اهمية بعض العوامل السريرية مع تقدير المؤشرات الحيوية المناعية إنترلوكين (IL-1B و IL-6 و IL-10 و INF-ϒ) في سرطان الثدي

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الملخص

الخلفية والهدف: تم إجراء الارتباط بين الفئات العمرية ومؤشر كتلة الجسم ودرجة السرطان مع التركيزات المصلية للواصمات الحيوية المناعية المناعية إنترلوكين (IL-1B و IL-6 و IL-10 و INF-ϒ) بين الإناث المصابات بسرطان الثدي في هذا. دراسة.

الطريقة: بلغ العدد الإجمالي لمرضى سرطان الثدي المشاركين في هذه الدراسة (80) فرد تم أخذهم من مركز الأورام بالبصرة بمحافظة البصرة، وتتراوح أعمار المرضى من 27-76 سنة و (80) فرد اعتبروا مجموعة ضابطة بعد أن أصبحوا. تم فحصه والتأكد من خلوه من أي مشاكل في الصدر أو أي مشاكل صحية أخرى.

النتائج: تم تحديد مستويات إنترلوكين IL-1B و IL-6 و IL-10 و INF-gamma (pg / ml) بين مرضى سرطان الثدي ومجموعات التحكم في مختلف الفئات العمرية ، ومراحل سرطان الثدي ومؤشرات كتلة الجسم المختلفة .

الخلاصة: هناك ارتباط احصائي بين العوامل السريرية المختلفة والمراحل المختلفة لسرطان الثدي

الكلمات المفتاحية: سرطان الثدي، درجة السرطان ، مؤشر كتلة الجسم ، الفئة العمرية ، IL-1B، IL-6، IL-10 و INF-ϒ .

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The importance some clinical factors with estimation of immunological biomarkers

(interleukin IL-1B, IL-6, IL-10 and INF-ɣ) in breast cancer

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Abstract

Background and aim: The correlation between age groups, body mass index and cancer grade with serological concentrations of immunological biomarkers (interleukin IL-1B, IL-6, IL-10 and INF-ɣ) among females with breast cancer was carried out in this study.

Methods: The total number of breast cancer patients involved in this study are (80) individual were taken from Basrah oncology center in Basrah province, the age of patients range from 27-76 yrs and (80) individuals considered as control group after they were checked and confirmed to be free from any chest or any other health problems.

Results: The levels of interleukin IL-1B, IL-6, IL-10 and INF-gamma (pg/ml) were determined among patients with breast cancer and control groups in various age groups, stages of breast cancer and various body mass indices .

Conclusion: there are statistical correlation between various clinical factors and various stages of breast cancer

Key words: breast cancer , cancer grade , BMI , age group , IL-1B, IL-6, IL-10 and INF-ɣ , statistical analysis

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Introduction

In Iraq, breast cancer is the most common type of malignancy in women, it accounts for one third of the registered female cancers according to the latest Iraqi Cancer Registry (ICR , 2009) and this showed a trend for the disease to affect younger age groups (Lawn, 2010). There was an increase in the incidence rates of breast cancer within the last two decades, which became one of the major threats to Iraqi female health (Hussain , 2016). Worldwide, breast cancer is the second most common type of cancer and the fifth most common cause of cancer death. The National Cancer Institute estimated that in 2008, there would have been 182,460 (female) and 1,990 (male) new cases and, from these, 40,480 (female) and 450 (male) deaths in the United States (NCI , 2009). Breast cancer usually originates in the cells of the lobules (lobular carcinoma) or ducts (ductal carcinoma) of the milk glands, or, much less commonly, in the stromal tissue (fatty and fibrous connective tissue of the breast); (Jobe, 2009). The term "breast cancer", therefore, refers to cancer of the milk glands. Similar to other cancers, breast cancer is caused by genetic abnormalities that are either inherited, accounting for 5-10% of all cases, or occurs as a result of *de novo* mutation during aging accounting for over 90% of all cases (BCO, 2009 and Raheem, 2014). Cancer immune surveillance is an important process by which the immune system is able to monitor, recognize, and eliminate nascent tumor cells (Swann, *et al.,* 2007 and kim, *et al.,* 2007). There are three essential phases to this process termed elimination, equilibrium, and escape. Initially, innate and adaptive immune responses are able to control tumor growth. In this phase—elimination, acute inflammatory responses triggered by tumor-associated 'danger signals' initiates tumor cell recognition, the secretion of proinflammatory cytokines (notably, interleukin-12 (IL-12) and interferon-γ (IFN- γ), and killing by innate immune cells (e.g. natural killer (NK) cells, dendritic cells (DCs), and macrophages). Upon maturation, DCs migrate to nearby lymph nodes (LN), where they present tumor antigens and activate tumor-specific CD4+ and CD8+ T cells. These tumorspecific T cells will then migrate to the tumor site and facilitate killing. At this point, tumor cells are completely eradicated or resistant clonal variants develop. The clonal variants can develop resistance by decreasing their immunogenicity and/or secreting and recruiting immunosuppressive factors (several mechanisms of which are covered here). During this phase of equilibrium, if another cycle of immune responses is unable to eliminate the nascent cancer cells, then the phase of immune escape is reached, eventually leading to clinical manifestation. These phases together describe the theory of cancer immunoediting. Ample evidence proves that neoplastic lesions are under immunosurveillance. Early proof of this was noted by pathologists who recognized that many patient tumors were densely infiltrated by innate and adaptive immune cells (Joshua,*et al.,*2018 and Hanahan,*et al.,*2011). Recent studies demonstrate that these immune cells are indeed mounting an antitumor response and that tumors develop mechanisms to combat an immune response (Finn, *et al.,* 2008 and Vinay, *et al.,* 2015). It has also been shown that mice lacking various components of the immune system have a greater risk of developing cancer than their immune competent counterparts (Joshua, *et al.,* 2018). Following recognition of a specific antigen presented

by an appropriately activated antigen-presenting cell (APC), $CD4^+$ T cells can differentiate into different subsets determined by the cytokine milieu present when the cell encounters an antigen. Cytokines are biomolecules whose biological properties suggest a key role in infections, hematopoiesis, and homeostasis, revealing their multifunctional role that controls response against infectious diseases and even tumorigenesis by controlling tissue renewal, cellular sprouting, and growth. ILs are secretory immunomodulatory proteins that belong to the superfamily of cytokines and, as cytokines, present complex immunological functions. The main objective of ILs is to mediate intercellular communication in the immune system, including cell migration, proliferation, maturation, and adhesion, which, as mentioned, plays a vital role in the inflammatory response (Dmitrieva, *et al.*, 2016). Interleukins are involved in both acute and chronic inflammatory responses (Fasoulakis, *et al.*, 2018).

The present study aimed to determine the Statistical association between age groups, body mass index and cancer grade with serological concentrations of immunological biomarkers (interleukin IL-1B, IL-6, IL-10 and INF-ɣ) among females with breast cancer.

Materials and methods

Breast cancer Cases

Breast cancer cases in this study (case\control study) have been collected in Basra province particularly from Basra oncology center, period time of this study was extended from January 2021 to May 2021. During samples collection process all medical information for all patients which involved in the study were recorded in questionnaire paper that included name, age, residency and marital status Breast Cancer females who are in the early stages, the second stages, and the last stages after taking all the treatments, Both patients and control samples that investigated in this study have age ranged between 20 to 80 year. All informations were collected from patients after the accurate diagnosis by the oncologist which confirmed by other findings (Cystoscopy, biopsy, ultra sound and CT scan).

Serological detection of IL-1B, IL-6, IL-10 and INF-Gamma

Sandwich ELISA test was used for detection the titer of IL-1B, IL-6, IL-10 and INF- Gamma Ag's in serum samples for both patients and controls. By using Elabscience American ELISA kit composed of 96 well microtiter plate. The procedure of this test was done according to kit manufacture
instructions.

Statistical analysis Statistical analysis was carried out by using SPSS VER.23 two way T test (student's T-test) and chi square to find out the statistical differences between all variables. probability less than 0.05 is significant ($P<0.05$).

The results

1- Distribution of patients within various age groups.

Table (1) show the minimum number of breast cancer females was found within the age groups 20-29 yrs (2.5%), followed by age group 30-39 yrs (12.5%, above 40-49yrs (31.25%), the age group 50-59 yrs (32.5%) and >60 yrs (21.25%). P-value <0.0163.

Table (1): The number and percentages of patient with breast cancer and control group according to age groups.

ge Groups	Breast Cancer patients		Mean of IL-1B pg/ml	Control group		Mean of IL-1B in control pg/ml	Standard value pg/ml
	No.	%		No.	%		
20 -29 year	2	2.5	2.87	2	2.5	(0.2 – 0.57)	0.5
30 -39 year	10	12.5	3.485	8	10.00		
40 -49 year	25	31.25	4.168	23	28.75		
50-59 year	26	32.5	4.733	24	30.00		
>60 year	17	21.25	5.049	23	28.75		
Total	80	100%		80	100%		

IL-1B various stage of patients with breast cancer

Table (2) and figure (1) show the levels of IL-1B with stages of breast cancer in IA and IIIC 2 mean of IL-1B are (0.4) and (1.400) pg/ml respectivly , IB recorded (2.60 Pg/ml), followed by IIA (1.709 pg/ml), IIB (1.523 pg/ml), and IIIA (1.405Pg/ml), the IIIB (2.166 pg/ml) and the last stage IV was (2.675 pg/ml) with P-value= 0.4998.

Table (2) shown IL-1B levels and various stage of patients with breast cancer.

Stages of Breast Cance	Breast Cancer No	%	Mean of IL-1B Pg/ml
IA	2	2.5	0.4
IB	1	1.25	2.60
IIA	22	27.5	1.709
IIB	21	26.25	1.523
IIIA	19	23.75	1.405
IIIB	3	3.75	2.166
IIIC	2	2.5	1.400
IV	10	12.5	2.675

F Ratio= 0.9153 , DF= 7 , P-value = 0.4998

IL-1B various body mass index of patients with breast cancer

Table (4) show the mean of IL-1B Pg/ml various body mass index, overweight group recoreded (0.1536 pg/ml), followed by underweight (0.1850 pg/ml) and normal (0.1806 pg/ml) with P-value = 0.9648

Table (3) IL-1B various body mass index of patients with breast cancer

Body Mass Index	Breast C No	%	Mean of IL-1B Pg/ml
Underweight	3	3.75	0.1850
Normal	27	33.75	0.1806
Overweight	36	45.0	0.1536
Obese	14	17.5	0.1592

Interleukin 6 (IL-6)

IL-6 Concentration among studied groups in relation to age group

Table (3) shows the levels of interleukin IL-6 Pg/ml among patients with breast cancer and control groups in various age group that show the age group (20-29) yrs (4.69 pg/ml) followed by (30-39) yrs (4.97 pg/ml), continued with (40-49) yrs (6.30 pg/ml), (50-59) yrs (6.28 pg/ml) and (>60) yrs (6.65 pg/ml) with P-value = 0.01599.

Table (3):IL-6 concentration among patients with breast cancer and control groups according to various age group.

Age Groups	Breast Cancer patients		Mean of IL-6 pg/ml	Control group		Mean of IL-6 in control **pg/ml**	Standard value pg/ml
	No.	%		No.	%		
20 -29 year	2	2.5	4.69	2	2.5	(0.48 – 0.82)	(0.15-0.5)
30 -39 year	10	12.5	4.97	8	10.00		
40 -49 year	25	31.25	6.30	23	28.75		
50-59 year	26	32.5	6.28	24	30.00		
>60 year	17	21.25	6.65	23	28.75		
Total	80	100%		80	100%		

P value = 0.01599 , F ratio = 1.97 , DF= 1

IL-6 concentration among patients with breast cancer according to cancer stage

Table (4) and show the levels of IL-6 with stages of breast cancer in IA and IIIC 2 recorded concentration mean IL-6 (5.6950) and (18.5650) pg/ml respectively, IB show (0.9800 pg/ml), followed by IIA (6.0977 pg/ml), IIB (5.8324 pg/ml), and IIIA shows (7.8189 pg/ml), the IIIB (3.9100 pg/ml) and the last stage IV (6.0370 pg/ml) with P-value = 0.3100.

Table (4) show IL6 levels in various stage of patients with breast cancer.

Stages of Breast Cancer	Breast Cancer No	%	Mean of IL-6 Pg/ml
IA	2	2.5	5.6950
IB	1	1.25	0.9800
IIA	22	27.5	6.0977
IIB	21	26.25	5.8324
IIIA	19	23.75	7.8189
IIIB	3	3.75	3.9100
IIIC	2	2.5	18.5650
IV	10	12.5	6.0370

F Ratio = 1.2072 , DF = 7 , P –value = 0.3100

IL-6 concentration among patients with breast cancer according to various body mass index

Table (5) show the mean of IL-6 pg/ml various body mass index, shown overweight (6.797 pg/ml), followed by underweight (2.831 pg/ml) and normal (4.629 pg/ml) with P-value = 0.5018.

Table (5) means of IL-6 concentrations patients with breast cancer according to various body mass index

Body Mass Index	Breast C No	%	Mean of IL-6 Pg/ml
Underweight	3	3.75	2.831
Normal	27	33.75	4.629
Overweight	36	45.0	6.797
Obese	14	17.5	8.719

F Ratio = 0.7924 , DF= 3 , P value = 0.5018

Interleukin 10 (IL-10)

IL-10 Concentrations among studied groups in relation to age group

Table (6) shows the levels of interleukin IL-10 pg/ml among patients with breast cancer and control groups in various age group show the age group (20-29) yrs recorded (2.18 pg/ml) followed by (30-39) yrs (22.15 pg/ml), continued with (40-49) yrs (24.16 pg/ml), (50-59) yrs (26.33 pg/ml) and (>60) yrs (26.87 pg/ml) with P-value = 0.0042.

Table (6):IL-10 concentration among patients with breast cancer and control groups in various age group.

Age Groups	Breast Cancer		Mean of IL-10 Pg/ml	Control		Mean of of IL-10 In control Pg/ml	Standard value pg/ml
	No.	%		No.	%		
20 -29 year	2	2.5	2.18	2	2.5	(1.6 – 7.0)	(4.8-9.8)
30 -39 year	10	12.5	22.15	8	10.00		
40 -49 year	25	31.25	24.16	23	28.75		
50-59 year	26	32.5	26.33	24	30.00		
>60 year	17	21.25	26.87	23	28.75		
Total	80	100%		80	100%		

P- value = 0.0042 , F ratio = 8.21 , DF = 1

IL-10 concentration among patients with breast cancer according to cancer stage

Table(7) and figure (3) show the levels of IL-10 with stages of breast cancer in IA and IIIC recorded concentration mean (26.6775) and (29.0600) IL-10 pg/ml respectively, IB (12.6000 pg/ml), followed by IIA (29.2464 pg/ml), IIB (20.8810 pg/ml), and IIIA (28.9226 pg/ml), the IIIB contain (23.1400 pg/ml) and the last stage IV (32.4991pg/ml) with P-value = 0.3100.

Table (7) show IL-10 levels in various stage of patients with breast cancer.

Stages of Breast Cancer	Breast Cancer No	%	Mean of IL-10 Pg/ml
IA	2	2.5	26.6775
IB	1	1.25	12.6000
IIA	22	27.5	29.2464
IIB	21	26.25	20.8810
IIIA	19	23.75	28.9226
IIIB	3	3.75	23.1400
IIIC	2	2.5	29.0600
IV	10	12.5	32.4991

IL-10 concentration among patients with breast cancer according to various body mass index

Table (8) show the mean of IL-10 pg/ml various body mass index , shown overweight (22.493 pg/ml), followed by underweight (24.119 pg/ml) and normal (23.487 pg/ml) with P-value = 0.133

Table (8) Means IL-10 concentration among patients with breast cancer according to various body mass index

Age Groups	Breast Cancer		Mean of INF-ɣ Pg/ml	Control		Mean of INF-ɣ in control Pg/ml	Standard value pg/ml
	No.	%		No.	%		
20 -29 year	2	2.5	3.4	2	2.5	(0.1 – 4.1)	(0.11-1.4)
30 -39 year	10	12.5	5.31	8	10.00		
40 -49 year	25	31.25	5.90	23	28.75		
50-59 year	26	32.5	6.48	24	30.00		
>60 year	17	21.25	6.72	23	28.75		
Total	80	100%		80	100%		

F Ratio = 1.9205 , DF= 3 ,P value 0.1334

Interferon gamma (INF-gamma)

INF-gamma Concentrations among studied groups in relation to age groups

Table (9) show the levels of INF-gamma pg/ml among patients with breast cancer and control groups in various age group that show the age group (20-29) yrs recorded concentration of (3.4 pg/ml) followed by (30-39) yrs (5.31 pg/ml), continued with (40-49) yrs (5.90 pg/ml), (50-59) yrs (6.48 pg/ml) and (>60) yrs (6.72 pg/ml) with P-value = 0.0042.

Table (9):INF-gamma concentrations among patients with breast cancer and control groups in various age group.

Body Mass Index	Breast C No	%	Mean of IL-10 Pg/ml
Underweight	3	3.75	24.119
Normal	27	33.75	23.487
Overweight	36	45.0	22.493
Obese	14	17.5	32.942

P value=0.0136 , F ratio=1.83 , Df=1

INF-gamma concentration among patients with breast cancer according to cancer stage

Table (10) and figure (4) show the levels of INF-G with stages of breast cancer in IA and IIIC recorded concentration mean of (6.1800) and (14.4800) INF-gamma pg/ml respectively , IB (8.4700 pg/ml), followed by IIA (7.8168 pg/ml), IIB (2.8410 pg/ml), and IIIA (9.0616 pg/ml), the IIIB contain (8.9167 pg/ml) and the last stage IV (5.0900 pg/ml) with P-value = 0.5175.

Table (10) show INF-gamma levels in various stage of patients with breast cancer

Stages of Breast Cancer	Breast Cancer No	%	Mean of IFN-ɣ Pg/ml
IA	2	2.5	6.1800
IB	1	1.25	8.4700
IIA	22	27.5	7.8168
IIB	21	26.25	2.8410
IIIA	19	23.75	9.0616
IIIB	3	3.75	8.9167
IIIC	2	2.5	14.4800
IV	10	12.5	5.0900

F Ratio = 0.8920 , DF=7 , P-value = 0.5175

INF-gamma concentration among patients with breast cancer according to various body mass index

Table (11) show the mean of INF-G Pg/ml various body mass index, shown overweight 36 (5.965Pg/ml), followed by underweight 3 (4.050Pg/ml) and normal 27 (6.358Pg/ml) with P-value < 0.9431 .

Table (11) Means of INF-gamma concentration among patients with breast cancer according to various body mass index

Body Mass Index	Breast C No	%	Mean of IFN-ɣ Pg/ml
Underweight	3	3.75	4.050
Normal	27	33.75	6.358
Overweight	36	45.0	5.965
Obese	14	17.5	6.557

F Ratio = 0.1282 , DF = 3 P value 0.9431

Discussion

From total number of (80) patients with breast cancer , the age of patients was between 20->60 yrs and (80) individuals regarded as control group without any chest problems were also studies. ,The majority of disease was in age group 50-59 yrs (32.5%), followed by age group 40-49 yrs (31.25%), and the lowest infection was in age group 20-29 yrs (2.5%) with significant differences (P≤0.05). This results was similar with other studies (Benz, 2009; Andres, *et al*., 2010, and Siegel, *et al.*, 2017). This result is due to modifiable risk factors; and non-modifiable risk factors that is increased risk of breast cancer among participants from Asia was associated with older age. Breast cancer is the malignant tumor that forms from the uncontrolled growth of abnormal breast cells. It usually affects tissues involved in milk production (Ductal and lobular tissues). It is the most common malignancy in women and it remains one of the greatest health threats facing women around the world as we enter the 21st century.

Interleukin IL-1B is a pro-inflammatory cytokine whose expression in primary tumors has been identified as a potential biomarker for predicting breast cancer patients at increased risk for developing bone metastasis. Interleukin- (IL-) 1β is the prototypical proinflammatory cytokine (Filipi, et al., 2015)and its expression in most tumors correlates with tumor invasiveness and metastasis, as well as with angiogenesis(Voronov ,et al., 2003and Naldini ,et al.,2005).Several studies have shown how IL-1β may contribute to breast cancer development and metastasis among it multiple effects, IL-1β activates ahypoxia-angiogenesis (Filippi, et al., 2015 and S-uswam, et al., 2005) program by upregulating the hypoxia-inducible Factor - (HIF-) 1α, the pivotal mediator of cellular responses to hypoxia (Jung, et al., 2003). The finding of IL-1B concentration was higher (5.049 pg/ml) in age group (>60) yrs

comparative with (2.87pg/ml) and (0.2-0.57) in age group (20-29) and control group respectively with significant differences. Similar study showed that the highest mean level of IL-1 beta was found in women with breast cancer comparing with healthy control women (36.92±11.1 and 10.1±3.7 pg/ml) respectively (P: ≤0.05) (Sulaiman, et al. 2019). Cheung, et al., (2015) recorded higher level of IL-1β in patients with breast cancer which agreement with our study.

IL-6 in particular has been shown to play a large role in the inflammatory process following nerve injury and has been implicated in the initiation and maintenance of neuropathic pain (Jongh,et al,2003 , Lee,et al.,2004 and Obreja,et al.,2002). However, IL-6 activity is dependent upon the distribution of receptors on specific cell types to which it can bind. The distribution of membrane-bound (IL-6R) receptors, to which IL-6 can bind directly, is fairly limited throughout the body, existing mainly on hepatocytes and certain subsets of leukocytes. In contrast, IL-6 can complex with soluble receptor IL-6R (sIL-6R) to activate the signal transducing receptor, gp130, which is expressed nearly ubiquitously among all cell types (Kaplanski,et al.,2003 and Heinrich,et al.,2003). This means that cells capable of responding to IL-6 alone are restricted to IL-6R + cells whereas virtually all cells respond to the IL-6/sIL-6R complex. Unlike other soluble cytokine receptors that inhibit cytokine signaling, such as those for TNF-alpha, sIL-6R prolongs the half life of IL-6 and amplifies its inflammatory actions by allowing gp130 + cells to respond to IL-6 (McLoughlin,et al.,2003 and Ernst,et al., 2004). Soluble gp130 (sgp130) can inhibit IL-6 activity by binding to the sIL-6R/IL-6 complex thereby preventing its attachment with gp130 within the cellular membrane. Thus, a higher number of sIL6-R receptors could increase IL-6 activity, thereby increasing inflammation and sensitization of the peripheral nerves, whereas higher sgp130 levels could impair IL-6 activity (Starkweather, 2010). Our study revealed that the mean of IL-6 was (5.77pg/ml) in patients with breast cancer women high than healthy control women (0.48-0.8) pg/ml, and the mean was elevated to (6.65pg/ml) in age group > 60 yrs

IL-10 is an important immunoregulatory cytokine mainly produced by activated T cells, monocytes, B cells and thymocytes. As an immune response modulator, IL-10 can both stimulate and suppress the immune response (Mocellin,et al.,2005). Numerous studies have shown that IL-10 may be involved in the pathogenesis of cancer, but the results were inconsistent. On the one hand, increased serum IL-10 levels could facilitate development of cancer by suppressing expression of MHC class I and II antigens (Kong,et al.,2010) and preventting tumor antigen presentation to CD8-cytotoxic T lymphocytes. On the other hand, antiangiogenic effects of IL-10 are supposed to play a protective and preventive role against tumor (Kong,et al.,2010). The mean value of IL-10 concentration was found significant between age groups which found the aged >60 yrs (26.8pg/ml) higher than other group and the control (6.7pg/ml.) in the current study. The stages of breast cancer show the concentration of IL-10 elevated in stage IV (32.49pg/ml.) than other stages and have high value (32.9pg/ml.) in obese. These result were agreement with same study done in Baghdad that have shown the high significant relation of elevated IL-10 levels, increasing in 98.6% of infected women with breast cancer (Althwani, and Najim,

2011). These increasing of IL-10 inhibits the generation of immune responses at the tumor site. The results of 27 patients with breast cancer were detected by immunohistochemistry, showed that 23 breast cancer samples showed a strong expression for IL-10 (Langsenlehner, et al. 2005). Similar results recorded with our study that show elevated the concentration of IL-10 in all of 80 cases studying. Langsenlehner, et al. (2005) conclude that the IL-10 −592C > A promoter polymorphism may be associated with a reduced the risk of breast cancer. In another study they investigated the roles of IL-10 in regulating of vascular invasion and their prognostic related significance in breast cancer, High expression of IL-10 was related with clinicopathological criteria e.g. hormone receptor status (Ahmad, et al. 2018).

IFN-g is a cytokine that exhibits immune regulator and antiproliferative effects (Karakus,et al.,2011) .IFN-g inhibits the growth of many cell lines that originated from tumors, including breast cancer (Karakus,et al.,2011 and Ruiz,et al.,2000). Low level IFN-g expression promotes tumor growth, but high level IFN-g expression shows antitumor activity (He,et al., 2005). IFN-g gene is located on the long arm of chromosome 12 (12q24.1) (Tegoshi,et al., 2002).One of the variants identified in IFN-g gene is a T>A polymorphism at the þ874 position in the first intron of the gene (Pravica,et al.,2000). This polymorphism coincides with a putative NF-kB binding site, which might have functional consequences for the transcription of the human IFN-g gene (Pravica,et al.,2000). The þ874T and A alleles are associated with high and low the IFN-g production، respectively (Rossouw, et al., 2003). The IFN-g þ874 polymorphism is associated with many diseases, includingbreast cancer (Lio, et al., 2002, Ari, et al., 2003, Daher, et al., 2003, Sarvestani,et al., 2005 and Karakus, et al., 2011). The values of INF- G were studied with relation of age groups which finding the higher value (6.7pg/ml) in ages > 60 years compared with (3.4pg/ml.) in age group (20-29)years and control group (2pg/ml) with significant differences. The levels of IFN-G concentration were fluctuated with the stage of disease and BMI with non-significant P=0.5).

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