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ORIGINAL ARTICLE

Immunohistochemical Study of IL-6 and IL-10 Expression in Breast Cancer Correlations with ER, PR, Her-2, Ki-67, and Clinicopathological Features in Iraqi Women

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ABSTRACT:

Background: Breast cancer was the most prevalent malignancy identified in the studied female population. and is also the primary reason for cancer-related fatalities among women. Releasing inflammatory cytokines, such as IL-6 and IL-10, induces inflammation and subsequent tissue damage.

Aim: The research aims to learn more about the connection between IL-6 and IL-10 immunohistochemical expression "ER, PR, and Her-2 status in breast cancer in association with Ki-67 status ".

Materials and Methods: A total of 36 breast cancer cases were analyzed and reviewed; ER, PR, Her-2, Ki-67, IL-6 and IL-10 were examined in tumor cells.

Results: The high percentage of IL-10 expression was positive and showed statistically significant associations with ER and PR (P = 0.005). Similarly, cells with a high Ki-67 concentration but a low Her-2 level with a low percentage of IL-6 had positive expression.

Conclusion: This research established a link “between IL-6, IL-10 expression with the presence of ER, PR, Ki-67, and a lack of Her-2”.

KEYWORDS: IL-6, IL-10, breast cancer, immunohistochemistry

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INTRODUCTION:

The recent research of breast cancer, published by the World Health Organization, demonstrates that it is the foremost dominant form of malignancy across 154 of the total 185 countries in the world. Additionally, it stands as the main reason for death is the cancer-related fatalities in over 100 countries. Breast cancer continues to be the most prevalent form of cancer among women worldwide, constituting 25% of female cancer diagnoses (Al-Shiekh et al., 2020, Shaheen et al., 2021).

In 2018, there were around 2.1 million newly reported cases of this disease (Bray et al.,2018). It is deemed the primary contributor to mortality rates among American women. This year, there are 268,600 new breast cancer diagnoses and an estimated 41, 760 fatalities, resulting from this condition (Siegel et al.,2018).

According to the Iraqi Ministry of Health and the Iraqi Cancer Registry, cancer of the breast is the foremost among the top ten malignant neoplasms in females in Iraq, accounting for 19.5% of the total (4996 cases) and 34.3% of female cancers (4922 cases). In 2016, it was recorded about 897 deaths among women due to that, and it is registered as the primary reason for cancer mortality amongst females with a ratio of about 24% in Iraq, along with a ratio of 12.1% recorded among females and males (Iraqi cancer registry: 2016). The variations in incidence rates typically show that there are risk factors related to a higher prevalence, particularly among transitioning regions in Asia, Africa and South America (Bray et al.,2004).

A heterogeneous disease is caused by serious genetic and epigenetic events that result in cell growth dysregulation and apoptosis circumvention. In addition, the capacity that gets through the basement membrane and enters the underlying tissue. Although epidemiological studies have identified environmental, genetic, and lifestyle factors as risk contributors, the precise mechanisms underlying breast cancer development remain unclear. (Antonio et al., 2006). Many clinical and pathological characteristics, such as tumor size, lymph node metastasis, differentiation, histological form, vascular invasion, age and menstruation, have been used to predict prognosis and direct treatment of breast cancer (Watanabe et al., 2010). The identification and characterization of biomarkers associated with breast cancer, such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal receptor-2 (HER-2), have substantially contributed to the advancement of novel therapeutic interventions (Kwa et al., 2017). The proliferation markers of Ki-67, ER, PR, and

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Her2 status are used as surrogates to determine the intrinsic subtype of breast cancer (Goldhirsch et al., 2013). Hormones primarily influence cancer risk by regulating cell division, differentiation and the proliferation of susceptible cells (Jarallah et al ., 2023).

The host's immune system has a substantial influence on the onset and spread of cancer of breast (Gil Del Alcazar et al., 2017). Plans for breast cancer treatment focusing on enhancing the body's natural antitumor defenses are currently under investigation (D. Hammerl et al., 2017). Inflammatory cytokines that are linked to cancer have a big effect on the growth and environment of the tumor. Cancer's life cycle includes tumor cell transformation, angiogenesis, invasion, stopping apoptosis, immunosurveillance, drug resistance, and metastasis (S I Grivennikov et al., 2017). Interleukin-6 (IL-6) is a cytokine that is very important for breast cancer. This cytokine is involved in both normal and abnormal ways that cells work. IL-6 can act as an autocrine or paracrine cancer cell growth factor, which makes breast cancer spread (Heikkilä et al ., 2008; Knupfer et al., 2007). According to these findings, blocking the interaction between IL-6 and its receptor with certain antibodies has been suggested as an extra way to treat cancer in cancer cells that have been exposed to IL-6 or that make the cytokine on their own (Conze et al., 2001; Sehgal et al., 1991 Selander et al., 2004).

Interleukin-10 (IL-10) is a well-established cytokine with immunosuppressive properties that play a significant role in regulating the cellular immune response. Its actions include inhibition of the secretion of pro-inflammatory factors and promotion of tumor cell proliferation and metastasis via immunosuppression (Yuan et al., 2020). The immunosuppressive effects of IL-10 are mediated by the synthesis of aspects related to tumor necrosis, IL-1, IL-12, and chemotactic aspects, as well as the down regulation of stimulatory molecules CD86 and CD80 found onto the surface of the tumor (Ortiz et al., 2020). Furthermore, IL-10 can prompt the expression and synthesis of IL-6 and up-regulate B-cell lymphoma-2, which consequently brings about alterations in the proliferation and apoptosis of neoplastic cells. In addition, IL-10 suppresses the creation of TNF-α, IL-1b, IL-6 and MMP-9 within tumors using down-regulating vascular endothelial growth factors (Fröschen et al., 2020). IL-10 has an attribution that includes tumor-promoting and tumor-inhibiting, and it was found that the effects of therapeutic through distinct mechanisms present its agonists and antagonists evoke (Sheikhpour et al., 2018). Within the tumor microenvironment, immune cells can release a significant quantity of IL-10 and tumor cells can also produce this cytokine. In the context of ovarian cancer, the expression of hypoxia-inducible factor 1α is upregulated by tumor-associated macrophages through the secretion of IL-10. This process subsequently facilitates the invasion and metastasis of cancer cells (Levin et al., 2024). Similarly, upregulated IL-10 expression is related to capsule invasion and the metastasis of

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lymph nodes in papillary thyroid carcinoma (Wang et al., 2019).

The signal transducer and activator of transcription 3(STAT3) is the common pathway between IL-6 and IL-10(Braun et al., 2013). Although they both signal through STAT3, IL-6 is pro-inflammatory whereas IL-10 is anti-inflammatory and suppresses the expression of other cytokines by immune cells. In dendritic cells, these reactions have been explained by the transient stimulation of STAT3 via IL-6 as opposed to the protracted effect of IL-10 via activation of the suppressor of cytokine signaling (SOCS3) (Braun et al., 2013).

It was reported that there is a potential prognostic biomarker in the cancer of the breast. This leads to the growth and progression of the tumor. Hence, cytokine and chemokine blockade were necessary for their usage. Additionally, there is a crucial need for examining anti-inflammatory drugs in the chemoprevention and dealing with malignant diseases (Braham et al., 2017).

This study examined IL-6 and IL-10 countenance in the cancer of breast and its connection to prognostic indicators, including age of the patient beside tumor stage, grade of tumor, lymph node metastasis, and ER, PR, Her-2, and Ki-67 status.

MATERIALS and METHODS

The study analyzed 36 formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue samples. The research study was conducted between November 2014 and March 2016. The central public health lab provided all of the samples. After examining hematoxylin and eosin-stained tissue sections, the best paraffin blocks were selected from each specimen for immunohistochemistry preparations in the central public health lab's histopathology unit. Each tissue block was sectioned into seven 4-µm slides and stained with hematoxylin and eosin (H& E) and immunohistochemically for IL-6, IL-10, Ki-67, ER, PR, and HER-2.

Immunohistochemistry

Following a standardized procedure, immunohistochemistry was used to detect the IL6, IL-10, Ki67, ER, PR, and HER-2. A total of four m-thick paraffin sections were formed. Tissues fixed in formalin were deparaffinized in xylene and eventually rehydrated by dipping them in 100 percent for 5 min, 95 percent for 3 min, 70 percent alcohol for 3 min and distilled water for 1 min. Microwave treatment in citrate buffer was used to retrieve antigen (10 min, PH-6.0). After serum blocking, the primary antibody IL6, IL-10(Abcam, company, UK) was applied in a diluted (1:100) concentration "and incubated overnight at four °C." The secondary antibody application and staining were carried out

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according to a standard protocol using the Universal Detection Kit (Abcam, UK) and the Diaminobenzidine (DAB) substrate in a dark room and incubated in a humidity chamber for 10 min (Abcam ab80436 Kit). Then, units were treated with hematoxylin, dehydrated, and placed in an aqueous solution before being examined.

For IL-6, IL-10 a negative control was used in which the primary antibody was removed and replaced with buffered-phosphate saline. For precision and standardization of the elaborated IHC results of this marker primary and secondary antibodies, IL-6 was added to the process with the breast tissue sections in the same run.

Each field was assessed for its IL-6 immunoreactive population. "Both the percentage of positive cytoplasm and the degree of staining were used to categorize the neoplastic cells":
The percentage was categorized as follows: 0 if less than 1%, 1 if greater than 1% but less than 25%, 2 if greater than 25% but less than 50%, 3 if greater than 50% but less than 75%, and 4 if greater than 75%. The intensity of staining was rated as 1(weak), 2(moderate), and 3 (strong). The last classification was determined by multiplying mean values of percentage with intensity. Resulting in scores ranging from (0 to 12). The classifications were as follows: score 0 (value= 0); score 1(0.1-1); score 2(>1 but <4); score 3(>4 but <8) and score 4(>8) (Azare et al., 2011). Interleukin 10 expression in tumor tissue was observed as positive once 10% or more cells of tumor were cytoplasmic positive (Bhattacharjee et al., 2016).

STATISTICAL ANALYSIS:

The results were presented as percentages and statistically analyzed by T-test analysis using the Statview 0.5 program. Values were considered significant if the p values considered significant if the p value was less than 0.05.

RESULTS

Regarding Table (1), the findings in this study showed of 36 patients with breast cancer 26 patients (72%) were negative expression of IL-6. 21 (58%)>40, 5 (14%) <40. While, there was a high percentage of IL-6 positive expression 8 patients (22%) among patients >40 compared to 2 patients (6%) among patients ≤ 40 with a significant difference in p. value (0.012).
Seven out of twenty-two patients (19%) in stage 2 and grade 2 breast cancer showed positive

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expression of IL-6 by immunohistochemistry, which was statistically significant (p. values:0.035 and 0.032, respectively).

Among breast cancer histotypes, IL-6 positivity was observed in 19% (7 cases) of invasive ductal carcinoma and 8% (3 cases) of invasive lobular carcinoma. The result of regional L. N in Table 1 showed a significant difference p.value:0.052. The markers investigated are presented in this table. It was shown that there is statistically noteworthy correlation between ER and PR expression and positive IL-6 expression; (p.value: 0.005), with 7 instances (19%) exhibiting positive ER and PR.

As shown in Table 1, expression of IL-6 in cancer cells was associated with Her-2. The highest percentage of IL-6 positive "expression was 9 cases (25%) in Her-2 negative expression", while the lowest rate of positive IL-6 was 1 case (3%) in Her-2 positive expression with a significant difference p.value:0.029. Regarding IL-6 positive expression of breast cancer patients with ki-67 had a higher percentage of expression in 9 cases (25%) in positive ki-67 than negative expression in 1 case (3%) p.value:0.015. IL-10 positivity was observed in 69% (25 cases) of patients older than 40 years and 17% (6 cases) of patients ≤40 years old.

Table 1: Expression of IL-6 in patients with breast cancer about the clinical pathological characteristics, ER, PR, and HER's-2 and Ki-67 expression.

	IL-6+		**IL-6-**		
	nr	**%**	**nr**	**%**	**P-value**
age					
≤40	2	6%	5	14%	0,095
>40	8	22%	21	58%	0,026
P-value		0,012		0,032	
grade					
1	0	0%	3	8%	0,044
2	7	19%	19	53%	0,023
3	2	6%	3	8%	0,050
high	1	3%	1	3%	0,964
P-value		0,035		0,021	
stage					
1	0	0%	5	14%	0,021
2	7	19%	13	36%	0,028
3	3	8%	5	14%	0,060
4	0	0%	3	8%	0,044
P-value		0,032		0,026	
histotype					
invasive ductal carcinoma	7	19%	25	69%	0,008

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invasive lobular carcinoma	3	8%	1	3%	0,065
P-value		0,047		0,005	
regional LN					
metastasis	3	8%	8	22%	0,041
no metastasis	7	19%	18	50%	0,030
P-value		0,052		0,029	
hormone receptor status					
ER%+	7	19%	14	39%	0,043
ER%-	3	8%	12	33%	0,038
P-value		0,052		0,076	
PR+	7	19%	13	36%	0,043
PR-	3	8%	13	36%	0,026
P-value		0,052		0,996	
HRE2%+	1	3%	4	11%	0,066
HRE2%-	9	25%	22	61%	0,022
P-value		0,029		0,006	
Ki67%+	9	25%	21	58%	0,021
Ki67%-	1	3%	5	14%	0,500
P-value		0,015		0,006	

The high percentage of positive expression of IL-10 was in stage 2 (T2; 16 patients, 44%) while a low percentage of positive expression of IL-10 was in stage 4 (T4; 3 patients, 8%) with a significant difference P.value:0.031. According to grade, the high percentage of positive expression of IL-10 was in grade 2 (grade 2; 24 patients, 67%) compared with the low percentage of positive expression in grades 1, and 4 (grade 1,4; 2 patients, 6%) with difference significant p. value: 0.022. Regarding histology, 28 cases (78%) invasive ductal carcinoma and 3 (8%) lobular carcinomas were positive expression of IL-10, value: 0.002. The majority of tumors in our study were no metastasis 25 cases (21 out of 25) 58 % were positive expression for IL-10 while (10 out of 11) metastasis cases were IL-10 positive expression, p.value:0.024 as shown in table 2.

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Table 2: Expression of IL-10 in patient with breast cancer relation to the clinico pathological characteristics, ER, PR, and HER's-2 and Ki-67 expression

	il 10+		**il10-**		
	nr	**%**	**nr**	**%**	**P-value**
age					
≤40	6	17%	1	3%	0,034
>40	25	69%	4	11%	0,003
P-value		0,026		0,033	
grade					
1	2	6%	1	3%	0,062
2	24	67%	2	6%	0,002
3	3	8%	2	6%	0,052
high	2	6%	0	0%	0,041
P-value		0,022		0,079	
stage					
1	5	14%	0	0%	0,024
2	16	44%	4	11%	0,016
3	7	19%	1	3%	0,023
4	3	8%	0	0%	0,013
P-value		0,031		0,027	
histotype					
invasive ductal carcinoma	28	78%	4	11%	0,006
invasive lobular carcinoma	3	8%	1	3%	0,070
P-value		0,002		0,032	
regional LN					
metastasis	10	28%	1	3%	0,031
no metastasis	21	58%	4	11%	0,024
P-value		0,024		0,043	
hormone receptor status					
ER%+	17	47%	4	11%	0,031
ER%-	14	39%	1	3%	0,042
P-value		0,059		0,078	
PR+	16	44%	4	11%	0,034
PR-	15	42%	1	3%	0,039
P-value		0,098		0,078	
HRE2%+	4	11%	1	3%	0,078
HRE2%-	27	75%	4	11%	0,036
P-value		0,007		0,078	
K167%+	25	69%	5	14%	0,035
K167%-	6	17%	0	0%	0,037
P-value		0,008		0,037	

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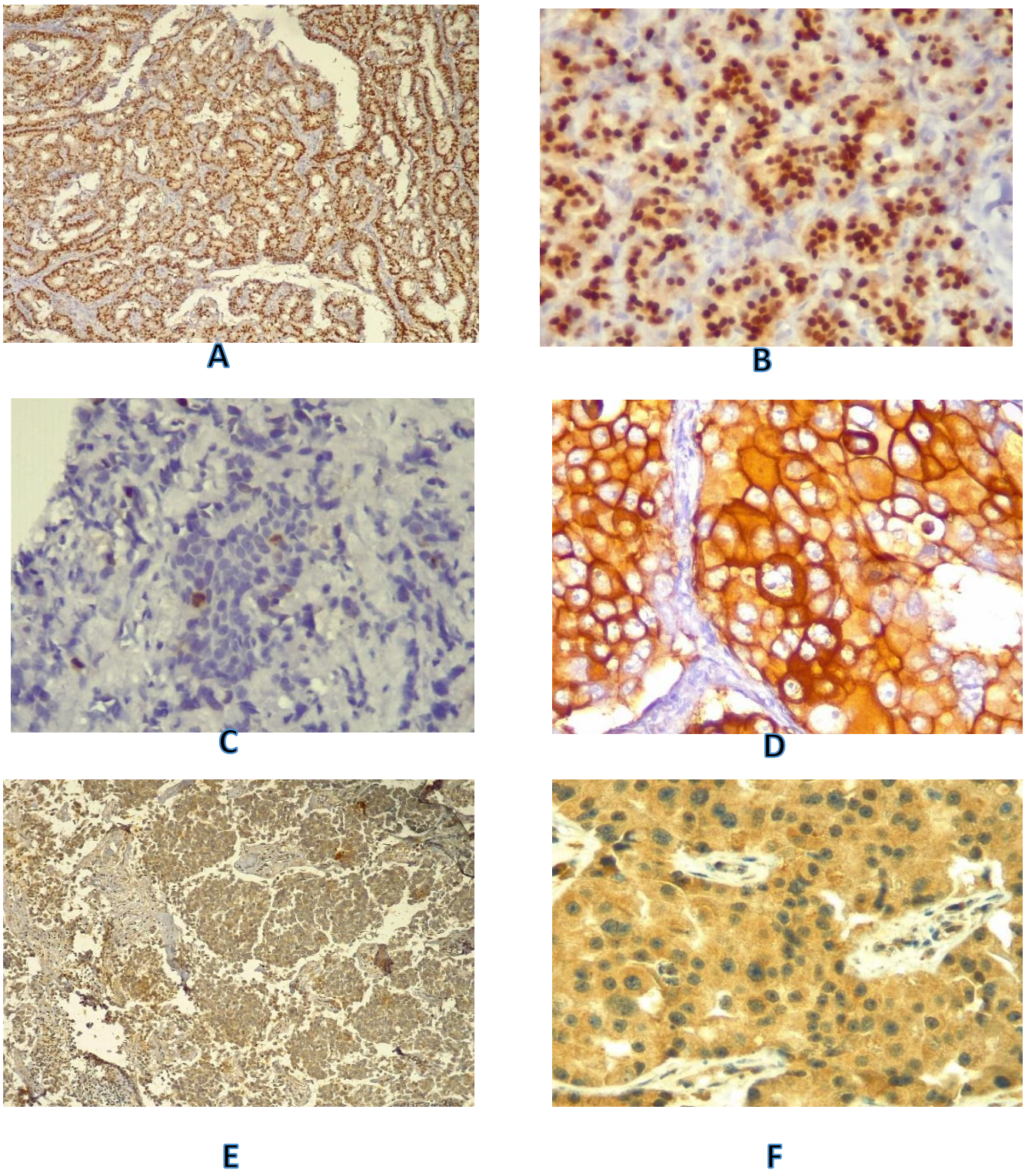

Figure 1: Immunohistochemical staining of (A) strong nuclear expression of PR, (B) strong diffused ER expression in 10 HPF, (C) focal moderate expression of Ki67 at HPF, (D) strong positive expression of her-2 score 3 at 40 HPF, (E) diffused cytoplasmic expression of tumor cells of IL-6 at 10 HPF. (F) diffused cytoplasmic expression of tumor cells of IL-10 at HPF.

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DISCUSSION:

The association of IL-10 with ER, PR, Her-2 and Ki67 is shown in Table 2. Positive expression of IL-10 in 17 cases (17%) from ER-positive tumors compared to ER-negative tumors which were 14 cases (39%) IL-10 positive expression with significant difference p: value 0.059. Expression of IL-10 in PR+, PR- was 16 cases (44%), 15 cases (42%) respectively with no significant difference P. value: 0.098. As shown in Table 2 expression of IL-10 was positive in 27 cases (75%) in Her-2 negative expression while positive expression of IL-10 was in 4 cases (11%) of Her-2 positive expression with high significant p.value:0.007. In this study, IL-10 expression was positive in 25 cases (69%) from ki-67 positive expression compared to ki-67 negative expression with a high significant difference p.value:0.008.

The multitasking cytokine that controls immunological response, inflammation, and blood cell production is IL-6. Normal cells, including monocytes and macrophages, survive (Ásgeirsson et al., 1998). Breast, prostate, colon, and ovarian cancers all express this gene to variable degrees (Chung et al., 2006; Coward et al., 2011; Hobisch et al., 2000; Karczewska et al., 2000).

IL-6 plays a crucial role in multiple oncogenic processes, including apoptosis resistance, tumor proliferation, migration, invasion, angiogenesis, and metastasis. Among other tumor characteristics (Qu et al., 2015). Both a cell-surface receptor of IL-6 (IL-6R) and a soluble variation known as the soluble IFN- receptor (sIFNR) are involved in the signaling of "IL-6 (SIL-6R)" (Scheller et al.,2006) , which activates the STAT3 transcription factor (Ma et al., 2020). IL-6 and IL-6R were overexpressed in BC (Garcia-Tunon et al., 2005), and high serum IL-6 levels were associated with a worse prognosis. In addition to bone metastases (Sanguinetti et al., 2015), high serum level of IL-6 is connected with breast cancer and might have detecting and indicative helpfulness (Al.Thwani et al., 2012). In addition, multidrug-resistant cancer cell lines (Pu et al., 2006; Wang et al.,2010) and the basal-like BC phenotype (Sanguinetti et al.,2015) express IL-6 at considerably higher levels. Overexpression of the transcription factor STAT3 has also been associated with poorer permanence in solid tumor patients (Wu et al, 2016) and higher treatment resistance in cancer cells (Spitzner et al., 2011).

IL-6 expression was positive in 28% of breast cancer patients in our sample. However, there is a connection between IL-6 expression observer and age >40, grade 2, stage 2, and invasive ductal carcinoma with no metastasis, ER+, PR+, Her-2 –ve, and Ki67+ve. High IL-6 expression was observed in patients older than 40, with ER-positive, PR-positive, and HER-2 .

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Ahmad et al. found a correlation between high IL-6 expression and ER positivity. Despite its ability to inhibit autocrine downstream signaling in cancer cells, stromal cells may exhibit ER-active signaling. It is plausible that, unlike in BC, the ER pathway plays a lesser role in malignancies, in which IL-6 has been shown to promote tumor growth (Ahmed et al 2018). According to a paper by Tripsianis et al., the functional contact between their molecular pathways can enhance breast cancer invasiveness and metastasis by enhancing their molecular pathways' interaction (2013). This discovery contradicts our findings. Our findings comparable with those of Cho et al., (2013), who found a correlation between IL-6 and IL-8 levels and breast cancer recurrence in patients with her-2-negative tumors alone. When overexpressed, the type 1 transmembrane receptor tyrosine kinase HER2 may bind to any receptors of tyrosine kinase binding copartner independent of the presence or absence of a ligand (Elster et al., 2015). Signals from passageways like the phosphoinositide-3-kinase pathway stimulate the growth of cells, replication, and metastasis (Subbiah et al., 2014). By interfering with the development of any of these pathways, it can inhibit HER2-positive illness.

Fontanini et al., (1999) also identified a link between IL-6 expression and ER, which is inconsistent with these findings. The ER-positive MCF 7 cell line does not generate IL-6 but the ER-negative MD MBA 231 cells can do. Chavey et al., (2007) used whole breast cancer (BC) tissue lysates to reveal a negative association between IL-6 expression in ER-positive BC. There may be a similarity between the current research's findings and those of Chavey's study and the technique employed to quantify IL-6 “i.e., tumoral vs. whole tissue lysates”. In BC cell lines, there is proof that ER activation decreases the level of STAT3 signaling (Yamamoto et al., 2000).

Estrogen and progesterone receptor expression are the most important and useful predictors we have right now. When IL-6 is used to target estrogen receptor (ER)-positive breast cancer cells, it stops them from growing. However, the main effect of IL-6 in breast cancer is to give a bad prognosis. Several studies have linked high levels of circulating IL-6 to a bad prognosis. In keeping with recent research on “BC patients (n=149) (Fontanini et al., 1999), we find no indication of a significant correlation between IL-6 expression and lymph node metastasis. Recent results by Ahmad et al., support IL-6's link with decent prediction biomarkers in BC by establishing a considerable correlation between IL-6 expression and negative lymph node status in our study (Ahmad et al., 2018). The precise mechanisms underlying the anti-inflammatory properties of

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interleukin-10 in relation to breast cancer remain incompletely elucidated and subject to ongoing debate (Al Ameri et al., 2020).

Our research looked at the relationship between IL-10 expression in breast cancer cells and clinicopathological characteristics and found that a high percentage of positive IL-10 expression was associated with age > 40, grade 2, stage 2, and invasive ductal carcinoma with metastasis. Positive ER, PR, negative Her-2, and positive Ki-67 expression were also related. In this study, 31 of 36 patients had IL-10 expression in their breast cancer tissue. Similar findings have been found in a few other studies. In a study of 60 breast cancer patients, immunohistochemistry revealed that 60% of the patients had IL-10 expression (Bhattacharjee et al., 2016). IHC revealed high expression of IL-10 in 85 percent of the breast cancer (Lianes-Fernandez et al., 2006). In addition, a connection between IL-10 and Her-2, ER-positive expression has been discovered. Our findings support previous research on the connection between IL-10 and ER positively.

High expression of IL-10 was found to be significantly linked to the lower tumor grade, positive ER, positive PR, and negative Her-2 in a recent study by Ahmed *et al* (2018), which agrees with our findings. In breast cancer tissues, Bhattcharjee et al. (2016) discovered a statistically important association between IL-10 expression and Her-2+ve, ER-ve, and PR-ve. This disagrees with our findings, which found a statistically significant connection between IL-10 expression in breast cancer and Her-2-, PR+, and ER+ status. Another research by Chavey *et al* found no connection between IL-10 expression and tumor Her-2 status.

According to MA and KONG, (2021), the countenance of IL-10 in the tissues of breast cancer significantly increases, which is consistent with our findings and indicates a positive correlation with lymph node metastasis in patients have breast cancer. Despite clinical parameters of ER, PR, and HER-2 that linked to this cancer, the observations of MA and Kong did not reveal a distinct correlation between these parameters and the expression of IL-10 ($P>0.05$). Additionally, the expression of IL-10 did not exhibit a conspicuous correlation with patient age, tumor size, or pathological grade ($P>0.05$). This finding is not congruent with our results (MA, T. and Kong, 2021).

IL-10, initially identified as the cytokine synthesis inhibitory factor, has been observed to hinder the generation of specific cytokines. It is expressed by a variety of immune cells such as TH0, TH1, TH2, Treg, cytotoxic T cells, mast cells, and activated monocytes. IL-10 is recognized as the most extensively studied and recognized anti-inflammatory cytokine (Ekmekciogl et al., 2008).

Numerous investigations have elucidated elevated IL-10 mRNA expression in breast tumor cells, indicating that IL-10 plays a significant role in the development of mammary carcinogenesis.

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Additionally, its multifaceted functions in the pathogenesis, metastasis, progression and the growth of this type of cancers (Joimel et al., 2010; Kozłowski et al., 2000; Lyon et al., 2003) have been demonstrated to encompass various roles, including immunosuppressive and antiangiogenic functions. In breast cancer subtypes ER-positive, non-triple-negative, non-basal and progesterone (PR)-positive, it was indicated that the IL-10 is a potent predictor of disease-free survival (Ahmed et al., 2018).

It was reported that the role of the estrogen receptor during cytokine production and regulation is still unknown (Kassi et al., 2010). Besides, Interleukin-10 is found within tumor cell cytoplasm and stroma. Additionally, cytokines counting IL-10 were existing over-expressed in estrogen receptor (ER)-negative breast carcinoma (Cho et al., 2013).

IL-10 was only stated in ER-negative tumors (Khan et al., 2012), and it was found further the expression of the transcription factor of activator protein (AP) -1 that is greater in ER-negative than in ER-positive tumors. The enlarged AP-1 appearance relates to augmented IL-10 (Khan et al., 2012). This indicates that IL-10 assists as a prognostic marker in the cancer of breast (Li et al., 2014). Likewise, G1, a G protein-coupled estrogen receptor (GPER) agonist and Thalidomide encourage IL-10 appearance through effecting on Th17 or hybrid T-cell populations (Khan et al., 2012). It was also stated that enlarged IL-10 appearance in the cell of tumor cytoplasm is linked to the lower grade and positive estrogen receptor (Li et al., 2014) which is consistent with our results. IL-10 can counteract the effects of IL-6 by inhibiting its production and signaling. This balance between pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines is crucial in determining the immune response to breast cancer and may limit tumor growth in some contexts

Interleukin (IL)-10, a multifunctional immune-regulatory cytokine with both immunosuppressive and antiangiogenic functions, is produced by cells of immune such as T lymphocytes, macrophages and cells of natural killer. It was presented that IL-10 promotes tumor cell proliferation and metastasis through immune-suppression are resulted from those effects. Interleukin-10-mediated immunosuppression is simplified by the synthesis of tumor necrosis features such as chemokines, IL-1, IL-12 and down-regulation of surface co-stimulatory molecules CD80 and CD86 onto tumors.

Also, Interleukin-10 indorses IL-6 appearance and synthesis, resulted in the proliferation of cells through B cell lymphoma-2 (Bcl-2) up regulation and deviations the proliferation/apoptosis toward neoplastic cell proliferation. Besides, IL-10 obstructs tumorigenesis by down-regulating of VEGF, IL-1b, TNF-α, IL-6, and MMP-9. Interleukin-10 also inhibits nuclear factor-κB (NF-κB). Interleukin-10

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has been reported to have both tumor-promoting and -inhibiting properties. It appears that IL-10 agonists and antagonists may have therapeutic effects through different mechanisms. Besides, IL-10 gene polymorphisms may determine breast cancer susceptibility ((Sheikhpour et al., 2017)). However, the conflicting effects of IL-10 make therapeutic manipulation challenging.

Based on a reported work, the role of IL-6 and IL-10 in BC is mostly studied in whole tissue extracts or serum regardless the localization of tissue of the tumor. The appearance of IL-10 and IL-6 (macrophage-associated cytokines) in tumor tissue was researched with small patient cohorts and limited information about prognostic significance (Ahmed et al., 2017). The discrepancy between our research findings and those of other studies can attributed to Using different research methods. The variation in sample characteristic size, demographics, and environment, can also contribute to different results.

CONCLUSION:

These findings provide insight into the role of IL-10 and IL-6 in the progression of breast cancer. Our results present that a high expression level of IL-10 in Breast cancer tissues is linked to specific clinical pathological criteria, such as the presence of the (ER), the presence of the (PR), the absence of the (Her-2) gene, and a high Ki-67 index". The presence of high levels of IL-10 could indicate an adaptive mechanism by which tumors evade immune-suppressing pro-inflammatory signals like those from IL-6. A deep understanding of cellular functions and molecular mechanisms of IL-10 support us to establish potential therapeutic agents for controlling the IL-10-related immune response to cells of tumor. Finally, the usage of IL-10 agonists and antagonists proposals benefits in treating breast cancer. However, further extensive research is necessary to develop novel targeted therapies and enhance patient outcomes.

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REFERENCES

1. Ahmad, N., Ammar, A., Storr, S. J., Green, A. R., Rakha, E., Ellis, I. O., & Martin, S. G. (2018). IL-6 and IL-10 are associated with good prognosis in early stage invasive breast cancer patients. *Cancer Immunology, Immunotherapy, 67*(4), 537–549.

ISSN:2523 - 0522
Volume 8, Issue 1, April 2025
ISRAA UNIVERSITY JOURNAL OF APPLIED SCINCE (IUJAS)

2. Al-Ameri, S., Al-Ezzi, A., Zhao, H., Yali, L., Nana, D., Nsenga, A., Gabriel, A., Yvette, U., & Haiting, M. (2020). Function and regulation of interleukin-10 in breast cancer. *Annals of Research, 3*, 162–183.
3. Al-Shiekh, S. S. A., Ibrahim, M. A., Etewa, B. B., & Alajerami, Y. S. (2020). The effect of a training program on female breast self-examination knowledge and practice among Al-Azhar University students, Gaza Strip, Palestine. *Israa University Journal of Applied Sciences, 4*(1).
4. Antoniou, A. C., & Easton, D. F. (2006). Models of genetic susceptibility to breast cancer. *Oncogene, 25*(43), 5898–5905.
5. Ásgeirsson, K. S., Ólafsdóttir, K., Jónasson, J. G., & Ögmundsdóttir, H. M. (1998). The effects of IL-6 on cell adhesion and e-cadherin expression in breast cancer. *Cytokine, 10*(9), 720–728.
6. Azare, J., Doane, A., Leslie, K., Chang, Q., Berishaj, M., Nnoli, J., Mark, K., et al. (2011). Stat3 mediates expression of autotaxin in breast cancer. *PLOS ONE, 6*(11), e27851.
7. Bhattacharjee, H. K., Bansal, V. K., Nepal, B., Srivastava, S., Dinda, A. K., & Misra, M. C. (2016). Is interleukin 10 (IL10) expression in breast cancer a marker of poor prognosis? *Indian Journal of Surgical Oncology, 7*, 320–325.
8. Braham, J. A., Balti, M., Mouna, B. A., Manel, A., Zribi, A., Sonia, B. N., Sana, F., & Haddaoui, A. (2017). The role of IL10, IL6 and TNFα as potential prognostic markers in breast cancer in Tunisia. *Annals of Oncology, 28*, xi13.
9. Braun, D. A., Fribourg, M., & Sealfon, S. C. (2013). Cytokine response is determined by duration of receptor and signal transducers and activators of transcription 3 (STAT3) activation. *Journal of Biological Chemistry, 288*(5), 2986–2993.
10. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians, 68*(6), 394–424.
11. Bray, F., McCarron, P., & Parkin, D. M. (2004). The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Research, 6*, 1–11.
12. Chavey, C., Bibeau, F., Bourgade, S. G., Burlinchon, S., Boissière, F., Laune, D., Roques, S., & Lazennec, G. (2007). Oestrogen receptor negative breast cancers exhibit high cytokine content. *Breast Cancer Research, 9*, 1–11.
13. Cho, Y. A., Sung, M., Yeon, J., Ro, J., & Kim, J. (2013). Prognostic role of interleukin-6, interleukin-8, and leptin levels according to breast cancer subtype. *Cancer Research and Treatment: Official Journal of Korean Cancer Association, 45*(3), 210–219.
14. Chung, Y., Chaen, Y., & Hsu, C. (2006). Clinical significance of tissue expression of interleukin-6 in colorectal carcinoma. *Anticancer Research, 26*(5B), 3905–3911.
15. Conze, D., Weiss, L., Regen, P. S., Bhushan, A., Weaver, D., Johnson, P., & Rincón, M. (2001). Autocrine production of interleukin 6 causes multidrug resistance in breast cancer cells. *Cancer Research, 61*(24), 8851–8858.
16. Coward, J., Kulbe, H., Chakravarty, P., Leader, D., Vassileva, V., Leinster, D. A., Thompson, R., et al. (2011). Interleukin-6 as a therapeutic target in human ovarian cancer. *Clinical Cancer Research, 17*(18), 6083–6096.
17. Ekmekcioglu, S., Kurzrock, R., & Grimm, E. A. (2008). Hematopoietic growth factors and cytokines. In *The molecular basis of cancer* (pp. 605–619).
18. Elster, N., Collins, D. M., Toomey, S., Crown, J., Eustace, A. J., & Hennessy, B. T. (2015). HER2-family signalling mechanisms, clinical implications and targeting in breast cancer. *Breast Cancer Research and Treatment, 149*, 5–15.

ISSN:2523 - 0522
Volume 8, Issue 1, April 2025
ISRAA UNIVERSITY JOURNAL OF APPLIED SCINCE (IUJAS)

19. Fontanini, G., Campani, D., Roncella, M., Cecchetti, D., Calvo, S., Toniolo, A., & Basolo, F. (1999). Expression of interleukin 6 (IL-6) correlates with oestrogen receptor in human breast carcinoma. *British Journal of Cancer, 80*(3), 579–584.
20. Fröschen, F. S., Schell, S., Schildberg, F. A., Klausing, A., Kohlhof, H., Gravius, S., & Randau, T. M. (2020). Analysis of synovial biomarkers with a multiplex protein microarray in patients with PJI undergoing revision arthroplasty of the hip or knee joint. *Archives of Orthopaedic and Trauma Surgery, 140*, 1883–1890.
21. García-Tuñón, I., Ricote, M., Ruiz, A., Fraile, B., Paniagua, R., & Royuela, M. (2005). IL-6, its receptors and its relationship with bcl-2 and bax proteins in infiltrating and in situ human breast carcinoma. *Histopathology, 47*(1), 82–89.
22. Gil, D. A., Huh, S. J., Muhammad, B. E., Trinh, A., Liu, L. L., Beca, F., Zi, X., et al. (2017). Immune escape in breast cancer during in situ to invasive carcinoma transition. *Cancer Discovery, 7*(10), 1098–1115.
23. Goldhirsch, A., Winer, E. P., Coates, A. S., Gelber, R. D., Piccart-Gebhart, M., Thürlimann, B., Senn, H.-J., et al. (2013). Personalizing the treatment of women with early breast cancer: Highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Annals of Oncology, 24*(9), 2206–2223.
24. Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell, 140*(6), 883–899.
25. Hamidullah, B. C., & Konwar, R. (2012). Role of interleukin-10 in breast cancer. *Breast Cancer Research and Treatment, 133*, 11–21.
26. Hammerl, D., Smid, M., Timmermans, A. M., Sleijfer, S., Martens, J. W. M., & Debets, R. (2018). Breast cancer genomics and immuno-oncological markers to guide immune therapies. *Seminars in Cancer Biology, 52*, 178–188.
27. Heikkilä, K., Ebrahim, S., & Lawlor, D. A. (2008). Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. *European Journal of Cancer, 44*(7), 937–945.
28. Hobisch, A., Rogatsch, H., Hittmair, A., Fuchs, D., Bartsch, G., Jr., Klocker, H., Bartsch, G., & Culig, Z. (2000). Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. *The Journal of Pathology, 191*(3), 239–244.
29. Jarallah, S. A., Al-Fartusie, F. S., & Zageer, D. S. (2023). Assessment of insulin and cortisol levels in Iraqi women with breast cancer. *Al-Mustansiriyah Journal of Science, 34*(1), 32–36.
30. Joimel, U., Gest, C., Soria, J., Pritchard, L., Alexandre, J., Laurent, M., Blot, E., et al. (2010). Stimulation of angiogenesis resulting from cooperation between macrophages and MDA-MB-231 breast cancer cells: Proposed molecular mechanism and effect of tetrathiomolybdate. *BMC Cancer, 10*, 1–13.
31. Karczewska, A., Nawrocki, S., Bręborowicz, D., Filas, V., & Mackiewicz, A. (2000). Expression of interleukin-6, interleukin-6 receptor, and glycoprotein 130 correlates with good prognoses for patients with breast carcinoma. *Cancer, 88*(9), 2061–2062.
32. Kassi, E., & Moutsatsou, P. (2010). Estrogen receptor signaling and its relationship to cytokines in systemic lupus erythematosus. *BioMed Research International, 2010*, Article 317452.
33. Knüpfer, H., & Preiß, R. (2007). Significance of interleukin-6 (IL-6) in breast cancer. *Breast Cancer Research and Treatment, 102*, 129–135.
34. Kozłowski, L., Zakrzewska, I., Tokajuk, P., & Wojtukiewicz, M. Z. (2003). Concentration of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. *Roczniki Akademii Medycznej w Bialymstoku, 48*, 82–84.

ISSN:2523 - 0522
Volume 8, Issue 1, April 2025
ISRAA UNIVERSITY JOURNAL OF APPLIED SCINCE (IUJAS)

35. Kwa, M., Makris, A., & Esteva, F. J. (2017). Clinical utility of gene-expression signatures in early stage breast cancer. *Nature Reviews Clinical Oncology, 14*(10), 595–610.
36. Levin, G., & Gotlieb, W. H. (2024). Interleukin-10 in ovarian cancer. *Chinese Clinical Oncology, 13*(4), 48.
37. Li, Y., Yu, H., Jiao, S., & Yang, J. (2014). Prognostic value of IL-10 expression in tumor tissues of breast cancer patients. *Chinese Journal of Cellular and Molecular Immunology, 30*(5), 517–520.
38. Llanes-Fernández, L., Goyanes, R. I. Á., Arango Prado, M. d. C., Alcocer-González, J. M., Mojarrieta, J. C., Pérez, X. E., López, M. O., et al. (2006). Relationship between IL-10 and tumor markers in breast cancer patients. *The Breast, 15*(4), 482–489.
39. Lyon, D. E., McCain, N. L., Walter, J., & Schubert, C. (2008). Cytokine comparisons between women with breast cancer and women with a negative breast biopsy. *Nursing Research, 57*(1), 51–58.
40. Ma, J., Qin, L., & Li, X. (2020). Role of STAT3 signaling pathway in breast cancer. *Cell Communication and Signaling, 18*, 1–13.
41. Ma, T., & Kong, M. (2021). Interleukin-18 and -10 may be associated with lymph node metastasis in breast cancer. *Oncology Letters, 21*(4), 253.
42. Ortiz, W. J. M., Olexen, C. M., Errasti, A. E., Schattner, M., Rothlin, C. V., Correale, J., & Silva, E. A. C. (2020). GAS6 signaling tempers Th17 development in patients with multiple sclerosis and helminth infection. *PLOS Pathogens, 16*(12), e1009176.
43. Pu, Y., Hour, T., Chuang, S., Cheng, A., Lai, M., & Kuo, M. (2004). Interleukin-6 is responsible for drug resistance and anti-apoptotic effects in prostatic cancer cells. *The Prostate, 60*(2), 120–129.
44. Sanguinetti, A., Santini, D., Bonafè, M., Taffurelli, M., & Avenia, N. (2015). Interleukin-6 and pro inflammatory status in the breast tumor microenvironment. *World Journal of Surgical Oncology, 13*, 1–6.
45. Scheller, J., Ohnesorge, N., & Rose-John, S. (2006). Interleukin-6 trans-signalling in chronic inflammation and cancer. *Scandinavian Journal of Immunology, 63*(5), 321–329.
46. Sehgal, P. B. (2010). Thirty years after "interferon-β2: interleukin-6 at the host-tumor interface. *Contemporary Oncology/Współczesna Onkologia, 14*(4), 237–241.
47. Selander, K. S., Li, L., Watson, L., Merrell, M., Dahmen, H., Heinrich, P. C., Müller-Newen, G., & Harris, K. W. (2004). Inhibition of gp130 signaling in breast cancer blocks constitutive activation of Stat3 and inhibits in vivo malignancy. *Cancer Research, 64*(19), 6924–6933.
48. Shaheen, B. M., Etewa, B. B. E., & El Bilbeisi, A. H. H. (2021). Quality assessment of the current cancer control plan at the governmental hospitals in Gaza Strip, Palestine. *Israa University Journal of Applied Sciences, 5*(1).
49. Sheikhpour, E., Noorbakhsh, P., Foroughi, E., Farahnak, S., Nasiri, R., & Neamatzadeh, H. (2018). A survey on the role of interleukin-10 in breast cancer: A narrative. *Reports of Biochemistry & Molecular Biology, 7*(1), 30.
50. Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians, 68*(1), 7–30.
51. Spitzner, M., Ebner, R., Wolff, H. A., Ghadimi, B. M., Wienands, J., & Grade, M. (2014). STAT3: A novel molecular mediator of resistance to chemoradiotherapy. *Cancers, 6*(4), 1986–2011.
52. Subbiah, I. M., & Angulo, A. M. G. (2014). Advances and future directions in the targeting of HER2-positive breast cancer: Implications for the future. *Current Treatment Options in Oncology, 15*, 41–54.

ISSN:2523 - 0522
Volume 8, Issue 1, April 2025
ISRAA UNIVERSITY JOURNAL OF APPLIED SCINCE (IUJAS)

53. Thwani, A. N. Al, & Mohsin, S. M. (2012). Serum level of interleukin-6 in breast cancer Iraqi women. *Iraqi Journal of Cancer and Medical Genetics, 5*(1), 42–45.
54. Tripsianis, G., Papadopoulou, E., Romanidis, K., Katotomichelakis, M., Anagnostopoulos, K., Kontomanolis, E., Botaitis, S., Tentes, I., & Kortsaris, A. (2013). Overall survival and clinicopathological characteristics of patients with breast cancer in relation to the expression pattern of HER-2, IL-6, TNF-α and TGF-β1. *Asian Pacific Journal of Cancer Prevention, 14*(11), 6813–6820.
55. Wang, L., Zhang, X., Farrar, W. L., & Yang, X. (2004). Transcriptional crosstalk between nuclear receptors and cytokine signal transduction pathways in immunity. *Cellular & Molecular Immunology, 1*(6), 416–424.
56. Wang, X., Li, J., Lu, C., Wang, G., Wang, Z., Liu, X., Liu, B., Wang, G., Zhang, Q., & Yang, Q. (2019). IL-10-producing B cells in differentiated thyroid cancer suppress the effector function of T cells but improve their survival upon activation. *Experimental Cell Research, 376*(2), 192–197.
57. Wang, Y., Niu, X. L., Qu, Y., Wu, J., Zhu, Y. Q., Sun, W. J., & Li, L. Z. (2010). Autocrine production of interleukin-6 confers cisplatin and paclitaxel resistance in ovarian cancer cells. *Cancer Letters, 295*(1), 110–123.
58. Watanabe, M. A. E., Oda, J. M., Amarante, M. K., & Voltarelli, J. C. (2010). Regulatory T cells and breast cancer: Implications for immunopathogenesis. *Cancer and Metastasis Reviews, 29*, 569–579.
59. Wu, P., Wu, D., Zhao, L., Huang, L., Shen, G., Huang, J., & Chai, Y. (2016). Prognostic role of STAT3 in solid tumors: A systematic review and meta-analysis. *Oncotarget, 7*(15), 19863.
60. Yuan, X., Li, Y., Shen, Y., Yang, J., & Jin, Y. (2020). Clinical and Th1/Th2 immune response features of hospitalized children with human rhinovirus infection. *Journal of Medical Virology, 92*(1), 26–33.