colon - 6	
	Pancreas - 2	Esophagus -1	
	HCC - 2		
	Esophagus -1		

percentage of each band was determined automatically.

Data analysis

Data were analyzed by use of the SPSS statistical package for personal computers (SPSS 10.0, Inc., Chicago. IL). Differences between disease-grouped data were studied by non-parametric tests (Kruskall-Wallis and median tests). Cut off value was chosen for HMW-ALP on the basis of the receiver- operator characteristics curve (ROC) by use of the Medical Calculator statistical package. The HMW-ALP cut off value was determined with reference to the distributions of its activity among patients with and without hepatic metastasis. Any value above the cut off was regarded as positive. Sensitivity was defined as the percentage of positives among patients known to have hepatic metastasis. Specificity was defined as the percentage of negatives among patients known not to have hepatic metastasis. Accuracy was defined as the percentage of all correct test results among all patients.

RESULTS

Charactenstics of patients and healthy volunteers are shown in Table 1.

There were 10 female and 15 male patients with hepatic metastasis (Group I) and the mean age was 58 (range 31-75) years. Patients without hepatic metastasis (Group II) included eight female and 17 male patients with a mean age of 55 (range 32-78) years, while Group III comprised 11 female and nine male patients with a mean age of 53 (range 32-78) years. There was no significant difference between groups in terms of sex, age, GGT, AST, ALT and total ALP levels ($p > 0.05$). When HMW-ALP values were compared with these test results (Table 2), a significant difference was observed ($p = 0.0002$). The differences were observed between patients with metastasis and the control group and also between patients with and without metastasis.

When 0 U/L was used as a cut-off value, the sensitivity, specificity and accuracy of this test for hepatic metastasis was 60%, 72%, and 66% respectively. It seemed, however, that the specificity was too low for practical use, because the patients whose HMW-ALP activities were slightly raised without hepatic metastasis were erroneously judged as positive. When the pathological lower limit was taken as 10 U/L as suggested by Viot (13), the sensitivity, specificity and accuracy of this test was 48%, 92% and 85.7%, respectively.

In the present study, a new cut-off value of 7.1 U/L was set for study group, according to which the sensitivity, specificity and accuracy were 54.2%, 88.5%, and 70%, respectively (Table 3, Figure 1,2). Positive predictive value was 81.2%, and negative

Table 2. Comparison of biochemical parameters in patients with metastasis, patients without metastasis and control group.

	AST	*ALT*	*GGT*	*ALP*	*HMW-ALP*
Group I Met +	24.8±17.7	24.6±22.7	43.1±34.1	197.0±119.7	13.1±15.0
Group II Met -	24.7±30.4	27.2±44.6	69.5±129.7	172.2±210.8	2.1±5.8
Group III Con.	21.4±6.2	16.7±6.7	26.0±12.5	102.3±39.9	2.1±5.8
p	> 0.05	> 0.05	> 0.05	> 0.05	P=0.0002

Table 3. Sensitivity, specificity and accuracy at different cutoff values

Cutoff value	*Sensitivity (%) (TP / TP + FN)*	*Specificity (%) (TN / TN + FP)*	*Accuracy (%) (TP + TN / All)*
0 U/L	60	72	66
10 U/L	48	92	85.7
7.1 U/L	54.2	88.5	70

TP: True-positive; TN: True-negative; FP: False-positive; FN: False-negative

predictive value was 64.7%.

When HMW-ALP values were evaluated according to the type of primary cancer, no significant differences were found (Table 4). Further series are needed in order to evaluate the relationship between HMW-ALP and type of primary cancer.

DISCUSSION

HMW-ALP appears in the sera of patients with a wide variety of liver diseases including metastatic liver cancer (10-12). Viot et al reported that HMW-ALP was a useful marker for hepatic metastasis because of its excellent sensitivity (96%) and specificity (93%) (13) .

Mayne et al, in their research evaluating the levels of total ALP and HMW-ALP in breast carcinoma, found the sensitivity of HMW-ALP to be lower than other studies while specificity value was 85% for total ALP; this level was thought to be due to the early phase of the disease levels and that the result was caused by high number of false negative results (14).

Traynor et al found HMW-ALP, GGT and total ALP levels to be higher in liver metastasis found in 26 of 42 colorectal carcinomas cases. When positive predictive value is taken into consideration, HMW-ALP was found to have best positive predictive value (87%) (15).

Nishio et al investigated the diagnostic value of HMW-ALP in 126 patients with lung carcinoma and 15 patients with benign lung disease and high levels of the enzyme were observed in patients with liver metastasis. This study found sensitivity and specificity of the test to be 71% and 85% respectively. In 6 patients in whom no metastasis was found by imaging techniques, determination of HMW-ALP aided an early diagnosis of liver metastasis (16).

In the present study, the diagnostic value of measuring HMW-ALP to detect hepatic metastasis in patients with gastrointestinal cancer was investigated. The sensitivity, specificity and accuracy were 54.2%, 88.5%, and 70% respectively. HMW-ALP was compared with total ALP, GGT, AST and ALT levels and significant differences were observed (p=0.0002). It is therefore concluded that HMW-ALP is a useful marker of hepatic metastasis in patients with gastrointestinal cancer and these results are concordant with those in the literature. The low sensitivity was thought to be due to high false negativity (48%). The location, size and spread of metastasis is thought to have a role in the activity of HMW-ALP and it is also thought that HMW-ALP could be transformed into low molecular weight ALP by the detergent activity of deoxycolate (16). In our study, the location, size and spread of metastasis was not used in evaluation.

The of false positivity rate was 11%. In the literature, cytostatic drugs, abdominal radiotherapy,

Table 4. Comparison of HMW-ALP levels in patients with metastasis and patients without metastasis, according to type of primary cancers

Primary cancer	*Group I Met +*	*Group II Met -*	*Total*
Stomach	10.4±12.8 n=11	1.3±2.6 n=11	5.8±10.1 n=22
Colon	7.5±10.5 n=4	3.6±6.1 n=6	5.1±7.9 n=10
Rectum	14.5±13.9 n=5	2.6±6.2 n=7	7.5±11.3 n=12
Esophagus	..... n=1	..... n=1	_
Pancreas	..... n=2	_	_
HCC	..... n=2	_	_
p	-	-	>0.05

alcohol abuse and hematological malignancies are reported to cause false positive results (13,15) but in the two patients in our control group with higher HMW-ALP levels than pathological lower limit, none of these causes were established.

No difference was found in total ALP and GGT levels between groups which may have been due to the early stages of liver metastasis or might indicate that liver function tests can be normal for a long time. This is an important consideration in HMW-ALP enzyme determination.

To conclude, HMW-ALP determination may be a useful tool both in the diagnosis of liver metastasis and following the results of treatment.

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