Immunological study of CA-15.3 and CEA tumor markers among post-operative breast cancer patients

Hammadi Al-Hilaly[1] Aws Rasool[2] and Rawaa Magid[3]

Address: [1] Professor, Department of Medical Microbiology, College of Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq, Email: hammadi.abttan@qu.edu.iq,[2] Assist Professor, Department of pathology , college of medicine , University of AlQadisiyah ,Diwaniyah, Iraq, Email: drawsrasool@qu.edu.iq and [3]Department of medical microbiology, College of Medicine, University of Al-Qadisiya, Diwaniyah, Iraq, Email:rawaamagid@qu.edu.iq

الخلاصة: سرطان الثدي هو ورم خبيث يحدث في خلايا الثدي , والورم الخبيث هو مجموعة من الخلايا السرطانية تنمو داخل أو حول النسيج أو تنتشر إلى مناطق مختلفة من الجسم. إن ثقل مرض سرطان الثدي يبدأ في جميع دول العالم حيث يعتبر هو أكثر مرض تتعرض إليه الإناث من بين بقية أنواع السرطانات حيث يشكل حوالي 18% من بين السرطانات التي تصيب الإناث ويشكل حوالي خمس حالات الوفيات الناجمة عن أمراض السرطان حول العالم. ترتبت الدراسة الحالية على التحقق من دور بعض المعلمات السرطانية في عينات عشوائية للمرضى الوافدين إلى مستشفى الديوانية التعليمي في محافظة الديوانية . تم جمع 10 ملي لتر دم من 50 مريضة مصابة بسرطان الثدي في مرحلة ما بعد العملية الجراحية كمجموعة مرضى و 50 أنثى لا تعاني من أي نوع من أنواع السرطانات كمجموعة سيرة في الدراسة الحالية, تراوحت أعمار كلا المجموعتين بين 18 إلى 80 سنة. في مجال العلاقة بين مرض سرطان الثدي وبعض المعلمات السرطانية أظهرت نتائج دراستنا وجود علاقة إحصائية ($p<0.05$) بين الإصابة بسرطان الثدي وارتفاع تركيز CA-15.3 و CEA في مصل المرضى إلى مستويات عالية بالمقارنة مع مجموعة السيطرة P <0.0001. كذلك أظهرت نتائج دراستنا عدم وجود علاقة إحصائية بين CA-15.3 و CEA ($p=0.185$) لكن هنالك علاقة موجبة طفيفة ($r = 0.2432$)

Abstract :

Breast cancer is a malignant tumor that starts from cells . of the breast. A malignant tumor is a group of cancer cells that may grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women; comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality. This case-control study was arranged to investigate the possible role of selected genetic and immunological parameters in a random samples of patients with breast cancer in the Al-Diwanyia province. Five ml blood samples obtained from fifty females with breast cancer in post-operative stage attending the outpatient department of psychiatry in Al-Diwaniyia teaching hospital have been recruited in the study and compared to 50 health control females without any cancer types, ages of patients and control were ranged between 18-80 years. 6ml blood samples were assessed for serum measurement CA-15.3 and CEA tumor markers by using Fully-auto chemiluminescence immunoassay – CMIA. In the association between breast cancer and selected immunological markers our results showed a significant association between serum concentration of tumor markers (CA-15.3 and CEA) and breast cancer disease ($p<0.05$) in comparison with control group. The mean serum CA-15.3 and CEA were significantly higher in patients as compared to both control groups P value <0.0001. The statistical results show no significant association between CA 15-3 and CEA in patients group ($p= 0.185$) , but moderate positive correlations is appeared ($r = 0.2432$). In the other hand the association between patients age and selected immunological markers study in our study (CA-15.3 and CEA) and the results showed to the serum concentrations of CA-15.3 and CEA not effected strongly by Age ($r= 0.20$ and $r= 0.114$ respectively).

Introduction:

Breast cancer is the most common type of non-skin cancer and one of the most common cause of cancer death for women in Western countries[1]. About 1.2 million women will be diagnosed with breast cancer annually worldwide and about 411.000 will die from this disease [2]. According to American Cancer Society 95% of new breast cancer cases and 97%

of breast cancer deaths occur in women aged 40 and older[3]. Also, 50% of women who developed breast cancer are age 61 or younger at the time of diagnosis[4]. Lifetime risk of developing this malignancy is 12.2% and a lifetime risk of death is 3.6%4,5. Multiple factors are associated with an increase in breast cancer risk, including genetic and familial predisposition, hormonal factors, diet, benign breast diseases and environmental factors[5]. Over the past decade many improvements and new discoveries in the diagnosis, staging and treatment of breast cancer patients, resulted in increased survival of breast cancer patients. Breast cancer is a heterogeneous and progressive disease and its early detection remains one of the most urgent issues in cancer research[6]. Because many breast cancers still escape early detection, identification of biological tumor markers able to reveal early stage disease may greatly reduce related mortality [7]. Furthermore, an effective follow-up is needed for all treated patients who may develop progression recurrence of the disease during their life[8].In parallel, the immune system is recognized as an extrinsic tumor-suppressor that can eliminate epithelial cells that have transformed to breast cancer cells and limit their growth when they have escaped intrinsic tumor suppression mechanisms [9]. Therefore, it has been suggested that the immune system plays dual host-protective and tumor-promoting roles in breast cancer initiation and progression, by mechanisms that may shield breast cancers from immunosurveillance and enable breast cancer cells to evade immune cell induced apoptosis and produce an immunosuppressive tumor microenvironment [10]. Although the measurement of tumor markers in breast cancer has been studied for nearly 20 years, their usefulness remains unclear. In patients with metastatic breast carcinoma, tumor markers appear to be useful during follow-up, but a wide range in rates of marker positivity has been reported: 50%–80% (1-3). Breast cancer is the most common malignancy in women. Successful treatment of breast cancer relies on a better understanding of the molecular mechanisms involved in breast cancer initiation and progression [11]. The CA 15-3 concentrations increase was observed in various malignant tumors, but this is a useful marker for breast cancer metastasis and is determined in monitoring disease progression and success of therapy, It is not used as screening test or as a test for primary diagnosis because it has low diagnostic sensitivity. CA 15-3 alone, however, is not recommended as a marker for either diagnosis or detection of early recurrence of breast cancer according to the American Society of Clinical Oncology (ASCO) guidelines, because of insuffi cient data, the ASCO also does not recommend the use of CA 15-3 alone as a marker for monitoring response. It should be noted that the elevation of CA 15-3 between 4 and 6 weeks after initiation of a new therapy, i.e. spurious early rise (surge), indicates poor prognosis[12].

Aim of Study: Study of some predisposing genes and tumor markers to reach to more frequent and dangerous factor among breast cancer patients through the following objective:

Study and measure some standers of tumor markers(CA-15.3 & CEA) which is used in identification and diagnosis of disease by using Fully-auto chemiluminescence immunoassay – CMIA

Materials and Methods

Subject : The current study was conducted on 100 females (50 patients group and 50 controls group) .The patients were females who had a breast cancer (post-operative stage) . Both groups include females with 18-80 years old. The patients were referred to Al-Diwanya Teaching hospital , department of oncology, during the period March-November 2016. The diagnosis was made

by the consultant medical staff, all patient in after surgery stage (post-operative). Demographical and risk factor data were collected using a short structured questionnaire, that included information on age, weight, height, marital status, number of pregnancies and children, age at first child birth, average lactation term, family history of breast cancer or other cancers (first degree relatives), age at menarche, age at marriage, menopausal status and age at onset. Furthermore, the patients were also followed-up after the surgical operation to define the histopathology classification of breast tumor, and on which, lymph node metastases and cancer stage at the time of testing were recorded. Another group include healthy females without any history family of breast cancer also included in this study as a control group.

Tumor marker analysis

Fully-auto chemiluminescence immunoassay – CMIA

1-Test Procedure for CEA and CA-15.3

Use an anti-CEA and ant-CA15.3 monoclonal antibodies to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control are mixed thoroughly with FITC Label and nano magnetic microbeads in a cuvette incubated at 37°C, then cycle washing adding for 1 time. Then added ABEI Label and incubated to form a sandwich, after sediment in a magnetic field, sucked the supernatant then cycle washing for the 2nd time. Subsequently, Starter1+2 substrates are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of CEA and CA15.3 present in samples

2-Test Procedure of CEA and CA-15.3 Tests

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the reagent Integral. refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions, procedure steps in ready kit maglomi, germany.

Calculation of Results

The analyzer automatically calculates the CEA and CA15.3 concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating 3/4 Instructions.

Statistical analysis: Statistical analysis was performed by Social Science Statistics and the Statistical Package For Social Sciences version 19 for Windows Software and Microsoft Excel 2010. Continuous random variables of age and serum concentration of immunological makers that normally distributed are described by mean, SD (standard deviation), SE (standard error), and the parametric statistical tests of significant. ANOVA test are used to analysis the statistical significance of difference in mean between more than 2 groups and when ANOVA model shows statistically significant differences, additional exploration of the statistical significance of difference in mean between each 2 groups was assessed by Bonferonni t-test The statistical significance, direction and strength of linear correlation between 2 quantitative variables was measured by Spearman's rank and Pearson linear correlations coefficient as in state of serum markers. Moreover measure the strength of association between 2 categorical variables, such as the presence of certain genotype and disease status the odds ratio (OR) and Chi-square ($\chi 2$) test were used. P value calculate from different tests depend on variables and that less than the 0.05 level of significance was considered statistically significant [13, 14].

Results

1-The Association Between Breast Cancer And Selected Immunological Marker

Tables (1) show significant association between Immunological Marker (CA 15-3) and breast cancer ($p<0.05$). Patients have high concentration of CA 15-3 mean (18.396) in compare with controls Mean (8.136).

Table(1): The case-control difference in mean serum concentration of tumor antigen CA 15-3

serum concentration of tumor antigen CA 15-3 (ng/ml)	Case (breast cancer)	healthy controls	P – value
Range	3.9 – 42	28 – 0.5	< 0.05
Mean	18.396	8.136	
SD	8.871	6.58	
SE	1.255	0.931	
N	50	50	

❖ **Significant ($p < 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number**

Tables (2) show significant association between Immunological Marker (CEA) and breast cancer ($p< 0.05$). Patients have high concentration of CEA mean (11.66) in compare with controls Mean (5.086).

Table(2): The case-control difference in mean serum concentration of CEA

Serum concentration of CEA (ng/ml)	Case (breast cancer)	Healthy controls	P – value
Range	1.9 – 24.2	0.5 – 16.5	< 0.05
Mean	11.66	5.086	
SD	5.693	4.085	
SE	0.81	0.578	
N	50	50	

❖ Significant ($p < 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number

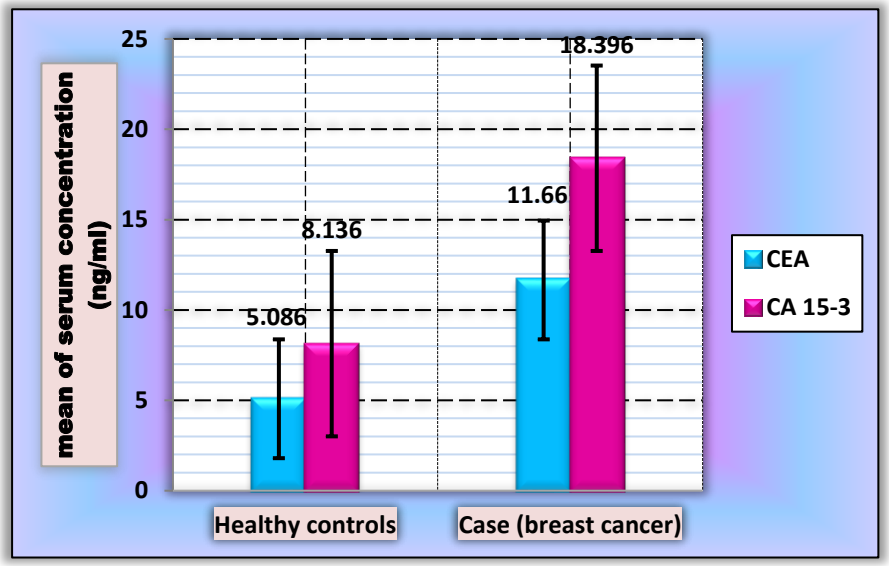

Figure (1): Bar chart show mean differences of CA 15-3 and CEA in patient and control

2-Correlation Between CA 15-3 and CEA In Breast Cancer Patients

In our present study we try to found relationship between both tumor markerswhich included CEA and CA.15.3, Figure (1) show not significant association between CA 15-3 and CEA in breast cancer patients (P=0.185) ,but moderate positive correlation is appeared (r = 0.2432).

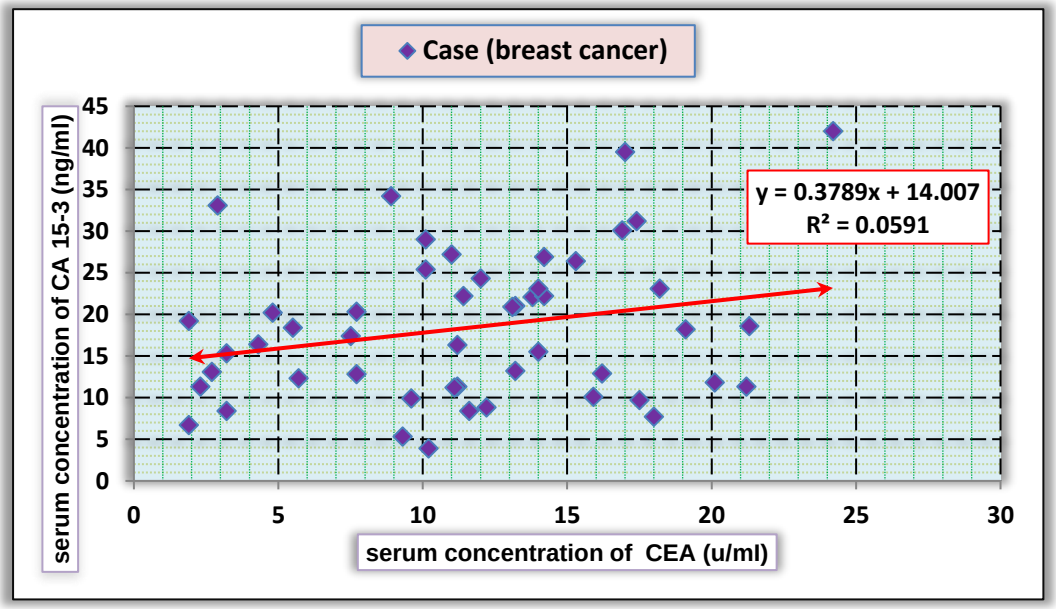

Figure (2): Scatter diagram showing the linear correlation between serum concentration of tumor antigen CA 15-3 and CEA (r= 0.2432, p= 0.185) in tumor patients

Discussion

1-The Association Between Breast Cancer And Selected Immunological Markers

The results of this study indicate that of the tow tumor markers tested are cancer antigen 15.3(CA-15.3) and cytoembryonic antigen (CEA) on 50 females with breast cancer as a patients group and 50 healthy (without any type of cancers) females as a control group. The results of present study show significant association between serum concentration of CA-15.3 and breast cancer disease ($p<0.05$) , Patients have high concentration of CA 15-3 (mean 18.396) and this result considered as a high ratio in compare with concentrations of control group (mean 8.136) this results agreed with [15] who found significant association of 150 breast cancer patients(mean 16.94) in compared with for control group (mean 7.21). So our present results matched with results stated by [16] who found serum CA-15.3 of 100 females with breast cancer have significant association with 100 females in control group ($p<0.0001$).

The results in present study show a significant association between elevation CEA and breast cancer disease ($p<0.05$) , breast cancer patients have a elevated concentration of CEA (mean 11.66) in compare with control group (mean 5.086), this result agreed with results of [17] who found a significantly association in concentrations of CEA between breast cancer patients group and controls group ($p<0.05$) , and so who found elevated concentration of CEA in 140 post-operative breast cancer as a patients group whom visited most China hospitals (mean 13.54) compared with 280 females as a control group (mean 3.87) , the results of our present study so agreed with [18] and [19] who did a comparison between post-operative breast cancer females as a patients group and healthy females as a control group, and who found the levels of CEA in patients serum elevated to highest concentrations (means 16.43 and 19.08

respectively) compared with controls (mean 4.03 and 6.91 respectively) , and appeared a significant association between both groups ($p<0.05$).The utility of these serum biomarkers may be served as effective prognostic indicators for post-operative breast cancer patients, further researches are needed to determine the effectiveness of these serum biomarkers in formulating treatment strategies in clinical practice, therefore, CEA concentrations greater than 7.5 μg/L are associated with high probability of subclinical metastases [20] .Prognosis of patients whose CEA level was within the normal range at the time of diagnosis is significantly better than those with elevated CEA levels [21].

Baseline CA 15-3 might be value in the identification of higher risk of relapse, where adjuvant chemotherapy must be introduced. In other words an explicitly that presence of an abnormal CA 15-3 pre-surgical value is associated with an increased risk of recurrence and death [22] .The prognostic value of preoperative serum tumor markers CEA and CA 15–3 was evaluated in our present study including 50 patients compared with low concentrations in 50 females among control group , the results showed that preoperative serum CEA and CA15-3 levels were independent factors affecting prognosis. The utility of measuring CEA and CA15-3 levels in patients with breast cancer remains controversial. Due to their low sensitivity, they cannot be recommended for screening or early diagnosis, but serial levels may be useful in the early diagnosis of distant metastases , European Group on Tumor Markers has recommended the CEA and CA15-3 levels be used for assessing prognosis, the early detection of disease progression, and treatment monitoring in breast cancer markers should be measured prior to every chemotherapy course and at least every 3 months for patients receiving hormone therapy [23].

The American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) guidelines do not currently recommend the use of serum CA 15–3 and CEA for breast cancer screening and directing treatment [24]. On the one hand, this may partly due to the conflicting conclusions of different researches [25]. CA 15-3 increase of 5-10 times above normal upper limit can predicts breast cancer, however, a low value cannot exclude metastasis making , CA 15-3 more of prognostic rather than diagnostic marker [26]. On the other hand, the low positive rate of serum tumor markers is also the possible reason, The incidence of breast cancer has been steadily increasing in the last two decades, however, due to the early detection and increased use of more effective systemic therapy, the survival rates of breast cancer have improved in recent years, and early breast cancer accounted for a large proportion. Previous researches demonstrated that the CEA and CA15-3 levels are associated with tumor burden indicators including tumor size and lymph node status and patients with locally advanced breast cancer exhibit significantly higher levels of CEA and CA-15.3[27]. The sensitivity of tumor markers is significantly higher in patients with advanced disease , and is related to the site of recurrence [25]. CA 15.3 and CEA are not useful in the early diagnosis of loco regional recurrence, for which clinical examination is superior. However, abnormal CEA and CA 15.3 levels are founding 40–50 and 50–70% of patients with distant metastases[28]. Since elevated levels of CA-15.3 and CEA are related to the tumor burden and higher levels may indicate an increased likelihood of systemic metastases Studies by Lee et.al showed that elevated tumor marker levels are more frequently observed in metastatic breast cancer patients than in primary breast cancer, and patients who had elevated tumor marker levels before surgery also showed more frequent elevation at recurrence [22]. Since markers are relatively easy and inexpensive to measure, regular measurement of serum

tumor marker levels could provide useful information for earlier detection of recurrence [29].

2-Correlation Between CA 15-3 and CEA In Breast Cancer Patients

In field of relationship between both tumor markers among our study ,our results show there are no relationship and association in nature and work between cancer antigen 15.3 and cytoempryonic antigen through the concentrations of this markers in serums of breast cancer patients , and the statistical results in this study show no significant association between CA 15-3 and CEA in patients group (p= 0.185) , but moderate positive correlations is appeared (r = 0.2432), this result agreed with [30] who study on 70 Iranian women who observed a significant increased value of CA15-3 and CEA in patients group of breast cancer females as compared with healthy control females and no significant association between both biomarkers among patients group (p= 0.112). And as well agreed with [31] who study on 150 females with breast cancers who visited Ramses Medical Center in Narew and reached to no linear correlation was found between both CEA and CA15-3 and the other variables which showed a mild (R=0.57) linear relationship , Moreover, no linear correlation was found between the values of CA15-3and CEA (R=0.29) and no significant association (P=134). As well as our results matched with [32] who showed did not identify significant differences in CA 15-3 and CEA levels between different sites of metastasis in patients with breast cancer.CA 15-3 in combination with CEA is also relevant tumor markers in breast cancer , and the serum concentration of marker CA 15-3 has superior prognostic relevance in relation to CEA, but unlike these authors, [33] reported that prognostic value of CEA is higher than that of CA 15-3, which demonstrated that this marker has conflicting implications in breast carcinogenesis. [34] measurement of tumor markers is a tool for detection of distant metastases, and the marker CA 15- 3 seems more efficient when compared to CEA and no linear relation between it . Monitoring of breast cancer patients after surgical treatment using only this tumor markers is insufficient, however, simultaneous use of both serum markers (CA 15-3 and CEA) allows the early diagnosis of metastasis in up to 60–80% of patients with breast cancer [35]. A better understanding of the correlation between serum CA 15-3 and CEA levels and the intrinsic biological characteristics of breast cancer may affect the selection of adjuvant therapy, the combined measurement of both serum markers allows early diagnosis of metastases in up to 60-80% of breast cancer patients [36]. Elevated serum CA 15-3 and CEA levels at recurrence suggest increased tumor burden and may be prognostic for survival for metastatic breast cancer patients [37].

References:

1.JEMAL A, SIEGEL R,WARD E, HAO Y, XU J, THUN MJ, CA Cancer J Clin, 59 (2009) 225.

2. J. FERLAY, F. BRAY, P. PISANI AND D.M. PARKIN, GLOBOCAN 2002: Cancer Incidence, Mortality and PrevalenceWorldwide IARC CancerBase No. 5. Version 2.0, IARC Press, Lyon, 2004.

3. AMERICAN CANCER SOCIETY, Breast Cancer Facts & Figures 2009-2010, accessed 08.02.2010. Available from: URL: http://www.cancer. Org/acs/groups/content/@nho/documents/document/f861009final90809pdf.

4. JEMAL A, TIWARI RC, MURRAY T, GHAFOOR A, SAMUELS A, WARD E, CA Cancer J Clin, 53 (2003) 4. —

5. EUROPEAN GROUP ON TUMOR MARKERS, Tumour markers in breast cancer – EGTM recommendations, accessed 08.02.2010. Available from: URL: http://www. Egtm.eu/tumour_markers_in_breast_cancer.

6. HARRIS J, MORROW M, NORTON L, Malignant tumors of the breast. In: DEVITA JR VT, HELLMAN S, ROSENBERG SA (Eds) Cancer: Principles and Practice of Oncology (Lippincott-Raven, Philadelphia, 1997).

7. SWENSON LI, Progress in tumor marker research. In: LUMACHI F, BASSO SMM (Eds)

Serum tumor markers in breast cancer (Nova Science Publisher, 2007).
8. LUMACHI F, ERMANI M, BRANDES AA, BASSO SMM, BASSO U, BOCCAGNI P, Anticancer Res, 21 (2001) 4105.
9. Schreiber, R.D., Old, L.J. and Smyth, M.J. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*, 331: 1565-1570.
10. Jiang, X. and Shapirob, D. J. (2013). The immune system and inflammation in breast cancer. *Mol. Cell Endocrinol*., (Corrected proof).
11. Shpyleva S.I., Tryndyak V.P., Kovalchuk O., Starlard-Davenport A., Chekhun V.F., Beland F.A., Pogribny I.P. Role of ferritin alterations in human breast cancer cells. Breast Cancer Res Treat. 2011; 126(1):63-71.
12. Kumpulainen E.J., Keskikuru R.J., Johansson R.T. Serum tumor marker CA 15.3 and stage are the two most powerful predictors of survival in primary breast cancer. Breast Cancer Res Treat 2002;76:95-102.
13. Sheskin, D. J., (2004). Handbook of parametric and nonparametric statistical procedures. 3rd ed. Boca Raton: Chapman & Hall /CRC, 1-77.
14. Viera, A. J., (2008). . South Med J., 101 (7): 730-734.
15. Maric P, Ozretic P, Levanat S, Oreskovic S, Antunac K, Beketic-Oreskovic L. Tumor markers in breast cancer—evaluation of their clinical usefulness. Coll Antropol 2011; 35:241–247.
16. Nicolini A, Carpi A, Ferrari P, Pieri L: Utility of a serum tumour marker panel in the post-operative follow-up of breast cancer patients with equivocal conventional radiological examinations. Tumor Biol. 2003, 24: 275-280. 10.1159/000076458.
17. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin 2014; 64:52–62.
18. Lee JS, Park S, Park JM, Cho JH, Kim SI and Park BW: Elevated levels of serum tumor markers CA 15-3 and CEA are prognostic factors for diagnosis of metastatic breast cancers. Breast Cancer Res Treat 141: 477-484, 2013.
19. Wu SG, He ZY, Zhou J, Sun JY, Li FY, Lin Q et al. Serum levels of CEA and CA15-3 in different molecularsubtypes and prognostic value in Chinese breast cancer. Breast 2014; 23:88–93.
20. Molina R, Barak V, van Dalen A, Duffy MJ, Einarsson R, Gion M et al. Tumor markers in breast cancer- European Group on Tumor Markers recommendations. Tumour Biol 2005; 26:281–293.
21. United Kingdom Office for National Statistics. *Cancer Survival in England: Patients Diagnosed 2007–2011 and Followed Up to 2012*; United Kingdom Office for National Statistics: Newport, UK, 2013.
22. Sharan SK, Pyle A, Coppola V, Babus J, Swaminathan S, Benedict J, Swing D, Martin BK, Tessarollo L,Evans JP, Flaws JA, Handel MA. BRCA2 deficiency in mice leads to meiotic impairment and infertility. Development. 2004;131(1):131-42.
23. Moline S., Nritschoy A, Shirja H, Chan DW: Tumor Markers, Physiology, Pathobiology, Technology and Clinical Applications. Washington, AAVC Press, 2012, pp 148–169.
24. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, et al. American Society of Clinical Oncology 2007 Update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol. 2007;25(33):5287-312.
25. Maric P, Ozretic P, Levanat S, Oreskovic S, Antunac K, Beketic-Oreskovic L. Tumor markers in breast cancer—evaluation of their clinical usefulness. Coll Antropol 2011; 35:241–247.
26. Kumar R, Kumar AN, Srivastava A. Breast cancer tumor markers. J Solid Tumor 2012;2:43-46.
27. Hashim ZM. The significance of CA15-3 in breast cancer patients and its relationship to HER-2 receptor status. Int J Immunopathol Pharmacol 2014; 27:45–51.
28. Jager R, Jhony S, Lili B, Alex S: Serial CEA and CA 15.3 during follow- up of breast cancer patients. Anticancer Res 2013; 30: 2139–2182.
29. Di Gioia D, Heinemann V, Nagel D, Untch M, Kahlert S, Bauerfeind I et al. Kinetics of CEA and CA15-3 correlate with treatment response in patients undergoing chemotherapy for metastatic breast cancer (MBC). Tumour Biol 2011; 32:777–785.
30. Mousavi S, Karemi M, Hassani M, Nahres A, (2011). Study of breast cancer in Iran: a study of the Tehran population based cancer registry. *Asian Pac J Cancer Prev*, 18, 3121-2341.
31. Moline B, Kaber C, Zaman G *et al*: The evaluation of tumor markers (CEA and CA 15.3) in postoperative patients with breast cancer. Anticancer Res *25*: 1033-1040, 2013.
32. Yerushalmi R, Tyldesley S, Kennecke H, *et al*: Tumor markers in metastatic breast cancer subtypes: frequency of elevation and correlation with outcome. Ann Oncol 23: 338-345, 2012.
33. Ebsani G., Sabrena R., Komech X., Manowel H.: Serum CEA and CA 15-3 as prognostic factors in patients with metastases breast cancer. N. KCV. Cancer 2012; 76: 1417–1312.
34. Bruna Karina Banin Hirata, JulieMassayo Maeda Oda, Roberta Losi Guembarovski, Carolina Batista Ariza, Carlos Eduardo Coral de Oliveira, and Maria Angelica Ehara Watanabe. Molecular Markers for Breast Cancer: Prediction on Tumor Behavior, "Disease Markers".Volume 2014, Article ID 513158, 12 pages.
35. Meyer UA. Pharmacogenetics and adverse drug reactions. Lancet. 2000 Nov 11; 356(9242):1667-71.

36. BIAO GENG1, MAN-MAN LIANG2, XIAO-BING YE1 and WEN-YING ZHAO1. Association of CA 15-3 and CEA with clinicopathological parameters in patients with metastatic breast cancer. MOLECULAR AND CLINICAL oncology. 3: 232-236, 2015.
37. Lee JS, Park S, Park JM, Cho JH, Kim SI, Park BW. Elevated levels of preoperative CA 15–3 and CEA serum levels have independently poor prognostic significance in breast cancer. Ann Oncol 2013; 24:1225–1231.