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Review Article

Oncology Section

Activated Leucocyte Cell Adhesion Molecule (CD166): A Biomarker in Diagnosis and Prognosis of Breast Cancer

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ABSTRACT

The global incidence of breast cancer and associated mortality is rising annually despite advanced diagnostic aids and treatment methods. This is due to the failure and difficulty in detecting the disease at an early stage. Due to an increase in the breast cancer mortality rate, biomarkers associated with early and prompt detection are being extensively studied, owing to their sensitivity, specificity, cost-effectiveness, and minimally invasive or non invasive analysis. Among those carbohydrate antigens, carcinoembryonic antigens, and circulating tumour cells are the most commonly observed serum biomarkers in the detection of cancer. In recent years, extensive research is carried out on cell adhesion molecules and their role in the process of cell adhesion which is essential for the development and homeostasis in multicellular organisms. These cell adhesion molecules accelerate appropriate cell response and intercellular communication. Activated Leucocyte Cell Adhesion Molecule (ALCAM) or Cluster of Differentiation 166 (CD166) is a transmembrane glycoprotein and emerging as a promising biomarker for primary detection of tumour cells with metastatic potential and is also upregulated in breast tumours. This review ascertains the usefulness of the ALCAM as a potential diagnostic and prognostic marker for early detection, classification, and prognosis of breast cancer and also elucidates the role of the various other biomarkers.

Keywords: Cluster differentiation 166, Early detection, Transmembrane glycoprotein

INTRODUCTION

Breast cancer accounts for nearly 25% of female malignancies and their prevalence is higher in developed countries in recent years [1]. According to the World Health Organisation (WHO), 2.3 million women were diagnosed with breast cancer in 2020, with 685000 deaths worldwide; by the end of 2020, there were 7.8 million women alive who had been diagnosed with breast cancer in the previous five years, making breast cancer the world's most prevalent cancer [2]. In India, an estimated 1,62,468 women were newly diagnosed with breast cancer in 2018. In India, 87,090 women died of breast cancer in 2018, the second-highest number in the world for that year [3]. Breast cancer in young women is associated with relatively aggressive pathophysiology and also has a poor prognosis when compared to that of older women. A mammogram is a vital tool for breast cancer screening, but it has concerns about radiation exposure and high false positive and false negative results [4]. In addition, mammograms cannot detect small tumours and give inaccurate results in women with dense breasts [5].

Biopsy, either as a needle or surgical invasive procedure is done for confirmation of breast cancer, which is not required in benign tumours [6]. The current trend of usage of biomarkers for breast cancer detection has brought a safe solution to this problem [7]. Much research is being conducted for the development of non invasive biomarkers to aid in early breast cancer detection. Circulating biomarkers like Carcinoma Antigens (CA), Circulating Tumour Cells (CTC), micro-Ribonucleic Acid (miRNA), cell-free tumour nucleic acids {Deoxyribonucleic Acid (DNA)/Ribonucleic Acid (RNA)}, in the peripheral blood, sweat, urine, tears, saliva, nipple aspirate fluid, as well as Volatile Organic Compounds (VOC) in breath, are all potential candidates of non invasive early breast cancer detection methods [8]. Carbohydrate Antigen (CA15-3) and Carcinoembryonic Antigen (CEA) are the commonly used serum biomarkers in breast cancer detection and prognosis assessment [9]. Extensive research is being carried out in this area and significant progress has been made.

Many studies report that these biomarkers lack sensitivity and specificity in detecting the early stages of carcinoma [8]. Activated Leucocyte Cell Adhesion Molecules (ALCAM) or Cluster of Differentiation (CD166), a member of the immunoglobulin superfamily, mediates cell to cell clustering and also influences cellular growth, cell differentiation, junction formation, and polarity. This has different names depending on the species and laboratory that described it: chicken neural adhesion molecule BEN/SC-1/DM-GRASP, rat KG-CAM, fish neurolin, human melanoma metastasis cloned, Mouse/Human CD166 and rat HB2 [10]. This is found to be expressed in many types of cancer and is proved to possess high specificity in breast cancer [10-11]. Studies show a positive correlation between high levels of ALCAM and an early prognosis in breast cancer patients [12]. Early detection and prognosis assessment play a pivotal role in reducing mortality associated with breast cancer. The development of a simple and convenient non invasive test is the need of the hour to overcome the shortcomings of the existing invasive and painful breast cancer detection methods.

The present review summarises the various non invasive biomarkers in breast cancer detection and also ascertains the usefulness of ALCAM as a diagnostic and prognostic biomarker by comparing multiple authenticated published articles which might aid in the development of a promising biomarker in the near future. The current progress of research in this area has also been discussed.

BREAST CANCER

Epidemiology and Global Burden

Breast cancer ranks first among Indian females when compared with other types of cancer and has an incidence of 41 per one lakh women in Delhi. The other Indian cities with a high incidence of breast cancer are Chennai (37.9), Bengaluru (34.4), and Thiruvananthapuram (33.7) [12]. A 2020 projection of breast cancer in India says that the numbers are as high as 1797900 [13]. The 2022 report of the National Breast cancer Foundation, United States says that one in eight women may develop breast cancer

in their lifetime. If diagnosed and treated early, breast cancer is a potentially curable disease [14].

Surveillance, Epidemiology, End Results programme (SEER programme) has reported that if breast cancer is diagnosed at stage I/II, the five year survival rate will increase by 85% to 98%. The worldwide economic impact of premature death and disability from breast cancer was $447 billion in 2008. This figure represents 0.75% of the world's Gross Domestic Product (GDP) [15]. Therefore, if early intervention and treatment are both efficient and cost-effective, the economic burden, as well as mortality rates, can be offset.

Classification of Breast Cancer and Biological Characteristics

Breast cancer has diverse etiological and pathological features; it can show slow growth but excellent prognosis in some cases, and a highly aggressive nature in other cases; based on this difference breast cancer can be classified based on tumour grade, morphology, and molecular classification [16]. According to the tumour grade, breast cancer can be grade 1 or well-differentiated (slow-growing cells, looks more like normal breast tissue), grade 2 or moderately differentiated (cells grow at a speed and look like cells somewhere between grades 1 and 3), and grade 3 or poorly differentiated (cancer cells look very different from normal cells, proliferate and spread faster) [17]. The two main morphologic umbrellas are based on the invasiveness and place of origin of breast cancer and are classified as invasive or in situ carcinoma; ductal and lobular carcinoma. The schematic classification based on morphology can be found in [Table/Fig-1]. This grading index is called Nottingham Prognostic Index (NPI) and is a vital tool in assessing the prognosis [18]. Based on its molecular genetics, breast cancer can be classified as Estrogen Receptor positive (ER+) and Estrogen Receptor negative (ER-). These are the two major molecular subtypes and many other subtypes do exist, discussing which will be out of the scope of this review. The other oncogenes associated with breast cancer are Human Epithelial Receptor-2 (HER2), c-MYC, Retrovirus Associated DNA Sequence (RAS) genes, cyclins D1 and E, and tumour suppressor genes like Retinoblastoma (RB), TP53, PTEN, Breast Cancer gene 1 (BRCA1) and Breast Cancer gene 2 (BRCA2) [19].

Risk Category and Risk Factors of Breast Cancer

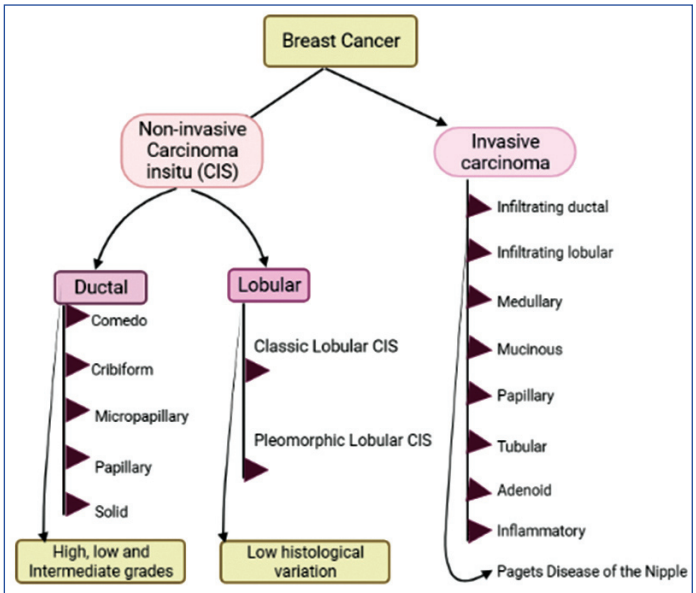
There are two categories of breast cancer risk factors: major risk factors and minor risk factors. Major risk factors for breast cancer include being a woman, getting older, having a family history of the disease, and having the BRCA1 or BRCA2 gene mutation; minor risk factors include early menarche (before age 12), late menopause (after age 55), nulliparity, having a first child after 30, taking hormone replacement therapy, and eating a high-fat diet [19].

Early Detection and Screening

Mammography is the quintessential imaging technique of the present day for breast cancer evaluation that use X-ray say to produce images. However, owing to the high false-positive results, overdiagnosis and radiation exposure mammography may not be the gold standard [20]. Clinical breast examination and self-breast examination have shown some benefits in early breast cancer screening [4]. Ultrasound, Magnetic Resonance Imaging (MRI), biopsy, and additional lab tests may be required for further analysis as described in [Table/Fig-2] [20-22]. In addition, biomarkers supplement clinical diagnosis in the past few decades. A study by Kazarian A et al., and his team members on blood biomarkers for early detection and screening of breast cancer found that the biomarkers lacked sensitivity in predicting the disease and even the combined candidates showed consistent changes in levels [23]. This is in agreement with the previous cohort studies, where a combination of up to 10 biomarkers {including Cancer Antigen 15-3 (CA15-3) and Osteopontin (OPN)} could not discriminate the breast cancer cases from control [10]. Therefore, a novel biomarker that can effectively identify early-stage breast cancer still needs to be found.

BIOMARKERS OF BREAST CANCER

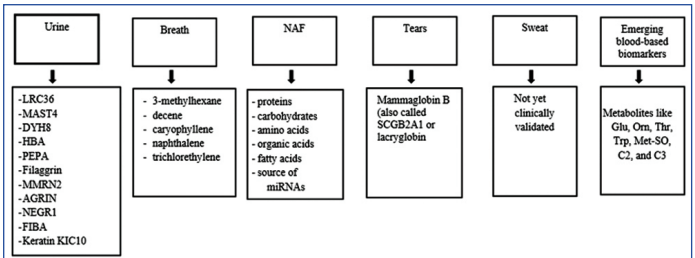
Despite the high incidence rate of breast cancer, the mortality rates have steadily decreased due to the better treatment options and timely early diagnosis as a result of the development of non invasive diagnostic methods that tested the body fluids for biomarkers [24]. The detection of breast cancer through biomarkers is simple, economic, more feasible when compared with other detection methods. Blood, tears, sweat, Nipple Aspirate Fluid (NAF) and Volatile Organic Compounds (VOC) in the exhaled breath, saliva, and urine are potential body fluids that can supplement early breast cancer detection [25]. A detailed tabulation of the non invasive biomarkers, and blood-based biomarkers and how they are sampled is shown in [Table/Fig-3] and [Table/Fig-4] respectively.



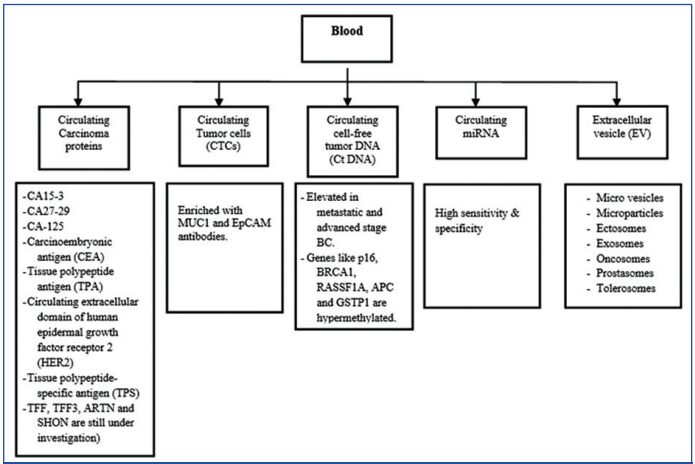
[Table/Fig-1]: Morphological classification of breast cancer [16].

Mammography	Ultrasound	MRI	Biopsy	ELISA	Fluorescent in-situ hybridisation (FISH Test)	Radioimmuno assay	Immunohis-tochemistry
• 70% Accuracy • Low sensitivity/ selectivity • Sensitivity proportional to breast tissue density • Frequent false Positive • Low energy X-ray cause mutation • Painful • Not suitable for women under the age of 30 years.	• Low sensitivity • Requires professional • Sound energy used • Size and position of tumour can be found • Suitable for pregnant women • Microcalcification are not seen.	• Highly Sensitive • Expensive • Requires professional to read the MRI • Suitable for pregnant women. • Indicated in high-risk category patients and occult breast carcinoma, preoperative and postoperative staging of breast carcinoma, implant assessment, response to neoadjuvant chemotherapy.	• Chance to miss tumour (sampling of non cancerous region) • May cause tumour metastasis • Require professional • Expensive • Time taking procedure and result	• Insensitive to low biomarker concentration • Risk of false positive • Requires professional • Accurate diagnosis with less sample	• Used for understanding of various chromosomal abnormalities and gene mutations • Not generally recommend • If in the IHC test does not clearly show whether the cells are HER2-Positive or Negative • FISH is more accurate method of HER2 testing	• Radiation hazardous • High cost of waste disposal • Require professional and special environment conditions • Extremal sensitive assay as it can measure antigen upto picogram quantities • Time consuming and complex	• Complex and Time consuming • Used to see the hormonal receptors on breast cancer cells • Require professional

[Table/Fig-2]: Screening/Diagnostic modalities/methods available for breast cancer detection [20-22].
ELISA: Enzyme-linked immune sorbent assay; MRI: Magnetic resonance imaging; RIA: Radioimmune assay; IHC: Immunohistochemistry



[Table/Fig-3]: Non invasive biomarkers/non blood-based biomarkers.



[Table/Fig-4]: Blood-based biomarkers.

Circulating serum carcinoma proteins like CA15-3, CA-125, CEA and HER2 are associated with the proliferation, invasiveness, and oncogenic signalling of tumour cells and are effective markers of advanced cancer detection [26]. They have low diagnostic sensitivity and specificity and are not used alone for cancer screening. Circulating cell-free tumour DNA (ctDNA) was found in levels ≥ 0.75% in advanced metastatic breast cancer and has a sensitivity of over 90% and specificity over 99% and is a promising biomarker [27]. The miRNAs (small regulatory RNA molecules) are upregulated in the serum of breast cancer patients and could differentiate diseased patients from healthy controls. Many studies have shown the effectiveness of miRNAs in distinguishing breast cancer from healthy control and also to differentiate malignant lesions from benign lesions [28]. But a panel of circulating miRNAs that is ready to be used in a clinical setting is still unavailable. Extracellular Vesicles (EV) comprise microparticles, microvesicles, prostasomes, endosomes, and tolerosomes and are secreted from normal as well as cancer cells. Although, EVs are not specific for cancer diagnosis, their elevated levels in breast cancer and their ability to mirror the origin and tumour state brought them attention as breast cancer biomarkers [29]. Phospholipids like Phosphatidylethanolamine (PE), Phosphatidylcholine (PC), and sphingomyelin are present in abundant quantities in the cell membrane and their metabolism is increased in cancer tissues. When urine samples of breast cancer patients were analysed by nanoflow liquid chromatography/electrospray ionisation tandem mass spectrometry, the concentrations of PCs and PEs increased by 44% and 71% respectively when compared with that of healthy controls [30]. Additionally, a panel of 11 upregulated proteins was also identified in the urine samples of breast cancer patients as mentioned in [Table/Fig-3], which were stage-specific [31]. Nipple Aspirate Fluid (NAF) is another rich source of breast cancer biomarkers. Apart from a high concentration of proteins, carbohydrates, and metabolites, it also contains exfoliated breast epithelial cells from which breast cancer originates. The Thomsen-Freiden reich (TF) antigen and its precursor Tn antigen are expressed in higher amounts in NAF of breast cancer patients and the TF concentrations could differentiate precancerous and cancerous lesions from benign disease [32]. Saliva is an easily accessible body fluid and has elevated levels of salivary fluid protein biomarkers in breast cancer as measured by ELISA. Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF) and Carcinoembryonic Antigen (CEA) levels were significantly increased in breast cancer and saliva can serve as a useful tool to supplement the current methods of breast cancer detection [28].

Activated leucocyte cell adhesion molecule is one such non invasive biomarker that detects the metastasis and prognosis of breast cancer with high sensitivity [24]. In recent years, extensive research is being done on ALCAM about its usefulness as a potential non invasive biomarker of breast cancer. ALCAM is associated with tumour growth and metastasis and also protects the breast cancer cells from cell death and autophagy [33]. It is gaining more attention because of its extremely high levels in breast cancer. Apart from being simple, quick, and selective the detection of ALCAM is also cost-effective and this has been a breakthrough in early breast cancer screening and prognosis assessment [34].

Activated Leucocyte Cell Adhesion Molecule (ALCAM)

Adhesion molecules are classified into immunoglobulins, cadherins, selectins, integrins, and mucins and they can involve in tumour cell-tumour cell adhesion, tumour cell-matrix adhesion, and tumour cell-endothelial adhesion. These adhesions play a role in primary tumour formation and tumour metastasis and can be upregulated or downregulated during this process. Adhesion molecules influence cellular growth, differentiation, and junction formation and can contribute to unrestrained cell growth [19,35].

ALCAM is a glycoprotein of the immunoglobulin superfamily of adhesion molecules and is mapped to chromosome 3q13 [8]. Research has revealed that cancer biomarkers are not specific and sensitive in detecting early disease stages. ALCAM which is a transmembrane glycoprotein receptor has five extracellular immunoglobulin-like domains that can mediate cell-cell clustering employing homophilic (ALCAM-ALCAM) and heterophilic (ALCAM-CD166) interaction [11]. ALCAM is involved in the maintenance of tissue architecture, immune response, and tumour progression.

The sALCAM is soluble form of ALCAM that is formed by ADAM17/TACE metalloprotease activity and its levels were found to increase in certain types of cancer. There are also shreds of evidence of expression of ALCAM in colon cancer and in metastatic melanoma where they are responsible for local cell spread to tissue invasion [10,12]. There were contradictory reports about the expression of ALCAM in breast cancer. But recent studies have proved that ALCAM is expressed in extremely high levels in breast cancer and is associated with disease prognosis. The high membranous expression causes weakened adherence of the malignant cells and promotes tumour development [24]. Zhou et al., in their study also demonstrated that ALCAM is associated with tumour progression and metastasis. A low ALCAM expression can cause apoptosis and autophagy of the cancer cells and its presence protects the breast cancer cells against these two programmed cell death processes [36].

Studies have assessed that ALCAM shows a pattern in its expression according to the grade, stage, and invasive nature of the tumour, and the prognostic value varies depending on the type of cancer. ALCAM may be upregulated in the early stages of prostate cancer but down-regulated with tumour progression. On the contrary, in melanoma, oesophageal carcinoma, and breast carcinoma, the levels of ALCAM expression are directly related to invasiveness and metastasis [10,37-38]. Many controversies still exist in the study results of breast cancer. The expression of ALCAM has been detected in the breast cancer cell lines of MCF10A, MCF10AT, MCF10CA CI-A, MCF10CA CI-D, and MDA-MB-23146 according to the unpublished results of study by Ofori-Acquah SF and King JA [10]. The study finding also showed that the cell lines MCF-7 and MDA-MB-435 had very weak ALCAM expressions [10]. The study also demonstrated the first analysis of ALCAM mRNA expression in breast cancer. Low levels of ALCAM m RNA correlated with nodal

metastasis, high-grade tumour, and poor clinical outcomes in a study of 120 primary breast carcinomas where ALCAM was measured by PCR and analysed concerning clinical data from a six year follow-up period [10]. [Table/Fig-5] shows the levels of ALCAM expressions among different types of carcinomas.

Type of condition	ALCAM status
Breast cancer [10,36,38-40]	Increased ALCAM expression in breast cancer condition.
Cervical cancer [10]	Serum ALCAM maybe associated with moderate, compared to poorly differentiated cervical cancer.
Melanoma [10]	Increased expression.
Prostate cancer [39]	Enhanced levels of tissue ALCAM are associated with metastasis and serum ALCAM proved to have a comparable diagnostic power to prostate specific antigen.
Bladder cancer [40]	Novel prognostic biomarker
Gastric cancer, hepatocellular carcinoma, oesophageal carcinoma [37]	Elevated levels are indicative of cancer poorer prognosis.
Non small cell lung carcinoma [41]	Elevated levels of ALCAM
Laryngeal squamous cell carcinoma [42]	Increased ALCAM expression seen in condition.
Endometrial cancer [43]	Upregulation of ALCAM expression and potential of ALCAM as a recurrence biomarker.
Lupus nephritis [44]	Upregulated ALCAM levels seen in urine samples.
Thyroid tumours [45]	Overexpressed tissue ALCAM.
Colorectal cancer [46]	ALCAM Expression correlated significantly with shortened patient survival [10].

[Table/Fig-5]: ALCAM expression in various types of conditions [10,36,38-46].

Splice Variants of ALCAM

ALCAM has nine identified splice variants, out of which only two isoforms have been confirmed on both mRNA and protein levels. ALCAM-Iso1is composed of all 15 coding exons and ALCAM-Iso2 lacks exon 13, which corresponds to 13 amino acids in the stalk region of the protein. The functional and biochemical differences between the two isoforms have not yet been explored [47]. The variant ΔN-ALCAM is caused by a deletion mutation and disturbs the ligand-receptor binding function and cognitive properties of ALCAM. It mostly conserves the oligomerisation properties and the ectopic expression of this variant is involved in the intercellular networks of ALCAM. An overexpression of ΔN-ALCAM worsens the invasive behaviour of the cells by interfering with the homophilic interactions of endogenous ALCAMs [48].

Similarly, sALCAM is a shortened form of ALCAM transcript in endothelial cells that consists of the first three axons and encoding the NH2- terminal sequence with the first variable domain. sALCAM was first isolated by Ikeda et al and his study reported that the expression of sALCAM and ALCAMs in Human Microvascular Endothelial Cells (HMVEC) is differently regulated by Tumor Necrosis Factor Alpha (TNF-α) stimulation. sALCAM can also independently affect the cell migration process [48]. Yet, another form released from the ectodomain of ALCAM structure through proteolytic cleavage is so ALCAM. The actual role of these molecules is yet to be discovered, but they serve as molecular buffers capable of neutralising excess ligands.

Comparative Studies on ALCAM

Several studies have been conducted so far about the effectiveness of ALCAM in breast cancer detection and prognosis assessment. The expression of ALCAM in advanced and metastatic disease directly and positively correlated with a poor prognostic value. The results were also specific when compared to the other biomarkers. Witzela I et al., conducted a cohort study among 157 primary breast cancer patients and compared the results with ALCAM protein and mRNA expression in the tumour tissue of those patients using cDNA arrays and Western blot analysis. The association between ALCAM levels and clinicopathological parameters and survival data was also assessed. In univariate analysis, high sALCAM levels were significantly associated with shorter Disease-free Survival (DFS). Also, 36% of the patients have disease recurrence and 25% of the patients died in a median follow-up of 87 months [49].

Ihnen M et al., in their study in 1997-2005 among 25 tissue samples of primary breast cancer and 84 samples of the advanced disease reported found that ALCAM staining was found in 24 out of 25 primary breast cancer patients. But in metastatic samples (except two), no ALCAM staining was seen [50]. This finding is similar to the study results of Jezierska A et al., who reasoned that aggressive breast cancer correlates with low ALCAM values as the malignant cells could dissolve out the primary tumour and spread easily due to the absence of connecting adhesion molecule [33]. Kulasingam V et al., measured the concentration of ALCAM in serum using a highly sensitive and specific non-competitive "sandwich-type" ELISA among 150 patients with breast cancer. Their study results showed evidence that serum ALCAM concentration has potential utility as a diagnostic tool. In addition, the combination of ALCAM with CA15-3 improved the diagnostic sensitivity and an elevated ALCAM level was associated with increasing age [51].

Fawziah S, in his study compared the effects of ALCAM, CA 15-3, and CEA and their results revealed that the p-value of ALCAM was highly significant in grade II and grade III tumours by 90% and 127% respectively, while CA15-3 was significant by 40%, 72%, and CEA was significant by 33%, 156%, respectively. They demonstrated ALCAM as the most sensitive and specific biomarker in breast cancer detection when compared to CA15-3 and CEA [52]. Ajeed Am et al., in their study compared the expression of ALCAM, CEA, and CA15-3 in benign and malignant breast carcinoma and their results revealed that the p-value between patients with benign tumour and control was 0.015, p-value between malignant tumour and control was <0.001, p-value between patients with malignant tumours and benign tumours was 0.044 and p-value among all studied group by Analysis of Variance (ANOVA) was <0.001 [53]. The ROC curve results revealed that CEA and CA15-3 and also the combination between them had low AUC, sensitivity, and specificity values whereas ALCAM showed higher AUC values with acceptable sensitivity and specificity in patients with a malignant tumour when compared with benign tumours [40]. Lal N et al., in their study report in 2016 stated that a positive association between serum ALCAM levels and disease grading was observed. The median ALCAM levels in grade I patients was 91.350 and in grade II it was 215.743 in their study. The median serum ALCAM levels were highest in grade III 490.773 (p-value=0.005). There was no significant difference in the levels of serum and salivary CA-15-3 across the three grades. In regards to the correlation with morphological parameters and grade, the values of serum ALCAM significantly correlated with the nuclear size and thereby, the histopathological grade. The values of sALCAM increased proportionately with higher grades [54]. The study finding results of Lal N et al., were similar to that of Mohammed AS et al., findings. This study author compared the expression of ALCAM, CEA, and CA15-3 in breast cancer women and concluded that the serum ALCAM may represent a diagnostic biomarker for early detection of breast cancer [55]. [Table/Fig-6] shows a list of studies done on ALCAM expression in breast cancer patients [49,51-56].

It was believed that no single cancer biomarker can provide all necessary information for optimal cancer diagnosis and the focus has been on the identification of biomarkers that can be used in combination. This review provides evidence that serum ALCAM displays a higher diagnostic sensitivity for breast cancer detection when compared with the other tumour markers. ALCAM is expressed at certain levels in melanoma, prostate, and bladder cancer but shows 95% specificity to breast cancer detection. These findings represent ALCAM as a single potential candidate for breast cancer detection and prognosis assessment.

Author	No. of patients/ samples (N)	Study findings	p-value
Witzel I et al., 2012 [49]	Serum samples from 157 Breast Cancer patients, 48 Healthy women	Median ALCAM expression: Breast cancer=24.2 ng/mL (range 3-108) Healthy=18.9 ng/mL (range 10.3-32.6)	0.044
Kulasingam et al., 2009 [51]	Serum samples: Breast cancer, N=150 Healthy women, N=100 Healthy Men, N=50	Median ALCAM values: Breast cancer=74 µg/L Healthy women=60 µg/L Healthy men=58 µg/L	<0.0001
Fawziah S et al., 2015 [52]	Serum samples: Breast cancer, N=119 Grade II=58 Grade III=61 42 Healthy subjects	ALCAM (Mean±SE) Healthy subjects: ALCAM (ng/mL)=79±2.5189 Grade 2=92.416±1.7074 Grade 3=110±2.8242 Breast cancer=101.208±2.26	0.0001
Ajeed AM et al., 2020 [53]	60 patients with breast cancer and 60 patients with benign breast tumour, 75 Healthy volunteers	Mean levels (pg/mL) of ALCAM in: Benign=85.87±12.38 Malignancy=91.1±9.74 Control=80.1±12.91	<0.001
Lal N et al., 2017 [54]	Serum samples: Breast cancer, N=25 Controls, N=25	ALCAM: Case: Serum ALCAM ±SD=244.490±239.958 Control: Serum ALCAM-27.106±12.017	<0.001
Mohammed AS et al., 2013 [55]	Serum samples: 20 Healthy women 41 Breast cancer patients	ALCAM: Breast cancer patients=97±10.65 µg/L Healthy controls=86.41±9.81	<0.05
Ihnen M et al., 2010 [56]	Tissue samples from 25 primary breast cancer and 84 Metastatic breast cancer samples	ALCAM staining was found in 24 out of 25 primary breast cancer patients	<0.001

[Table/Fig-6]: Studies on ALCAM expression in Breast cancer [49,51-56].
p-value <0.05 is considered as statistically significant

CONCLUSION(S)

A lot of invasive and non invasive biomarkers for early breast cancer detection have been described in the last few decades to overcome the limitations of mammogram screening and other modalities of tumour detection. Few studies suggest that level of ALCAM expression is more when compared to CA15-3 and CEA. Studies suggest that decreased ALCAM expression is an indication for a bad prognosis of malignancy in few cancers but elevated levels of ALCAM were seen found in breast carcinoma. The proteomic nature of ALCAM can facilitate its expression in other biological fluids like urine and saliva. But, many studies researched the expression of ALCAM in both breast tumour tissue and serum of breast cancer patients but no studies were conducted on non invasive methods of ALCAM expression in breast cancer patients. Despite limitations, serum ALCAM is a promising biomarker for early breast cancer detection and exhibits higher specificity and sensitivity. Further extensive studies focussing on the detection methods of ALCAM and comparing its effectiveness with other cancer biomarkers would prompt for an in-depth understanding of its exclusivity in breast cancer detection.

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