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Activated leukocyte cell adhesion molecule serum levels as a marker in the diagnosis of patients with breast cancer

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ABSTRACT

Objectives: To assess the possible role of activated leukocyte cell adhesion molecule (ALCAM) in the diagnosis of a tumor with the evaluation of its importance in differentiating between benign and malignant tumors in comparison with the classical tumor markers for breast carcinoma, CEA and CA15-3.

Methods: A case-control research has been achieved at Al-Nahrain University, in the Department of Chemistry and Biochemistry, College of Medicine, Baghdad, Iraq. This work has been done for 60 female patients with the cancer of the breast and 60 female patients with benign breast tumors who were recruited from Al Imamain Al-Kadhimayn medical city and Oncology teaching Hospital, Baghdad between May 2018 and December 2018. The ALCAM, CEA, and CA15-3 levels have been calculated from sera of women with either malignant or benign breast tumors and compared to the age of 75, BMI, and sex-matched control subjects.

Results: The levels of ALCAM in the patients with benign and malignant tumors were 85.87±12.38, 91.1±9.74 pg/mL; respectively, and significantly higher ($p<0.05$) than that of controls (80.1±12.91 pg/mL). CA15-3 levels exhibited significant ($p<0.05$) increase in cancerous patients in comparison with controls (29.21±9.96, 22.74 ±8.67; respectively) whereas CEA levels showed nog significant differences among the studied groups. Levels of ALCAM were positively and significantly correlated with levels of CA15-3 in the patients with malignant tumors while CEA showed no correlations with other parameters. Analysis of ROC (Receiver Operating Characteristic) showed that ALCAM can be considered as an excellent marker for diagnosis of the differentiation between malignant and benign breast tumor and excellent marker for the diagnosis of the cancer of the breast in comparison with controls, whereas CEA, CA15-3 and there combinations.

Conclusion: The possibility of using ALCAM in the diagnosis of breast cancer and differentiating between malignant and benign tumors.

Keywords: Breast cancer; tumor marker; Activated leukocyte cell adhesion molecule; Benign tumor; carcinoembryonic antigen; malignant tumor; Cancer antigen 15-3.

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INTRODUCTION

The cancer of the breast is considered as one of the most common women cancers globally, accounting for about 570,000 deaths in the year 2015. More than 1,500,000 women (25% of the women with cancers) were diagnosed with the cancer of breast annually in the global [1]. In the USA, it is estimated that in 2017 about 30% of all new cases of cancers (252,710) among women were the cancer of the breast [2]. The cancer of the breast is one of the metastatic cancers and can usually transfer to distant organs like the liver, bone, brain, and lung, which generally accounts for its incurableness. For this reason, it becomes important to diagnose the tumor in an early stage which is performed by tumor markers evolved previously [3].

Tumor biomarker is defined as a molecule, which is produced in response to the tumor or by the tumor. Biomarkers can be detected from any tissue in the body including the breast. They may have prognostic, diagnostic, and/or predictive values. The currently used serological breast cancer markers include CEA (carcinoembryonic antigen) and carbohydrate antigen 15-3 (CA 15-3) [4]. Briefly, serum assays of BR 27.29 and CA 15-3 (also called CA 27.29) detected the same antigen, for example, the protein of MUC-1 and provided similar clinical data. However, CA 15-3 had more widely tested than BR 27.29. Levels of CEA and CA 15-3 in the serum were associated with the size of the tumor and nodal involvement and were advised by international bodies like ASCO (American Society of Clinical Oncology) to monitor the patients with metastatic diseases through efficient treatment [5].

However, these markers were advised to be employed in conjunction with diagnostic imaging, physical, and historical examination. Generally, levels of the serum marker reflect the burden of the tumor and for this reason, these markers aren't sensitive enough to be employed for early diagnosis and screening the primary cancer of the breast [6]. So, new biomarkers with higher sensitivity were involved such as molecules of activated leukocyte cell adhesion (ALCAMs).

Also, ALCAMS called MEMD or CD 166, it is considered as a member of the super-family of immunoglobulin with 5

extracellular immunoglobulin-like domains, which assists cell-cell clustering during heterophilic interactions (ALCAM– CD 6) and homophilic interactions (ALCAM–ALCAM) [7]. Its role has been implicated in the genesis of cancer. ALCAM is expressed mostly in tissues involved in active growth or migration. A recent sign proposes that ALCAM expression maybe reflect the attack of a cellular program for homeostatic control of saturation of growth, which enhances either migration of cells or arrest of growth. Recently conducted studies have found that ALCAM can represent a potential biomarker for the diagnosis of the cancer of the breast [8].

This research has been aimed to calculate the levels of ALCAM as a new biomarker in addition to CA15-3 and CEA which are considered as routine tumor markers for the breast in women with benign and malignant tumors and the results obtained have been compared as the control.

Method

Case-Control research has been done on 60 female patients with the cancer of the breast and 60 female patients with benign breast tumors who were recruited from Al Imamain Al-Kadhemayn medical city and Oncology teaching Hospital, Baghdad, Iraq between May 2018 and December 2018. Ages of the malignant group ranged between 30 and 48 years (mean± SD of 42.83±4.27 years) and benign group's ages were ranged between 27 and 44 years (mean± SD of 41.73±5.09 years). The group of control comprised 75 age, BMI, and sex-matched healthy females with mean± SD age of 41.32±4.77 years.

The practical part of the study was conducted at the Department of Chemistry and Biochemistry and Department of Pathology, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

In the current research, women were eligible for this study if they had a suspicious breast lesion (newly diagnosed) which was recorded by clinical breast examination and/or imaging technology. Patients were subjected to physical breast examination and mammography.

Exclusion criteria

1. Subjects that had a history of any kind of cancer.
2. Subjects that had a history of any chronic or serious diseases.
3. Patients received hormonal treatment or chemotherapy.

The research has approved by the local Ethical Committee of the College of Medicine, University of Al-Nahrain, Baghdad, Iraq. Additionally, informed written consent for participation in this research was signed by tested subjects depending on the principles of Helsinki.

Blood samples

About 5ml of samples of blood has been obtained from fasting patients and controls. These samples have been transferred to serum separating tubes (SST) and left for clotting at room temperature for 30 minutes then have been centrifuged at 4000 rpm (1252 x) g for 10 minutes. The separated sera were divided into small aliquots and stored at -20 ^{o}C until assayed for CEA evaluation, CA15-3, and ALCAM. Levels of serum CEA, CA15-3, and ALCAM were estimated by enzyme-linked immunosorbent assays (ELISAs) with kits obtained from the Elabscience (China), Cell Biolabs (USA) and RayBiotech Inc. (USA); respectively according to the manufacturer instructions.

Statistical analysis

The obtained results in the current work have been expressed as mean±Standard Deviation, and statistical comparisons have been used with an independent t-test to compare 2 independent groups (controls and patients). Test of ANOVA (Analysis of variance) for comparison among more than two groups by the test of Tukey HSD Post-Hoc for assessing the significant differences between the studied sub-groups; statistically considered $p < 0.05$ as significant. Correlations among all the studied parameters have been investigated using the Pearson correlation test. However, all the statistical analyses used in the current work have been achieved by IBM SPSS Statistics for Windows system, Ver.24 (IBM, NY: Corp Armonk,) [9]. The normality of the distribution has been checked by the tests of Kolmogorov- Smirnov, and Shapiro-Wilk.

However, analyses of ROC (Receiver operating characteristic) have been completed as a comprehensive method for determining the accuracy of the markers employed in the current study. An analysis of ROC, AUC (the area under the curve) can be considered as a powerful statistical tool to compare various bio-markers given the value of AUC that becomes closer to one referee that parameters can be considered as predictive biomarkers and excellent diagnostic, the curve obtained in this statistical test maybe indicate the significance of the marker. So, the parameter's curve that lies close to the diagonal (AUC =0.5) indicates no diagnostic significance. The value of AUC that is closer to 1 is usually coupled with specificity and sensitivity satisfactory values [10].

Results

The levels of CEA, CA15-3, and ALCAM were compared between patients with benign and malignant tumors in comparison with age-matched control subjects. Results illustrated in the table (2) revealed that there were non-significant differences in the levels of CA15-3 between females suffered from benign breast tumor and control ($p= 0.08$) and also between patients with benign and malignant breast tumor ($p=0.08$). On the other hand, a significant increase ($p<0.001$) in the level of CA15-3 in patients with malignant tumors was observed in a comparison with control subjects. Furthermore, the ANOVA test revealed that there was a significant difference ($p<0.001$) among all studied groups.

Furthermore, CEA levels exhibited non-significant differences ($p>0.05$) among all studied groups. Slightly non-significant increases in the level of CEA were observed in benign and malignant patients in comparison with healthy controls.

Moreover, there were significant increases in ALCAM levels in both patients with benign and malignant breast tumors in comparison with controls ($p=0.015$, $p<0.001$; respectively). Additionally, there was a significant increase ($p=0.044$) in the ALCAM level in patients with malignant tumors when compared with benign ones. Furthermore, the ANOVA test revealed that ALCAM levels showed significant variations ($p<0.001$) among all the studied groups.

Table 1. Demographic characteristics of the patients with a malignant and benign tumor in comparison with controls.

	Control	Benign breast tumor	Malignant breast tumor
n	75	60	60
Age	41.32±4.77	41.73±5.09	42.83±4.27
P-value with control		0.13	0.57
Weight	70.28±9.06	69.82±8.93	73.37±9.87
Height	160.15±14.73	159.42±13.6	159.7±14.55
BMI	27.63±5.04	28.13±5.22	28.53±5.34
P-value with control		0.61	0.47

Results obtained in the present study clarified that there were non-significant correlations between all studied markers among healthy controls and patients with benign breast tumors whereas results illustrated in the table (3) and figure (1) revealed that there was a significant positive correlation between CA 15-3 and ALCAM.

Table 2. CA15-3, CEA, and ALCAM levels in controls, patients with benign and malignant breast tumors.

	Group	mean±SD	P^a	P^b	P^c	P^d
CA 15-3 (U/ml)	Control n= 75	22.74±8.67	0.08	<0.001	0.08	<0.001
	Benign tumor n=60	25.81±6.67				
	Malignant Tumor n=60	29.21±9.96				
CEA (ng/ml)	Control n= 75	1.42±0.5	0.32	0.06	0.72	0.07
	Benign tumor n=60	1.53±0.41				
	Malignant Tumor n=60	1.59±0.4				
ALCAM (pg/ml)	Control n= 75	80.1±12.91	0.015	<0.001	0.044	<0.001
	Benign tumor n=60	85.87±12.38				
	Malignant Tumor n=60	91.1±9.74				

P^a value between patients with benign tumor and control

P^b value between patients with malignant tumor and control P^c value between patients with benign tumor and patients with a malignant tumor P^d value among all studied group (ANOVA test)

Table 3. Correlations between the levels of all studied biochemical parameters among patients with malignant tumors.

		CEA	CA153	ALCAM
CEA	**R**	1	0.166	0.182
	P		0.205	0.165
CA153	**R**	0.166	1	0.669*
	P	0.205		<0.001
ALCAM	**R**	0.182	0.669*	1
	P	0.165	<0.001	

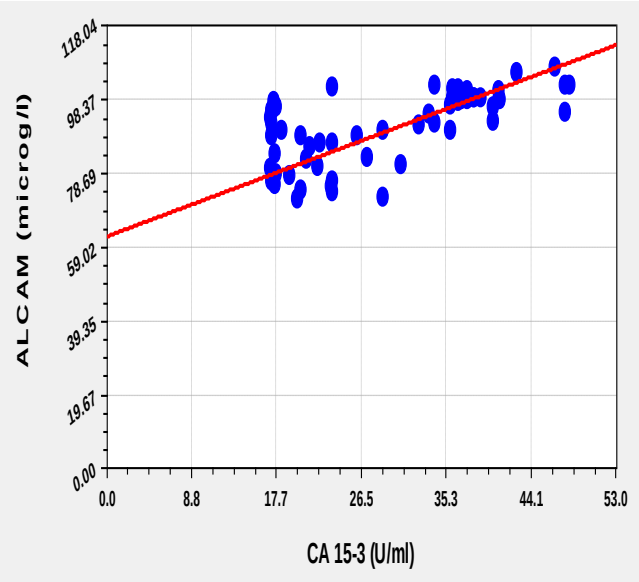

Figure 1. Correlation between CA15-3 and ALCAM in malignant tumor group.

ROC curve results illustrated in table (4) revealed that CEA and CA 15-3 and also the combination between them had a low AUC, sensitivity and specificity values Whereas ALCAM showed higher AUC values with acceptable sensitivity and specificity in patients with a malignant tumor when compared with benign ones as illustrated in figure (2). Interestingly, Combining CA15-3 and ALCAM showed a slight improvement in AUC and a decrease in sensitivity with a valuable increase in specificity when compared with ALCAM alone as shown in figure (2).

Table 4. ROC curve results for all studied parameters in the patients with malignant breast tumor comparing with benign breast tumor patients

Parameters	AUC	Sensitivity (%)	Specificity (%)
CEA	0.555	48.3	60
CA153	0.582	63.3	51.7
ALCAM	0.842	76.7	63.7
Combining CEA and CA15-3	0.589	56.7	60
Combining CEA and ALCAM	0.842	70	90
Combining CA15-3 and ALCAM	0.846	73.3	80

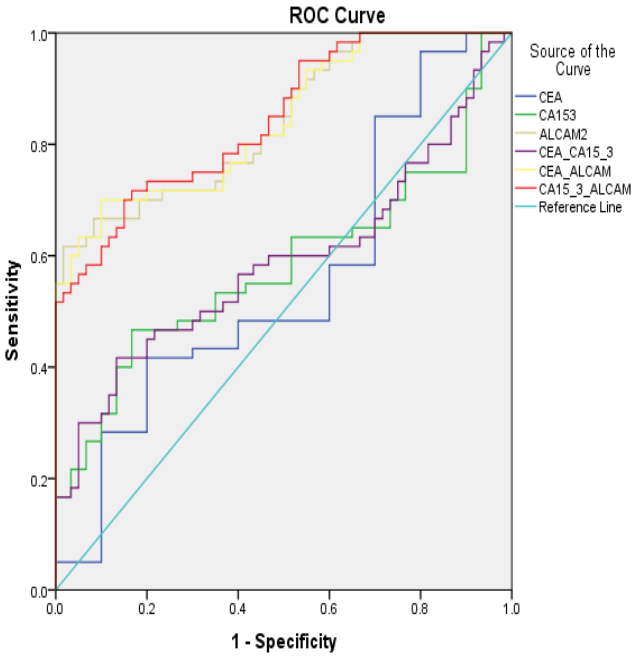

Figure 2. ROC curve for CEA, CA15-3, ALCAM, and their combinations in patients with malignant tumors compared with benign ones.

Results obtained in table (5) revealed that AUC, specificity, and sensitivity of CEA, CA 15-3, and a combination of both were low while ALCAM showed high AUC values with excellent sensitivity (figure 3). Moreover, the results obtained from the combinations between all subjected parameters showed that the combination between ALCAM and either of CEA or CA 15-3 did not affect the ROC curve results significantly

Table 5. ROC curve results for all studied parameters in the patients with malignant breast tumor comparing with controls

Parameters	AUC	Sensitivity (%)	Specificity (%)
CEA	0.613	58.3	58.7
CA153	0.714	75	53.3
ALCAM	0.968	91.7	85.3
Combining CEA and CA15-3	0.7	55	84
Combining CEA and ALCAM	0.968	91.7	85.3
Combining CA15-3 and ALCAM	0.971	91.7	84

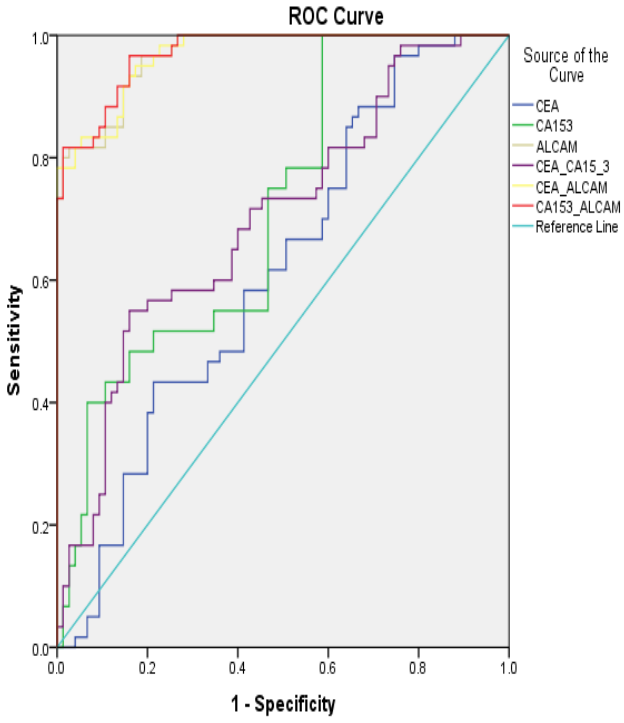

Figure 3. ROC curve for CEA, CA15-3, ALCAM, and their combinations in patients with malignant tumors compared with controls.

Discussion

Breast cancer is one of the heterogeneous diseases with widespread molecular, histological, and clinical presentations. Deplorably, other than definitive diagnosis using histopathology and biopsy, no screening or diagnostic test is currently adequate for early detection of the cancer of the breast [11]. The capability for detection of human malignancy by a simple method to test blood has long been a main objective in the medical screening.

CEA and CA15-3 discovered more than four and two decades ago, respectively, are the most generally employed markers of the tumor for the cancer of the breast [12,13]. Levels of CA15-3 and CEA in the serum are

advised to monitor the treatment of advanced breast cancer [11]. Nevertheless, these biomarkers of cancer have proven to be ineffective in detecting the early stages of the disease because of low diagnostic specificity and sensitivity [6,14–17]. These findings confirmed by the results obtained by the current study in which non-significant differences were obtained between patients with benign tumor and control in agreement with results obtained previously [18] and also between patients with malignant and benign tumors which is in agreement with results obtained by Fu and li in 2016 [19].

Results obtained in this study revealed that CEA and CA15-3 were non-significantly correlated in all studied groups that are also consistent with Fu and li results that indicate a non-significant correlation between these markers and the stage of the tumor [19]. Furthermore, the low sensitivity and specificity obtained by ROC curve results in this study were in agreement with many previous studies stated that "Because CEA lacks disease sensitivity and specificity, it cannot be used for screening the general asymptomatic population" he also stated that CA15-3 has low sensitivity and study conducted by Wang and his colleagues who found that CEA, CA15-3, and combination of these two markers sensitivity for a malignant tumor in comparison with controls were 56.7%, 44.5%, and 68.9%; respectively, whereas in the current study the sensitivity of CEA, CA15-3, and combination of them was 58.3, 75 % and 55%; respectively [17,20].

Additionally, levels of CA15-3 and CEA showed to be non-useful parameters for differentiation between malignant and benign tumor patients as they showed non-significant differences (table 2) beside the low sensitivity and specificity of these two parameters between these two subgroups (table 4). The possible explanation of the poor deferential ability of CA15-3 may be owned to the fact that it can be raised in benign breast tumor as well as malignant which make it difficult to be used as a deferential tool as mentioned previously in many studies [19–22]. Moreover, Zhao and his co-workers found non-significant differences in CEA level in nipple discharge of patients with a benign and malignant tumor that confirm results obtained in this study given that the measurement of local CEA levels can be considered as more predictor of breast tumor than systemic measurements used in this study [22].

However, results obtained in this research revealed that there were significant increases in levels of CA 15-3 in women with the cancer of the breast in comparison with controls that agree with several last studies [18,23].

In this study, levels of ALCAM were significantly increased in benign patients in comparison with healthy volunteers, and results obtained revealed that there was a significant elevation in the malignant group compared with benign one as illustrated in the table (2). The results obtained in the current study were in agreement with several previous studies which demonstrated a significant elevation in the levels of ALCAM in cancer patients when compared with healthy volunteers [7,8,24]. There was no previous research that targeted ALCAM levels in women with benign mass but results of some previous studies revealed that ALCAM levels may increase in some benign tumors other than breast tumors [25,26] but this increment still significantly lower than that in malignant patients as demonstrated in the current work which is owned to the possible role of ALCAM in metastasis. ALCAM showed to be widely expressed in tissues but restricted to particular subsets of cells that have a role in migration processes and the dynamic growth [27,28].

According to previous literature, ALCAM levels considered as a controversial parameter given that some studies reported that high level reflects a good prognostic value while others conclude an opposite finding [7,8,29,30]. These controversial findings might be probably caused by the variation in ALCAM function per the type of cell and the micro-environment that surround cancerous cells. The possible explanation of the elevated level of ALCAM in tumorigenesis is the role of sheddases metalloproteinase (ADAM-17) that increases in cancerous tissues which in turn participate in the shedding of ALCAM (considered as a substrate for it) from the site of the tumor into the circulation [31–34].

ROC curve results obtained in the current study confirm the previously mentioned significant increase in ALCAM levels in malignant patients sera in comparison with controls, given that AUC was high with excellent sensitivity and good specificity (0.968, 91.7%, 85.7%; respectively). Furthermore, ALCAM levels also showed good AUC with acceptable sensitivity and specificity in women with malignant breast tumors in comparison with benign groups which are agreed with several previous works [7,24,35]. Moreover, a combination between either CEA or CA15-3 with ALCAM showed no valuable improvement in sensitivity and specificity when compared with ALCAM alone in agreement with other previous researches [25].

Biochemical markers subjected to the present study in this group showed correlations manner different from those obtained in the benign group. The newly emerged biomarker studied in this study namely ALCAM showed significant positive correlations with CA15-3 as indicated in the table (3).

There were conflicting studies that either in the agreement or not consistent with results obtained by the current study in which Kulasingam et al., 2009 denoted that there was a weak correlation between CA15-3 and ALCAM while Al-Shehri and EL Azeem, 2015 demonstrated a correlation between CA15-3 and ALCAM since they highly elevated in women with the cancer of breast compared with controls.

Conclusion

ALCAM levels in the patients with benign and malignant tumors have been significantly higher than those in the controls that may be used as a new sensitive marker to detect breast tumors in addition to its ability in differentiation between benign and malignant tumors as it increased significantly in the cancerous group in comparison with women having benign masses. The data obtained support the assumption that ALCAM can be considered an important tumor marker superior to the classical markers CEA and CA15-3.

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REFERENCES

1 F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA. Cancer J. Clin. 68 (2018) 394–424. https://doi.org/10.3322/caac.21492.

2 R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2017, CA. Cancer J. Clin. 67 (2017) 7–30. https://doi.org/10.3322/caac.21387.

3 A.J. Redig, S.S. McAllister, Breast cancer as a systemic disease: a view of metastasis, J. Intern. Med. 274 (2013) 113–126. https://doi.org/10.1111/joim.12084.

4 M. Nagpal, S. Singh, P. Singh, P. Chauhan, M.A. Zaidi, Tumor markers: A diagnostic tool, Natl. J. Maxillofac. Surg. 7 (2016) 17–20. https://doi.org/10.4103/0975-5950.196135.

5 D.C. Lin, J.R. Genzen, Concordance analysis of paired cancer antigen (CA) 15-3 and 27.29 testing, Breast Cancer Res. Treat. 167 (2018) 269–276. https://doi.org/10.1007/s10549-017-4513-0.

6 J.L. Khatcheressian, A.C. Wolff, T.J. Smith, E. Grunfeld, H.B. Muss, V.G. Vogel, F. Halberg, M.R. Somerfield, N.E. Davidson, American Society of Clinical Oncology 2006 Update of the Breast Cancer Follow-Up and Management Guidelines in the Adjuvant Setting, J. Clin. Oncol. 24 (2006) 5091–5097. https://doi.org/10.1200/JCO.2006.08.8575.

7 F.S. Al-Shehri, E.M. Abd El Azeem, Activated leukocyte cell adhesion molecule (ALCAM) in Saudi breast cancer patients as prognostic and predictive indicator, Breast Cancer Basic Clin. Res. 9 (2015) 81–86. https://doi.org/10.4137/BCBCR.S25563.

8 N. Lal, S. Irfan, N. Zaidi, O. Musa, A. Mishra, I. Rizvi, Role of Biomarkers ALCAM and CA-15-3 in the Diagnosis of Breast Cancer: A Case-Control Study, Online, 2017. www.ijcmr.com (accessed October 31, 2019).

9 IBM Corp., IBM SPSS Statistics for Windows, Version 24.0, 2016. (2016).

10 K. Hajian-Tilaki, Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation, Casp. J. Intern. Med. 4 (2013) 627–635. https://www.ncbi.nlm.nih.gov/pubmed/24009950.

11 L. Harris, H. Fritsche, R. Mennel, L. Norton, P. Ravdin, S. Taube, M.R. Somerfield, D.F. Hayes, R.C. Bast, American society of clinical oncology 2007 update of recommendations for the use of tumor markers in breast cancer, J. Clin. Oncol. 25 (2007) 5287–5312. https://doi.org/10.1200/JCO.2007.14.2364.

12 P. Gold, S.O. Freedman, SPECIFIC CARCINOEMBRYONIC ANTIGENS OF THE HUMAN DIGESTIVE SYSTEM, J. Exp. Med. 122 (1965) 467 LP – 481. https://doi.org/10.1084/jem.122.3.467.

13 J. Hilkens, F. Buijs, J. Hilgers, P. Hageman, J. Calafat, A. Sonnenberg, M. Der Van Vlak, Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors, Int. J. Cancer. 34 (1984) 197–206. https://doi.org/10.1002/ijc.2910340210.

14 F.G. Ebeling, P. Stieber, M. Untch, D. Nagel, G.E. Konecny, U.M. Schmitt, A. Fateh-Moghadam, D. Seidel, Serum CEA and CA 15-3 as prognostic factors in primary breast cancer, Br. J. Cancer. 86 (2002) 1217–1222. https://doi.org/10.1038/sj.bjc.6600248.

15 F. Lumachi, S.M.M. Basso, Serum tumor markers in patients with breast cancer, Expert Rev. Anticancer Ther. 4 (2004) 921–931. https://doi.org/10.1586/14737140.4.5.921.

16 N. Serdarevic, R. Serdarevic, A. Memic, Comparison of the performance of three cancer antigen (CA) 15-3 immunoassays, J. Heal. Sci. 6 (2016). https://doi.org/10.17532/jhsci.2016.381.

17 W. Wang, X. Xu, B. Tian, Y. Wang, L. Du, T. Sun, Y. Shi, X. Zhao, J. Jing, The diagnostic value of serum tumor markers CEA, CA19-9, CA125, CA15-3, and TPS in metastatic breast cancer, Clin. Chim. Acta. 470 (2017) 51–55. https://doi.org/10.1016/J.CCA.2017.04.023.

18 Z.M. Hashim, The Significance of CA15-3 in Breast Cancer Patients and its Relationship to HER-2 Receptor Status, Int. J. Immunopathol. Pharmacol. 27 (2014) 45–51. https://doi.org/10.1177/039463201402700107.

19 Y. Fu, H. Li, Assessing Clinical Significance of Serum CA15-3 and Carcinoembryonic Antigen (CEA) Levels in Breast Cancer Patients: A Meta-Analysis, Med. Sci. Monit. 22 (2016) 3154–3162. https://doi.org/10.12659/msm.896563.

20 A.M. Kabel, Tumor markers of breast cancer: New prospectives, J. Oncol. Sci. 3 (2017) 5–11. https://doi.org/10.1016/j.jons.2017.01.001.

21 A. Bahrami-Ahmadi, F. Makarian, M.R. Mortazavizadeh, M.F. Yazdi, M. Chamani, Symptomatic metastasis prediction with serial measurements of CA 15.3 in primary breast cancer patients, J. Res. Med. Sci. 17 (2012) 850–854. https://www.ncbi.nlm.nih.gov/pubmed/23826012.

22 S. Zhao, X. Gai, Y. Wang, W. Liang, H. Gao, K. Zhang, H. Wang, Y. Liu, J. Wang, R. Ma, Diagnostic Values of Carcinoembryonic Antigen, Cancer Antigen 15-3 and Cancer Antigen 125 Levels in Nipple Discharge., Chin. J. Physiol. 58 (2015) 385–92. https://doi.org/10.4077/CJP.2015.BAD336.

23 H.Q. Ali, N.K. Mahdi, M.H. Al-Jowher, The value of CA15-3 in diagnosis, prognosis and treatment response in women with breast cancer., J. Pak. Med. Assoc. 63 (2013) 1138–41. http://www.ncbi.nlm.nih.gov/pubmed/24601193

24 I. Witzel, C. Schröder, V. Müller, H. Zander, M. Tachezy, M. Ihnen, F. Jänicke, K. Milde-Langosch, Detection of Activated Leukocyte Cell Adhesion Molecule in the Serum of Breast Cancer Patients and Implications for Prognosis, Oncology. 82 (2012) 305–312. https://doi.org/10.1159/000337222.

25 S.L. Kulasingam, R. Rajan, Y. St Pierre, C.V. Atwood, E.R. Myers, E.L. Franco, Human papillomavirus testing with Pap triage for cervical cancer prevention in Canada: A cost-effectiveness analysis, BMC Med. 7 (2009) 69. https://doi.org/10.1186/1741-7015-7-69.

26 T.A. Stamey, J.A. Warrington, M.C. Caldwell, Z. Chen, Z. Fan, M. Mahadevappa, J.E. McNeal, R. Nolley, Z. Zhang, Molecular genetic profiling of Gleason grade 4/5 prostate cancers compared to benign prostatic hyperplasia., J. Urol. 166 (2001) 2171–7. http://www.ncbi.nlm.nih.gov/pubmed/11696729 (accessed November 2, 2019).

27 G. Carbotti, A.M. Orengo, D. Mezzanzanica, M. Bagnoli, A. Brizzolara, L. Emionite, A. Puppo, M.G. Centurioni, M. Bruzzone, P. Marroni, A. Rossello, S. Canevari, S. Ferrini, M. Fabbi, Activated leukocyte cell adhesion molecule soluble form: a potential biomarker of epithelial ovarian cancer is increased in type II tumors, Int. J. Cancer. 132 (2013) 2597–2605. https://doi.org/10.1002/ijc.27948.

28 R. Tavaré, W.H. Wu, K.A. Zettlitz, F.B. Salazar, K.E. McCabe, J.D. Marks, A.M. Wu, D. Neri, Enhanced immunoPET of ALCAM-positive colorectal carcinoma using site-specific ^{64}Cu-DOTA conjugation, Protein Eng. Des. Sel. 27 (2014) 317–324. https://doi.org/10.1093/protein/gzu030.

29 M. Burkhardt, E. Mayordomo, K.-J. Winzer, F. Fritzsche, T. Gansukh, S. Pahl, W. Weichert, C. Denkert, H. Guski, M. Dietel, G. Kristiansen, Cytoplasmic overexpression of ALCAM is prognostic of disease progression in breast cancer, J. Clin. Pathol. 59 (2006) 403–409. https://doi.org/10.1136/jcp.2005.028209.

30 Y.J. Jeong, H.K. Oh, S.H. Park, J.G. Bong, Prognostic significance of activated leukocyte cell adhesion molecule (alcam) in association with promoter methylation of the ALCAM gene in breast cancer, Molecules. 23 (2018) 131. https://doi.org/10.3390/molecules23010131.

31 F. Miccichè, L. Da Riva, M. Fabbi, S. Pilotti, P. Mondellini, S. Ferrini, S. Canevari, M.A. Pierotti, I. Bongarzone, Activated Leukocyte Cell Adhesion Molecule Expression and Shedding in Thyroid Tumors, PLoS One. 6 (2011) e17141. https://doi.org/10.1371/journal.pone.0017141.

32 D. Narita, E. Seclaman, S. Ursoniu, A. Anghel, Increased expression of ADAM12 and ADAM17 genes in laser-capture microdissected breast cancers and correlations with clinical and pathological characteristics, Acta Histochem. 114 (2012) 131–139. https://doi.org/https://doi.org/10.1016/j.acthis.2011.03.009.

33 K. Ieguchi, Y. Maru, Savior or not: ADAM17 inhibitors overcome radiotherapy-resistance in non-small cell lung cancer, J. Thorac. Dis. 8 (2016) E813—5. https://doi.org/10.21037/jtd.2016.07.56.

34 L. Devis, E. Martinez-Garcia, C.P. Moiola, M.T. Quiles, M.A. Arbos, T.V. Stirbat, F. Brochard-Wyart, Á. García, L. Alonso-Alconada, M. Abal, B. Diaz-Feijoo, W. Thomas, S. Dufour, G. Mancebo, F. Alameda, J. Reventos, A. Gil-Moreno, E. Colas, L. Devis, E. Martinez-Garcia, C.P. Moiola, M. Teresa Quiles, M. Antonia Arbos, T. Vasilica Stirbat, F. Brochard-Wyart, Á. García, L. Alonso-Alconada, M. Abal, B. Diaz-Feijoo, W. Thomas, S. Dufour, G. Mancebo, F. Alameda, J. Reventos, A. Gil-Moreno, E. Colas, ALCAM shedding at the invasive front of the tumor is a marker of myometrial infiltration and promotes invasion in endometrioid endometrial cancer, Oncotarget. 9 (2018) 16648–16664. https://doi.org/10.18632/oncotarget.24625.

35 P.M. McGowan, B.M. Ryan, A.D.K. Hill, E. McDermott, N. O'Higgins, M.J. Duffy, ADAM-17 Expression in Breast Cancer Correlates with Variables of Tumor Progression, Clin. Cancer Res. 13 (2007) 2335 LP – 2343. https://doi.org/10.1158/1078-0432.CCR-062092.