Journal of Population Therapeutics & Clinical Pharmacology

Assessment Of Serum Levels of Zeb2 and Fat10 and Tumor Necrosis Factor-Alpha as Potential Novel Biomarkers in Breast Cancer Patients

Abdalnaser K. A. Al-Zahrawi[1*], Firas Faris Rija[2]

[1,2] Department of Biology, college of science, University of Tikrit, Tikrit, Iraq

*Corresponding author: Abdalnaser K. A. Al-Zahrawi, Department of Biology, college of science, University of Tikrit, Tikrit, Iraq, Email: abdalnaser.k.ahmed.bio2022230@st.tu.edu.iq

ABSTRACT

Background: Breast cancer is the primary and most frequent type of cancer that kills women, ranking fifth among all malignancies that result in fatalities globally and second behind lung cancer in terms of prevalence.

Objective: In this study, zeb 2 and fat10 proteins and pro-inflammatory cytokines (TNF-α) were evaluated in Iraqi women with breast cancer.

Patients and methods: In this study, serum concentrations of zeb 2 ,fat 10 and TNF-α were determined using samples from 90 Iraqi women (60 patients and 30 healthy volunteers) Among them are healthy and patients, premenopausal and postmenopausal woman **.**

Results: According to the findings of the current study, FAT10 and TNF- α were both considerably elevated in breast cancer patients ($P < 0.05$), and they also had a tight relationship, and ZEB2 was also high on the probability level ($P < 0.01$).

Conclusions: This study discovered a link between elevated serum levels of pro-inflammatory cytokines TNF-α, ZEB2, and FAT10.

Keywords: *Five Minute Preceptor, critical thinking, nursing profession students*

INTRODUCTION

Breast cancer is the first and most common cancer that causes death in women and the fifth among the types of cancers that cause death around the world, and the second most common around the world after lung cancer, and despite the development of detection and treatment methods for breast cancer, which led to a decrease in the death rate in developed countries, There is an increase in the incidence of the disease and the resulting deaths in middle and low-income countries, and in Iraq, breast cancer represents one-third of cancer cases in women (1,2). The American Cancer Society's estimates for breast cancer in the United States for 2022

that Breast cancer mainly occurs in middle-aged and older women. the incidence of breast cancer rises after age 40. A very small number of women diagnosed with breast cancer are younger than 45 (3).Men rarely get breast cancer, but it mostly affects women and it is believed that the reason is due to the large tissue mass in women compared to men(4). The breast tissue in women consists of the lobes that produce milk and the ducts that transport it, fatty tissue and the connective tissue surrounding the ducts, lobes, blood vessels and lymphatic vessels (5).Many cells increase their proteolytic activity in response to inflammatory cytokines, such as tumor necrosis factor α (TNF-α), which is

frequently upregulated in human epithelial malignancies as breast cancer.

TNF-α exerts diverse functions in the biology of cancer. In addition to causing cell death, it can stimulate cancer cell survival and proliferation as well as promote angiogenesis, tumor cell migration and invasion (6).

In other side ,Emerging data indicate that ZEB2 plays a pivotal role in EMT-induced processes such as development, differentiation, and malignant mechanisms, for example, drug resistance, cancer stem cell-like traits, apoptosis, survival, cell cycle arrest, tumor recurrence, and metastasis. Zinc finger E-box binding homeobox 2 (ZEB2) is a DNA-binding transcription factor, which is mainly involved in epithelial-to-mesenchymal transition (EMT) a pathological process leading to specific morphological and phenotypic alterations during cancer metastasis in tumor cells(7) .

FAT10,is an ubiquitin-like protein , is involved in several biological activities, Multivariate analyses also revealed that FAT10 overexpression was independent prognostic factors for poor outcome of patients with breast cancer. FAT10 plays a crucial oncogenic role in Breast Cancer metastasis, According to some studies, that FAT10 knockdown significantly inhibited the metastasis abilities and the epithelial-mesenchymal transition (EMT) of breast cancer cell. Further investigation revealed that FAT10 directly bound ZEB2 and decreased its ubiquitination to enhance the protein stability of ZEB2 in breast cancer cells.(8). In this study, we review recent findings about the biological properties of ZEB2 , fat 10, protien, TNF-alph and their relationships with each other .

SUBJECTS AND METHODS

Ninety women were involved in the study, 60 of them were breast cancer patients who visited Al-Amal National Hospital for Cancer Treatment in Baghdad, and the remaining 30 were female volunteers who seemed to be in good health and They were divided into two groups, the premenopausal group and the postmenopausal group.

Ethical consent

Written informed consent was obtained from each patient to participate in the current study. The Central Scientific Research Ethics Committee at Tikrit University approved this research.

Inclusion criteria

Patients with malignant or invasive breast tumors who were newly diagnosed with breast cancer, prior to partial mastectomy or mastectomy and who did not undergo any type of cancer treatment such as chemotherapy or radiotherapy before and after menopause, And patients after radiation therapy before and after menopause And control womans before and after menopause and patients who agreed to participate in this study.

Exclusion criteria

Cases with other types of cancer such as colon, rectum, stomach, brain, lung and others, cases where the consent was refused.

Sampling

The study's patients provided five milliliters of blood, which was drawn, put in gel tubes, allowed to clot for 20 minutes, and then centrifuged for 15 minutes at a speed of 4,000 revolutions per minute to extract serum. Following the storage of the serum in three Eppendorf tubes in a deep freezer at 20 °C, samples were once more warmed to room temperature before these assays were carried out.

Evaluation of FAT10,ZEB2 and TNF-alpha serum concentrations

Commercial kits were used to evaluate the concentrations of FAT10,ZEB2 and TNF-α using Enzyme-Linked Immunosorbent Assay technique.

Study enrollment procedures

Detailed information was recorded for all cases, including age, gender, the duration of the injury

for breast cancer, Determine chemotherapy, radiotherapy or primary injury and others. The presence of breast cancer was confirmed through the medical history taken from the patients and the tests they performed, such as imaging and tissue biopsy.

Statistical analysis

A statistical analysis of the results was carried out by applying the minitab program according to the analysis of variance (ANOVA) test. The arithmetic means were compared to Duncan's multiple ranges test under the probability level of 0.05(9,10).

RESULTS

Figure (1) and table (1) showed a significant increase in serum level of FAT10 protein, in newly diagnosed women before menopause and after menopause compared to the rest of the groups at the level of probability (P < 0.05) in the women patients with breast cancer.

The results of the current study also showed, as shown in Figure (2) and Table (1), the existence of statistical differences between groups at the level of probability (P<0.01). in the blood serum of breast cancer patients of the premenopausal age group and the postmenopausal age

Also, the present study showed as shown in Figure (3) and Table (1) in serum level of TNF-α that there were statistical differences between groups at the level of probability (P <0.05) in the serum of breast cancer patients of the age group before menopause and the age group after menopause.
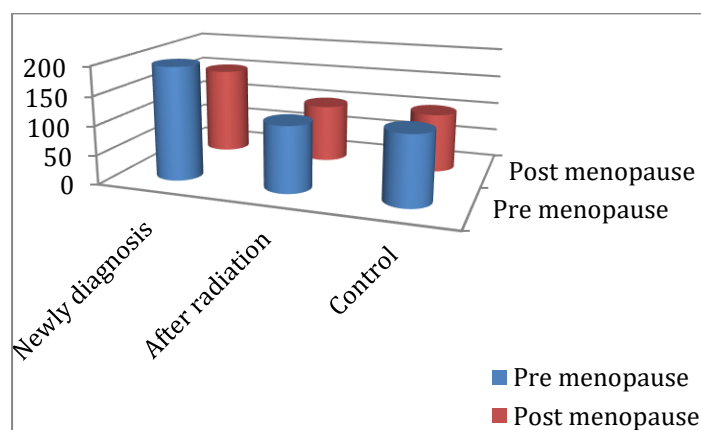

FIGURE 1: Assessment Serum FAT10 (pg/ml) in studied groups

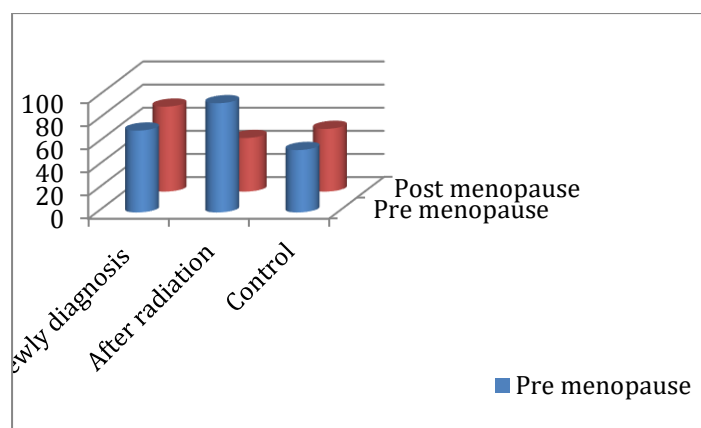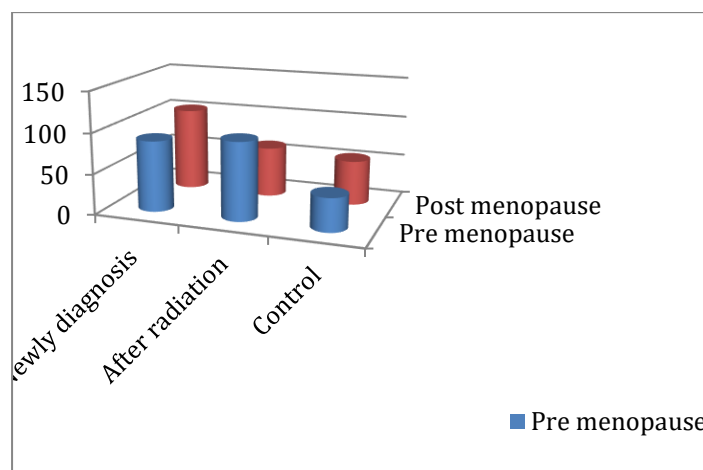**FIGURE 2:** Assessment Serum ZEB2 (ng/L) in studied groups

FIGURE 3: Assessment Serum TNF-α (pg/ml) in studied groups

TABLE 1: Concentration of serum FAT10,ZEB2 and TNF-α in Women with breast cancer compared to healthy women subject.

No. of individual	group	FAT10	ZEB2	TNF-α
Newly diagnosis females	Pre menopause	194.8±26.1% a	70.82±7.42% b	87.6±21.0% a
	Post menopause	151.3±20.3% b	73.50±9.70% b	102.9±23.9% a
After radiation	Pre menopause	113.0±16.9% c	94.50±9.50% a	96.3±16.1% a
	Post menopause	99.6±19.1% c	46.37±8.81% c	62.2±15.0% b
Control	Pre menopause	118.5±22.7% c	53.98±9.33% c	41.1±9.96% c
	Post menopause	101.2±16.3% c	54.27±8.50% c	55.0±11.3% bc
P-value		*0.028	**0.008	*0.051

DISCUSSION

The results of the current study agreed with the findings of (Chen, et al., 2018) who indicated that an increase in the level of FAT 10 in premenopausal and postmenopausal women was in response to DNA damage (DDR), and the role of pcna is an important role as a positive regulator and as a scaffold protein It is associated with bypassing DNA damage and repair pathways by serving as a platform for the recruitment of associated components and that fat10 binds to PCNA stimulated by ultraviolet (or ionizing radiation therapy) and Vp-16 in HeLa cells. Moreover, DNA damage promotes fat10 and as The result of our study showed that the level of fat10 gives a clear improvement after radiotherapy for its role in stimulating pcna (proliferating cell nuclear antigen .)

An immunohistochemistry experiment suggested that the expression levels of FAT10 and PCNA improve in HCC tissues compared with healthy liver tissues; However, FAT10 expression is repressed in regenerated liver tissues, which express high levels of PCNA, indicating that the association between FAT10 expression and PCNA appears only in tumor tissues. The results of the current study indicate that FAT10 may be involved in DDR (DNA damage response) and thus the development of tumors. Another study found that high FAT10 expression was frequently detected in primary breast cancer tissues, and was strongly associated with

malignant phenotype and shorter survival among breast cancer patients (8,11) .

In line with previous investigations and findings (Safaee et al.,2021), our results indicated a critical oncogenic role for ZEB2 overexpression in breast cancer tumours. These properties make ZEB2 an essential molecule for further studies in the treatment of cancer .ZEB 2 protein is involved in tumour invasion and metastasis in the invasive front of carcinomas by EMT induction and In solid tumors, the loss of cell–cell adhesion results in a disruption of normal tissue architecture, promoting progression and dissemination of the malignancy. Further research indicated that ZEB2 was directly bound by FAT10, which reduced its ubiquitination and improved ZEB2's protein stability in BC cells. Additionally, our data indicate that ZEB2 amplification plays a role in the pro-metastasis action of FAT10 in BC. Our findings provide novel evidence that FAT10 may be a prognostic and therapeutic target for BC patients and indicate that FAT10 plays a significant oncogenic role in BC metastasis(12).

Tumor necrosis factor-alpha (TNF-α) plays important roles in chronic inflammation-associated tumorigenesis . therefore, cytokines can play an essential role in in many diseases(13). TNF-a, had been shown to play an important role in promoting inflammation-associated tumorigenesis through the NF-kB pathway More than 150 target genes of NF-kB have been identified, of which some might be involved in tumorigenesis by regulating the balance between apoptosis and cell survival upon TNF-a induction and we demonstrate that FAT10, the ubiquitinlike protein that is overexpressed in the tumors of various cancers can be induced by TNF-a through the NF-kB pathway mediated via TNFR1 which is one of two receptors that TNF-a can act through The action of TNF-a through the TNFR1 was implicated to play important roles in tumorigenesis because deletion of TNFR1 resulted in lower cancer incidence upon carcinogen treatment The action of TNF-a through the TNFR2 was suggested to mediate tumor suppression probably by enhancing TNFR1-induced apoptosis Hence, the induction of FAT10 expression by TNF-a through the

TNFR1 strongly implicates potential roles of FAT10 in promoting tumorigenesis(14).

CONCLUSION
Present study concluded that both FAT10,ZEB2 and TNF-α are increased and highly correlated during breast cancer.

CONFLICT OF INTEREST
No conflict of interest.

Sources of funding
No.

Author contribution
Authors contributed equally in the study.

REFERENCES
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. (2018).Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. Nov;68(6):394-424.
2. Sung H , ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F.(2020).Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. Nov; 71(3):209-249.
3. Giaquinto AN , Sung H ,Miller, K. D., Kramer, J. L., Newman, L. A., Minihan, A., ... & Siegel, R. L. (2022). Breast cancer statistics, 2022. CA: A Cancer Journal for Clinicians, 72(6), 524-541.
4. Yalaza M , İnan A, Bozer M.) 2016) .Male Breast Cancer. J Breast Health. Jan 1;12(1):1-8.
5. Lawrence, R. A. (2022). Anatomy of the breast. In Breastfeeding (pp. 38-57). Elsevier.
6. Wolczyk, D., Zaremba-Czogalla, M., Hryniewicz-Jankowska, A., Tabola, R., Grabowski, K., Sikorski, A. F., & Augoff, K. (2016). TNF-α promotes breast cancer cell
7. migration and enhances the concentration of membrane-associated proteases in lipid rafts. Cellular Oncology, 39(4), 353-363.
8. Li J, Riedt T, Goossens S, Carrillo García C, Szczepanski S, Brandes M, Pieters T, Dobrosch L, Gütgemann I, Farla N, et al. (2017) The EMT transcription factor Zeb2 controls adult murine hematopoietic differentiation by regulating cytokine signaling. Blood.;129:460–472.

9. Zou Y, Ouyang Q, Wei W, Yang S, Zhang Y, Yang W (2018) .FAT10 promotes the invasion and migration of breast cancer cell through stabilization of ZEB2. Biochem Biophys Res Commun. Nov 30;506(3):563-570.

10. Krishnamoorthy D, Sankaran M. Modulatory effect of Pleurotus ostreatus on oxidant/antioxidant status in 7, 12-dimethylbenz (a) anthracene induced mammary carcinoma in experimental rats--A dose-response study. J Cancer Res Ther. 2016 Jan-Mar;12(1):386-94.

11. Rae S, Spillane C, Blackshields G, Madden SF, Keenan J, Stordal B. The EMT-activator ZEB1 is unrelated to platinum drug resistance in ovarian cancer but is predictive of survival. Hum Cell. 2022 Sep;35(5):1547-1559.

12. Chen, Z., Zhang, W., Yun, Z., Zhang, X., Gong, F., Wang, Y. ... Leng, L. (2018). Ubiquitin-like protein FAT10 regulates DNA damage repair via modification of proliferating cell nuclear antigen. Molecular Medicine Reports, 17, 7487-7496.

13. Safaee S, Fardi M, Hemmat N, Khosravi N, Derakhshani A, Silvestris N, Baradaran B. Silencing ZEB2 Induces Apoptosis and Reduces Viability in Glioblastoma Cell Lines. Molecules. 2021 Feb 9;26(4):901.

14. Rija, F. F., Hussein, S. Z., & Abdalla, M. A. (2021). Physiological and immunological disturbance in rheumatoid arthritis patients. Baghdad Sci J, 18(2), 247-252.

15. Ren, J., Wang, Y., Gao, Y., Mehta, S. B., & Lee, C. G. (2011). FAT10 mediates the effect of TNF-α in inducing chromosomal instability. Journal of cell science, 124(21), 3665-3675