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ORIGINAL ARTICLE

SIGNIFICANT ROLE OF NF-KB, NF-AT, ANNEXIN A5 AND TRAIL IN IRAQI FEMALE WITH BREAST CANCER

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ABSTRACT

Background: Breast cancer is a common cancer in women and especially in developing countries the incidence rates of this cancer are rising, including Iraq. There is need to identify the molecular basis of the disease and its progression with a view of discovering better treatment regimens. This study aimed to investigate the role of NF-KB, NF-ATc1, Annexin A5 and TRAIL in pathogenicity of breast cancer in order to detect a new therapeutic target.

Materials & Methods: A pilot study was carried out on samples at Iraqi women with breast cancer in hospitals of Baghdad during the period from August 2023 until December 2023. A total of 88 blood samples were collected (60 blood samples which included 30 samples for newly diagnosed patients and 30 samples for patients under treatment with chemotherapy (Adriamycin and cyclophosphamide) at a dos-age of (60mg/M2) with age range 25-65 years, in addition to 28 blood samples for apparently healthy women serve as control group with age range match to patients group. The current research focuses on the real participation of NF-κB, NFATc1, ANXA5, and TRAIL in the progression among the Iraqi women with Breast cancer. Expression of NF-κB and NFATc1 was determined by quantitative PCR. AnnexinA5 and TRAIL levels were determined by ELISA technique. The findings confirmed that NF-κB and NFATc1 are over expressed in newly diagnosed breast cancer patients compared with the control and under treatment patients suggesting their implication in tumor advancement.

Results: It was observed that serum Annexin A5 and TRAIL levels were higher in newly diagnosis patients and a significant lowering of all the parameters was noted under treatment thus establishing the efficacy of the treatment strategies. It was also found that NF-κB and NFATc1 genes are co-regulated because of their positive correlation, r=0. 668, p=0. 001. However, no correlation was observed between the gene expression of NF-κB or NFATc1 and serum levels of AnnexinA5 or TRAIL, indicating distinct regulatory mechanisms. These findings underscore the importance of NF-κB and NFATc1 as potential therapeutic targets, while the reduction of all markers under treatment highlights the efficacy of current interventions.

Conclusion: In conclusion, targeting these molecular pathways could offer new therapeutic strategies for improving breast cancer outcomes, particularly in Iraqi women.

KEY WORDS: Cancer; Breast cancer; NF-KB; NF-AT; Annexin A5; TRAIL; Gene expression; ELISA.

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INTRODUCTION

Breast cancer remains one of the most common cancers in women and has a significant impact on global health.[1,2] The disease is caused by a complex interplay of genetic, epigenetic and environmental factors that lead to the development of heterogeneous tumor subtypes.[3,4] Although advances in early detection and treatment have improved survival rates, breast cancer remains the leading cause of cancer-related deaths in women worldwide, particularly in low- and middle-income countries. [5,6] Recent cancer registries point to rising incidence rates in several developing countries, including Iraq, where breast cancer will account for 36% of newly diagnosed cancers in women between 2020 and 2022.[7, 8]

In Iraq, the incidence rate of breast cancer has increased, which has been linked to environmental and lifestyle factors. There are a few reasons that could be a cause of cancer, namely political instability, environmental pollution and the use of chemical weapons in the warfare that affected the young women. [9, 10] However, the mortality rate persists due to various complications in medical care, which is

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why the mortality rate for breast cancer is 20%.[8,11]

This emphasises the urgent need for molecular studies to decipher the causes of breast cancer and find potential targets for intervention. NF-κB signalling is one of the molecular pathways identified as being involved in the development of breast cancer; it regulates immune responses, inflammation and cell proliferation. Up-regulation of NF-κB has been found to increase tumour growth, tumour survival and metastasis in various cancers, including breast cancer.[12,13]

Similarly, the nuclear factor of activated T cells (NF-AT) family, particularly the member NF-ATc1, is involved in the regulation of tumourigenic cytokines and the immune defence microenvironment.[14,15]

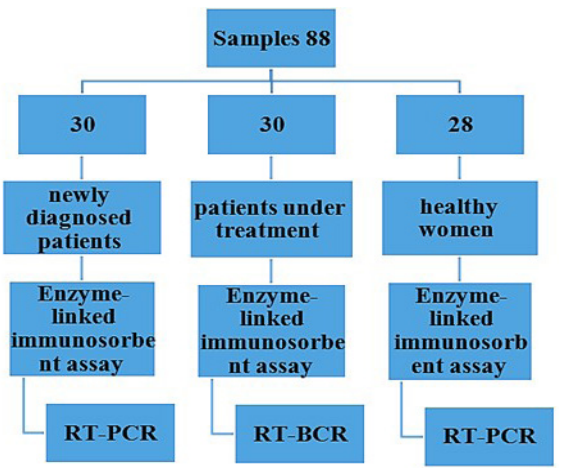
Annexin A5, a calcium-dependent phospholipid-binding protein belonging to the annexin family, has become the centre of interest due to its role in apoptotic cell death and cancer metastasis. As the results showed that annexin A5 is overexpressed in breast cancer cells and can help to assess the aggressiveness of the disease, it could be used as a biomarker.[16,17]

Similarly, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) acts as both a sensitiser and an inducer of apoptosis in breast cancer cells, while it is non-toxic to normal cells. Because the effects of TRAIL on apoptosis are convoluted and complicated, and because TRAIL is poorly responsive to breast cancer chemoresistance, TRAIL is an important molecule to study.[16,18]

Since these molecules are related to the biology of breast cancer, the present work attempted to identify their expression patterns and biological significance in Iraqi women with breast cancer. The aim was to identify new proteins or molecules that could be targeted to stop or slow the progression of the disease and improve the quality of life of patients. By focussing on these key molecules. The aim of this study was to investigate the role of NF-KB, NF-ATc1, Annexin A5 and TRAIL in the pathogenicity of breast cancer in order to find a new therapeutic target that can halt the progression of the disease or even cure it.

MATERIALS AND METHODS

Subject collection (Inclusion and exclusions criteria): A pilot study was carried out on samples at Iraqi women with breast cancer who attended to Iraqi medical city (oncology teaching hospital) in Baghdad during the period from August 2023 until December 2023. A total of 88 blood samples were collected (60 blood samples which included 30 samples for newly diagnosed patients and 30 samples for patients under treatment with chemotherapy (Adriamycin and cyclophosphamide) at a dosage of (60mg/M2) with age range 25-65 years, in addition to 28 blood samples for apparently healthy women serve as control group with age range match to patients group. The patients were chosen and diagnosed by doc-tors who specialize in breast cancer. The diagnosis was verified by mammography and histological findings. The following conditions were not considered for inclusion in the study: other types of cancer, autoimmune illnesses, infectious diseases, pregnancy, breastfeeding, and other serious acute or chronic medical conditions. Women made up the entire sample for this research.



Blood collection: Five ml of peripheral blood was withdrawn from each patient and control, collected by disposable syringe, and then blood samples were divided into two parts:

- 4 ml were set in a gel tube for the ELISA study and centrifuged to separate serum in several Eppendorf tubes to prevent repeated freeze-thaw cycles.

- 0.25 mL of blood was added to 0.5mL of TRIzol Rea-gent mixed in 1.5ml Eppendorf tube for the molecular study, and the Eppendorf tube was stored at -20ºC for further analysis.

Enzyme-linked immunosorbent assay (ELISA): The assay employs the "Double Antibody Sandwich" method, which is available from ELISA Kits, USNF, USA. Annexin A5 and TRAIL concentrations in the samples have to be measured. To do this, we compare the samples' optical densities (O.D.) to a pre-calculated standard curve. The accuracy and consistency of the results were guaranteed by carefully preparing all standards, samples, and reagents according to the instructions provided in the kit brochure.

The primers of RT-PCR: These primers were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/ul as a stock solution. A working solution of these primers was prepared by adding 10ul of primer stock solution (stored at freezer -20 C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl.

Table 1. The primers used in RT-PCR.

Primer Name	Seq.	References
β-Globin-F	A C A C A A C T G T-GTTCACTAGC	19
β-Globin-R	CAACTTCATCCAC-GTTCACC	
N F A T C 1 _ exp-F	CACCAAAGTCCTG-GAGATCCCA	20
N F A T C 1 _ exp-R	T T C T T C C T C - CCGATGTCCGTCT	
NF-κB_exp-F	G C A G C A C - TACTTCTTGAC-CACC	(Primer3) program
N F - κ B _ exp-R	TCTGCTCCTGAG-CATTGACGTC	

Extraction and determination of RNA: Isolated RNA was done according to TRIzolTM Rea-gent's protocol (Thermo Scientific, USA). Quantus Flu-orometer detected the quality of extracted RNA for downstream applications.

Real-time polymerase chain reaction and gene expression: Analysis and Calculation of regulatory element levels of one or more genes depend on miRNA concentration after conversion it to cDNA. All processes including total RNA purification, qPCR amplification and data analysis. The specific components of the One Step RT-PCR kit used in this study are as follows: 5 μl of qPCR Master Mix, 0.25 μl of R.T. mix, 0.25 μl of MgCl2, 0.5 μl of Forward primer, 0.5 μl of Reverse primer, 2.5 μl of Nu-clease Free Water, and 1 μl of RNA, for a total volume of 10 μl. Mix 1 μl of the template with 9 μl of the Master mix and add it to an aliquot for a single reaction. It was then tested using Real-time PCR. The first stage was a preliminary denaturation at 95 °C for 5 minutes and a 15-minute cycle at 37 °C for cDNA synthesis. In Step 2, 40 cycles included denaturing the template at 95 °C for 20 seconds (A), annealing the primers to the template at 60°C and 65°C for NF-KB and NF-ATC1 and β-globin, respectively, for 20 seconds (B), and, finally, extending the primers at 72°C for 20 seconds. Step three involved heating the green material from 72 to 95 degrees Celsius. The Livak Method was used to determine the levels of gene expression.

Folding = $2^{-\Delta\Delta CT}$

ΔCT =CT gene - CT House Keeping gene

ΔΔCT =ΔCT Treated - ΔCT Control

Statistical Analysis: The collected data were analyzed using SPSS program using ONE ANOVA test by obtaining LSD between studied groups. The data were presented as Mean ± S.E and significant differences was considered at P≤ 0.05.

Ethics approval and consent to participate:

Ethical permission has been obtained from the Iraqi Ministry of Health, specifically the Department of Med-ical Teaching City, Oncology Teaching Hospital (per-mission No. 26512, dated 17/7/2023). The subject to the agreement of the patient. The oncologist directed suitable patients for referral.

RESULTS

The gene expression and serum level of studied parameters in patients and control:

The gene expression and serum levels of NF-κB, NFATc1, Annexin A5, and TRAIL showed significant differences across the studied groups. NF-κB expression was markedly higher in newly diagnosed patients (1.203±0.404)fold compared to controls (0.054±0.020) fold and under treatment patients (0.019±0.015)fold, with a high significance (p=0.001). Similarly, NFATc1 levels were elevated in newly diagnosed patients (1.093±0.285)fold compared to controls (0.190±0.093)fold and under treatment patients (0.103±0.027)fold, with p-values of <0.001 in both cas-es.

Serum levels of Annexin A5 (ANXA5) were significantly increased in newly diagnosed patients (34.18±3.28) Pg/mL compared to controls (20.26±1.72) Pg/mL and under treatment patients (10.93±1.04) Pg/mL, with a p-value of 0.001. TRAIL levels followed a similar trend, with higher levels in newly diagnosed patients (0.96±0.24) ng/mL compared to controls (0.21±0.05) ng/mL and under treatment patients (0.23±0.04) ng/mL, also showing high significance (p=0.001).

The difference between newly diagnosed and under treatment patients for all parameters was also highly significant (p=0.001). These results highlight a strong association between disease progression and the studied biomarkers. As showed in (Table 2).

Distribution of studied parameters according to grade of disease in all studied groups: The mean NF-κB gene expression level was significantly increased in newly diagnosed breast cancer patients with respect to normal, healthy women, and increased progressively with grade of malignancy from Grade-I (1. 371±1. 187) to Grade II (1. 143±0. 442) to Grade III (0. 724±0. 036). As in the case of NADPH and CD68, NFATc1 was also upregulated in osteoclasts and its expression was highest in Grade III specimens 2. 356±0. 404. Under Treated patients showed a significant reduction in NF-κB and NFATc1 levels across both Grade II (0.032±0.027 for NF-κB, 0.085±0.028 for NFATc1) and Grade III (0.004±0.002 for NF-κB, 0.123±0.049 for NFATc1), with significant differences observed between newly diagnosed and under treatment patients, especially in Grade II (p=0.013* for NF-κB, p=0.004** for NFATc1) and Grade III (p=0.03* for NF-κB, p=<0.001** for NFATc1).

Serum levels of Annexin A5 (ANXA5) were elevated in newly diagnosed patients, with the highest concentration observed in Grade II (37.93±4.17) Pg/mL. However, under treatment levels significantly decreased, particularly in Grade II (11.37±1.48) Pg/mL, with p-values of <0.001. TRAIL levels followed a similar pattern, with newly diagnosed patients in Grade II showing the highest levels (1.17±0.37) ng/mL, which decreased under treatment, but with less significant changes compared to other parameters. As showed in (Table 3).

Distribution of studied parameters according to stage of disease in all studied groups: In newly diagnosed breast cancer patients, NF-κB expression was highest in Stage I (1.968±1.474) and significantly decreased in Stage II (0.972±0.352), though the difference was not statistically significant (p=0.3). NFATc1 followed a similar trend, with higher expression in Stage I (2.157±0.802) compared to Stage II (0.868±0.243), showing a near-significant difference (p=0.06). In under treatment patients, NF-κB and NFATc1 levels were significantly reduced, particularly in Stage III (0.004±0.00001 for NF-κB, 0.137±0.119 for NFATc1), with significant differences between newly and under treated patients in Stage II (p=0.01* for NF-κB,

Table 2. The gene expression and serum levels of NF-κB, NFATc1, Annexin A5, and TRAIL in patients and control groups.

Groups	Parameter Folding (Mean±S.E.)		Parameter Concentration (Mean±S.E.)	
	NF-κB	NFATc1	Annexin A5 (ANXA5) (Pg/mL)	TRAIL (ng/mL)
Control	0.054±0.020	0.190±0.093	20.26±1.72	0.21±0.05
Newly Diagnosed patients	1.203±0.404	1.093±0.285	34.18±3.28	0.96±0.24 a
Under Treatment patients	0.019±0.015	0.103±0.027	10.93±1.04	0.23±0.04
P value	0.001**	<0.001**	0.001**	0.001**
Between newly diagnosed and under treatment P value	0.001**	0.001**	0.001**	0.001**

Table 3. Gene expression and serum levels of NF-κB, NFATc1, Annexin A5, and TRAIL across cancer grades.

Patients groups	Grade groups	Folding (Mean±S.E.)		**Concentration (Mean±S.E.)**	
		NF-κB	**NFATc1**	**Annexin A5 (ANXA5) (Pg/mL)**	**TRAIL (ng/mL)**
Newly Diagnosed patients	I	1.371±1.187	1.085±0.893	26.48±7.12	0.71±0.19
	II	1.143±0.442	0.743±0.205	37.93±4.17	1.17±0.37
	III	0.724±0.036	2.356±0.404	29.16±7.58	0.44±0.22
P value		0.9 NS	0.17 NS	0.32 NS	0.5 NS
Under Treatment patients	II	0.032±0.027	0.085±0.028	11.37±1.48	0.20±0.04
	III	0.004±0.002	0.123±0.049	10.44±1.49	0.25±0.07
P value		0.36 NS	0.52 NS	0.66 NS	0.58 NS
Between newly diagnosed & under treatment	II	-	-	-	-
P value		0.013*	0.004**	<0.001**	0.01*
Between newly diagnosed & under treatment	III	-	-	-	-
P value		0.03*	<0.001**	0.05*	0.3 NS

NS= no significant, **= high significant

Table 4. Gene expression and serum levels of NF-κB, NFATc1, Annexin A5, and TRAIL in patients across cancer stages.

Patients groups	Stage groups	Folding (Mean±S.E.)		Concentration (Mean±S.E.)	
		NF-κB	NFATc1	Annexin A5 (ANXA5) (Pg/mL)	TRAIL (ng/mL)
Newly Diagnosed patients	I	1.968±1.474	2.157±0.802	42.38±9.08	1.11±0.60
	II	0.972±0.352	0.868±0.243	32.13±3.40	0.92±0.27
P value		0.3 NS	0.06 NS	0.22 NS	0.76 NS
Under Treatment patients	I	-	-	12.05±0.95	0.62±0.24
	II	0.021±0.017	0.098±0.028	10.46±1.21	0.19±0.03 a
	III	0.004±0.00001	0.137±0.119	13.23±2.90	0.22±0.17 a
P value		0.7 NS	0.64 NS	0.65 NS	0.01*
Between newly diagnosed & under treatment	I	-	-	-	
P value		-	-	0.02*	0.7 NS
Between newly diagnosed & under treatment	II	-	-	-	
P value		0.01*	0.02*	<0.001**	0.01*

[a] *vs. stage I in treated group, NS= no significant, **= high significant*

Table 5: The correlation between q-PCR results and the serum level of studied parameters.

Parameter		NF-κB	NFATc1	ANXA5	TRAIL
NF-κB	Pearson Correlation	1	0.668**	-0.008	-0.126
	Sig. (2-tailed)		0.001	0.959	0.405
NFATc1	Pearson Correlation	0.668**	1	0.093	-0.005
	Sig. (2-tailed)	0.001		0.537	0.972

p=0.02* for NFATc1).

Serum levels of Annexin A5 were highest in newly diagnosed patients in Stage I (42.38±9.08) Pg/mL but de-creased in Stage II (32.13±3.40) Pg/mL, though this was not statistically significant (p=0.22). In under treatment patients, Annexin A5 levels were lower across all stages, with the lowest levels observed in Stage II (10.46±1.21) Pg/mL, and the differences between newly and under treated patients in Stage II were highly significant (p<0.001). TRAIL levels in newly diagnosed patients were slightly higher in Stage I (1.11±0.60) ng/mL compared to Stage II (0.92±0.27) ng/mL, and under treatment levels were significantly lower in Stages II and III, with p=0.01* when comparing the stages. As showed in (Table 4).

The correlation of ELISA parameters and q-PCR expression: The current study also detected if any correlation is found between the gene expression and serum levels of other parameters measured using ELISA technique. As shown in Table 5, a significant strong positive correlation was noticed between the gene expressions of NF-κB and NFATc1 (as r= 0.668, p=0.001). In contrast, no significant correlation was found between NF-κB or NFATc1 and other parameters (ANXA5, TRAIL) (Table 6), suggestion no correlation cab be found between q-PCR results and the serum levels of the studied parameters.

Receiver Operating Characteristics (ROC) test: Table 6 and Figure 1, presents the ROC curve analysis for various parameters, highlighting their diagnostic performance. NF-kB demonstrates an impressive Area Under the Curve (AUC) of 0.91, with a cutoff value of 0.28 folding, achieving 80% sensitivity and 100% specificity, indicating excellent predictive accuracy (P<0.001**). NFATc1 presents an AUC of 0.88 with a cutoff at 0.88 folding, with a sensitivity of 87% and specificity of 88%, suggesting a moderate predictive value (P=0.001**). Annexin A5 (ANXA5) presents an AUC of 0.76 with a cutoff at 33.19 Pg/mL, with a sensitivity of 57% and specificity of 93%, suggesting a moderate predictive value (P=0.001**). TRAIL exhibits a high AUC of 0.89, with a cutoff value of 0.156 ng/mL, showing 100% sensitivity and 76% specificity, reflecting strong diagnostic performance (P<0.001**).

DISCUSSION

The study, observed significant upregulation of both

Table 6: The ROC curve of studied parameters that summarized the performance and prediction results.

Parameters	AUC	cutoff	Sensitivity	Specificity	P Value
NF-kB	0.91	0.28	80%	100%	<0.001**
NFATc1	0.88	0.14	87%	88%	<0.001**
ANXA5	0.76	33.19	57%	93%	0.001**
TRAIL	0.89	0.156	100%	76%	<0.001**

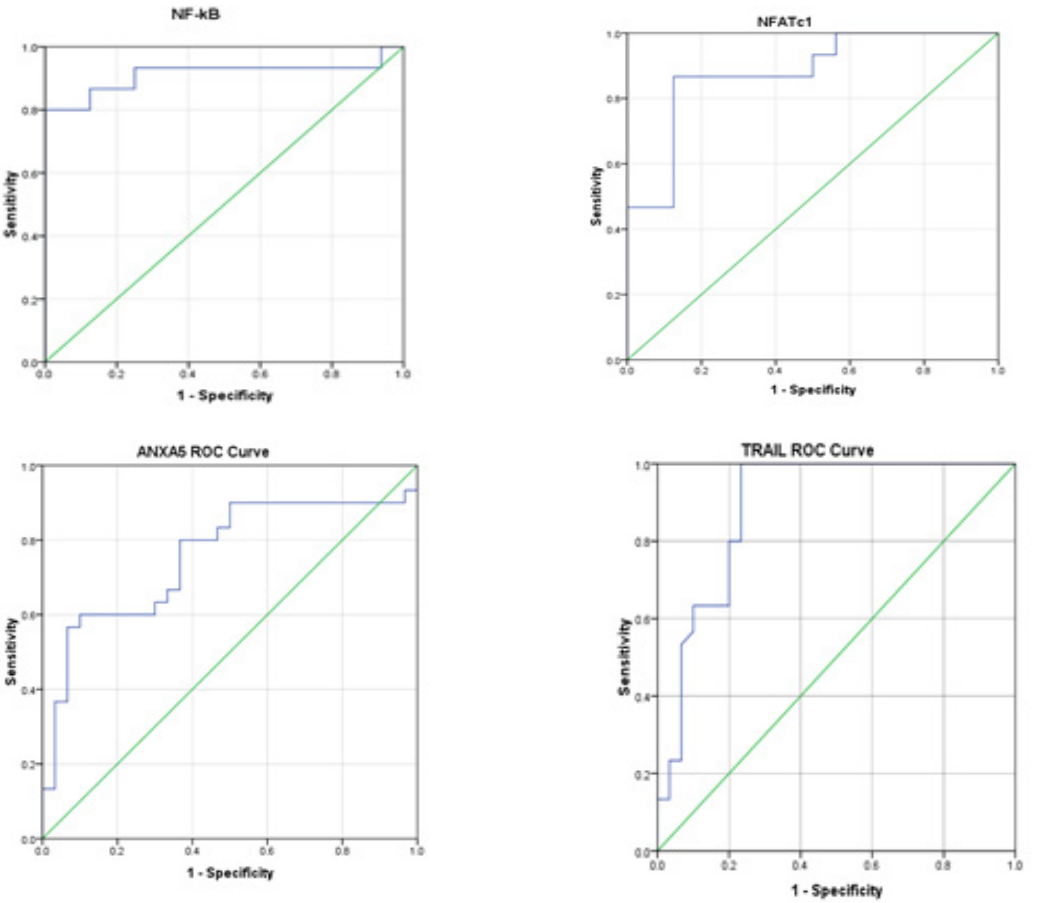


Figure 1. ROC curve of study parameters.

NF-κB and NFATc1 gene expression in newly diagnosed breast cancer patients compared to both control and under treatment groups. Expression of NF-κB in newly diagnosed of patients also involve in inflammation and tumor progression that is in correlation with NF-κB activation and cancer development and metastasis.[21] This results was agreed with previous studies. [22,23], suggest that NF-κB expression could serve as a prognostic marker in these patients.

Likewise, the immune response and cancer-related NFATc1transcription factor was significantly upregulated in the newly diagnosed patients and it has been proposed to play a role in tumorigenesis.[24] This results was agreed with previous studies.[25,26] it was suggested that high NFATc1 expression was present in breast cancer and it might be involved in the process of cancer development and related to poor prognosis of breast cancer.

Serum levels of Annexin A5 (ANXA5) were also significantly up-regulated in newly diagnosed patients and similar to previous studies, this protein maybe involved in apoptosis regulation and cancer cells survival[27]. Analysis of the results reflects the levels of ANXA5 in patients who have undergone therapy, which supports the conclusion on the efficacy of treatment. Likewise, the levels of TRAIL, which are implicated in the process of apoptosis in cancer cells, were higher in newly diagnosed patient and came to normalcy as soon as the treatment was over Therefore, there are studies suggesting that TRAIL has potential in cancer treatment. [28]

The practical decrease of NF-κB, NFATc1, ANXA5, and

TRAIL in treated patients indicates that currently used treatments are effective in decreasing pro-tumorigenic markers consistent with prior therapeutic research.[29]

The study findings indicate that NF-κB and NFATc1 expression progressively increased with disease grade in newly diagnosed breast cancer patients, particularly in Grades II and III, suggesting their role in advancing tumor aggressiveness. This aligns with prior studies showing that NF-κB and NFATc1 are key drivers of inflammation and tumor growth[30]. The significant decrease in these markers following treatment highlights the efficacy of therapeutic interventions in reducing inflammation and immune response, especially in higher-grade tumors[31].

Annexin A5 was significantly elevated in newly diag-nosed patients, particularly in Grade II, reflecting its role in inhibiting apoptosis, which may contribute to cancer cell survival.[32] The drop in Annexin A5 levels under treatment further supports its relevance as a marker for treatment efficacy. While TRAIL levels were elevated in newly diagnosed patients, the under treatment normalization suggests its potential role in cancer therapy.[33]

This study shows that NF-κB and NFATc1 expression were elevated in newly diagnosed breast cancer, especially in Stage I, indicating the role of these factors in the initial phases of cancer progression. These results are consistent with the previous studies highlighting the role of NF-κB in facilitating the tumor initiation and immune response.[34] It is observed that even after the treatment both NF-κB and NFATc1 levels are reduced notably and specially in stage II indicating that inflammation and immune response is well under control. [35]

Indeed, newly diagnosed patients had significantly increased Annexin A5 levels particularly in Stage I samples indicating that the protein is involved in apoptotic suppression during early carcinogenesis[36]. The low Annexin A5 value noted under treatment especially stage II viewed from therapeutic interventions supports the exercise that apoptotic relate treatments are effective in restoring apoptotic sequences. There was higher level of TRAIL in Stage I newly diagnosed patients but the level was significantly lower in under treated patients especially in Stage II and III which clearly demonstrated efficacy of apoptosis-inducing treatments.[37]

The current study revealed a significant positive correlation between the gene expressions of NF-κB and NFATc1, with a strong Pearson correlation coefficient ($r=0.668$, $p=0.001$). This finding aligns with previous research indicating that NF-κB and NFATc1 are both involved in regulating inflammation and immune response, suggesting a functional relationship between these transcription factors in cancer progression.[38] The co-activation of NF-κB and NFATc1 could indicate their synergistic roles in promoting tumorigenesis, particularly through pathways that involve chronic inflammation and immune cell activation.[39] On other hand, no significant correlation was found be-tween NF-κB or NFATc1 gene expression and the serum levels of Annexin A5 (ANXA5) or TRAIL. The absence of relationship between gene expression levels estimated by q-PCR and the protein levels quantified by ELISA ensures that they reflect different pathways of regulation. Although, it can be considered that NF-κB and NFATc1 may regulate the transcriptional profile, the serum level of ANXA5 and TRAIL can be regulated through other post-transcriptional factors such as protein stability, secretion or degradation pathway.[40]

In breast cancer, NF-κB (particularly the p65/RelA and p50 subunits) is often over activated and is linked with tumor growth, therapy resistance, and poor prognosis. Studies using ROC curves to evaluate NF-κB-related molecules have shown excellent diagnostic performance, often with high area under the curve (AUC) values, indicating strong discriminative power between cancerous and non-cancerous tissues. For example, high expression of NF-κB-related genes like IL-8 and MMP-1 has been associated with a worse prognosis in breast cancer patients. [41, 42]

Similarly, NFATc1, another transcription factor involved in immune response regulation, is increasingly recognized for its role in cancer. Its dysregulation can contribute to metastasis and immune evasion in tumors. While specific ROC curve data for NFATc1 in breast cancer are limited, its interaction with NF-κB signaling in tumor cells suggests that combined analyses of both pathways could improve the diagnostic accuracy and prognostic predictions. [41]

Annexin A5 (ANXA5) did present a moderate diagnostic performance with an AUC equal to 0.76. The screening criterion used was a score of 33 or above. While 19 Pg/mL gave 57% sensitivity and 93% specificity for CVD. The high value of specificity in this case means that the ANXA5 test is good in excluding non-affected individuals but the low value of sensitivity reveals that the test might miss a large number of affected individuals. This performance is in conformity with emerging evidence by[43] and [44] that identified ANXA5 a marker for stresses and apoptosis but with contrasting diagnostic value in different diseases. A high diagnostic performance was obtained by TRAIL with an AUC value of 0. 89 percentile which is considered good so there is good overall accuracy. The concentration of 156 ng/mL accompanied by sensitivity at 100% and specificity at 76% suggests that TRAIL has the ability to confirm affected persons at a high rate. This is in line with 75% of the available literature where.[45,46], among others have acknowledged the importance of TRAIL in apoptosis , as well as its prospect in diagnostics of cancer and other diseases.

CONCLUSION

This study establishes that NF-κB, NFATc1, Annexin A5, and TRAIL are critical in breast cancer development in women of Iraq. The strong relationship between NF-κB and NFATc1 indicates that these proteins have cooperative effect of enhancing tumor progression.

In contrast, the lack of correlation between gene expression and serum protein levels indicates complex regulatory mechanisms. Therapeutic interventions successfully reduced the expression and serum levels of these markers, highlighting their potential as molecular targets for treatment.

Study limitation

The study encountered a critical limitation, namely, the sample size of breast cancer. Although breast cancer has been a disease for a long time, its causes and treatment are still unknown.

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REFERENCES

1. Almohaidi AMS, Al-Abassi HM, Abdulkareem ZJ, Al-Sayyid MM. Sera level and polymorphism of interleukin-33 gene in Iraqi females patients with breast cancer. Ibn AL-Haitham J Pure Appl Sci. 2021;34:1-11. https://doi.org/10.30526/34.1.2806
2. Zinatizadeh MR, Schock B, Chalbatani GM, Zarandi PK, Jalali SA, Miri SR. The nuclear factor kappa B (NF-κB) signaling in cancer development and immune diseases. Genes Dis. 2021;8:287-97. https://doi.org/10.1016/j.gendis.2020.06.005
3. Barnabei L, Laplantine E, Mbongo W, Rieux-Laucat F, Weil R. NF-κB: at the borders of autoimmunity and inflammation. Front Immunol. 2021;12:1-27. https://doi.org/10.3389/fimmu.2021.716469
4. Salman AO, Al-Abassi HM, Mahod WS. Demographic and clinico-pathological characteristics of some Iraqi female patients newly diagnosed with breast cancer. Ann Roman Soc Cell Biol. 2021;25:8264-78. http://annalsofrscb.ro/index.php/journal/article/view/7018
5. Zhang T, Ma C, Zhang Z, Zhang H, Hu H. NF-κB signaling in inflammation and cancer. Med Comm. 2021;2:618-53. https://doi.org/10.1002/mco2.104
6. Ali HK, Mahood WS. Expression of transketolase TKTL1 in Iraqi breast cancer females. J Popul Therap Clin Pharmacol. 2023;30:55-61. https://doi.org/10.47750/jptcp.2023.30.08.006
7. M-Amen K, Abdullah OS, Amin AMS, Mohamed ZA, Hasan B, Shekha M, et al. Cancer incidence in the Kurdistan region of Iraq: results of a seven-year cancer registration in Erbil and Duhok governorates. Asian Pac J Cancer Prev. 2022;23(2):601-15. https://doi.org/10.31557/APJCP.2022.23.2.601
8. Dare AJ, Knapp GC, Romanoff A, Olasehinde O, Famurewa OC, Komolafe AO, et al. High-burden cancers in middle-income countries: a review of prevention and early detection strategies targeting at-risk populations. Cancer Prev Res (Phila). 2021;14(12):1061-74. https://doi.org/10.1158/1940-6207.CAPR-20-0571
9. Alrawi N. A review on breast cancer in Iraq and future therapies insights. Baghdad J Biochem Appl Biol Sci. 2022;3:4-16. https://doi.org/10.47419/bjbabs.v3i01.64
10. Alkashaf KH, Mohammed SI. Impact of clinical pharmacist intervention on chemotherapy knowledge, attitude, and practice among breast cancer women. J Fac Med Baghdad. 2024;66:103-9. https://doi.org/10.47419/bjbabs.v3i01.64
11. Abdullah MR, Alabassi HM. The potential influence of immune modulatory molecules (TGF-βIII and CTLA-4) in pathogenicity of PCOS. Karbala Int J Mod Sci. 2024;10(2):363-75. https://doi.org/10.33640/2405-609X.3361
12. Hashim HT, Ramadhan MA, Theban KM, Bchara J, El-Abed-El-Rassoul A, Shah J. Assessment of breast cancer risk among Iraqi women in 2019. BMC Womens Health. 2021;21:1-9. https://doi.org/10.1186/s12905-021-01557-1
13. Abbas MM, Alkhazrajy LA. Knowledge, attitudes and practices regarding the screening of colorectal cancer among primary care physicians in Baghdad during 2022. J Fac Med Baghdad. 2024;66:45-50. https://doi.org/10.32007/jfacmedbagdad.2162
14. Lin Y, Song Y, Zhang Y, Shi M, Hou A, Han S. NFAT signaling dysregulation in cancer: Emerging roles in cancer stem cells. Biomed Pharmacother. 2023;165:115167. https://doi.org/10.1016/j.biopha.2023.115167
15. Rasheed RA, Al-Abassi HM. TGF-BETA3 (gene expression) as a new biological marker for diagnosing the gastric and colorectal cancer. Biochem Cell Arch. 2021;21:1539-43. http://dx.doi.org/10.13140/RG.2.2.29988.81281
16. Gounou C, Bouvet F, Liet B, Prouzet-Mauléon V, d'Agata L, Harté E, et al. Annexin-A5 and annexin-A6 silencing prevents metastasis of breast cancer cells in zebrafish. Biol Cell. 2023;115(6):e202200110. https://doi.org/10.1111/boc.202200110
17. Khalifa MF. Impact of psychological distress in women upon coping with breast cancer. Iraqi Nat J Nurs Spec. 2022;35:82-87. https://doi.org/10.58897/injns.v35i1.565
18. ALAbady AA, Al-Majidi SMH. Preparation of some new pyrazine and triazine derivatives and study of their antimicrobial and anticancer activities. Iraqi J Sci. 2024;65:2357-72. https://doi.org/10.24996/ijs.2024.65.5.1
19. Al Shami MA, Saud AM. Assessing gene expression of factor XIII-A1 in Iraqi patients with FXIII deficiency. J Biosc Appl Res. 2024;10(1):103-10. https://doi.org/10.21608/jbaar.2024.268467.1038
20. Ismael AK, Alabassi HM. PD-1 and NFATc1 as promising immunotherapy for SLE patients. J Popul Therap Clin Pharmacol. 2023;30:260-66. https://doi.org/10.47750/jptcp.2023.30.05.025
21. Pavitra E, Kancharla J, Gupta VK, Prasad K, Sung JY, Kim J, et al. The role of NF-κB in breast cancer initiation, growth, metastasis, and resistance to chemotherapy. Biomed Pharmacother. 2023;163:1-14. https://doi.org/10.1016/j.biopha.2023.114822
22. Ma Q, Hao S, Hong W, Tergaonkar V, Sethi G, Tian Y, et al. Versatile function of NF-κB in inflammation and cancer. Exp Hematol Oncol. 2024;13(1):68. https://doi.org/10.1186/s40164-024-00529-z
23. Guo Q, Jin Y, Lin M, Zeng C, Zhang J. NF-κB signaling in therapy resistance of breast cancer: Mechanisms, approaches, and challenges. Life Sci. 2024;348:122684. https://doi.org/10.1016/j.lfs.2024.122684
24. Wang SF, Tseng LM, Lee HC. Role of mitochondrial alterations in human cancer progression and cancer immunity. J Biomed Sci. 2023;30(1):61. https://doi.org/10.1186/s12929-023-00956-w
25. Patil S, Forster T, Reutlinger K, Kopp W, Versemann L, Spitalieri J, et al. Chromatin-independent interplay of NFATc1 and EZH2 in pancreatic cancer. Cells. 2021;10(12):3463. https://doi.org/10.3390/cells10123463

26. Zhang X, Song W, Gao Y, Zhang Y, Zhao Y, Hao S, et al. The role of tumor metabolic reprogramming in tumor immunity. Int J Mol Sci. 2023;24(24):17422. https://doi.org/10.3390/ijms242417422
27. Jing J. The relevance, predictability, and utility of annexin A5 for human physiopathology. Int J Mol Sci. 2024;25(5):2865. https://doi.org/10.3390/ijms25052865
28. Hein T, Krammer PH, Weyd H. Molecular analysis of annexin expression in cancer. BMC Cancer. 2022;22(1):994. https://doi.org/10.1186/s12885-022-10075-8
29. Lee S, Lee SL, Min B, Oh SC. Prognostic implication of systemic inflammatory markers in young patients with resectable colorectal cancer. Ann Surg Treat Res. 2021;100:25-32. https://doi.org/10.4174/astr.2021.100.1.25
30. Cruz-Reyes N, Radisky DC. Inflammation, infiltration, and evasion-tumor promotion in the aging breast. Cancers. 2023;15:1836. https://doi.org/10.3390/cancers15061836
31. Bower JE, Ganz PA, Irwin MR, Cole SW, Carroll J, Kuhlman KR, et al. Acute and chronic effects of adjuvant therapy on inflammatory markers in breast cancer patients. JNCI Cancer Spectr. 2022;6:1-9. https://doi.org/10.1093/jncics/pkac052
32. El Hagary HM. Study of the serum level of annexin A5 as a marker for hepatocellular carcinoma in hepatitis C virus cirrhosis patients. Afro-Egypt J Infect Endem Dis. 2023;13(3):165-73. https://doi.org/10.21608/aeji.2023.211128.1291
33. Woodward A, Faria GNF, Harrison RG. Annexin A5 as a targeting agent for cancer treatment. Cancer Lett. 2022;547:215857. https://doi.org/10.1016/j.canlet.2022.215857
34. Zhu B, Zhang X, Sun S, Fu Y, Xie L, Ai P. NF-κB and neutrophil extracellular traps cooperate to promote breast cancer progression and metastasis. Exp Cell Res. 2021;405:1-12. https://doi.org/10.1016/j.yexcr.2021.112707
35. Surai PF, Surai A, Earle-Payne K. Silymarin and inflammation: food for thoughts. Antioxidants. 2024;13(1):98. https://doi.org/10.3390/antiox13010098
36. Ghasemi M, Reiazi N, Behzad-Behbahani A, Takhshid MA. Annexin-A5 overexpression increases sensitivity of MCF-7 and MCF-7/ADR cells to epirubicin. Avicenna J Med Biotechnol. 2023;16:34-9. https://doi.org/10.18502/ajmb.v16i1.14169
37. Liu X, Wang L, Duan C, Rong Y, Liang Y, Zhu QX, et al. Daurisoline inhibits proliferation, induces apoptosis, and enhances TRAIL sensitivity of breast cancer cells by upregulating DR5. Cell Biol Int. 2024;48:951-63. https://doi.org/10.1002/cbin.12162
38. Hasani S, Fathabadi F, Saeidi S, Mohajernoei P, Hesari Z. The role of NFATc1 in the progression and metastasis of prostate cancer: a review on the molecular mechanisms and signaling pathways. Cell Biol Int. 2023;47:1895-904. https://doi.org/10.1002/cbin.12094
39. Lv F, Qin J, Kong S, Pan L, Ding R. MicroRNA-506 ameliorates breast cancer-induced osteolytic bone metastasis via the NFATc-1 signaling pathway. J Biochem Mol Toxicol. 2022;36:1-10. https://doi.org/10.1002/jbt.23156
40. Abidalkareem A, Ibrahim AK, Abd M, Rehman O, Zhuang H. Identification of gene expression in different stages of breast cancer with machine learning. Cancers. 2024;16:1-16. https://doi.org/10.3390/cancers16101864
41. Ren C, Han X, Lu C, Yang T, Qiao P, Sun Y, et al. Ubiquitination of NF-κB p65 by FBXW2 suppresses breast cancer stemness, tumorigenesis, and paclitaxel resistance. Cell Death Differ. 2022;29(2):381-92. https://doi.org/10.1038/s41418-021-00862-4
42. Zuo X, Zhao X, Zhang X, Li Q, Jiang X, Huang S, et al. PTPN20 promotes metastasis through activating NF-κB signaling in triple-negative breast cancer. Breast Cancer Res. 2024;26(1):155. https://doi.org/10.1186/s13058-024-01910-w
43. Yang D, Chen H, Zhou Z, Guo J. ANXA5 predicts prognosis and immune response and mediates proliferation and migration in head and neck squamous cell carcinoma. Gene. 2024;931:148867. https://doi.org/10.1016/j.gene.2024.148867
44. Pimentel JM, Zhou JY, Wu GS. The role of TRAIL in apoptosis and immunosurveillance in cancer. Cancers (Basel). 2023;15(10):2752. https://doi.org/10.3390/cancers15102752
45. Yuan J, Amin P, Ofengeim D. Necroptosis and RIPK1-mediated neuroinflammation in CNS diseases. Nat Rev Neurosci. 2018;20:19-33. https://doi.org/10.1038/s41583-018-0093-1
46. Kundu M, Greer YE, Dine JL, Lipkowitz S. Targeting TRAIL death receptors in triple-negative breast cancers: challenges and strategies for cancer therapy. Cells. 2022;11:1-37. https://doi.org/10.3390/cells11233717

CONFLICT OF INTEREST
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

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All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.