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Research Article

Immunohistochemical aspects on (IL-6) and its relationship to pathological parameters and hormones receptors (ER, PR, HER-2) in breast cancer (BC)women from south of Iraq

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ABSTRACT:

The aim of this study was to review the observations and information on relationship of preoperative level of (IL-6) with hormones receptors (ER,PR,HER-2) and the expression of these receptors in biopsies from patients with different stages and grades of disease by immunohistochemical method. In this study total number of (130)cases was analyzed and most patients diagnosed with stage (S111) then (S11)and (S1) with highly significant difference at ($p \leq 0.05$) and the same results was obtained from classified tumor grading with (BC)patients. The recent study evaluated level of (IL-6) in all (BC)patients ,the data showed highly significant difference and the mean level was increased in (71)case compared to control ,also we evaluated the relationship between tumor staging and grading with level of (IL-6) and data revealed to high level in (S111) patients while no signification between (S1,S3) ,the same results recorded between tumor grading and level (IL-6). Hormones receptors expression (ER,PR,HER-2) and its correlation with (IL-6)level was also determined ,the(IL-6) with (ER+)compared to (ER-) with high significance (p=0.00),the same results shown with (PR+) and(HER-2) expression and (IL-6)in all (BC)patients. The role of (IL-6) in (BC) immunity ,carcinogenesis ,cancer pathogenesis ,tumor initiation ,growth and metastasis .Results also explained correlate of (IL-6)with clinical staging and grading ,these findings Pointed to an important role in the progression of (BC) at least in patients with advanced disease stages.Data determined that the (ER+)positive tumor more reactive with(IL-6)then (PR+),(HER-2) which explained the effect of this factor on hormones receptors. Moreover present study found that the level of (IL-6) in (BC)recurrence may be differ according to(HER-2)status ,positive association with(BC) recurrence only among (HER2-)tumor with high mean level as compared to patients with(HER2+).Immunhistochemical staining was used in this study and results showed variable degree of staining Within invasive ductal and lobular carcinoma types compared to control.

1- Introduction

Breast cancer is a complex and multifactorial disease resulting from abnormal growth of cells begins in the lining layer of duct or lobe of the breast. that leads to malignant tumor formation . It is the commonest malignancy in women, affecting women during their lifetime .Bc, considered the most common cancer, is now leveled as the first among all cancers diagnosed in women (Kolahdoozan, *etal*, 2010; Ferlay *et al*., 2015).(IL-6)is a cytokines and consider as pleiotropic inflammatory cytokines produced by neutrophil and from macrophages, monocytes, T-cells, B-cells and fibroblasts, endothelial cells, osteoblasts and tumor cells(Salgado,*etal*,2003 Kishimoto 2006; Fields , 2009; Guillermo, and Descoteaux,2014)( ). IL-6 plays a chief role in pathogenesis and progress of malignancies. It helps tumor to grow through inhibiting apoptosis of cancer cells and the stimulation of tumor angiogenesis,(Salgado, et al.,2003),also IL-6 contributes to the proliferation of many cancer cells and, especially those at the advanced stage of development, IL-6 concentrations can depend on the clinical tumor stage and histological tumor grade (Brozek ,*etal*.,2005).most commonly elevated in patients with endometrial cancer, lung cancer, colorectal cancer breast cancer and ovarian carcinoma.(Zakrzewska and Pozański,2001 Salgado, *et al*.,2003;Songur,*etal*.,2004;Bellone,*etal*.,2005).However IL-6 overexpression was found in breast carcinoma and in prostate cancer (Garcia,*etal*.,2005;Culig,*etal*.,2005). The ovarian steroid hormones progesterone and estrogen play critical roles in the development and progression of breast cancer and endometriosis(Shao *et al*., 2014).Estrogen receptor and progesterone receptor, along with human epidermal growth factor receptor 2 (HER2), are used to classify phenotypes in breast cancers and to predict response to specific therapies(Cadoo *et al* .,2013).Bc is a heterogeneous disease, and its prognosis may depend on characteristics of tumor (Onitilo,*etal*.,2009).Therefore, the roles of IL-6 related markers in mediation of tumor growth and metastasis could be influenced by distinct subtypes that have been identified on the basis of gene or protein expression in tumor tissue (Seruga ,*etal*.,2008),different breast cancer subtypes may produce distinct inflammatory mediators, which may affect their distinct tumor progression pathways (Gonzalez,*etal*.,2011; Levano,*etal*.,2011),so this study have focused on the use of IL-6 as a prognostic factor for breast cancer.

Material and mothed

Sample collection

In this study total number of (130)cases was analyzed and (60) cases were subjected to Histopathological and

Immunohistochemcal studies. The recent study evaluated level of (IL-6) in all (BC) patients and (16)control ,the data showed highly significant difference and the mean level was increased in (71)case compared to control

Immunological studies: In this study we measured IL-6 level in the serum of the breast cancer patients by using Enzyme-Linked Immuno Sorbent Assay (ELISA) , human (IL-6) ELISA kit.

Histopathological studies: the histopathological study of tumor stage was assessed according to the criteria established by the seventh edition of the American Joint Committee on Cancer (AJCC) staging manual as SI, SII and SIII , while the tumor grade was determined according to the Scarff-Bloom-Richardson classification modified by Elston and Ellis also as GI,GII and GIII.

Immunohistochemstiry: Hormone receptors(ER,PR and HER-2) were assessed via immunohistochemistery mothed (IHC) .

Statistical analysis: The data of this study were analytic with one way anova ,t-test and chi-square by using Spss program version 22.

Results:

1- Clinical staging and histologic grading

Dependent on Tumor-node-metastasis (TNM) system was used to classified the stage of BC, in our patients out of (130) cases , staging system can be applied only to (60) cases , this study had reported (1)cases (1.7%) diagnosed as stage I, (28) cases with (46.7%) are clarified as stage II and (31) cases with (51.7%) are stage III, there were a significant differences among different stages at($P \leq 0.05$),(table1).

Table (1) : Types of tumor histologic staging in (BC) patients.

Staging	no. of patients	Percentage rate%
Stage I	1	1.7%
Stage II	28	46.7%
Stage III	31	51.7%
Total	60	100%

Chi-square = 27.30 df= 3 p- value = 0.00

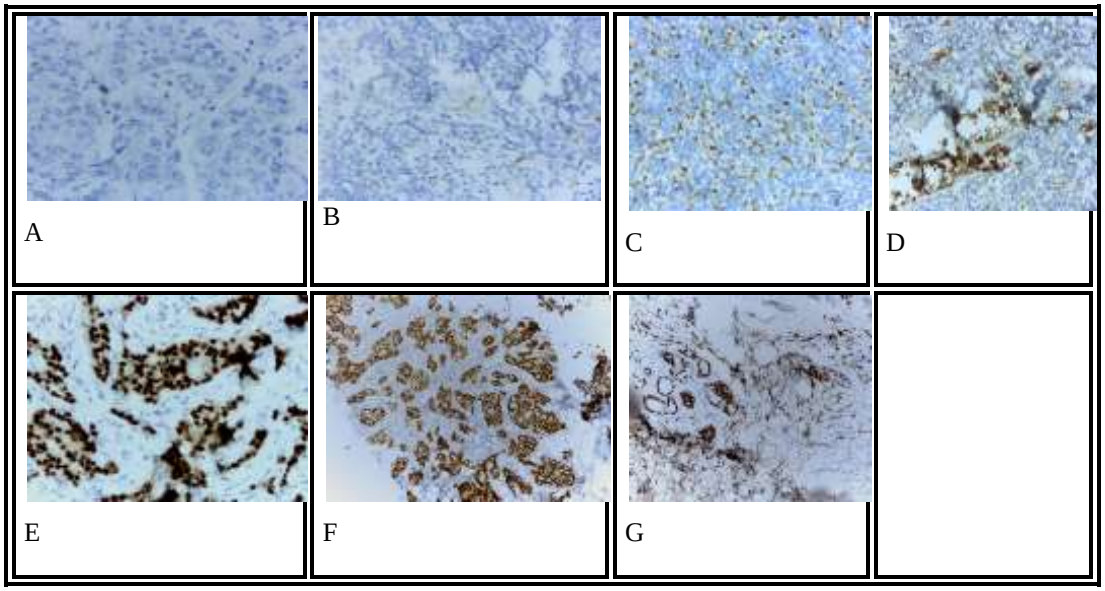
In this study grading system can be applied only to 60 cases , and we find that 2 cases (3.33%) were diagnosed as grade I, 30 cases with (50 %) are grade II, and 20 cases with (46.67%)recorded as grade III. There was a significant differences among different grades ($P \leq 0.05$) , table (4-10).

Table (2): Tumor grading in (BC) patients.

Grade	no. of patients	Percentage rate %
Grade I	**2**	**3.33%**
Grade II	**30**	**50%**
Grade III	**28**	**46.67%**
Total	**60**	**100%**

Chi-square = 24.40 df= 2 p- value = 0.00

2-Estrogen and progesterone receptors



There are (40) patients were positive for ER,(66.7%) distributed according scoring system, while only (20) of patients were negative for this receptor(33.3%), the difference between the two percentages was significant (P > 0.05), Table(3) Figure(1).

Figure 1. Breast Invasive Duct Carcinoma, LuminalA Subtype Showing Positive Nuclear Immunostainingfor ER (A) Negative (B), 1+ (C)2+(D)3+(E)4+(F)5+(G)6+ Magnification x400

Table (3): BC patients distributed by the expression of estrogen receptor.

Hormone receptor	**No.**	**Percentage rate%**
ER positive	**40**	**66.7%**
ER negative	**20**	**33.3%**
Total	**60**	**100%**

X^2= 6.67 df= 1 p- value = 0.010

While (45)cases were positive for PR (75%) and (15) cases were (25%) significant (P > 0.05), Table (4)Figure(2).

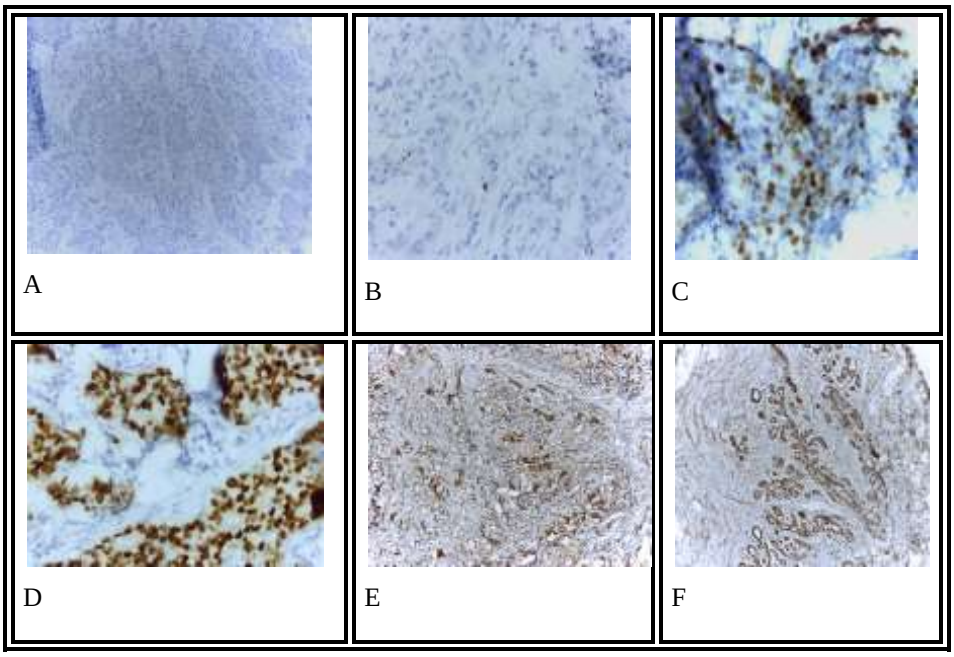


Figure 2. Breast Invasive Ductal Carcinoma, LuminalA Subtype Showing Positive Nuclear Immunostainingfor PR (A) Negative (B), 1+ (C)2+(D)3+(E)4+(F)5+ Magnification x400

Table (4): BC patients distributed by the expression of progesterone receptor.

Hormone receptor	**No.**	**Percentage rate%**
PR positive	45	75%
PR negative	15	25%
Total	60	100%

X^2= 15.00 df= 1 p- value = 0.00

3- HER-2

Result found (33)cases were positive (55%),and(27) cases were negative (45%) of patients, the percentage of cases of breast tumor were significant (P > 0.05) Table (5) Figure(3)

Table (5): BC patients distributed by the expression of human epidermal growth factor-2 receptor.

HER-2	No.	Percentage rate%
Positive	33	55%
Negative	27	45%
Total	60	100%

X^2= 0.60 df= 1 p- value = 0.43

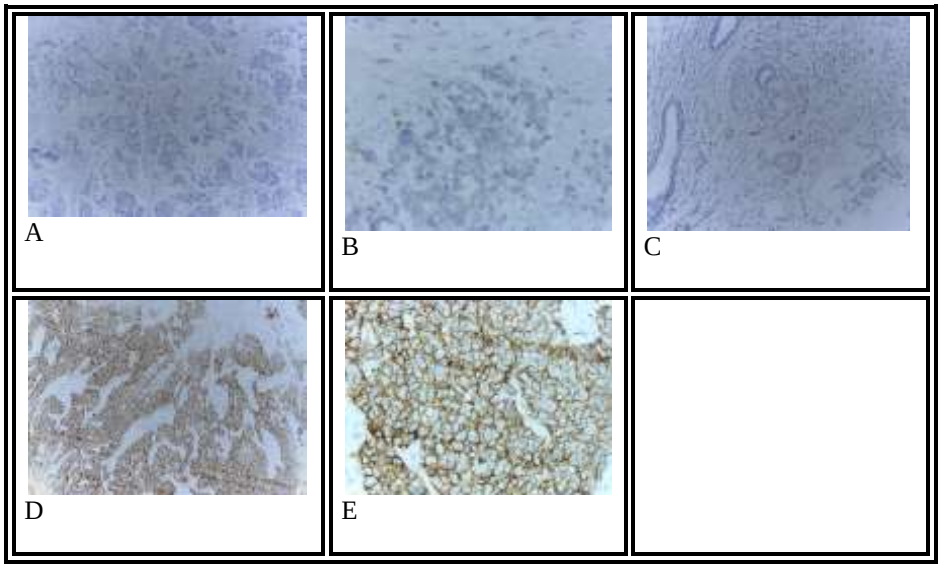


Figure 3. Breast Invasive Ductal Carcinoma, LuminalA Subtype Showing Positive Nuclear Immunostaining for HER2 (A) Negative (B), 1+ (C)2+(D)3+(E)4+ Magnification x400

3- Evaluation of 6 (IL-6) levels

The data showed elevated level of IL-6 in (BC) patient reached to (41.2732 pg/ml), as compared to control that the level of (IL-6)was (.2254pg/ml), with significant difference (p >0.05) Table (6).

Table (6) Serum levels of(IL-6) in BC patients groups and healthy the result expressed as (mean± SE) .

Groups	No.	Mean of IL-6 pg/ml	SE±	Percentage rate %
patient	71	41.2732	6.99329	.000
control	16	.2254	.00907	

4- Correlation between (IL-6) level and staging and grading

Also the study determined the relationship between tumor staging and level of (IL-6) mean levels The results showed highest level was in patients at stage3(S3) that the (IL-6) level reached to (76.5465pg/ml), while the patients from S2 the (IL-6)level was (13.9293pg/ml), and reached to (8.3400 pg/ml) in patient from (S1), statistically no significant difference observed between (S1,S2)and no significant difference also between (S1 , S3) when (IL-6) level compared ,but high significant difference was recorded between (S2,S3)at (P=0.000), Table(7).

Table(7):Concentration of IL-6 in relation to tumor stage in (BC) patients. values expressed as **(Mean ± S.E.**)

Tumor staging	no .of patient	Concentrate of(IL-6) pg/ml	Sig. between groups	P value
Stage1(S1)	2	8.3400± .01000	S1-S2	.896
Stage 2(S2)	28	13.9293± 4.86047	S1-S3	.114
Stage 3(S3)	31	76.5465± 13.88997	S2-S3	.000

Also the results showed high significant increased with(IL-6) levels in G3 patients that the mean level was (57.1746pg/ml) ,(19.9030 pg/ml) and(19.9030 pg/ml) in patient whose diagnosed with(G2,G1) , respectively, also no significant difference between (G1,G2) and(G1,G3) but high significant difference recorded between (G2,G3)at (p=0.010) (Table8).

Table(8): Concentration of IL-6 in relation to tumor stage in (BC) patients. values expressed as **(Mean ± S.E.**)

Tumor grading	no. of cases	**Concentrate of(IL-6) pg/ml**	**Sig.between groups**	**P value**
Grade1(G1)	2	5.0050±3.32500	G1-G2	.705
Grade2(G2)	30	19.9030±5.14490	G1-G3	.189
Grade3(G3)	28	57.174±13.63024	G2-G3	.010

5-Correlation between (IL-6) level and (ER,PR,HER2) receptor expression

Data and figures from this study indicated to the relationship between (IL-6) level and expression of each receptor in (BC) patients ,the result showed increased with(IL-6) level reached (56.3145 pg/ml± 11.14360pg/ml) with(ER+)comparative with(ER-) which the (IL-6)level was (19.1690±6.53831 pg/ml) at high significant differences(p=.000), Table(4) and increased in PR+(62.5028 pg/ml) as compared with PR-(11.6953 pg/ml) also with high significant differences(p=.000), Table(5) while the result showed increased mean level of (IL-6) with Her2-(62.6413 pg/ml) as compared with Her2+(27.8280 pg/ml) Table(9) and Table(10)

Table(9)increased mean serum level of(IL-6) with(ER) receptor. Result expressed as (mean ±SE)

Receptors type	no.of patient	(IL-6) levels pg/ml	SE	T	Df	P value
ER+	40	56.3145	11.14360	2.254	58	.000
ER-	20	19.1690	6.53831			

Table(10): increased mean serum level of(IL-6) with PR receptor. Result expressed as(mean ±SE)

Receptors type	no.of patient	(IL-6) levels pg/ml	SE	T	Df:	P. value
PR+	45	62.5028	10.35058	2.781	58	.000
PR-	15	11.6953	5.16183			

Table(6): Level of(IL-6) and (HER2) receptor. Result expressed as (mean ±SE)

Receptors type	No.of patien.	(IL-6) levels pg/ml	SE	T	Df	P value
HER2+	33	27.8280	8.40926	2.235	58	=.007
HER2-	27	62.6413	13.83570			

Disscution

IL-6 in breast cancer

Our data was found high level of (IL-6) with signification difference in all(BC) patients compared with its concentration in healthy women , this may be to the role of (IL-6) with (BC) and the cancerous cells that product it in addition to the inflammatory cells from the surrounding tissue ,this results in agreement with other studies by CG,*etal*.,(2003) which displayed the patients with Bc had showed high level of IL-6 and more less than in healthy group,also agreed with other study made by Victor P.,*etal* .,(2015) who observed an association of levels IL-6, and IL-10 in (BC) patients with or without receiving chemotheraphy, the high proinflammatory cytokine IL-6 mRNA expression in most patients without chemotherapy was(10.8447) due to the production of cancer cells and cells surrounding the tumor tissue. Furthermore our findings close to study of Hong DS,*etal*.,(2007) and Salgado R ,*etal*,(2003) they found high levels of circulate IL-6 associate with the numeral of metastatic sites and IL-6 promotes the formation of distant metastasis, previous study done by ChoYA ,*etal*.,(2013) indicated that inflammation within the tumor microenvironment may play an important role in breast cancer progression this displayed the mean value of IL-6 of breast cancer patients without receiving chemotheraphy was statistically significant higher than in patients with chemotheraphy, The role of IL-6 in breast cancer immunity and carcinogenesis has been well established, its play diverse roles in cancer pathogenesis, with increasing evidence suggesting their involvement in tumor initiation, growth and metastasis Smyth MJ. *etal*.,(2004),the (IL-6) produced from tumor cells and normal cells and it is considered as stimulators for angiogenesis as well as factor for cancer cell growth and proliferation (Salgado *etal*,2003;victor *etal*,2015).

IL-6 levels correlation with clinical stage and histologic grade

Present study proved the relationship between mean level of (IL-6)in serum of all (BC) patients that diagnosed with S(III),then S(II) and the lowest level was measured in patients with (SI), this results may be related to late stage of tumor growth and all patients(mastectomy)so this factor correlate with clinical stage and this in agreement with other researchers who showed that there was detectable correlation between stage and the levels of (IL-6),and high level was frequently in S(III) than in S(I and II),it produced by tumor cells and it has able to promote tumor growth by up-regulating anti apoptotic and angiogenic proteins in tumor cells Ordemann *etal*., (2002); Kozlowski *et al*., (2003);Fuksiewicz M,*etal*.,(2006), the level of IL-6 has a positive correlation with TNM staging system of breast cancer thus indirectly correlating with the prognosis of the patient with breast cancer, and this agree with Chavey C,*etal*.,(2007) .may be illustrated that the (IL-6) level was increased with tumor grading and invasiveness of tumor cells and associated with tumor progression to be more malignant, these results deal with other studies indicated to high level of (IL-6) with G3(Kozlowski *et al*., (2003); Mousa,(2014) ; Sherif A.,*etal*.,(2016); furthermore these results support with study made by Mousa,(2014) on patients with bladder cancer who showed a high significant increased mean levels of IL-6 in patients with G3 tumor as compared with patients with G1and G2,IL-6 is produced by a variety of tumor cell lines and is associated with many changes as auto and paracrine stimulation of tumor cells proliferation, with the upregulation of anti-apoptotic proteins, and with the induction of pro-angiogenic cytokines, these changes may parallel the tumor cell progression toward a more malignant phenotype, secrete of IL-6, which stimulates the growth and invasiveness of Michigan Cancer Foundation-7 cells(MCF-7) are isolated breast cancer cells (Studebaker ,etal.,2008; Baumgarten and Frasor, 2012),high level of IL-6 in MCF-7 cells induces the epithelial-to-mesenchymal transition (EMT), and increases their invasiveness (Sullivan and others 2009) . Further, IL-6 is associated with angiogenesis by optimize of its ability to induce the mRNA of vascular endothelial growth factor (VEGF), which is typically adirect angiogen Chavey C,*etal*.,(2007) Also another study showed the displayed role of(IL-6)that induced the expression of vascular endothelial growth factor and referred to over expression of (IL-6) in human basal cell carcinoma lines increases antiapoptotic activity and tumorigenic potency(Cohen T.,*etal*.,(1996) Jee SH, etal.,(2001).Relationship between IL-6 with vascular endothelial growth factor in cervical cancer the, these findingss point to an important role of IL-6 in the progression of breast cancer at least in patients with advanced disease(Wei LH,*etal*.,2001).

Level of IL-6 relation to hormone receptors (ER,PR and HER2)

In the current study we investigated the relationship of (IL-6) hormone receptors in (BC) samples ,we observed an association of levels of IL-6 with ER,PR the result showed increased mean level of (IL-6) with positive (ER+)as compared with negative (ER-) with high significant differences at(p=.000),and increased in (PR+) receptors as compared with (PR-) also with high significant differences(p=.000),these results may be regarded that the (IL-6) associated with (ER)(PR) and (HER2) receptors which it was mammary markers, and this results agreed with (Fontanini G.,*etal*.,1999) who observed ,level of IL-6 was expression directly associated with the expression of ER , PR that symbolize important mammary differentiation markers, ER-positive tumors were also reactive with IL-6 (P =.02), and the percentage of ER-positive cells was directly related to that of IL-6-positive cells (P.00005),same the results were obtained with regard to the percentages of PR-positive cells (P =025). The possible contact between interleukins and steroid hormones has been suggested by (Reed ,*et al*,1992) on breast cyst fluid, they were observed that IL-1 and IL-6 caused increase of estrogen synthesis through stimulating aromatase

and estradiol dehydrogenase activities in (BC) cells,(ER-) positive human breast cancer cells express the IL-6 receptor and their proliferation was inhibited by addition of IL-6, Chen *et al*,(1988) and Chiu *et al*,(1996), Also study by (Young A.,*etal*.,2013)whose observed an association on (IL-6 and IL-8) level with (BC) recurrence; these associations differed according to tumor subtype. other studies have established that cytokines can enhance, inhibit, or have no effect on cell proliferation and differentiation depending on the cell type examined, implying that the role of cytokines in mediation of tumor growth could be affected by tumor subtype every tumor subtype, may correspond differently with the immune system and produce a distinct cytokine profile these findings estimate that IL-6 may stimulate tumor cell motility and invasion for enhancement of metastasis of tumor cells Bazzoni and Beutler ,(1996); Salgado R.,*etal*.,(2003); Knupfer and Preiss ,(2007) ; Also(IL-6) chemoattracting and mitogetic for promotion of tumor growth, like IL-6, role in stimulates the growth and invasiveness of (MCF-7) breast cancer cells (Studebaker ,etal.,2008; Baumgarten and Frasor, 2012), High level of IL-6 in MCF-7 cells induces the epithelial-to-mesenchymal transition (EMT), and increases their invasiveness (Sullivan and others 2009).(Gilbert and Slingerland,2013),also IL-6 can increase aromatase activity, promote estrogen production, and, thus, stimulate progression of (ER+) (BC) (Chiu *et al*,1996) ; (Catalano S,*etal*.,2003) and (Catalano S ,*etal*.,2004) Recently(Iliopoulos, *et al*.,2011) demonstrated that IL-6 links inflammation to malignant transformation by constitutively activating the(**nuclear factor kappa-light-chain-enhancer of activated B cells**) NF-κB pathway which in turn drives further IL-6 production creating a positive feedback loop and (Sasser ,*etal*., 2007),they referred that cancer cell lines produce IL-6, of which ER-negative cells secrete more levels than ER-positive cells , IL-6 induces proliferation and a more aggressive phenotype in ER-positive cells ,also while (Buyse *et al.*, 2006); Sorlie *et al.*, (2001), they displayed A clear and well-characterized inverse correlation exists between breast cancer ER status and IL-6. This result may be clarified the role of (IL-6) with cell growth, proliferation ,differentiation and the over expression of(HER2) interact with immune system and lead to induced tumor growth , this result agreed with the studies showed increased with (IL-6) level in relation to patients with (HER2-) negative receptor (Young Ae.,*etal*.,2013; Sherif A.,*etal*.,2016). Moreover, the recent study found that the levels of IL-6 in breast recurrence may differ according to HER2 status ,as well as levels of IL-6 showed a positive association with breast cancer recurrence only among patients with (HER2–) tumors with high mean as compared to patients with HER2+ ,our results deal with study made by Salhia B.,*etal*.,(2011) whose observed high level of IL-6 associated with HER2- in patients of BC ,other study done by Young Ae.,*etal*.,(2013) showed that increased level of IL-6 in HER2- with BC patients ,similarly ,our results agree with Egyptian study done by Sherif A.,*etal*.,(2016) ,they observed level of IL-6 may increased and correlate with HER2-,As known HER2 is a transmembrane tyrosine kinase receptor that mediates growth, differentiation, and survival of cells; overexpression of HER2 at the cell membrane may lead to activation of multiple signaling complexes Kumar S,*etal*.,(2003).Some studies have reported different immune-mediated mechanisms according to patients' HER2 status and implied that abnormal expression of HER2 in breast tissue may affect the complex interaction between cancer and the immune response (Chavey C,*etal*.,2007). Moreover (IL-6) is able to induce epithelial-mesenchymal transitions (EMT) which has been implicated in generation of stem cell phenotype (Iliopoulos *et al*., 2011; Mani *et al*., 2008; Sullivan et al.,2009). Our results disagree with result of other researchers that referred to high level of (IL-6) associated with (HER2+) as compared with (HER2-) (Fontanini G.,*etal*.,1999; Hartman ,*et al*., 2011).

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