Separation with Estimation of High Molecular Weight Isoenzyme in Breast Cancer Patients

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ABSTRACT

Total alkaline phosphatase (ALP) was measured in two groups of breast cancer patients, group 1 consisted of 27 patients with axillary metastases, group 2 consists of 4 patients with liver metastases .In addition , a group of control subjects was included . The results showed that total serum ALP was especially increased in group 2. ALP was purified from sera of patients with breast cancer by gel filtration on a sepharose CL-6B(106+30)cm and high molecular weight alkaline phosphatase isoenzyme (HALP) was isolated at pH 7.4.

Key words: tumor markers; Alkaline phosphatase.

INTRODUCTION

A variety of substances could be used as tumor marker in breast cancer, one of these marker is a alkaline phsophatase (1). Alkaline phosphatase [ALP, orthophosphoric-monoester phosphohydrolase (alkaline optimum); Ec 3.1.3.1] is present in human serum primarily as unbound tissue-specific isoenzymes (2). In sera of patient with hepatobiliary disease, a minor phosphatase component is frequently present, which because high relative molecular mass, has low or zero mobility in starch or polyacrylamide gel. It has been noted regularly since the earliest electrophortic studies of serum ALP (3)(4)(5). This property and its association with liver disease account for its being termed the "fast liver fraction" (6)(7)(8)(9), later, high molecular weight alkaline phosphatase (HALP) confired in serum and tissue by cellulose acetate electrophoresis(10). In other studies, HALP has been called origin, stationary, particulate(11), bile, cholestatic and pathological biliary ALP(12). It is generally believed that HALP is part of a multienzyme membrane particle (13). It is thought to be associated with phosphatidyl choline and / or lipoproteins.This lipid – alkaline phosphatase complexes are associated with liver cell fragments(14). Whatever its nature or origin, HALP seems to be more sensitive marker of cholestases than the regular liver isoenzyme of ALP (15). HALP is commonly increased in patients with metastatic liver cancer even in the absence of jaundice (17). in practice, although it is possible to quantify HALP based on its highly negatively charged character,

these measurements have not gained wide spread acceptance as adjuncts for cancer monitoring (16),(17),(18). We developed a method for HALP

PATIENTS

In this study two groups of breast cancer patients were included : Group 1 contained 27 patients with axillary metastases , with an age men + SD (44 ± 7.7) . Group 2 consisted of 4 patient with liver metastases with on age (39 ± 1.4) . All patients were historically proven , none of them had a history of hypertension , diabetes , anemia or any other disease that may interfere with our study . The blood was withdrawn by venipuncture , the amount of blood was not fixed , but usually was in the range (2.5 – 5) ml . Blood samples were left at room temperature for about half an hour then the sera were separated by spinning for 15 minutes at 3500 rpm , at room temperature . A analysis on sera were always performed on the same day of sample collection.

MATERIALS AND METHODS

Determination of total alkaline phosphatase activity :

The activity of ALP was measured in sera according to the method of bowers and McCom (19) in which the colorless substrate , (P – Nitrophenyl phosphate , P - NPP) was convert at alkaline pH to yellow P-Nitrophenoxide ion . The reaction was followed by measurement of the increase in absorbance at 403 nm , in spectrophotometer (LKB Ultra spec 4050)

Separation and measurement of high M.wt alkaline phosphatase activity:

A. Gel preparation and column pacing :

Gel filtration was carried in a (1.6×30) cm column . The gel was allowed to swell in an excess of 0.2 M Tris buffer. Then the slurry was carefully poured into a vertical glass column down the wall . After the gel had settled, the column was equilibrated with Tris buffer PH 7.4 .

Void volume determination: The void volume was measured by blue dextran 2000 with a concentration of 2 mg /ml in a Tris buffer (0.2 M , PH 7.4) .

1.2 ml of blue dextran was applied to the column and eluted by the same buffer, with a flow rate of 22 ml /hour. Fractions of 2 ml were collected and their absorbance was measured at 600 m .

B. sample application and elution :

One ml of serum was applied to the surface of the gel and left for a few minutes until it was absorbed . Elution was started by the addition of Tris buffer pH 7.4 and a number of fraction were collected , with a volume of 2 ml each at a flow rate of 22 ml / hour . This was carried out in the cold room at 4□c .

C. The activity of ALP :

For each fraction was determined as described above for total ALP activity , and the protein amount of each fraction was measured by the method of lowery , using bovine serum albumin (BSA) as standard (20) .

RESULTS

The normal value of serum ALP activity is in the range of 25-74 U/L. The values of ALP activity of 31 patients with breast cancer are illustrated in table (1). An increase in the activity was found in patients with liver metastases.

Table (1) Total ALP activity in sera of patients with breast cancer. The kinetic method was used to assay total serum ALP.

Patients	Number	Age	Type Of Tumors (cell Type)	Metastases	ALP activity (U/L)	
					Mean	Range
Group 1	27	44 ± 7.7	22 infiltrating ductal carcinoma & lobular carcinoma.	Axillary	58.8	25.9 - 78.9
Group 2	4	39 ± 1.4	Infiltrating ductal carcinoma.	Liver	131	82.7 - 181
Control	15	45 ± 4			57	25 - 74

H.wt ALP was separated by gel filtration on a sephorose CL-6B column. The serum applied to the gel filtration column was from a patient with breast cancer. The void volume (Vo) was determined and found to be equal to 22ml at a flow rate of 22ml/ hour. As shown in figure (1), ALP activity eluted as five peaks. The first peak (peak1) represent fractions number (1-6) was considered as a high M.wt ALP. The first six fractions were mixed together. Then the activity of concentrated fractions representing peak1 was measured and found to be 15.8 U/L . Table (2) summarize the results of gel filtration . The table shows that the yield of the first peak is equal to 18% of the ALP activity of crude serum . The protein concentration of peak1 was found to be 2.74mg/L. The specific activity and purification factor were also calculated and found to be 5.766 U/mg and 19.8 respectively.

Figure (1). Gel filtration profile of serum ALP on sepharose CL-6B column (1.6 x 30) cm of breast cancer patient with liver metastases.

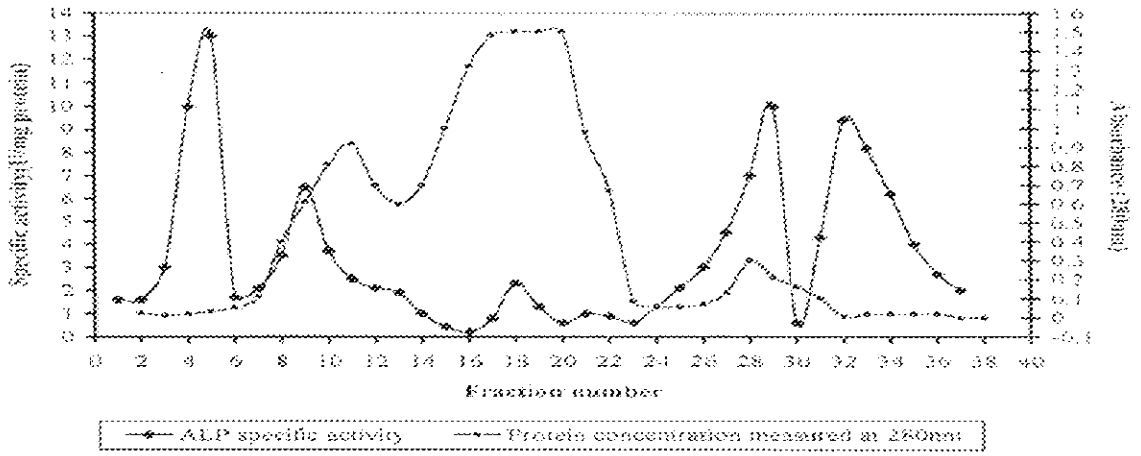

Table (2) Separation of high M.wt ALP from serum of breast cancer patient with liver metastases by gel filtration chromatography.

Purification step	Protein concentration (mg/L)	Activity (U/L)	Specific activity (U/mg)	Yield (percent)	Fold Of Purification
1 Crude Serum	299	86.9	0.290	100	1
2 Gel filtration Peak 1 (fractions 1-6)	2.74	15.8	5.766	18	19.8

DISSCUTION

The rise in ALP activity in patients with liver metastases comes in agreement with other studies. Most of the rise in activity is due to the liver isoenzyme (ALP). An elevation in liver ALP in serum is almost invariable result of malignant infiltration of the liver (21).

The serum of breast cancer patient may contain a high molecular weight form of ALP. The results indicate that high –molecular mass ALP is present in patient's serum in condition associated with liver metastases. In this study, we have used the sepharose CL-6B gel filtration in separation of high molecular weight alkaline phsphatase

The presence of the high - molecular - mass fraction in serum is regarded as valuable evidence of obstructive liver disease , particularly extra hepatic obstruction (24) , (25) ,(26), and has been claimed to be superior to other enzymatic tests in distinguishing liver metastases from other forms of liver diseases (27),(28),(29) .

The origin of high molecular mass fraction is not yet completely clear; it probably does not represent a single entity. Other membrane bound enzymes such as 5_nucleotidase (Ec 3.1.3.5) are present in the same fraction and association with lipoprotein has been demonstrated in some cases, but not in all (6) , (7) . In view association of membrane enzymes with lipid material and morphological appearances of membrane structure , the particles or complex making up the high molecular weight fraction have been identified as fragments of cell membranes from biliary- tract cells or hepatocytes released into the blood as a result of cell damage (22) . however , enzyme complex of high relative molecular mass also occur in bile , and these may be regurgitated into the circulation in biliary obstruction (23).

The values of HALP obtained from healthy individuals was 8.5 U/L a value we defined as the "normal" value (30). As shown in table(2), the breast cancer patient with liver metastases had HALP value 15.8 U/L, which is more than the normal's value. Several groups have reported that HALP is useful marker for hepatic metastases because of its excellent sensitivity and specificity (31),(32); .

Conclusion

An increase in total serum ALP activity is obtained in breast cancer patients with liver metastases over those with axillary metastases, the isolation of high molecular weight alkaline phosphatase in sera of breast cancer with liver Metastases is a valuable evidence of liver metastases.

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فصل وتقدير متناظر انزيم الفوسفاتيز القاعدي عالي الوزن الجزيئي في مرضى سرطان الثدي

الخلاصة :-

تم قياس فعالية انزيم الفوسفاتيز القاعدي في مجموعتين من مرضى سرطان الثدي تتألف المجموعة الاولى من المرضى الذين تم انتشار المرض لديهم الى منطقة الابط والمجموعة الاخرى تتألف من مرضى سرطان الثدي الذين تم انتشار المرض لديهم الى منطقة الكبد ، بالاضافة الى ذالك تم قياس فعالية انزيم الفوسفاتيز القاعدي في الاشخاص الاصحاء . اضهرت النتائج ان فعالية انزيم الفوسفاتيز القاعدي قد زادت بصورة ملحوظة في المجموعة الثانية من مرضى سرطان الثدي . تم تنقية انزيم الفوسفاتيز القاعدي الموجود في مصل المرضى المصابين بسرطان الثدي بواسطة تقنية الترشيح الهلامي وتم عزل متناظر انزيم الفوسفاتيز القاعدي عالي الوزن الجزيئي وقياس فعاليته .