C5aR2 in Breast Cancer and Its Relationship with Clinicopathological Features

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ABSTRACT

Objective: To determine the relation between C5aR2 and clinicopathological parameters of breast cancer.
Study Design: Observational study.
Place and Duration of the Study: Department of Histopathology, PNS Shifa Hospital, Karachi, from January to June 2023.
Methodology: One hundred and twenty-eight women, aged 24-90 years with histologically proven diagnosis of breast cancer, were included in the study. Immunohistochemistry (IHC) was performed to evaluate the C5aR2 expression by its percentage and intensity. The C5aR2 staining was observed in membranes and/or cytoplasm. The immunoreactive score (IRS) was obtained and its association was analysed with clinicopathological parameters of breast cancer. SPSS version 27 was used to analyse the data.
Results: The C5aR2 expression was higher in tumour cells (90.6%) compared to stromal cells (53.1%), predominantly exhibiting cytoplasmic localisation. Higher C5aR2 expression was observed in older age groups, higher-grade tumours, and ER/PR/HER2 and HER2 positive tumours. Moreover, tumours with poor treatment response also showed increased C5aR2 expression compared to those with good treatment response. Although no significant association was identified between C5aR2 expression and Ki67, increased C5aR2 expression has been found in tumours with high cell proliferation rates.
Conclusion: In this study, an association between tumour and stromal cell C5aR2 expression and age, grade, receptor status, proliferation rate, and post-treatment response was identified.

Key Words: *Breast cancer, C5aR2, Cancer associated fibroblasts, Immunohistochemistry, Ki67*.

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INTRODUCTION

Breast cancer is the most common cause of death for women all over the world and Pakistan has the highest incidence of breast cancer among Asian countries.[1] The high incidence of breast cancer is attributed to its heterogeneous nature, apparent at both morphological as well as molecular levels, and demands different therapeutic regimens, based on the molecular subtype. The complex nature of breast cancer is intricately linked with the tumour microenvironment (TME) and is not solely dependent on the intrinsic features of tumour cells alone.[2] Therefore, understanding the TME in breast cancer is crucial owing to its role in tumour behaviour and treatment response.[3]

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TME comprises of a heterogeneous collection of resident and infiltrating cells, extracellular matrix, and secreted factors.[4] The composition of TME determines its positive or negative effect on the outcome of cancer patients.[5] Among different cells, complement proteins constitute an important part of TME. The complement components are not only infiltrated systemically[5] but are also aberrantly activated locally by tumour cells. This is evident from the presence of activation products of complement in breast and lung tumour tissue[6] and its elevated levels in the sera of patients with neoplastic diseases, thus proving its obvious role in tumorigenesis. The activation of the complement system generates many components, among which C5a is one of the most powerful pro-inflammatory mediators.[7]

C5a generates its response by binding with G-protein-coupled receptors C5aR1 (CD88) and C5aR2 (GPR77). The tumorigenic role of C5aR1 has been established but the role of C5aR2 is still uncertain. It is generally considered that the complement system plays an important role in the tumour growth and progression. On the contrary, there are researches demonstrating its anti-tumoural role.[8] Recent studies have highlighted its tumorigenic role in breast cancer development, proliferation, migration, and chemoresistance.

C5aR2 is mainly expressed in human and rat cells and tissues of both myeloid and non-myeloid origin, most abundantly in neutrophils. Its localisation is mainly intracellular in many tissues.[9] C5aR2 was initially considered a mere decoy receptor, scavenging excess C5a to mitigate inflammation. However, emerging evidence suggests that C5aR2 has its own unique signalling pathways and immunomodulatory effects.[10] It has been found through bio-informatic analysis that different cancerous and non-cancerous cells and tissues show variable C5aR2 expression levels and tumours with high C5aR2 expression have a poor prognosis.[11]

Zhu *et al*. conducted an extensive analysis of human breast cancer samples to elucidate the expression, prognosis, immune infiltration, and correlation with signalling pathways of C5aR2 and revealed several signalling pathways or biological processes through which C5aR2 regulates tumour immunity and impacts the fate of the tumour. Moreover, they also demonstrated that C5aR2 promotes the proliferation, migration, invasion, and activation of oncogenic pathways in breast cancer cells.[11] In a recent study, Su *et al*. found that cancer-associated fibroblasts (CAF) have different subsets that noticeably affect the prognosis of tumours. Among different CAF subpopulations, they explored a strong association of C5aR2 expressing CAFs with chemoresistance and poor prognosis among various cohorts of breast and lung cancer patients, coupled with IL-10. The authors alluded that these new markers can analyse the response of cancer patients to neoadjuvant chemotherapy and its possible consequences.[12] In another study conducted by Tong *et al*., the relationship between C5aR2 and other CAFs biomarkers and chemoresistances was explored clinically in locally advanced gastric cancer. They observed that increased post-C5aR2 expression was associated with a poor pathological response indicating its relation with drug resistance.[13] The mechanism by which C5aR2-labelled CAFs drive gastric cancer progression, leading to neoadjuvant chemotherapy (NCT) resistance and poor prognosis is through the induction of epithelial to mesenchymal transition (EMT) and cancer stem cells (CSCs) in the gastric cancer cells of patients with locally advanced disease.[14]

In contrast, research on a melanoma-bearing murine model has revealed that C5aR2 restricts tumour growth.[15] Similar anti-tumorigenic effects of C5aR2 were seen in C5aR2 deficient, Azoxymethane / Dextran Sodium Sulfate (AOM/DSS)-induced colorectal carcinoma showing increased tumour growth.[16]

A research on C5aR inhibitor (W-54011) was conducted by Wang Yuxuan, using human gastric cancer cell lines, obtained from different variants of gastric cancer such as MKN1 (adenosquamous carcinoma), MKN7 (tubular adenocarcinoma of well-differentiated type), NUGC3, and AGS (poorly differentiated tubular adenocarcinoma). Increased expression of C5aR and C5L2 (C5aR2) was observed in gastric cancer cell lines MKN1 and MKN7, which upon stimulation by recombinant human complement component C5a (rC5a), showed a marked increase in invasion.[17]

The C5aR2 analysis in human breast cancer is scarce and needs further exploration to ascertain its role in breast cancer. Therefore, this study aimed to evaluate the association between C5aR2 expression and breast cancer in the local population.

METHODOLOGY

It was a cross-sectional study, conducted in the Department of Histopathology, PNS Shifa Hospital, Karachi, Pakistan, in the period between January and June 2023 after obtaining ethical approval from the Ethical Review Committee (ERC) of Bahria University Health Sciences campus. The retrospective and prospective breast cancer samples were obtained through convenient sampling (non-probability) technique. The breast cancer samples included modified radical mastectomy (MRM), excisional, incisional, and trucut biopsy specimens. The specimens confirmed as primary breast cancer and post-neoadjuvant cases with available primary samples were included in the study. Metastatic tumours, male breast cancer samples, poorly-fixed tissues, and patients not willing to participate were excluded from the study.

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of primary breast cancer cases were retrieved from PNS Shifa Histopathology Department archives for retrospective cases. For prospective cases, all the gross specimens were initially examined thoroughly, following the guidelines set forth by the College of American Pathologists and then FFPE tissue blocks were made. H&E staining of the sections obtained from FFPE tissue blocks of both retrospective and prospective cases was done for histological diagnosis. Parameters including age, grade, stage, Ki67, receptor status, molecular classification, post-treatment response, Ki67, lymph node involvement (LNI), lymphovascular invasion (LVI), perineural invasion (PNI), and ductal carcinoma *in-situ* (DCIS) were recorded.

Immunohistochemistry (IHC) was performed using GPR77 Rabbit/IgG Polyclonal Antibody (RRID-AB_2853667, Thermofisher) as the primary antibody. The primary antibody was utilised at a working dilution of 1:100. Normal tissue samples from the intestine and kidney were taken as controls. Four µm thick tissue sections were taken on Poly-L-Lysine coated slides. De-paraffinisation, hydration, and antigen retrieval were done automatically through the Dako PT Link pretreatment system in 40-45 minutes using citrate buffer. Samples were blocked with blocking buffer (1.5 hour) and incubated with primary GPR77 polyclonal antibody (1:100 dilution) in a humidity chamber for 1.5 hour at 22°C. This was followed by application of Horseradish Peroxidase (HRP) as the secondary antibody to indirectly detect C5aR2 to which the primary antibody was bound. The secondary antibodies were conjugated with chromogen 3,3'-Diaminobenzidine (DAB). DAB, an HRP substrate, was used for secondary HRP-conjugated antibody detection. After counterstaining with haematoxylin stain, slides were mounted and examined.[18] The C5aR2 staining was observed in the membrane and/or cytoplasm. The percentage of stained cells and the intensity of staining was recorded and immunore-

active score (IRS) was obtained by multiplying both. IRS score of 0-1 was considered as negative, 2-3 as mild, 4-8 as moderate, and 9-12 as strong.[19,20]

Data analysis was performed by IBM SPSS Statistics version 27. Mean and standard deviation were calculated for quantitative variables while frequency and percentages were presented for qualitative variables. The association between qualitative variables was determined by the Chi-square / Fisher's exact test. Odds ratios were calculated by univariate binary logistic regression. A p-value of less than 0.05 was considered as significant.

RESULTS

The study was conducted on 128 cases of invasive carcinoma of female breasts with the age range of 24-90 years (mean = 49.04 ± 13.92 years). The demographic details of the study are mentioned in Table I. Of the total cases, 86.7% were invasive breast carcinoma no special type (IBC-NST). Most of the patients (53.9%) were under the age of 40. Of the 128 patients, 73.4% were determined to have grade I/II, 26.6% had grade III, and 11.2% had stage I, 47.2% had stage II, and 41.6% had stage III breast carcinoma.

Among 128 patients, 17.9% of patients had luminal A type, 50.4% had luminal B type, 14.5% had HER2 positive, and 17.1% had triple-negative breast cancer. In 48.3% of patients, the right side of the breast was involved and 51.7% had left-sided breast cancer, 81.9% showed high Ki67, and 18.1% of patients had low Ki67. Lymphovascular invasion was seen in 35.2% of patients, 8.5% had PNI, and 46.9% had DCIS. C5aR2 expression in tumour cells was higher (90.6%) than C5aR2 expression in the stromal cells (53.1%). Figure 1 represents the levels of C5aR2 expression in tumour cells and stromal cells of breast cancer.

The images of mild, moderate, and strong C5aR2 staining of breast tumour and stromal cells are shown in Figure 2.

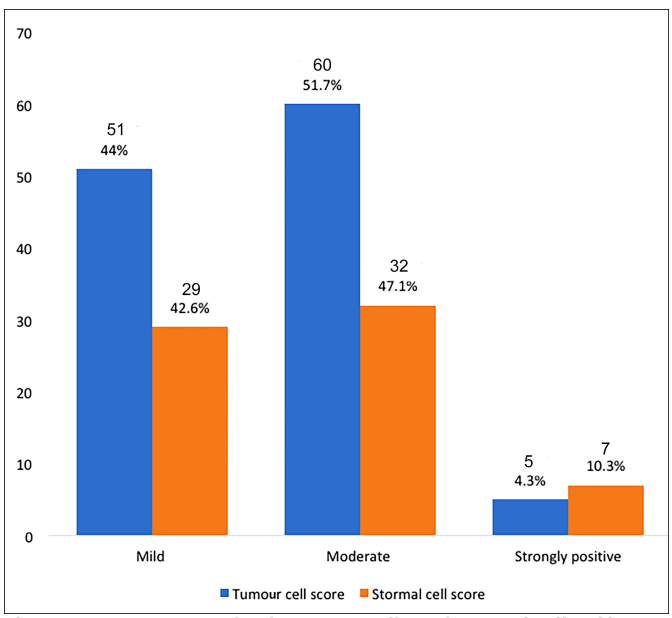

Figure 1: C5aR2 expression in tumour cells and stromal cells of breast cancer.

Table I: Baseline Characteristics of study population (n = 128).

Parameters	n (%)
Age (years)	
Mean ± std. dev	49.04 ± 13.92
Age groups	
≤40 years	69 (53.9)
>40 years	59 (46.1)
Biopsy	
MRM	87 (68)
Excisional	8 (6.3)
Incisional	5 (3.9)
Trucut	19 (14.8)
NOS	9 (7)
Histology	
IBC-NST	111 (86.7)
ILC	9 (7)
DCIS	4 (3.1)
Others	4 (3.1)
Tumour grade	
Grade-I / Grade-II	94 (73.4)
Grade-III	34 (26.6)
Tumour stage (n = 89)	
Stage-I	10 (11.2)
Stage-II	42 (47.2)
Stage-III	37 (41.6)
Receptor (n = 117)	
ER+	11 (9.4)
ER/PR+	51 (43.6)
ER/PR/Her2+	9 (7.7)
TNBC	20 (17.1)
ER/Her2+	6 (5.1)
PR/Her2+	2 (1.7)
Her2+	16 (13.7)
PR+	2 (1.7)
Molecular status (n = 117)	
Luminal A	21 (18)
Luminal B	59 (50.4)
HER2+	17 (14.5)
TNBC	20 (17.1)
Residual tumour	
<30%	5 (3.9)
30-50%	8 (6.3)
51-80%	4 (3.1)
>80%	11 (8.6)
No TM	100 (78.1)
Laterality (n = 120)	
Right	58 (48.3)
Left	62 (51.7)
Ki67 (n = 105)	
Low (≤14%)	19 (18.1)
High (>14%)	86 (81.9)
LVI (n = 94)	
Present	45 (47.9)
Absent	49 (52.1)
PNI (n = 91)	
Present	8 (8.8)
Absent	83 (91.2)
LNI (n = 88)	
Present	53 (60.2)
Absent	35 (39.8)
DCIS (n = 98)	
Present	46 (46.9)
Absent	52 (53.1)
C5aR2 expression in tumour cells	
Positive	116 (90.6)
Negative	12 (9.4)
C5aR2 expression in stromal cells	
Positive	68 (53.1)
Negative	60 (46.9)

Table II: Association of tumour cell C5aR2 expression and clinicopathological parameters of breast cancer.

Parameters	C5aR2 expression in tumour cells n (%)		‡p-value	Univariate analysis	
	Positive (n = 116)	Negative (n = 12)		OR (95% CI)	‡p-value
Age groups					
≤40 years	60 (51.7)	9 (75)	0.124	0.357 (0.092-1.387)	0.137
>40 years	56 (48.3)	3 (25)		Ref	
Biopsy					
MRM	80 (69)	7 (58.3)	0.150	5.714 (1.169-27.928)	0.031
Excisional	7 (6)	1 (8.3)		3.500 (0.284-43.161)	0.328
Incisional	5 (4.3)	0 (0)		NA	0.999
Trucut	18 (15.5)	1 (8.3)		9.000 (0.781-103.724)	0.078
NOS	6 (5.2)	3 (25)		Ref	
Histology					
IBC-NST	99 (85.3)	12 (100)	0.820	NA	0.999
ILC	9 (7.8)	0 (0)		NA	>0.99
DCIS	4 (3.4)	0 (0)		NA	>0.99
Others	4 (3.4)	0 (0)		Ref	
Tumour grade					
Grade-I / Grade-II	86 (74.1)	8 (66.7)	0.732	1.433 (0.402-5.105)	0.579
Grade-III	30 (25.9)	4 (33.3)		Ref	
Tumour stage (n = 89)					
Stage-I	10 (12.2)	0 (0)	0.068	NA	0.999
Stage-II	41 (50)	1 (14.3)		7.935 (0.908-69.348)	0.061
Stage-III	31 (37.8)	6 (85.7)		Ref	
Receptor					
ER+	9 (8.5)	2 (18.2)	0.455	4.500 (0.190-106.823)	0.352
ER/PR+	47 (44.3)	4 (36.4)		11.750 (0.613-225.353)	0.102
ER/PR/Her2+	9 (8.5)	0 (0)		NA	0.999
TNBC	18 (17)	2 (18.2)		9.000 (0.392-206.530)	0.169
ER/Her2+	6 (5.7)	0 (0)		NA	0.999
PR/Her2+	2 (1.9)	0 (0)		NA	0.999
Her2+	14 (13.2)	2 (18.2)		7.000 (0.302-162.202)	0.225
PR+	1 (0.9)	1 (9.1)		Ref	
Molecular status (n = 117)					
Luminal A	17 (16)	4 (36.4)	0.226	0.472 (0.076-2.921)	0.420
Luminal B	56 (52.8)	3 (27.3)		2.074 (0.321-13.408)	0.444
HER2+	15 (14.2)	2 (18.2)		0.833 (0.104-6.646)	0.863
TNBC	18 (17)	2 (18.2)		Ref	
Residual tumour					
<30%	1 (0.9)	4 (33.3)	<0.001*	0.013 (0.001-0.141)	<0.001
30-50%	8 (6.9)	0 (0)		NA	0.999
51-80%	4 (3.4)	0 (0)		NA	0.999
>80%	8 (6.9)	3 (25)		0.140 (0.028-0.697)	0.016
No TM	95 (81.9)	5 (41.7)		Ref	
Laterality (n = 120)					
Right	52 (47.7)	6 (54.5)	0.665	0.760 (0.219-2.640)	0.666
Left	57 (52.3)	5 (45.5)		Ref	
Ki67 (n = 105)					
Low (≤14%)	17 (17.7)	2 (22.2)	>0.99	0.753 (0.144-3.947)	0.737
High (>14%)	79 (82.3)	7 (77.8)		Ref	
LVI (n = 94)					
Present	41 (47.7)	4 (50)	>0.99	0.911 (0.214-3.881)	0.900
Absent	45 (52.3)	4 (50)		Ref	
PNI (n = 91)					
Present	7 (8.4)	1 (12.5)	0.536	0.645 (0.069-6.018)	0.700
Absent	76 (91.6)	7 (87.5)		Ref	
LNI (n = 88)					
Present	46 (57.5)	7 (87.5)	0.138	0.193 (0.023-1.645)	0.133
Absent	34 (42.5)	1 (12.5)		Ref	
DCIS (n = 98)					
Present	45 (50)	1 (12.5)	0.063	7.000 (0.827-59.237)	0.074
Absent	45 (50)	7 (87.5)		Ref	

*Chi-square/Fisher's exact test‡ Binary logistic regression↕ Significant association**

Table III: Association of stromal cell C5aR2 expression and clinicopathological parameters of breast cancer.

Parameters	C5aR2 Expression in Stromal Cells n (%)		ǂp-value	Univariate analysis	
	Positive	Negative		OR (95% CI)	↕p-value
Age groups				0.553 (0.273-1.119)	0.099
≤40 years	32 (47.1)	37 (61.7)	0.098	Ref	
>40 years	36 (52.9)	23 (38.3)			
Biopsy				2.258 (0.565-9.030)	0.249
MRM	56 (82.4)	31 (51.7)	0.002*	0.750 (0.107-5.238)	0.772
Excisional	3 (4.4)	5 (8.3)		0.313 (0.024-4.024)	0.372
Incisional	1 (1.5)	4 (6.7)		0.333 (0.060-1.854)	0.210
Trucut Bx	4 (5.9)	15 (25)		Ref	
NOS	4 (5.9)	5 (8.3)			
Histology				3.529 (0.356-39.984)	0.281
IBC-NST	60 (88.2)	51 (85)	0.792	3.750 (0.274-51.373)	0.322
ILC	5 (7.4)	4 (6.7)		3.000 (0.150-59.890)	0.472
DCIS	2 (2.9)	2 (3.3)		Ref	
Others	1 (1.5)	3 (5.0)			
Tumour grade				0.619 (0.278-1.379)	0.241
Grade-I / Grade-II	47 (69.1)	47 (78.3)	0.239	Ref	
Grade-III	21 (30.9)	13 (21.7)			
Tumour stage (n = 89)				0.682 (0.168-2.772)	0.593
Stage-I	5 (8.8)	5 (15.6)	0.338	1.705 (0.668-4.353)	0.265
Stage-II	30 (52.6)	12 (37.5)		Ref	
Stage-III	22 (38.6)	15 (46.9)			
Receptor				NA	0.999
ER+	2 (3.3)	9 (16.1)	0.176	NA	0.999
ER/PR+	26 (42.6)	25 (44.6)		NA	0.999
ER/PR/Her2+	7 (11.5)	2 (3.6)		NA	0.999
TNBC	12 (19.7)	8 (14.3)		NA	0.999
ER/Her2+	3 (4.9)	3 (5.4)		NA	0.999
PR/Her2+	1 (1.6)	1 (1.8)		NA	0.999
Her2+	8 (13.1)	8 (14.3)		Ref	
PR+	2 (3.3)	0 (0)			
Molecular status (n = 117)				0.606 (0.176-2.091)	0.428
Luminal A	10 (16.4)	11 (19.6)	0.871	0.690 (0.246-1.932)	0.480
Luminal B	30 (49.2)	29 (51.8)		0.750 (0.203-2.770)	0.666
HER2+	9 (14.8)	8 (14.3)		Ref	
TNBC	12 (19.7)	8 (14.3)			
Residual tumour				NA	0.999
<30%	0 (0)	5 (8.3)	0.093	2.660 (0.512-13.822)	0.244
30-50%	6 (8.8)	2 (3.3)		0.887 (0.120-6.545)	0.906
51-80%	2 (2.9)	2 (3.3)		1.552 (0.427-5.636)	0.504
>80%	7 (10.3)	4 (6.7)		Ref	
No TM	53 (77.9)	47 (78.3)			
Laterality (n = 120)				1.245 (0.605-2.564)	0.552
Right	34 (50.7)	24 (45.3)	0.552	Ref	
Left	33 (49.3)	29 (54.7)			
Ki67 (n = 105)				1.253 (0.459-3.420)	0.660
Low (≤14%)	11 (19.6)	8 (16.3)	0.660	Ref	
High (>14%)	45 (80.4)	41 (83.7)			
LVI (n = 94)				0.797 (0.345-1.842)	0.595
Present	27 (45.8)	18 (51.4)	0.595	Ref	
Absent	32 (54.2)	17 (48.6)			
PNI (n = 91)				1.882 (0.358-9.902)	0.455
Present	6 (10.5)	2 (5.9)	0.705	Ref	
Absent	51 (89.5)	32 (94.1)			
LNI (n = 88)				0.698 (0.284-1.720)	0.435
Present	32 (57.1)	21 (65.6)	0.434	Ref	
Absent	24 (42.9)	11 (34.4)			
DCIS (n = 98)				0.685 (0.305-1.540)	0.360
Present	25 (43.1)	21 (52.5)	0.360	Ref	
Absent	33 (56.9)	19 (47.5)		0.553 (0.273-1.119)	0.099

*Chi-square/Fisher's exact test‡ Binary logistic regression↕ Significant association**

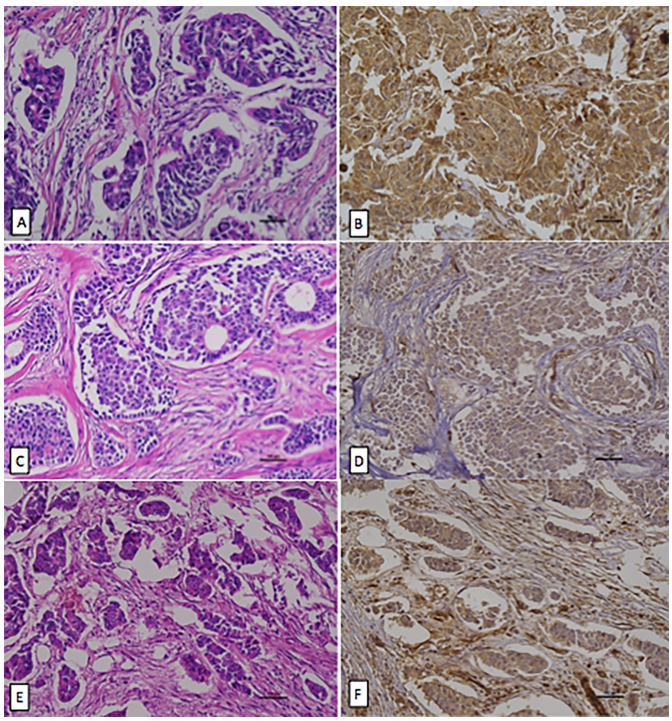

Figure 2: IBC-NST (A) H&E staining (B) IHC of the same case, showing strong C5aR2 staining in tumour cells and moderate C5aR2 staining in stromal cells (20X). IBC-NST (C) H&E staining (D) IHC of the same case, showing moderate C5aR2 staining in tumour cells (20X). IBC-NST (E) H&E staining (F) IHC of the same case, showing strong C5aR2 staining in stromal cells (20X).

Table II showed no significant association between C5aR2 expression in tumour cells and age group (p = 0.124), biopsy (p = 0.150), histology (p = 0.820), tumour grade (p = 0.732), tumour stage (p = 0.068), receptor (p = 0.455), molecular status (p = 0.226), laterality (p = 0.665), Ki67 (p = >0.99), LVI (p = >0.99), PNI (p = 0.536), LNI (p = 0.138), and DCIS (p = 0.063). However, a significant association was determined between C5aR2 expression in tumour cells and residual tumour (p = <0.001).

Through univariate logistic regression, it was found that patients under 40 years old had a lower probability of positive C5aR2 expression in tumour cells compared to those over 40 years old (OR = 0.357, p = 0.137). Compared to patients with grade-III tumours, patients with grade-I/II tumours were more likely to have positive C5aR2 expression in tumour cells (OR = 1.433, p = 0.579). Detailed odds for C5aR2 expression in tumour cells are shown in Table II.

The results of Table III showed no significant association between C5aR2 expression in stromal cells and age group (p = 0.098), histology (p = 0.792), tumour grade (p = 0.239), tumour stage (p = 0.338), receptor status (p = 0.176), molecular status (p = 0.871), residual tumour (p = 0.093), laterality (p = 0.552), Ki67 (p = 0.660), LVI (p = 0.595), PNI (p = 0.705), LNI (p = 0.434), and DCIS (p = 0.360).

Compared to patients over 40, those under 40 had a lower likelihood of positive C5aR2 expression in stromal cells (OR = 0.553, p = 0.099), according to univariate logistic regression analysis. In contrast to patients with grade-III tumours, patients with tumours grade I/II had a lower probability of positive C5aR2 expression in stromal cells (OR = 0.619, p = 0.241). Detailed odds for C5aR2 expression in stromal cells are presented in Table III.

DISCUSSION

Progressively increasing discoveries regarding the existence and significance of complement proteins and their receptors in the tumour microenvironment have established their role in tumour progression.[9] Recent studies have evolved the role of complement receptor C5aR2 in breast cancer development and progression. However, the scarce analysis of C5aR2 has prompted us to explore its association with various clinicopathological parameters of breast cancer.

In the present study, the expression of C5aR2 in primary breast cancer samples was evaluated by IHC and its association was identified with clinicopathological parameters of breast cancer. C5aR2 expression was observed in both tumour cells and stromal cells of breast cancer. This is in accordance with the immunohistochemically detected protein expression of C5aR2 in breast cancer tissue by Zhu *et al*. and C5aR2 expression seen in cancer-associated fibroblasts (CAFs) found in breast cancer by Su *et al*.[11,12] In the current study, the expression was more pronounced in tumour cells (90.6%) than in stromal cells (53.1%).

The association between C5aR2 expression and age was analysed through univariate logistic regression analysis. The results showed higher C5aR2 expression in tumour cells of advanced age group breast cancer patients compared to younger breast cancer patients. Although insignificant, stromal cells of older age group breast cancer patients also exhibited increased C5aR2 expression than younger breast cancer patients. Since this study represents the inaugural investigation to evaluate the association between C5aR2 expression and age, therefore comparable data are unavailable.

Tumour cells in low-grade breast tumours demonstrated higher C5aR2 expression than those in high-grade breast cancer patients. On the contrary stromal cells in high-grade breast cancer cases showed increased C5aR2 expression compared to low-grade lesions. Su *et al*. revealed an association between grade and C5aR2 expression in stromal cells, indicating poor survival in high-grade breast cancer patients exhibiting abundant C5aR2 expression.[12]

Most of the stage I/II and stage III breast cancer patients revealed positive C5aR2 expression in both tumour and stromal cells. Su *et al*. reported poor patient survival in both stage I/II and stage III breast cancer patients with high C5aR2 expression in stromal cells.[12]

In this study, increased C5aR2 expression was observed in tumour and stromal cells of patients with luminal B type,

HER2-positive, and TNBC. Conversely, Zhu *et al*. illustrated elevated C5aR2 expression in tumour cells of breast cancer patients with luminal A Type and luminal B Type compared to HER2 positive and TNBC cases.[11] Su *et al*. also revealed increased C5aR2 expression in stromal cells of TNBC, luminal A Type and luminal B Type breast cancer patients.[12]

Both tumour and stromal cells of breast cancer cases positive for HER2 and ER showed the most pronounced C5aR2 expression. Zhu *et al*. demonstrated a significant association between high C5aR2 expression in tumour cells and poor prognosis in ER-positive breast cancer patients.[11]

The patients with poor treatment response showed increased C5aR expression in both tumour and stromal cells, indicating its potential role in chemoresistance. This observation was supported by the minimal expression of C5aR2 in breast cancer patients showing favourable response to the treatment. Su *et al*. also illustrated comparable results, indicating that in breast cancer patients C5aR2 positive stromal cells are not only chemoresistant themselves but also induce chemoresistance in tumour cells.[12] The chemoresistant role of C5aR2 was supported by the study conducted by Tong *et al.* on locally advanced gastric cancer cases which revealed an association of increased on C5aR2 expression on CAFs with poor pathological response indicating its relation with drug resistance.[13]

The majority of breast cancer patients with positive C5aR2 tumour cells displayed high Ki67 values indicating increased proliferating capacity in C5aR2-expressing cells. Zhu *et al*. also validated the enhanced proliferating potential of C5aR2-overexpressed breast cancer cells using the CCK8 assay.[11]

The C5aR2 expression in breast cancer cells was observed in the majority of patients with lymphovascular invasion specifying its invasive ability. The increased invasive capacity of C5aR2-positive breast cancer cells was also revealed by Zhu *et al*. through the transwell assay.[11]

The current study has certain limitations. It was a single-centred study with a small sample size, so generalisation of results was not possible. There is limited research on C5aR2 in breast cancer, so good comparable data were not available.

CONCLUSION

The study demonstrates that elevated C5aR2 expression is associated with advanced age, high tumour grade, and proliferation rate which corroborates its role in tumorigenesis and suggests its potential as a biomarker for disease progression and prognosis. The differential expression of C5aR2 in tumour and stromal cells of breast cancer emphasises its complex role in the tumour microenvironment. The upregulation of C5aR2 in chemoresistant cases indicates its potential as a therapeutic target which may prove helpful in hampering tumour progression and reversing chemoresistance. An association between HER2 and ER-positive breast cancer cases and increased C5aR2 expression was also observed. This could have implications for the prognosis and treatment regimens for patients with HER2 and ER-positive breast cancer.

DISCLOSURE:
This study was part of the author's MPhil thesis.

ETHICAL APPROVAL:
The study was conducted after obtaining ethical approval from the Ethical Review Committee (ERC) of the Bahria University Health Sciences campus (Reference No: 111/2022).

PATIENTS' CONSENT:
In prospective cases, the patients' informed consent was taken before the commencement of the study.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
EK: Conception of the idea, data collection, analysis and interpretation of data, and critical revision.
SS: Analysis and interpretation of data, and critical revision.
NJ: Analysis and interpretation of data and critical revision.
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REFERENCES

1. Saeed S, Asim M, Sohail MM. Fears and barriers: Problems in breast cancer diagnosis and treatment in Pakistan. *BMC Women's Health* 2021; **21(1)**:151. doi: 10.1186/s12905-021-01293-6.
2. Mehraj U, Dar AH, Wani NA, Mir MA. Tumor microenvironment promotes breast cancer chemoresistance. *Cancer Chemother Pharmacol* 2021; **87(2)**:147-58. doi: 10.1007/s00280-020-04222-w.
3. Li JJ, Tsang JY, Tse GM. Tumor microenvironment in breast cancer-updates on therapeutic implications and pathologic assessment. *Cancers (Basel)* 2021; **13(16)**:4233. doi: 10.3390/cancers13164233.
4. Truffi M, Sorrentino L, Corsi F. Fibroblasts in the tumor microenvironment. *Adv Exp Med Biol* 2020; **1234**:15-29. doi: 10.1007/978-3-030-37184-5_2.
5. Revel M, Daugan MV, Fridman CS, Fridman WH, Roumenina LT. Complement system: Promoter or suppressor of cancer progression? *Antibodies (Basel)* 2020; **9(4)**:57. doi: 10.3390/antib9040057.
6. Niculescu F, Rus HG, Retegan M, Vlaicu R. Persistent complement activation on tumor cells in breast cancer. *Am J Pathol* 1992; **140(5)**:1039-43.
7. Pio R, Corrales L, Lambris JD. The role of complement in tumor growth. *Adv Exp Med Biol* 2014; **772**:229-62. doi: 10.1007/978-1-4614-5915-6_11.
8. Qian X, Yang Z, Gao L, Liu Y, Yan J. The role of complement in the clinical course of hepatocellular carcinoma. *Immun Inflamm Dis* 2022; **10(3)**:e569. doi: 10.1002/iid3.569.

9. Zhang T, Garstka MA, Li K. The controversial C5a Receptor C5aR2: Its role in health and disease. *J Immunol Res* 2017; **2017**:8193932. doi: 10.1155/2017/8193932.
10. Li XX, Lee JD, Kemper C, Woodruff TM. The complement receptor C5aR2: A powerful modulator of innate and adaptive immunity. *J Immunol* 2019; **202(12)**:3339-48. doi: 10.4049/jimmunol.1900371.
11. Zhu Y, Wang X, Xu Y, Chen L, Ding P, Chen J, Hu W. An integrated analysis of C5AR2 related to malignant properties and immune infiltration of breast cancer. *Front Oncol* 2021; **11**:736725. doi: 10.3389/fonc.2021.736725.
12. Su S, Chen J, Yao H, Liu J, Yu S, Lao L, *et al*. CD10+GPR77+ cancer-associated fibroblasts promote cancer formation and chemoresistance by sustaining cancer stemness: *Cell* 2018; **172(4)**:841-56. doi: 10.1016/j.cell.2018.01.009.
13. Tong Y, Zhao Z, Zhang J, Wang W, Zhu Y. High expressions of CD10, FAP and GPR77 in CAFs are associated with chemoresistance and worse prognosis in gastric cancer. *Front Oncol* 2022; **12**:984817. doi: 10.3389/fonc.2022.984817.
14. Zhao Z, Zhu Y. FAP, CD10, and GPR77-labeled CAFs cause neoadjuvant chemotherapy resistance by inducing EMT and CSC in gastric cancer. *BMC Cancer* 2023; **23**:507. doi: 10.1186/s12885-023-11011-0.
15. Nabizadeh JA, Manthey HD, Panagides N, Steyn FJ, Lee JD, Li XX, *et al*. C5a receptors C5AR1 and C5AR2 mediate opposing pathologies in a mouse model of melanoma. *FASEB J* 2019; **33(10)**:11060-71. doi: 10.1096/fj.201800980RR.
16. Ding P, Li L, Li L, Lv X, Zhou D, Wang Q, *et al*. C5aR1 is a master regulator in colorectal tumorigenesis *via* immune modulation. *Theranostics* 2020; **10(19)**:8619-32. doi: 10.7150/thno.45058.
17. Yuxuan W. Important roles of C5a and C5aR in tumor development and cancer treatment. *E3S Web of Conferences*, *ICBTE* 2019; **136**:06012. doi: 10.1051/e3sconf/201913606012.
18. Magaki S, Hojat SA, Wei B, So A, Yong WH. An Introduction to the performance of immunohistochemistry. *Methods Mol Biol* 2019; **1897**:289-98. doi: 10.1007/978-1-4939-8935-5_25.
19. Nurge B, Schulz AL, Kaemmerer D, Sanger J, Evert K, Schulz S, *et al*. Immunohistochemical identification of complement peptide C5a receptor 1 (C5aR1) in non-neoplastic and neoplastic human tissues. *PLoS One* 2021; **16(2)**:e0246939. doi: 10.1371/journal.pone.0246939.
20. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - A review. *Diagn Pathol* 2014; **9**:221. doi: 10.1186/s13000-014-0221-9.