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IL-5 & IL-4 Serum Levels in female Breast Cancer patients in Baghdad

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Abstract: **Background**: It has been proven that cytokines have a role in cancer immunity and carcinogenesis in general, which have a major role in causing many types of solid cancer. This study aimed to estimate the levels of IL-5 and IL-4 in the blood of some diagnosed breast cancer patients, and to study the relationship of these interleukins to the development of human breast cancer. **Patients and methods**: The study included 75 women, divided into two groups, including 50 breast cancer patients and 25 other apparently healthy women. The ELISA method was used to estimate the level of IL-5 and IL- 4 in the blood serum of the two groups studied. **Results**: The current study showed an increase in the level of interleukin 4 in the blood of breast cancer patients, with significant differences between breast cancer and the control group ($p<0.005$), and this increase was associated with tumor development. In addition, it was found that high levels of IL-4 in the blood were directly associated with increasing of Ca15.3. On the other hand, a significant decrease in the average level of interleukin 5 in blood serum was observed in affected patients compared to control groups. **Conclusions**: The study showed an association between high levels of IL- 4 in the blood and breast cancer, and it was found that this increase is related to the advanced stage of the disease. In addition, from our study there is no statistically significant association between a statistically significant decrease in the level of serum IL-5 and the advanced stage of human breast cancer.

Keywords: Breast cancer, Cytokines, ELISA method, IL5, IL4.

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INTRODUCTION

Breast cancer is the most common cancer in women World Cancer Research Fund International [1]. It's the most diagnosed cancer that causes death in women and it is noticed to be a higher incidence in developed countries with higher death rates in developing countries [2]. Breast cancer (BC) is a complex multiple contributing factors. Both the innate and acquired components of the immune system are believed to play crucial roles in the body's response against tumor development [3]. Extensive research condition with has focused on understanding how the host's immune system interacts with cancer cells over the past few decades [4]. One important prognostic factor is the absence of estrogen receptors (ER), which is associated with a poorer outlook for BC patients [5]. ER is typically found in most human breast cancers, indicating a hormone-dependent nature [6]. Circulating tumor cells (CTCs) in women with breast cancer are an indication of prognosis before starting systemic treatment [7]. In addition to ER, cytokines are emerging as potential contributors to breast cancer development. Cytokines, produced by various cell types, are primarily supplied by T-helper cells and macrophages [8]. Two subsets of T-helper cells, IL-4 and IL-5, release different cytokines with distinct functions [9]. The cells secrete IL-4 and IL-5, which activate cytotoxic lymphocytes and macrophages, promoting cellular immunity and inflammation [10]. The cells release IL-4 and IL-5, stimulating antibody production by B cells. It has also been observed that cancer tissues themselves produce cytokines [11]. Some of these cytokines may inhibit an effective immune response and could contribute to local or metastatic spread of the disease [12]. The aim of this study is an estimation serum levels of IL-5 and IL-4 in breast cancer patients in Baghdad.

PATIENTS AND METHODS

The study included 75 women, divided into two groups, including 50 breast cancer patients and 25 other apparently healthy women. The ELISA method was used to estimate the level of IL-5 and IL- 4 in the blood serum of the two groups studied.

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Methods

Interleukin-5 and IL-4 have been estimated by using sandwich enzyme immunoassay (ELISA) technique (BIOSOURCE, Europe S.A., Belgium, Lot No. 051501/B; 060601). The procedure was done according to the manufacturer instruction as supplied with kit from BioSource Europe S.A. Company, Belgium. Statistical analysis The Kruskal-Wallis test was used to assess the statistical significance of difference in median between the 3 study groups.

Statistical analysis

For statistical analysis, SPSS 22.0 (SPSS Inc., IBM, Chicago, IL- USA) was used. The relationship between each cytokine of the Th2 group (IL-5 and IL- 4) and each factor studied was determined using the non-parametric Spearman correlation coefficient (p-values reported). All the statistically significant results by Spearman correlation coefficient were furthermore confirmed with the non-parametric Mann–Whitney U-rank sum test. Variables were examined by using box-plot analysis. All statistical tests were considered significant at $p<0.05$.

RESULTS

The current study showed an increase in the level of interleukin 4 in the blood of breast cancer patients, with significant differences between breast cancer and the control group ($p<0.005$), and this increase was associated with tumor development as shown in (Table I). In addition, it was found that high levels of IL-4 in the blood were directly associated with increasing of Ca15.3. On the other hand, a significant decrease in the average level of interleukin 5 in blood serum was observed in affected patients compared to control groups.

Table-I: shows the level of protein Ca15.3 according to studied groups

Groups / Parameters	Mean ± SD		P-Value
	Control N=25	Patients N=50	
Ca15.3	9.34 ± 8.91	36.14 ± 21.06	< 0.05
IL-5	164.37 ± 133.51	156.58± 128.31	> 0. 05
IL-4	188.99±74.12	289.46 ±154.54	< 0.05

Ca 15-3 = Carbohydrate Antigen.

The current study showed a decrease in the level of interleukin 4 in the blood of Smokers, with significant differences between Smokers and the Nonsmokers group ($p>0.05$), and this decrease was associated with tumor development as shown in (Table II). On the other hand, a significant decrease in the average level of interleukin 5 in blood serum was observed in affected Smokers compared to Nonsmokers groups ($p<0.05$).

Table-II: Shows differences between serum markers of breast cancer women according to smoking status

Groups / Parameters	Mean ± SD		P-Value
	Non smokers	Smokers	
IL-4	281.46± 185.51	275.32± 91.30	> 0. 05
IL-5	192.1 ± 165.86	109.5± 38.61	< 0. 05

(IL-4) = Interleukin 4, (IL - 5) = Interleukin 5.

In addition, it was found that high levels of IL-4 in the blood were directly associated with increasing of family history. On the other hand, a significant decrease in the average level of interleukin 5 in blood serum was observed in affected family history compared to nonfamily history groups.

Table-III: Shows differences between tissue markers of breast cancer women according to breast cancer family history

Groups / Parameters	Mean ± SD		P-Value
	Non Family History for Brest Cancer	Family History for Brest Cancer	
IL-4	259.33± 105.59	302.54± 173.98	> 0. 05
IL-5	161.64±140.36	136.77±114.42	> 0. 05

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Table-IV: Shows Correlations between Ca15.3 , IL5 and IL4

				Ca15.3	IL5	IL4
Ca15.3	Pearson Correlation			1	-.265-	.047
	Sig. (2-tailed)				.063	.744
	Sum of Squares and Cross-products			21735.362	-35089.323-	7550.973
	Covariance			443.579	-716.109-	154.101
	N			50	50	50
	Bootstrap[c]	Bias		0	-.001-	-.013-
		Std. Error		0	.119	.172
		95% Confidence Interval	Lower	1	-.484-	-.305-
			Upper	1	-.020-	.353
IL5	Pearson Correlation			-.265-	1	.029
	Sig. (2-tailed)			.063		.843
	Sum of Squares and Cross-products			-35089.323-	806750.573	27935.840
	Covariance			-716.109-	16464.297	570.119
	N			50	50	50
	Bootstrap[c]	Bias		-.001-	0	-.013-
		Std. Error		.119	0	.198
		95% Confidence Interval	Lower	-.484-	1	-.326-
			Upper	-.020-	1	.413
IL4	Pearson Correlation			.047	.029	1
	Sig. (2-tailed)			.744	.843	
	Sum of Squares and Cross-products			7550.973	27935.840	1170322.132
	Covariance			154.101	570.119	23884.125
	N			50	50	50
	Bootstrap[c]	Bias		-.013-	-.013-	0
		Std. Error		.172	.198	0
		95% Confidence Interval	Lower	-.305-	-.326-	1
			Upper	.353	.413	1
c. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

Figure (I), Figure (II) and Figure (III) shows the relationship between Ca15.3 and IL5, Ca15.3 and IL4 and between IL4 and IL5. It showed that the probability value for the three relationships is greater than (> 0.05) this means that there is no correlation.

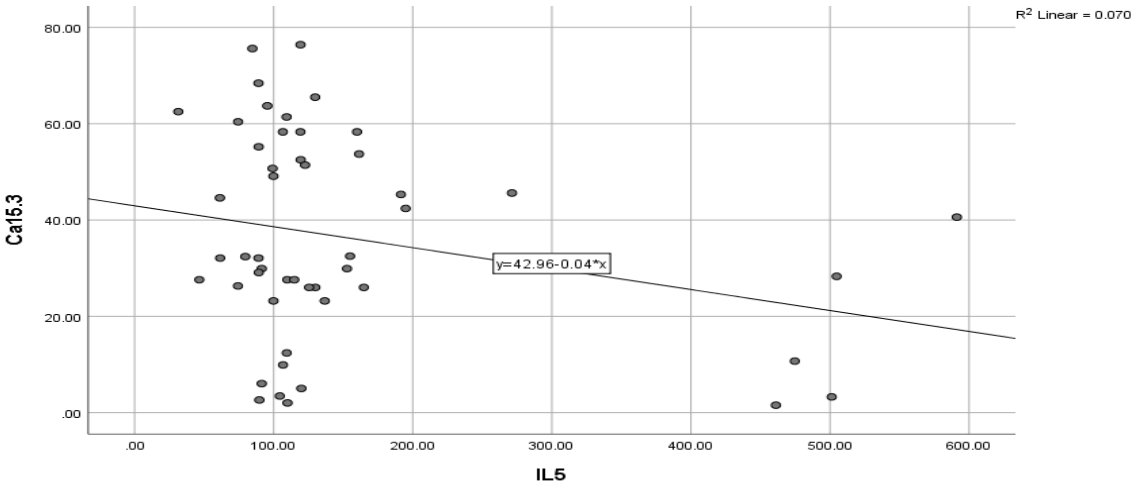

Figure-I: represents a diagram between Ca15.3 and IL5

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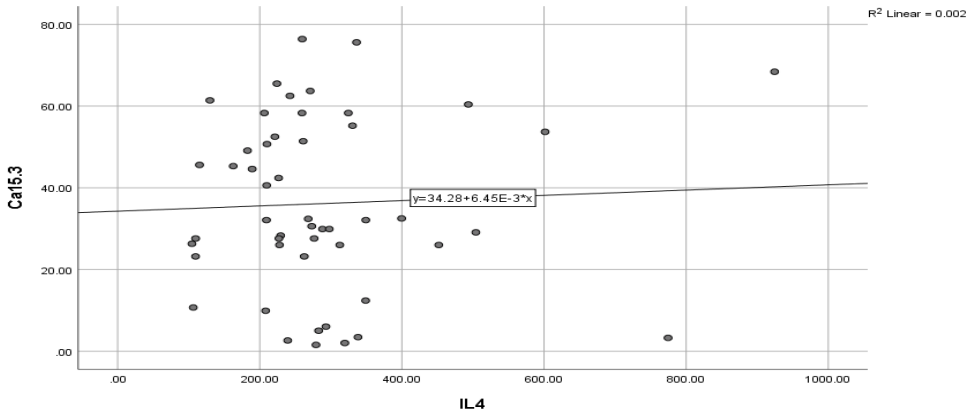

Figure-II: represents a diagram between Ca15.3 and IL4

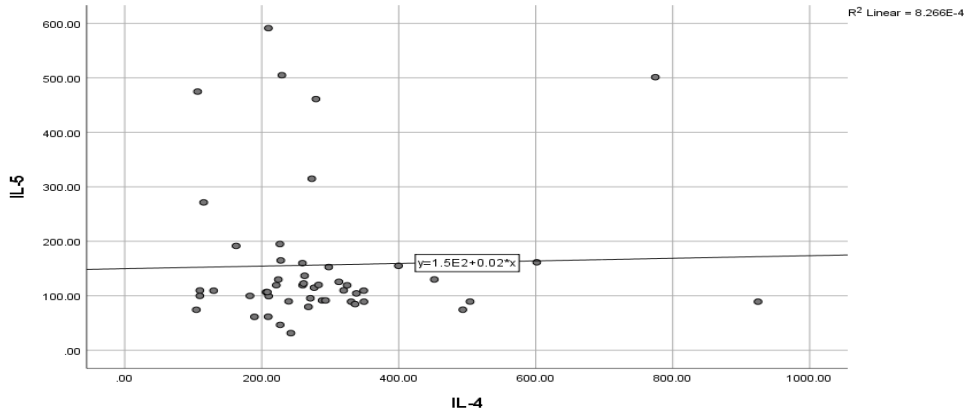

Figure-(III: represents a diagram between IL4 and IL5

DISCUSSION

This study showed a highly significant P value (<0.05) increase breast cancer women in comparison with control group as shown in (Table I). This increase was due to increased secretion of Ca15 – 3 from cancer cells and this result agrees with Hassan *et al*. [13] that demonstrated elevated levels of Ca 15-3 in breast cancer patients. The normal levels of Ca15–3 were considered< 30 U / ml [14]. The increase of Ca 15-3 after mastectomy confirmed the advantage from this marker for progression of disease [15]. Bacalbasa *et al*. and Othman *et al*. showed higher concentration of Ca 15-3 in breast cancer patients than healthy women [16,17] also increased level of Ca 15-3 after surgery > 30 U / ml in comparison with control group. The increase in Ca 15-3 was accompanied with cancer development and this elevation indicated that patients in advanced stage of disease [18]. Singh et al. [19] showed that high concentrations of Ca 15-3 in patients with breast cancer as compared with control group [19]. Smoking is one of the factors that affect breast cancer. Smoking induced mechanism underlying tumor progression and metastasis. Kispert, and McHowat [20], demonstrated that increased motility and Epithelial to Mesenchymal Transition (EMT) in breast tumor cells exposed to cigarette smoking [20]. Kalluri, and Weinberg [21] also showed that EMT has the central mechanism for acquiring malignant phenotype by epithelial cancer cells and subsequently acquirement of invasiveness and begin invasion process by passing through basement membrane and ultimately lead to metastatic propagation [21]. The invasive ductal carcinoma can metastasize from the primary site to many other organs and commonly to bone, lung and liver [20]. Noonan *et al*. [22] showed increasing level of Ca Ca15.3 in lung adenocarcinoma patients when comparison with other tumor markers [22]. Present study agreed with results of Xian-long *et al*. [24]on breast cancer that showed no significant correlation between family history [23]. Also Yang et al. showed that there was no significant correlation between family history for breast cancer and the patients are underwent surgery and did not receive any chemo and/or radiotherapy, immune - modulatory or hormonal therapy prior surgery or through time of follow-up [24].

CONCLUSION

The study showed an association between high levels of IL- 4 in the blood and breast cancer, and it was found that this increase is related to the advanced stage of the disease. In addition, from our study there is no statistically significant association between a statistically significant decrease in the level of serum IL-5 and the advanced stage of human breast cancer.

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