Alkaline Phosphatase and Simple Gray Scale Ultrasonography in Detecting Liver Metastasis in Breast Cancer Patients: A Comparison between Bone Scan Positive and Bone Scan Negative Patients

**Hoor Fatima[1]*, Amir Maqbool[2], Marium Siddiqui[3], Habiba Sohail[4],
Omem Saleem[5] and Zainab Hasan[6]**

[1]*Jinnah Medical and Dental College, Karachi, Pakistan.*
[2]*Department of Oncology, Karachi Institute of Radiology and Nuclear Medicine, Karachi, Pakistan.*
[3]*Baqai University, Karachi, Pakistan.*
[4]*Jinnah Medical and Dental College, Karachi, Pakistan.*
[5]*Dow International University, Karachi, Pakistan.*
[6]*Department of Community Medicine, Jinnah Medical and Dental College, Karachi, Pakistan.*

Authors' contributions

This work was carried out in collaboration between all authors. Author HF designed the study and wrote the first draft of the manuscript. Author AM wrote the protocol. Author MS managed the literature searches. Author HS wrote the last draft of the manuscript. Author OS managed the experimental process and author ZH performed the statistical analyses of the study. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Alkaline Phosphatase (s-ALP) has been extensively used to monitor the disease and screen the patients for cancer metastasis. Both the baseline s-ALP and changes in s-ALP have been reported as prognostic factors for treatment effect and survival in bony and liver metastatic breast cancer.

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Corresponding author: Email: hoorimran@yahoo.com;

Aims: To signify the role of serum Alkaline Phosphatase in combination with simple gray scale ultrasound in detecting and confirming liver metastasis in breast cancer patients with and without bone metastasis.

Study Design: It was a cross sectional comparative study.

Place and Duration of Study: Conducted at Karachi Institute of Radiology and Nuclear Medicine between 2011 and 2012.

Methodology: Histopathologically confirmed cases of breast cancer were identified and bone scans were conducted. 100 females with positive and 100 females with negative bone scan belong to any age group were included. Liver ultrasound of all subjects was conducted, using simple gray scale ultrasound machines. Serum Alkaline Phosphatase was measured with the help of semiautomatic biochemical analyzer.

Results: Liver metastasis was found by ultrasound in both bone scan positive and bone patients. Serum ALP levels found very high (*P* value< 0.001) in patients with liver metastasis.

Conclusion: Positive liver ultrasound, using simple gray scale and high serum ALP levels strongly correlated with positive liver metastasis in breast cancer. Liver metastasis was found more frequent among bony metastatic breast cancer. A combination of routinely done, low cost test in determination of liver metastatic breast cancer will prove better detecting tools especially in poor resources settings.

1. INTRODUCTION

Cancer is a major health hazard as well as burden on the economy all around the world. Globocan provided rough estimates, approximately 14 million cancer cases were newly diagnosed and 8.2 million deaths occurred in 2012 all around world. In coming era, the victims of cancers are developing countries, which currently give a measure about 57% of cases and 65% of cancer deaths in world. Cancer prevalence is still high in developed countries and mortality rates are 8% to 15% higher in first world [1]. Growing number of breast cancer cases are the leading cause of cancer death among females in first world as well as in less developed countries [2]. Spread of cancerous cells to distant organs is major cause of mortality [3]. Bone metastases may complex a number of cancers, which are responsible of great health risks. Types of receptors appeared, aggressiveness of tumor, vascularity, turnover of regional cancerous cells and characteristics of tissues where they spread, are main features that influence the pattern of cancer spread [4,5]. In many patients, metastasis to bones takes several years and diversity of treatment provided, enables the patient to survive for a long time. The survival of patient depends upon kind of tumor. The average survival rate after the secondary metastasis in breast cancer is usually many years [6,7]. The breast cancer with bone metastases is less aggressive as compare to other visceral metastasis. As studied by Coleman and Ruben's, the diagnosed patients of cancers in 1970s and 1980s showed an average survival rate up to 24 months only. Detection of the risk factors responsible for increasing mortality may appear helpful in prediction of prognosis and survival in patients of breast cancer with metastasis.

Alkaline Phosphatases (ALP) is a collective term for isoenzymes with lesser specificity for the substrate that catalyzes the cleavage of phosphate esters at an alkaline pH. Site of alkaline phosphatases are, bone, intestine, kidney, liver, placenta and white blood cells. It is not necessary that all persons with disturb levels of liver function test are related to abnormalities of liver. In case of raised ALP, differential diagnosis of disease related to liver or due to other clinical problems is a critical matter for clinicians. This problem is complicated by introduction of automated routine laboratory testing. Diseases of the liver can be identified by the abnormality of different enzymes however liver enzymes may be raised in secondary liver disease as well and hence primary and secondary disease cannot be differentiated and needs further investigative modalities. Serum Alkaline Phosphatase (s-ALP) is widely used screening test for the screening of metastasis secondary to cancer. The reason might be its sensitivity, low cost, rapid result and easy availability. A lot of research regarding the prognostic role of s-ALP has been carried out among different kinds of malignancies with secondary metastasis. The normal range of s-ALP in serum and fluctuation in its level is a

prognostic factor to evaluate the treatment effect and survival rate [8,9]. Serum ALP greater than 500 u/l is alarming for further analysis. Serum ALP was found elevated in majority of breast cancer patients with liver metastasis. Serum ALP levels found toward high normal in breast cancer patients with no metastasis. Admitted patients of breast cancer for surgery showed a double triple increased in serum ALP levels [10]. CA 15-3 level in serum of patients with recurrence of breast cancer is proved a good indicator of metastasis than ALP, but altogether use of both biomarkers gives an early sign of recurrence. Whether routine use of these biomarkers improves overall survival remains an open question [11]. According to the report by breast cancer study group, ALP, Aspartate Transaminase AST) and C-Glutamyltransferase (GGT) were measured in serum and their sensitivity was observed in detecting breast cancer recurrence. The role of AST and GGT for that purpose did not show any beneficial result although only ALP was abnormal among most of case of breast cancer with bone metastases and/or liver metastases, and ALP is thought to be more superior to AST and GGT in distinguishing patients with recurrence [12]. Serum ALP proved success full to monitor disease course [13]. Advanced imaging scans and staging laparoscopy in association with Ultrasound guidance during surgery can be used to lower the hazards of open surgery in patients with breast cancer complicated by liver metastasis [14]. Simple gray scale sonography can be used only for screening purposes but cannot alone confirm diagnosis. Gray scale with color Doppler, is highly associated with better results and diagnosis. Because of less harms, cheap and easy availability, simple Ultrasound is the way of choice for the initial detection of isolated liver lesions. Simple Ultrasound is also useful to differentiate the surgical patients with diffuse and isolated lesion. Following simple and routine Ultrasound it becomes easy to decide whether the patient is suitable for surgery or not [15]. Breast cancer related liver metastasis is a blood born spread of the primary tumor. It may show a recurrence [16].

Robert WC and et al. mentioned that in breast cancer multi organ metastases is common [17]. According to Robert WC and et al, systemic metastasis limited to bone are less frequent in breast cancer [18,19,20]. Female patients with intraductal and pathological stage I tumors, do not require bone scanning, liver Ultrasonography and chest radiography for baseline staging. Female patients with pathological stage II tumors require postoperative bone scan for baseline staging. Routine liver Ultrasonography and chest radiography are only necessary for patients with four or more positive lymph nodes. Female patients with pathological stage III tumors need bone scan, liver ultrasound and chest radiography as essential tools for prospective baseline staging [21]. Use of screening programs is a better step in diagnosis of early-stage disease with better results [22,23].

We checked and compared serum Alkaline Phosphatase levels along with imaging techniques (ultrasound) as a predictor of liver Metastasis inpatients of breast cancer. This study may be beneficial for physicians using expensive and invasive imaging techniques to rule out metastasis. Economic burden on cancer patients may be minimized especially in low resources centers.

2. MATERIALS AND METHODS

2.1 Study Design

It is a cross sectional comparative study.

2.2 Inclusion Criteria

Histopathological diagnosed cases of breast cancer belong to any age group. Patients were premenopausal, perimenopausal, postmenopausal and passing through different stages of cancer. Married, unmarried, lactating, non- lactating were included. All cases were of invasive ductal carcinoma.

2.3 Exclusion Criteria

Diagnosed cases of primary liver disease have been excluded from study.

2.4 Data Collection

Study was conducted at Karachi Institute of Nuclear Medicine. We explained the objectives and important features of the study to all patients prior to the start of study and their consent was taken. Trained medical physician, nurses and laboratory persons collected data. General information from each subject was collected through a standard questionnaire including participant's name, age, education, monthly income and living style, ethnicity, gravidity, para, abortions, clinical sign and symptoms, material

status, family history, etc. Bone scans were conducted using Siemens E Can scanner using intravenous dye 20 mci technetium 99 MDP has been used. 100 patients with histopathological diagnosed breast cancer and positive bone scan and 100 patients of histopathological diagnosed breast cancer and negative bone scan were selected for the study. Simple gray scale ultrasound was used to determine liver metastasis and blood samples were taken for measurement of serum ALP levels, Bilirubin and SGPT tests from both groups.

2.5 Blood Collection

Before interviewing and blood collection a verbal consent was taken from each respondent. Venous blood was withdrawn for the investigation. First the skin was cleaned thoroughly with sterilized with 70% Isopropyl Alcohol swab and dried before puncturing. 2 ml of blood taken from the antecubital vein with a 5 cc disposable syringe (Becton Dickinson, Pak pvt Ltd). The blood sample was transferred to labeled test tube. And then tubes were marked with codes and immediately taken to the lab.

2.6 Liver Function Test

ALP, SGPT and BILIRUBIN done on. SELECTRA –E, XL Semi Auto Biochemical Analyzer. Vital Scientific and Diagnostic Machine, Netherland, Holand Latest Model By Roch.

2.7 Determination of Serum Alkaline Phosphatase (ALP)

Serum ALP was determined by in vitro test for the quantitative determination of Alkaline Phosphatase in human serum and plasma on Roche automated clinical chemistry analyzer. Kit Cat. No. 14858 Ecoline (Panteghini et al., 2006).

2.8 Ultrasound

Simple Grey scale ultrasound was done on Toshiba machine model no Aplio 500.

During the study chemo therapy and radiotherapy were allowed accordingly and most of patients have passed through the first three cycles of chemotherapy and radiotherapy.

2.9 Statistical Analysis

The database was developed and analyzed using SPSS (statistical packages of social sciences) version 11.0. Clinical characteristics were summarized in terms of frequencies and percentages for qualitative/categorical variables, mean ± S.D for quantitative variables (biochemical parameter ALP, SGPT, Bilirubin total). Student t-test was used for comparison of quantitative variables and chi-square test for qualitative variables in between bone scanning positive and negative groups.

3. RESULTS

Sample size was n=100 in bone scan negative group and n=100 in bone scan positive group.

Liver metastasis by ultrasound was found more frequent in bone scan positive patients as compare to bone scan negative cases (Table 1). Elevated serum ALP levels were found extremely statistical significant, (P= .0001) in positive bone scan group with positive liver metastasis (Table 2). Serum total Bilirubin and serum Alanine Transaminase (SGPT) were found comparatively higher (high normal) in patients of liver metastasis and Serum Alkaline Phosphatase levels were found high in all patients of breast cancer with liver metastasis (Table 3). Elevated levels of serum ALP levels were found extremely statistical significant, (P= .0001) in negative bone scan group with positive liver metastasis (Table 4). Fig. 1 shows serum ALP levels in both groups. ALP levels were found highly significant in bone scan positive group. Frequency of liver metastasis in both groups detected by simple gray scale ultrasound is high in both groups as shown in Fig. 2.

Serum levels of total Bilirubin in bone scan positive and negative groups is shown in Fig. 3. Serum total Bilirubin found within normal range but high normal in all patients of age group below 39 years and above 70 years in bone scan negative group. Age group in between 40 years to 69 showed high serum total Bilirubin in bone scan negative group. Serum levels of SGPT in both groups of bone scan are shown in Fig. 4 and found higher levels in all age groups of bone scan positive patients except age group 30-39 years.

Table 1. Biophysical parameters in bone scanning positive and negative cases

	Bone scanning		P-value
	Positive (n=100)	Negative (n=100)	
Liver metastasis	22	12	0.060

*** Statistically significant p<0.001*

Table 2. Serum alkaline phosphatase levels in subjects of positive and negative bone scan with positive liver metastasis

No of liver metastasis positive in bone scan positive patients n = 22			No of liver metastasis positive in bone scan negative patients n = 12			P-value
Mean	S. Dev	SEM	Mean	S. Dev	SEM	
480.81	246.15	24.61	314.88	216.10	20.5	0.0001

*** Statistically significant p<0.001*

Table 3. Biochemical parameters in bone scanning positive and negative cases

	Bone scanning (Positive) (n=100)			Bone scanning (Negative) (n=100)			P-value
	Mean	S.D	SEM	Mean	S.D	SEM	
Bilirubin total	1.65	10.14	1.01	0.60	0.52	0.05	0.302
SGPT (Alanin transaminase)	36.4	21.04	2.11	32.5	33.52	3.35	0.331
Alkaline phosphatase	227.2	183.91	18.39	201.6	107.03	10.70	0.231

*** Statistically significant p<0.001*

Table 4. Serum alkaline phosphatase levels in subjects of negative bone scan with positive and negative liver metastasis

	No of liver metastasis positive in bone scan negative patients n = 12			No of liver metastasis negative in bone scan negative patients n = 88			P-value
SERUM ALP	Mean	S. Dev	SEM	Mean	S. Dev	SEM	0.0001
	314.88	216.10	20.5	180.95	71.05	7.1	

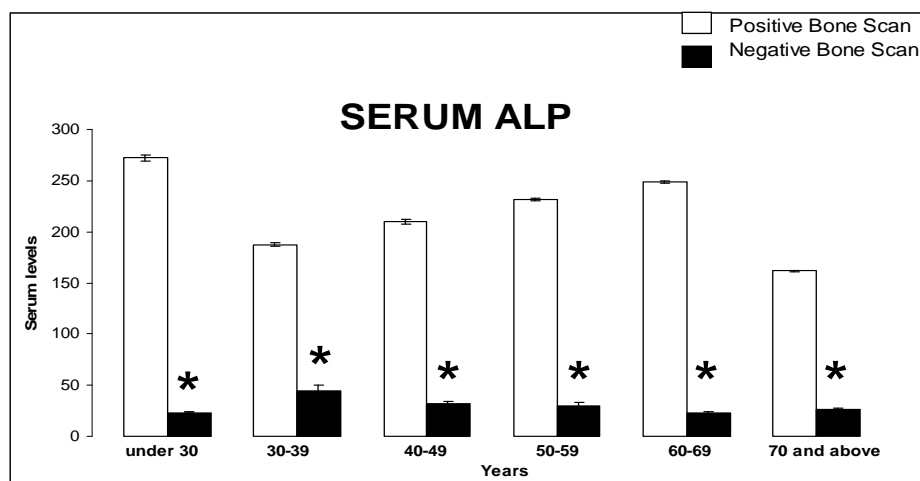*** Statistically significant p<0.0001*

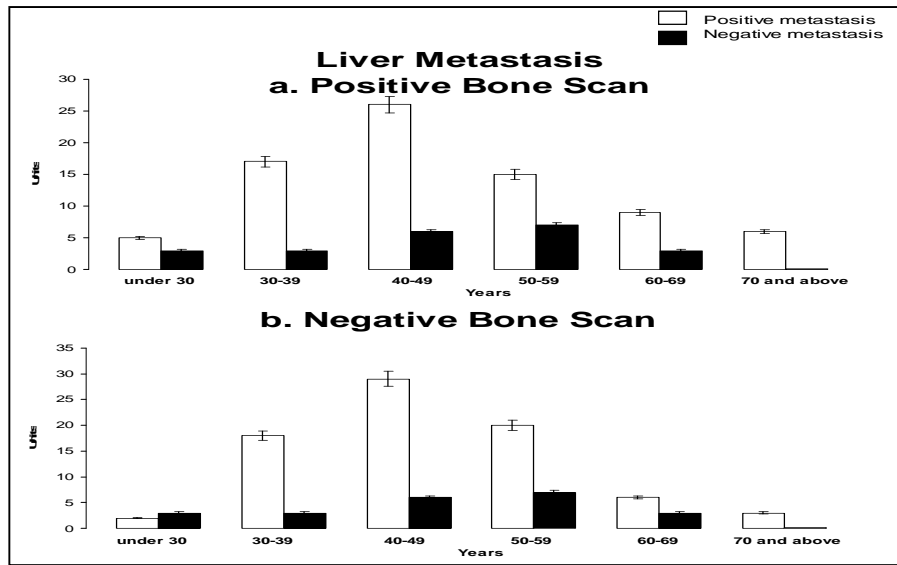**Fig. 1. Serum ALP levels in subjects with positive- and negative bone scan**

Fig. 2. Liver metastasis diagnosed by simple gray scale ultrasound in subjects with positive- and negative bone scan
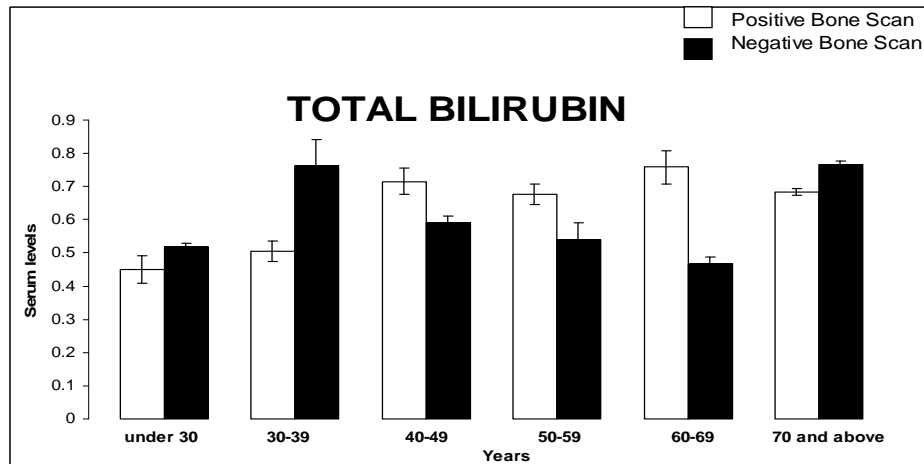**Values represented, as means ± SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found *Values represented as means ± SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found*

Fig. 3. Serum Bilirubin levels in subjects with positive- and negative bone scan
*Values are represented as means ± SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found

4. DISCUSSION

This study is carried out to find out the role of tumor marker Alkaline Phosphatase as a predictor of breast cancer metastasis to liver in combination with simple grey scale ultrasound.

During the study we observed that liver metastasis was positive in bone scan positive patients in significant number of cases. Among 100 cases of positive bone scan, 22 cases of liver metastasis were found (P = 0.05). On the other hand only 12 cases of liver metastasis were found among 100 cases of negative bone scan, (*P*= .06). Serum ALP levels were found significantly high in patients with liver metastasis in both bone scan positive and bone scan negative patients (*P* = .0001). The cases of
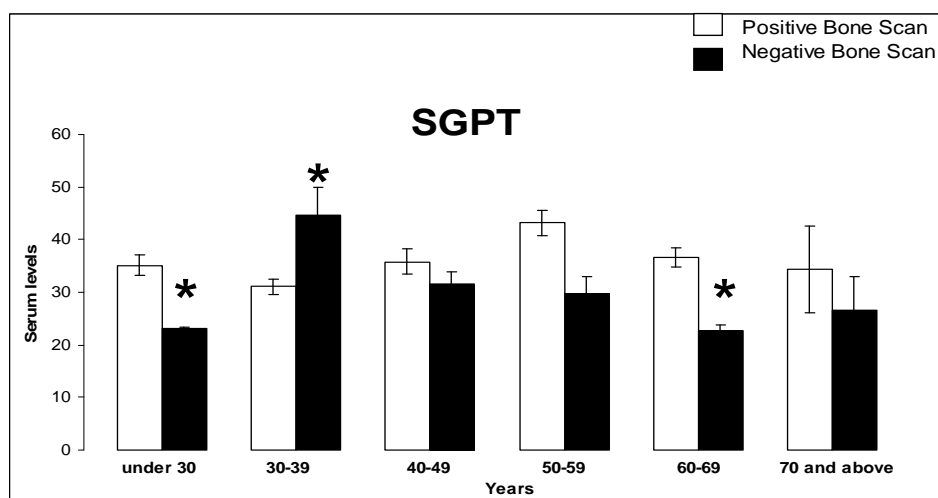
Fig. 4. Serum SGPT levels in subjects with positive- and negative bone scan
*Values are represented, as means ± SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found

breast cancer those were negative for any metastasis showed high normal levels of serum ALP. The results indicate that the breast cancer patients have a higher tendency of metastasis to liver. This metastasis is initially can be detected with the help of serum ALP levels in association with simple and cheaper imaging techniques such as gray scale Ultrasound. These tests are routinely used in most of oncology centers. Serum total Bilirubin and serum Alanine Transaminase (SGPT) were found comparatively higher in patients of liver metastasis. Other comparative studies support that high scanning sensitivity by simple Ultrasound to evaluate liver lesions, because it is cheap but not highly efficient, is still a question mark in the routine preoperative staging for the purpose of for liver surgery. Ct scan and MRI thought to be superior as a routine, first line imaging technique over the simple Ultrasound [24].

Blood dissemination of breast cancer most commonly results in liver metastasis. This dissemination may occur with the appearance of primary tumors and may take several years. Metastases are often showing the relapse of cancer. Previous research shows the rate of liver metastasis is only 1-2% [25,26]. This spread to liver might be restricted to liver only. If the tumor can be removed successfully by surgery so the surgery is treatment. Research shows 30-47%, 5 year survival rate after curative surgical treatment [27].

ALP is important to detect all body cancer spread. A small prediction of liver metastasis is shown by raised ALP level [28,29]. ALP with CA 15-3 indicates bone metastasis and with CEA (Carcino Embryonic Antigen) indicates liver metastasis [24]. A retrospective cohort study of 146 patients with secondary metastasis did not confirm the importance of liver U/S scans as necessary tool for the routine screening in patients with liver metastases especially when the ALP level is within normal range. Comparatively ALP levels were not correlating with the bone scan results. All patients with a double or triple increase in the ALP level had abnormal bone, liver, or bone and liver scans. Routine ALP assays give the accurate information about the staging and their routine use can lowers the expenses of initial and follow-up examination in patients with breast cancer [25]. Fluctuation in serum levels of biochemical markers like Alkaline Phosphatase (ALP) may be of great importance in diagnosis of breast carcinoma. By spectrophotometer serum ALP activity was observed in 388 histopathological diagnosed cases of breast cancer and its association was monitored with cancer stages. By the logistic regression study a significant result was obtained (p\0.001) after the comparison of group's ALP levels (500 Iu/l) with metastasis. This study highlighted the benefits of ALP to pick the breast cancer stages and secondary metastasis [26]. Archanaetal after comparing the activities of serum ALP and GGT in case control study published that serum ALP

AND GGT levels were significantly high in cancer cases as compare to control group [27].

5. CONCLUSION

High serum ALP level with positive liver scan is predictive of secondary liver metastasis in breast cancer patients.

ALP and simple liver ultrasound are of low cost and easily available diagnostic facilities through which secondary liver metastasis can be detected, especially in poor resources centers.

ETHICAL APPROVAL

Authors have all necessary ethical approval obtained from ethical committee of Board of advance studies, University of Karachi.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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