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Immunohistochemical expression of Cytokeratin 17 in Histological section of Sudanese Female with breast Cancer

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Abstract:

Background:- *Cytokeratin 17 is the main product of breast cancer in grading in this study we collect 40 patient of breast cancer-Invasive ductal carcinoma from RICK (Radio Isotope Center of Khartoum) using IHC (Immunohistochemistry) and our results show highly significant of P. =0.001and highly association of estrogen ER, progesterone PR receptor , Her2 and Cytokeratin 17(CK17) of 95 % of confidence and association between ER ,PR ,and Her2 in the gradient of expression in grade 3 with 30.8% meaning that the association and significant are good .*

Methods:- *paraffin embedded sections were studies retrospectively from RICK in Khartoum state (Sudan)*

Immunohistochemistry were done in 40 samples of patients with breast cancer Cytokeratin 17 including ER, PR and Her2.

Results: *Using of 40 samples invasive ductal carcinoma, which was determined using subtype was typically immunohistochemical grade and estrogen receptor ER and progesterone receptor PR and HER2 and basal Cytokeratin 17,. Using breast carcinoma tissue IHC representing 40 patients with 49.8 year mean basal Cytokeratin 17 expression was associated with low disease-specific survival.*

Conclusions*: to detect CK 17 in tissue sections, suitable staining procedure and careful examination will increase sensitivity. The immunohistochemistry is method of choice.*

Key words: Cytokeratin 17, Estrogen Receptor, Progesterone Receptor, Invasive ductal carcinoma, grading, Immunohistochemistry

INTRODUCTION:

Breast cancer is the most commonly diagnosed cancer in women worldwide, and in the second only to lung cancers leading cause of cancer death. African women are more likely than women in developed world to be diagnosed at latter stages of disease and more likely to die from it, this is due to the lack of awareness by women the most people detected in late stage (stage III) Because absence of screening program. Cytokeratin 17 is expressed we can show latter stage of carcinoma in the breast If the stage of CK 17 appear we follow this stage not transfer to me tastes Invasion carcinoma[5][6][9]. The incidence rate of breast cancer (25.1 per 100,000) [1], was substantially higher than the other primary cancer sites. The most common primary. Cytokeratin 17 (CK 17) studies on breast tumors have identified distinct subtypes of breast carcinomas that are associated with different clinical outcomes [1, 9]. Using an intrinsic set on genes. [1] Analyzed the expression profiles of 40 independent breast tumor samples and categorized breast

tumors into four groups: basal CK 17; estrogen receptor (ER); Progesterone receptor (PR); epidermal growth factor receptor (HER2) over expressing. spanning three independent data sets, breast cancers of the basal subtype comprised 19% of the tumors and had poor prognoses as assessed by relapse-free survival [1– 2]. Therapies targeting the ER or HER2 oncogene would not be expected to be effective on basal breast cancers because this subtype typically expresses neither of these proteins. Although diagnostic antibodies that work in formalin-fixed, paraffin-embedded archival commercial antibodies to Cytokeratin 17 are available. The prevalence and poor prognosis of basal breast cancers has been validated immunohistochemical on a 40 case tissue IHC with few month mean outcome data using overall survival as an end point [14]; 16% of tumors in cross section stained. positive for Cytokeratin 17., another independent IHC study of basal Cytokeratin expression and related immunohistochemical markers was published. In this study, breast cancers that were Cytokeratin 17 positive were found to be associated with expression of the epidermal growth factor receptor (HER1) [7][8]. In our study, the basal subtype, as defined by Cytokeratin 17expression by immunohistochemistry (IHC), was also found to be common among breast cancer patients with hereditary. BRCA1 mutations [12]. Basal breast cancers represent a poorly characterized subtype of tumor with no validated clinical assay to identify them[11]; therefore, in this report, we first improved our immunohistochemical definition of basal breast cancers by comparison to our gene expression data. Next, based on across sectional, we show that the basal breast cancer tumors show poor disease-specific survival times and show that HER1 expression is a marker that helps to distinguish basal breast cancers. These data identify a simple set of IHC markers that can be routinely used in the clinical setting to accurately identify basal breast cancers.

OBJECTIVES:

General objective:

To correlate between immunohistochemistry (IHC) expression of Cytokeratin 17 and histological grading of invasive ductal carcinoma of breast cancer among Sudanese female.

Specific objective:

1-To assess the presence of CK 17 in barest cancer affected tissue

2-To estimate the grade of marker expression

MATERIAL AND METHODS:

Study design:- Retrospective cross sectional hospital basal study

Study area:- (RICK) radio isotope centre at Khartoum.(Sudan)

Study population: Tissue block in invasive ductal carcinoma from female affected by breast cancer at (RICK), May 2015.

Sample size:- Forty samples of IHC collected from RICK hospital.

Protocol:-

The immunohistochemical procedure will be done as follows:

Sections (3μm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto Stalinized slides (Fisher brand). Following deparaffinization in xylene, slides will be rehydrated through a graded series of alcohol and will be placed in running water. Samples will be steamed for antigen retrieval forck17 using PT link. Briefly, slides will be placed in slide tank containing enough sodium citrate buffer (pH 9.0) to cover the sections, then will be boiled at high Temp for 20minutes then will allow sections to cool at RT. Endogenous peroxidase activity will be blocked with 3% hydrogen peroxidase and methanol for 10 min, then Slides will be incubated with 100-

200 µl of primary antibodies for 20 min at room temperature in a moisture chamber, and then will be rinsed in Phosphate buffer saline. The primary antibody ck17, (monoclonal)will be ready to use (Thermo). After washing with PBS for 3 min, binding of antibodies will be detected by incubating for 20 minutes with dextran labeled polymer (Thermo kit).Finally, the sections will be washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine 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. Slides will be counterstained with haematoxylin. For each run of staining, positive and negative control slides will be also prepared. The positive control slides will contain the antigen under investigation and the negative control slides will be prepared from the same tissue block, but will be incubated with PBS instead of the primary antibody. Each slide will be evaluated with investigator Positive ck17staining will be identified in form of brown membranous staining. The obtained results and variables will be arranged in standard master sheet, then will be entered a computer program SPSS and analyzed.

Ethical Consideration:-

*Consent will be obtained from all participants and /or their guardians .The approval to carry out this study will be obtained from university research committee of collage of medical laboratory science Alneelian University.

*Approval should be taken from ministry of health and related hospitals.

*The objectives of the study should be explained to the participants in simple clear words.

*Voluntary consent will be obtained from participants .

*Any participants has to a right to withdraw at any time.

* Any participants has a right to benefit and no harm.

Statistical analysis:- Data were analyzed using SPSS software package (version 16 for windows 7).

RESULTS:

Using of 40 samples invasive ductal carcinoma, which was determined using subtype was typically immunohistochemical grade and estrogen receptor ER and progesterone receptor PR and HER2 and basal Cytokeratin 17,. Using breast carcinoma tissue IHC representing 40 patients with 49.8 year mean basal Cytokeratin 17 expression was associated with low disease-specific survival.

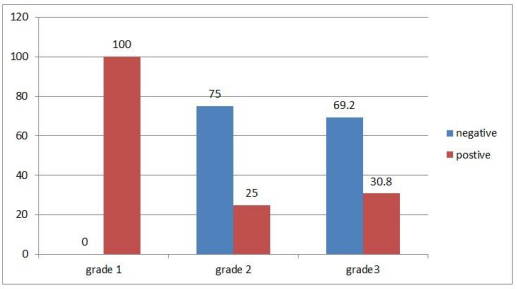
Table (1) figure (1):-Summarize is expression of CK17 and tumor grade , classified grade (I) and grade (II) and grade (III) ,grade (I) CK17 –ve (0 OF 27) (0%)and CK17+(2 of 13) (100%) and grade II of CK17 –ve (9 OF 27) (75%) and CK17+(3 of 13) (25%) ,grade III with CK17-ve (18 of 27) (69.2%) and CK17+ve (8 of 13) (30.8%). Histological grade could not be used in this multivariate analysis because accurate grading information was unavailable for the majority of cases.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
AGE	40	24,00	90,00	49,8500	14,09410
Valid N (list wise)	40				

GRADE * CYTO17 Cross-tabulation

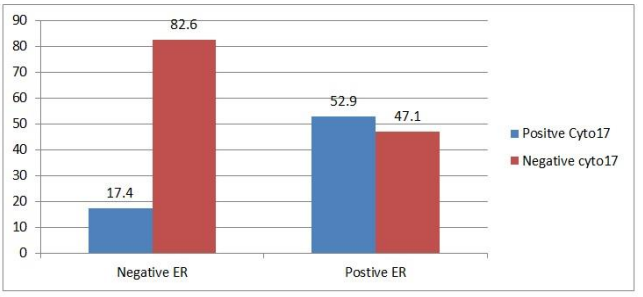
			CYTO17		Total
			negative	positive	
GRADE	grade 1	Count	0	2	2
		% within GRADE	,0%	100,0%	100,0%
	grade 2	Count	9	3	12
		% within GRADE	75,0%	25,0%	100,0%
	grade 3	Count	18	8	26
		% within GRADE	69,2%	30,8%	100,0%
Total		Count	27	13	40
		% within GRADE	67,5%	32,5%	100,0%



In table 2 (figure 2) :- compression between CK-17 and ER the following results are show as if ER- CK17 of (N=27) 19 patient are negative with 82.6% and (4 of 13) are positive with 17.4% , where ER+,CK17 8 patient(47.1%) are negative out of 27 and 9 patient(52.9%) of CK17 are positive out of 13 patient.

ER * CYTO17 Cross tabulation

			CYTO17		Total
			negative	positive	
ER	negative	Count	19	4	23
		% within ER	82,6%	17,4%	100,0%
	positive	Count	8	9	17
		% within ER	47,1%	52,9%	100,0%
Total		Count	27	13	40
		% within ER	67,5%	32,5%	100,0%

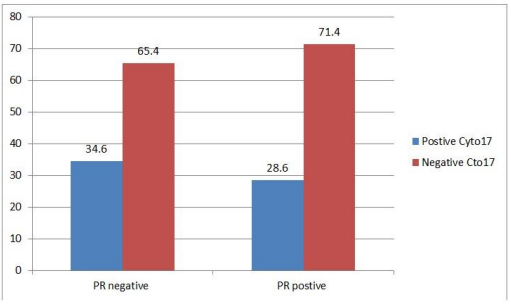


In table 3 (figure 3):- in this table we summarize the PR and CK17, in PR negative the CK17 17 patient with 65.4% out of 27 are negative and 9 patient out of 13(34.6%) are positive of

CK17, where PR positive the CK17 10 patient (71.4%) are negative out of 27 patient and 4(28.6%) are positive out of 13 patient.

PR * CYTO17 Cross-tabulation

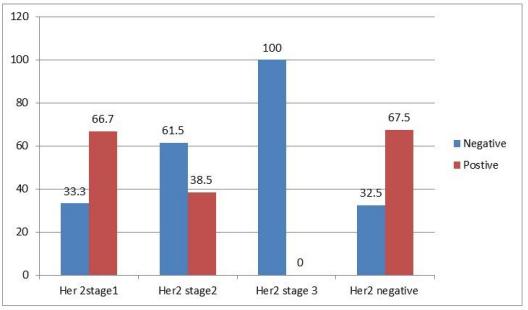
			CYTO17		Total
			negative	positive	
PR	NEGATIVE	Count	17	9	26
		% within PR	65,4%	34,6%	100,0%
	POSITIVE	Count	10	4	14
		% within PR	71,4%	28,6%	100,0%
Total		Count	27	13	40
		% within PR	67,5%	32,5%	100,0%



In table 4(figure 4):- HER2 (type1) expression was observed in 33.3% of 19 cases 2 are negative for basal Cytokeratin 17 is and 4 patient(66.7%)out of 19 are positive. In type 2 ,8 patient with percentage of (61.5%) are negative and 5 patient with (38.5%) are positive. In type 3 (100%)of 9 patient are negative , with no associated with poor survival independent of stage 3 of Her2(0) where CK 17 negative than in other breast cancers but did not influence prognosis. Association of the Basal-Like Subtype with HER2 and basal breast cancers immune-profile was expression of HER2 positive On the current HER2 expression was significantly less common among the basal Cytokeratin negative cases (40 of 40) of 100%,with *P.* 0.001 by the test of IHC.

HER2 * CYTO17 Cross tabulation

			CYTO17		Total
			negative	positive	
HER2	Type 1	Count	2	4	6
		% within HER2	33,3%	66,7%	100,0%
	Type 2	Count	8	5	13
		% within HER2	61,5%	38,5%	100,0%
	Type 3	Count	9	0	9
		% within HER2	100,0%	,0%	100,0%
	negative	Count	8	4	12
		% within HER2	66,7%	33,3%	100,0%
Total		Count	27	13	40
		% within HER2	67,5%	32,5%	100,0%



One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
GRADE	40	2,6000	,59052	,09337
CYTO17	40	1,3250	,47434	,07500

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
GRADE	27,846	39	,000	2,6000	2,4111	2,7889
CYTO17	17,667	39	,000	1,3250	1,1733	1,4767

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
CYTO17	40	1,3250	,47434	,07500
ER	40	1,4250	,50064	,07916

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
CYTO17	17,667	39	,000	1,3250	1,1733	1,4767
ER	18,002	39	,000	1,4250	1,2649	1,5851

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
CYTO17	40	1,3250	,47434	,07500
PR	40	1,3500	,48305	,07638

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
CYTO17	17,667	39	,000	1,3250	1,1733	1,4767
PR	17,676	39	,000	1,3500	1,1955	1,5045

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
CYTO17	40	1,3250	,47434	,07500
HER2	40	2,6750	1,07148	,16942

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
CYTO17	17,667	39	,000	1,3250	1,1733	1,4767
HER2	15,789	39	,000	2,6750	2,3323	3,0177

CONCLUSIONS:

A panel of four antibodies (ER, PR ,HER2, and Cytokeratin 17) can accurately identify basal tumors using standard available clinical tools and shows high specificity. These studies show that many basal- tumors express CK17, in these patients. In this study of tumors (ER and PR tumors with HER2 expression) were not statistically different, suggesting that poorly differentiated carcinomas with hormone receptors correspond to the basal type of tumor. Among poorly differentiated breast carcinomas, the classic profile associated with basal-CK identifies distinct subtypes equivalent to those seen by genetic classification. CK -17 with ER- with (N=27) where(19 of 27) 82.6% and , ER + CK17 are (4 of 13) 17.4% are positive ,ER+ (N=13) CK17 + is positive about (9 of 13) 52.9% suggesting that poorly differentiated carcinomas with hormone receptors correspond to the basal type of tumor. Among poorly differentiated breast carcinomas, the classic profile associated with basal-CK identifies distinct subtypes equivalent to those seen by genetic classification. The overall frequency of basal-like subtype (ER-/ PR-/ Her-2 / basal CK+) was only 10% of 113 cases examined at the Histopathology and Cytopathology Department at RICK. This frequency is low compared to breast cancer cases from East and West Africa but much higher than the frequencies reported for Caucasian and African-American women in the United States of America [(3/4)]. Furthermore, in a previous study about 75% and 55% of breast cancer cases from women (n = 40) presented at Khartoum Teaching Hospital during the period of 2000–2001 were estrogen receptors-positive and progesterone receptors-negative, respectively [(12).] Recently, few studies have attempted to identify biomarkers for breast cancer in Sudanese women [(10)(12)]. High levels of Se, Zn, and Cr elements in breast cancer cases (N = 40) compared to matched normal breasts (N = 40) were suggested as candidate markers

for early detection of breast cancer [13]. Preliminary proteomic examination of Sudanese breast cancer and normal tissues ($n = 24$) identified Peroxiredoxin V (PrdxV) protein as differentially expressed in tumor tissues. Immunohistochemistry analysis of tumor ($n = 77$) and control ($n = 68$) tissues revealed that PrdxV protein was not expressed in 88.3% of breast cancer and majority of control tissues. Loss of this protein was suggested as a tumor marker of population specificity [13].

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