Relation of Serum Level of Lipocalin 2 to Her2/neu in Women with Breast Cancer

Atheer R. Atia[1], Salam S. Ahmed[2]

[1]Asst. Lec. Kirkuk General Hospital, Kirkuk Governorate/Iraq, [2]Prof Dr. Professor of Clinical & Medical Biochemistry–A General Pediatrician, College of Medicine–University of Tikrit/Iraq

Abstract

Background: Breast cancer is the most common type of cancer worldwide, being one of the leading causes morbidity in the female.(1) Lipocalin is a neutrophil gelatinase-associated lipocalin (NGAL) or lipocalin 2 that is a 25 KDa glycoprotein, which was originally identified as a covalent complex with matrix metalloproteinase-9 (MMP-9) in human neutrophil.(2) Lipocalin acts as bone-derived hormone which crosses the blood brain barrier (BBB) and acts on the hypothalamus in the brain. The human epidermal growth factor receptor 2 (HER-2/neu), has shown to have a prognostic value for the treatment with the monoclonal antibody in breast cancer.(3)

Material and Method: This is a cross sectional, hospital based study. This study was carried out at the Oncology Center in Kirkuk City-Iraq from the 1st of November 2018 to the end of June 2019. Forty-two women with breast cancer were considered as a study group. Forty-four apparently healthy women without breast cancer (relative of patients) and with a negative family history for the first and second degree relatives of breast cancer were consider as a control group, their ages were from 23 to 70 year. By using a sterile disposable syringe 10 mls of venous blood sample was drawn from each woman at morning and was kept in a plain tube and allowed to clot at room temperature, then each sample was centrifuged at 6000 rpm to obtain serum. Serum of the patients and controls had assay for lipocalin 2 and Her2/neu by ELISA.

Results: The mean serum level of lipocalin 2 was significantly elevated in breast cancer women compared to control women (62.77 ± 6.1 and 23.98 ± 5.9 ng/l) respectively at a P value of 0.001. This study also reveals that the mean serum level of Her2/neu was higher in breast cancer women (33.96 ± 2.7 ng/ml) as compared with the control group (9.76 ± 1.6 ng/ml). This result was highly significant at a P value of 0.001. This study found that there was a moderate positive correlation between lipocalin with Her2/neu in breast cancer women (R: 0.33), which means that lipocalin 2 was proportionally elevated with increasing of Her2/neu level.

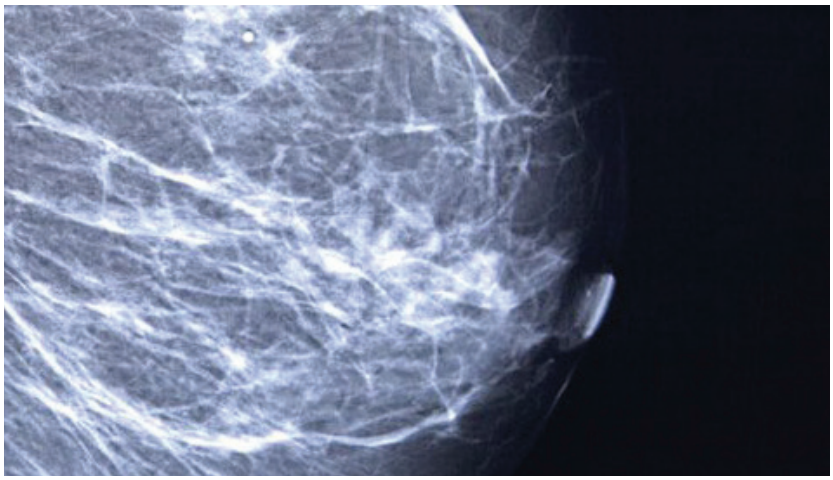
Conclusion: This study reveals that there was a significant positive correlation between lipocalin and Her2/neu in breast cancer women, the r value was 0.33.

Keywards: *Breast cancer, lipocalin 2, and Her2/neu.*

Introduction

Breast cancer is the most dangerous disease that threats women lives in Iraq for the last twenty years ago. Every year 1000-1200 new cases are recorded, 98% of which affect women, and 2% of which affect men. Breast cancer occupies 14% of the whole disease in different cancers and a rate of 1-6 of women are affected from every 100000 women.(4) It is important to distinguish the biochemical profiles of cells that have been transformed to malignant cells. Generally these cells, are different from that of normal, tend to concentrate almost upon growth by maximizing the anabolic processes and put down the catabolic functions.(5)

Corresponding Author:
Atheer R. Atia
Asst. Lec. Kirkuk General Hospital, Kirkuk Governorate/Iraq
e-mail: mohammedtalal8883@gmail.com



Figure(1): Breast cancer photo of mammography.[6]

Lipocalin (LCN2), a member of the lipocalin protein family, is a secreted as a glycoprotein that transports small lipophilic ligands. The protein part of lipocalin are part of a group of more than 20 diverse proteins that exhibit limited amino acid sequence similarity but share a highly conserved 3D- structure. LCN2 is a prominent member of the lipocalin super family and was originally identified as a 25-kDa neutrophil glycoprotein based on its covalent binding to matrix metalloproteinase-9 (MMP-9) in human neutrophils.[7,8] LCN2 has gained attention as a potential biomarker and a modulator of human cancer. LCN2 protein expression levels have been demonstrated to be increased in various human epithelial cancer types, including breast cancer.[10,11]

The human epidermal growth factor receptor 2, is a transmembrane tyrosine kinase receptor. This protein is encoded by the HER2gene, which is located on the long arm of chromosome 17 (17q12–21.32). Clinically, HER2-positive breast cancer occurs in 15–20% of breast cancer patients and is characterized by the over expression of the HER2 receptor and/or *HER2* gene amplification.[12] HER2-positive breast cancer patients have a particular worse prognosis.[9]

Patients Materials and Method:

A cross sectional, hospital based study. The protocol of this study was approved by the scientific committee of Tikrit University-College of Medicine, and the agreement of the attendance to Kirkuk Oncology Center to collect the sample from the patients was approved by the Kirkuk Health Directorate. This study was carried out at the Oncology Center in Kirkuk City- Iraq from the 1st of November 2018 to the end of June 2019. A verbal consent was taken from each women included in this study whether considered as a case or control. Forty-two women with breast cancer were considered as a study group, their ages were between 23 to 70 years, and they were from the center and periphery of Kirkuk City, while 44 apparently healthy women without breast cancer and with negative family history for the first and second degree relatives of breast cancer were consider as a control group, their ages were from 23 to 70 year.

By using a sterile disposable syringe 10 mls of venous blood sample was drawn from each woman included in this study at the morning and was kept in a plain tube and allowed to clot at room temperature, then each sample was centrifuged at 6000 rpm to obtain serum. The serum was aspirated then divided into aliquots in plastic tubes and stored at -20^{0}C until the time of estimation. Serum of the patients and controls had assay:

1. Lipocalin 2 by ELISA.
2. Her2/neu by ELISA.

Statistical Analysis: All the date collected in this study were analyzed by using the student t-test, the mean, standard deviation, and P-value was also considered. The significance was considered at a P value of less than 0.05. While the correlation was considered as follow:

Interpretation of R value (correlation coefficient)

- 0.70. A strong negative correlation.

- 0.50. A moderate negative correlation.

- 0.30. A weak negative correlation.

- 0.3<R <0.3: no correlation.

+ 0.30. A weak positive correlation.

+ 0.50. A moderate positive correlation.

+ 0.70. A strong positive correlation.

Results

The total number of a subject that participate are 88 (42 patient and 44 control). The highest rate of breast cancer women (42.86%) was within the age group 41 - 50 years, followed by those within the age group of 51 - 60 years (21.44%). The least rate of breast cancer women (9.52%) was within the age group of more than 60 years, as see in the Figure 2.

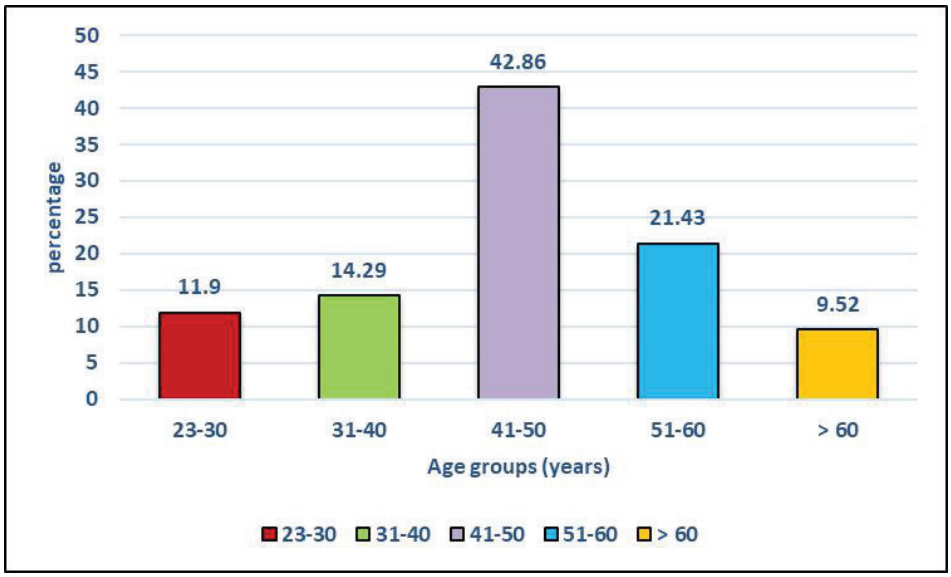


Figure (2): Relation of breast cancer with age.

This study show that the mean of BMI was recorded in breast cancer women when compared with healthy women was 28.5±2.19 *versus* 24.12±0.99 Kg/m^2 at a P value P: 0.01, as shown in the following figure 3

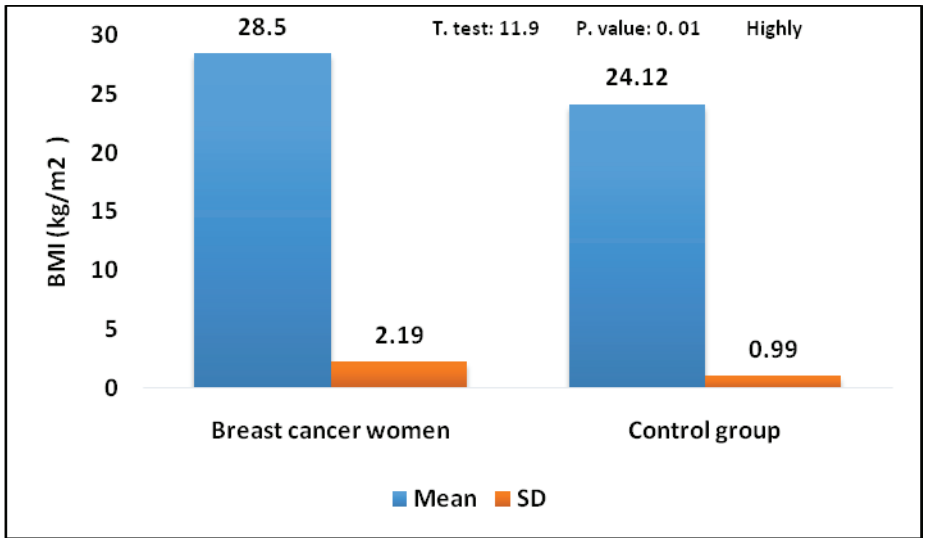


Figure 3: Relation of BMI to breast cancer

The mean serum level of lipocalin 2 was significantly elevated in breast cancer women compared to control women (62.77 ± 6.1 and 23.98 ± 5.9 ng/l) respectively at a P value of 0.001.

Table (1): The mean and standard deviation (SD) of lipocalin 2 level in breast cancer women and the control group.

Lipocalin 2 level (ng/L)	Breast cancer women	Control group
No.	42	44
Mean	62.77	23.98
SD.	6.1	5.9
T. test: 29.4 P. value: 0. 001 Highly Significant (HS)		

This study also shows that the mean serum level of Her2/neu was higher in breast cancer women (33.96 ± 2.7 ng/ml) as compared with the control group (9.76 ± 1.6 ng/ml). This result was highly significant at a P value of 0.001, see the table 2.

Table (2): Determination of Her2/neu level in breast cancer women and thecontrol group.

Her2/neu level (ng/ml)	Breast cancer women	Control group
No.	41	44
Mean	33.96	9.76
SD.	2.7	1.6
T. test: 35.15 P. value: 0. 001 Highly Significant		

This study found that there was a moderate positive correlation between lipocalin 2 with Her2/neu in breast cancer women (r: 0.33), which means that lipocalin 2 was proportionally elevated with increasing of Her2/neu level, see the figure 4.

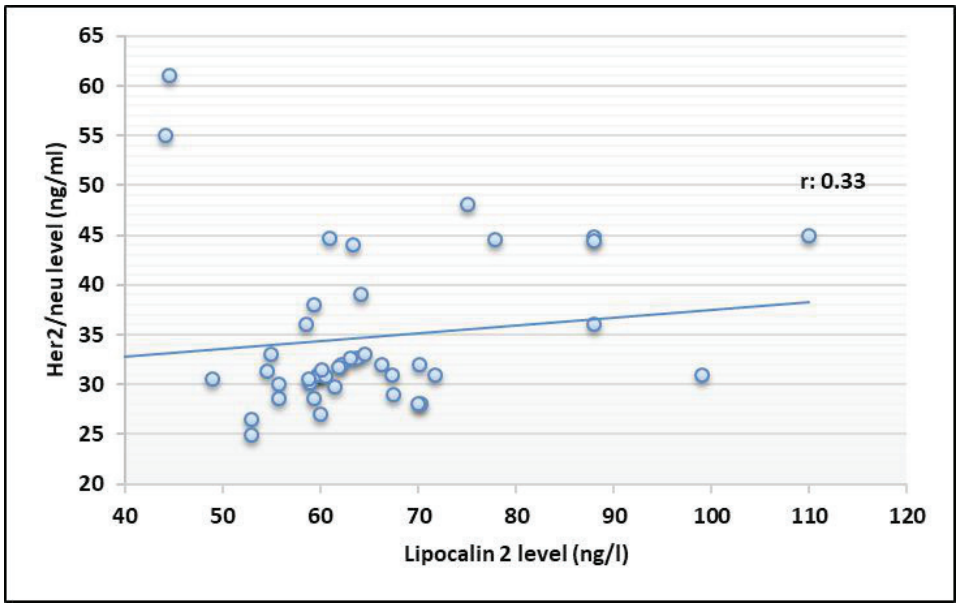


Figure (4): Correlation of lipocalin 2 with Her2/neu in breast cancer women.

Discussion

This study reveals that, he highest rate of breast cancer in women was within the age group 43 - 52 years and the least was within the age group of more than 63 years. Numerous results obtained by other studies were focused on the age of women with breast cancer, e.g. the study that carried by Ghanim H *et al*[13], who found that the mean age of breast cancer women was 42.2 ± 10.41 years, while the study by Armstrong K[14] who found that the mean age was 50.4± 12.45 years with range of 22 to 80 years.

The study showed that 76.19% of breast cancer women in this study were belonged to urban area comparing with 23.81% were from rural area, this finding could be attributed to that the majority of cases included in this study were from the urban area. Previous study found that malignant lesions of breast cancer were more common in rural areas as compared to urban.[15] This could be due to poor medical aid in rural areas, lower socioeconomic status, and illiteracy.[16] These studies not agree with the present study, this disagreement may be related to a non-homogenous samples.

Body mass index is known to be a modify risk factor for breast cancer. [17] In current study malignant case with BMI > 30.0 kg/m^2 represent 50% of malignant tumor that agree with other studies.[18,19] Epidemiological studies have shown that postmenopausal obesity causes an increase in the risk for breast cancer. [20] Since obesity has a close relationship with increased levels of insulin and insulin like growth factors, although increased BMI, elevated blood glucose, dyslipidemia and hypertension are components of metabolic syndrome. Insulin resistance reduces sex hormone-binding globulin levels, causing an increase in free estrogen and androgen levels, hence increasing proliferation of breast epithelial cells.[21]

In the present study the mean serum level of lipocalin 2 for patients with malignant tumor was higher than the control at a P value of 0.001. LCN2 has gained attention as a potential biomarker and a modulator of human cancer, its protein expression levels have been demonstrated to be increased in various human epithelial cancer types, including breast cancer.[22,23] The finding of Hu *et al* [7] were in agreement with finding of this study, who found that there was a significant relation between LCN2 and breast cancer and provided strong evidence for the role of LCN2 in aggressive subtypes of breast cancer. Additionally, Yang *et al* [24] reported that lipocalin 2 promoted breast cancer progression, and found that LCN2 level was consistently associated with invasive breast cancer in human tissue. Ören*et al*[25] also demonstrated that LCN2 was highly related to breast cancer as compared with healthy women. Bratt[26] reported that level of LCN2 also significantly higher in breast cancer women than normal breast stroma.

The present study showed that the mean serum level of Her2/neu was higher in breast cancer women compared to the control women. Previousstudywasperformed on 64 women with breast cancer; 25 (39.1%) had elevated serum HER2/neu levels accompanied with increased tissue expression of HER2/neu receptors,[27]their results was in a greement with the present study. These results suggest that the elevated in serum Her2/neu level was associated with a clinicopathological aggressive phenotype of breast carcinoma and was related to tissue Her2/neu overexpression. Therefore, serum Her2/neu may be useful for monitoring the course of the disease and response to treatment.[28] Other studies found that, patients with distant metastases (liver, lung and ovary) had markedly elevated serum HER2/neu levels as compared to those without metastases.[29,30]

The positive correlation between lipocalin 2 and Her2/neu, this finding could be attributed to that both of them were elevated in the sera of women with breast cancer.

Conclusion

There were an increase in serum levels of lipocalin 2 and Her2/neu in women with breast cancer.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

Funding: Self-funding

References

1. Miller B. Don't Lose Your Breast To Cancer: What Every Healthy Woman Must Know & Do To Keep Breast Cancer At Bay. Oak Publication SdnBhd; 2016.
2. Nair A, Ingram N, Verghese ET, Wijetunga I, Markham AF, Wyatt J, Prasad KR, Coletta PL. Neutrophil Gelatinase-associated Lipocalin as a Theragnostic Marker in Perihilar Cholangiocarcinoma. Anticancer research. 2018 Dec 1;38(12):6737-44.
3. Park BW, Rushmore TH, Morton MR,et al. Preoperative serum RNAs levels as predictor for breast cancer outcomes. Annals of oncology. 2016;19(4): 675-681.
4. Iraqi cancer board. Results of Iraqi cancer registry 1995-1997. Ministry of Health 1997. Baghdad.
5. Seth LR, Kharb S, Kharb DP. Serum biochemical markers in carcinoma breast. Indian J Med Sci. 2003,57(8):350-4.

6. Schroeder SM. Photography: The Silent Savior of Breast Cancer Patients. Scholarly Horizons: University of Minnesota, Morris Undergraduate Journal. 2015;2(1):5.
7. Hu C, Yang K, Li M, Huang W, Zhang F, Wang H. Lipocalin 2: a potential therapeutic target for breast cancer metastasis. OncoTargets and therapy. 2018;11:8099.
8. Wenners AS, Mehta K, Loibl S, et al. Neutrophil gelatinase-associated lipocalin (NGAL) predicts response to neoadjuvant chemotherapy and clinical outcome in primary human breast cancer. PLoS One. 2012;7(10):e45826.
9. Provatopoulou X, Gounaris A, Kalogera E, et al. Circulating levels of matrix metalloproteinase-9 (MMP-9), neutrophil gelatinase-associated lipocalin (NGAL) and their complex MMP-9/ NGAL in breast cancer disease. BMC Cancer. 2009;9:390.
10. Yang J, Moses MA. Lipocalin 2: a multifaceted modulator of human cancer. Cell Cycle. 2009;8(15):2347-2352.
11. Shi H, Gu Y, Yang J, Xu L, Mi W, Yu W. Lipocalin 2 promotes lung metastasis of murine breast cancer cells. J Exp Clin Cancer Res. 2008;27:83.
12. Segerstolpe A, Lundkvist P, Osheim YN, et al. Mrd1p binds to pre-rRNA early during transcription independent of U3 snoRNA and is required for compaction of the prerRNA into small subunit processomes. Nucl Acids Res.2008; 36: 4364–80.
13. Ghanim H,Mishra S,Sharma D and Sharma P. Studies of biochemical parameters in breast cancer with and without metastasis. Indian Journal of Clinical Biochemistry, 2004, 19 (1) 71-75.
14. Armstrong K, Eisen A & Weber B. Primary care: Assessing the risk of breast cancer. The New England Journal of Medicine.2016; 342(8), 564-571.
15. Shrivastava, V, Pratibha Meena, Radhika Nandwani. A study of Serum levels of LDH and ALP in carcinoma breast: An Observational study". Journal of Dental and Medical Sciences. 2016; 15(9): 26-31.
16. Amritpal Kaur, Mridula Mahajan and Sudhir Khichy. Biomarkers of carcinoma breast in females. International Journal of Recent Scientific Research. 2015,6(2) 2625-2629
17. Seth RK, Kharb S, Kharb DP. Serum biochemical markers in carcinoma breast. Ind J Med Sci. 2003:57;350-4.
18. Platet N, Cathiard A, Gleizes M and Garcia M. Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. Critical rev. in Onco./Heamato. 2014. 51:55-67.
19. Rebbeck R, Allredl C, Mohsin C and Zhang O. Modification of BRCA1- and BRCA2-associated breast cancer risk by AIB1 genotype and reproductive history. Cancer. Res. 2015,61: 5420-5424.
20. Signletary KW and GapsturSMA.lcohol and breast cancer: review ofepidemiologic and experimental evidence and potential mechanisms. JAMA. 2011,286: 2143-2151.
21. Jiralerspong S, Kim ES, Dong W et al. Obesity, diabetes, and survival outcomes in a large cohort of early-stage breast cancer patients. Ann Oncol 2013; 24:2506–2514.
22. Lee MM, Lin SS. Dietary fat and breast cancer. Annu Rev Nutr,2000; 20, 221–8.
23. Shaukat N, Jaleel F, Moosa FA, et al. Association between Vitamin D deficiency and breast cancer. Pak J Med Sci,2017 33, 645–9.
24. Yang J, Bielenberg DR, Rodig SJ, Doiron R, Clifton MC, Kung AL, Strong RK, Zurakowski D, Moses MA. Lipocalin 2 promotes breast cancer progression. Proceedings of the National Academy of Sciences. 2009 Mar 10;106(10):3913-8.
25. Ören B, Urosevic J, Mertens C, Mora J, Guiu M, Gomis RR, Weigert A, Schmid T, Grein S, Brüne B, Jung M. Tumour stroma-derived lipocalin-2 promotes breast cancer metastasis. The Journal of pathology. 2016 Jul;239(3):274-85.
26. Bratt T. Lipocalins and cancer. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology. 2000 Oct 18;1482(1-2):318-26.
27. Shukla., S., Bhawana Komal Singh1, Om Prakash Pathania. Evaluation of HER2/neu oncoprotein in serum & tissue samples of women with breast cancer. Indian J Med Res. 2016; 143 (Supplement): 52-58.
28. Sorensen PD, Jakobsen EH, Langkjer ST, Bokmand S, Olsen DA, Madsen JS, et al. Serum HER-2

concentrations for monitoring women with breast cancer in a routine Oncology setting. Clin Chem Lab Med 2009; 47 : 1117-23.

29. Lee SB, Lee JW, Yu JH, Ko BS, Kim HJ, Son BH, et al. Preoperative serum HER 2 extracellular domain levels in primary invasive breast cancer. BMC Cancer 2014; 14 : 929.
30. Tchou J, Lam L, Li YR, Edwards C, Ky B, Zhang H. Monitoring serum HER 2 levels in breast cancer patients. Springer Plus 2015; 4 : 237.