ORIGINAL ARTICLE

Prevalence of Basal-like Breast Cancer among Indigenous Black Zambians at the University Teaching Hospital, Lusaka, Zambia

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ABSTRACT

***Background**:* Breast cancer comprises a group of very diverse diseases, which can be demonstrated at the molecular, histopathological and clinical levels. Gene expression studies using RNA microarray studies have categorised breast carcinomas into several classes. Of these basal-like tumour class has showed poor prognosis and their insensitivity to hormonal therapy makes therapeutic management difficult. Immunohistochemistry using certain Cytokeratins such as CK 5/6 has made it easier to identify these tumours allowing for better management. We conducted this study to determine whether the Zambian women presenting with breast cancer at the University Teaching Hospital express CK5/6 protein.

***Materials and methods**:* This was a cross-sectional study conducted to evaluate 44 conveniently sampled breast tissue diagnosed with breast cancer on histology at the University Teaching Hospital. The breast tissue was stained with CK 5/6 antibody (Dako, Glostrup, Denmark). The Labelled Streptavidin Binding (LSAB) staining was used to amplify and view the reaction. Pearson-chi-square test was used to indicate statistical significance of the findings.

***Results**:* Results revealed that 23 (52%) of the 44 samples tested negative for CK5/6 while 21(48%) were positive as shown in table 2. Out of the 23 samples that tested positive, 18 were ductal carcinoma while 5 were lobular carcinoma ($P = 0.023$).

Conclusion: In our study close to half of the tissue specimen were positive for the monoclonal antibody CK 5/6. This is highly suggestive of the presence of Basal like tumours in Zambian patients. These basal-like tumours are highly aggressive and have a greater propensity to metastasize. These tumours are also associated with BRCA ½ genes suggesting a hereditary role in the pathogenesis. There is need therefore to do more studies in breast cancer characterisation in order to develop better strategies in the management of patients with these tumours.

INTRODUCTION

Breast cancer comprises a group of very diverse diseases, which can be demonstrated at the molecular, histopathological and clinical levels[1]. The heterogeneity is shown at molecular level by reproducible variations in the frequencies and magnitudes of genomic abnormalities and by variation of gene expression among breast carcinomas, even those whose histology is alike [1, 2]. This heterogeneity means that the classification of these tumours should include the histopathological classification and grade as well as immunohistochemical parameters to allow for better treatment options for the patients[3]

The breast ducts of a normal female are made up of three types of epithelial cells; luminal cells, basal /myoepithelial cells and stem cells [4]. The luminal

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epithelial cells make up the outer layer in the form of polarized epithelial cells and myoepithelial cells make up the outer layer that end at the terminal duct lobular units forms the edge of the structure [5]. The breast also contains somatic stem cells (SCs) in low numbers that are labile and multipotent. These cells are closely regulated and located along the breast ducts in areas called SC niches[6].

The great majority of breast cancers are carcinomas that originate from malignant transformation of epithelial cells; the most common type being the invasive ductal carcinoma (IDC)[7]. Classification of these cells has provided information of markers that allow for early diagnosis and prognosis of breast cancers [7]. Originally it was thought that the majority of breast cancer originated from the luminal epithelial cells alone [8]. However, recent findings have shown that basal and myoepithelial cells also contribute to breast cancer and that some subtypes may possibly originate from distinct normal precursors that have distinct clinical outcomes [7]. Other studies have also elucidated that SC number and function are important determinants of breast cancer risk as well progression [9]. Additionally, that factors that raise breast SC number such as high birth weight and growth rate appear to increase breast cancer risk[6].

Gene expression studies using RNA microarray studies have categorised breast carcinomas into five (5) distinct types which include; luminal A [estrogen receptor (ER) +]; luminal B (ER+); HER2 overexpressing; normal breast-like; and basal-like 10[]. These subtypes showed varying clinical outcomes and also different response of therapy especially to hormone therapy[11].

An assessment by relapse-free survival showed that the basal-like tumours showed poor prognoses and most of them were negative for ER and HER2 expression making therapeutic management difficult [11,12]. Gene expression studies are quite expensive and cannot be done routinely in all healthcare facilities and further more paraffin-embedded archival tissue do not express most genes present in the basal-like breast cancer gene profile. Fortunately, these basal-like tumours have been shown to express cytokeratin 5/6 and 17 (CK 5/6 and CK17) using immunohistochemistry. It has been shown that breast cancers that express CK5/6 do have a poor clinical outcome[13].

Cytokeratin 5 and 6 (CK5/6) are type 2 medium sized neutral polypeptides that are part of the cytokeratin family of polypeptides. They are expressed in keratinized and non-keratinized squamous epithelium of prostate, mammary, and salivary glands. CK5/6 is a sensitive marker for squamous differentiation [14,15]. It is expressed in both benign and malignant tumours found in the epithelium, squamous mucosa, and myoepithelium [16]. The expression of CK5/6 is used in the differential diagnosis of basal-like breast cancer from other triple negative (TN) breast cancer. And its expression in TN breast cancer is correlated with poor prognosis, high grade differentiation and axillary lymph node metastasis [17,18]. The primary function of keratins is to protect epithelial cells from mechanical and non-mechanical stresses that result in cell death []. CK 5/6 expression has been linked to phenomenon known as epithelial-mesenchymal transition (EMT). This is a process by which cells of epithelial origin lose epithelial characteristics and polarity, and acquire a mesenchymal phenotype with increased migratory behavior that is associated with increased aggressiveness, and invasive and metastatic potential[11,20].

Hence the aim of this study was to assess the expression of CK 5/6 in 44 formalin fixed-paraffin embedded breast tissue diagnosed with breast cancer as a pilot for further analysis into prevalence and molecular characteristics of basal-like tumours among Zambian women with breast cancer.

METHODOLOGY

Methods and protocols

This was a laboratory based cross-sectional study and was done at the University Teaching Hospital (UTH), department of Pathology and Microbiology (Histopathology laboratory) in Lusaka, Zambia for a period of six months. The histopathology component of this study (tissue sectioning, H & E staining, cover slipping and microscopic examinations) was done in the UTH histopathology laboratory.A convenient sampling method was used to select the specimen sample size of forty four (44) formalin fixed-paraffin embedded tissue blocks (FFPE) of breast tissue diagnosed with breast cancer on histology. Cytokeratins 5/6 (CK 5/6) staining was done on four μm thick sections which were cut from formalin-fixed paraffin-embedded as previously described[21]

Statistical Analysis

Findings were analyzed using statistical software SPSS for Windows, Version 20. Age, histology and immunohistochemical results were variables under

consideration. The differences in the distribution of the study variables were evaluated by the use of Pearson Chi-square and when necessary, by Fisher's Exact test. All statistical tests were performed at 5% significance level, and differences were considered significant if two-tailed $P < 0.05$.

Ethical considerations

Ethical clearance was sought from the University of Zambia Biomedical Research Ethics Committee (UNZABREC). Permission to conduct a study was obtained from the Senior Medical Superintendent at UTH.

RESULTS

The specimens were from patients aged between 21 and 70 years old and breast cancer was more prevalent in the samples from patients that were aged between 30-49 years than in those that were 50 years and above as shown in table 1. In this study, results revealed that 23 (52%) of the 44 samples tested negative for CK5/6 while 21(48%) were positive as shown in table 2. Out of the 23 samples that tested positive, 18 were ductal carcinoma while 5 were lobular carcinoma ($P = 0.023$).

Table 1: Age frequency participants (n = 44)

Age	Frequency	Per cent
20 – 29 years	2	4.5
30 – 39 years	11	25
40 – 49 years	11	25
50 - 59 years	10	22.7
60 – 70 years	10	22.7
Total	**44**	**100**

Table 2: Immunohistochemistry of CK5/6 (n = 44)

CK5/6	Frequency	Per cent
Negative	23	52.3
Positive	21	47.7
Total	**44**	**100**

DISCUSSION

Despite the difficult in classification of tumour type and clinical course that the heterogeneity of breast cancer poses, it can be used as a breakthrough for the creation of target specific therapy for the patients. This would be achieved if the pathology report included the tumour morphology, the histopathological grade as well as the immunohistochemical parameters, which would provide a wholesome picture of the disease and systemic interventions[3,22].

Basal-like tumours are classified into another distinct entity of breast tumours known as the triple negative (TN) tumours. These TN tumours are an aggressive class of breast carcinoma considered for their estrogen receptor, progesterone receptor, and HER2 negativity [23]. The TN tumours are categorised as basal-like and non-basal-like, though not all basal-like tumours are TN. The TN basal-like tumours have been associated with expression of the cytokeratin's (CK) 5/6, CK14, and CK17, and epidermal growth factor receptor (EGFR). However those that express CK5/6 and EGFR have persistently poorer prognosis in the longer term. Because the expression of CK5/6 and EGFR is significantly associated with Nodal and Distant Metastases[13,23-25].

While the use of CK5/6 as a single marker can successfully identify the subset of patients with poor outcome, it can only detect about half of the basal-like tumours. Therefore for a more comprehensive analysis that includes CK14, CK17, SMA and P63 would give a more accurate diagnosis that shows more concordance with the gene expression studies[18,26].

Some studies have shown that basal-like TN tumors are associated with increased tumor size and positive axillary lymph node. With bigger tumours and a high proportion of positive lymph nodes projecting metastatic disease and necessitating a more aggressive treatment strategy. Furthermore it was shown that the TN breast cancer expression correlated with a younger age, in most studies affecting women less than 45 years [23, 27, 28]. On the issue age our study findings where comparable with those seen in the published literature.

Furthermore, there has been an association found between basal-like breast cancer patients who are immunoreactive for CK 5/6 also expressing Breast cancer 1gene (BRCA 1)

[29]. About 5–10% of all newly diagnosed breast cancers in Western nations are hereditary, attributable mainly to inherited mutations in the BRCA1 and BRCA2 (BRCA1/2) gene which have also been implicated in ovarian cancer [30]. Of those newly diagnosed with the mutations (BRCA1/2) only about 20% end up with TN breast cancer. However among BRCA1 mutation carriers at least one-third have TN breast cancers. Which shows that BRCA1 mutation carriers have a higher risk of developing TN breast cancer [13, 30]. The TN breast cancer patients diagnosed with BRCA1 mutations were mainly those diagnosed below the age 45 [30]. It was therefore noted that women with early-onset TN breast cancer, and if possible all TN breast cancer patients need to be tested for BRCA1 genes even in the absence of a family history of breast or ovarian cancer [31]. Studies have shown that TN breast carcinomas account for approximately 10% to 17% of all breast carcinomas and are more prevalent among young African, African American, and Latino women [32, 33]. In Zambia the majority of the population is made up of indigenous Africans, hence the need to do more studies.

In the face of these improved morphological, immunohistochemical, and molecular characterisation and identification of these TN tumours, diagnostic practice does not separately recognise these tumours, hence, they are managed as other less aggressive types. Even when research has clearly shown that basal-like tumours are associated with a lower endocrine therapy sensitivity which uses drugs such as Tomoxifen [34, 35]. The TN breast cancer patients have shown encouraging response to chemotherapy, however, they still present with a higher risk of relapse and a relatively poor outcome [36]. The treatment challenges of TN tumours are highly suggestive of their heterogeneity, which prompts need for further research to allow for more target specific therapy [37].

CONCLUSION

Our study showed that breast cancer was more prevalent in women between the ages of 30-49. Furthermore, close to half of the tissue specimen were positive for the monoclonal antibody CK 5/6 meaning that half of the breast cancer presenting in Indigenous Zambian patients may be of TN tumours and therefore not very responsive to Tomoxifen treatment. Hence this would justify histogenetic confirmation using immunohistochemistry in order to determine the patients' response to treatment by ruling-out the presence of TN breast cancer cases. It may also be necessary to screen as well as raise awareness about BRCA1/2 genes due to their association with early onset TN breast cancer.

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Conflicts of interests

The authors declare no conflict of interest.

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