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Anti- Cytomegalovirus antibodies, TNF- α, IL-19, CD4, CD163, and VEGF-A serum levels in Patients with Breast Cancer

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ABSTRACT:

Breast cancer is the most prevalent malignant tumor detected in women globally. Cytomegalovirus (CMV) infection has been linked to immune system modulation, potentially altering cytokine levels and establishing a tumor-promoting microenvironment. The aim of the study was to determine the effect of CMV infection and cytokine levels in breast cancer progression. A total of 80 blood samples were analyzed, comprising 40 samples from breast cancer patients admitted to Nanakaly Hospital's / oncology department and 40 samples from healthy controls selected randomly. CMV-IgG and IgM levels were tested using a Cobas analyzer to determine seropositivity among the participants in the study. ELISA technique was used for determining serum cytokine levels including interleukin-19 (IL-19), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and soluble CD163 and CD4. Statistical analysis was used to determine the associations between CMV seropositivity, cytokine levels, and breast cancer progression. TNF-α emerged as the most diagnostically accurate biomarker due it is high area under curve (AUC). It demonstrated statistically significant odds ratios as a risk factor for breast cancer development (P= 0.048). CD4, IL-19, exhibited minimal impact on breast cancer development (p=0.659 and p=0.564 respectively), indicating weak associations. Vascular endothelial growth factor A (VEGF A) did not show a significant association with the development of the breast cancer. Similarly, CD 163 showed no relationship with breast cancer progression. In addition, in this study the serum concentrations of all immunological and tumor markers were elevated in CMV IgG positive controls as detected in breast cancer patients. It is concluded that high levels of TNF-α in both CMV infected and breast cancer patients indicates a positive correlation between the progression of breast cancer and CMV infection. Slight increase in the other estimated markers may also confirm this correlation in this study.

Keywords: Cytomegalovirus, Breast cancer TNF-a, CD4, CD 163, IL-19

1 INTRODUCTION

The most common carcinoma and a major contributor to deaths caused by cancer in women is breast cancer. Initiation of breast cancer, development, progression, and metastasis are complex physiologically, immunologically, and hormonally [1, 2]. The body's immune response has conflicting roles in the initiation and metastasis of cancers. Although surveillance of immune system provides a crucial first defense towards malignant cells, immune response can also influence malignant growth by altering tissue microenvironments as well as selecting more virulent cells via immunoediting [3]. The human cytomegalovirus (CMV), is one of the factors related to invasive breast cancer [4, 5]. CMV causes a latent infection that lasts a lifetime in 70–90% of the general population [6]. CMV reactivates but only affects immunocompromised individuals, causing clinical illness. With time, CMV could disrupt immunological function [7, 8] and leads to chronic diseases and accelerate growth of the tumors [9, 10]. Breast cancer growth and progression are regulated by numerous cytokines, which may enhance cancer cell survival, aid in tumor immune evasion, and activate the epithelial mesenchymal transition (EMT) process, leading to invasion, angiogenesis, and breast cancer metastasis [11]. IL-19, a cytokine from the IL-10 family, has various functions in immune control and illness [12, 13]. The IL-19 protein promotes fibronectin (FN) production and assembly, the spread of cancer cells and cellular division in breast cancer cells [14]. Studies show that IL-19 expression is higher in breast cancer tissue compared to healthy tissue, indicating its potential role in breast cancer pathogenesis [15].

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Shabo et al (2015) revealed that CD163 which is a scavenger receptor expressed on tumors-associated M2 macrophages, are increased in an anti-inflammatory tumor microenvironment [16]. Angiogenesis is a crucial stage in the progression of a malignant tumor. Vascular endothelial growth factor (VEGF) plays an essential role in angiogenesis primarily because it stimulates the cells of endothelial tissue. In many types of malignant tumors, including breast cancer, elevated VEGF levels and a higher density of micro vessels are associated with a later stage of the disease and a poor prediction [17]. T and B lymphocytes are immune cells that aid in the elimination of malignant cells and contribute to immunological surveillance [18, 19]. Besides to their well-known helper roles, CD4 cells play a part in antitumor responses when the majority of tumor cells do not express MHC class II [20]. TNF-α, a pro-inflammatory cytokine, is increased in several cancer types. In breast cancer, it is related with greater proliferation of tumor cells, higher frequency of metastases, advanced stage of the cancer and overall worse outcome for the patient [21]. Investigating the effects of CMV infection on the imbalance of these important cytokines and immunological markers and assess its correlation with disease severity in breast cancer patients. Knowing this connection may give new insights on the ways in which CMV and immunological imbalance influence tumor growth and suggest possible treatment targets to enhance the outcome of breast cancer.

2 MATERIAL AND METHODS

2.1 STUDY DESIGN

This study included 80 patients divided into two groups. Forty patients were diagnosed with breast cancer and visited Nanakaly Hospital's / oncology department in Erbil, regularly. The control group contains 40 participants with no breast cancer or any other cancer. inclusion criteria included adults between the age of (30 to 56 years) with no autoimmune disease, chronic inflammatory and infectious diseases. Exclusion criteria included individual with chronic disease, immunosuppressive medications and chronic disease This study was carried out in the Science and Health Research Center at Koya University.

Table 1. Participant characteristics include family history of cancer, smoking, and CMV status.

Variable	Breast Cancer with CMV (n=40)	CMV without Breast Cancer (n=40)
Age (mean ± SD)	47.48 ± 7.418	45.23 ± 7.940
Occupation		
• **Employed (%)**	Number (35%)	Number (75%)
• **Housewife (%)**	Number (65%)	Number (25%)
Smoking status (%)		
• **Smoker**	Number (12.5%)	Number (5%)
• **Non-smoker**	Number (87.5%)	Number (95%)
Family history of cancer	Number (75%)	Number (25%)
Anti-CMV IgG seropositive	Number (100%)	Number (100%)
Anti-CMV IgM seropositive	Number (0%)	Number (0%)

2.2 BLOOD SAMPLE COLLECTON

Blood samples were taken from the participants over the period from August 19, 2024 to January 27, 2025. Blood samples were withdrawn into a 3ml syringe and placed in a yellow tube. The serum was then prepared for measuring CMV status and cytokine analysis by centrifuging blood samples for 10 minutes at 5000 rpm. To assess cytokine concentrations, serum was placed into a 1.5 ml Eppendorf tube and stored at -80 °C freezer [31].

2.3 DETECTION OF ANTI-CMV IGG AND IGM ANTIBODIES

Anti-CMV IgG and IgM antibody levels were determined using the Cobas immunoassay analyzer (Roche Diagnostics, Switzerland). This approach uses CMV-specific antigen-coated wells to quantify the amounts of IgG and IgM antibodies in serum samples. this approach was performed based on the manufacture's instruction (Roche Diagnostics) [32]. The Cobas system provides great sensitivity and specificity for detecting CMV antibodies [33].

2.4 DETERMINATION OF IL-19, CD163, VEGF A, CD4, and TNF-α CONCENTRATIONS

Human IL-19 ELISA Kit (cat. no. SL1906HuSoluble CD163 ELISA Kit (cat. no. SL1761Hu), VEGF ELISA Kit (cat. no. SL1811Hu), Soluble CD4 ELISA Kit (cat. no. SL0465Hu), TNF-α ELISA Kit (cat. no. SL1761Hu) from Sunlong Biotech Co., Ltd (China) were used to determine these markers' serum concentrations. Thekits used the sandwich-ELISA testing approach. An ELISA reader (BioTek Instruments, Inc.,United states of America) was used to spectrophotometrically measure the optical density (OD) at a wavelength of 450 nm. Washing steps were performed manually using wash buffer, according to the manufacture protocol (Sunlong Biotech Co.,Ltd, China). The levels of TNF-α, VEGF, CD4, IL-19, and CD163 were determined by measuring OD using a microplate reader, based on the instruction of each ELISA kit. The concentrations of these markers were determined by comparing the optical density of the samples with the standard curves.

2.5 STATISTICAL ANALYSIS

The concentration of each ELISA test was estimated with GraphPad Prism (version 10). In addition, descriptive statistics, including the mean, standard error of the mean and standard deviation, were performed using GraphPad αPrism (version 10). Although commercially ELISA kits were used, the manufacture give specificity and sensitivity and cutoff values are determined based on the reference population that are differ from our study in term of clinical characteristic and demographics. for that to measure the diagnostic performance of each marker within our study population, we recalculated the sensitivity, cutoff values and specificity using Medcalc statistical program (version20.215). To assess the diagnostic performance of serological measures, the area under curve (AUC) was employed to measure each marker's overall accuracy. cutoff value were determined by comparing CMV seropositive without breast cancer group and breast cancer group with CMV seropositive , in order to evaluate biomarker associated with breast cancer risk.. Furthermore, the risk variables like CD4, CD163, TNFα linked to CMV infection and cytokine levels in the evolution of breast cancer were evaluated using binary logistic regression analysis using SPSS Statistics (version 25).

3 RESULTS

The serum concentrations of inflammatory and tumor biomarkers are shown in Table 2. As shown, the serum concentration of the studied immunological and tumor markers was elevated in breast cancer and control with CMV IgG positive participants. All of the participants tested positive for CMV-IgG (100%), although CMV-IgM was negative in both the breast cancer (n=40) and control groups (n=40), further details about participant data, including demographic, clinical, and serological information, are shown in Table 1. TNF-α levels were significantly increased in breast cancer group with CMV seropositive and have an area under curve of 0.798 (95% CI 0.641 to 0.908, p= 0.0001), sensitivity of 100% and specificity of 68.18% (Figure 1).This was followed by CD4 with an area under curve of 0.784 (95% CI: 0.678 to 0.868, p <0.0001), sensitivity of 80% and specificity of 75% Table 3. Other markers were also evaluated, with their corresponding ROC curves shown in Figure 1. A binary logistic regression analysis was used to assess how the predictors and outcome likelihood were related. Only the TNF-α predictor among the variables under analysis was statistically significant (p = 0.048, Exp (B) = 1.203), suggesting that greater values of this variable would slightly enhance the probabilities of the outcome. The remaining predictors did not reach statistical significance, including variables with odds ratios near 1, like IL-19 Exp (B) = 1.024 (p = 0.564) and CD4 Exp (B) = 1.109 (p = 0.659), which suggested minimal effects, and others with lower odds ratios, like smoking Exp (B) = 0.017 (p = 0.067) and serious illness Exp (B) = 0.007 (p = 0.116), which also failed to reach statistical significance. These results indicate that there could be a positive relation between CMV seropositivity and breast cancer severity by increasing the levels of TNF-α and CD4 in the serum of participants.

Table 2. The serum concentration of CMV IgM, IgG, immunological and tumor markers

Parameters (Serum Concentration)	Breast cancer (Mean± SE)	Control group (Mean± SE)	Normal range
CMV IgG (IU/mL)	335.3± 25.83	378.6±24.34	0.7-1 IU/mL
CMV IgM (IU/mL)	0.2585± 0.008705	0.2454± 3.860	0.7-1 IU/mL
TNF-a (pg/mL)	95.46±52.20	67.50± 2.450	0-8.1 pg/mL
IL-19 (pg/mL)	121.8± 3.599	112.7±2.802	<21 pg/mL
CD4 (ng/mL)	1.688± 0.5628	1.767± 0.1982	< 10 ng/mL
CD163 (ng/mL)	13.23± 0.3139	12.15± 0.3430	< 10 ng/mL
VEGF A (pg/mL)	110.6±12.30	85.30± 10.36	24.7 – 467.7 pg/mL

Table 3. Diagnostic Metrics of CD4, VEGF-A, IL-19, CD163, TNF-α and in Breast Cancer progression.

Parameters	AUC Mean ± SE	95% C.I. for AUC	P-values	Cut off	Sensitivity (95% CI)	Specificity (95% CI)
TNF-α	0.798 ± 0.0769 pg/mL	0.641-0.908 pg/mL	0.0001	>52.90443 pg/mL	100% (81.5 -100%)	68.18% (45.1 - 86.1%)
CD 4	0.784 ± 0.0552 ng/mL	0.678-0.868 ng/ml	<0.0001	>1.348407 ng/mL	80% (64.4 - 90.9%)	75% (58.8 - 87.3%)
IL-19	0.618 ± 0.0638 pg/mL	0.503-0.725 ng/mL	0.0641	≤113.5217 pg/mL	57.5 % (40.9% - 73)	65% (48.3 - 79.4%)
CD 163	0.646 ± 0.0623 ng/mL	0.531-0.750 ng/mL	0.0190	≤11.86158 ng/mL	50% (33.8 - 66.2%)	77.5% (61.5 - 89.2%)
VEGF A	0.590 ± 0.0649 pg/mL	0.475-0.699 pg/mL	0.1639	≤115.5725 pg/mL	80% (64.4 - 90.9%)	45% (29.3 - 61.5%)

DISCUSSION

These data presented here determines that CMV seropositive may have a potential role in the severity of breast cancer. In this study several inflammatory and immune biomarker were assessed to determine their patterns in CMV seropositive individuals with breast cancer or without breast cancer.in general the breast cancer group demonstrated higher levels of TNF α, IL-19 ,CD163 ,VEGF A compered to CMV seropositive group without breast cancer .most of these markers were higher than normal range detected in healthy individuals , indicating a state of increased inflammation or immune activation. TNF-α is a pleiotropic cytokine that is generated by T cell, B cell, NK cell, neutrophils, and macrophages and tumor cells. TNF- α, can function as an endogenous tumor promoter [23-25]. Levels of TNF α were increased in breast cancer group with CMV seropositive (95.46 pg/ml) compared to the positive control group (67.50 pg/ml) . both group means were higher than normal range of 0-8.1 pg/mL. this may indicate pro-inflammatory environment linked to the presence of breast cancer. One member of the IL-10 family is interleukin-19 (IL-19). This biomarker expression is linked to poor survival, greater metastases, advanced tumor stage, and enhanced mitotic figures in breast cancer. It directly stimulates proliferation and migration while indirectly providing an environment for tumor growth 12,28]. In this investigation, we discovered that serum interleukin-19 levels were higher in breast cancer group by mean value of 122.2 pg/ml compered to CMV seropositive group without breast cancer (112.7 pg/ml), both groups were higher than normal range of (<21 pg/mL). These findings are consistent with prior research indicating that interleukin-19 is a predictive marker in breast cancer severity and may play a role in tumor growth [28]. CD163 is a macrophage- receptor, and overexpression of CD163 is one of the primary modifications in macrophage switching to alternate active phenotype during inflammation in the body [29]. The scavenger receptor CD163 is elevated in an anti-inflammatory tumor microenvironment by tumor-associated M2 macrophages [16]. In human cancers, including breast cancer, a low survival rate was linked to the existence of CD163+ M2 macrophages in the tumor stroma [30]. In this study, serum level of sCD163 in the CMV seropositive group with breast cancer(13ng/ml) was higher than CMV seropositive without breast cancer group (12ng/mL) and both mean groups are higher than normal range. These levels are substantially higher, indicating increased macrophage activation. In this study VEGF A levels were elevated in breast cancer group (110.6 pg/ml) compared to CMV seropositive group without breast cancer (85.30 pg/ml). but both mean groups were in normal

range (24.7 – 467.7 pg/mL). CD4 T cells play a crucial role in the establishment of antitumor responses; enhancing the tumoricidal activity of other effector cells such CD8 T cells and macrophages [26,27]. In this research, CD4 demonstrated a weak positive association with disease risk (Exp B) = 1.109). This implies a minimal effect on the severity of breast cancer. In this study, the serum concentration of IgG for CMV are increased in both groups (<300 IU/mL) in comparison to the standard reference range 0.7–1 IU/mL, in which may indicate an enhanced humoral immune response due to previous antigenic exposure like past CMV infection. The increased levels in the breast cancer group are consistent with prior research [23]. This study is limited by CMV seropositivity (only IgG presence) which indicate past exposure, it does not indicate recent CMV infection. absence of CMV IgM limited our ability to evaluate biomarker change during CMV infection.

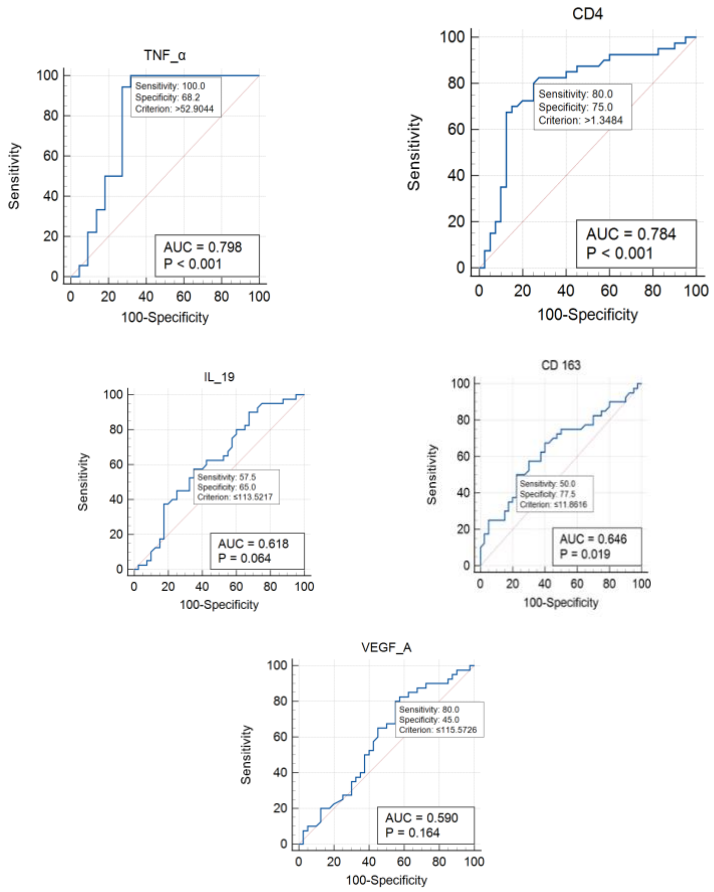

Figure 1. ROC curves displaying the diagnostic performance of TNF-α, CD4, IL-19 and CD163 and VEGF A for Breast Cancer Progression.

CONCLUSION

This study showed elevated levels of TNF α , CD 163 , IL-19 in breast cancer group with CMV seropositive and their role as supportive biomarker Furthermore, examining gene polymorphisms could provide more information about whether the examined cytokines have a significant impact on disease progression. These finding could lead to more focused methods of diagnosing and treating breast cancer.

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CONFLICTS OF INTREST

The author declares no conflict of interest.

DATA AVAILABILITY

All data generated or analyzed during this study, including ELISA results, patient demographic details, and logistic regression analyses, are included in this published article.

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