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The relationship between cellular mediators (IL-1β, IFN–γ, MMP-9) in Iraqi women with breast cancer

Muna Shukri Mahmood Al-Mashhadani
Post Graduate, Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq
Email: mshmukri@gmail.com

Hazima Mossa Al-Abassi
Prof. Dr., Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq
Email: hazema_mosa@yahoo.com

Wafaa Sabri Mahood
Assit Prof. Dr., Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq
Email: wafa.sabry@yahoo.com

***Abstract*---**Breast cancer is the most common type of cancer in Iraq. The incidence of breast cancer has increased among Iraqi women in the past two decades, as it represents one of the main threats to women's health. This steady aimed To evaluate the level of some immune factors Interleukin-1β (IL-1β), Interferon- γ (IFN–γ), and Matrix metalloproteinase-9 (MMP-9), and find the relationship between these immune factors in Iraqi women diagnosed with breast cancer. Material and method Fifty women diagnosed with breast cancer were enrolled in this study in addition to 25 healthy individuals as a control group. Level (IL-1β, IFN-γ, MMP-9) was measured using ELISA technology. Conclusion The expression level of (IL-1β, IFN-γ, MMP-9) increased in women with breast cancer in different age groups, but the age group (40-49) also recorded the highest expression of these cellular mediators. Regarding the relationship of (MMP-9), it was not significant with (IL-1β), but it was significant with (IFN-γ) ($p \leq 0.05$), and about the relationship between (IL-1β) and (IFN-γ) it was no significant negatively, that Increased level of (IL-1β, IFN-γ, MMP-9) expression could be a predictive marker of breast cancer.
***Keywords*---**Breast cancer, IL-1β, IFN-γ, MMP-9.

Introduction

Breast cancer is one of the most common cancers among women (Abdulkareem and Al-Abassi, 2019) that causes abnormal and uncontrolled proliferation of breast cells (Salman, 2021). Breast cancer is one of the most common types of cancer in Iraq (Alwan, Tawfeeq and Muallah, 2017). The Iraqi Ministry of Health (2019) reported that the total number of cancer deaths amounted to 10,957 in 2019, with a death rate of 10.28/100,000. While the incidence of bronchial and lung cancer was 4.48/100,000 F at a rate of 15.99%, the mortality rate for females with cancer was Breasts 6.22/100,000 Fahrenheit have the highest mortality rate of 22.58%.

IL-1β

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine and is an acidic endotoxin-induced protein secreted by activated macrophages, monocytes, and cells within the hypothalamus. Inflammation-induced overexpression of IL-1β may lead to carcinogenesis, as its elevated level has been observed in lung adenocarcinomas (Milosevic *et al.*, 2020). IL-1β invades pathogens and signals danger and has been found to exert vascular functions, so inhibiting IL-1β signaling is a new therapeutic method to inhibit angiogenesis and inhibit inflammation, and this leads to the weakening of bone malignancy in breast cancer (Tulotta and Ottewell, 2018; Tulotta *et al.*, 2019). it is a prognostic marker for many tumors, including breast cancer and its progression (Templeton *et al.*, 2015), through its role in many physiological events and its ability to modulate gene expression, cytokine production, regulation of cell adhesion and migration, angiogenesis, and immune response (Rébé and Ghiringhelli, 2020)

IFN-γ

IFN-γ, a type II interferon, is a soluble bipolar cytokine involved in cytotoxicity and tumor immune surveillance (Imai *et al.*, 2019) with an important role in innate and adaptive immunity against viral and bacterial infections. IFN-γ activates macrophages and stimulates MHC class II. Abnormal IFN-γ expression is associated with several diseases such as autoinflammatory and autoimmune diseases, IFN-γ can directly inhibit viral replication, and also can modulate immunity. IFN-γ is produced by natural killer (NK) cells and natural killer (NKT) T cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocytes (CTL) as part of the adaptive immune response (Artis and Spits, 2015). human interferon (IFN-γ) plays a role in immune evasion and tumor growth, as it stimulates the expression of immunosuppressive molecules, such as B7-H1 (PD-L1), arginase, and indoleamine 2,3-dioxygenase (IDO), in the tumor microenvironment (Diskin *et al.*, 2020). IFN-γ signaling plays a therapeutic role because it enhances the immune system against cancer by inhibiting the growth of many cancer cells including breast cancer cells (García-Tuñón *et al.*, 2007), and It has also a role in promoting cancer development and immune evasion, so IFN-γ is considered to have a dual role (Du *et al.*, 2021).

MMP-9

The MMP-9 gene is located on chromosome 20 and encodes for gelatinase protein B. MMP-9 expression in normal tissues is little or absent, but it is elevated in the case of inflammation and wound healing (Opdenakker, Van den Steen and Van Damme, 2001)**.** The main substrates for this enzyme include gelatin and collagen IV and V (Dofara, Chang and Diorio, 2020)

MMP-9 plays a major function in degrading and removing ECM molecules from tissues (Lachowski *et al.*, 2019), thus degrading the extracellular matrix, thus affecting the adhesion capacity of cancer cells (Li *et al.*, 2013; Huang, 2018) and also plays a role in angiogenesis leading to tumor development tumor cell invasion, and metastasis (Almalki and Agrawal, 2016)

MMP-9 is secreted by a large number of cell types, such as macrophages, neutrophils, and fibroblasts (Li *et al.*, 2021). The amount of MMP-9 expression is closely related to breast cancer tumor stage, lymph node metastasis, and tumor staging, and it is also a prognostic criterion and contributes to guiding breast cancer treatment (X. Li *et al.*, 2017).

Material and Method

Blood was collected in gel tubes from the first group (50 Iraqi patients) newly diagnosed with breast cancer, their ages ranged (30-65 years), with an average age of 48 years, from the Oncology Teaching Hospital in Medical City in Baghdad and Al-Alawiya Hospital during the period from March to September 2021. Ethical permission was obtained from all participants in this study. Patients were selected and diagnosed under the supervision of the consultant medical staff and pathology committee of the Medical City Teaching Oncology Hospital and Al Alawi Hospital. Cases were diagnosed by mammography and histological findings. It was found that the lesion was early in patients and none of the patients had received chemotherapy, radiotherapy, or mastectomy before blood collection. Blood was also collected from the second group (the control group) which included 25 healthy women of the age group (30-67) years with an average age of 49 years. The main data collected from the patients in this study were: age, weight, Body Mass Index (BMI), primary tumor site, family history, smoking, hormone use, educational level, menstrual history, etc. All the women were of Arab descent, Residents in different areas of Baghdad and the provinces, Tubes were placed in a centrifuge at 3000 rpm, and the level (IL-1β, IFN- γ, MMP - 9) was measured using ELISA technology (Sandwich ELISA). The following cats have calculated the Human Interleukin-1β (IL-1β) (HCUSABIO) ELISA Kit (cat . MMP9) ELISA Kit MYBioSource) (cat number. MB5025304).

Statistical Analysis

Statistical analysis was performed using (Steel and Torrie, 1960) using spss version 25.

Results and Discussion

The results of the current study in a table (1) showed a significant increase in the level of IL-1β in patients with age (<40, 40-49, ≥50) where it reached (12004.801±2317.567, 10001.550±1284.939, 15490.030±4075.269) and the highest significant difference was at the age of 40-49 compared with the control group (1242.787±27.570, 1270.579±126.603, 1147.073±67.94) at the probability level (p≤0.01, p≤0.001, p≤0,05) respectively. while there was no significant difference in IL-1β level among patients with ages (<40, 40-49, **≥50**), and despite that, the highest value for it, was (15490.030 ± 4075.269) in the age group (**≥50**) at probability (p≤0.05).

These results were supported by the study by Ebbing et al., (2019), in which the pro-inflammatory cytokines TNF-a, Il-6, and IL-1B that is a prognostic markers for breast cancer progression (Templeton *et al.*, 2015) as they stimulate angiogenesis that aids its growth, these cytokines are produced by tumor cells and stromal cells in many cancers that lead to the development of drug resistance and metastasis (Hernandez-Vargas *et al.*, 2020), this explains the reason for the high level of IL-1B in patients with breast cancer, and the high level at the age of **≥50**, post-menopause, is due to run away adipokines, cytokines, IL-1β, IL-6, TNF-α, and chemokines such as IL-8 and MCP-1 is from adipose tissue and this has a role in cancer development (Lee *et al.*, 2007), adipose tissue also increases the level of estrogen induced by aromatase activity (Awatef *et al.*, 2011). Studies have shown that the risk of developing breast cancer increases by 40% for every 10 increase in body mass post-menopause that fibroblasts in old age acquire "Secretory phenotype associated with aging" (SASP), which causes the production of pro-inflammatory cytokines (IL-6, IL-1β), chemokines (IL-8, MCP-1, GRO-1/α), MMPs, integrins, and adhesion molecules (Szulc-Kielbik and Klink, 2022).

Table 1: The association of the IL-1β Level with age in the patients and control groups

Age	Level of IL-1β in Patients Group (No. 50)			Level of IL-1β in Control Group (No. 25)			T-test	p-value
	No.	%	Mean ± SE	No.	%	Mean ± SE		
<40	7	14	12004.801±2317.567	5	20	1242.787±27.570	3.870	0.003**
40-49	26	52	10001.550±1284.939	10	40	1270.579±126.603	4.174	0.000***
≥50	17	34	15490.030±4075.269	10	40	1147.073±67.942	2.677	0.013*
F-test	1.252			0.475				
P-value	0.295NS			0.628NS				

NS: Non significant, Pa.: patient, Co.: control
*: significant (p≤0.05), **: significant (p≤0.01), ***: significant (p≤0.001)

The results of Table (2) showed that there were no significant differences in the expression of IL-1β at different levels of Grads, and the results showed that the expression was increased with the highest grades and that the highest expression was recorded in grade III, the result was (2218.337± 14.000, 12134.45± 1662.146, 22391.55± 2.996) in grade I, II, III respectively under the probability level (p≤ 0.05). also, there were no statistically significant differences in the level of IL-1β among different stages, but the highest level was (15342.822 ± 2919.430) which was recorded in stage I, while the results were (13030.782± 3115.887, 10268.547± 1383.792) in stage II, III respectively at the probability level (p ≤ 0.05).

This resalt is agree with (Szulc-Kielbik and Klink, 2022) interleukin (IL-1B) is a pro-inflammatory cytokine that is overexpressed in primary tumors, and in the current study, its expression was elevated in stage I which represents early stages and is a prognostic indicator for breast cancer patients at risk for bone metastases, changes bone rotation in favor of the vicious circle (Tulotta and Ottewell, 2018), Breast cancer tissues are therefore characterized by a high rate of IL-1B expression compared to the corresponding parental line (Nutter *et al.*, 2014). It is closely related to inflammation and has a major role in the development of cancer (Tulotta and Ottewell, 2018). It is produced by tissue macrophages, monocytes, microglia in the brain, and dendritic cells in the skin (Garlanda, Dinarello and Mantovani, 2013). IL-1B is synthesized upon stimulation by PAMPs, (DAMPs), and invading pathogens (Baroja-Mazo *et al.*, 2014).

IL-1B downregulates breast cancer cell invasion through induction of MMP-9 production via focal adhesion kinase 1 (FAK) and the primary oncogene tyrosine-protein kinase SRC (Mon, Senga and Ito, 2017). so there is a close relationship between IL-1B and inflammation, which plays a major role in the development of cancer, the increasing IL-1B level, and IL1R1a is also important in controlling and reducing inflammation, which leads to improvement, inhibition of IL-1B also impairs the recruitment of immune cells to the cancer site, thus inhibiting angiogenesis by decreasing the production of its component cells (Tulotta and Ottewell, 2018).

Table 2: The association of the IL-1β Level with Grad and Stage in the patients and control groups

	Level of IL-1β in Patients Group (No. 50)			
Grad		No.	%	Mean ± SE
	I	2	4	2218.337± 14.000
	II	46	92	12134.45± 1662.146
	III	2	4	22391.55± 2.996
	F-test	1.673		
	P-value	0.199NS		
Stage	I	6	12	15342.822± 2919.430
	II	23	46	13030.782± 3115.887
	III	21	42	10268.547± 1383.792

	F-test	0.603
	P-value	0.551NS

NS: Non significant, Pa.: patient, Co.: control

The results in Table (3) showed the highest significant increase in the level of IFN-γ in breast cancer serum was (259.844 ± 26,205) in patients age group (40-49) compared with the control group (83.267 ± 11.513) at the probability level (0.001). The difference was also significant in the age group (<40, ≥50) which reached (155.771 ± 15.743, 160.848 ± 22.958) compared to the control group (74.278 ± 9.652, 71.138 ± 4.625) respectively (p≤0.01). these results were in agreement with the results of Rasheed (2021) with a significant increase in the level of IFN-γ in Gastrointestinal Tract patients compared to the control group, while the current results disagree with (J. Li *et al.*, 2017).

There was also a significant difference in its level between patients compared to age groups (<40, 40-49, ≥50) where it reached (155.771 ± 15.743, 259.844 ± 26.205, 160.848 ± 22.958) respectively and the highest level was recorded in the age group (40-49) where (p≤0.05) breast cancer patients. this result agreed with (Lee *et al.*, 2014), a study performed on GC patients, who found that there were no significant differences in the level of IFN–γ between different age groups of patients. this is because there is no difference in NK activity between GC patients and healthy subjects of the same age (Lee *et al.*, 2014).

The results of Rasheed (2021) also showed that there was no significant difference in gastro-intestinal expression compared to the three age groups among gastrointestinal patients, These results did not agree with (Lee *et al.*, (2014), where a significant decrease in the level of NKA-IFNγ was observed in GC. patients in different age groups compared to the control group, the reason for the decrease was attributed to the change in the balance of BAX/BCL-2 (García-Tuñón *et al.*, 2007), although the current study agreed with him that there were no significant differences in the level of IFN-γ in different age groups of patients.

The difference in the expression level of IFN-γ results from abnormalities in the functioning of natural killer cells in cancer patients during the early stages of GC, and this defect may be a substrate for tumor progression. Therefore measurement of NK function may be useful in screening for GC (J. Li *et al.*, 2017), IFN–γ has an antitumor effect so it inhibits the growth of cancer cells like breast cancer by regulating the expression of p21 to bring the cell to the resting state and thus prevent the growth and proliferation of cancer cells, as found In a study of prostate cancer, IFN-γ mediated TNFRI stimulation and apoptosis by stimulating p21, which suppresses the cell cycle in the G1 and S phases But if IFN–γ cannot activate p21, the cytoplasmic product p21 will not be able to enter the nucleus and therefore will not be able to stop the cell cycle, and the cancer cell will continue to develop (García-Tuñón *et al.*, 2007) scattering the ability of macrophages to secrete reactive oxygen mediators and reactive nitrogen mediators are related to their ability to perform two important functions: to kill and analyze intracellular microorganisms for cancer cells, Hydrogen peroxide production by macrophages in aged mice was 50% lower than that of young mice, whereas production of macrophage-stimulated cytokines IFN-γ was not diminished in spleen cells in the elderly group. In addition, there was no

difference in the expression of IFN-γ receptors in both old and young mice. Macrophage responses to IFN-γ decreased with age, IFN-γ-induced hydrogen peroxide and nitric oxide release were reduced by 50% in old mice compared to young mice. therefore, a diminished response of macrophages to activation signals is an indicator of a poor immune response in aged mice and this was observed at a lower IFN-γ level in the ≥50 age group compared to the 40-49 age group, IFN-γ can also enhance the protection of cancer cells from natural killer cells mediated by cytolysis by increasing HLA-G and its soluble analog (Aquino-López *et al.*, 2017; Salman, 2021).

Table 3: The association of the IFN-γ Level with age in the patients and control groups

Age	Level of IFN-γ in Patients Group (No. 50)			Level of IFN-γ in Control Group (No. 25)			T-test	P-value
	No.	%	Mean ± SE	No.	%	Mean ± SE		
<40	7	14	155.771±15.743	5	20	74.278±9.652	3.973	0.003**
40-49	26	52	259.844±26.205	10	40	83.267±11.513	4.087	0.000***
≥50	17	34	160.848±22.958	10	40	71.138±4.625	2.953	0.007**
F-test	4.939			0.539				
P-value	0.011*			0.591NS				

NS: Non Significant, Pa.: patient, Co.: control
*: significant (p≤0.05), **: significant (p≤0.01), ***: significant (p≤0.001)

Through the results of Table (4) no statistically significant differences were observed in the level of IFN-γ among the different grades (I, II, III), as it was noted that its expression increased at grade II and then decreased again at grade II The results were (70.6605 ±). 0.499, 219.7647 ± 18.113, 165.1325 ± 1.000) respectively. on the contrary, a significant difference was observed in its expression compared to the different levels of stages (I, II, and III) in breast cancer patients. These results confirmed that the level of IFN-γ decreased at stages II and then its expression increased again at stages III and the results were (311,888 ± 51.942, 165.433 ± 19,743, 233,546 ± 27.971) respectively (p≤0.05).

Natural killer (NK) cells are an important part of innate immunity, and they have an important role in tumor development especially in its early stages (Zhu *et al.*, 2015) This study is in agreement with the current findings where an elevated IFN-γ level was observed in stag I due to NK activity that may adhere to malignant natural killer cells Through Granzyme, perforin and IFN-γ secretion, as well as by antibody-dependent cytotoxicity (ADCC), cell death is enhanced by glycolysis, therefore, any disfunction of natural killer cells causes the development of malignant diseases (Wan et al., 2017) this occurs during the advanced stages of the disease, in the current study, the decreased level of IFN-γ was observed in

stage II compared to the stageI, and this was in agreement with (J. Li *et al.*, 2017) indicated that the level of NKA-IFNγ decreased in the advanced stages of TNM, so the stage IV of TNM recorded the lowest level of IFN–γ compared to third stage II or stage III, as the activity of natural killer cells negatively correlated with the stages of GC (J. Li *et al.*, 2017) . Therefore, decreased T and NK cell activity causes a decreased level of IFN-γ and thus contributes to tumor progression (Ayers *et al.*, 2017) (Gato-Cañas *et al.*, 2017).

Table 4: The association of the IFN-γ Level with Grad and Stage in the patients

	Level of IFN-γ Patients Group (No. 50)			
Grad		No.	%	Mean ± SE
	I	2	4	70.6605±0.499
	II	46	92	219.7647±18.113
	III	2	4	165.1325±1.000
	F-test	1.630		
	P-value	0.207NS		
Stage	I	6	12	311.888±51.942
	II	23	46	165.433±19.743
	III	21	42	233.546±27.971
	F-test	4.627		
	P-value	0.015*		

NS: Non significant, Pa.: patient, Co.: control
*: significant (p≤0.05)

The results in Table (5) showed the highest significant difference in the level of MMP-9 in breast cancer patients as it reached (237.142±21.503)pg/ml in the age group (≥ 50) compared with the control group (41.727±3.764)pg/ml (P≤0.001). The difference was also significant in the age group (<40, 40-49), where it reached (298.605±43.541, 346.268±60.726) compared with the control group (52.576±1.777, 56.097±2.860), respectively, and the level of probability (P≤0.01). however, there was no significant difference in its level among all age groups of patients (<40, 40-49, 50) patients.

The results of the current study are in agreement with those of (Somiari *et al.*, 2006), in which it was found that the level of MMP-9 was elevated in breast cancer patients compared to healthy controls, Matrix Metalloproteinases (MMPs) contribute to ECM degradation of the extracellular matrix as they degrade basement membrane components such as type IV collagen and increase vascular permeability (Quintero-Fabián *et al.*, 2019) thus facilitating cancer invasion, its concentration rises in the case of inflammation and wound healing(Augoff *et al.*, 2022), It also has important physiological and pathological roles, such as fetal tissue remodeling. wound healing and other cellular processes, such as activating and directing adhesion molecules, TNF-α, and fas ligand (Javadian *et al.*, 2019).

As for its concentration in normal tissues, it is little or absent. It encodes the protein gelatinase B. The primary substrates for this enzyme are gelatin and

collagen IV and V (Dofara, Chang and Diorio, 2020). therefore, endopeptidases contribute not only to invasion but also to cell proliferation, immune response, and angiogenesis (Fouad and Aanei, 2017). Also, the present results showed no significant differences in their expression in age groups and this was in agreement with one study that confirmed that abnormal expression levels of MMP-2 and MMP-9 are not related to the age of the patient (H. Li *et al.*, 2017).

However, other studies have shown that its level increases with age (Fiotti *et al.*, 2005). The reason for this is that the level of MMPs rises with aging, as fibroblasts acquire a 'senescence-associated secretory phenotype (SASP), which produces pro-inflammatory cytokines (IL-6, IL-1β), chemokines (IL-8, MCP-1, GRO-1/α), MMPs, and integrins, adhesion molecules (Szulc-Kielbik and Klink, 2022).

Table 5: The association of the MMP-9 Level with age in the patients and control groups

Age	Level of MMP-9 Patients Group (No. 50)			Level of MMP-9 Control Group (No. 50)			T-test	P-value
	No.	%	Mean ± SE	No.	%	Mean ± SE		
<40	7	14	298.605±43.541	5	20	52.576±1.777	4.707	0.001**
40-49	26	52	346.268±60.726	10	40	56.097±2.860	2.937	0.006**
≥50	17	34	237.142±21.503	10	40	41.727±3.764	6.878	0.000***
F-test	1.106			5.760				
P-value	0.339 NS			0.01**				

NS: Non significant, Pa.: patient, Co.: control
: significant (p≤0.01), *: significant (p≤0.001)

As for the level of MMP-9, it was found that there are no significant differences between the different levels of Grad (I, II, III) in patients who reached (105.735 ± 0.501, 313.291 ± 35.690, 250,887 ± 0.495) respectively, But the highest level was recorded (313.291 ± 35.690) in grade II, but a significant difference in its expression compared to the different levels of stages (I, II, and III) was observed in breast cancer patients (526.588 ± 204.061, 272.639 ± 25.790, 271.161 ± 43.553)pg/ml respectively, and the highest values recorded in the first stage and below the probability level (p≤0.05).

Previous studies have shown an association between a high level of MMP-9 expression with high histological grades including HER2-negative and triple-positive breast cancer (Yousef *et al.*, 2014). Where it was observed that its level increased in the second and third grades, although there were no intestinal differences, also, its level was significantly higher in breast cancer patients than in the benign disease group (Wu *et al.*, 2008), and this confirms its higher level with disease progression, Studies have confirmed that MMP expression is associated with a poor prognosis for invasive breast cancer, It has been found

that there is a relationship between increased expression of MMP and poor prognosis of patients in different types of cancers, especially breast cancer, abnormal level of MMP-9 expression is also associated with lymph node metastasis in addition to its association with tumor stage (H. Li *et al.*, 2017). This is because MMP-9 is the main component of the basement membrane (Sand *et al.*, 2013), and it can smash all kinds of ECM and type IV collagen (Weaver, 2006). facilitate the invasion of tumor cells into the local blood vessels and tissues and thus the formation of new metastases, MMPs contribute to the biological functions of the cell such as programmed cell death, cell proliferation, differentiation, migration, and host defense, in healthy cases, there is a physiological balance between activation and inhibition of protein degradation through the expression of TIMPs and MMPs, but this balance is broken in the case of cancer. In addition, the presence of CD44 on the surface of the cancer cell facilitates cancer cell invasion and aids in angiogenesis (Chetty *et al.*, 2012).

MMP also modulates the tumor immune microenvironment. (Kessenbrock, Plaks and Werb, 2010) . MMP is a diagnostic biomarker for aggressive and metastatic types of breast cancer (Yousef *et al.*, 2014), it also has prognostic significance, as its level was significantly decreased after 1 month and 6 months post-surgery (Quaranta *et al.*, 2007).

Table 6: The association of the MMP-9 Level with Grad and stage in the patients

	Level of MMP-9 Patients Group (No. 50)			Mean ± SE
		No.	%	
Grad	I	2	4	105.735± 0.501
	II	46	92	313.291± 35.690
	III	2	4	250.887± 0.495
	F-test	0.785		
	P-value	0.462NS		
Stage	I	6	12	526.588± 204.061
	II	23	46	272.639± 25.790
	III	21	42	271.161± 43.553
	F-test	3.378		
	P-value	0.043*		

NS: Non significant, Pa.: patient, Co.: control
*: significant (p≤0.05)

The Correlation Between Immunological Mediateres

The results in the table (7) show the transformation of the poitive relationship between IL-1β and IFN–γ in the healthy group to a negative relationship in breast cancer patients and the reason for this:

The inflammatory state in cancer leads to the secretion of inflammatory mediators, including cytokines such as IL-1β and chemokines. Chronic inflammation causes approximately 15% of cancers, and NO synthase and ROIs are produced, both of which are capable of DNA damage (Szulc-Kielbik and Klink, 2022). Ras-induced ROS production also causes DNA damage and genomic instability and is an important feature of tumor enabling through oncogene activation (Algire *et al.*, 2012), also, iNOS which is one of the NOS isoforms involved in carcinogenesis.

It is expressed after cytokine exposure where it downregulates tumor activity such as tumor transformation, angiogenesis, metastases, and angiogenesis (Vannini, Kashfi and Nath, 2015). in addition, the expression of HLA-G receptors on the surface of T cells and NK cells causes dysregulation of these cells (Chauvin *et al.*, 2015), which leads to decreased infiltration of NK cells and T cells into the tumor environment. As a result, IFN-γ expression is reduced. (Szulc-Kielbik and Klink, 2022).

Whereas, the association of IL-1β was insignificantly positive with MMP-9 in both patient and control groups. Studies have confirmed that both IL-1β and MMPs are positively elevated in the senescence stage of cancer fibroblasts as they acquire a senescence-associated secretory phenotype. (SASP), which causes the production of pro-inflammatory cytokines (IL-6, IL-1β), chemokines (IL-8, MCP-1, GRO-1/α), MMPs, and integrins, adhesion molecules (Szulc-Kielbik and Klink, 2022) Therefore, we note a positive relationship between them.

Concerning the relationship of IFN–γ with MMP-9, it was a significantly positive relationship under the level of protection ($p \leq 0.05$) in the group of patients, as well as the relationship in the control group, but it was not significant. This could be explained by the fact that tumor cells increase the expression of IFN-γ to reduce the expression of natural killer (NK) cells and T cells, thus weakening the body's immunity.

Also, tumor cells increase the expression of Matrix Metalloproteinases (MMPs) contribute to ECM degradation of the extracellular matrix as they degrade basement membrane components such as type IV collagen and increase vascular permeability facilitating cancer invasion and progression (Wan et al., 2017).

Table 7: the correlation of immunological mediate among themselves in patients and controls

		IFN–γ	MMP-9
IL-1β	Patient No.50	-0.155	0.181
	Control No.25	0.493*	0.300
IFN–γ	Patient No.50		0.303*
	Control No.25		0.329
*. Correlation is significant at the 0.05 level (2-tailed).			

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