

**SERUM ALKALINE PHOSPHATASE ALONG WITH IMAGING
TECHNIQUES AS A PREDICTOR OF LIVER AND BONE METASTASIS
IN BREAST CANCER**

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ABSTRACT:

Our objective was to find out the role of serum Alkaline Phosphatase in combination with gray scale ultrasound and bone scan as a predictor of liver and bone metastasis in breast cancer patients. For the above mentioned purpose 100 female patients of any age group, with and without bone and liver metastasis were selected. Serum alkaline phosphatase levels of all patients were measured and compared. Liver metastasis detection done by gray scale ultrasound machines. Bone metastasis was detected by bone scanners after injection of Technetium dye. Globocan provided rough estimates, regarding breast cancer prevalence and approximately 14.1 million cancer cases were reported as newly diagnosed and 8.2 million deaths occurred in 2012 all around world. Approximately 40,000 females die per year in USA due to complicated breast cancer. The major complication is bone and liver metastasis respectively. Osteolytic bone lesions cause 85% morbidity. Alkaline Phosphatase (s-ALP) has been extensively used to monitor the disease and screen the patients for cancer metastasis. Hospitalized patients for breast surgery due to cancer showed a significant elevated level of ALP. The prognostic role of s-ALP has been explored in various types of malignancies with bony and liver metastatic disease. 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. Breast cancer patient shows high normal levels of serum ALP. In this study, levels of serum Alkaline Phosphatase were measured in patients of breast cancer with and without bone and liver metastasis and were compared. Findings are suggestive of great importance for serum ALP level in prediction of metastasis either to bones or liver. During the study it was found that serum ALP levels were very high (statistical significant , p value= 0.0001) in patients of breast cancer with liver and bone metastasis . Liver metastasis was found more pronounced in cases where bone metastasis was positive. Positive liver Ultrasound accompanied with High serum ALP levels are correlated with positive liver metastasis. While positive bone scans along with elevated serum ALP is suggestive of bone metastasis in breast cancer patients. A combination of easily available, cheap imaging techniques and serum ALP in determination of metastasis of cancer to common sites like bones and liver will be better detecting tools. Poor resources center can take the benefit of these tests.

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

INTRODUCTION:-

Cancer is a major health hazard as well a burden on the economy all around the world. The reason for raise in cancer rate is mainly enormous increase in rate of population and wide distribution of established risk factors especially smoking of any type, obesity, and sedentary life style, use of contraceptives and desire of short family size, for the sake of civilization. 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. 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. Cancer prevalence is still high in developed countries and mortality rates are 8% to 15% higher in more in first world. 1. Breast cancer develops in over 7000 women each year in Ontario. 2. According to a study included 105 patients the age ranged from 16year 70 year, majority of patients were perimenopausals,the left breast was affected in 58 patients while right breast in 45cases. Major pattern of presentation was appearance of breast mass. 3. Alkaline Phosphatases is a collective term for isoenzymes with lesser specificity for the substrate that catalyze 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 thought that this enzyme may be involved in the active transport of substances across the membranes where they exist. Diagnosis of disease related to liver or due to other clinical problems is a critical matter for clinicians in case of a raised or disturbed liver function test. This problem is complicated by introduction of automated routine laboratory testing. It is not necessary that all persons with disturbed levels of liver function test are related to abnormalities of liver. The abnormality of different enzymes reflects the isolated disturbed function of liver. Most of the time the enzymes level is found elevated in primary liver disease but may be disturbed in other pathological conditions. Simple gray scale sonography can be used only for screening purposes and not associated with confirm diagnosis. Gray scale with color Doppler, is highly associated with better results and diagnosis. Introduction of micro bubble contrast agents for sonography is a miracle in this imaging field. 4. Because of less harms, cheap and easy availability, simple Ultrasound is the way of choice for the initial detection of isolated liver lesions. Also useful to differentiate the surgical patients with diffuse lesions and isolated lesion. Following simple and routine Ultrasound it becomes easy to decide whether the patient is suitable for surgery. CT scan or MRI can be done for surgical planning. 5. Breast cancer related liver metastasis is a blood born spread of the primary tumor. It may show a recurrence. 6. Spread of cancerous cells to distant organs is a major cause of mortality. 7. 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. 8. Bone metastases may complicate a number of cancers, which are responsible for great health risks. By research the axial skeleton is proved to be the first site of bone metastases following breast cancer. The axial skeleton is subject to initial metastasis because it contains the red marrow, having high vascularity and circulation. It has been proved by research that blood from some anatomic sites may reach directly to the axial skeleton. In postmortem studies of animals and humans, Batson, 9, et al supported the statements about drainage of deoxygenated blood from the breasts and pelvis into the Venae Cava as well as into a vertebral-venous plexus of Pelvis extended throughout the epidural and paravertebral veins. This pattern of drainage of blood to the main skeleton through the vertebral-venous plexus is a basic theme that how breast and other cancers spread to the axial skeleton, Pelvic and Pectoral girdles. Types of receptor 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. 10, 11, 12. 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 bone metastasis in prostate cancer or breast cancer is usually many years. 13, 14, 15. The breast cancer with bone metastases is less aggressive as compared 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 with breast cancer and bone-only-metastasis. 16.

Robert WC and etal, mentioned that in breast cancer multi organ metastases is common. 17. According to Robert WC and etal, systemic metastasis limited to bone are less frequent in breast cancer.18, 19, 20. Approximately 40,000 females die per anum in USA due to complicated breast cancer the major complication is bone metastases; osteolytic bone lesions cause 85% morbidity due to hypercalcemia, fracture, severe bone pain, and nerve compression. Median survival rate in breast cancer patients after bone involvement is 2 to 3 years, and about 10% in patients with breast cancer with metastases at diagnosis. 21. Female patients with intraductal and pathological stage I tumors, not required bone scanning, liver ultrasonography and chest radiography for baseline staging. Female patients with pathological stage ii tumors required, 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. Serum Alkaline Phosphatase (s-ALP) is widely used screening test for the evaluation of bone involvement 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 bone metastasis. The normal range of s-ALP in serum and fluctuation in its level has been appeared as prognostic factors to evaluate the treatment effect and survival rate.25, 26. 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. 27.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. 28. 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.29. Serum ALP proved success full to monitor disease course.30.We checked and compared Serum Alkaline Phosphatase levels along with imaging techniques as a predictor of liver and bone Metastasis inpatients of breast cancer.

MATERIAL AND METHOD:-

Participants:

Data were collected by trained medical physician, nurses and laboratory persons. 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,

170 abortions, clinical sign and symptoms, material status, family history, etc. We explained the
171 objectives and important features of the study to all patients prior to the start of study and their
172 consent was taken.

173 **Blood Collection**

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

180 Only histopathological diagnosed cases of breast cancer were selected for study. Study was done
181 in KARACHI INSTITUTE OF NUCLEAR MEDICIN. They were of any age group
182 premenopausal, perimenopausal, postmenopausal, passing through any stage of cancer, married,
183 unmarried both, lactating, non- lactating. Bone scan was done to check the metastatic
184 involvement of bones. During the study chemo therapy and radiotherapy were allowed
185 accordingly and most of patients have passed through the first three cycles of chemotherapy and
186 radiotherapy.

187 188 189 **Bone Scan**

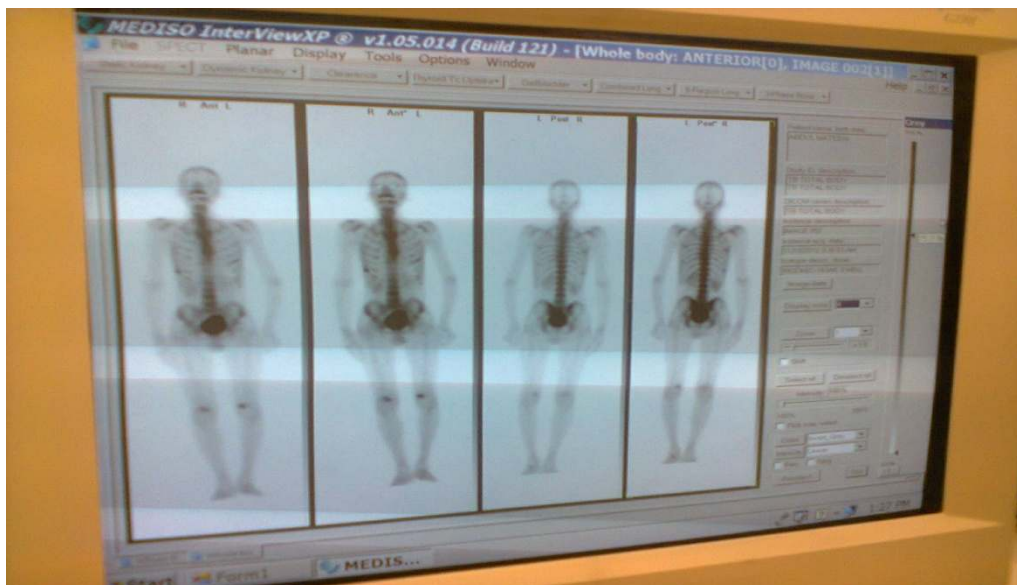
190 Bone scan done on Siemen E Cam scanner with accessories. (Figure B&C).Intravenous
191 dye technetium 99 mdp has been used. This test helps to detect if a cancer has
192 metastasized to bones and is useful because it provides a picture of the entire skeleton.
193 For this purpose, 20/mci (dose) of radioactive material (technetium 99) was injected into
194 a vein (intravenously or IV). The substance settles in areas of damaged bone
195 throughout the entire skeleton over the course of a couple of hours. (Six hours to twenty
196 four hours). Patient lied on a table for about 30 minutes while a special camera detected
197 the radioactivity and created a picture of the skeleton. FIG. A.
198
199



200
201

Siemens E Cam Bone Scan Machine with Accessories

With the permission of Kiran Hospital, Karachi. Fig- B&C



Bone Scan film showing skeleton after dispersion of radioactive dye, affected areas appeared as dark spots. With the permission of Kiran Hospital, Karachi. Fig -A

LFTS: (TOTAL BILIRUBIN, ALANINTRANSAMINASE, ALKALINE PHOSPHATASE).

SERUM ALKALINE PHOSPHATASE:-

ALP, SGPT AND BILIRUBIN DONE ON. SELECTRA –E, XL SEMI AUTO BIOCHEMICAL ANALYZER. VITAL SCIENTIFIC AND DIAGNOSTIC MACHINE, NETHERLAND, HOLLAND LATEST MODEL BY ROCH. (FIG H).



Selectra –E XL Semi Auto biochemical Analyzer. Fig- H

STATISTICAL ANALYSIS:

The data feeding and analysis was done on computer package SPSS (statistical packages of social sciences) version 11.0. Clinical characteristics will be summarized in terms of frequencies and percentages for qualitative/categorical variables (ultrasound and liver metastasis), 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. In all statistical analysis only p-value <0.05 are considered significant.

RESULT:

Serum Alkaline Phosphatase levels were found highly statistical significant in patients of breast cancer with bone metastasis and liver metastasis as shown in table 3. Liver metastasis was coexisting with bony metastasis in sufficient numbers of cases included in the study. Statistical analysis of the study was highly significant. Serum total Bilirubin and serum Alanine Transaminase (SGPT) were found comparatively higher in patients of liver and bone metastasis.

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 $p < 0.01$

Table 2- 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	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 $p < 0.01$

Table 3- SERUM ALKALIN PHOSPHATASE LEVELS IN SUBJECTS OF POSITIVE AND NEGATIVE BONE SCAN WITH POSITIVE LIVERMETASTASIS.

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

** Statistically significant $p < 0.01$

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

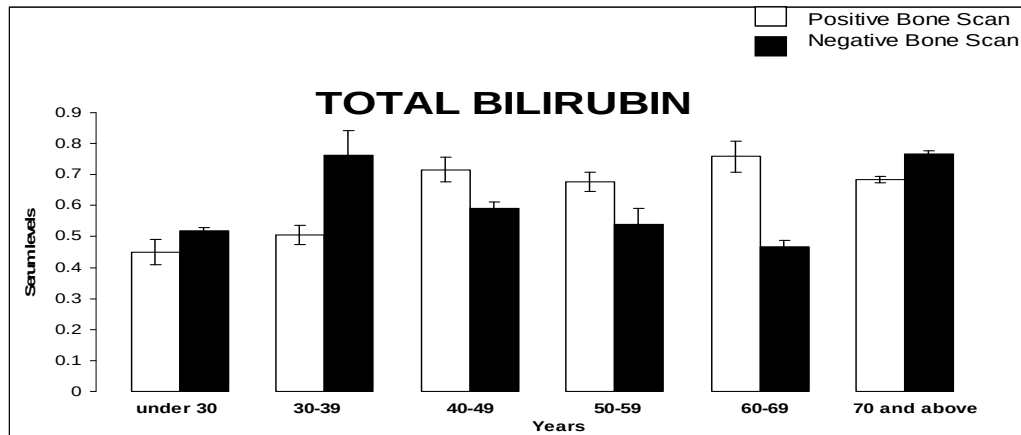


FIGURE -1

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

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

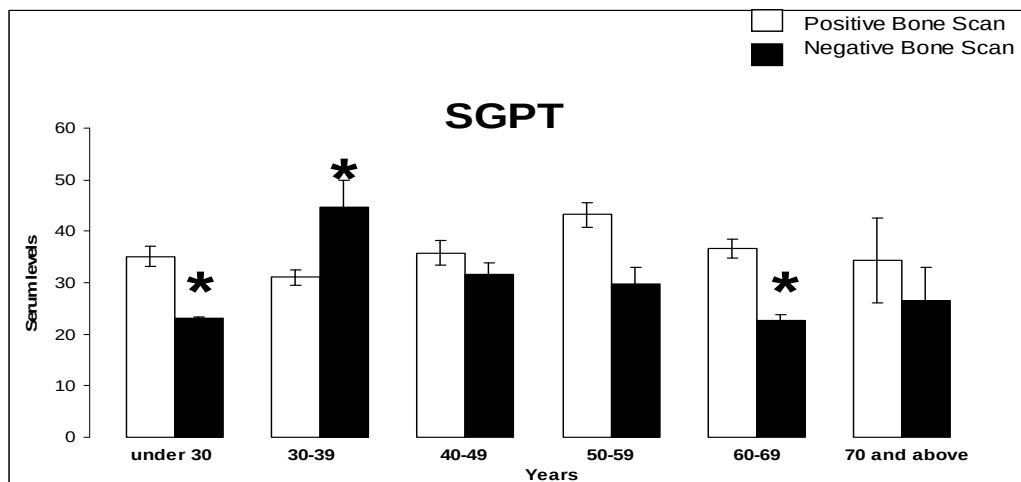


FIGURE -2 Values are represented as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found.

SERUM ALP LEVELS IN SUBJECTS WITH POSITIVE- AND NEGATIVE BONE SCAN

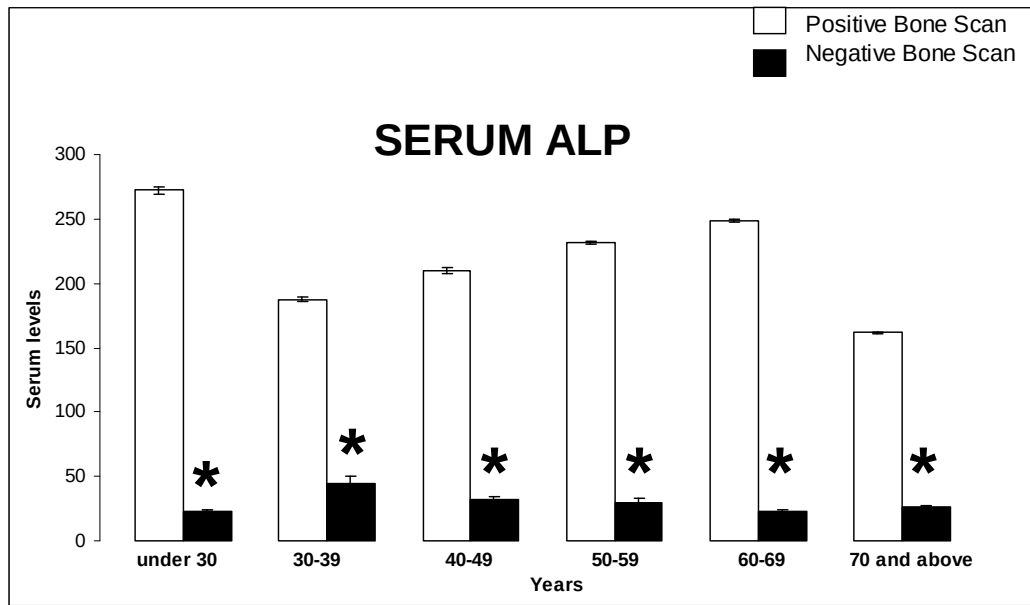


FIGURE -3
Values are represented as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found.

LIVER METASTASIS IN SUBJECTS WITH POSITIVE- AND NEGATIVE BONE SCAN

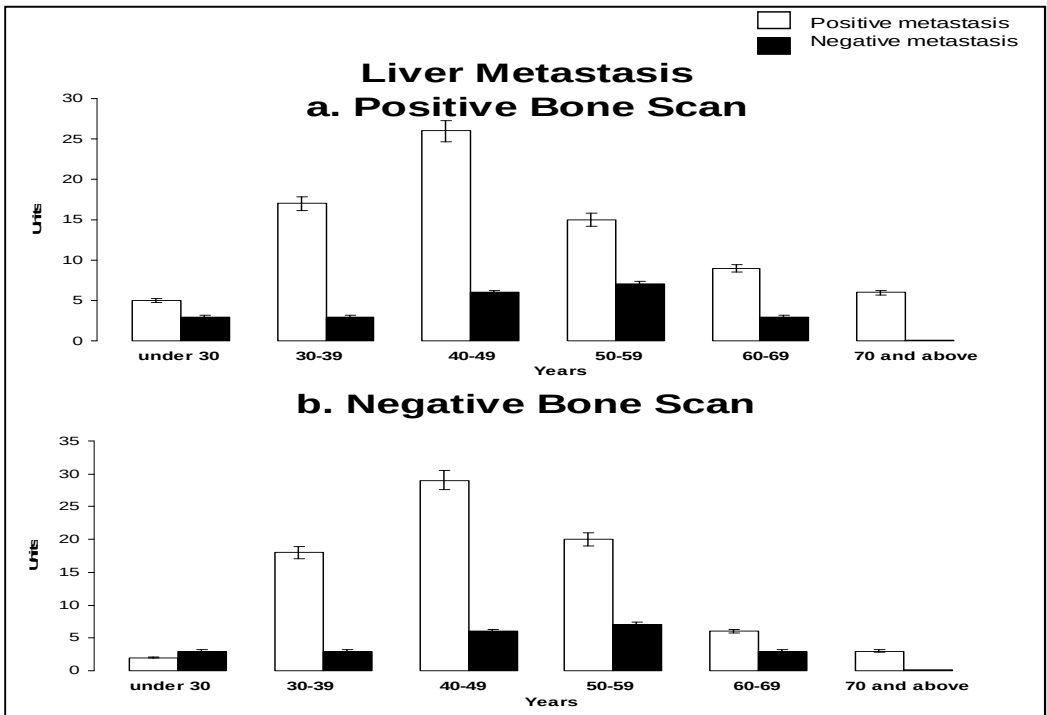
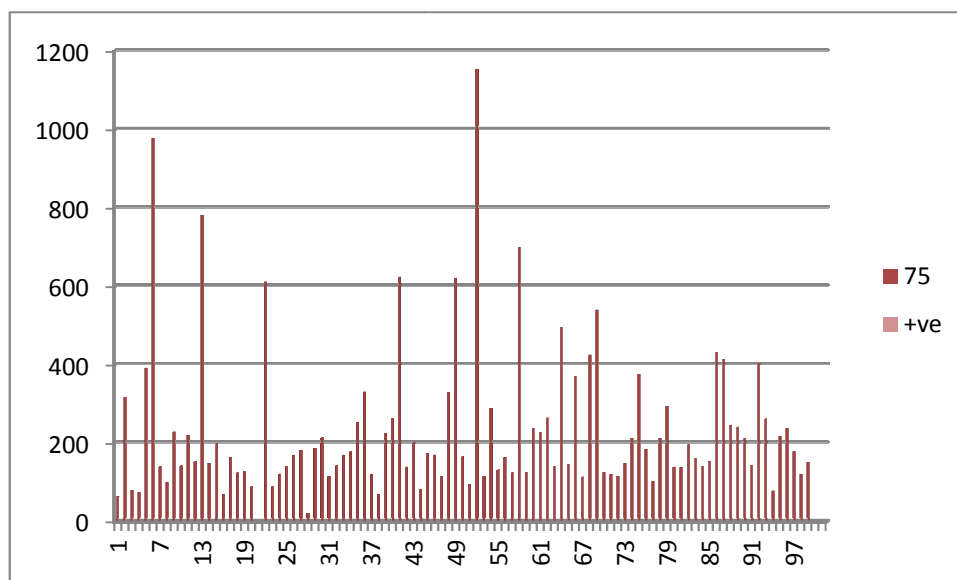


FIGURE -4
Values are represented as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found.



Levels of ALP in patients of breast cancer with liver metastasis in light red color lines and in patients without liver metastasis in dark red color lines.

Patients with liver metastasis showing comparatively higher serum levels of ALP.

DISCUSSION:

This study is carried out to find out the role of tumor marker Alkaline Phosphatase as a predictor of breast cancer metastasis to bones and liver in combination with imaging techniques like bone scan and ultrasound. The degree of metastasis to bones and liver in breast cancer patients was also evaluated.

For the study purpose 100 case of breast cancer with bone scan positive and 100 cases of breast cancer with negative bone scan were checked for their serum levels of ALP. Along with the tumor marker ALP other chemical parameters like SGPT and total Bilirubin were checked and compared. Ultrasound whole abdomen of all patients was done to reveal the liver metastasis. Bone scan was done to detect bone metastasis.

During the study we observed that liver metastasis was coexisting with bone metastasis in significant number of cases. Among 100 cases of positive bone scan, 22 cases of liver metastasis were found. On the other hand only 12 cases of liver metastasis were found among 100 cases of negative bone scan, p value is 0.06 which is closer to statistical significant value (0.05). Serum ALP levels were found significantly high in patients with bone and liver metastasis p value is 0.0001. The cases of breast cancer those were negative for any metastasis showed high normal levels of serum ALP. The results indicates that the breast cancer has a higher tendency of metastasis to bones and then 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 and bone scan. 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 and bone 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.³¹. 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%.^{32, 33}. 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. ³⁴

ALP is important to detect all body cancer spread. A small prediction of liver metastasis is shown by raised ALP level.^{35, 36}. ALP with CA 15-3 indicates bone metastasis and with CEA (Carcino Embryonic Antigen) indicates liver metastasis.³⁷. 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. ³⁸. 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 histopathologically 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 metastasis.³⁹. 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.⁴⁰.

CONCLUSION:

Serum ALP in combination with bone and liver scan is a predictor of secondary bone and liver metastasis in breast cancer patients.

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

High serum alkaline phosphatase level with positive bone scan is indicative of bone metastasis in breast cancer patients.

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

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