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Expert Opinion Article

Evaluation of Components Associated with the Complement Pathway as Potential Biomarkers for Breast Tumors

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Abstract
The study was designed to investigate the variations in complement system components (C1q, Properdin, C3, and C4) expression patterns among breast tumor types that are benign, malignant, and normal. Eighty samples were collected from women who attended the Early Detection of Breast Diseases Center at Al-Hussein Medical City in Karbala from 1/12/2024 to 20/12/2025. The women were examined clinically by a specialist consultant. The tissue sample was collected by fine needle aspirate or tissue biopsy, and then analyzed by immunohistochemistry to detect its type, degree of cancer, as well as the expression rate of hormones. This study used ELISA to evaluate C1q, Properdin, C3, and C4 levels in the serum of 68 female participants: ductal (n = 25), lobular (n = 8), benign (n = 18), and healthy controls (n = 17). Samples were obtained prior to treatment, stored at -20°C, and ethically approved.
Age and residence showed no significant differences between groups, but BMI, socioeconomic status, and education did. Most patients were overweight and had lower socioeconomic and educational levels. Hormone receptor positivity was highest in lobular carcinomas, while ductal tumors showed more triple-negative and basal-like subtypes. C1q levels were significantly higher in ductal carcinoma, while C3 was lower in benign lesions. C4 showed no significant variation. Properdin was reduced considerably in all pathological groups compared to controls, but did not differ among them. Correlations suggested complex immune-hormonal interactions. Findings support the potential of C1q and Properdin as diagnostic markers, though not for subtype distinction.

Keywords: Breast cancer, Complement, C1q, C3, Properdin

Introduction

Statistics from the American Cancer Society, 310,720 invasive breast cancer cases and 56,500 cases of ductal carcinoma in situ (DCIS) will be diagnosed in2024. Breast cancer is the second most common cancer in women, and a US woman faces a 1:8 chance of developing invasive carcinoma in her lifetime [1]. The investigation involved breast cancer diagnosis with advanced tests like genetic detection of breast cancer genes (BRCA 1 and BRCA 2), and non-BRCA mutations which increase the risk for developing BC in women The study showed that among this group of Iraqi women with breast cancer, the frequency of BRCA1 mutations was higher (48%) than that of BRCA2 mutations (12%)[2]. The second important test is the immunohistochemical IHC intended to detect the hormonal estrogen receptor ER, progesterone receptor PR and growth factor human epidermal growth factor receptor 2 (Her2/neu) expressions on breast tissue, these markers are involved in determination of prognosis and molecular subtypes of breast cancer [3]. The most important female sex hormones are estrogen and progesterone, which are responsible for many female physiological and physical characteristics, like breast tissue growth, and are also involved in the menstrual cycle and its regulation, along with an important role during pregnancy [4]. It has been confirmed that other females hormones affect the possibility of getting BC, Prolactin (PRL) levels increased significantly ($P < 0.01$) in breast cancer patients compared to normal values [5]. ER and PR expression have been associated with more aggressive tumors and poorer prognosis BC, Comprehending the correlation between breast cancer and hormone receptor status, together with pertinent prognostic variables, is of significant importance [6]. And a good prognosis BC that with ER^+ and PR^+ [4]. Females exhibited elevated levels of EGF, HER2, and CA-13 in malignant breast cancer compared to benign breast cancer [7].
According to IHC, the BC can be classified according to hormonal receptor expression status, luminal A subtype characterized by ER+, PR+ (at least 20%), HER2-, and Ki-67 >14 %. It is the commonest subtype of BC but the least aggressive, with an excellent prognosis [8]. Luminal B, a unique subset of BC type which characterized by ER+/PR- or +/Her2- with an invasive nature,[9] [10]. This subtype is E-receptor

positive, although these are usually expressed in less quantity, they can be PR-positive or not, HER2-negative. Chemotherapy can benefit this BC type, also high levels of Ki-67 result in faster growth than Luminal A, and so have a worse prognosis[11]. Growing evidence has been used to investigate the association between the histopathological investigation of breast tissue tumors and IHC markers, ER, PR, HER2 receptors[12]. It is widely accepted that patients who show a high expression of both ER and PR can benefit from chemotherapy with higher survival rates compared to other phenotypes[10 13]. Her2+ subtype accounts for about 15% to 20% of newly diagnosed BC[14]. In this type, the HER2 receptor is highly expressed (more than 10%) with negative estimation of both ER and PR (< 1% and < 20% respectively) and an increase inKi-67 (> 20%)[15]. Triple negative BC (TNBC) is a highly proliferative tumor and constitutes about 10% to 20% of breast cancer. It shows a negative expression for ER (< 1%), PR (< 20%), and HER2 (≤ 10%)[15].; it's important to mention that this subtype was more prevalent in patients with BRCA1 mutations and young women with a higher histological grade[16].

There is evidence that all complement pathways are activated in malignant tumors. However, it is unclear which pathway is primarily responsible for complement activation inside tumors[17]. The situation is further complicated by the fact that serine proteases attached to the membrane of cancer cells can cleave C5 and produce C5a without complement activation[18]. As demonstrated for C1q in a syngeneic murine model of melanoma, where C1q expression impacted angiogenesis, tumor growth, and metastasis, complement proteins produced in tumors may also contribute to cancer progression independently of complement activation [19]. In this mouse model, C1q was expressed independently of C4 in tumor-infiltrating myeloid cells, spindle-shaped fibroblasts, and endothelial cells. The absence of C4 co-expression in tumors expressing C1q suggests that C1q plays a part in tumor progression outside the traditional pathway.

Carcinogenesis and the advancement of cancer are significantly influenced by inflammation that promotes tumors[20 21 22]. Several sophisticated studies demonstrated that complement system activation is a key element of inflammation that promotes tumor growth. Because of decreased inflammation, Bonavita et al. demonstrated that C3-deficient animals were shielded from chemical carcinogenesis in mesenchymal and epithelial tissues[23]. Recent studies revealed that depending of the cancer type, complement can be pro or anti-tumoral and, even for the same type of cancer, different models presented opposite effects[24]. This idea contradicts the initial hypothesis that complement plays a role in tumor immunosurveillance by causing cancer cells to become cytotoxic. Although it has been shown that complement activates solid tumors[25], there is insufficient proof that complement can eradicate a sizable portion of tumor cells due to the evasion strategies used by cancer cells[26]. Furthermore, cancer cells use posttranslational alterations to modify the complement regulators to avoid complement-mediated lysis. Lin et al. demonstrated that ST3GAL1-mediated sialylation of CD55 improves complement system inhibition at the C3 level, while ST3GAL1 silencing increases C3 deposition and CDC-mediated breast cancer cell death[27]. Fibroblasts appear to be a significant source of C3 and C3a in patients with breast cancer[28]. It was discovered that C3expression was greater in primary tumors than in lymph node metastases from patients with luminal breast cancer[29]. In line with previous research, the association between poor prognosis and decreased C3 expression in lymph node metastases suggests that the function of tumor-derived C3 may change in primary tumors compared to the metastatic niche and that C3 may be involved in EMT or MET conversion[30]. A recently published study indicated that C3a levels were significantly elevated in both the (ER & PR)-negative group compared to the (ER & PR)-positive group. C5a is not significantly correlated with the expressions of ER and PR[31].

In addition, many studies have revealed elevated levels of complement activity in biological samples taken from cancer patients[32], as well as a connection with the amount of tumor burden[33]. Complement activation has been thought to harm cancer cells by complement-dependent cytotoxicity (CDC) and phagocytosis of complement-coated tumor cells. On the other hand, complement activation has been seen as benign. One of the most essential strategies utilized to eliminate tumor cells is the induction of complement-dependent cell death (CDC) by utilizing antibodies that activate complement[34]. On the other hand, the accumulation of evidence has also highlighted the complement system's significance in advancing cancer. Even more specifically, many studies have shown that specific complement components can change the tumor microenvironment (TME) into a setting that supports tumor growth. The stage of carcinogenesis, the location of the tumor, the constituents of the tumor microenvironment (TME), or the sensitivity of tumor cells to complement activation and attack may be the factors that define these conflicting effects in cancer [35].

Materials and Methods

This cross-sectional study was done on women who attended the Center for Early Detection of Breast Diseases affiliated with Imam Hussein Medical City, where peace be upon him, in Karbala Governorate. Firstly, the patients were diagnosed according to

clinical examination by a consultant with the aim of mammography, and then a pathological protocol was run to confirm or exclude the case. A questionnaire paper was filled out by direct interview with the patients, including demographic information like age, address, social status, and marital status. Also, the patient's medical history and family history were recorded. The tumor type, grade, and other pathological data obtained from the tissue biopsy examination by immunohistochemistry confirmed the patient's health state.

This study included one hundred female patients who complained of palpable breast lumps. Three assessment tests were administered to all, comprising a physical examination, imaging (mammography and/or ultrasonography), and FNA. The study followed a prudent mechanism in the patient selection process and set special criteria for selecting the sample in line with the study's objectives and expected results. 1)Patients with other cancers or metastasis.2) Treated patients with chemotherapy, radiation, hormonal, or other anticancer drugs. 3) Presence of any other autoimmune or chronic disease. 4) Taking any biological agents. 5) Recent blood transfusions (during the last 6 months). A pathologist who specializes in breast cancer pathology evaluated the hormone receptor status. According to the Allred scoring criteria[36] for ER and PR, scoring was achieved by examining every tumor cell on the slide. Five grades constitute the proportion score (PS), which was determined by estimating the percentage of tumor cells with positive nuclear staining. The Allred score is a semi-quantitative approach incorporating staining intensity (with a score of 0-3) and the percentage of positive cells (scored on a scale of0-5). Four categories represent the intensity score (IS), which has been determined using the average staining intensity of all positive tumor cells. PS and IS taken in tandem yield the total score (TS). TS ≥ 3, mild positivity (scoring3–4), moderate positivity (scoring 5–6), and strong positivity (scoring 7–8) are considered beneficial outcomes for both ER and PR.

Serum samples from four groups of female participants were used in this study: ductal carcinoma (n = 33) and lobular carcinoma (n = 10) patients, patients with benign breast lesions (n = 20), and healthy controls (n = 17). The institutional review board gave its ethical approval, and all participants gave informed consent. Before treatment, blood samples were taken, processed to separate serum, and kept at -20°C until analysis. Using commercially available enzyme-linked immunosorbent assay (ELISA) kits, the concentrations of complement proteins C1q, Properdin, C3 and C4 were determined by the manufacturer's instructions for 68 samples: ductal carcinoma (n = 25), lobular carcinoma (n = 8) patients, benign (n = 18), and healthy controls (n = 17).

The Statistical Package for Social Science (SPSS 26) program was employed to analyze the data. The ER, PR, and HER2/neu receptor expression rates were determined using descriptive statistics. Means and Standard Deviations (SD)were applied to summarize continuous variables. For categorical variables, percentages and frequencies were employed. Frequencies, percentages, means, and standard deviations were acquired whenever necessary. The Pearson Chi-square test assessed the relationship between IHC stains and clinic-pathological features. A p-value below 0.05 was considered significant.

Results

This cross-sectional study was conducted on 80 women (63 with breast tumors and 17 healthy controls) who were diagnosed for the first time. In table 1, age distribution revealed no significant difference between cases and controls (χ^2 = 3.307, df =5, P = 0.653), suggesting that the two groups' age distributions were similar across the identified categories. This implies that age could not be a significant confounding factor within the study population. However, there was a statistically significant difference between the Body Mass Index (BMI)categories (χ^2 = 13.367, df = 3, P = 0.004). Interestingly, 58.3% of patients were overweight, compared to just 11.8% of controls, indicating a high positive correlation between disease risk and being overweight. Obesity, on the other hand, was more common in controls (52.9%) than in cases (18.3%), a finding that merits greater research because it might indicate misclassification or even reverse causality. There was no significant correlation between residence (rural vs. urban) and illness status (χ^2 = 0.288, df = 1, P = 0.591), suggesting that regional living conditions may not determine disease prevalence in this cohort.

There was a significant difference in socioeconomic position between the groups (χ^2 = 18.827, df = 2, P < 0.001). Most cases fell into the fair (58.7%) or poor (6.3%) categories, with only 34.9% reporting a good socioeconomic position, compared to 94.1% of controls. This suggests that social determinants of health are important and highlights a possible inverse link between socioeconomic position and disease risk. Also, the results show no significant difference in work status (χ^2 = 0, df = 1, P = 0.986), suggesting that employment was not distributed differently and probably had no bearing on illness risk in this population. A significant difference in educational attainment was found (χ^2 = 27.755, df = 2, P < 0.001), with most patients having just primary (44.4%) or secondary (27%) education, whereas all controls had college-level education. This notable disparity suggests that poor educational attainment may be a risk factor or correlate for disease onset,

potentially mediated by behavioral variables, health literacy, or access to care.

Table 1: Demographic Characteristics of Study Groups.

Variable	Category	Controls n (%)	Cases n (%)	Chi-Square	df	P-value
Age period, year	**25-34**	2 (11.8)	5 (7.9)	3.307	5	0.653
	35-44	5 (29.4)	21 (33.3)			
	45-54	7 (41.2)	20 (31.7)			
	55-64	3 (17.6)	8 (12.7)			
	65-74	0 (0)	7 (11.1)			
	≥75	0 (0)	2 (3.2)			
BMI Categories	**Under weight**	0 (0)	0 (0)	13.367	3	**0.004****
	Normal	6 (35.3)	13 (21.7)			
	Over weight	2 (11.8)	35 (58.3)			
	Obese	9 (52.9)	11 (18.3)			
	Extreme obese	0 (0)	1 (1.7)			
Residence	**Urban**	6 (35.3)	18 (28.6)	0.288	1	0.591
	Rural	11 (64.7)	45 (71.4)			
Socio Status	**Poor**	0 (0)	4 (6.3)	18.827	2	**< 0.001****
	Good	16 (94.1)	22 (34.9)			
	Fair	1 (5.9)	37 (58.7)			
Employment	**Yes**	14 (82.4)	52 (82.5)	0	1	0.986
	No	3 (17.6)	11 (17.5)			
Educational Level	**Primary**	0 (0)	28 (44.4)	27.755	2	**< 0.001****
	Secondary	0 (0)	17 (27.0)			
	College	17 (100)	18 (28.6)			

Note: **Significant at $p < 0.01$.

The histopathological results show that the positive cases for hormonal receptors were 74% of cases estrogen receptor (ER), 65% progesterone receptor (PR), 35% human epidermal growth factor 2 (HER2), and only 19% of cases were positive for E-Cadherin as illustrated in table 2. The molecular subtypes and immunohistochemical profiles of breast lesions in the benign, ductal, and lobular categories showed significant distributional tendencies. However, none of the correlations were statistically significant ($p > 0.05$ for all variables). The most significant percentage of lobular cases (80.0%) had estrogen receptor (ER) positivity, which was followed by benign (66.7%) and ductal (56.3%) lesions. This suggests that hormone-responsive tumors, especially lobular carcinomas, predominate. Lobular tumors again displayed the most significant positive rate (80.0%) for progesterone receptor (PR) expression, confirming their luminal-like character. Although the small sample sizes hampered the discriminatory ability, HER2 overexpression was more common in lobular lesions (40.0%) than in ductal (28.1%) or benign lesions (33.3%).

E-cadherin, a molecule that promotes cell adhesion and is frequently lost in lobular carcinoma, was negative in all benign cases and most ductal (81.3%) and lobular (80.0%) lesions. Since the loss of E-cadherin usually characterizes lobular histology, this points to a non-classical expression pattern. It lacks in ductal cancers could be due to technical differences in detection or tumor heterogeneity. According to molecular subtyping, lobular tumors had the highest prevalence of Luminal A and Luminal B subtypes (each 40.0%). In comparison, ductal tumors had a higher proportion of triple-negative (28.1%) and basal-like (15.6%) phenotypes, suggesting greater heterogeneity and the potential for aggressive behavior. A surprising result that reflects the small number of benign samples examined immunohistochemically was the equal distribution of benign lesions among Luminal A, Luminal B, and triple-negative classifications. These results show significant physiologic differences in receptor status and molecular subtypes across histologic classifications, which should be confirmed in larger, powered cohorts, even though they did not reach statistical significance.

Table 2: Association of biomarkers and molecular subtypes with breast cancer cases

Variable	Category	Benign	Ductal	Lobular	p-value
		n (%)	n (%)	n (%)	
ER	**Positive**	2 (66.7)	18 (56.3)	8 (80.0)	**0.395**
	Negative	1 (33.3)	14 (43.8)	2 (20.0)	
PR	**Positive**	2 (66.7)	16 (50.0)	8 (80.0)	**0.233**
	Negative	1 (33.3)	16 (50.0)	2 (20.0)	
Her2	**Positive**	1 (33.3)	9 (28.1)	4 (40.0)	**0.775**
	Negative	2 (66.7)	23 (71.9)	6 (60.0)	
E-Cadherin	**Positive**	0 (0.0)	6 (18.8)	2 (20.0)	**0.704**
	Negative	3 (100.0)	26 (81.3)	8 (80.0)	
Molecular subtypes	**Luminal A**	1 (33.3)	4 (12.5)	4 (40.0)	**0.478**
	Luminal B	1 (33.3)	14 (43.8)	4 (40.0)	
	Basal-like	0 (0.0)	5 (15.6)	0 (0.0)	
	Triple negative	1 (33.3)	9 (28.1)	2 (20.0)	

Table 3 provided correlation matrix sheds light on potential immuno-hormone interplay within the tumor microenvironment by displaying the correlations between adhesion molecules, complement system components, and hormonal receptors in breast tissue. Estrogen receptor (ER) and progesterone receptor (PR) expression showed a statistically significant and high positive correlation ($r = 0.911$, $p < 0.01$), which is in line with their known co-expression in hormone-dependent breast malignancies. In line with the established inverse association between HER2 overexpression and hormone receptor positivity in specific breast cancer subtypes, HER2 expression displayed modest and non-significant correlations with ER ($r = 0.029$) and PR ($r = -0.009$), indicating an independent regulatory system.

While there were no significant correlations between E-cadherin and the primary receptors, there was a weak negative correlation with HER2 ($r = -0.061$) and a slight positive association with ER ($r = 0.123$) and PR ($r = 0.044$), suggesting that different molecular subtypes may have different cell adhesion characteristics. C1q showed modest positive correlations with ER ($r = 0.291$) and PR ($r = 0.335$) among the complement components, indicating a possible connection between hormone receptor signaling pathways and classical complement activation. A potential regulatory imbalance between the alternative and classical/lectin pathways was highlighted by the substantial inverse correlation between C4 and Properdin, a stabilizer of the alternative complement pathway ($r = -0.416$, $p < 0.05$). Furthermore, properdin exhibited weak negative associations with E-cadherin, HER2, PR, and ER, indicating a more extensive antagonistic or suppressive interaction with adhesion and hormonal markers. Although largely non-significant, the observed correlation patterns highlight the intricate immune-hormonal interactions in the biology of breast cancer and encourage more research in larger, mechanistically focused studies to fully understand the immunomodulatory roles of complement in the development of breast tumors.

Table 3: Correlation Matrix Between Hormonal Receptors, E-Cadherin, and Complement System Components in Breast Tissue

Variable	ER	PR	Her2	E-Cadherin	C1q	C3	C4
ER							
PR	**0.911****						
Her2	0.029	-0.009					
E-Cadherin	0.123	0.044	-0.061				
C1q	0.291	0.335	-0.35	0.01			
C3	0.131	0.083	-0.122	-0.231	0.018		
C4	0.081	0.212	0.09	-0.016	0.383	-0.013	
Properdin	-0.243	-0.283	-0.123	-0.041	-0.316	0.155	**-0.416***

C1q expression was significantly higher in Ductal Carcinoma than in benign or normal tissues, as evidenced by the statistically significant differences in C1qlevels between the Benign and Ductal Carcinoma groups ($p = 0.001$) and between the Control and Ductal Carcinoma groups ($p = 0.001$). Comparing Lobular Carcinoma to Benign, Control, or Ductal Carcinoma, on the other hand, did not provide statistically significant findings ($p > 0.05$) as show in table 4 and figure 1, indicating that this subtype has a more varied or intermediate pattern of C1q expression. These results imply that increased C1q

levels might be primarily linked to the ductal subtype of breast cancer, which could be a reflection of its unique tumor microenvironment or more aggressive immune profile. Further research is necessary to clarify the underlying mechanisms causing the heterogeneity inC1q expression among breast cancer subtypes, as there are no discernible distinctions between Lobular Carcinoma and other groups.

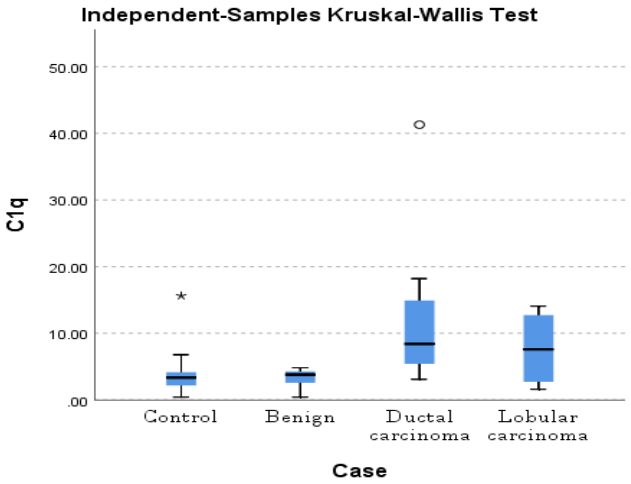

Figure 1. Variation in C1q Levels Among Breast Tumor Subtypes and Control

Table 4: Pairwise Comparison Results for C1q Levels Between Groups

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Benign-Control	.780	4.947	.875
Benign-Lobular carcinoma	-8.423	7.344	.251
Benign-Ductal carcinoma	-16.615	5.038	.001
Control-Lobular carcinoma	-7.643	7.282	.294
Control-Ductal carcinoma	-15.835	4.947	.001
Lobular carcinoma-Ductal carcinoma	8.192	7.344	.265

According to the findings, there are statistically significant variations in C3levels between Benign and Control tissues (p = 0.019) and between Benign and Ductal Carcinoma (p = 0.011) table 5 and figure 2. These results imply that, in contrast to the Ductal Carcinoma and Control groups, C3 expression is markedly lower in benign tissues. Remarkably, neither Lobular Carcinoma nor Ductal Carcinoma and Control showed statistically significant differences from one another (p =0.978), suggesting that C3 expression levels were comparable in these comparisons.

This trend implies that benign lesions have a distinct immune profile, defined by decreased C1q activity, which could differentiate them from healthy and malignant tissues. The absence of notable variations in Lobular Carcinoma suggests more variation or overlap in expression profiles within this group, which may reflect tumor biology heterogeneity. Our results point to a distinct function of C1q in the pathophysiology of breast tissue, which may have consequences for its potential use as a biomarker to differentiate between benign and malignant diseases.

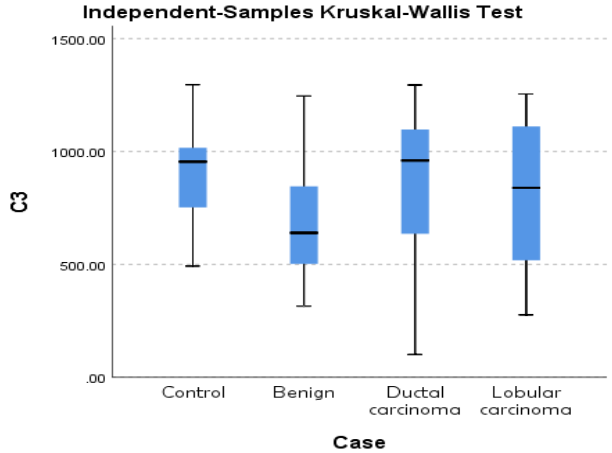

Figure 2. Variation in C3 Levels Among Breast Tumor Subtypes and Control

Table 5: Pairwise Comparison Results for C3 Levels Between Groups

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Benign-Lobular carcinoma	-10.764	8.402	.200
Benign-Ductal carcinoma	-15.509	6.112	.011
Benign-Control	15.683	6.687	.019
Lobular carcinoma-Ductal carcinoma	4.745	8.032	.555
Lobular carcinoma-Control	4.919	8.478	.562
Ductal carcinoma-Control	.174	6.216	.978

Statistical analysis using the Kruskal-Wallis's test produced a p-value of 0.367, showing no significant difference in C4 levels among the groups as illustrated in figure 3, despite visual diversity in median and range values. Individual C4 responses may vary, as seen by the interquartile ranges and outliers, especially within the cancer subtypes; nonetheless, these variations fall short of statistical significance. According to the results, there is no differential regulation of C4 expression in the setting of benign or malignant breast disease. This lack of relevance suggests that C4could not have a clear or quantifiable function in systemically differentiating between normal and diseased breast tissues, in contrast to other complement system components. As a result, C4 is not likely to be a valid biomarker for the categorization or advancement of breast disease in this group. More research may be necessary to examine local tissue-level C4 activity or its interactions with other complement components to elucidate its functional significance in breast carcinogenesis.

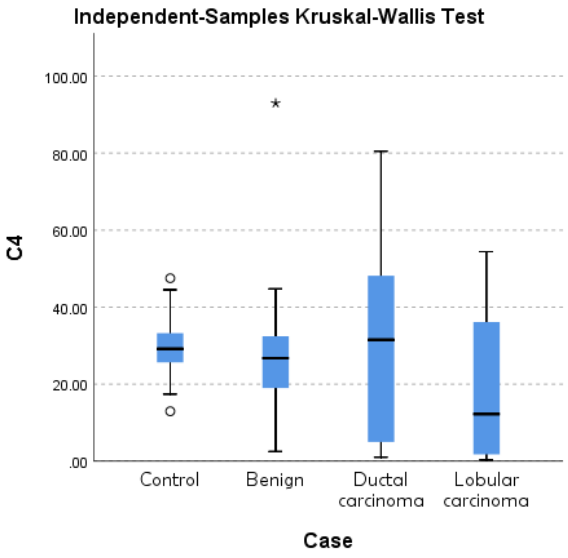

Figure 3. Variation in C4 Levels Among Breast Tumor Subtypes and Control

According to the research, all three pathological groups—Benign, Ductal Carcinoma, and Lobular Carcinoma—showed significantly lower levels of Properdin expression than the Control group ($p < 0.001$) as show in table 6 and figure 4. Properdin, a crucial positive regulator of the alternative complement pathway, may be downregulated in benign and malignant breast lesions compared to normal tissue, as suggested by this steady and significant decline. The pathological groups themselves, however, did not show any statistically significant differences, such as Ductal vs Lobular Carcinoma ($p = 0.897$), Benign versus Ductal Carcinoma ($p = 0.259$), or Benign versus Lobular Carcinoma ($p = 0.345$). These results suggest that although Properdin expression is dramatically decreased in disease states, neither the benign nor malignant situations nor the carcinoma subtypes are meaningfully distinguished by its level. These findings suggest that Properdin suppression may play a part in breast tissue pathology early on or frequently, maybe due to immunological dysregulation or changes to the complement system in the tumor microenvironment. Though it may help differentiate between healthy and sick tissue, its inability to discriminate across cancer subtypes may restrict its use as a biomarker for malignancy grading.

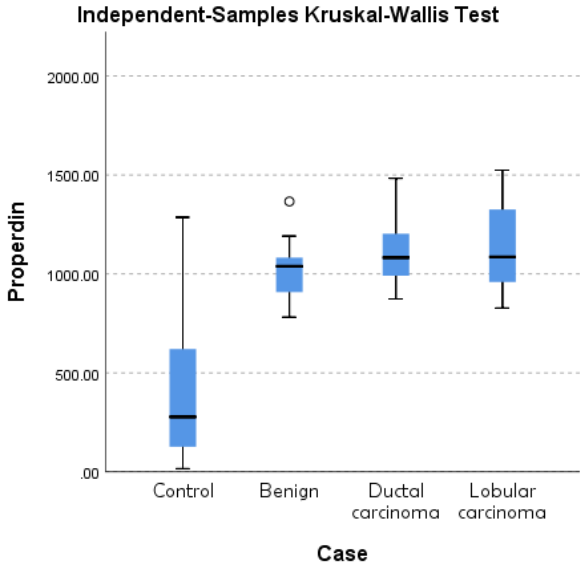

Figure 4. Variation in properdin Levels Among Breast Tumor Subtypes and Control

Table 6: Pairwise Comparison Results for Properdin Levels Between Groups

Sample 1-Sample 2	Test Statistic	Std. Error	Sig.
Control-Benign	-23.650	6.687	.000
Control-Ductal carcinoma	-30.546	6.216	.000
Control-Lobular carcinoma	-31.581	8.478	.000
Benign-Ductal carcinoma	-6.896	6.112	.259
Benign-Lobular carcinoma	-7.931	8.402	.345
Ductal carcinoma-Lobular carcinoma	-1.035	8.032	.897

Discussion

The breast cancer hormonal receptor markers have been confirmed to be significant in management and prognosis determinants. Also, they are influenced by genetic inheritance and changeable environmental attributes, and their expression differs across ethnic and geographical areas[37]. The present study recorded that more than half of the women aged between 40 and 50 years were diagnosed with BC and confirmed with ductal carcinoma. Gore CR, et. al recorded that 85% of BC cases were DC and in the age group 40 – 50 years[38]. Recently published studies were reported similar result as a study in Bangladesh,[39] Qatar[40], Pakistan[41] and Iran[42]. According to these statistics, compared to women in other regions, breast cancer is more common in our population among younger women. This might be brought on by the nation's protracted conflict, the stress of fighting, life-threatening circumstances, detrimental psychological effects, environmental contamination, and other genetic factors[43] [44].

According to BC molecular subtypes, our study recorded 71.4% of ER+/PR+, HER2+, and 28.6% of ER+/PR-, HER2+, which belong to luminal A subtypes. Also, the results showed that 76.9% of DC were ER+/PR+, HER2-. This conforms with the results of previous findings. Keshgegian found that ER-/PR +phenotype BC composed 1.5% of all cases, but he also demonstrated that ER-/PR + phenotype is an impartially identified rare subtype[45] [46]. an extensive cohort study previously reported similar findings, with 67.2% of the BC patients being ER-positive/PR-positive[47]. With 76% ER positivity, Shushan Shweta et al. also mentioned the same thing. However, Sarmah et al. revealed that only 44% of cases were ER+ [48]. They also documented a low occurrence of ER positivity in India compared to Western countries. A study conducted by Sarmah et al.[48] revealed a 48% PR positive rate comparable to our study. Few earlier studies in Iraq have shown PR and ER positivity in 91% and 57% of the cases, respectively[49]. Recently published research in Iraq showed that 70 % from cases were ER and PR positive[50]. According to regular tissue tests,70–90% of all patients diagnosed with breast cancer are assumed to beHER2/neu negative [51]. The activity of tumor cells and the clinical progression or regression of the disease are reflected in the overexpression of HER2/neu. The overexpression of HER2/neu appears to be associated with an increase in the proliferative activity of breast cancer cells, and it plays a significant role in cell differentiation and proliferation[52] [53]. Younger age, advanced stage, larger tumor size, stronger HER2 expression, and lower PR expression are all linked to ER^{low} breast cancer in comparison to ER^{high}[54] to ER^{high}.

A growing body of research suggests the complement system is a significant regulator of the TME. It

appears that cancer cells can co-opt complement-mediated processes in order to alter the tumor microenvironment (TME) and facilitate the growth of the tumor, metastasis, and evasion of the immune system[55 56]. According to the findings of Vijayakumar et al., cancer samples that include C3 and C4 deposits, which are related to C5b-9 deposits, indicate that the complement component has been activated through the presence of the classical pathway[57]. Factors connected to complement may also correlate with responses to alternative therapeutic approaches. Elevated amounts of circulating C3activation-derived fragments and tumor immunostaining of these fragments alongside CD55 indicated chemotherapy response in breast cancer patients[58 59]. According to the findings of Ferda et al.[60], which confirm that malignant tumors lead to elevation of complement component levels, the results agreed with those findings. C3 andC4 have notable differential expressions across four stages of breast cancer compared to healthy circumstances, with C3 demonstrating a substantial mutation rate[61].

C1q is a multifunctional protein that significantly influences tumor cells, positively and negatively affecting their biology. C1q is an apoptosis inducer with an anti-tumor effect in human prostate, breast cancer, and neuroblastoma[62 63]. Variations in the expression of complement proteins can be significantly associated with the prognosis, condition, and survival of breast cancer[64]. Although there is an increase in C1q mRNA levels in breast cancer tissue relative to control tissue, in this instance, these levels are favorably correlated with breast cancer patient survival[65]. Accordingly, it was discovered that C1q causes apoptosis in a variety of cancer cell types, including breast cancer[66 67]. While the other complement components were only weakly visible, the staining was positive for C1q, suggesting that C1q deposition occurred independently of complement activation. Specifically, stroma, ECs, and infiltrating leukocytes showed C1qstaining[68].

In basal-like breast cancer, increased levels of C1q have been shown to have a favorable prognosis index for disease-free survival. In HER2-positive breast cancer, it has been shown to have a favorable predictive index for overall survival[69]. Wilson et al. discovered that C1q chain genes were prevalent in the stromal compartment. Serum properdin levels were found to be significantly higher in all breast disease groups, benign, ductal carcinoma, and lobular carcinoma, than in healthy controls ($p < 0.001$). In contrast, there were no discernible differences across the disease subtypes. This indicates that properdin overexpression is a universal response to breast tissue disease, potentially signifying early activation of the complement system. Properdin, the sole identified positive regulator of the alternative complement pathway, increases the stability of C3 convertase and can attach to modified self-surfaces, augmenting immune responses[70].

Elevated levels of properdin may signify an initial innate immune response to atypical epithelial proliferation, even in benign tumors. Recent findings indicate that properdin expression is associated with immune cell infiltration and positive outcomes in several malignancies, including breast cancer[71], and may have direct anti-tumor effects via neutrophil-mediated cytotoxicity[72]. Properdin significantly influences the quantities of C5a and CCL2 generated in this mouse melanoma model and may be instrumental in coordinating immunosuppressive cells within the tumor microenvironment and surrounding tissues[73].

Nonetheless, its failure to differentiate between benign and malignant tumors restricts its independent diagnostic utility. Additional investigation is necessary to ascertain its function in tumor immunology and its potential as a therapeutic or prognostic biomarker[74 75]. Increased serum properdin in all breast lesions indicates that alternative-pathway activation is an early and persistent response to pathological alterations in mammary tissue, rather than a result of malignant transformation[76].

Conclusions:

This study identified substantial correlations between breast cancer risk and many demographic, genomic, and immunological variables. Obesity, poorer socioeconomic level, and inadequate educational achievement were more common among patients, indicating that social determinants and metabolic health significantly influence illness vulnerability. Hormonal receptor analysis revealed a prevalence of estrogen and progesterone receptor-positive tumors, especially in lobular carcinomas, whereas ductal carcinomas had more molecular heterogeneity. Analysis of the complement system revealed increased C1q levels in ductal carcinomas and significantly reduced Properdin expression across all clinical categories, underscoring possible roles in tumor immunobiology. These findings highlight the intricate relationship among socioeconomic status, tumor biology, and immune regulation in breast cancer, necessitating additional research into their clinical and prognostic significance.

Authers declaration:

Conflicts of Interest: None

We hereby confirm:

- All the figures and tables in the manuscript are ours. Any figures and images that are not ours have been included with the necessary permission for

- re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Authors signed on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at Al-Furat Alawsat Technical University

References:

1. Giaquinto AN, Sung H, Newman LA, Freedman RA, Smith RA, Star J, Jemal A, Siegel RL. Breast cancer statistics. *CA Cancer J Clin.* **2024;** 74(6): 477-495. https://doi.org/10.3322/caac.21863.
2. AL-Thaweni, Amina N.; Yousif, Waleed H.; and Hassan, Sarah Salih. Detection of BRCA1and BRCA2 mutation for Breast Cancer in Sample of Iraqi Women above 40 Years. Baghdad Science Journal. **2010;** 7(1): 14. https://doi.org/10.21123/bsj.2010.7.1.394-400.
3. Tsang, J. Y. S. & Tse, G. M. Molecular Classification of Breast Cancer. *Adv Anat Pathol*. **2020;** 27 (1): 27–35. https://doi.org/10.1097/PAP.0000000000000232.
4. Poorolajal J, Heidarimoghis F, Karami M, Cheraghi Z, Gohari-Ensaf F, Shahbazi F, Zareie B, Ameri P, Sahraee F. Factors for the Primary Prevention of Breast Cancer: A Meta-Analysis of Prospective Cohort Studies. *J Res Health Sci*. **2021;** 21(3): e00520. https://doi.org/10.34172/jrhs.2021.57.
5. Ali, J., Hassan, S. & Merzah, M. Prolactin serum levels and breast cancer: Relationships with hematological factors among cases in Karbala Province, Iraq. *Medical Journal of Babylon*. **2018;** 15, 178. DOI:10.4103/MJBL.MJBL_40_18
6. Gore R W, Patil B U. Hormone Receptor Status in Breast Cancer and its Relation to Age and Other Prognostic Factors at Tertiary Care Hospital at Central India. **2020;** 11(2): 164-169. https://dx.doi.org/10.21088/nijs.0976.4747.11220.14.
7. Hussein Hameedi, B., Hussain Mahdi, A. A. Al & Shalash Sultan, A. Estimation of Epidermal growth factor (EGF), HER2, CA15-3 and Acid phosphatase in Iraqi breast cancer women. *Bionatura*. **2022;** 7(3) :1–6. https://doi.org/10.21931/RB/2022.07.03.40.
8. Orrantia-Borunda E, Anchondo-Nuñez P, Acuña-Aguilar LE, et al. Subtypes of Breast Cancer. In: Mayrovitz HN, editor. Breast Cancer. Brisbane (AU): Exon Publications; **2022**; Chapter 3: 31-42 https://doi.org/10.36255/exon-publications-breast-cancer-subtypes.
9. Dembinski, R., Prasath, V., Bohnak, C. et al. Estrogen Receptor Positive and Progesterone Receptor Negative Breast Cancer: the Role of Hormone Therapy. *HORM CANC.* **2020;** 11(3-4): 148–154. https://doi.org/10.1007/s12672-020-00387-1.
10. Viehweger F, Gusinde J, Leege N, Tinger LM, Gorbokon N, *et al*. Estrogen receptor expression in human tumors: A tissue microarray study evaluating more than 18,000 tumors from 149 different entities. *Hum Pathol*. **2025**; 157: 105757-105767. https://doi.org/10.1016/j.humpath.2025.105757.
11. Carvalho E, Canberk S, Schmitt F, Vale N. Molecular Subtypes and Mechanisms of Breast Cancer: Precision Medicine Approaches for Targeted Therapies. *Cancers*. **2025;** 17(7): 1102. https://doi.org/10.3390/cancers17071102.
12. Kumar RV, Panwar D, Amirtham U, Premalata CS, Gopal C, Narayana SM, Patil Okaly GV, Lakshmaiah KC, Krishnamurthy S. Estrogen receptor, Progesterone receptor, and human epidermal growth factor receptor-2 status in breast cancer: A retrospective study of 5436 women from a regional cancer center in South India. *South Asian J Cancer*. **2018;** 7(1):7-10. https://doi.org/10.4103/sajc.sajc_211_17.
13. Yousef EM, Alswilem AM, Alfaraj ZS, Alhamood DJ, Ghashi GK, Alruwaily HS, Al Yahya SS, Alsaeed E. Incidence and Prognostic Significance of Hormonal Receptors and HER2 Status Conversion in Recurrent Breast Cancer: A Retrospective Study in a Single Institute. *Medicina*. **2025**; 61(4): 563. https://doi.org/10.3390/medicina61040563.
14. Y.N. Khatamovna. 40P Molecular subtypes and imaging phenotypes of breast cancer: MRI. *Annals of Oncology.* **2020;** 31: S1255-S1256. https://doi.org/10.1016/j.annonc.2020.10.060.
15. Khaled, H., Nada, Y.W., Ramadan, K.M. *et al.* Primary therapy of early breast cancer: Egyptian view of 2021 St. Gallen consensus. *J Egypt Natl Canc Inst*. **2022;** 34(1): 56. https://doi.org/10.1186/s43046-022-00156-x.
16. Almansour NM. Triple-Negative Breast Cancer: A Brief Review About Epidemiology, Risk Factors, Signaling Pathways, Treatment and Role of Artificial Intelligence. *Front. Mol. Biosci*. **2022;** 9: 836417. https://doi.org/10.3389/fmolb.2022.836417.
17. Merle, N.S. and Roumenina, L.T. The complement system as a target in cancer immunotherapy. *Eur. J. Immunol*. **2024;** 54(10): 2350820. https://doi.org/10.1002/eji.202350820.
18. Nitta H, Murakami Y, Wada Y, Eto M, Baba H, Imamura T. Cancer cells release anaphylatoxin C5a from C5 by serine protease to enhance invasiveness. *Oncol Rep*. **2014;** 32(4): 1715–1719. https://doi.org/10.3892/or.2014.3341.
19. Chen LH, Liu JF, Lu Y, He XY, Zhang C, Zhou HH. Complement C1q (C1qA, C1qB, and C1qC) May Be a Potential Prognostic Factor and an Index of

Tumor Microenvironment Remodeling in Osteosarcoma. *Front Oncol*. **2021;** 17(11): 642144. https://doi.org/10.3389/fonc.2021.642144.

20. Ma S, Song W, Xu Y, Si X, Zhang D, Lv S, Yang C, Ma L, Tang Z, Chen X. Neutralizing tumor-promoting inflammation with polypeptide-dexamethasone conjugate for microenvironment modulation and colorectal cancer therapy. *Biomaterials*. **2020;** 232: 119676. https://doi.org/10.1016/j.biomaterials.2019.119676.
21. Pęczek, P., Gajda, M., Rutkowski, K. *et al.* Cancer-associated inflammation: pathophysiology and clinical significance. *J Cancer Res Clin Oncol*. **2023;** 149: 2657–2672. https://doi.org/10.1007/s00432-022-04399-y.
22. Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discov*. **2022;** 12(1): 31-46. https://doi.org/10.1158/2159-8290.CD-21-1059.
23. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, *et al.* PTX3 Is an Extrinsic Oncosuppressor Regulating Complement-Dependent Inflammation in Cancer. *Cell.* **2015;** 160(4): 700–714. https://doi.org/10.1016/j.cell.2015.01.004.
24. Revel M, Daugan MV, Sautés-Fridman C, Fridman WH, Roumenina LT. Complement System: Promoter or Suppressor of Cancer Progression? *Antibodies (Basel).* **2020;** 9(4): 57. https://doi.org/10.3390/antib9040057.
25. Senent Y, Tavira B, Pio R, Ajona D. The complement system as a regulator of tumor-promoting activities mediated by myeloid-derived suppressor cells. *Cancer Lett*. **2022;** 549: 215900. https://doi.org/10.1016/j.canlet.2022.215900.
26. Fishelson, Z. & Kirschfink, M. Complement C5b-9 and Cancer: Mechanisms of Cell Damage, Cancer Counteractions, and Approaches for Intervention. *Front Immunol*. **2019;** 10: 752. https://doi.org/10.3389/fimmu.2019.00752.
27. Lin WD, Fan TC, Hung JT, Yeo HL, Wang SH, Kuo CW, Khoo KH, Pai LM, Yu J, Yu AL. Sialylation of CD55 by ST3GAL1 Facilitates Immune Evasion in Cancer. *Cancer Immunol Res*. **2021;** 9(1): 113–122. https://doi.org/10.1158/2326-6066.CIR-20-0203.
28. Shu C, Zha H, Long H, Wang X, Yang F, Gao J, Hu C, Zhou L, Guo B, Zhu B. C3a-C3aR signaling promotes breast cancer lung metastasis via modulating carcinoma associated fibroblasts. *Journal of Experimental & Clinical Cancer Research*. **2020;** 39(1): 11. https://doi.org/10.1186/s13046-019-1515-2.
29. Popeda M, Markiewicz A, Stokowy T, Szade J, Niemira M, Kretowski A, Bednarz-Knoll N, Zaczek AJ. Reduced expression of innate immunity-related genes in lymph node metastases of luminal breast cancer patients. *Sci Rep.* **2021;** 11: 5097. https://doi.org/10.1038/s41598-021-84568-0.
30. Mamoor S. C3 Is Differentially Expressed in Both Lymph Node and Brain Metastases in Human Breast Cancer. *OSF Preprints*. **2021;** 21. https://doi.org/10.31219/osf.io/r89pn_v1.
31. Hameed, B. H., Abdulsatar Al-Rayahi, I. & Muhsin, S. S. The Preoperative Serum Levels of the Anaphylatoxins C3a and C5a and Their Association with Clinico-Pathological Factors in Breast Cancer Patients. *Arch Razi Inst*. **2022;** 77(5): 1873–1879. https://doi.org/10.22092/ARI.2022.358193.2173.
32. Ajona, D., Ortiz-Espinosa, S., Pio, R. & Lecanda, F. Complement in Metastasis: A Comp in the Camp. *Front Immunol*. **2019;** 10: 669. https://doi.org/10.3389/fimmu.2019.00669.
33. El-Maboud Suliman, Lucy A. Moawad, Amr A. Elshahawy, Heba Abdalla, Dina. Role of complement activation product C4d as a predictor biomarker in lung cancer diagnosis: a case–control study. *The Egyptian Journal of Chest Diseases and Tuberculosis.* **2021;** 70(2): 231-235. https://doi.org/10.4103/ejcdt.ejcdt_92_20.
34. Golay, J. & Taylor, R. P. The Role of Complement in the Mechanism of Action of Therapeutic Anti-Cancer mAbs. *Antibodies.* **2020;** 9 (4): 58. https://doi.org/10.3390/antib9040058.
35. Roumenina, L. T., Daugan, M. V., Petitprez, F., Sautès-Fridman, C. & Fridman, W. H. Context-dependent roles of complement in cancer. *Nat Rev Cancer.* **2019;** 19: 698–715. https://doi.org/10.1038/s41568-019-0210-0.
36. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, *et al*. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Unabridged Version). *Arch Pathol Lab Med*. **2010;** 134(7): e48–e72. https://doi.org/10.5858/134.7.e48.
37. Sadeghi, M., Vahid, F., Rahmani, D., Akbari, M. E. & Davoodi, S. H. The Association between Dietary Patterns and Breast Cancer Pathobiological Factors Progesterone Receptor (PR) and Estrogen Receptors (ER): New Findings from Iranian Case-Control Study. *Nutr Cancer*. **2019;** 71(8): 1290–1298. https://doi.org/10.1080/01635581.2019.1602658.
38. Walter V, Fischer C, Deutsch TM, Ersing C, Nees J, Schütz F, Fremd C, Grischke EM, Sinn P, Brucker SY, Schneeweiss A, Hartkopf AD, Wallwiener M. Estrogen, progesterone, and human epidermal growth factor receptor 2 discordance between primary and metastatic breast cancer. *Breast*

Cancer Res Treat. **2020;** 183:137-144. https://doi.org/10.1007/s10549-020-05746-8.

39. Roshed, M. M., Kamal, S., Hossain, S. M. & Akhtar, S. Evaluation of Breast Cancer Subtypes Based on ER/PR and Her2 Expression: A Clinicopathologic Study of Hormone Receptor Status (ER/PR/HER2-neu) in Breast Cancer. *Faridpur Medical College Journal*. **2020;** 14(1): 8–12. https://doi.org/10.3329/fmcj.v14i1.46158.
40. Aman NA, Doukoure B, Koffi KD, Koui BS, Traore ZC, Kouyate M, Effi AB. HER2 overexpression and correlation with other significant clinicopathologic parameters in Ivorian breast cancer women. *BMC Clin Pathol*. **2019;** 19: 1-6. https://doi.org/10.1186/s12907-018-0081-4.
41. Sohail, S. K., Sarfraz, R., Imran, M., Kamran, M. & Qamar, S. Estrogen and Progesterone Receptor Expression in Breast Carcinoma and Its Association with Clinicopathological Variables Among the Pakistani Population. *Cureus.* **2020;** 12(8): e9751. https://doi.org/10.7759/cureus.9751.
42. Aldaz-Roldán P, Pardo-Vásquez DF, Chamba-Morales GN, Aguirre-Reyes DF, Castillo-Calvas JM, Noblecilla-Arévalo G. Immunohistochemical subtype and its relationship with 5-year overall survival in breast cancer patients. *Ecancermedicalscience*. **2023;** 16(17): 1509. https://doi.org/10.3332/ecancer.2023.1509.
43. Bowen DJ, Fernandez Poole S, White M, Lyn R, Flores DA, Haile HG, Williams DR. The Role of Stress in Breast Cancer Incidence: Risk Factors, Interventions, and Directions for the Future. *Int J Environ Res Public Health.* **2021;** 18(4): 1871. https://doi.org/10.3390/ijerph18041871.
44. Koval, L. E., Dionisio, K. L., Friedman, K. P., Isaacs, K. K. & Rager, J. E. Environmental mixtures and breast cancer: identifying co-exposure patterns between understudied vs breast cancer-associated chemicals using chemical inventory informatics. *J Expo Sci Environ Epidemiol*. **2022;** 32: 794–807. https://doi.org/10.1038/s41370-022-00451-8.
45. Michał Kunc, Marta Popęda, Michał Bieńkowski, Marcin Braun, Aleksandra Łacko. Estrogen receptor-negative progesterone receptor-positive breast cancer is a molecularly distinct group characterized by the down-regulation of genes controlled by ESR1 and SUZ12. *Cancer Res.* **2023;** 83(5_Supplement): 2–23–06. https://doi.org/10.1158/1538-7445.SABCS22-P2-23-06.
46. He Dou, Fucheng Li, Youyu Wang, Xingyan Chen, Pingyang Yu. *et al.* Estrogen receptor-negative/progesterone receptor-positive breast cancer has distinct characteristics and pathologic complete response rate after neoadjuvant chemotherapy. *Diagn Pathol.* **2024;** 19: 5. https://doi.org/10.1186/s13000-023-01433-6.
47. Li Y, Yang D, Yin X, Zhang X, Huang J, Wu Y, Wang M, Yi Z, Li H, Li H, Ren G. Clinicopathological Characteristics and Breast Cancer–Specific Survival of Patients with Single Hormone Receptor–Positive Breast Cancer. *JAMA Netw Open.* **2020;** 3(1): e1918160. https://doi.org/10.1001/jamanetworkopen.2019.18160.
48. Sarmah, A., Das, A. & Datta, D. A study to determine the incidence of estrogen receptor (ER) and progesterone receptor (PR) expression in different histological grades of breast cancer. *J Histotechnol*. **2014;** 37(2): 54–59. https://doi.org/10.1179/2046023614Y.0000000042.
49. Ruqayah Ali Salman. Immunohistochemical Determination of Estrogen and Progesterone Receptors in Women Breast Cancer Patients. *J. Med. Chem. Sci.* **2022;** 5(7): 1224-1230. https://doi.org/10.26655/JMCHEMSCI.2022.7.11.
50. Hussain, Abeer M.; Ali, Alia Hussein; and Mohammed, Haider Latif. Correlation between Serum and Tissue Markers in Breast Cancer Iraqi Patients. *Baghdad Science Journal*. **2022;** 19(3): 501-514. https://doi.org/10.21123/bsj.2022.19.3.0501
51. Quaquarini E, Grillo F, Gervaso L, Arpa G, Fazio N, Vanoli A, Parente P. Prognostic and Predictive Roles of HER2 Status in Non-Breast and Non-Gastroesophageal Carcinomas. *Cancers*. **2024;** 16(18): 3145. https://doi.org/10.3390/cancers16183145.
52. Mukhtar Z, Faisal A, Mudassir G, Mamoon N. Correlation between HER2/neu protein overexpression on Immunohistochemistry and Fluorescent in Situ Hybridization (FISH) in breast carcinoma: Problems in developing countries. *Pak J Med Sci*. **2023;** 39(6): 1814-1817. https://doi.org/10.12669/pjms.39.6.6704.
53. Shukla S, Singh BK, Pathania OP, Jain M. Evaluation of HER2/neu oncoprotein in serum & tissue samples of women with breast cancer. *Indian J Med Res*. **2016;** 143(Suppl.1): S52-S58. https://doi.org/10.4103/0971-5916.191769.
54. Poon IK, Tsang JY, Li J, Chan SK, Shea KH, Tse GM. The significance of highlighting the oestrogen receptor low category in breast cancer. *Br J Cancer*. **2020;** 123: 1223–1227. https://doi.org/10.1038/s41416-020-1009-1.
55. Roumenina, L. T., Daugan, M. V., Petitprez, F., Sautès-Fridman, C. & Fridman, W. H. Context-dependent roles of complement in cancer. *Nat Rev Cancer*. **2019;** 19: 698–715. https://doi.org/10.1038/s41568-019-0210-0.
56. Ajona, D., Ortiz-Espinosa, S., Pio, R. & Lecanda, F. Complement in Metastasis: A Comp in the Camp. *Front Immunol.* **2019;** 10: 669. https://doi.org/10.3389/fimmu.2019.00669.

57. Hammody, R. H., M. Q. Al-ani, and F. A. Turkey. "serum immunoglobulin and complement levels in patients with breast cancer in Iraq". *Asian Journal of Pharmaceutical and Clinical Research*. **2018;** 11(6): 473-5, https://doi.org/10.22159/ajpcr.2018.v11i6.25060.
58. Michlmayr A, Bachleitner-Hofmann T, Baumann S, Marchetti-Deschmann M, Rech-Weichselbraun I, *et al.* Modulation of plasma complement by the initial dose of epirubicin/docetaxel therapy in breast cancer and its predictive value. *Br J Cancer*. **2010;** 103: 1201–1208. https://doi.org/10.1038/sj.bjc.6605909.
59. Lu Y, Zhao Q, Liao JY, Song E, Xia Q, Pan J, Li Y, Li J, Zhou B, Ye Y, Di C, Yu S, Zeng Y, Su S. Complement Signals Determine Opposite Effects of B Cells in Chemotherapy-Induced Immunity. *Cell.* **2020;** 180(6): 1081-1097.e24. https://doi.org/10.1016/j.cell.2020.02.015.
60. Anna Felberg, Michał Bieńkowski, Tomasz Stokowy, Kamil Myszczyński, Zuzanna Polakiewicz, *et al*. Elevated expression of complement factor I in lung cancer cells associates with shorter survival–Potentially via non-canonical mechanism. *Translational Research*. **2024;** 269: 1-13. https://doi.org/10.1016/j.trsl.2024.02.003.
61. Lin CH, Huang RY, Lu TP, Kuo KT, Lo KY, Chen CH, Chen IC, Lu YS, Chuang EY, Thiery JP, Huang CS, Cheng AL. High prevalence of APOA1/C3/A4/A5 alterations in luminal breast cancers among young women in East Asia. *NPJ Breast Cancer.* **2021;** **7**(1): 88. https://doi.org/10.1038/s41523-021-00299-5.
62. Hong Q, Sze CI, Lin SR, Lee MH, He RY, Schultz L, Chang JY, Chen SJ, Boackle RJ, Hsu LJ, Chang NS. Complement C1q Activates Tumor Suppressor WWOX to Induce Apoptosis in Prostate Cancer Cells. *PLoS One*. **2009;** 4(6): e5755. https://doi.org/10.1371/journal.pone.0005755.
63. Bandini S, Macagno M, Hysi A, Lanzardo S, Conti L, Bello A, Riccardo F, Ruiu R, Merighi IF, Forni G, Iezzi M, Quaglino E, Cavallo F. The non-inflammatory role of C1q during Her2/neu-driven mammary carcinogenesis. *Oncoimmunology*. **2016;** 5(12): e1253653. https://doi.org/10.1080/2162402X.2016.1253653.
64. Zabihi, M. R., Farhadi, B. & Akhoondian, M. Complement protein expression changes in various conditions of breast cancer: in-silico analyses—experimental research. *Ann. Med. Surg*. **2024;** 86(9): 5152–5161. https://doi.org/10.1097/MS9.0000000000002216.
65. Mangogna A, Agostinis C, Bonazza D, Belmonte B, Zacchi P, Zito G, Romano A, Zanconati F, Ricci G, Kishore U, Bulla R. Is the Complement Protein C1q a Pro- or Anti-tumorigenic Factor? Bioinformatics Analysis Involving Human Carcinomas. *Front Immunol*. **2019;** 10: 865. https://doi.org/10.3389/fimmu.2019.00865.
66. Bandini S, Macagno M, Hysi A, Lanzardo S, Conti L, Bello A, Riccardo F, Ruiu R, Merighi IF, Forni G, Iezzi M, Quaglino E, Cavallo F. The non-inflammatory role of C1q during Her2/neu-driven mammary carcinogenesis. *Oncoimmunology*. **2016;** 5(12): e1253653. https://doi.org/10.1080/2162402X.2016.1253653.
67. Kaur A, Sultan SH, Murugaiah V, Pathan AA, Alhamlan FS, Karteris E, Kishore U. Human C1q Induces Apoptosis in an Ovarian Cancer Cell Line via Tumor Necrosis Factor Pathway. *Front Immunol*. **2016;** 21(7): 599. https://doi.org/10.3389/fimmu.2016.00599.
68. Bulla R, Tripodo C, Rami D, Ling GS, Agostinis C, Guarnotta C, Zorzet S, Durigutto P, Botto M, Tedesco F. C1q acts in the tumour microenvironment as a cancer-promoting factor independently of complement activation. *Nat Commun*. **2016;** 1(7): 10346. https://doi.org/10.1038/ncomms10346.
69. Mangogna A, Agostinis C, Bonazza D, Belmonte B, Zacchi P, Zito G, Romano A, Zanconati F, Ricci G, Kishore U, Bulla R. Is the Complement Protein C1q a Pro- or Anti-tumorigenic Factor? Bioinformatics Analysis Involving Human Carcinomas. *Front Immunol*. **2019;** 3(10): 865. https://doi.org/10.3389/fimmu.2019.00865.
70. van Essen, M.F., Schlagwein, N., van den Hoven, E.M.P., van Gijlswijk-Janssen, D.J., Lubbers, R., van den Bos, R.M., van den Born, J., Ruben, J.M., Trouw, L.A., van Kooten, C. Initial properdin binding contributes to alternative pathway activation at the surface of viable and necrotic cells. *Eur. J. Immunol*. **2022;** 52: 597-608. https://doi.org/10.1002/eji.202149259.
71. Mangogna A, Varghese PM, Agostinis C, Alrokayan SH, Khan HA, Stover CM, Belmonte B, Martorana A, Ricci G, Bulla R, Kishore U. Prognostic Value of Complement Properdin in Cancer. *Front Immunol*. **2021;** 19(11): 614980. https://doi.org/10.3389/fimmu.2020.614980.
72. Uday Kishore, Praveen M Varghese, Alessandro Mangogna, Lukas Klein, Mengyu Tu, *et al.* Neutrophil-derived complement factor P induces cytotoxicity in basal-like cells via caspase 3/7 activation in pancreatic cancer. *bioRxiv preprint*. **2023;** 10(28): 564512. https://doi.org/10.1101/2023.10.28.564512.
73. Al-Rayahi, I. A. M., Machado, L. R. & Stover, C. M. Properdin Is a Modulator of Tumour Immunity in a Syngeneic Mouse Melanoma Model. *Medicina (B Aires)*. **2021;** 57(2): 85. https://doi.org/10.3390/medicina57020085.

74. Saeed, Z., Greer, O. & Shah, N. M. Is the Host Viral Response and the Immunogenicity of Vaccines Altered in Pregnancy? *Antibodies*. **2020;** 9(3): 38. https://doi.org/10.3390/antib9030038.
75. Reis, E. S., Mastellos, D. C., Ricklin, D., Mantovani, A. & Lambris, J. D. Complement in cancer: untangling an intricate relationship. *Nat Rev Immunol*. **2018;** 18(1): 5–18. https://doi.org/10.1038/nri.2017.97.
76. Murugaiah V, Varghese PM, Beirag N, De Cordova S, Sim RB, Kishore U. Complement Proteins as Soluble Pattern Recognition Receptors for Pathogenic Viruses. *Viruses*. **2021;** 13(5): 824. https://doi.org/10.3390/v13050824.