

ORIGINAL ARTICLE

**ALKALINE PHOSPHATASE AND SIMPLE GRAY
SCALE ULTRASONOGRAPHY IN DETECTING LIVER
METATATASIS IN BREAST CANCER PATIENTS: A
COMPARISON BETWEEN BONE SCAN POSITIVE AND
BONE SCAN NEGATIVE PATIENTS.**

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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.

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.

Key words: Breast Cancer, Serum Alkaline Phosphatase, Liver Metastasis, Ultrasound.

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. 5. 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.^{7, 8, 9} 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. ^{10, 11, 12} The breast cancer with bone metastases is less aggressive as compare to other visceral metastasis. As studied by Coleman and Ruben's ³⁰, 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

73 screening of metastasis secondary to cancer. The reason might be its sensitivity, low cost, rapid result
74 and easy availability. A lot of research regarding the prognostic role of s-ALP has been carried out among
75 different kinds of malignancies with secondary metastasis. The normal range of s-ALP in serum and
76 fluctuation in its level is a prognostic factors to evaluate the treatment effect and survival rate.^{25, 26.}
77 Serum ALP greater than 500 u/l is alarming for further analysis. Serum ALP was found elevated in
78 majority of breast cancer patients with liver metastasis. Serum ALP levels found toward high normal in
79 breast cancer patients with no metastasis. Admitted patients of breast cancer for surgery showed a
80 double triple increased in serum ALP levels. 27.CA 15-3 level in serum of patients with recurrence of
81 breast cancer is proved a good indicator of metastasis than ALP, but altogether use of both biomarkers
82 gives an early sign of recurrence. Whether routine use of these biomarkers improves overall survival
83 remains an open question. 28According to the report by breast cancer study group, ALP, Aspartate
84 Transaminase AST) and C-Glutamyltransferase (GGT) were measured in serum and their sensitivity was
85 observed in detecting breast cancer recurrence. The role of AST and GGT for that purpose did not show
86 any beneficial result although only ALP was abnormal among most of case of breast cancer with bone
87 metastases and/or liver metastases, and ALP is thought to be more superior to AST and GGT in
88 distinguishing patients with recurrence.^{29.} Serum ALP proved success full to monitor disease course.³⁰
89 Advanced imaging scans and staging laparoscopy in association with Ultrasound guidance during surgery
90 can be used to lower the hazards of open surgery in patients with breast cancer complicated by liver
91 metastasis. 6. Simple gray scale sonography can be used only for screening purposes but cannot alone
92 confirm diagnosis. Gray scale with color Doppler, is highly associated with better results and diagnosis.
93 Because of less harms, cheap and easy availability, simple Ultrasound is the way of choice for the initial
94 detection of isolated liver lesions. Also useful to differentiate the surgical patients with diffuse lesions and
95 isolated lesion. Following simple and routine Ultrasound it becomes easy to decide whether the patient is
96 suitable for surgery or not. 3. Breast cancer related liver metastasis is a blood born spread of the primary
97 tumor. It may show a recurrence.^{4.}
98 Robert WC and et al, mentioned that in breast cancer multi organ metastases is common. 13. According
99 to Robert WC and et al, systemic metastasis limited to bone are less frequent in breast cancer.^{14, 15, and}
100 16. 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 4 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. 22. Use of screening programs is a better step in diagnosis of early-stage disease with better results. 23, 24.

We checked and compared serum Alkaline Phosphatase levels along with imaging techniques (ultrasound) as a predictor of liver Metastasis in patients 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

MATERIAL AND METHOD:

Study design: It is a cross sectional comparative study.

Inclusion criteria:

Histopathological diagnosed cases of breast cancer coming to Karachi Institute of Radiology and Nuclear Medicine belonging to any age group premenopausal, perimenopausal, postmenopausal, passing through any stage of cancer, married, unmarried both, lactating, non- lactating.

Exclusion criteria:

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

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.

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. Then 2 ml of blood taken from the antecubital vein with a 5cc 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.

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.

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)

Ultrasound:

Simple grey scale ultrasound 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.

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 $\pm$ 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.

RESULT:

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). Serum ALP levels were found extremely statistical significant among bone scan negative cases with positive liver metastasis ($P = .0001$) (Table 2) Elevated serum ALP levels were highly statistical significant, ($P= .0001$) in positive bone scan group with positive liver metastasis (Table 3). 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). Figure 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 figure 2.

Serum levels of total Bilirubin in bone scan positive and negative groups is shown in figure 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 figure 4 and found higher levels in all age groups of bone scan positive patients except age group 30-39 years.

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173 Table 1- BIOPHYSICAL PARAMETERS IN BONE SCANNING POSITIVE AND NEGATIVE
174 CASES

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

175

176

177 Table 2- COMPARISON OF SERUM ALKALINE PHOSPHATASE LEVELS IN SUBJECTS OF
178 NEGATIVE BONE SCAN WITH POSITIVE AND NEGATIVE LIVER METASTASIS

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☐ Bone scan negative cases n = 100						
Liver metastasis positive n = 12			Liver metastasis negative n = 88			
MEAN	S.D	S.E.M	MEAN	S.D	S.E.M	P--value
314.8	216.1	20.5	180.5	71.0	7.1	0.0001

181 ** Statistically significant $p < 0.0001$

182 Table 3- SERUM ALKALINE PHOSPHATASE LEVELS IN SUBJECTS OF POSITIVE AND
 183 NEGATIVE BONE SCAN WITH POSITIVE LIVER METASTASIS

184

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			
ALP	480.81	246.15	24.61	339.41	204.94	20.59	0.0001

185 ** Statistically significant $p < 0.0001$

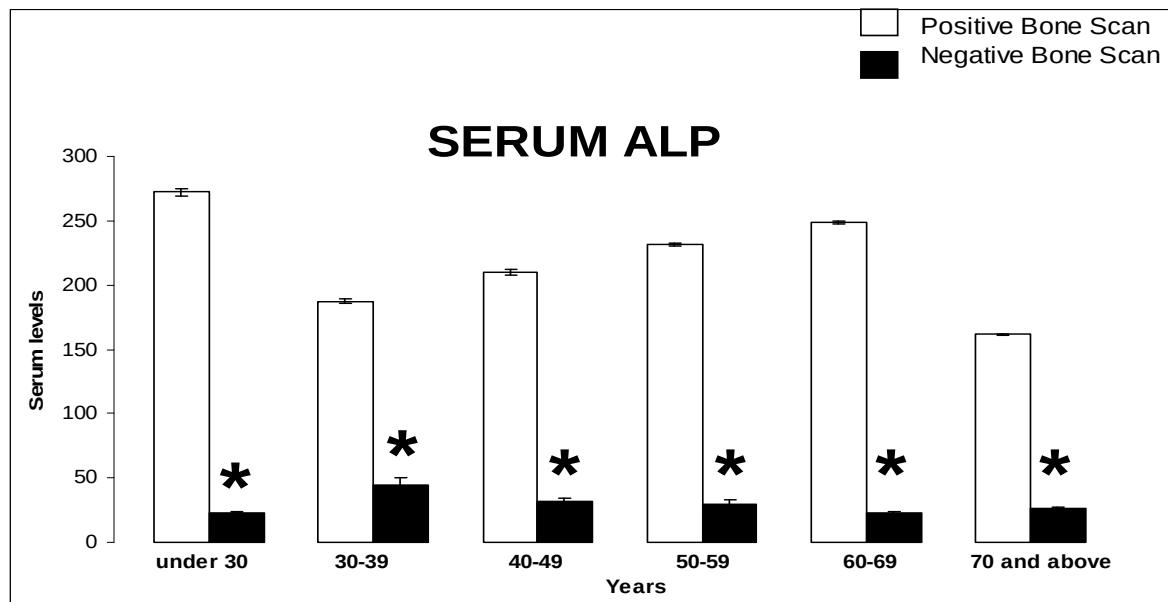
186

187 Table 2- BIOCHEMICAL PARAMETERS IN BONE SCANNING POSITIVE AND NEGATIVE
188 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

189 ** Statistically significant $p < 0.05$.

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191

192 FIGURE 1: Serum ALP Levels In Subjects With Positive- And Negative Bone Scan

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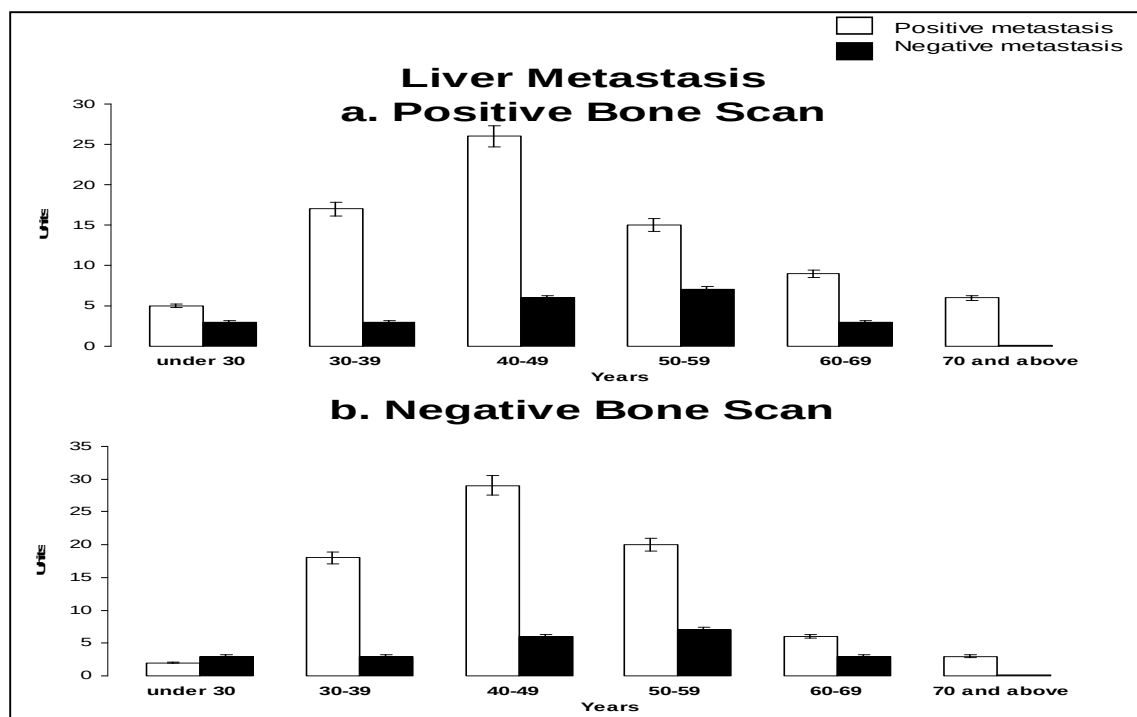


FIGURE 2: LIVER METASTASIS DIGNOSED BY SIMPLE GRAY SCALE ULTRASOUND IN SUBJECTS WITH POSITIVE- AND NEGATIVE BONE SCAN

*Values represented, as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found*Values represented as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found.

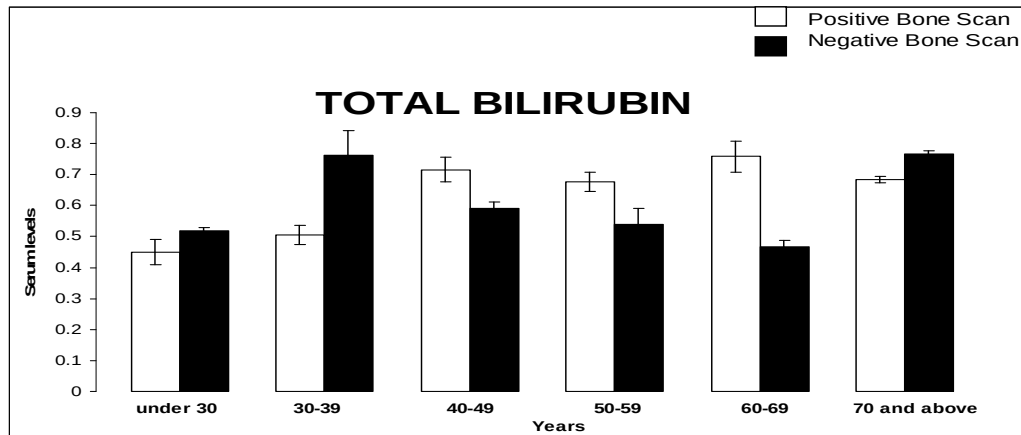


FIGURE: 3 SERUM BILIRUBIN LEVELS IN SUBJECTS WITH POSITIVE- AND NEGATIVE BONE SCAN

*Values are represented as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found.

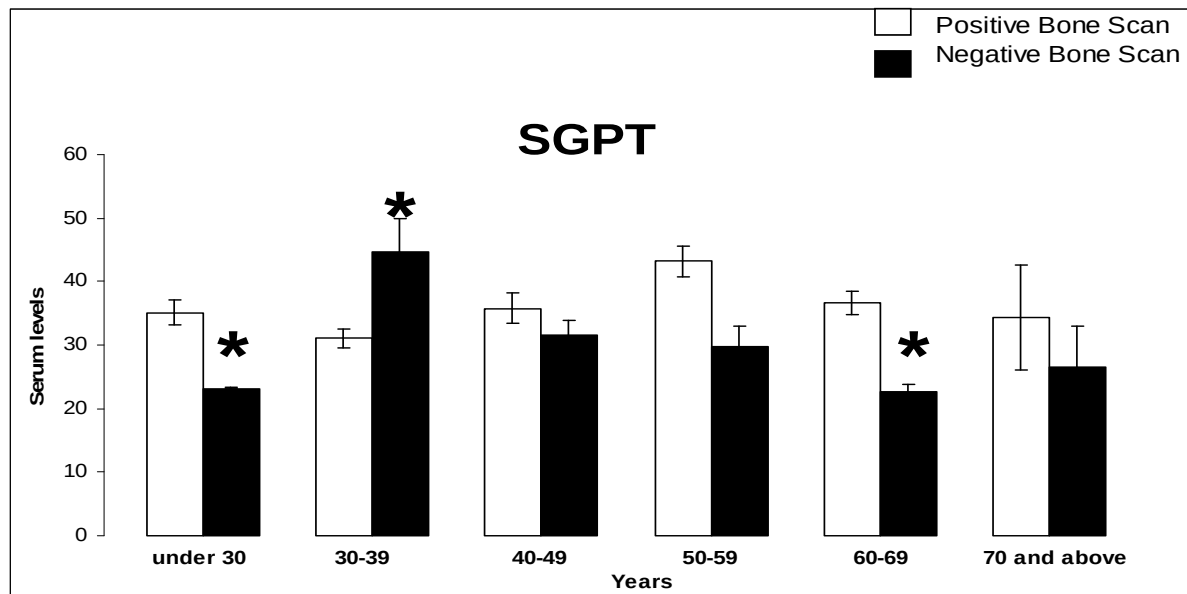


FIGURE 4: SERUM SGPT LEVELS IN SUBJECTS WITH POSITIVE- AND NEGATIVE BONE SCAN

*Values are represented, as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found

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 ($P = .0001$). Cases of negative bone scan with positive liver metastasis showed an extremely high statistical significant p

223 value as compare to cases of negative bone scan with negative liver metastasis ($P =$
224 .0001). Serum ALP is proved a significant marker for liver metastasis evaluation in
225 breast cancer patients. The cases of breast cancer those were negative for any
226 metastasis showed high normal levels of serum ALP. The results indicate that the
227 breast cancer patients have a higher tendency of metastasis to liver. This metastasis is
228 initially can be detected with the help of serum ALP levels in association with simple and
229 cheaper imaging techniques such as gray scale Ultrasound. These tests are routinely
230 used in most of oncology centers. Serum total Bilirubin and serum Alanine
231 Transaminase (SGPT) were found comparatively higher in patients of liver metastasis.
232 Other comparative studies support that high scanning sensitivity by simple Ultrasound
233 to evaluate liver lesions, because it is cheap but not highly efficient, is still a question
234 mark in the routine preoperative staging for the purpose of for liver surgery. Ct scan
235 and MRI thought to be superior as a routine, first line imaging technique over the simple
236 Ultrasound.²⁶

237 Blood dissemination of breast cancer most commonly results in liver metastasis. This
238 dissemination may occur with the appearance of primary tumors and may take several
239 years. Metastases are often showing the relapse of cancer. Previous research shows
240 the rate of liver metastasis is only 1-2%.^{27,28} This spread to liver might be restricted
241 to liver only. If the tumor can be removed successfully by surgery so the surgery is
242 treatment. Research shows 30-47%, 5 year survival rate after curative surgical
243 treatment.²⁹

244 ALP is important to detect all body cancer spread. A small prediction of liver metastasis
245 is shown by raised ALP level.^{30, 31} ALP with CA 15-3 indicates bone metastasis and

with CEA (Carcino Embryonic Antigen) indicates liver metastasis. 32.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.

33. 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 ($[500 \text{ lu/l}]$) with metastasis. This study highlighted the benefits of ALP to pick the breast cancer stages and secondary metastasis³⁴. Archana et al 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.³⁵

CONCLUSION:

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

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- 271 • ALP and simple live ultrasound of low cost and easily available diagnostic
272 facilities through which secondary liver metastasis can be detected, especially in
273 poor resources centers.

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275 **ETHICAL APPROVAL:**

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

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