RESEARCH ARTICLE

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Immunohistochemical Study of the Expressed Cluster Differentiation Markers Proteins Type 20 And 56 in Breast Tissues from a Group of Iraqi Patients with Breast Cancers

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Abstract

Background: Tumor-infiltrating lymphocytes (TIL) are important immunological components in response to cancers. Patients with higher numbers of TIL in breast cancerous tissues, comprising T- cytotoxic and T – helper cells along with B- and rare natural killer (NK) cells, have more favorable clinical outcomes. **Objective:** To analyze the rate of the expressed surface biomarker proteins of CD20-B cells and CD56- NK cells on the infiltrative lymphocytic subpopulations in a group of breast tumorous tissues (invasive and benign) from female patients in Iraq and explore the relations to the grade of the invasive breast cancerous tissues. **Patients and methods:** One hundred and 75 archived breast tissues were enrolled in this retrospective research: 100 archived breast from female patients with invasive breast cancers (BC) [20 well differentiated BC tissues; 48 moderately differentiated BC and 32 poorly differentiated BC tissues]; 50 tissue biopsies from female patients with benign breast tumors and 25 apparently normal individuals with healthy breast tissues (included as the control group for this study). Immunohistochemistry was achieved for the detection of the expressed surface biomarker proteins related to B cell CD20 and NK cell CD56 present on the infiltrative lymphocytic subpopulations in breast tissues by using specific primary antibodies for these proteins via utilizing an immune-enzymatic antigen detection system. **Results:** The detection of IHC reactions for the expressed B cell CD20 - cell surface (CD) biomarker proteins were observed in 53 out of 100 (53.0%) BC tissues, and in 24 out of 50 (48.0%) benign breast tumorous tissues, while CD20- positive cell surface markers was detected in apparently healthy breast tissues of the control group in a percentage of 32.0% (8 out of 25 tissues). Statistical significant differences ($P<0.05$) between both groups of malignant and benign breast tumors and the control group were found. However, between breast malignant and benign tumor groups, no significant difference was found ($p >0.05$). Detection of CD56- IHC reactions revealed in 14% (14 out of 100 BC tissues), in 16% (8 out of 50 benign breast tissues) and none of control breast tissues revealed CD56- IHC reactions. Among all the enrolled groups, no significant differences ($P>0.05$) were detected. **Conclusions:** The observed significant rates that showed highly significant differences between both studied groups of breast malignant and benign tumor in comparison to the control group indicate that the CD20- positive infiltrative B cell- lymphocytic subpopulations might contributed in the defense against these subsets of benign and malignant breast tumors. However, the observed rates of NK cell CD56 present on the lymphocytic subpopulations infiltrating the examined malignant and benign breast tumorous tissues seeming to play irrelevant roles in the defense against these studied breast tumor groups.

Keywords: Breast cancers- benign breast tumors- CD56- CD20- NK cells- B lymphocytes- IHC

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Introduction

Globally, breast cancer is the most prevalent cancer. World-wide, 2.3 million women in 2020 as well as 7.8 million were diagnosed in the past 5 years to have breast cancers and reporting 685,000 deaths from such cancers (WHO, 2021). The incidence of female breast cancers continued to have a slow increase (annually as 0.5%) from 2014 through the year of 2018. In 2022, death was estimated to comprise a total of 43,250 women as well as 530 men because of their breast cancers (ASC, 2021).

A rare, highly aggressive, inflammatory breast cancer (IBC), is still poorly understood although it accounts for < 3% of all breast cancers forms and constituted one tenth of their related deaths. Improvements of survival of patients with IBC were achieved by many therapeutic approaches, yet, inflammatory breast cancer has a 5-year survival of ~30% which is still worse than stage-matched non-IBC counterparts (Çakar et al., 2018). Hence, developing biomarkers in favor of better prognostic as

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well as predictive purposes are constituting an urgent and an important need for identifying those patients who will potentially get benefit from applying different treatment interventional modalities for IBC. The tumor-infiltrating lymphocytes (TIL) are regarded as crucial immunological components in response to cancers. Their lymphocytes partners are recognized by the specific expression of biomarkers on their cell surface (Whiteside, 2022). B-cells drive the humeral immune response as well as their role in the antibody-dependently drive response and are often co-localized with T- cells counterpart immune response to the tumors (Laumont et al., 2022).

Previous researchers potentially predicted the presence of infiltrated lymphocytes with CD3-, CD8-, and CD20- positive markers to have relation to an improved response to both chemo- as well as monoclonal antibody therapies (Dunn et al., 2004; Vesely et al., 2011; Luque et al., 2022). The CD20 is a member of the MS4A family, recognized as trans-membrane protein composed of non-glycosylated 33–37 kDa phosphoprotein and was recognized as a surface marker of B-cell that activating and proliferating B-cells as well as involved in calcium channeling (Hong et al., 2021). The expression of CD20 was found to be initiated at pre-B cells developmental stage and up to plasma cells development. It is worthy to mention that such CD20- positive cellular population were previously being considered as cancer stem-like cells or tumor-initiating cells fulfilling some tumor stemness criteria (Pavlasova and Mraz, 2020).

CD56 is a cluster of differentiation of neural cell adhesion molecule (NCAM) that is expressed abundantly in the cells of neuronal origin where multiple isoforms were identified, the most abundant are the NCAM120, NCAM140 and NCAM180. In addition, NCAM plays other important non-immune cellular roles as those found in disorders of learning, memory and behavior.Also, this NCAM was also found to be expressed in liver, kidney, heart, muscles, and on hematopoietic cells, as on natural killer and non- killer T cells as well as the dendritic cells (Gunesch et al., 2020). Among the human circulating lymphocytes, the NK cells, which are defined within them as CD56+CD3− cells, constitute approximately 10% of these lymphocytes where the CD56dim cellular subset of are found as predominant peripheral blood circulating NK cells whereas their counterparts of CD56 bright cells are the minor subset among these circulating NK cells. The distinction of the phenotypic and functional capacities of both subsets are defined through the expression of their unique surface cellular receptors as well as transcription factors and their intracellular effector molecules. However, human CD56-NK cells role in the immune function is poorly However, human CD56-NK cells role in the immune function is poorly defined (Justin et al., 2020).

Human natural killer (NK) cells are important components in the innate immune system and having importantly shared roles both in clearing tumorous and virally- infected cells (Cooper et al., 2009). The present study was designed to describe the rates of human natural killer (NK) cells expressing CD56 proteins as well as the B cells expressing CD20 proteins in the a group of breast tissues obtained from patients in Iraq with both benign breast tumors as well as primary invasive breast cancer enrolled in this study.

Materials and Methods

This retrospective research was designed as a case-control study on a total enrolled number of 175 archival female breast tissue blocks, for the detection of the expressed CD20-cell surface differentiation protein biomarker of B cells as well as that of the CD56-cell surface differentiation proteins biomarker of NK- T cells which were present among the infiltrative lymphocytic subpopulations in the examined breast tissues by using specific primary antibodies for these proteins by using the immunohistochemistry technology and via utilizing an immune enzymatic antigen detection system.

Following the accurate application of the instructions of the manufacturing company, the results obtained of the properly enhanced IHC detection reagents are the observed detection of a brown precipitate in the positive cells in each examined tissue sections. This study analyzed the measurement of the density of each specifically defined CD20- positive and CD56-positve-TILs per high-power field, where quantification of the immunohistochemical reactions of the expressed proteins of CD20- positive and CD56-positve-TILs were counted in ten different fields of each samples under light microscopy at X1000, where an average of positive cells in 10 high power fields has assigning the results to one of the 3 following categories: 1. Score 1: 1-25%; 2. Score 2: 26-50%; and 3. Score 3: >50% . Based on the intensity of positive staining and the number of stained cells, they have being determined as an intensity and percentage scores, respectively, where the scales 0-3 allocated for the relative intensity where scale 0 is corresponding to no detectable IHC reaction, while scales 1, 2 and 3 are equivalent to low, moderate and high intensity, respectively (Zlobec et al., 2006).

Results

Distribution of female patients with breast cancers, benign breast tumors and apparently healthy control female counterparts according to their age

The archival breast specimens enrolled in this research study were related to female patients with breast tumors whom age ranged from sixteen years to seventy- eight years while the age of their apparently healthy control female counterparts ranged from nineteen years to sixty-eight years. The mean age of the patients with breast cancers (43.5 + 10.7 years) was higher than the mean age of the benign group (40.3 + 12.2 years) and the mean age of those females in the group of healthy control (39.9+ 11.1 years). However, there are non-significant statistical differences (P>0.05) among the studied groups according to the age of different female individuals (Table 1).

Histopathological grading of breast carcinomatous tissues

Using Scarf-Bloom-Richardson system for grading of breast cancers, the results of the present study showed that breast cancers with moderately differentiated grade constituted 48% (48 of total 100 tissues), whereas breast

Table 1. Age Distribution of Female Patients with Breast Tumors

	Enrolled Numbers					
Female Patients Groups		Mean Age	S.D	S.E	Minimum	Maximum
Breast Cancers	100	43.5	10.7	1.6	18	78
Benign Breast Tumors	50	40.3	12.2	2.1	16	75
Individuals with Healthy Breast Tissues (Control Group)	25	39.9	11.1	2.8	19	68
Statistical Analysis						P= 0.06: (P>0.05)

Table 2. Scarf-Bloom-Richardson System for Grading of the Studied Breast Cancers Tissues

Breast Cancers (Grading / Differentiation)	N	%
Well *	20	20
Moderately **	48	48
Poorly	32	32
Total	100	100

cancers tissues with poorly and well differentiated grades were constituting 32% (32 out of 100 tissues) and 20% (20 out of 100 tissues), respectively. The statistical analysis of grading distribution of breast cancers showed significant differences (p<0.05) between poorly and well differentiated grades, while non-significant difference was noticed between poorly and moderately differentiated grades of breast cancers (Table 2).

Results of signal scoring of IHC testing for CD markers in breast tissues of female patients with breast tumors

A: The Rsults of IHC Testing for Cell Surface CD 20 Markers:

*Signal scoring distribution of immunohistochemical staining for CD20 protein in breast tumor*s

The immune staining of CD20- positive cell surface (CD) markers resulted in a brownish discoloration at the specified cell surface localization. The CD20- positive cell surface markers were revealed by IHC in 53% (53 out of 100) in the breast carcinomatous tissues group, while 48% (24 out of 50) of benign breast tumor tissues group and 32% (8 cases out of 25) of healthy (control) breast tissues group showed CD20- positive cell surface markers. The statistical analysis of the percentage of detection of CD20- positive cell surface markers in both breast cancers and benign tumor groups and as compared to their healthy breast tissues group have revealed statistically significant differences (P<0.05). However, the difference between breast cancers and benign tumor groups was statistically not significant (p >0.05) (Table 3).

Table 3. The Signal Scoring Distribution of Immunohistochemical Staining for CD20 Protein in Breast Tumors

State of CD20- Immunohistochemical signaling		Control breast tissues (n=25)		Benign breast tumors (n=50)		Breast Cancers (n=100)		P Value
		N	%	N	%	N	%	
Signal Scoring	Negative	17	68	26	52	47	47.0	0.038 (Significant differences = P<0.05).
	Positive	8	32	24	48	53	53	
	I	5/8	62.5	10/24	41.7	32/53	60.4	
	II	2/8	25	8/24	33.3	Nov-53	20.8	
	III	1/8	12.5	4/24	16.7	Jul-53	13.2	
	IV	0	0	2/24	8.3	Mar-53	5.7	
	Mean Rank		44		89.5		94	

Table 4. The Correlation of IHC Signal Scoring of CD20-Tumor Infiltrative Lymphocytes with Breast Cancer Typing

		Breast Cancer Types						
	CD20-TILs Status	Ductal (n==-70)		Medullary (n=19)		Lobular (n=11)		P value
		N	%	9/19	%	N	%	
Scoring	Negative	30/70	47.0	10/19	47.3	8/11	72.7	P= 0.049 (Significant differences =P<0.05)
	Positive	40/70	53.0	6/10	52.7	3/11	27.3	
	I	23/40	57.5	2/10	60.0	2/3	6672	
	II	10/40	25.0	1/10	20.0	1/3	33.3	
	III	6/40	15.0	1/10	10.0	0/3	0.0	
	IV	1/40	2.5	65.1	10.0	0/3	0.0	
	Mean rank	60.5		65.1		72.3		

Table 5. The Correlation of Immunohistochemical Signal Scoring of CD20-Positive Tumor Infiltrative Lymphocytes with the Grading of Breast Cancers

CD20-immunohistochemical Signal Status		Breast Cancer Types						P
		Well differentiated (n=45)		Moderately differentiated (n=30)		Poorly differentiated (n=25)		
		N	%	N	%	N	%	
	Negative	19/45	42.2	13/30	43.3	15/25	60.0	0.043
	Positive	26/45	57.8	17/30	56.7	10/25	40.0	[S]
Signal Scoring	I	8/26	30.8	9/17	52.9	6/10	60.0	
	II	14/26	53.8	4/17	23.5	3/10	30.0	
	III	4/26	15.4	3/17	17.7	1/10	10.0	
	IV	0/26	0.0	1/17	5.9	0/10	8.6	
	Mean rank	62.6		64.1		66.2		

Table 6. The Signal Scoring Distribution of Immunohistochemical Staining for CD 56 Protein in Breast Tumors

CD20-immunohistochemical Signal Status		Apparently healthy breast tissues (n=25)		Benign breast tumors (n=50)		Breast Cancers (n=100)		P Value
		N	%	N	%	N	%	
	Negative	25/25	100.0	42/50	84.0	86/100	86.0	P=0.7
	Positive	0	0.00	8/50	16.0	14/100	14.0	[Non- significant differences] (P>0.05)
Signal Scoring	I	0	0.0	6/8	75.0	11/14	78.6	
	II	0	0.0	1/8	25.0	2/14	1.4	
	III	0	0.0	1/8	25.0	1/14	7.1	
	IV	0	0.0	0.0	0.0	0	0.0	
	Mean rank	88.5		93		87.3		

The highest percentages of CD20- immunohistochemical signaling were found to have score I in 62.5% (5 of total 8 tissues), 41.7% (10 of total 24 tissues) and 60.4% (32 of total 53 tissues) of normal breast tissues group, benign breast tumors and breast cancers, respectively. The percentage of CD 20 was significantly higher in both benign and malignant breast tumor groups when compared to control group.

Typing of breast cancer in correlation with positivity of CD20- tumor infiltrative lymphocytes

Table 4 shows the correlation of CD20- IHC scoring of tumor infiltrative lymphocytes in correlation with cancer types. The results showed that positivity of CD20-IHC reaction found in 53 out of 100 breast cancer tissues. The highest percentages of CD4- IHC that showed low score (score I) were found in 32.9%; 31.6% and 18.2% of ductal, medullary and lobular breast carcinoma, respectively. The statistical analysis of CD20-IHC signal scoring, in correlation with breast cancer typing, showed significant differences (P<0.05).

The correlation of CD20-positive tumor infiltrative lymphocytes with breast cancer grading

It was found that 31.1% of breast cancer tissues that showed score II of IHC reaction for CD20-TILs have well differentiation while 30% of breast cancer tissues that have score I of IHC reaction for CD20 have moderate

Table 7. The Correlation of CD56- Positive TIL Scoring with Breast Cancer Typing

IHC-Signal Status of CD56-TILs		Breast Cancer Types						P Value
		Ductal (n==-70)		Medullary (n=19)		Lobular (n=11)		
		N	%	N	%	N	%	
	Negative	60/70	85.7	16/19	84.2	10/11	90.9	0.69
	Positive	10/70	14.3	3/19	15.8	1/11	9.1	[N.S]
Signal Scoring	I	9/10	90.0	2/3	66.7	1/1	100.0	
	II	1/10	10.0	1/3	33.3	0/1	0.0	
	III	0/10	0.0	0/3	0.0	0/1	0.0	
	IV	0/10	0.0	0/3	0.0	0/1	0.0	
	Mean rank	67.5		64.7		58.5		

Table 8. The Correlation of CD56-IHC Scoring with Grading of Breast Cancers

CD56-Immunostaining Signal Status		Breast Cancer Grading / Differentiation: Well differentiated (n=45)		Moderately differentiated (n=30)		Poorly differentiated (n=25)		P Value
		N	%	N	%	N	%	
	Negative	39/45	86.7	25/30	83.3	24/25	96.0	P=0.3
	Positive	6/45	13.3	5/30	16.7	1/25	4.0	[non- significant differences] (P>0.05
Signal Scoring	I	4/6	66.7	4/5	80.0	1/1	100.0	
	II	2/6	33.3	1/5	20.0	0/0	0.0	
	III	0/0	0.0	0/0	0.0	0/0	0.0	
	IV	0/0	0.0	0/0	0.0	0/0	0.0	
	Mean rank	78.5		70		65.5		

differentiation. Lastly, 24% of the breast cancer tissues that showed score I have presented with poor differentiation. The statistical analysis of CD20-IHC signal scoring in relation to breast cancer grades showed significant differences(P<0.05) (Table 5).

B: The Results of IHC Testing for Cell Surface CD56 Markers:

Signal scoring distribution of immunohistochemical staining for CD56 protein in breast tumors

In the present study, the immunostaining for the CD56 -cell surface marker protein was detected as a brownish discoloration at the specified cell surface localization of the specific CD56 - antibodies at the cellular sites of CD56 -cell surface marker protein (Figure 2). The CD56- IHC reactions detected in a percentage of 14% (14 of total 100 tissues) in the breast cancers group and in 16% (8 0f total 50 tissues) in the benign breast tumors group while none of normal breast tissues (in the control group) revealed CD56- IHC reactions. Non- significant differences (P>0.05) were found among the results of CD56 scoring in all of the studied groups (Table 6) .

CD56-Positive TILs in relation to breast cancer typing

The CD56 – IHC scoring results showed that 14 tissues out of 100 (14%) have positive CD56-IHC reaction. The highest percentages of CD56-IHC reaction that have low score (score I)[9 out of 10 tissues; 2 tissues out of 3; and 1 tissue out of 1] were found to be breast carcinoma of ductal, medullary, and lobular types, respectively. The statistical analysis of CD56-IHC signal scoring in relation to breast cancer typing showed non- significant differences (P>0.05) (Table 7).

The correlation of CD56-positive TILs with breast cancer grading

It was found that 8.9%, 13.4% and 4.0% of breast cancer tissues that showed score I of IHC reaction for CD56 have well differentiated, moderately differentiated and poorly differentiated grades, respectively. The statistical analysis of CD56-IHC signal scoring in relation to breast cancer grades showed non- significant differences(P>0.05) (Table 8).

Discussion

The pathogenesis of inflammatory breast cancer (IBC), the highly aggressive form among breast cancers, is still poorly understood, yet, the development of an urgent as well as important prognostic and predictive biomarkers constituting a crucial need to identify those patients for potential benefits by getting different treatment modalities for such BC (Feng et al., 2018).

In addition, it was found that the programmed cell death ligand (PD-L1) is expressed both on TILs as well as the tumor cells, where by binding to its programmed cell death receptor 1 (PD-1) and CD80 receptor, has played a crucial role in immune suppression, via suppressing activation of T cells and inducting apoptosis of T cells, and in their way of escaping tumor-specific T cell immunity, thus, blocker antibodies or inhibitors of these PD-L1 and PD-1 receptor therefore representing an approach in IBC- therapy since are increasing such specific T cell immunological response to different tumors, including BC (Rollins and Gibbons, 2017). Several other researchers, however, reported in their studies that PD-L1 associated with favorable outcome in breast cancer patients (Schmid et al., 2018; Parvathareddy et al., 2021).

According to Nottingham Bloom Richardson grading system for grading of breast cancers, the results of the current study showed that breast cancers with moderately differentiated grade constituted 48% whereas those tissues with poorly and well differentiated grades constituted 32% and 20%, respectively (Table 2). The researchers found that the histological grading is a critical risk parameter to assess patients with BC, where younger women with higher grade and high proliferated tumors had more vascular invasion as compared to the tumors in their older women counterparts. Also researchers found that the 10-years survival percentages of BC patients with grade I reached 80% but descended to 45% in BC patients with grade III (Harvey and Everett, 2004).

As an important grading system in making a clinical decision, the broadly used Nottingham Bloom Richardson grading system (NHG) is representing a well-established prognostic factor in breast cancer, where this NHG system allocated the scores of 0–6 mitotic figures per 10 high-power fields as grade 1, scores of 7–12 as grade 2,

and scores of >12 mitotic figures as grade 3 (corrected for field size of 0.183 mm2) (Meyer et al., 2005). However, 50% of breast cancer patients are classified to have grade 2 (as an intermediate risk group as well as with low clinical value) (Siegel et al., 2022). A heterogeneous population of tumor-infiltrating lymphocytes (TIL) are regarded as an important immunological component in response to cancers (Luque et al., 2022) and in breast cancers have been detected at different proportions by several studies, mainly comprising cytotoxic T cells, along with different proportions of T – helper cells as well as B cells and rare natural killer (NK) cells (Whitford et al., 1992; Chin et al., 1992; Shi-Chao et al., 2019).

For assessing TILs the following various methods are used: as TIL count; as density, which is measurement of TILs per high-power field; as semi-quantitative scales, and lastly the as the percentage of stromal infiltrate. TIL is assessed as intra-tuomral or stromal location (Verdicchio et al., 2023). Previous studies predicted that the lymphocyte infiltration have an improved response to chemotherapy as well as treatment by specific monoclonal antibodies, and CD20 along with CD3 and CD8 have represented as a potentially predicting biomarkers (Ruffell et al., 2012; Brown et al., 2014). The results of the present study showed that the IHC detection of the expressed B cell CD20 - cell surface differentiation(CD) proteins biomarker were observed in (53.0%) of BC tissues, and in (48.0%) benign breast tumorous tissues, while CD20-positive cell surface markers was detected in a 32.0% of apparently healthy breast control tissues. Statistically, significant differences ($P<0.05$) found between each breast tumor groups in comparison to control group, while non-significant difference between both breast tumor groups ($p >0.05$).

Previous reports had identified an incrementally encroachment of CD20+ B cells in the tumor cellular microenvironments as a clinically effective way that is strongly associated with both better breast cancer patient's outcome and their response to NEO adjuvant chemotherapy (Brown et al., 2014; Arias et al., 2018).

The lymphocytic subpopulations are recognized by their expression of specific cell surface biomarkers. NK cells are regarded as an important component in the innate immune system and are playing a central role both in defense against viral infections as well as their way in tumor surveillance. Among the human circulating lymphocytes, the NK cells, which are defined as CD56+CD3− cells, constitute approximately 10% of these lymphocytes (Lanier, 2008). The results of the current study also revealed that the IHC detection of the expressed CD56-cell surface differentiation (CD) proteins biomarker were revealed in 14% of 100 BC tissues and in 16% of 50 benign breast tissues while none of control breast tissues revealed CD56- IHC reactions.

Regarding the extent and type of lymphocyte infiltration in relation to subtypes of carcinoma of the breast, no clear conclusions reached for both the efficacy of T cell-dependent immune mechanisms and the tumor progression of these BC subtypes (Christina et al., 2019).

In this study, CD56-positive TILs in relation to breast cancer grading showed that 13.3%, 16.7% and 4.0% of breast cancer tissues expressed positive IHC reactions for CD56 that were having well, moderate and poor differentiations, respectively (Table 8). Tumor-infiltrating lymphocytes (TIL) are an important immune component of the response to cancer.Despite conserved expression of CD56 on the human NK cells, its role in the immune function is poorly defined (Gunesch et al., 2020).

The presence of higher numbers of tumor-infiltrating lymphocytes in breast cancerous tissues have been found to be associated with more favorable clinical outcomes of patients with this cancer (Nayeli et al., 2016). Among the drawbacks in assessment TILs by the various mentioned methods were the variable selections as well as the number of analyzed fields of view and moreover, including issues as TILs semi- quantitative assessed, inability to account for location, subjectivity and non-reproducibility (Chin et al., 1992; Brown et al., 2014; Verdicchio et al., 2023).

The active cellular immunity has an important role in targeting the cancer cells and eradicating the residual tumor cells and as such, a loss of the cellular immunity as well as humeral immunity imbalance have triggered cancer progression (Sin et al., 2019). It was reported that the function of tumor-infiltrating lymphocytes could be impaired by the effect of many factors , including, inhibitory cytokines, highly active regulatory T lymphocytes, altered MHC molecules on these tumor cells and Fas ligand aberrant expression (Christina et al., 2019).

On the other hand, and based on the previous HPVs, HCMV and EBV investigations in human breast cancers by a previous study in Syria (Akil et al., 2008) as well as by many Iraqi studies (Ali et al., 2017; Ali et al., 2017; Ali et al., 2018; Alajeely et al., 2019) , could point for important roles of the detected TILs against the infections of breast tissues by HPV , HCMV and EBV and their cooperation in human breast cancer, as well. However, the lower counts of NK detected in breast tissues from female patients with carcinoma and benign tumors could be related to that T lymphocytes and dendritic cells are mutually acting with NK cells, which can results in inhibition of NK in certain breast tissue microenvironments (Raulet, 2004).

Monocytes chemokines protein -1 (MCP-1) is over-expressed in breast tumor cells (Soria and Ben–Baruch, 2008), increasing the harmful tumor-associated macrophages and inhibiting the effects of anti-tumor T cells on the cancer (Feng et al., 2011). It can concluded that the higher percentages of the detection rates of CD20- and CD56- tumor infiltrating lymphocytes could point for a possible compatibility of their participation in the cellular immunity during the initiation and / or progression of breast cancers and benign tumors as well as against any possible associating and relating viral infections of the breast tissues.

Author Contribution Statement

All authors contributed equally in this study.

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Approval

If it was approved by the scientific committee of

microbiology department in the college of medicine university of Baghdad, Iraq.

Ethical Declaration

Approved by the ethical committee, college of medicine university of Baghdad, Iraq.

Study Registration

The study registered in registering dataset of University of Baghdad.

Conflict of Interest

This work has no conflict of interest.

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