Expression of P 16 ink4a in basal like triple negative breast carcinoma in Sudanese women

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Abstract:

Basal-like breast carcinoma (BLBC) has attracted considerable attention over the past few years. It has been suggested that tumors expressing basal markers have a more aggressive clinical behavior. However, a molecular basis for this disease remains unclear, and it lacks currently used therapeutic targets. Therefore developing a novel treatment strategy is crucial for improving the prognosis.

The aim of this study was to characterize the immunohistochemical (IHC) expression of p16 in patients with BLBC compared with non-BLBC.

Methods: A total of 30 cases of formalin –fixed – paraffin embedded blocks of Sudanese female with breast cancer with different grades were selected from archive of radiation and isotope center-Khartoum records, department of histopathology (Sudan).

Result: fifteen categorized as BLBC versus fifteen non – basal. Distant metastasis developed in (62% p. value 0.57) of cases of BLBC.

P16 had significant correlation moderate expression in triple negative breast carcinoma (p. value 0.003).

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*Conclusion: the expression of p 16 and histological grade
showed moderate significant difference in breast cancer with p.value
0.003*

Key words: Expression of P 16 ink4a, basal like triple negative
breast carcinoma, Sudanese women

INTRODUCTION:

Breast cancer (BC) is a common malignant neoplasm
comprising a large heterogeneous group of cancers with
variable histological type, biological and clinical characteristics
(Al-Moundhri, et a, 2003).[1]

Registrations of breast cancer in Sudan (1068) in 2010,
(1036) in 2011 representing 16% of total patients and (1242) in
2012 representing 17% of total patients according to radiation
and isotope center-Khartoum records (RICK, 2013).[2]

Prognostic factors of breast cancer include tumor size,
lymph node stage, tumor grade; tumor type and vascular
lymphatic invasion (David, 2006)[3].

Immunohistochemical studies in sporadic cancers have
lead to identification of novel targets with roles in diagnosis,
prognostic and therapeutics. Examples include hormones (e.g.
estrogen receptor) and tumor-specific oncogene products (e.g.,
HER-2) (Salmon, et al, 2001).

There is no consensus on how to define basal-like breast
carcinoma (BLBC) based on immunohistochemistry.
The majority of BLBCs lack the expression of ER, PR, and
HER2 protein overexpression [4, 5, 6] The prevalence of BLBC
ranges from 7.5% to 36.7% of breast cancer cases in different
patient cohorts [7, 8, 9,10–20]. These tumours were
predominantly grade-3, displaying a high mitotic count, tumour
necrosis, pushing margin of invasion, stromal lymphocytic

response, high rates of nuclear pleomorphism, and a lack of tubule formation [21–23].

The cyclin-dependent kinase inhibitor-2A gene (p16INK4a), located within the CDKN2A locus, is a strong and specific inhibitor of the progression through the G1 phase of the cell cycle by preventing phosphorylation of Rb protein [24]and is considered a major tumour suppressor gene. The inactivation of p16 seems a crucial event in the development of several human tumours [25]. Hui et al [26] demonstrated an inverse relationship between p16INK4a and ER mRNA levels in cell lines and primary breast cancers, suggesting that p16INK4a inactivation by hypermethylation and overexpression is a marker of poor prognosis.

Similarly, Milde-Langosch et al [27] have described high P16 INK4a reactivity (both nuclear and cytoplasmic) as indicative of more undifferentiated phenotype in mammary carcinoma.

MATERIAL AND METHOD:

This is non – interventional descriptive case study received from the radiation and isotope – center Khartoum. ALL 30 cases previously diagnosed as breast carcinoma.

The immunohistochemical procedure was done as follows: Sections (3µm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto salinized slides (Fisher brand). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in running water. Samples were steamed for antigen retrieval for p 16 using PT link. Briefly, slides were placed in slide tank containing enough sodium citrate buffer (pH 9.0) to cover the sections, then were boiled at high Temp for 20 minutes then were allow sections to cool at RT. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and

methanol for 10 minutes, then Slides were incubated with 100-200 µl of primary antibodies for 20 min at room temperature in a moisture chamber, and then were rinsed in Phosphate buffer saline. The primary antibody p 16, (monoclonal) was ready to use (Thermo). After washing with PBS for 3 min, binding of antibodies were detected by incubating for 20 minutes with dextran labeled polymer (Thermo kit). Finally, the sections were 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 were counterstained with haematoxylin. For each run of staining, positive and negative control slides were also prepared. The positive control slides contained the antigen under investigation and the negative control slides were prepared from the same tissue block, but were incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator.

Positive p 16 staining will be identified in form of brown nuclear staining.

The obtained results and variable were arranged in standard master sheet, then were entered a computer program SPSS and analyzed.

RESULTS INTERPRETATION:

Result obtained were detected by researcher and confirmed by experienced histopathologist.

The data were analyzed using version 16 SPSS computer program: frequencies, mean and chi square correlation were calculated.

RESULT:

The total number was 30 cases with different grade of breast carcinoma, they occur at moderately, but significantly in triple negative breast carcinoma figure (1). Although we found higher proportion of grade iii in basal like phenotype (40%) figure (2), this difference was not statistically significant (p=0.12)

In The diagnosis invasive ductal carcinoma represent (84%) from all type of breast carcinoma, figure (3).

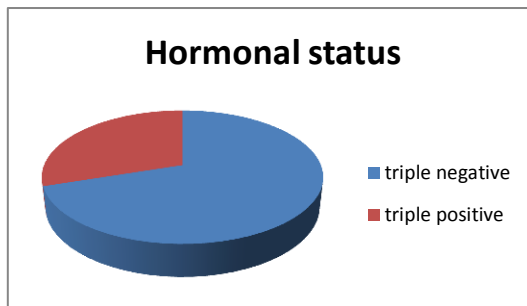There were no statistical significant regarding metastasis (p=0.57) figure (4).

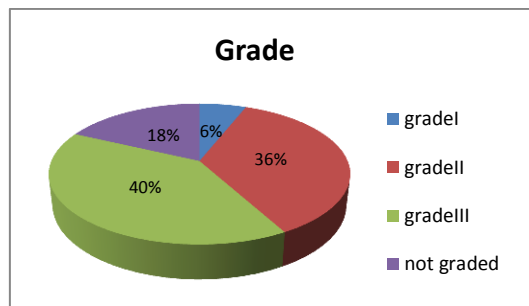**Figure (1) Correlation between p16 and Hormonal status**
P. value: 0.003

Figure (2): Correlation between p16 and Tumor grade
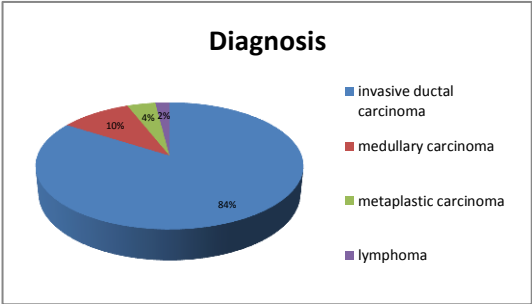**P.value:0.12**

Figure (3): Correlation between p16 and Tumor metastasis P.value:0.57
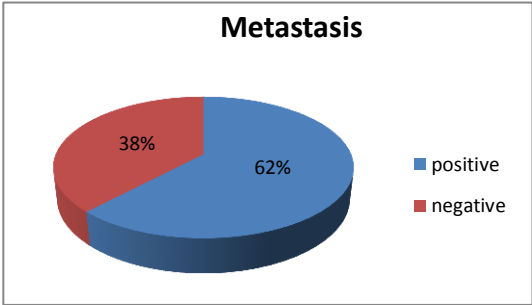

Figure (4)

DISCUSSION:

Basal –like tumours are gaining an increasing amount of attention in part to recognition as distinct entity, but most importantly owing to the overall poor prognosis that the diagnosis indicates.

Although no strong data is available, significant increase of the disease is noticed in Sudan. In this study the expression of p16 in 30 cases of breast carcinoma was evaluated the predictive value of the labeling index by p16 has special importance in the types of cancer in which it was poor prognosis. There are numerous studies that analyze this predictive value in carcinoma of the breast, although there is great variability of results. Our study found that there is no

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significant correlation with diagnosis, metastasis p.value0.57
and tumor grade p.value 0.12. while there was significant
correlation with triple negative breast carcinoma p.value 0.003
this finding is similar finding of Bohn et al [53] found a strong
positive reaction with p16 antibody in 16 out of (89%) basal
cases. The propose p16 as biomarker for identification of truly
basal- like cancer and raise the possibility that triple – negative
breast cancer with cytokertin, and p16 co-expression may
adequate identify these tumours and serve as potential
diagnostic / prognostic biomarker.

CONCLUSION:

BLBC are distinct clinical and pathological group represent
about (84%) of grade -3 invasive ductal carcinoma . they show
metastasis representing about (64%) of cases . p16 is frequent
occurrence in these cancer and play a role in the poor outcome.

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