

Assessment of Immunohistochemical Expression of CEA among Female with Invasive Ductal Breast Carcinoma by using Manual Tissue microarray Technique

Abdalraheem Ali Babiker¹, Amel Hassan Babeker¹, Elhadi A. Ahmed², GadAllah Modawe³

1- Department of histopathology and cytopathology Faculty of Medical Laboratories Sciences, University of Gezira, Wad Medani, Sudan

2- . Faculty of Medical Laboratories Sciences, University of Gezira, Wad Medani, Sudan

3. Omdurman Islamic University, Faculty of Medicine and Health Science, Department of Biochemistry, Omdurman, Sudan

Abstract : *Background: Immunohistochemistry is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions. CEA marker is one of the successful tumor markers in diagnosis of adenocarcinoma eg (breast cancer) in plasm and rarely applied in tissues. Objective: To assess of CEA among female with invasive ductal breast carcinoma by using manual tissue microarray technique Methods: Retrospective study was conducted at Alrahmah center, Khartoum state in the period from June to October 2021. Thirty-five were examined using immunohistochemistry technique. The data was analyzed using SPSS version (25). Results: In the results patient age ranged from 30 to 75 years, grade two breast cancer patients were equal 31.4% (n=11/35) while grade three was equal 68.6% (n=24/35). 85.7%(n=30/35) of examined tissues gave positive result for CEA marker while negative results 14.3% (n=5/35). There was no relation between grade , age and scoring because value of sig=.892 great than .05, and very weak correlate.CEA marker was appearance positive result in grade two and grade three of result. Conclusion: The study found no relation between age and IHC score . TMA can facilitate the IHC staining procedure and interpretation of result. CEA marker is not common using in breast cancer but appearance positive result in grade two and grade three of result, and given 85.7% result positive. Further studies should be done with large sample size to assessment of CEA in breast cancer by using IHC in different cancer grade .*

Key words: breast cancer , CEA , IHC ,TMA , Sudan ,IDBC

Introduction

The breast is a bilateral organ that in the female undergoes dramatic changes in size ,shape, and function in association with infantile growth, puberty, pregnancy, lactation, and postmenopausal regression (Russo IH *et al*,1998) . Breast cancer is the most common cancer occurring in women, with over 200,000 new cases diagnosed yearly in the United States alone, and it is the second leading cause of cancer-related deaths in women. Breast cancer and benign breast tumors can also rarely occur in men, adolescents and children. Breast cancer consists of a heterogeneous group of diseases, with many different subtypes of both invasive and non-invasive cancers that have different treatment options and prognoses. The diagnosis of breast cancer is increasing, in part due to early detection of disease through imaging such as screening mammography. Many women undergo breast biopsies to evaluate whether a radiologic abnormality is due to a benign or neoplastic (cancerous) process.(Goss, *et al*2011)Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, prior history of breast cancer, and family history. Breast cancer is a worldwide disease resulting in many deaths. Although breast cancer incidence is lower in Sub-Saharan African countries than in developed countries, African women are more likely than women in the developed world to be diagnosed at later stages of the disease and, thus, are more likely to die from it. This is due to the lack of awareness by women, accessibility to screening methods, and availability of African-based research findings that would influence decision making at the governmental level .(Lauby,Secretan 2011). CEA is one of the oncofetal antigens that is produced by the normal fetal cells and can be produced in appreciable amounts by some malignant, but not normal adult cells. The presence of such antigens is thought to be associated with behavior common to both fetal and malignant cells, enabling them to grow rapidly, spread, and escape destruction by the response of host tissue. Since its discovery, CEA has been evaluated in a wide range of malignancies, including breast cancer, and historically, it has been considered the standard to which new serum markers are compared. (Wang WSetal,2005).Tissue microarray (TMA) approach is a high throughput technology that enables analysis of multiple molecular targets simultaneously without causing any morphological alteration to tissue specimens. This high-throughput technique facilitates analysis of very large numbers of tumors at once, either at the DNA, RNA, or protein level. History of Tissue Microarray technology:The TMA technology had its beginning in 1986, when Hector Battifora introduced a 'sausage' block method. In this method 1-mm thick 'rods' of tissue, obtained from different specimens, were wrapped carefully in a sheet of small intestine. This sausage of tissue was embedded in a normal-sized paraffin block, which was then called the 'multi tumor tissue' block. This method was designed for immunohistochemical control and standardization between laboratories (Juhan , Kononen *etal* 1998). the objective of present study was to assess of CEA among female with invasive ductal breast carcinoma by using manual tissue microarray technique

Material and Method

This is retrospective cross section study, aimed to evaluation of CEA as diagnostic marker in breast cancer by using TMA was conducted at ELrahma ,Khartoum,Sudan , during the period from June to October 2021. among Sudanese patients womens with IDC.

Sample size Tissue blocks obtained from (35) samples were previously diagnosed as breast cancer was taken from patients with breast cancer in Khartoum-bahri ,Alrahma Center.

Inclusion criteria: Patients women of breast cancer IDC from different age .

Exclusion criteria: Women have not breast cancer

Datacollection: Patients identification information (age, gender, histopathological diagnosis, and malignant tumor grade) the available demographic, data according to the laboratory guidelines, were obtained from patients records using master sheet, (IDC was detected by TMA.

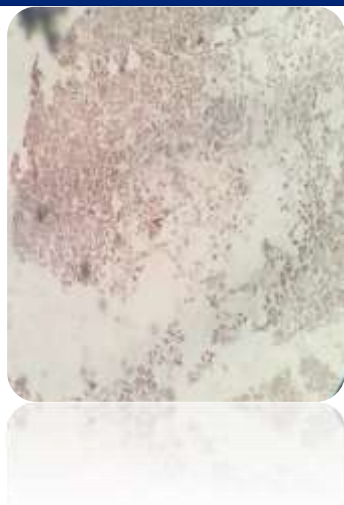
Data analysis: The obtained results and variables arranged in standard master sheet, then analyzed using statistical package for social science SPSS version (25). computer program. Frequencies, Means and Chi squire tests were calculated and presented in form figure and table.

Ethical considerations: This study were approved by faculty of medical approval permission department of histopathology and cytopathology. Permission this approve research committee from gezira university from Elrahma Medical Lab administration.

CEA Prepration: Applications and suggested dilutions: Immune precipitation (native and denatured) use protein A) (Ab 10 μ l/mg protein lysate) ,western blotting (Ab 1:200 for 2hrs at RT), immune histology (Formalin/paraffin) (Ab 1:500-1:1000 for 30 min at RT), (no special pretreatment is required for the immune histochemistry staining of formalin / paraffin tissues.) The optimal dilution for a specific application should be determined by the investigator. Cellular localization: cytoplasmic and luminal membrane ,in breast have been used to help monitor metastatic breast cancer .Storage and stability: store vial at 4°C. When stored at 2-8°C, this antibody is stable for 24 months .Supplied as: purified antibody fraction from rabbit anti serum prepared in 10mM PBS, pH 7.4, with 0.2% BSA and 0.09% sodium aside, pre diluted antibody which is ready-to-use for staining of formalin-fixed, paraffin-embedded tissues (Bergeron A; et al,1996).

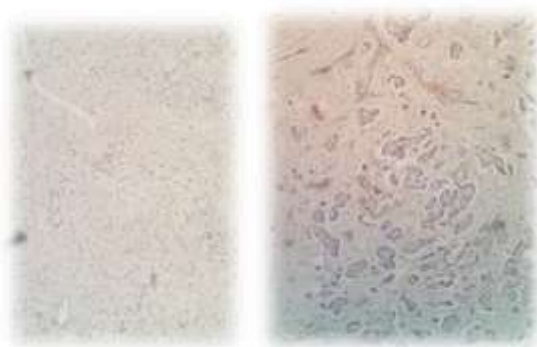
Methods: The immunohistochemical procedure was done as follows: one section (3 μ m) from formalin-fixed, paraffin-embedded tumors were cut and mounted onto salinized slides. Following de paraffinization in xylene, slides were rehydrated through a graded series of ethyl alcohol 100% 4 min, 90% 2 min, 70% 2 min , and water 2 min) and were placed in distilled water. Samples were steamed for antigen retrieval for section using high PH (0.9) by water bath at 95C for 40 min. After washing with PBS for 3 min Endogenous peroxides activity were blocked with 3% hydrogen peroxide and methanol for 10 min, and After washing with PBS for 3 min then Slides were incubated with (100 μ L) of mouse monoclonal antibody , for 30 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibodies will be detected by incubating for 20 min with dextrin labeled polymer. Finally, the sections washed in three changes of PBS, followed by adding 3, 3 di amino benzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distal water for 3 min Slides were counterstained with Mayer 's haematoxylin for one min were washed in running tap water for several minutes 7-10 (bluing), then dehydrate and, cleaned, mount in DPX. The diagnosis of breast cancer was established depending on clinical examination and histopathological features of the biopsy Investigator then the results were confirmed by consultant histopathologist.

Result Interpretation : Expression of CEA marker show cellular localization of: cytoplasmic and luminal membrane brown color in positive result ,in breast have been used to help monitor metastatic breast cancer.



(A) Photo light microscopic showing IDC, IHC using CEA marker strong positive ++++(10x)

(B) Photo light microscopic showing IDC, IHC using CEA marker strong positive +++(10x)



(C&D) Photo light microscopic showing IDC, IHC using CEA marker

negative (10x)

Results and Discussion

Results :

Table (1): distribution of study population according to their age frequency

Age	Frequency	Percent
30-45	17	48.6%
46-60	14	40%
61-75	4	11.4%
Total	35	100%

This table shows the majority (48.6%) of the study sample where ("30-45").

Table (2): distribution study population of according to their TMA

TMA	Frequency	Percent
CEA/Positive	30	85.7%
CEA/Negative	5	14.3%
Total	35	100%

This table shows the majority (85.7%) of the study sample three (CEA/Positive).

Table (3): distribution of study population according to their Tumor grade

Tumor grade	Frequency	Percent
Two	11	31.4%
Three	24	68.6%
Total	35	100%

This table shows the majority (68.6%) of the study sample (three).

This table shows the majority (85.7%) of the study sample where (CEA/Positive).

Table (4): Co relation between Grade and Age.

Chi-Square	Correlation	P.value
.228	- 0.02-	.892

This table shows the No Relation between Grade and Age because value of sig=.892 great than .05, and very weak correlate.

Table (5): co- relation between TMA and Age.

Chi-Square	Correlation	P.value
3.895	-.183-	.143

This table shows the No Relation between TMA and Age because P.value (.143) greater than Significance level (.05) and very weak correlated.

Table (6): corelation between Score and Age.

Chi-Square	Correlation	P.value
8.618	-.270-	.196

This table shows the No Relation between Score and Age because P.value (.196) greater than Significance level (.05) and very weak correlated.

Discussion:

CEA is one of the oncofetal antigens that is produced by the normal fetal cells and can be produced in appreciable amounts by some malignant, but not normal adult cells.⁽⁹⁾

(Wang WSet *al* 2005).A number of markers have been investigated in patientswith breast cancer including CEA, CA 15-3 and related, and c-erbB-2,here 2 p53oncoprotein, but it is generally agreed that CEA tumor markers in breast cancer is not common using in breast cancer but in my this study used CEA in IDC it was good result 85.6% positively in 35 patient. In the results patient age ranged from 30 to 75 years, grade two breast cancer patients was equal 31.4% (n=11/35) while grade three was equal 68.6% (n=24/35). 85.7% (n=30/35) of examined tissues gave positive result for CEA marker while negative results 14.3% (n=5/35). There was no relation between grade , age and scoring because value of sig=.892 great than .05, and very weak correlate.

Several studies have reported that positive serum CEA levels at the time of primary breast cancer diagnosis may represent a negative prognostic parameter .According to (Guadagnet *al* 2001). They also reported increase in CEA levels in case of recurrence of the disease and metastases. Pathak et al. observed that following successful treatment, serum CEA levels fellsignificantly below pre-treatment level.

(Duffy *et al*2002¹ , found potential use of serum CEA in early diagnosis of breast cancer, determining its prognosis, prospectively predicting response and resistance to specific therapies, surveillance after primary surgery, and monitoring therapy in patients with primary disease.Walker demonstrated inSerum carcinoembryonic antigen as a tumor marker in breast cancerstudy that serum CEA

is related to good histological differentiation of breast cancer but not to the histological type of breast cancer.(Faridi, et al 2006)(Agrawalet al2002). also found in their study that there was no significant correlation of serum CEA to the histological type of breast cancer.In our study CEA detect degree of breast cancer ,and H&E it detect type of breast cancer. Similar results were found in our study and there was no correlation of the histological type of breast cancer with the serum CEA .In our study out of 35cases, 30(85.7%) patients were breast cancer positive and 5(14.3%) were negative. Serum CEA values in IDC-positive cases were significantly higher than in negative cases⁽¹¹⁾.(Ghaffariet al2000).

According to some studies, serum CEA marker is not common using in breast cancer but appearance positive result in grade two and grade three of result,and given 85.7% result positive in 35 patients of IDC .

Conclusion and recommendation:

Conclusion

CEA marker is appearance positive result in grade two and grade three of result,and given 85.7% result positive.The study found no relation between TMA , age and score,IHC are good technique in histopathologyand good interpretation of result.

Recommendation

_The study recommended using big sample size to give better result and good study.

_Further studies should bedone to assessment of CEA in breast cancer by using IHC.

Reference

Al Kuraya K, Simon R, Sauter G (2004). *Tissue microarrays for high-throughput molecular pathology*. Ann Saudi Med **24**: PP 169-74.

Al-Alwan M, Olabi S, Ghebeh H, Barhoush E, Tulbah A, Al-Tweigeri T(2011). Fascin is a key regulator of breast cancer invasion that acts via the modification of metastasis-associated molecules ,PLos One,; 6(11):e27339

Alizadeh AA, Ross DT, Perou CM and van de Rijn M. (2001.,**195**,pp 41–52.

Bancroft JD, (2008). *Theory and practice of histological techniques*. 6th Edition,ElsevierPhiladelphia .

Barlund M, MonniO,Kononen J, Cornelison R, Torhorst J,(2000).Cancer Res.,**60**,pp 5340–5344

Bocker W, Hungermann D, Decker T (2009) *Anatomy of the breast*. Pathologe 30:612.

Böcker W, Moll R, Poremba C, Holland R, Van Diest PJ, Dervan P, ,et al,(2002), *Common adult stem cells in the human breast give rise to glandular andmyoepithelialcelllineages:anewcellbiologicalconcept*,Lab Invest**82**(6):pp737–746.

Hirsimaki P.(2011); *MMP-1 expression has an independent prognostic value in breast cancer BMC Cancer*,11:348 ,**61**:pp 69–90.

Camp, R. L., Charette, L. A., and Rimm, D. L. (2000) *Validation of tissue microarrayechnology in breast*,Med.**8**, 1285–9.

Camp, R. L., Chung, G. G., and Rimm, D. L. (2002) *Automated subcellular localization and quantification of protein expression in tis-sue microarrays*. Nat Med.**8**, 1323–7.

Carmichael AR,Mokbel K.(2002) Evolving Trends in Breast Surgery: Oncoplastic to Onco-Aesthetic Surgery.

Going JJ, (2006). Human breast duct anatomy, the 'sick lobe' *hypothesis and intraductal approaches to breast cancer*. Breast Cancer Res Treat. **97**(3):285-915ast carcinoma. Lab Invest.**80**,pp 1943–9.

Goss,P.E.(2011).Exemestane for Breast-Cancer Prevention in Postmenopausal Women.New England Journal of Medicine**364**(25): p. 2381-2391.

Gown AM.(2002) Genomic immunohistochemistry: *a newer a in diagnostic immunohistochemistry*.CurrDiagnPathol; 193-200.

Graham, A. D., Faratian, D., Rae, F., and Thomas, (2008) Tissue microarray tech- a prospective study of the use of immunohistochemistry and fluorescence in situ hybridization. Histopathology**52**, 847–55..

Guarneri V, Conte PF (2009)*Metastatic breast cancer: therapeutic options according to molecular subtypes and prior adjuvant therapy* , The Oncologist; 14:645 – 656 .

HarrisJR, Lippman ,MorrowM, Osborne CK,(2012).Diseasesofthe breast, 4th edition. Philadelphia (PA), USA: Lippincott Williams & Wilkins 16:345 – 456 .

Histochem(2014)*carcinoma*. Ann DiagnPathol. **15**(2):pp84-92,

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2000):Global cancerstatistics.(; 39: pp741-748TheLancet.;383 (9922):1041 - 1048.

Jordan RCK, Daniels TE, Greenspan JS,Regezi JA.(2002) Advanced diagnostic methods in oral and maxilla ofacial pathology. Pathol; **93**(1): p56-74.

Kumar V, Abbas AK, Fausto N. Robbins and Cotran (2005).pathologic basis of disease. 7th ed. Philadelphia: Elsevier Saunders,84: 185-93.

Lauby-Secretan B, Scoccianti C, LoomisD, Benbrahim-TallaaL, Bouvard V, BianchiniF, et al.; International Agency for Research on Cancer Handbook Working Group (2015). *Breast-cancer screening–viewpoint of the IARC Working Group*. N Engl J Med. **372**(24):p2353–8.

Lee JS; ActaCytologica, (1996), 40: 631-6.models. Biotech Histochem

Nocito A, Kononen J, Kallioniemi OP, Sauter G (2001). Tissue microarray(TMAs) for high-throughput molecular pathology research. Int J Cancer 94: p 1-5.

O'Hurley G, Sjostedt E, Rahman A, (2014) Garbage in, garbage out: acritical evaluation of strategies used for validation of immunohistochemical biomarkers. MolOncol; 8: p783-98.

Parkin DM, FernándezLMG(2006). Use of statistics to assess the global burden ofbreastcancer. *Breast J.* **12**(S1):ppS70–80.

Peppercorn J, Perou CM, Carey LA, (2008);. *Molecular subtypes in breast cancer evaluation and management: divide and conquer*, Cancer Invest p 26:1 – 1..

Ramos-Vara JA. (2005) *Technical aspects of immunohistochemistry*. Pathol; 42:p405-426.

Rusby JE(2007): Breast duct anatomy in the human nipple: three dimensional patterns and clinical implications. Breast Cancer Res Treat pp 77–93 .

Russo J, Russo IH (1987) Development of the human mammary gland. In: Neville MC, Daniel C (eds) The mammary gland. Plenum, New York, pp 67–93 .

Russo J, Russo IH (1998) *Development of the human breast*. In: Knobil E, Neill JD (eds) Encyclopedia of reproduction. Academic, New York. pp 120-140

Shi SR, Key ME, Kalra KL. (1991) Antigen retrieval in formalin fixed 2009; 84: 185-93.

Wang WS, Lin JK, Lin TC, Chiou TJ, Liu JH, Yen CC, *et al* (2005). Plasma von Willebrand factor level as a prognostic indicator of patients with metastatic colorectal carcinoma. World J Gastroenterol; 11: pp2166-70.

Weisenburger DD, Armitage JO, Warnke R, Staudt LM *et al.* (2000). Nature, 403, 503–

welves D (2012): *The anatomy of fluid-yielding ducts in breast cancer*. *Breast Cancer Res Treat.* **132**(2) pp:555-64

welves D (1998): The anatomy of fluid-yielding ducts in breast cancer. *Breast Cancer Res Treat.* **132**(2):555-64.

Wester K, Wahlund E, Sundström C, (2000) Paraffin section storage and immunohistochemistry Appl Immunohistochem Mol Morphol; 8: p 61-70.

Wilhelm. , (1992) *Int J Biol Markers*, **7**(3): pp148-53.