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Effect of Obesity on Plasma Alkaline Phosphatase Activity in Breast Cancer

Farah Aqeel Rashid*[1], Sarah Mahdi[1],
Shaymaa Abd-alkader Mahdy[2], Ahmed Thamer Salim[3]

Abstract

Background: Breast cancer is most common cancer in women. Obesity is one of related-risk factor in breast cancer. In obese normal subjects, alkaline phosphatase (ALP) has been studied. However, there is no previous study investigate the association between ALP and obesity in breast cancer and its correlation with other clinical characteristics. Therefore, the objective of present study is to investigate the association between ALP and clinical characteristics in generally and obesity in particularly.

Methods: A cross-study 111 new diagnosed breast cancer patients was included. Plasma ALP was measured in different subgroups: patients age <40 vs >40, premenopausal vs postmenopausal, estrogen receptor-positive (ER+) vs estrogen receptor negative (ER-), metastasis vs non-metastasis and obese vs non-obese patients.

Results: Significant increasing on plasma ALP were shown between groups of each age, menopausal status, metastasis, and obesity ($p< 0.05$, $p< 0.05$, $p< 0.01$ and $p< 0.05$) respectively. Positive correlation was observed between plasma ALP and age, menopausal status, metastasis, and obesity (r: 0.616, $p< 0.05$; r: 0.667, $p< 0.01$; r: 0.691, $p< 0.005$; and r: 0.627, $p< 0.01$). Multiple regression analysis was indicated that ALP can be determined by menopausal status, metastasis, and obesity (β-Coefficient = 0.428, $p< 0.01$; β-Coefficient = 0.534; $p< 0.001$; β-coefficient= 0.545; $p= 0.005$), respectively.

Conclusions: Together, the relation between ALP and obesity indicates that ALP could have a role in maturation of preadipocytes of breast cancer patients. Further investigations are needed to confirm that there could be a potential hormonal link between ALP and obesity in breast cancer patients.

Keywords: Alkaline phosphatase, Breast cancer, Metastasis, Obesity, Menopausal status.

Introduction

Breast cancer is one of the most frequent cancers in women. It is well known that breast cancer is second common and second related death in women (1). Globally, the total number of deaths is 535,341. In Iraq, the total number of death due to breast cancer is 2623 in 2016 (2). It was reported that 909 cases were died because of late diagnosis in Iraq (3-5). Therefore, investigation for a cheap and easily detectable biomarker, which can measure in simple laboratory, is an urgent need.

Obesity is one of the most important risk factor of breast cancer (6, 7). It is considered that adipose tissue is an endocrine organ which secretes many factors (8). Alkaline phosphatase (ALP) is an enzyme that hydrolyses phosphate in alkaline medium. ALP isoenzymes are expressed in different organs, such as bone, liver, intestine, bile duct, kidney, germ cell and placenta (9-11). In last decade, it was shown that ALP was expressed by preadipocytes which were isolated from abdominal subcutaneous adipose tissue in human and murine preadipocytes cell line 3T3 L1(12, 13). It was hypothesized that ALP may have a role in maturation of preadipocytes to adipocytes (14-16).

1: Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq.
2: College of Dentistry, Tikrit University, Tikrit, Iraq.
3: Department of Pharmaceutical Chemistry, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.
***Corresponding author: Farah Aqeel Rashid; Tel: +469870748900; E-mail: far@sc.nahrainuniv.edu.iq.**
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Furthermore, the presence of ALP in preadipocytes suggested that adipose tissue is one of the sources of ALP in blood circulation (17). In healthy subjects, it was found that serum ALP level is higher in obese in compared to non-obese (17). In cancer patients, it has been suggested that ALP as a potential tumor marker to predict bone metastasis in bladder (18) and breast cancer (19). However, there is limited clinical evidence about ALP and obesity in breast cancer patients. Therefore, the aim of the present study is to focus on the relation between ALP and clinical characteristics of breast cancer patients generally and ALP with obesity in particularly.

Materials and Methods

Study design

This study was conducted at Al-Amal National Hospital of Cancer Management in period from November 2018 to June 2019. The protocol of study was approved by ethics committee (27280) in the Ministry of Health in Baghdad, Iraq. The consent was taken from each patient before blood collection. A cross-sample, 111 new diagnosed breast cancer patients was included in the study. Patients, who treated with hormonal therapy, chemotherapy, and radiotherapy, were excluded from study. The clinical characteristics, which included age, menopausal status, breast cancer subtypes; estrogen receptor positive (ER+) and estrogen receptor negative (ER-), and metastasis, were informed form pathological report of each patient. BMI was measured by dividing the body weight in kilogram (kg) by body height in meter square (m^2). Patients, who have BMI more than or equal 30 kg/m^2, were categorized as obese and patients, who have BMI less than 30 kg/m^2, were categorized as non-obese.

Sample collection

Five ml of blood was collected from peripheral venous blood in heparin-plasma tube. The blood was centrifuged at 3000 g for 10 min. Plasma blood was stored at -20 ºC. The activity of total ALP was measured by Kinetic method. Twenty microliters of the plasma were incubated with 1000 µl solution which contains 10 mmole/l of p-Nitrophenyl phosphate, 0.25 mole/l of diethanolamine and 0.125 mmole/l of MgCl2 at pH 10.35±0.2. The absorbance of solution was measured after 1, 2, and 3 minutes. ALP activity was calculated by dividing the change in absorbance by time and then multiple by 2757 factor according to kit instruction of HUMAN company.

Statistical analyses

Normality of ALP measurement was tested by Kolmogorov-Smirnov test. ALP activity was expressed by mean±SD. The differences in ALP levels were performed by unpaired two-tailed t-student's test. The Pearson correlation was used for testing the relation between continuous variables and point bi-serial correlation for testing the relation continuous variable (ALP) and between dichotomous variables. The significant difference was considered when p value was less than 0.05. Statistical analyses were performed by IBM SPSS version 23.0 and GraphPad Prism 8.0.2.

Results

In the present study, some clinical features of breast cancer patients were investigated. The percentage of patients in each subclass of age, menopausal status, breast cancer subtypes, metastasis status and BMI are shown in Table 1.

The difference in ALP level between each clinical parameters subtype was tested by student t-test, as it was shown in Table 2. The significant difference ($p< 0.05$) was found in ALP level between breast cancer patients who their ages were less than 40 years old when compared to patients who their ages were more or equal 40 years old. In addition, ALP level was significantly increased in post-menopausal breast cancer when compared to pre-menopausal status ($p< 0.05$). ALP level was slightly higher in ER+ than in ER- breast cancer. Metastasis breast cancer patients have significantly higher ALP level than non-metastasis breast cancer patients ($p< 0.01$). In addition, a significant increase ($p< 0.05$) in ALP level was found in obese patients compared to non-obese patients.

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Table 1. Distribution of patients in some clinical characteristics.

Characteristics		Patient number n (%)
Age (year)	<40	36 (32.4)
	≥40	75 (67.6)
Menopausal status	Pre-menopausal	29 (26)
	Post-menopausal	82 (74)
Breast cancer subtype	ER-	60 (54.1)
	ER+	51 (45.9)
Metastases	Absent	57 (51.4)
	Present	54 (48.6)
BMI (kg/m^2)	Non-obese	60 (64)
	Obese	51 (46)
ALP (U/l)	<500	49 (44.1)
	≥500	62 (55.9)

Table 2. Association between ALP activity and some clinical characteristics of patients.

Clinical Characteristics	ALP activity (U/L)	p-value
Age (year)		0.0179
<40	421.39±188.75	
≥40	707.63±235.485	
Menopausal status		0.0373
Pre-menopausal	363.08±225.03	
Post-menopausal	577.86±150.68	
Breast cancer subtype		0.0621
ER-	382.57±181.96	
ER+	634.47±268.10	
Metastases		
Absent	419.97±194.58	0.0066
Present	688.35±91.734	
BMI		0.0199
Non-obese	422.86 ±215.39	
Obese	705.17±194.28	

To study the relation between ALP and some clinical characteristics of breast cancer patients, a Pearson correlation was performed. As it was shown in (Table 3, Fig. 1), positive correlations were found between ALP and age, menopausal status, metastasis, and BMI (r: 0.616, p< 0.05; r: 0.667, p< 0.01; r: 0.691, p< 0.005 and r: 0.627, p< 0.01), respectively. Interestingly, there is a slightly association between ALP level and breast cancer subtypes (presence and absence of ER) (r: 0.439, p> 0.05).

Table 3. Correlation between ALP and some clinical characteristics of patients.

Variables	r-value	p-value
Age (year)	0.616	0.011
Menopausal status	0.667	0.005
Breast cancer subtypes	0.439	0.089
Metastasis	0.691	0.003
BMI (kg/m^2)	0.627	0.009

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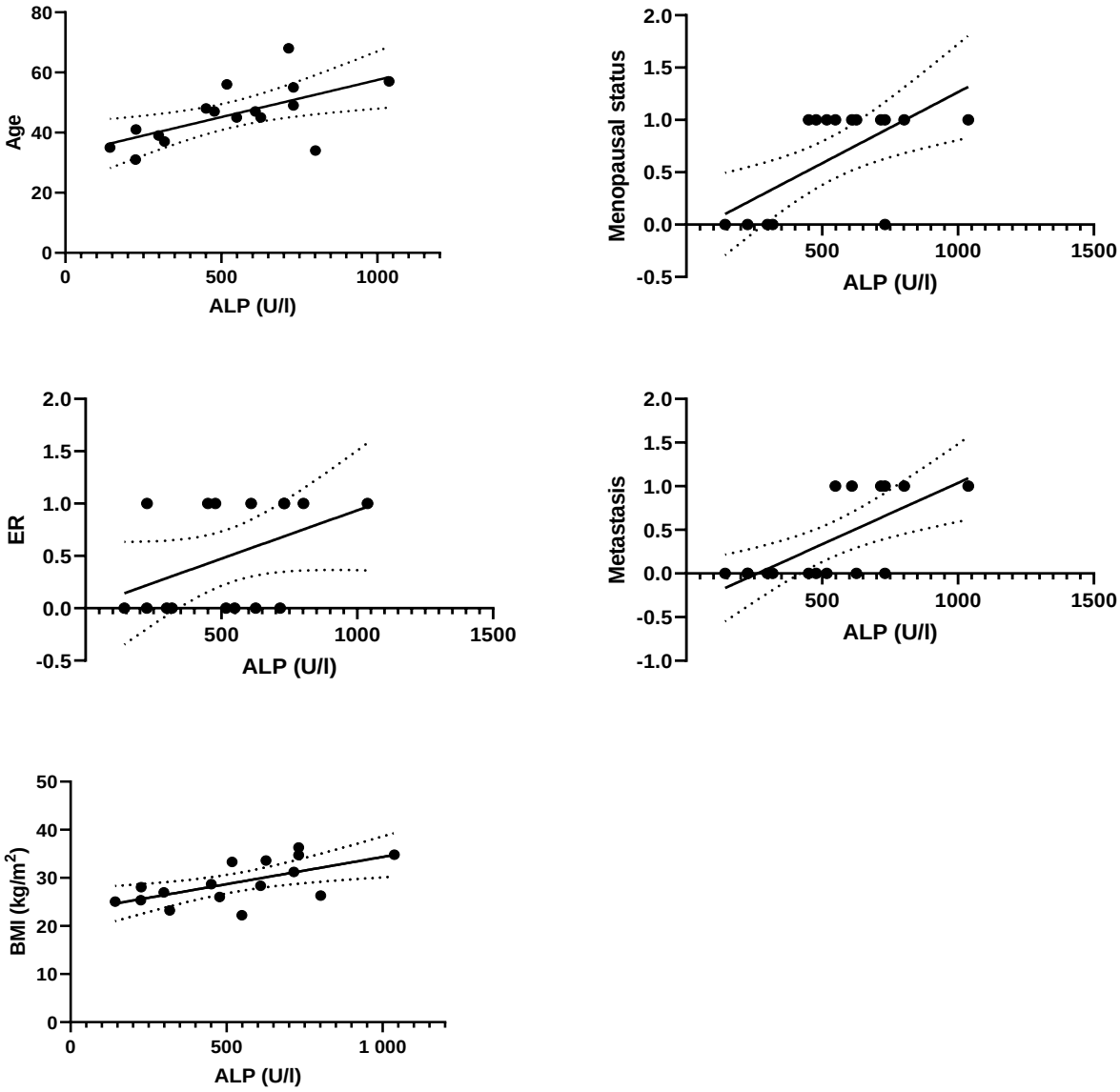

Fig. 1. Association between ALP and some clinical characteristic.

Table 4. Multiple linear regression analysis.

Dependent variable	Independent variable	β-Coefficient	p-value	Adjusted R^2 (p)
ALP	Age	-0.194	0.287	0.842 (0.000)
	Menopausal status	0.428	0.006	
	Breast cancer subtypes	0.042	0.728	
	Metastasis	0.534	0.001	
	BMI	0.545	0.005	

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Discussion

There are substantial evidences suggest that ALP is potential tumour marker in bladder and breast cancer (18, 19). However, there are limited studies investigate the relation between ALP and obesity in breast cancer patients.
Therefore, the present study aims to investigate the relation between ALP and clinical characteristics of breast cancer patients generally and between ALP and obesity in particularly.

Our results were found significant difference in ALP level between older breast cancer patients when compared to young patients. In addition, ALP level was significantly increased in post-menopausal breast cancer when compared to pre-menopausal status. This result is in consistent with previous studies which were done in normal subjects (17, 20, 21). The explanation of this could be because bone damage in postmenopausal women lead to increase ALP level (17, 21). Interestingly, there is slightly increasing difference between ALP levels in ER+ in comparison with ER- breast cancer patients. This data suggests that there might be a potential hormonal effect on ALP activity. Furthermore, it was shown that metastasis breast cancer patients have significantly higher ALP level than non-metastasis breast cancer patients. This data agrees with previous studies (19, 22) which suggested that measurement of ALP is an accurate and reliable marker.

Previous studies, which investigated ALP level in normal subjects, found that ALP level in obese is higher than in non-obese (17, 20). However, there are other studies reported that body weight has no effect on ALP level in normal subject (23, 24). This is the first study that investigates the effect of obesity on ALP level in breast cancer patients. The current study shows a significant increase in ALP level in obese patients compared to non-obese patients. This could be supporting the hypothesis that ALP has a potential role in maturation of preadipocytes (17, 25).

Furthermore, a positive correlation was found between ALP and age, and menopausal status These results confirmed the previous studies (19, 24). Moreover, a positive correlation between ALP and menopausal status was confirmed by multiple linear regression. The explanation of these results could be due to increasing age and after menopausal the bone damage increases leading to elevate ALP level (24).

Our study found a positive significant association between ALP level and metastasis. Furthermore, metastasis is mainly an independent variable that can be determined by ALP. This result could be explained that cancer cells may spread and settle in bone, liver, intestine, and brain in which ALP isoenzyme is expressed and led to elevate total ALP level.

Interestingly, there is a marginal association between ALP level and breast cancer subtypes. Furthermore, a positive correlation was shown between ALP level and obesity. These results suggest that an estrogenic hormonal effect could be link between ALP and both ER+ breast cancer and obesity. In addition, multiple regression analysis showed that ALP can be determined by BMI. The possibly explanation of these findings could be because adipose tissue is considered the main source of estrogen especially after menopausal (26). Furthermore, it was found previously that there is a potential regulation effect of estrogen on ALP expression in bone marrow stromal cell line (27). Further investigations are needed to confirm the relation between ALP and ER+ and between ALP and obesity.

The limitations in this study are firstly; there is high percent of postmenopausal breast cancer patients in compare to premenopausal patients. Secondly, it lacks the measurement of other ALP isoenzymes. Thirdly, it lacks study the estrogen level. Finally, the number of patients is small. Nevertheless, the current study highlights promising results which could help further in establish cheap and easily measurable biomarker for early detection obese breast cancer patients.

The activity of ALP increases with older, in postmenopausal patients and in metastasis breast cancer patients. Interestingly, the observed positive association between ALP and

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obesity indicates that ALP could have a role in maturation of preadipocytes of breast cancer patients. Further investigations are needed to confirm that there could be a potential hormonal link between ALP and obesity.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2. Hashim MJ, Fatima AA-S, Noura AA-M, Sarah SA-Q, Ali HM, Gulfaraz K. Burden of Breast Cancer in the Arab World: Findings from Global Burden of Disease, 2016. J Epidemiol Glob Health. 2018;8(1-2):54-58.
3. Kulhánová I, Bray F, Fadhil I, Al-Zahrani AS, El-Basmy A, Anwar WA, et al. Profile of cancer in the Eastern Mediterranean region: The need for action. Cancer Epidemiol. 2017;47:125-132.
4. Annual Statistical Report 2016. Planning Directorate, Ministry of Health /Environment, Republic of Iraq, 2017.
5. Annual Report of Cancer Diseases, Iraqi Cancer Registry 2013. Republic of Iraq: Iraqi Cancer Board, Ministry of Health, Iraq, 2017.
6. Munsell MF, Sprague BL, Berry DA, Chisholm G, Trentham-Dietz A. Body mass index and breast cancer risk according to postmenopausal estrogen-progestin use and hormone receptor status. Epidemiol Rev. 2014;36(1):114-36.
7. Gravena AAF, Romeiro Lopes TC, Demitto MdO, Borghesan DHP, Dell' Agnolo CM, Brischiliari SCR, et al. The Obesity and the Risk of Breast Cancer among Pre and Postmenopausal Women. Asian Pac J Cancer Prev. 2018;19(9):2429-2436.
8. Kershaw EE, Flier JS. Adipose Tissue as an Endocrine Organ. J Clin Endocrinol Metab. 2004;89(6):2548-56.
9. Weiss MJ, Henthorn PS, Lafferty MA, Slaughter C, Raducha M, Harris H. Isolation and characterization of a cDNA encoding a human liver/bone/kidney-type alkaline phosphatase. Proc Natl Acad Sci U S A. 1986;83(19):7182-7186.

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10. Berger J, Garattini E, Hua JC, Udenfriend S. Cloning and sequencing of human intestinal alkaline phosphatase cDNA. Proc Natl Acad Sci U S A. 1987;84(3):695-698.
11. Millán JL. Oncodevelopmental expression and structure of alkaline phosphatase genes. Anticancer Res. 1988;8(5a):995-1004.
12. Ali AT, Penny CB, Paiker JE, Psaras G, Ikram F, Crowther NJ. The effect of alkaline phosphatase inhibitors on intracellular lipid accumulation in preadipocytes isolated from human mammary tissue. Ann Clin Biochem. 2006;43(Pt 3):207-13.
13. Ali AT, Penny CB, Paiker JE, van Niekerk C, Smit A, Ferris WF, et al. Alkaline phosphatase is involved in the control of adipogenesis in the murine preadipocyte cell line, 3T3-L1. Clin Chim Acta. 2005;354(1-2):101-9.
14. Ali AT, Ferris WF, Penny CB, Van der Merwe MT, Jacobson BF, Paiker JE, et al. Lipid accumulation and alkaline phosphatase activity in human preadipocytes isolated from different body fat depots. Journal of Endocrinology, Metabolism and Diabetes of South Africa. 2013;18(1):58-64.
15. Ahmadiankia N, Bagheri M, Fazli M. Gene Expression Changes in Pomegranate Peel Extract-Treated Triple-Negative Breast Cancer Cells. Rep Biochem Mol Biol. 2018;7(1):102-109.
16. Raghunathachar Sahana K, Akila P, Prashant V, Sharath Chandra B, Nataraj Suma M. Quantitation of Vascular Endothelial Growth Factor and Interleukin-6 in Different Stages of Breast Cancer. Rep Biochem Mol Biol. 2017;6(1):33-39.
17. Ali AT, Paiker JE, Crowther NJ. The Relationship Between Anthropometry and Serum Concentrations of Alkaline Phosphatase

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Isoenzymes, Liver Enzymes, Albumin, and Bilirubin. Am J Clin Pathol. 2006;126(3):437-42.
18. Huang P, Lan M, Peng AF, Yu QF, Chen WZ, Liu ZL, et al. Serum calcium, alkaline phosphotase and hemoglobin as risk factors for bone metastases in bladder cancer. PLoS One. 2017;12(9):e0183835.
19. Yazdani A, Dorri S, Atashi A, Shirafkan H, Zabolinezhad H. Bone Metastasis Prognostic Factors in Breast Cancer. Breast Cancer (Auckl). 2019;13:1178223419830978.
20. Schiele F, Henny J, Hitz J, Petitclerc C, Gueguen R, Siest G. Total bone and liver alkaline phosphatases in plasma: biological variations and reference limits. Clin Chem. 1983;29(4):634-41.
21. Crilly RG, Jones MM, Horsman A, Nordin BE. Rise in plasma alkaline phosphatase at the menopause. Clin Sci (Lond). 1980;58(4):341-2.
22. Chen WZ, Shen JF, Zhou Y, Chen XY, Liu JM, Liu ZL. Clinical characteristics and risk factors for developing bone metastases in patients with breast cancer. Sci Rep. 2017;7(1):11325.
23. Munan L, Kelly A, PetitClerc C, Billon B. Associations with body weight of selected chemical constituents in blood: epidemiologic data. Clin Chem. 1978;24(5):772-7.
24. Pardhe BD, Pathak S, Bhetwal A, Ghimire S, Shakya S, Khanal PR, et al. Effect of age and estrogen on biochemical markers of bone turnover in postmenopausal women: a population-based study from Nepal. Int J Womens Health. 2017;9:781-788.
25. Rashid FA. Alhashemi WKH, Al-Saffar AT, Salman EA, Najeeb DA. Obesity and breast cancer risk in different menopausal status: a review. Biochem Cell Arch. 2020;20(1):1875-1878.
26. Fortner RT, Katzke V, Kühn T, Kaaks R. Obesity and Breast Cancer. Recent Results Cancer Res. 2016; 208:43-65.
27. Holzer G, Einhorn TA, Majeska RJ. Estrogen regulation of growth and alkaline phosphatase expression by cultured human bone marrow stromal cells. J Orthop Res. 2002;20(2):281-8.

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