Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5, a Potential Predictive Immunomarker in Invasive Ductal Breast Carcinoma

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ABSTRACT

Introduction: Breast cancer is on rise in Asian population. Identification of more prognostic and predictive factor in breast invasive carcinoma is need of the time in order to provide best possible management to improve the outcome.

Aim: the aim of the study is to observe Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) association with various clinicopathological parameters and molecular groups based on immunohistochemistry in invasive ductal breast carcinoma.

Methodology: Eighty-three patients undergoing modified radical mastectomy with primary microscopically proven invasive ductal carcinoma were recruited from two tertiary care hospitals, Pakistan. Grossing & reporting including Biomarker was performed as per College of American Pathologists (CAP) protocol. Molecular groups were formed. Data was entered and analyzed using IBM SPSS version 27.

Results: CEACAM5 was overexpressed in 78% of patients and negative in 22% of patient ($p < 0.001$) of invasive ductal carcinoma breast (Figure 1). The mean age $\pm$ SD of CEACAM5 positive patient was 49.94 ± 11.4 years. Statistically significant association was observed with age, tumour stage, glandular formation, mitosis, Nottingham histological grade and non-triple negative molecular group.

Conclusion: The increasing incidence of breast carcinoma in Pakistani population warrants dire need of new and cheap modalities for therapeutic assessments in order to provide the suitable treatment plan to patients. Significant association has been observed with various clinicopathological parameters and molecular group. CEACAM5 can be considered, as a predictive factor and follow-up marker, however, large-scale validation follow-up studies are required.

Key Words: Estrogen Nuclear Receptor, Mammary Ductal Carcinoma, Invasive Ductal Carcinoma, Breast, Receptor, Progesterone, Triple Negative Breast Cancer

INTRODUCTION

There is increase burden in late stages of breast cancer in Asian population.¹ Many risk factors has been observed in different population including micro and macro level health determinants.² Inter-observer variation and degree of heterogeneity exist from tumor to tumor and even intra-tumoural indicating that the histological classification is not sufficient for individual therapy selection.^{3,4} In Pakistan, being low-income country where most of the patients has to bear the cost of treatment out of their own pockets, opting for highly

expensive investigations like gene expression profiling tests might not be suitable, keeping in mind the economic conditions of the sufferer. Identification of novel immunomarker in daily practice can significantly benefit these patients.

The human Carcinoembryonic antigen (CEA) is one potential immunomarkers, which can be used in clinical practice as immunohistochemical assay as well as serological tumor marker. There are seven genes in CEA family. These subgroup members are linked with the cell membrane and display a complex expression pattern in normal and cancerous

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tissues. This protein, with a size of 100-200kDa, is a member of immunoglobulin superfamily with an N-terminal domain including 29 potential glycosylation sites and is attached to the membrane by a glycosyl phosphatidylinositol anchor.⁵ CEACAM5 takes part in cell adhesion, invasion and metastasis. CEACAM5 has been demonstrated to have both homophilic (CEA to CEA) and heterophilic (CEA binding to non-CEA molecules) interactions, suggesting that it is an intercellular adhesion molecule involved in cancer invasion and metastasis. CEACAM5 expression overcome apoptosis-inducing therapies.⁵ CEACAM5 (also known as cluster of differentiation (CD66e) was first described as a gastrointestinal oncofetal antigen in 1965. It is now observed to be overexpressed in a majority of carcinomas, including those of the gastrointestinal tract, the respiratory and genitourinary systems, and breast cancer. CEA antigen has been detected overexpressed in serum of oncological patients compared to healthy individuals. In symptomatic breast carcinoma patients, CEA sensitivity increases, and some authors evidenced that CEA levels at diagnosis are able to correlate with the stage of disease. As a prognostic tool, the pre-therapeutic CEA levels may be useful to identify patients with worse prognosis and increase risk of recurrence after primary carcinoma. As per guidelines from the European School of Oncology (ESO) that “if tumor markers such as CA15-3 and CEA are elevated at time of treatment initiation, they can be helpful for therapy monitoring and long-term surveillance but they cannot be used solely for decision making with respect to change of therapy”.⁵⁻⁷ CEA may be used for monitoring patients with metastatic disease undergoing active therapy in combination with imaging, history, and physical exam. Increasing CEA may be used to indicate treatment failure. It should be used with caution during first 4-6 weeks of a new therapy given possibility of a false early rise.⁸ CEA has been evaluated as serological marker and IHC but no conclusive results have been made till to date.

MATERIAL & METHOD

This is a prospective study comprised of 83 mastectomy/modified mastectomy specimens, microscopically confirmed primary invasive ductal carcinoma patients from two tertiary care hospitals of Lahore (Mayo Hospital & Shalamar Hospital). The cases who received neoadjuvant therapy were excluded. The study was approved as part of Ph.D research by advanced studies & Research Board, University of Health Science, Pakistan. After routine tissue processing and Hematoxylin & Eosin staining, Nottingham histological grading was done. Based of Nottingham Prognostic Index (NPI) was calculated to interpret the expected outcome.⁹ Two representative paraffin embedded sections of tumours cut at 4 µm were selected for CEACAM5 immunohistochemical staining. Primary antibody of Anti-Carcinoembryogenic rabbit

polyclonal antibody derived recombinant full length human CEACAM5 by AbCam with Code ab131070, using dilution of 1: 500 was used as per instruction manuals. Score was considered positive when cytoplasmic or membrane staining was present in > 10% of invasive tumor cells and negative when it is <10%. Slides were examined and evaluated by three-consultant histopathologist independently. In case of difference of opinion, two similar results out of the three were considered final. The data was entered and analysed using IBM SPSS version 27. Frequencies and percentages were reported for qualitative variables. Pearson Chi-square and Fisher Exact tests were applied to observe associations between qualitative variables. A p-value of less than 0.05 was considered as statistically significant. The ethical clearance number is UHS/Education/126-16/215.

RESULTS

CEACAM5 was overexpressed in 78% of patients and negative in 22% of patient ($p < 0.001$) of invasive ductal carcinoma breast (Figure 1). The mean age $\pm$ SD of CEACAM5 positive patient was 49.94 ± 11.4 years. The expression of CEACAM5 was higher in patient below than 50 years of age (48.2%) as compared to above 50 years, it was statistically significant. There was no significant association of expression of CEACAM5 in terms of laterality; however, frequency was bit increase on left side of tumours (47%). Overexpression of CEACAM5 was observed in node negative patients in 22.9% and nodal metastasis (N1-3) was present in 31.3% of patients. CEACAM5 was observed in with increase proportion in T2 (45.8%) as compared to T4 (12%) only, the association with lower stage was statistically significant ($p = 0.002$).

Cases which overexpressed CEACAM5 showed poor differentiation (Grade III) in 37.3% which is higher and statistically significant as compared with stage I. Highest frequencies of expression was recorded in less than 10% glandular formation (42.2%), moderate nuclear grade in 47% and 10-19 mitosis/HPF in 47% of patients. Increase expression was observed in all molecular groups as 32.5% in luminal A, 10.8% in luminal b, 15.7% in Her2-Enriched and 19.3% in triple negative group. After aggregating non-triple negative group together, its expression was found to be statistically significant in non-triple negative group with p-value of 0.038 (Figure 2). CEACAM5 was overexpressed in 45.8% of ER positive, 43.4% of PR positive and 26.5% of Her2 neu patients. However, no statistical significant association was observed with ER, PR, Her2neu, Ki67, p53 and NPI. Summarizing results the significant association was observed with age, tumour stage, glandular formation, mitosis and Nottingham histological grade only.

DISCUSSION

CEACAM5 functions in cell–cell adhesion.¹⁰ Level of expression of CEACAM5, have important bearings in the determination of response to chemotherapy.^{9, 11} Various controversial studies had been reported in literature regarding CEACAM5, like serum biomarker significance, correlation with worse outcome, lymph node metastasis associated with CEACAM5 expression, lower survival rate, inverse relationship between CEACAM5 and outcome and so on. There are discrepancies in relation to the clinical value of this protein but still consensus is lacking for its exact clinical & prognostic significance.⁵
¹² Elevated serum CEA levels has been observed in metastatic disease with significant worse survival.¹³

A study recently conducted on heterogeneity of CEACAM5 has reported its positivity in 70% of non-triple negative group (i.e., 41/60 Luminal group, 12/16 cases of Her2 enriched group) and 24% positivity in triple negative group (i.e., 8/34),¹² whereas in the present study, 88% positivity in non-triple negative group (i.e., 36/83, 43.3% cases in luminal group and 13/83, 15.7% cases in Her2 enriched group) and among triple negative group 16, 19.3% out of 83 cases showed positivity for CEACAM5. In our study group, 78 % of patients showed positivity for CEACAM5, which is very high as compared to study conducted in Denmark,¹² which could be interpreted as reason of treatment failure and poor prognosis in Pakistani population.

A statistically negative relationship between CEACAM5 and response to neoadjuvant chemotherapy has been found ($p= 0.004$),¹⁴ Blumenthal et al., in 2007 suggested that CEACAM5 can be a modulator of cancer cell chemosensitivity and can overcome apoptosis inducing therapies.⁵ High CEACAM5 expression correlates with reduced patient survival and in vivo experiments has shown it as a tumor and serum biomarker for metastatic progression and therapeutic agents that directly target CEACAM5 can be efficient in inhibiting metastatic tumour overgrowth.¹⁵

High level of CEACAM5-expression was significantly unfavorable for patients with ER-positive tumors, whereas it was more favorable for patients with Basal-like tumors.¹² It has been observed to be associated with malignant clinical behaviour and poor prognosis.¹⁶

In Pakistan, the relevant data of CEACAM5 is not available as per our extensive literature search. The present study is unique in its own kind in this region. Sundblad, et al. in their study quoted that it was not found to be associated with any of the clinicopathological parameter in stage I & II patients only, however, contrary to it we observed statistically significant association in Stage T2.¹⁷

Another study documented significant association of CEA in stage 4 as compared to other stages.¹⁸ Our results are in

correspondence with Zahraa, et al. results of statistical significant association in grade III patients.¹⁸

The use of CEA can provide valueable information in treatment assessments in breast cancer patients. Combination of CEA with other adhesion molecule has been proposed to have high discriminative power in evaluation prediction.¹⁹

Advancement in personalized medicine introduced Gene expression profiling as a better prognostic indicator than histological grading for evaluating disease-free and overall survival and has come up with identification of molecular subtypes with better correlation to clinical outcomes and sensitivity to adjuvant therapy, as well as elaborating understanding of the molecular basis of cancer development and progression.

Predictive gene expression signatures up till now includes Mammaprint (70 genes), Genomic Grade Index (97 genes), Oncotype diagnostic recurrence score (21 genes), Prosina PAM50 Risk of recurrence Score (50 genes), Breast cancer index (multi-gene assay), EndoPredict (12 genes), 7 subtype analysis, Sensitivity to endocrine therapy tests, Mammostrat®.^{8, 20-25} Unfortunately these signatures are highly sophisticated and expensive and not available as a routine modalities in developing countries. There is a great need of identification of cheap, effective and predictive immunomarker, which can be used as routine assessment tool.

CONCLUSION

The increasing incidence of breast carcinoma in Pakistani population warrants dire need of new and cheap modalities for therapeutic assessments in order to provide the suitable treatment plan to patients. Significant association has been observed with various clinicopathological parameters and molecular group. CEACAM5 can be considered, as a predictive factor and follow up marker, however, large-scale validation follow up studies are required.

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Authors' contribution: NH designed the project, data processing, collection, analysis, manuscript drafting; SI, FI,

FS performed analysis and interpretation of results; SA performed the laboratory work; AHN supervised the whole project and critical revision

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Table 1: Table shows association of CEACAM5 with various clinicopathological parameters.* significant p-values

		Negative, n (%)	Positive, n (%)	Total n (%)	Chi-Square (p value)
Age	<50 years	18 (21.7)	40 (48.2)	58 (69.9)	9.907 (0.002)*
	>50 years	0	25 (30.1)	25 (30.1)	
Laterality	Left	12 (14.5)	39 (47)	51 (61.4)	0.264 (0.607)
	Right	6 (7.2)	26 (31.3)	32 (38.6)	
N stage	No	5 (6)	19 (22.9)	24 (28.9)	2.130 (0.546)
	N1	7 (8.4)	22 (26.5)	29 (34.9)	

Table 1: (Continued)

		Negative, n (%)	Positive, n (%)	Total n (%)	Chi-Square (p value)
Tumor stage	N2	3 (3.6)	19 (22.9)	22 (26.5)	12.919 (0.002)*
	N3	3 (3.6)	5 (6)	8 (9.6)	
	T2	2 (2.4)	38 (45.8)	40 (48.2)	
	T3	9 (10.8)	17 (20.5)	26 (31.3)	
	T4	7 (8.4)	10 (12)	17 (20.5)	
Glandular component	>75%	0	(1.2)	1 (1.2)	9.936 (0.007)*
	10-75%	1 (1.2)	29 (34.9)	30 (36.1)	
	<10 %	17 (20.5)	35 (42.2)	52 (62.7)	
Nuclear grade	Mild	0	1 (1.2)	1 (1.2)	1.856(0.395)
	Moderate	8 (9.6)	39 (47)	47 (56.6)	
	Severe	10 (12)	25 (30.1)	35 (42.2)	
Mitosis	0-9	0	3 (3.6)	3 (3.6)	14.053 (0.001)*
	10-19	3 (3.6)	40 (48.2)	43 (51.8)	
	>19	15 (18.1)	22 (26.5)	37 (44.6)	
Nottingham grade	I	0	4 (4.8)	4 (4.8)	9.822 (0.007)*
	II	2 (2.4)	30 (36.1)	32 (38.6)	
	III	16 (19.3)	31 (37.3)	47 (56.6)	
ER	Negative	11 (13.3)	27 (32.5)	38 (45.8)	2.176 (0.140)
	Positive	7 (8.4)	38 (45.8)	45 (54.2)	
PR	Negative	11 (13.3)	29 (34.9)	40 (48.2)	1.536 (0.215)
	Positive	7 (8.4)	36 (43.4)	43 (51.8)	
Her2	Negative	13 (15.7)	43 (51.8)	58 (67.5)	0.237 (0.627)
	Positive	5 (6)	22 (26.5)	27 (32.5)	
Molecular groups	Luminal A	4 (4.8)	27 (32.5)	31 (37.3)	5.262 (0.154)
	Luminal B	1 (1.2)	9 (10.8)	10 (12)	
	Her2-enriched	4 (4.8)	13 (15.7)	17 (20.5)	
	Triple negative	9 (10.8)	16 (19.3)	25 (30.1)	
Ki67	<10%	4 (4.8)	18 (21.7)	22 (26.5)	0.217 (0.642)
	>10%	14 (16.9)	47 (56.6)	61 (73.5)	
P53	Low	14 (16.9)	42 (50.6)	58 (67.5)	1.113 (0.291)
	high	4 (4.8)	23 (27.7)	27 (32.5)	
NPI	Good	0	5 (6)	5 (6)	4.531 (0.104)
	Moderate	3 (3.6)	23 (27.7)	26 (31.3)	
	Poor	15 (18.1)	37 (44.6)	52 (62.7)

Figure 1: Pie Chart shows the expression of CEACAM5 in invasive ductal carcinoma breast with significant p-value ($p < 0.001$).

Figure 2: Bar chart shows the expression of CEACAM5 in triple negative and non-triple negative groups (Luminal & Her 2-enriched) with significant p-value of 0.038.

Figure 3: A: Microphotograph shows CEACAM5.2 in less than 10% of cells, considered negative (100x). B: Microphotograph shows CEACAM 5.2 positive (40x). C: Microphotograph shows CEACAM 5.2 cytoplasmic positive (200x). D: Microphotograph shows CEACAM 5.2 membranous and cytoplasmic positive (400x).