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Original Article

The Preoperative Serum Levels of the Anaphylatoxins C3a and C5a and Their Association with Clinico-Pathological Factors in Breast Cancer Patients

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Abstract

Breast cancer is a common women malignancy worldwide. Emerging evidence suggests that various complement molecules function as an immune suppressors in the tumor microenvironment (TME). Therefore this study aimed to investigate the role of the anaphylatoxins C3a and C5a in the propagation of breast cancer and their relationships with clinicopathological factors in Iraqi women suffering from breast tumors. ELISA technique was used to determine the serum anaphylatoxins C3a and C4a levels in 60 Iraqi women with breast tumors (30 with primary breast cancer and 30 with benign breast tumor) before surgery and treatment as 30 healthy controls. In addition, the clinicopathological factors of all the patients were collected. The result showed a significantly increased level of anaphylatoxins C3a and C5a (P<0.001) in primary breast cancer patients than in benign breast tumor and healthy control women. The area under the curve (AUC) of C3a and C5a for distinguishing patients with primary breast cancer and healthy control subjects was 0.878 (95% CI: 0.768 to 0.948) and 0.953 (95% CI: 0.865 to 0.991) respectively. C5a correlated with larger tumor size, lymph node metastasis, and histologic grade III, whereas C3a only with lymph node metastasis. In conclusion, preoperative levels of C5a exhibit high accuracy for primary breast cancer than C3a. C5a correlated with tumor burden more than C3a and suggested an enhancing effect of C5a on BC proliferation. Thus, suggesting prognostic value for C5a in primary BC.

Keywords: Breast cancer, C3a, C5a, Clinico-pathological factors, Prognosis

1. Introduction

Breast cancer (BC) is the second most common malignant tumor and the leading cause of cancer death among females worldwide, accounting for about 30% of all cancer cases (1). Breast cancer is the most common type of cancer in Iraq; GLOBOCAN reported 7515 new cancer cases in 2020; BC accounts for 22.2% of newly diagnosed cancer cases and 15.3% of cancer (2). The complement system is an important part of innate immunity; it is the first line of defense against pathogens or stressed host cells, consisting of more than thirty cell-bound or soluble proteins (3). The complement activation can be triggered, depending on the activator, by three different pathways, classical, lectin, and alternative (3). Once activated, the complement cascade generates several effectors molecules, resulting in the cleavage of C3 and C5 to their activated forms, C3a and C5a, respectively, and the terminal product C5b-9 (4).

These small cationic peptides, C3a and C5a, generated by complement activation, are termed anaphylatoxins and bind to their respective G-protein-

coupled receptors (GPCR), referred to as C3aR and C5aR1; these receptors are abundantly expressed on leukocytes of myeloid lineages, such as neutrophils, basophils, eosinophils, monocytes/macrophages and mast cells (5).

There is increasing evidence for the contribution of complement activation to cancer progression. Complement proteins can come inside the tumor via circulation and can also be produced by the tumor cells and the infiltrated immune cells (3). It may either act to kill antibody-coated tumor cells, assist local chronic inflammation or inhibit anti-tumor T cell responses supporting tumor progression. Recent studies demonstrate that these opposing effects depend upon the sites of complement activation, the composition of the TME, and the tumor cell susceptibility to complement attack (6).

Anaphylatoxins C5a and C3a trigger spurious tumor intracellular signaling pathways by binding to their cognate receptors (C3aR, C5aR1, and C5aR2) expressed in tumor and immune cells (7). The activation of these pathways enhances the proliferative, survival, and invasive properties of breast tumor cells (8). Therefore, the present study aimed to scout the relationships between preoperative serum C3a, C5a levels, and clinicopathological factors, as well as the prognostic value of these two serum biomarkers in breast cancer.

2. Materials and Methods

2.1. Participants

The study included sixty Iraqi females patients aged 22-80 years old suffering from breast tumors (30 with primary breast cancer (invasive ductal carcinoma) and 30 with benign breast tumor) who attended Kirkuk Oncology Center, Azadi Teaching Hospital, and Kirkuk General Hospital during a period from March/2021 to December/2021 for diagnostic or surgical operations without any prior history of receiving radiotherapy and chemotherapy and 30 healthy women with an age range from 22-56 years old were chosen as a control group from outside the hospital.

2.2. Study Design

The clinicopathological data, including tumor grade determination, histology, regional lymph node involvement, hormone receptor status, and HER2 status, were retrieved and tabulated from the patients' records after obtaining all the relevant ethical approvals. All the patients were informed about the sample processing steps and provided their written consent before the study enrollment.

2.3. Blood Sampling

Venous blood samples were collected from each subject in this study before surgery and treatment. The serum samples were obtained by centrifugation and immediately stored at -20 C until testing. Preoperative serum levels of C3a and C5a were quantified by the Enzyme-linked immunoabsorbent assay (ELISA) technique (Sunlong/ china) in accordance with the manufacturer's protocol. The expression of ER, PR, and HER-2 was detected by Immunohistochemistry (IHC). ER- and PR-positive was defined as the presence of >10% nuclear-stained cells. HER2-positivity was indicated by a 3+ or 2+ score from the immunohistochemical evaluation.

2.4. Statistical Analysis

Statistical Package for Social Sciences version (SPSS) version 26.00 was used for data analysis and the data are expressed as means ± standard deviation. Differences between study groups were evaluated by One-way analysis of variance (ANOVA) (Fisher's exact probability test), while the chi-square test was used to analyze the association of clinicopathological factors. P values less than 0.05 were considered statistically significant. The receiver operating characteristic curve (ROC) analysis was used to find the best parameter.

3. Results

This study was performed on 60 patients with breast tumors (30 with primary breast cancer and 30 with benign breast tumors) and 30 healthy control individuals. The preoperative serum levels of C3a and C5a in study groups are presented in table 1 and figure 1. C3a and C5a of patients with primary breast cancer

were significantly higher than those of patients with benign breast tumors and the healthy control group (P<0.001).

Table 1. The preoperative serum levels of C3a and C5a in study groups

Parameters	Study groups Mean ± STD		
	BCA (n=30)	**BBT (n=30)**	**HC (n=30)**
C3a	343.13±179.32*,**	205.77±47.69	169.76±21.75
C5a	237.90±109.58*,**	120.71±38.30	89.70±13.77

BBT, Benign breast tumor; BCA, breast cancer; HC, Healthy control; C3a, Complement component 3a; C5a, complement component 5a.
*Compared with Benign breast tumor, P<0.05
**Compared with Healthy control, P<0.05

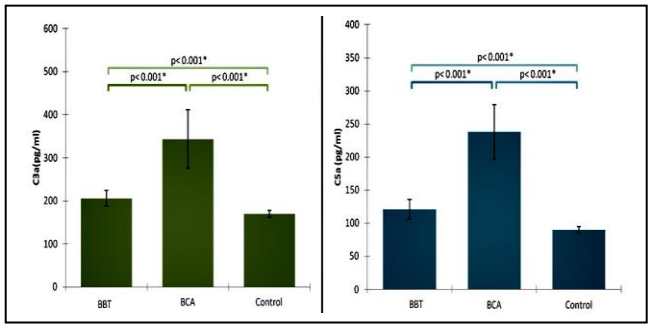

Figure 1. The preoperative serum levels of C3aand C5a in study groups

Receiver operating characteristic curve (ROC) analyses were performed to find out the best parameter that could be depended on to distinguish between primary breast cancer and benign breast tumor subjects and also to distinguish between primary breast cancer and healthy control subjects (as shown in figure 2). The ROC program showed different concentrations with each expected sensitivity, specificity, and accuracy. Between primary breast cancer and benign breast tumor subjects, it was noticed that the expected result of both C3a and C5a yielded high specificity with their optimum cut-off value (96.67% and 93.33%, respectively), although the sensitivity for both of them was low (56.67% and 70.0% respectively). While, between primary breast cancer and healthy control subjects, also it was noticed that the expected result of both C3a and C5a yielded high specificity with their optimum cut-off value (100.0% and 100.0%, respectively), although the sensitivity for both of them was (80.0%, 83.33% respectively). The area under the curve (AUC) of C3a and C5a for distinguishing patients with primary breast cancer and thus with benign breast tumors was 0.781 (95% CI: 0.655 to 0.877) and 0.846 (95% CI: 0.730 to 0.926) respectively. Furthermore, the AUC of C3a and C5a for distinguishing patients with primary breast cancer and healthy control subjects were 0.878 (95% CI: 0.768 to 0.948) and 0.953 (95% CI: 0.865 to 0.991), respectively.

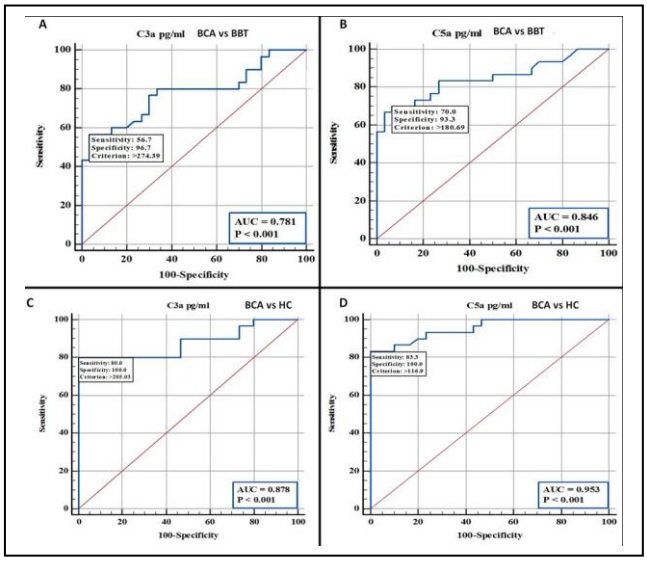

Figure 2. The receiver operating characteristic curve was constructed to evaluate the immunological parameters C3a and C5 a for breast cancer. (A & B) for distinguishing patients with primary breast cancer and patients with benign breast tumor; (C & D) for distinguishing patients with primary breast cancer and healthy control subjects

The relationship between serum C3a and C5a levels and clinicopathological factors of breast cancer patients is shown in table 2; the preoperative serum level of C5a had a rising trend more than C3a along with the development of tumor. Among patients with primary breast cancer, the level of C5a was associated with tumor size. Patients with tumor size (≥3cm) had

significantly higher C5a levels than patients with tumor size (<3cm). Regarding lymph node status, C3a and C5a were significantly elevated in patients with lymph node metastasis than in patients without metastasis (P<0.05). As well as, the levels of C5a have significantly elevated in grade III patients compared to grade I and II patients (P<0.05). There were no statistical differences in the C5a between (ER, PR, and HER-2)-positive group and (ER, PR, and HER-2)-negative group. While significantly elevated C3a was found in ER and PR-negative group compared to ER and PR-positive group (P<0.05).

Table 2. The relationship between serum C3a, C5a levels, and clinicopathological factors of breast cancer patients

Clinicopathological factors of BC patients (N=30)	Variable	No.	C3a (pg/ml) M±SD	*P* value	C5a (pg/ml) M±SD	*P* value
Age(Years)	≥45	18	370.94±192.43	0.301	253.28±107.43	0.375
	<45	12	301.41±165.25		214.82±118.07	
Family history	Yes	13	294.60±158.75	0.196	219.07±92.37	0.410
	No	17	380.24±194.98		252.29±124.92	
Menopausal status	Pre-	17	319.72±165.29	0.446	236.00±123.67	0.915
	Post-	13	373.75±205.36		240.38±98.05	
Histologic grade	I	2	257.34±114.74	0.08	168.68±94.77	0.039*
	II	16	303.42±179.67		204.78±96.23	
	III	12	410.37±176.03		293.59±112.30	
Tumor size	<3	18	319.13±185.05	0.385	197.34±104.71	0.011*
	≥3	12	379.12±180.07		298.72±95.26	
Lymph node metastasis	Positive	21	395.28±192.02	0.001*	271.74±110.04	0.002*
	Negative	9	221.44±67.93		158.93±68.78	
ES status	Positive	21	288.94±148.83	0.031*	213.58±96.69	0.115
	Negative	9	469.58±198.79		294.63±128.38	
PR status	Positive	16	259.38±90.67	0.010*	202.54±79.41	0.074
	Negative	14	438.84±215.00		278.30±130.90	
Herp status	Positive	10	331.53±144.13	0.789	225.45±74.82	0.618
	Negative	20	348.93±202.06		244.12±127.22	

C3a, complement component 3a; C5a, complement component 5a; ER, estrogen receptor; HER-2, human epidermal growth factor receptor; PR, progesterone receptor.*P<0.05 indicates a significant difference

4. Discussion

Breast cancer is the most common cancer among women worldwide (1). The complement system, a pivotal component of the innate immune system, is a potent inflammatory cascade (6). Although the role of the complement system as an effector system in killing cancer cells is known, Emerging evidence suggests that many complement proteins generate an immunosuppressive microenvironment that leads to tumor progression; it entirely belongs to the tumor microenvironment (TME) (9). Among which anaphylatoxins C3a and C5a are cleaved products of C3 and C5 proteins of the complement system, respectively (10), have been shown to play either pro-tumorigenic or anti-tumorigenic roles (7).

The current study evaluated preoperative anaphylatoxins C3a and C5a levels in patients with primary breast cancer and benign breast tumor compared to healthy control subjects and their usefulness as prognostic factors in breast cancer. The present findings of this study referred to the high level of anaphylatoxin C3a in patients' primary BC compared with benign breast tumors and healthy control women. Although far less studied about C3a, this result is in full agreement with a study conducted by Zhang and Sun (11), who found that the level of anaphylatoxin C3a in the sera of esophageal cancer patients was significantly higher compared with those in healthy participants. These authors also indicated that the anaphylatoxin C3a concentrations in the sera of treated patients are

significantly lower than those without treatment. It may be hypothesized that C3a is crucial in promoting esophageal tumorigenesis (11). Also, recent studies suggest that a C3a also preconditions a tumor-promoting microenvironment. Signaling mediated by C3a binding to C3aR (C3a-C3aR) in cancer-associated fibroblasts (CAFs) facilitates the metastasis of breast cancer (12).

The anaphylatoxin C3a props up the recruitment of C3aR+ macrophages, and perturbation of the C3a/C3aR axis disrupts immune infiltration, slowing tumor growth. C3a also promotes T-cell apoptosis, hampers T-cell proliferation and dendritic cell maturation, and raises the macrophage and MDSC recruitment, leading to a reduction in the number of CD8+T cells (13).

The present study's observed data also showed a high level of anaphylatoxin C5a in patients' sera of primary BC and benign breast tumor compared with healthy control women. The current result was supported by the facts mentioned by Lu and Hu, who found that breast cancer cells could proliferate in response to C5a stimulation much more efficiently than non-malignant breast cells (8).

C5a likely enhanced BC cell proliferation via C5aR (14). Signaling mediated by C5a binding to C5aR enhances the invasive properties proliferative and migration of tumor cells (15). Various studies have observed that elevation of C5a or C5aR1 levels in various human cancers leads to tumor progression so as breast (14), lung (15, 16), clear cell renal cancers (17), and gastric (18). The above-mentioned results were concomitant with the present result, which revealed that the high C5a level might promote breast carcinogenesis. On the contrary, Chen, Sun (19), and his colleagues reported that the C5a serum levels of patients with BC exhibited a remarkable reduction compared to healthy volunteers. The drop in serum C5a levels detected in the advanced BC patients was possibly due to the excessive activation and consumption of the complement in the early stages (19).

The anaphylatoxin C5a promotes the myeloid-derived suppressor cells (MDSC) recruitment, favors the generation of Treg and Th2 response that can suppress the anti-tumor CD8+ T cells (20), but also mediates production of the immunomodulators arginase-1(ARG1), CTLA-4, IL-6, IL-10, LAG3 and PDL (16). Then, immunomodulators induced by C5a/C5aR facilitated cancer metastasis by suppressing T cell responses (20).

The ROC analyses of the current study revealed that all the studied parameters were significantly differentiated between the sera of primary breast cancer subjects from the sera of the benign breast diseases subjects (*P* value=<0.0001 for both C3a and C5a) and also between the sera of primary breast cancer subjects from sera of the healthy control women (*P* value=<0.0001 for both C3a and C5a). However, C5a was considered the best marker with high AUC (0.846) for differentiation between women with breast cancer and the benign tumor and (0.953%) for differentiation between women with benign and healthy tumors.

Interestingly, C3aR and C5aR are likely expressed at the surface of most cell types in a tumor (5), suggesting that C3a and C5a could be used by the tumor cells to promote tumor growth. The activation of the complement system leads to the regulation of the immune system and amplifies tumor cell invasiveness by acting on proliferation, migration, and epithelial-mesenchymal transition (15).

The present study showed that the preoperative serum level of C5a had a rising trend more than C3a along with the development of breast cancer. As reported, a significantly elevated level of C5a was associated with large tumor size, lymph node metastasis, and high tumor grade III, suggesting an enhancing effect of C5a on BC proliferation. While significantly elevated C3a is only associated with lymph node metastasis. Therefore, it suggests a prognostic value for C5a. Consistent with

study results, the breast cancer cells are more sensitive to C5a stimulation than non-malignant breast cells.

In contrast, C3a did not significantly affect the proliferation of breast cancer cells (8). Furthermore, Imamura, Yamamoto-Ibusuki (14) reported that the tumor development and proliferation enhancing effect of C5a on C5aR-expressing BC cells suggest that the C5a–C5aR system is closely associated with BC progression. BC C5aR expression is related to larger tumor size, higher nuclear grade and Ki-67 labeling index, presence of lymph node metastasis, and advanced clinical stages.

Additionally, the present results of this study revealed that C3a levels were significantly greater in both (ER & PR)-negative group than in (ER & PR)-positive group. In contrast, C5a is not significantly associated with ER and PR expressions. A study in 2016 demonstrated that the C5aR-positive BC patient ratio was high in hormone receptor-negative patients (14). Breast cancer tumor diameter, histologic grade, lymph node status, hormone receptor status, and HER2 status are used to predict patients' outcomes and select appropriate systemic therapy for the patients (21). These results reveal that the C5a is critical for BC development; C5a correlates with tumor burden more than C3a and may be considered an effective biomarker for the early prognosis of breast cancer.

Authors' Contribution

Study concept and design: B. H. H.
Acquisition of data: I. A. A.
Analysis and interpretation of data: I. A. A.
Drafting of the manuscript: S. M.
Critical revision of the manuscript for important intellectual content: B. H. H.
Statistical analysis: I. A. A.
Administrative, technical, and material support: S. M.

Ethics

Ethical approval for this study was granted by ethical Committee of the Iraqi Ministry of Health (no. 12601).

Conflict of Interest

The authors declare that they have no conflict of interest.

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