

Case study**ROLE OF ALKALINE PHOSPHATASE IN DETECTING LIVER
METATATASIS IN BREAST CANCER****ABSTRACT:**

Our aim was to find out the role of serum alkaline phosphatase level in breast cancer patients with liver metastasis for that purpose 100 patients of any age, with and without bone 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 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 anum in usa due to complicated breast cancer the major complication is bone metastases. Osteolytic bone lesions cause 85% morbidity. Alkaline phosphatase (s-alp) has been extensively used to screen patients for bone metastasis. The prognostic role of s-alp has been explored in various types of malignancies with bony metastatic disease. Both the baseline s-alp and changes in s-alp have been reported as prognostic factors for treatment effect and survival such as in bony metastatic prostate cancer. By research the axial skeleton is proved to be the first site of bone metastases in breast cancer. The breast cancers with bone metastases are less aggressive as compare to other visceral metastasis. At diagnosis and that aspartate transaminase (ast) levels were increased inmost patients. The level of serum alkaline phosphatase in patients of breast cancer with and without bone and liver metastasis was compared and found that alp is a predictor of metastasis. High serum alp levels are correlated with positive liver scan for metastasis in breast cancer patients. Liver metastasis is predominant in cases with bone metastasis.

INTRODUCTION:-

cancer is a major heath hazard as well a burden on the economy all around the world. the reason of increasing cancer rate is mainly enormous increase in rate of population and wide distribution of established risk factors, especially smoking of any type, obesity, sedentary life style, and use of contraceptives and desire of short family size, for the sake of civilization. globocan provided rough estimates, approximately 14.1 million cancer cases were newly diagnosed and 8.2 million deaths occurred in 2012 all around world. in the 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. lung cancer is the top most cause of death due to cancers among males in whole of the world, and fastly growing number of cases of breast cancer as the leading cause of cancer death among females in first world;as well as in less developed countries. other leading causes of cancer death in first world include colorectal cancer among males and females and prostate cancer among males. in developing countries, liver and stomach cancer among males and cervical cancer among females are also top causes of cancer mortality? cancer prevalence is still high in developed countries and mortality rates are 8% to 15% higher in more in first world. this difference determines the lag in the mix of cancers, which is affected by health risk and way of diagnosis, the facilities of treatment. main risk factors are tobacco use (lung, colorectal, stomach, and liver cancer), overweight/obesity and sedentary life style (breast and colorectal cancer), and infection (liver, stomach, and cervical cancer). a huge burdon of cancer and its related mortality could be minimize by reducing the risk factors, such as tobacco control, vaccination, and early diagnosis 1breast cancer develops in over 7000 women each year in ontario 2 .according to a study included 105 patients with mean age of 41.9+ 10.9 year. the age ranged from 16 year 70 year. majority of patients were perimenopausals (n=31 - 29.5% and n=34 - 32.3% respectively). left breast was affected in 58 (57.6%) patients while right breast in 45 (42.8%) cases. major pattern of presentation was (n=91 - 86.8%) appearance of breast mass, mammograms were helpful in 70 (66%) patients while tru cut biopsy used as a diagnostic tool in 48 (45.7%) cases. 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. they are called isoenzymes as they catalyze the same chemical reactions, naturally exist in same species, like other isozymes differ in physicochemical properties. site of alkaline phosphatases are, bone, intestine, kidney, liver, placenta and white blood cells. the physiologic function of group of isozyme is unknown. it

is thought that they may be involved in the active transport of substances across the membranes where they are existing. alkaline phosphatase is found in serum as well.

diagnosis of disease related to liver or due to other clinical problems is a critical matter for clinicians in case of a raised or disturb liver function test. this problem is complicated by introduction of automated routine laboratory testing. it is not necessary that all persons with disturb levels of liver function test are related to abnormalities of liver. liver function tests include alanine transaminase (alt) and aspartate transaminase (ast), alkaline phosphatase (alp), gammaglutamyl transferase, serum bilirubin, prothrombin time, or neither international normalised ratio and serum albumin. the abnormality of different enzymes reflects the isolated disturb function of liver. the enzymes show the functions of the liver— as, to

excrete anions (bilirubin), normal hepatocellular function (transaminases), secretion and free flow of bile (bilirubin and alp), and protein synthesis (albumin). other functions of liver are also being tests including hepatitis serology, iron and copper studies, α 1-antitrypsin levels, and autoantibodies. they are related to the basic liver dysfunctions. most of time the enzymes level is found elevated in primary liver disease but may disturb in other pathological conditions. any abnormality in an asymptomatic patient indicates the abnormality at primary site, if it is statistically significant the tests should be reconfirmed and need a proper intervention.

simple gray scale sonography can be use only for screening purposes and not associated with confirm diagnosis. gray scale with color doppler, is highly associated to better results and diagnosis. introduction of microbubble contrast agents for sonography is a miracle in this imaging field. 6. contrast enhanced ultrasound (ceus) uses chemical agents that comprises of microbubbles of gas established by layer of a biocompatible surfactant or polymer like phospholipid or protein 7. these microbubble chemicals are highly intravascular, free from hazards and side effects, administration is not difficult, thus not harm full for the patient 8. normal renal function is not necessary for the excretion of dye because it is not excreted by kidneys, it is an advantage of use over ct and mri. 9. when ct and mri fail to characterize the lesion or provide accuracy the use of ceus is thought to be superior. ceus has been appeared as a solution in problems to characterize the isolated liver lesions. it presents the dynamic changes, due to its high temporal resolution, potential of real time scanning technique .10. because of less harms, cheap and easy availability, simple us is the way of choice for the initial detection of isolated liver lesions, and useful to differentiate the surgery patients with diffuse lesions and isolated lesion. following simple and routine us, it become easy to decide which patient is suitable for surgery, ct and/or mri can be done for surgical planning. 11.

breast cancer liver metastasis is a blood born metastases of the primary tumour. it may show a recurrence. 13. spread of cancerous cells to distant organs is major cause of mortality. 14. advanced imaging scans and staging laparoscopy in association with use of ultrasound guidance during surgery should be included for the sake to lowers the hazzard of open surgery in patients with breast cancer complicated by liver metastasis. 21. however, bone metastases may complex a number of cancers, which are responsible of great health risks and increase need of fascilities. malignancies related to thyroid, kidney, and bronchus are also main causes of bone metastases and according to a postmortem report 30% to 40% chances of bone involvement, gastrointestinal tract tumors shows only little tendency to spread to bones, (<10%). 22. by research the axial skeleton is proved to be the first site of bone metastases in breast cancer. the axial skeleton is subject to initial metastasis because 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 23 etal supported the statements about drainage of deoxygenated blood from the breasts and pelvis into the venae cavae as well as into a vertebral-venous plexus of vessels of pelvis extended throughout the epidural and perivertebral veins. this pattern of drainage of blood to the main skeleton through the vertebral-venous plexus is basics of theme, that how breast and prostate cancers, as well as those arising in kidney, thyroid, and lung, spread to the axial skeleton ,pelvic and pectoral girdles. however, the vertebral-venous plexus might not be the only responsible factor of cancers metastasis to the bones. types of receptor appeared, aggressiveness, risk factors, vascularity and turnover of regional cancerous cells as well as the characteristics of tissues to which they spread are main features that influence the pattern of cancer spread 24–26. 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. 27, 28, 29. comparatively, the average survival rate in patients of advanced lung cancer is only few months.

the breast cancer with bone metastases is less aggressive as compare to other visceral metastasis. as studied by coleman and rubens 30, the diagnosed patients with 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. 31

robert wc and etal determined that in breast cancer multi organ metastases is common. 32 according to robert wc and etal, systemic metastasis limited to bone are less frequent in breast cancer. rate of bone-only metastasis is 17-37% in patients with distant dissemination. distant spread restricted to the bones shows better prognosis than other kinds of secondary spread or multiple site involvement includings bone and the viscera. 33, 34, 35. 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. 36 .tabariès et al linked the immune response to metastasis in cancer and found increased recruitment of t lymphocytes (cd3+), myeloid-derived (gr-1+) cells and neutrophils (ly-6g+ or ne+) in lung and liver metastases, while decreased recruitment in bone metastatic lesions. infiltrating pattern of these cells are different within soft tissue during metastases. t lymphocytes and granulocytic immune cells infiltrates found around the periphery of liver metastases. in lung metastases the exhibit the dispersed pattern.they also found in gr-1+ cell-depletion studies that infiltration of myeloid-derived cells are essential part for the establishment of breast cancer liver metastases but not always for metastasis to the lung and bone. in ly-6g+ cell-depletion experiments, granulocytic component of the innate immune infiltrate exhibit markedly impaired formation of liver metastases. as a result they found that the cd11b+/ly-6g+ neutrophils that infiltrate and surround the liver metastases having ionic affinity toward an n2 phenotype, which are involved in an increase in tumor growth and its metastasis. interactions of infiltrating ly-6g+ cells within the liver microbiology are proved an essential component in metastasis of breast cancer to liver. 37

interaction between the tumor cells and surrounding stromal cells is a specific indicator of cancer cells potential for the metastasis to target organs. the tumor microenvironment finally composed on extracellular matrix proteins, resident fibroblasts and endothelial cells, as well as chemotactic (macrophages, neutrophils, myeloid-derived suppressor cells or natural killer cells) and adaptive (b and t lymphocytes) immune cells.38 leukocyte chemotaxis are also found in most of solid tumors; and playing a complex role and clinical outcomes.39 some times this inflammatory response appears very helpful to destroy tumor cells has a good prognosis.40, 41 .many research articles are contradictory about the role of inflammation in destroying tumor metastatic cells and exhibit the positive role in establishment of cancer development and its metastasis by utilizing their anti-tumor immune suppressor potential.42,43,44. among various studies regarding the role of cancer-related inflammation in primary tumor growth, it is evident that inflammatory cells and chemical mediators also participate in chemotaxis of malignant cells for secondary growth of cancer. 45 .female patients with intraductal and pathological stage i tumours, not required bone scanning, liver ultrasonography and chest radiography for baseline staging. female patients with pathological stage ii tumours, 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 tumours, bone scanning, liver ultrasonography and chest radiography are essential tools for prospective baseline staging.46. use of screening programs is a better step in diagnosis of early-stage disease with better out come.47.

udaya k. liyanage and et al found in tumor-infiltrating lymphocytes and lymph node lymphocytes, the distribution of t lymphocyte regulatory cells and calculated about 20.2% (se 3.93) and 20.1% (se 4.3), respectively. treg expressed ctla-4, cd45ro markers, secreted tgf and il-10 but did not secrete ifn. treg potently decreases the division and differentiation of cd8_ cells or cd4_25_ cells and secretion of interferon. udaya k. liyanage and et al presented the role of t regulatory cells in breast and pancreatic carcinoma and proved by the research that they were found in higher concentration in the peripheral blood as well as in the tumor microenvironment of sufferers with invasive breast or pancreas cancers. these treg may involve in the immune response against cancer, and may briefly explain the suppressed immune response against tumor ags.48 serum alkaline phosphatase (s-alp) is widely used screening test for the evaluation of bone involvement secondary to cancer,the reason might be its sensivity, 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, as in bony metastatic prostate cancer. 49, bony metastatic breast cancer 50, and clear cell chondrosarcoma of the bone 51.

ca15-3 level in serum of patients with recurrenceof breast cancer is proved a good indicator of metastasis than alp, but altogather use of both biomarkers gives an early sign of recurrence. whether routine use of these biomarkers improves overall survival remains an open question.52

according to the report by breast cancer study group (ibcsrg), 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. 53. serum alkaline phosphatase appears to be a significant independent prognostic index in patients with skeletal metastatic nasopharyngeal carcinoma, which could reflect the short-term treatment response of palliative chemotherapy and the long-term survival outcomes. serum alkaline phosphatase predicts survival outcomes in patients with skeletal metastatic nasopharyngeal carcinoma.54

comparison the level of serum alkaline phosphatase in patients of breast cancer with and without bone and liver metastasis was done.

MATERIAL AND METHOD:-

SELECTION OF PATIENT: - ONLY DIAGNOSED CASES OF BREAST CANCER WERE SELECTED FOR STUDY. THEY WERE OF ANY AGE GROUP PREMENOPAUSAL, PERIMENOPAUSAL, POSTMENOPAUSAL, PASSING THROUGH ANY STAGE OF CANCER, MARRIED, UNMARRIED BOTH, LACTATING, NONLACTATING, HAVING DIFFERENT BODY MASS INDEX. PASSING THROUGH ANY CYCLE OF CHEMOTHERAPY AND RADIOTHERAPY. A MEMORANDUM OF UNDERSTANDING (MOR) WAS SIGNED BY AUTHORIZED PERSONS OF UNIVERSITY OF KARACHI AND KIRAN HOSPITAL. CONSENT (SIGNATURE) OF EVERY PATIENT HAD BEEN TAKEN ON PATIENTS FORM. STUDY APPROVED BY BASR (UNIVERSITY OF KARACHI).

BONE SCAN

Bone scan done on siemen e cam scanner with accessories. (figure B&C) intravenous dye technetium 99 mdp has been used. This test helps to see if a cancer has metastasized to bones and is useful because it provides a picture of the entire skeleton. For this purpose, 20/mci (dose) of radioactive material (technetium 99) was injected into a vein (intravenously or iv). The substance settles in areas of damaged bone throughout the entire skeleton over the course of a couple of hours. (six hours to twenty four hours). Patient lied on a table for about 30 minutes while a special camera detected the radioactivity and created a picture of the skeleton. FIG. A.



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).



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

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198 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 sgpt, alp, bilirubin). 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:-

Results of liver metastasis express in terms of p value (statistically significant) after student t test in patients of breast cancer with positive and negative bone scan. Result of alp, sgpt& bilirubin total presents in terms of mean, standard deviation, standard error mean and 'p' value after student t test. Table 1 shows liver metastasis is more common in patients with bone metastasis and p value, (0.06) is closer to statistical significant value (<0.01). Table 2 shows the same results and alp, sgpt, total bilirubin were found comparatively increase in those patients who have secondary bone metastasis, especially alkaline phosphatase levels, but not statistically significant. Figure 1, 2, 3 shows the comparison of total bilirubin, sgpt and alp levels respectively in patients with positive and negative bone metastasis and found no significant difference. Figure no 4 shows the significant increase numbers of liver metastasis cases in patients of breast cancer with positive bone scan as compare to those with out bone metastasis. Figure no 5 shows comparatively higher serum levels of alp in bony metastatic breast cancer.

TABLES AND DIAGRAMS:-

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 phsphatase	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 WITH POSITIVE- AND NEGATIVE LIVER METASTASIS.

No of liver metastasis positive				No of liver metastasis negative			P-value
n = 22				n = 12			
ALP	MEAN 227.2	S.D 183.91	SEM 18.39	MEAN 201.6	S.D 107.03	SEM 10.70	0.231

** Statistically significant $p < 0.01$

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

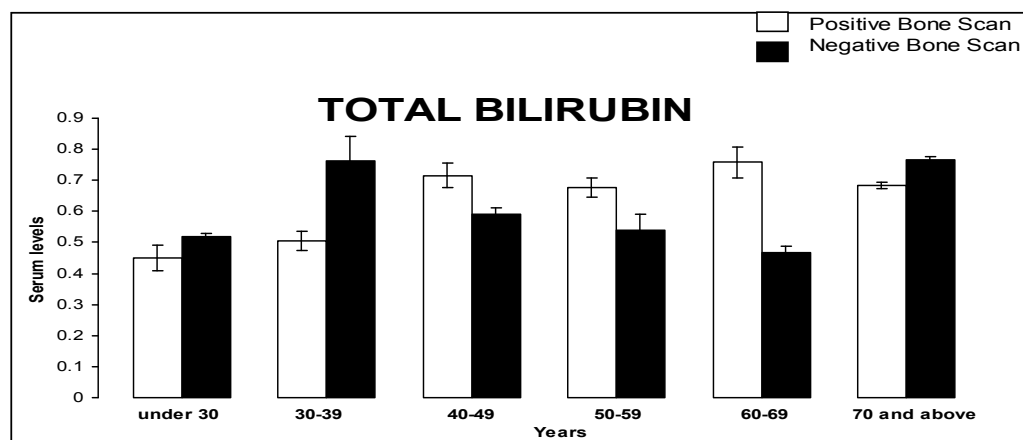


FIGURE -1

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

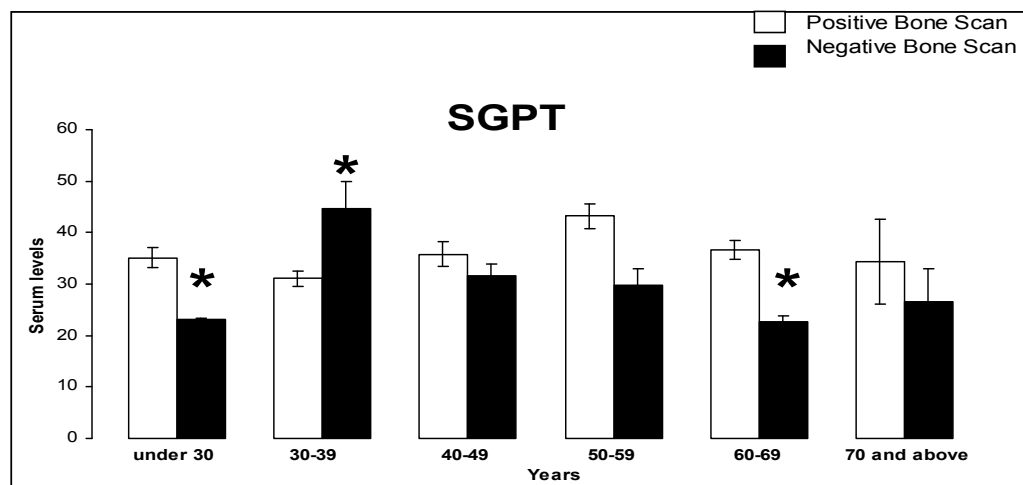


FIGURE -2 Values are represented as means $\pm$ SD. Statistically significant difference from patients with positive bone scan following student's t- test were not found.

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

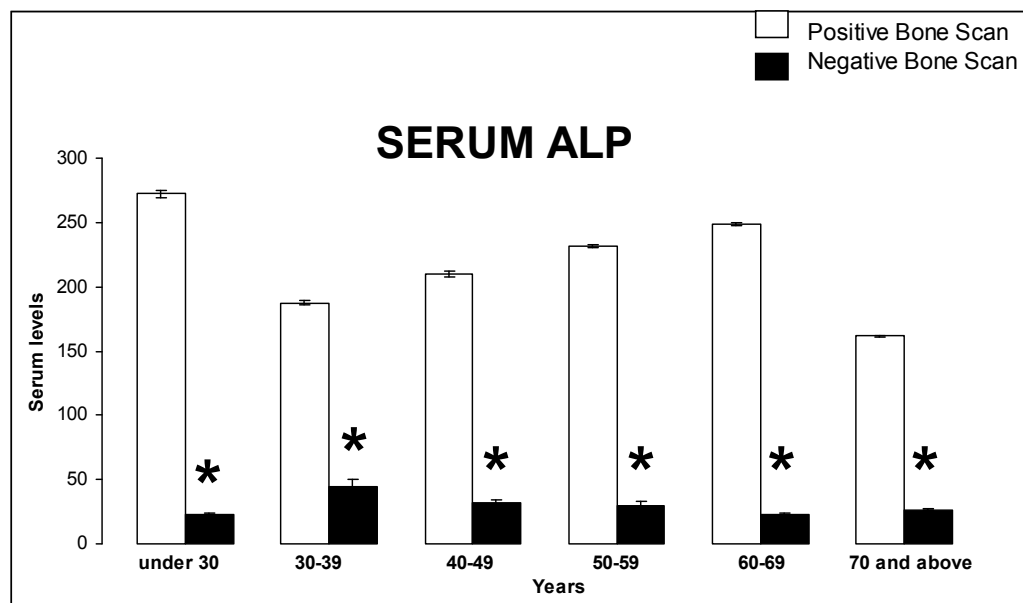


FIGURE -3

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

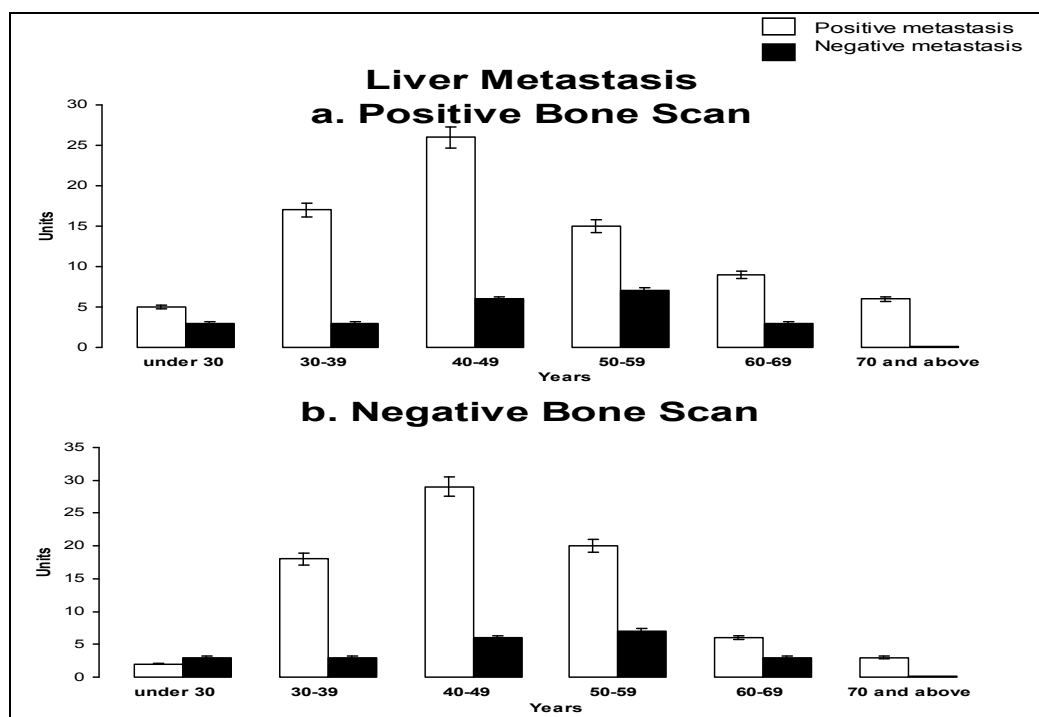
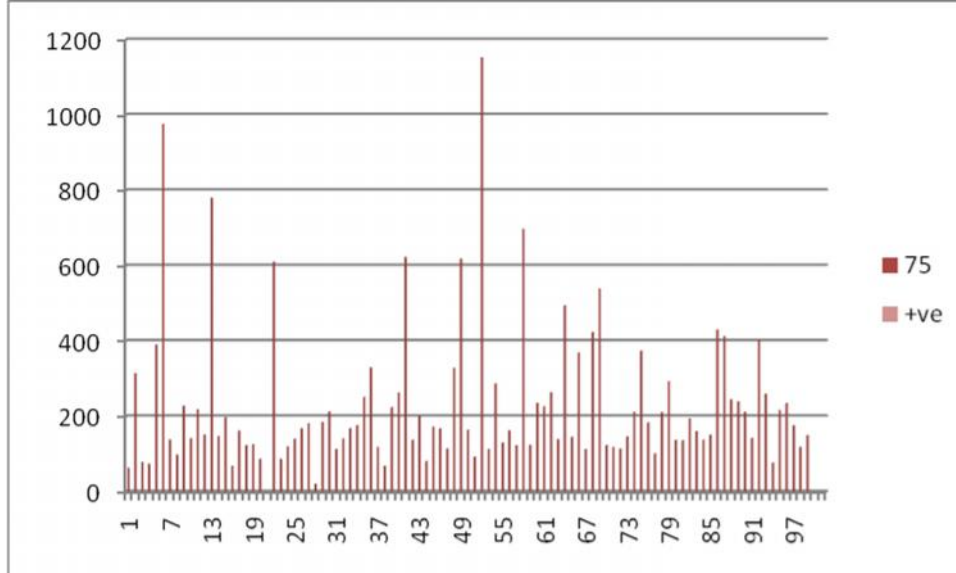


FIGURE -4

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 alkpo4 in combination with bone scan and ultrasound to determine the degree of metastasis to bones and liver in breast cancer patients.

For this 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 marker other parameters like sgpt and total billirubin. Ultrasound of whole abdomen was done to reveal the metastasis. Bone scan was done to detect bone metastasis.

During the study we observed that liver metastasis, (detected by simple ultrasound) was common or in greater number in case of breast cancer with bone metastasis (n=22) as compare to cases of cancer with negative bone metastasis (n=12), as well as the result (p=0.06) was found closer to statistical significant value (0.01). The result indicates that the cancer showing the tendency of metastasis to bone may also spread to liver meanwhile. The comparative high scanning sensitivity by simple us 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 us. 55

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%. 15, 16. 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.56

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 ap 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 expensis of initial and follow-up examination in patients with breast cancer. 57-

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 level ([500 iu/l) with metastatsis. This study highlighted the benefits of alp to pick the breast cancer stages and secondary metastasis.58

CONCLUSION:-

Alp Is A Predictor Of Secondary Bone And Liver Metastasis In Breast Cancer Patients Simultaneously And In Combination With Bone Scan Or Liver Scan Provides Specificity To Bone Or Liver Metastasis Alone.

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 and bone scan easily available diagnostic fascilities through which secondary liver and bone metastasis can br detected.

REFERENCES:-

- 1- Lindsey A. Torre, MSPH1; Freddie B, PhD2; Rebecca L. Siegel, MPH3; Jacques F, ME4; Joannie L- Tieulent, MSc5; Ahmedin J, DVM, PhD , Global Cancer Statistics, 2012; J Clin 2015;65:87-108. Vc 2015 American Cancer Society.
- 2- Robert E. M, Mary J, Kathy P, Mark L, Tom O; Baseline staging tests in primary breast cancer: a practice guideline † and the Breast Cancer Disease Site ,Group of the Cancer Care Ontario Practice Guidelines Initiative , CMAJ • MAY 15, 2001; 164 (10) 1439 © 2001 Canadian Medical Association or its licensors.
- 3-, Shahida P, Saeed A; Profile of Breast Cancer Patients Journal of Surgery Pakistan (International) 17 (3) July - September 2012.
- 4- M. Kaplan, M.D. N Engl J Med ;Alkaline Phosphatase Marshall ;1972; 286:200-202January 27, 1972DOI: 10.1056/NEJM197201272860407.
- 5-,J K Limdi, G M Hyde Evaluation of abnormal liver function tests ,Postgrad Med J2003;79:307–312.
- 6-Wilson, Burns. An algorithm for the diagnosis of focal liver masses using microbubble contrast-enhanced pulse-inversion sonography. AJR 2006;186:1401–12.
- 7-Tang M-X, Mulvana H, Gauthier T, Lim AKP, Cosgrove DO,Eckersley RJ, et al. Quantitative contrast-enhanced ultrasound imaging: a review of sources of variability. Interface Focus 2011;1:520–39.
- 8-Piscaglia F, Bolondi L. The safety of SonoVue in abdominal applications: retrospective analysis of 23188 investigations. Ultra- sound Med Biol 2006;32:1369–75.
- 9-Jang Hyun-Jung, Yu Hojun, Kim Tae Kyoung. Contrast-enhanced ultrasound in the detection and characterization of liver tumors. Cancer Imaging 2009;9:96–103.
- 10-Brannigan M, Burns Peter N, Wilson Stephanie R. Blood flow patterns in focal liver lesions at microbubble-enhanced US. Radiographics 2004;24:921–35.
- 11-Braga L, Guller U, Semelka RC: Modern hepatic imaging. *Surg Clin North Am* 2004, 84:375-400.
- 12-Marcela P C, Paulo H, Rubens C, Miriam RB P, Paula NV B and Almir GV B, Focused abdominal ultrasound in preoperative liver surgery staging: a prospective study Brazil *World Journal of Surgical Oncology* 2013, Department of Imaging, AC Camargo Cancer Center, Rua Professor Antônio Prudente, 211, São Paulo, Brazil ² Faculdade de Medicina, Universidade de São Paulo, Rua Eneas de Aguiar Carvalho, 255, São Paulo11:138 doi:10.1186/1477-7819-11-138
- 13- MalteWeinrich,1,2 ChristelWeiß,3 Jochen Schuld,2 and Bettina M. Rau1,2; Liver Resections of Isolated Liver Metastasis in Breast Cancer: Results and Possible Prognostic Factors; 2013
1 Department of General, Thoracic, Vascular and Transplantation Surgery, University Hospital Rostock, Schillingallee 35, 18057 Rostock, Germany 2Department of General, Visceral, Vascular and Paediatric Surgery, University Hospital of the Saarland, Kirrberger Straße, 66424 Homburg/Saar, Germany 3Department of Medical Statistics, Medical Faculty Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany; Published 19 January 2014
- 14- A. Jemal, R. Siegel, E. Ward, T.Murray, J. Xu, and M. J. Thun, “Cancer statistics, 2007,” *Ca-A Cancer Journal for Clinicians*, vol. 57, no. 1, pp. 43–66, 2007.
- 15-E. Viadana, I. D. Bross, and J. W. Pickren, “An autopsy study of some routes of dissemination of cancer of the breast,” 1973 *British Journal of Cancer*, vol. 27, no. 4, pp. 336–340,.

16-Y. T. N. Lee, "Breast carcinoma:pattern of metastasis at autopsy," 1983. *Journal of Surgical Oncology*, vol. 23, no. 3, pp. 175–180.

17- A. Sjövall, V. Järn, L. Blomqvist et al., "The potential for improved outcome in patients with hepatic metastases from colon cancer: a population-based study," 2004;*European Journal of Surgical Oncology*, vol. 30, no. 8, pp. 834–841,

18- J. Leporrier, J. Maurel, L. Chiche, S. Bara, P. Segol, and G. Launoy, "A population-based study of the incidence, management and prognosis of hepatic metastases from colorectal Cancer," 2006. *British Journal of Surgery*, vol. 93, no. 4, pp. 465–474.

19- P. C. Simmonds, J. N. Primrose, J. L. Colquitt, O. J. Garden, G. J. Poston, and M. Rees, "Surgical resection of hepatic metastases from colorectal cancer: , 2006; a systematic review of published studies," *Annals of Surgical Oncology*, vol. 13, no. 5, pp. 668–676.

20- A. C. Wei, P. D. Greig, D. Grant, B. Taylor, B. Langer, and S. Gallinger, "Survival after hepatic resection for colorectal metastases: a 10-year experience," *British Journal of Cancer*, vol. 94, no. 7, pp. 982–999, 2006.

21-M. A. Cassera, C. W. Hammill, M. B. Ujiki, R. F. Wolf, L. L. Swanstrom, and P. D.Hansen, "Surgical management of breast cancer liver metastases," 2011; *HPB*, vol. 13, no. 4, pp. 272–276.

22- Robert E. C; Clin; Clinical Features of Metastatic Bone Disease and Risk of Skeletal Morbidity; Cancer Res 2006; 12(6243s 20 Suppl) American Association.

23- Batson. O. The role of vertebral veins in metastatic processes. *Ann Intern Med* 1942; 16:38^45.

24- Bundred. N,Walker .R. A, Ratcliffe.W .A, et al. Parathyroid hormone related protein and skeletal morbidity in breast cancer. *EurJ Cancer* 1992; 28:690^ 2.

25- Coleman. R, Rubens. R. The clinical course of bone metastases in breast cancer.1987, *Br J Cancer*; 77: 336^40.

26- Koenders .P, Beex. L.V, Langens .R, et al. Steroid hormone receptor activity of primary human breast cancer and pattern of first metastasis. *Breast Cancer Res Treat* 1991; 18:27^32.

27- Coleman R, Rubens R. The clinical course of bone metastases in breast cancer. 1987 *Br J Cancer*; 77: 336^40.

28- Koenders P, Beex LV, Langens R, et al. Steroid hormone receptor activity of primary human breast cancer and pattern of first metastasis. 1991; *Breast Cancer Res Treat*; 18:27^32.

29- Fang K, Peng C. Predicting the probability of bone metastasis through histological grading of prostate carcinoma: a retrospective correlative analysis of 81 autopsy cases with ante-mortem transurethral resection specimens. *JUrol* 1983; 57:715^20.

30- Coleman R, Rubens R. The clinical course of bone metastases in breast cancer. *Br J Cancer* 1987; 77: 336^40.

31. Sung G .A, 1; Hak M. L, 1 Sang-H Cho, 2 Seung Ah. L, 1 Seung H. H, 1 Joon .J, 1 and Hy-D. L1; - Prognostic Factors for Patients with Bone-Only Metastasis in Breast Cancer 2013; 1Department of Surgery, Yonsei University Medical College, Seoul; 2Department of Statistics and Actuarial Science, Soongsil University, Seoul, Korea. <http://dx.doi.org/10.3349/ymj.2013.54.5>. pISSN: 0513-5796, eISSN: 1976-2437 ;Yonsei Med J 54(5):1168-1177.

32- Robert WC. Surveillance of patients following primary therapy. 2009 In: Harris JR, Lippman ME, Morrow M, Osborne CK, editors. *Disease of the breast*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; P.823-31.

33- Sherry MM, Greco FA, Johnson DH, Hainsworth JD. Metastatic breast cancer confined to the skeletal system. An indolent disease. 1986; *Am J Med*; 81:381-6.

34- Scheid V, Buzdar AU, Smith TL, Hortobagyi GN. Clinical course of breast cancer patients with osseous metastasis treated with combination chemotherapy. *Cancer* 1986; 58:2589-93.

35- Plunkett TA, Smith P, Rubens RD. Risk of complications from bone metastases in breast cancer. Implications for management; 2000. *Eur J Cancer*; 36:476-82.

36- Coleman RE, Smith P, Rubens RD: Clinical course and prognostic factors following bone recurrence from breast cancer. *Br J Cancer* 1998, 77:336? 340.].

37- Sébastien T1,2, Véronique O4, Brian E H1,2, Matt,hew G A1,2, April AN R1,2, Liliane M4, Euridice C 4, Christine E T 1,2, Anne-M. M -Masson4, 5 and Peter M Siegel1, 2, 3* Tabariès et al. Granulocytic immune infiltrates are essential for the efficient formation of breast cancer liver metastases *Breast Cancer Research* (2015) 17:45 DOI 10.1186/s13058-015-0558-3.

38- Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer*. 2009; 9:239–52.

39- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008; 454:436–44.

- 40- Andre F, Dieci MV, Dubsky P, Sotiriou C, Curigliano G, Denkert C, et al. Molecular pathways: involvement of immune pathways in the therapeutic response and outcome in breast cancer. *Clin Cancer Res*. 2013; 19:28–33.
- 41- de la Cruz-Merino L, Barco-Sanchez A, Henao Carrasco F, Nogales FernandezE, Vallejo Benitez A, Brugal Molina J, et al. New insights into the role of the immune microenvironment in breast carcinoma. *Clin Dev Immunol*. 2013; 2013:785317.
- 42- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008; 454:436–44.
- 43- Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol*. 2011; 29:235–71.
- 44- Sawant A, Ponnazhagan S. Myeloid-derived suppressor cells as osteoclast progenitors: a novel target for controlling osteolytic bone metastasis. *Cancer Res*. 2013; 73:4606–10.
- 45- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008; 454:436–44.
- 46- Robert E. M,* Mary J; Kathy. P, Mark .L, Tom O; Baseline staging tests in primary breast cancer: a practice guideline and the Breast Cancer Disease Site group of the Cancer Care Ontario Practice Guidelines Initiative, *CMAJ* • MAY 15, 2001; 164 (10) 1439.
- 47 Sheri & M. Dowsett* - Developments in Ki67 and other biomarkers for treatment decision making in breast cancer; 2012; Department of Academic Biochemistry, Royal Marsden Hospital, London SW3 6JJ, UK, and *Annals of Oncology* 23 (Supplement 10): x219–x227, 2012doi:10.1093/annonc/mds307
- 48- Udaya. K, Liyanag, Todd. T, Moore, Hong-G. J, Yoshiyuki. T, Virginia. H, Gerard .D, Jeffrey A. Drebin, Steven M. Strasberg, Timothy J. Eberlein, Peter S. Goedegebuure, and David C. Linehan2, Prevalence of Regulatory T Cells Is Increased in Peripheral Blood and Tumor Microenvironment of Patients with Pancreas or Breast Adenocarcinoma1 *The Journal of Immunology*, 2002, 169: 2756–2761.
- 49 -Sonpavde G, Pond GR, Berry. W.R, deWit R, Armstrong AJ, EisenbergerMA, et al. Serum alkaline phosphatase fluctuations strongly associated with prognosis regardless with the changes in PSA levels in cases of castration-resistant prostate cancer and bone Involvement, receiving anticancer drugs. *Urol Oncol*. 2012; 30(5):607-13, [http://dx. doi.org/10.1016/j.urolonc.2010.07.002](http://dx.doi.org/10.1016/j.urolonc.2010.07.002).
- 50- Kanakis I, Nikolaou M, Pectasides D, Kiamouris C, Karamanos NK. Determination and biological relevance of serum cross-linked type I collagen N- telopeptide and bone-specific alkaline phosphatase in breast metastatic cancer. *J Pharm Biomed Anal*. 2004; 34(4):827-32.
- 51- Ogoe A, Hotta T, Kawashima H, Hatano H, Umez H, Inoue Y, et al. Elevation of serum alkaline phosphatase in clear cell chondrosarcoma of bone. *Anticancer Res*. 2001; 21(1B):649-55.
- 52- A. Keshaviah1, S. Dellapasqua2, N. Rotmensz2, J. Lindtner3, D. Crivellari4, J. Collins5,M. Colleoni2, B. Thu“rlimann6,7, C. Mendiola8, S. Aebi7,9, K. N. Price1,10, O. Pagani7,11, E. Simoncini12, M. Castiglione Gertsch13, R. D. Gelber1,10,14, A. S. Coates15 & A. Goldhirsch2,11* CA15-3 and alkaline phosphatase as predictors for breast cancer recurrence: a combined analysis of sevInternational Breast Cancer Study Group trials *Annals of Oncology* 2007, 18: 701–708
- 53- Crivellari D, Price KN, Hagen M et al. Routine tests during follow-up of patients after primary treatment for operable breast cancer. International (Ludwig) Breast Cancer Study Group (IBCSG). *Ann Oncol* 1995; 6 (8): 769–776.
- 54-Ying .J, Mei.Q. Y, Jun.Q. C, Yi-Ping. Z. Zhejiang Cancer Hospital, Department of Medical Oncology, Hangzhou, China.. *Clinics*. January 27, 2015; 70(4):264-272
- .55-Marcela. P. C^{1*}, Paulo. H², Rubens. C¹, Miriam .R.B Poli¹, Paula N.V .B¹ and Almir .G.V B¹ ; Focused abdominal ultrasound in preoperative liver surgery staging: a prospective study; 2012³ Department of Imaging, AC Camargo Cancer Center, Rua Professor Antônio Prudente, 211, São Paulo, Brazil ;² Faculdade de Medicina, Universidade de São Paulo, Rua Eneas de Aguiar Carvalho, 255, São Paulo, Brazil ;*World Journal of Surgical Oncology* ; 11:138 doi:10.1186/1477-7819-11-138
- 57- Luft D, Richter A, Gunther S, et al., *A comparison of bone-related biomarkers and CA27.29 to assess response to treatment of osseous metastatic breast cancer*. *Anticancer Res* 2000;20:5099–105).
- 57-Crivellari D, Price KN, Hagen M et al. Routine tests during follow-up of patients after primary treatment for operable breast cancer. International (Ludwig) Breast Cancer Study Group (IBCSG). *Ann Oncol* 1995; 6 (8): 769–776.
- 58- A. K. Singh • A. Pandey • M. Tewari • R. Kumar • A. Sharma • K. A. Singh •H. P. Pandey • H. S. Shukla; Advanced stage of breast cancer hoist alkaline phosphatase activity: risk factor for females in India Received: 16 October 2012 / Accepted: 26 December 2012 / Published online: 6 January 2013_ The Author(s) 2013.