International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 3, Issue 1, pp: (151-164), Month: April 2015 - September 2015, Available at: www.researchpublish.com

Selective Prognostic Markers Of Breast Cancer Using Expressed CK5/6, CK14 And CK17 In Invasive Breast Cancer Tissue Of Indigenous Black Zambian Women Presenting At The University Teaching Hospital, Lusaka, Zambia

[1]John Nkhoma, [2]Trevor Kaile, [3]Clemence Marimo, [4]Aaron Shibemba

[1]Ndola College of Biomedical Sciences, Ndola Central Hospital
[2, 3] Department of Pathology and Microbiology, School of Medicine, University of Zambia
[4]Cancer Diseases Hospital, Lusaka, Zambia

Abstract: **Breast cancer is a heterogeneous disease with distinct biological subtypes that have a diverse natural history and presents with a varied spectrum of clinical, pathological and molecular features that have different prognostic and therapeutic implications. Amongst Zambian women, breast cancer is the second most frequent cancer and yet the prevalence of basal-like breast cancer, the subtype known to be associated with poor prognosis and resistant to chemotherapy is not known. It has been shown that a combined determination of CK5/6, CK14 and CK17 in breast cancer tissue samples by Immunonohistochemistry assists greatly in determining tumour growth behaviour, metastatases and outcome of the cancer. The objective of this study was to determine the frequency of expression of basal cytokeratins 5/6, 14 and 17 in breast cancer samples at the University Teaching Hospital (UTH) in Lusaka, Zambia.**

Methodology: Randomly selected cases for the period January 2012 – December 2013 were identified and samples retrieved from the archives (*n* = 50). Sections were cut at 4 µm and stained with Haematoxylin and Eosin to determine the histological types and were evaluated for expression of CK5/6, CK14 and CK17 using immunohistochemical staining with Vectastain Elite Kit. Expression was also correlated with age, histological grade and tumour size. Data was analysed using SPSS software version 17.

Results: The majority of samples examined were from patients below the age of 50 years (n=28, 56%) and 46% (n=22) were those from patients aged 50 years or older. The most frequent histological type of breast cancer was invasive ductal carcinoma (n=45, 90%). Of the 50 samples 22 (44%) expressed CK5/6, 11(22%) CK14, and 6 (12%) CK17. Combined basal cytokeratin expression was 32 (64%). There was significant statistical association between expression of CK5/6 and CK14 with larger tumour size equal or greater than 2.0 cm (p=0.05 and p=0.003 respectively).

Conclusion: The most prevalent histological type was invasive ductal carcinoma (90%) and CK5/6 was the most frequently expressed basal marker (44%). Expression of CK14 was strongly associated with larger tumours when the three markers were combined with high histological grade.

Keywords: **Breast Cancer, Basal-like breast cancer, Immunohistochemistry, CK5/6, CK14, CK17.**

1. INTRODUCTION

Management of breast cancer relies on the availability of robust clinical and pathological prognostic and predictive factors to guide decision making and selection of treatment options. At present, clinical management of breast cancer largely depends on three molecular markers namely oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) that are used as predictive and prognostic markers to select specific adjuvant therapies [1].

Epidemiology of Breast Cancer:

Basal-like tumours of the breast have been defined by their expression of CK5/6 and/or EGFR and this molecular signature is associated with cancers that are aggressive, resist chemotherapy, are associated with poor clinical outcomes, and contribute disproportionately to breast cancer-related mortality [2].

Globally breast cancer is the third most frequent cancer in the world, affecting more than one million patients annually and remains the most lethal malignancy and cause of death in women [3], [4]. Of the approximately 1.4 million women diagnosed with breast cancer in the world in 2008, there were 460,000 corresponding deaths. In Africa, approximately 68,000 women were diagnosed with the disease with a corresponding 37,000 deaths [5]. The exact incidence figures in Africa are however lacking mostly due to absence in cancer registration in most countries. According to recent GLOBOCAN data [6], it was estimated that in 2012, 94,000 women developed breast cancer and 48,000 died from it in sub-Saharan Africa. The precise mortality rate in Zambia is not well known, however according to Zyambo and collaborators [7], who used the cancer registry for notified cancer cases from 1990 to 2009 found that breast cancer was the second most frequent cancer in women after cervical cancer.

It has been indicated that African Americans experience higher rates of mortality and survive less long once diagnosed at each diagnostic stage as compared to White women in the United States of America [8]. The differences are believed to be multifactorial, and may in part be due to inequalities in access to, and receipt of, adequate health care and/or due to group differences in comorbidity [8]. However, evidence also exists, that links aggressive tumour characteristics that are more common among African American women than Whites and more specifically, basal-like tumour subtypes have been reported to be more prevalent among premenopausal African American women [9], [10]. Basal like tumours are more likely to contain mutations of the tumour suppressor gene p53, which could be the contributing factor to poorer survival among African American women.

Classification of breast cancer:

Since breast cancer is a heterogeneous disease with diverse morphological features, variable clinical outcome and response to different therapeutic options, it is necessary to devise a clinically meaningful classification of the disease, which has to be scientifically sound, clinically useful and widely reproducible.

Histopathological classification:

Classical pathology has segregated breast tumours into various categories, based on their overall morphology and structural organization. The most common invasive tumour type observed and reported is invasive ductal carcinoma, not otherwise specified (IDC NOS; constituting about 75% of cases), while invasive lobular carcinoma (ILC) represents the next most frequent histologic type of breast tumour (about 10% of cases) [11]. The combined two categories make up the vast majority (about 90%) of breast cancers, while the remainder are categorized as medullary, neuroendocrine, tubular, apocrine, metaplastic, mucinous (A and B), inflammatory, comedo, adenoid cystic, and micropapillary types [11], [12].

Molecular classification:

It is envisaged that the existing histological classification systems for breast cancer are far from being accurate in predicting the prognosis or selecting the appropriate treatment of a given patient [13]. It has been shown that the variations in clinical behaviour are due to molecular differences between histologically similar tumours. Consequently, molecular classification can be more powerful than histopathology as a predictive factor for the different treatments. This is advantageous in that it can result in less frequent use of chemotherapy with considerable benefits in reducing toxicity and costs [14]. DNA microarray technology, Immunohistochemistry (IHC), Fluorescent in situ hybridization (FISH), and quantitative reverse transcription polymerase chain reaction (RT-PCR) are ideally suitable techniques to reveal molecular differences among the same or different groups of histopathological specimens. By using a hierarchical clustering analysis of gene expression profiling, Perou and colleagues were able to identify molecularly defined classes of breast cancer (luminal, HER2 -enriched, basal-like and normal-like) which have distinctive biological and clinical features [15], [16], [17]. The results of the studies performed by Perou et al. and Sorlie et al. concluded that the HER2 overexpressing tumours and the basal-type tumours were the two subgroups associated with the shortest disease free survival and overall survival, emphasizing that the basal-like tumours may represent a distinct clinical entity. Furthermore in a study by Rouzier et al. [18], it was demonstrated that pathologic complete response to preoperative chemotherapy differed significantly between the molecular classes where the basal-like and the HER-2+ subgroups were associated with the highest rate of pathologic complete response.

Treatment of breast cancer:

Treatment modalities for breast cancer include targeted chemotherapy, endocrine therapy, radiotherapy and surgery, inhibitors of certain proteins and more recently immune therapy (monoclonal antibodies) and miRNA therapy. Studies have shown that improved survival for breast cancer patients is attained when individual cases are discussed by specialised multidisciplinary teams (involving surgeons, radiologists, pathologists, radiation oncologists, medical oncologists and specialist nurses) to ensure the best line of treatment [19], or using prognostic markers.

For efficient treatment of breast cancer various targeted genetic and molecular agents have been developed and include mutations of breast cancer susceptibility genes type 1, 2 (BRCA1/BRCA2) [20] and abnormal activation of human epidermal growth factor receptors (EGFR) [21]. The development of targeting molecular agents happens to be among major goals of current research for efficient treatment of advanced breast cancer.

Basalness in Breast Cancer:

Classification of breast cancer subtypes is achieved by standard microarray-based transcriptional profiling, requiring fresh frozen tissue, but this is currently not feasible for routine practice. A more practical approach is the use of immunohistochemistry (IHC) to identify protein expression surrogates. There is great controversy and confusion about what basal means in the context of breast pathology. In reality this term has been used to refer to myoepithelial cells, which are basally located that express high molecular weight basal cytokeratins. Basal-like breast carcinomas were so named because the neoplastic cells composing this tumour type express genes usually found in normal basal/myoepithelial cells of the breast and they account for up to 15% of all breast cancers [22]. The prevalence of BLBC ranges from 8% to 37% [23], depending on the patient population studied. The term basal-like is often used when referring to cDNA microarray based classification. The luminal cells and myoepithelial cells (basal cells) can be distinguished by their location, by their immunophenotype, and also by their gene expression profile [24], [25]. However, direct comparisons between the proposed immunohistochemical markers and the microarray-defined molecular subtypes are scarce [26], [27]. Immunohistochemical marker panels that have been proposed to define basal-like breast cancers include: (1) lack of ER, PR, and HER2 expression ('triple-negative'immunophenotype); (2) expression of one or more high-molecular-weight/basal cytokeratins(CK5/6, CK14, and CK17); (3) lack of expression of ER and HER2 in conjunction with expression of CK5/6 and/or EGFR [27]; and (4) lack of expression of ER, PR, and HER2 in conjunction with expression of CK5/6 and/or EGFR [28].

Immunohistochemical staining for CK5/6 and/or EGFR is one of the best approaches as it offers 76% sensitivity and 100% specificity for BLBC as identified by gene expression profile which is used to classify BLBC [27]. Based on the frequent expression of basal CKs in basal-like cancer most authors have included in their IHC definitions of basal-like immunopositivity, CK14 and/ or CK17 in addition to CK5/6 to define basal-like breast cancer [29], [30], [31]. The distinction between BLBC and TNBC is important because triple-negative tumours that express basal markers have distinct molecular lesions, such as p53 stabilisation and higher mitotic indices and as such are associated with worse survival than triple-negative tumours that lack basal-like markers [23], [28].

The significant observation is that despite the different definitions for basal-like breast cancers, it has been shown that these tumours have characteristic clinical presentations [9], histological features [26], [32], response to chemotherapy [31], [33], [34], [35], [36], [37], [38], sites of distant relapse, and outcome [16], [39], [40], [41], [42].

The aim of this study was to determine the frequency of breast cancers that express CK5/6, CK14 and CK17, in histological samples diagnosed with breast cancer at the University Teaching Hospital.

2. METHODOLOGY

The study design and research site:

This was a laboratory-based retrospective descriptive study and was conducted at the Histopathology Laboratory of the University Teaching Hospital, the main referral hospital located in Lusaka, the capital city of Zambia.

The sample size and sampling Framework:

Archival histological samples from breast cancer patients for the period January 2012 to December 2013 were retrieved and selected by random sampling. Fifty (50) randomly sampled formalin fixed-paraffin embedded (FFPE) tissue blocks from cases diagnosed with invasive breast carcinoma were collected from the archives.

Specimen sections:

All sections were cut at 4 µm thickness and floated in a water bath at 40°C and mounted on electrostatic slides. They were all dried in a hot air oven at 60°C for 30 minutes.

Haematoxylin and Eosin (H&E) staining:

The staining was carried out on the Leica Automatic stainer XL which is used for routine samples in the histopathology laboratory. Slides were mounted; in Distrene Plasticiser Xylene (DPX) and allowed to settle for 1 hour before microscopic examination

Immumohistochemical Staining Protocol:

Tissue sections were deparaffinised in two changes of xylene (5 minutes in each) and rehydrated through a series of graded alcohols and finally rinsed in tap water for five minutes. The sections were then incubated in 1% hydrogen peroxide in methanol for 20 minutes to quench endogenous peroxidise activity. The sections were washed in three changes of distilled water followed by antigen retrieval in citrate buffer for 20 minutes in the water bath. Slides were then cooled down at room temperature whilst in antigen retrieval buffer for 30 minutes. This was followed by a phosphate buffered saline (PBS) rinse for 5 minutes and the sections were then ringed with a Dako pen. The sections were then incubated for 30 minutes in diluted normal blocking serum (150µl stock to 10 ml buffer) which was supplied in the ABC Kit. Excess serum was then flicked off from the slides and then the sections were incubated for 30 minutes with the primary antibody (Anti-cytokeratin CK5/6, 14 or 17) diluted in PBS. Cytokeratin 5/6 and CK 14 were optimised for a dilution of 1 in 100. CK 17 was procured in a pre-diluted form and was only diluted 1 in 5. After incubation in primary antibody the sections were washed in PBS for 5 minutes and thereafter the sections were incubated for 30 minutes with biotinylated secondary antibody solution (150µl normal blocking serum, 10 ml PBS, 50µl biotinylated antibody stock). Sections were once more washed in PBS and then incubated for 30 minutes with Vectastain® Elite ABC Reagent (100µl avidin DH, 5 ml PBS, 100µl biotinylated horseradish peroxidise, mix).

Slides were thereafter washed in PBS and sections treated with peroxidise substrate solution (DAB) until the desired stain intensity in the control sections were developed. Sections were then rinsed in tap water and counterstained with haematoxylin for 20 seconds. After washing the sections in running tap water for five minutes, they were then dehydrated in ascending grades of alcohols, cleared in xylene and mounted in DPX and examined.

Microscopic examination of slides:

Classification and confirmation of histological types of the breast carcinomas was done by an independent Histopathologist. Reading of slides for immunohistochemistry was carried out by two independent readers, one being a histopathologist.

Statistical Analysis:

The data was entered into Microsoft excel and then exported to SPSS version 17 for coding and descriptive analysis. Clinicopathological parameters including age, tumour size, and histological grade were evaluated. Descriptive statistics were presented in tables and graphs while association between the expressions of CK5/6, CK14 and CK17 with age, tumour grade, tumour size and histological types was analysed using the Chi squared statistic. Prevalence estimates were presented with 95% confidence interval while statistical estimates were considered statistically significant when the p-value was ≤ 0.05.

Ethical Approval:

This study was a retrospective descriptive laboratory based study, with no direct contact with patients. It was submitted to the University of Zambia Biomedical Research Ethics Committee (UNZABREC) (IRB00001131 of IORG0000774) before data collection for approval which was granted. Written permission for use of laboratory samples and reports was given by the Senior Medical Superintendent of the University Teaching Hospital.

3. RESULTS

Histological types:

The histological types of breast cancer (N=50) as determined from the haematoxylin and eosin stained sections is shown in table 1. Table 1 shows that the most frequent invasive breast cancer type by histological classification is invasive ductal carcinoma (n=45, 90%, C.I. 78.6-95.7) in indigenous Zambians as presented at UTH.

Table.1 Frequencies of tumour types by histology

Tumour type	N	Frequency (%)	95% C.I.
Invasive ductal carcinoma	50	45 (90)	78.6 – 95.7
Invasive lobular carcinoma		04 (8)	3.2 – 18.8
Invasive papillary carcinoma		01 (2)	– 10.5

Abbreviations: n, sample size, C.I., Confidence Interval

CK 5/6, CK 14 and CK 17 immunostaining:

CK5/6 and CK14 showed more or less membranous staining while CK17 revealed cytoplasmic staining in basal epithelium and carcinoma cells (Figure 1). The surrounding tissue such as stroma cells exhibited little or no staining.

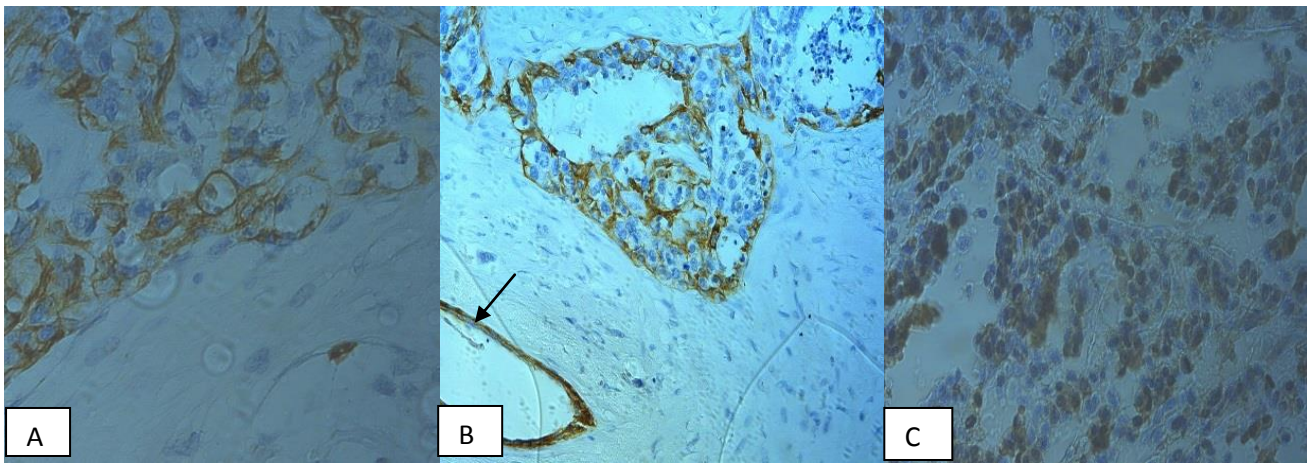

Fig. 1. Immunostaining of CK5/6, CK14 and CK17 in carcinomas. A) Positive immunostaining of CK5/6 in an infiltrating ductal carcinoma of breast, x 400 magnification. B) Positive immunostaining of CK14 in another infiltrating ductal carcinoma with positive staining in normal ductal epithelium, x 200 magnification. C) Positive immunostaining of CK17 in yet another ductal infiltrating breast carcinoma, x 400 magnification. Arrow indicating staining of normal basal epithelium.

Table.2 Frequency of expression of individual basal cytokeratins by all the tumour types

Tumour Type	No.	CK 5/6		CK 14		CK 17	
		Pos	Neg	Pos	Neg	Pos	Neg
IDC	45	22 (48.9)	23 (51.1)	8 (17.8)	37 (82.2	6 (13.3)	39 (86.7)
ILC	04	0 (0)	4 (100)	3 (75)	1 (25)	0 (0)	4 (100)
IPC	01	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)

Abbreviations: IDC, invasive ductal carcinoma, ILC, invasive lobular carcinoma, IPC, invasive papillary carcinoma

Table 2 shows that CK5/6 was the most highly expressed CK (n=22, 48.9%) among the IDCs and was only positive in these tumours. The non-expression of CK5/6 amongst the ILC and IPC could be due to the small sample size representing these tumours. The table also reveals that ILCs only expressed CK14 (n=3, 75%). Interestingly only CK14 was expressed by all the tumour types.

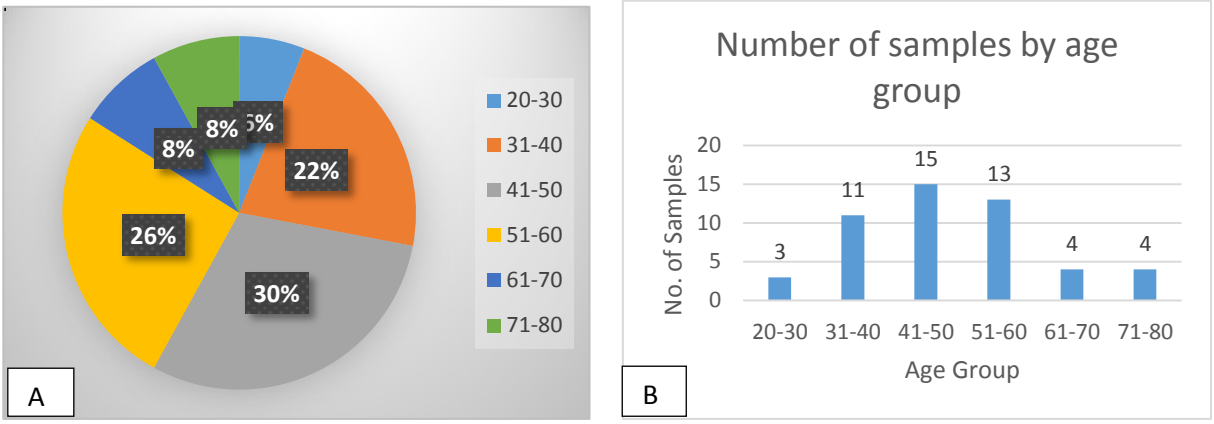

Fig.2 Number of breast cancer samples in relation to age category. 2a – frequency for each age category, 2b – number of samples for each age group

The samples used in the study were from patients with an age range of twenty five (25) years to eight (80) years with a mean age of forty nine (49) years. Figure 2a and 2b shows that the majority of the samples with invasive breast cancer were from patients aged between thirty one (31) years and sixty (60) years (n=39, 78%) with the 41-50 age category at the peak (n=15, 30%).

Table.3 Frequencies of each basal cytokeratin within age categories

Age Category	Frequency %	CK5/6		CK14		CK17	
		Neg	Pos	Neg	Pos	Neg	Pos
20 -30	3 (6)	1 (3.6)	2 (9.1)	2 (5.1)	1 (9.1)	3 (6.8)	0 (0.0)
31 – 40	11 (22)	7 (25)	4 (18.2)	10 (25.6)	1 (9.1)	8 (18.2)	3 (50.0)
41 – 50	15 (30)	8 (28.6)	7 (31.8)	11 (28.2)	4 (36.4)	15 (34.1)	0 (0.0)
51 – 60	13 (26)	7 (25)	6 (27.3)	9 (23.1)	4 (36.4)	11 (25.0)	2 (33.3)
61 – 70	4 (8)	3 (10.7)	1 (4.5)	4 (10.3)	0 (0.0)	3 (6.8)	1 (16.7)
71 -80	4 (8)	2 (7.1)	2 (9.1)	3 (7.7)	1 (9.1)	4 (9.1)	0 (0.0)
Total	50 (100)	28 (100)	22 (100)	39 (100)	11 (100)	44(100)	6 (100)

Abbreviations: Neg = negative, Pos, positive

Table 3 indicates that of the twenty two (22) positive samples, a high proportion expressed CK5/6 especially in samples from patients in the age range of 31 – 60 years (n=17, 77.3%) with highest expression in the 41-50 years category (n=7, 31.8%). CK14 was highly expressed in the 41 – 50 (n=4, 36.4) and the 51 – 60 (n=4, 36.4) from the eleven (11) positive samples. CK17 was only positive in six (6) samples with highest expression in the 31 – 40 age category (n=3, 50%). This may suggest that basal cytokeratin expression is more common in breast cancer patients aged 31-60 years.

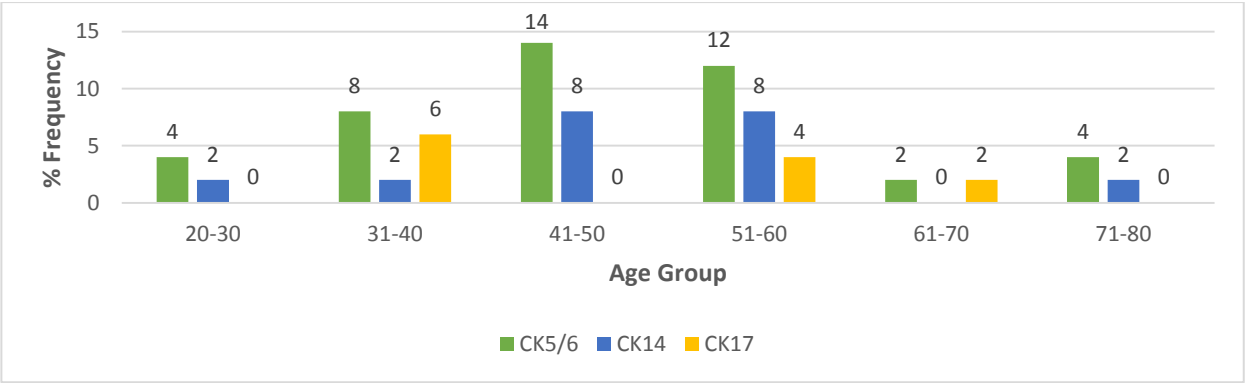

Fig.3 Frequency of basal cytokeratin expression by age group

Figure 3 summarises the frequency of CK expression for each age group and shows that the frequency of CK5/6 was highest in the 41–50 years age group (14%) of the 50 samples, whereas for CK14 it was in the 41–50 (8%) and 51–60 (8%) age groups, whilst CK17 showed highest expression in the 31–40 age category (6%). Figure 3 showed that CK5/6 was the most highly expressed basal marker across all age groups. This suggests that it was the best single representative marker for establishing the basalness of breast tumours.

Table.4 Association between individual basal cytokeratins and age categories

Age (years)	CK5/6 (%)		*P* value	CK14 (%)		*P* value	CK17 (%)		*P* value
	Neg	Pos		Neg	Pos		Neg	Pos	
20 – 30	1 (33.3)	2 (66.7)		2 (66.7)	1 (33.3)		3 (100)	0 (0.0)	
31 – 40	7 (63.6)	4 (36.4)		10(90.9)	1 (9.1)		8(72.7)	3(27.3)	
41 – 50	8 (53.3)	7 (46.7)		11(73.3)	4 (26.7)		15(100)	0 (0.0)	
51 – 60	7 (53.8)	6 (46.2)	0.90	9 (69.2)	4 (30.8)	0.67	11(84.6)	2(15.4)	0.29
61 – 70	3 (75.0)	1 (25.0)		4 (100)	0 (0.0)		3 (75.0)	1(25.0)	
71 – 80	2 (50.0)	2(50.0)		3 (75.0)	1 (25.0)		4 (100)	0 (0.0)	
Total	28(56.0)	22(44.0)		39(78.0)	11(22.0)		44(88.0)	6(12.0)	

Table 4 shows that there was no significant statistical difference between the expression of any of the three (3) basal CKs and the age groups (CK5/6, p=0.9, CK14, p=0.67, CK17, p=0.29) across the stratified age groups.

Table 5 shows that the frequency of combined basal CK+ status was 64% (n=32) with a peak in the 41 – 50 age category (n=10) and also indicates expected proportions in this population within 95% confidence intervals. The probability that patient will have a basal-like breast cancer in this series of breast cancer samples is high between the 31 and 60 years.

Table.5 Combined frequencies for CK+ status and CK- status within age category

Age Category	CK5/6+, CK14+, or CK17+ (Basal)	95% C.I.	CK5/;86-, CK14-, or CK17- (Non-basal)	95% C.I.
20 -30	2 (66.7)	1.1 – 13.5	1 (33.3)	0.4 – 10.5
31 – 40	7 (63.6)	7.0 – 26.2	4 (36.4)	3.2 – 18.8
41 – 50	10 (66.7)	11.2 – 33.0	5 (33.3)	4.4 – 21.4
51 – 60	9 (69.2)	9.8 – 30.8	4 (30.8)	3.2 – 18.8
61 – 70	2 (50)	1.1 – 13.5	2 (50)	1.1 – 13.5
71 – 80	2 (50)	1.1 – 13.5	2 (50)	1.1 – 13.5
Total	32 (64)	50.1 – 75.9	18 (36)	24.1 – 49.9

Table 6 indicates that the number of samples from patients below 50 years (the young) were more (n=28, 56%) compared to those equal to or greater than 50 years (the old) (n=22, 44%). There was no significant statistical association between these age categories and expression of CK5/6 (χ^2=0.34, p=0.85), or CK14 (χ^2=0.12, p=0.91), or CK17 (χ^2=0.10, p=0.75). Basal cytokeratin expression was not related to the age of the patient.

Table.6 Correlation between age and basal cytokeratin expression

Age (years)	CK5/6 (%)		*P* value	CK14 (%)		*P* value	CK17 (%)		*P* value
	Neg	Pos		Neg	Pos		Neg	Pos	
< 50	16(57.1)	12(42.9)		22(78.6)	6 (21.4)		25(89.3)	3(10.7)	
≥ 50	12(54.5)	10(45.5)	0.85	17(77.3)	5 (22.7)	0.91	19(86.4)	3(13.6)	0.75
Total	28(56.0)	22(44.0)		39(78.0)	11(22.0)		44(88.0)	6(12.0)	

Table.7 Correlation between histological grade and basal cytokeratin expression

Histological grade	CK5/6 (%)		*P* value	CK14 (%)		*P* value	CK17 (%)		*P* value
	Neg	Pos		Neg	Pos		Neg	Pos	
I / II	15(65.2)	8 (34.8)		19(82.6)	4 (17.4)		21(91.3)	2 (8.7)	
III	13(48.1)	14(51.9)	0.23	20(74.1)	7 (25.9)	0.47	23(85.2)	4(14.8)	0.51
Total	28(56.0)	22(44.0)		39(78.0)	11(22.0)		44(88.0)	6(12.0)	

Table 7 shows that there were more samples expressing basal cytokeratins in higher grade tumours than in ones with lower grades for all the three basal markers. However, there was no statistical significant association between tumour grade and the expression of any of the three basal cytokeratins (CK5/6, χ^2=1.47, p=0.23; CK14, χ^2=0.53, p=0.47; CK17, χ^2=0.44, p=0.51) when considered as single entities.

Table.8 Correlation between tumour size and basal cytokeratin expression

Tumour size	CK5/6 (%)		*P* value	CK14 (%)		*P* value	CK17 (%)		*P* value
	Neg	Pos		Neg	Pos		Neg	Pos	
< 2 cm	14(73.7)	5 (26.3)		19(100)	0 (0.0)		17(89.5)	2(10.5)	
≥ 2 cm	14(45.2)	17(54.8)	0.05	20(64.5)	11(35.5)	0.003	27(87.1)	4(12.9)	0.802
Total	28(56.0)	22(44.0)		39(78.0)	11(22.0)		44(88.0)	6(12.0)	

Table 8 reveals that expression of CK14 was only associated with larger tumours (≥ 2 cm) and likewise most of the samples expressing CK5/6 were associated with larger tumour size (17 against 5). There was a strong statistical significant association between CK14 expression and larger tumour size (χ^2=8.64, p=0.003) and a weak one for CK5/6 expression (χ^2=3.89, p=0.05). This may suggest that basal CK5/6 and 14 expression is associated with rapidly growing tumours.

Table 9 shows that positive basal status was strongly associated with higher histological grade (χ^2=4.39, p-0.03) and larger tumour size (χ^2=9.81, p=0.002). There was no statistical significant association between positive basal status and age (χ^2=0.002, p=0.96). This may indicate that basal cytokeratin expression is associated with aggressive and highly proliferative breast tumours respectively.

Table.9 Association between combined expression of CK+ and CK- tumours with clinical parameters

Parameter	CK5/6+, CK14+, or CK17+	CK5/6-, CK14-, or CK17-	Total (%)	*P* value
	Basal	Non-basal		
Age				
< 50	18 (64.3)	10 (35.7)	28 (100)	
≥ 50	14 63.6)	8 (36.4)	22 (100)	0.962
Total	32 (64.0)	18 (36.0)	50 (100)	
Grade				
I or II	11 (47.8)	12 (52.2%)	23 (100)	
III	21 (77.8)	6 (22.2)	27 (100)	0.03
Total	32 (64.0)	18 (36.0)	50 (100)	
Size				
< 2 cm	7 (36.8)	12 (63.2)	19 (100)	
≥ 2 cm	25 (80.6)	6 (19.4)	31 (100)	0.002
Total	32 (64.0)	18 (36.0)	50 (100)	

4. DISCUSSION

Breast cancer represents tumours that are morphologically, molecularly and prognostically heterogeneous. To provide a deeper understanding of the complexity of breast cancer, Perou et al [15] used gene expression profiling to classify these tumours into intrinsic molecular subtypes: Luminal-like, HER2 enriched, Basal-like and Normal-like. Because cDNA microarray is too expensive to be applied to routine practice, a very similar classification of breast cancer has been characterised using immunohistochemistry to analyse patterns of protein expression in tumour sections and suggests that a few protein biomarkers (e.g., ER, PR, HER-2, HER-1, and basal CKs) can be used to stratify breast cancers into different groups [27], [43].

This study specifically investigated the basal-like subtype in view of the interest elicited by clinicians and researchers because of their characteristically poor prognosis and resistance to existing molecularly-targeted treatment modalities, leaving cytotoxic chemotherapy as the principal systemic treatment [44], [45]. Breast cancers expressing basal markers CK5/6 and CK 14 have also been linked to presence of intrapulmonary and/or brain metastases and are also associated with poorer survival after metastatic presentation [46]. In the current study basal-like breast cancers were classified by virtue of their expression of either CK5/6 and/or CK14 and/or CK17.

Histology types and expression of basal cytokeratins:

In the present study, the most frequent histological type was invasive ductal carcinoma representing 90% (C.I. 78.6–95.7) of cases, which is in agreement with previous studies [47], [48], [49].

In this study, the tumours were divided into two major subgroups based on their CK status: CK 5/6 positive and/ or CK14 positive and/or CK 17 positive and those negative for any of the CKs. According to this definition, tumours which were positive with any of the CKs were defined as basal type, and those which were CK-negative as non-basal type.

The proportion of samples expressing basal CKs when combined was 64%, representing 44% for CK 5/6, 22% for CK14 and 12% for CK17, which is consistent with data reported by earlier studies [23], [26], [27], [28], which obtained prevalences of 71, 61 62%, and 53% respectively. However the high prevalence in these studies could be due to the fact that they had used known triple negative cases. It is important to note that none of these studies had used all the three markers. Nielsen et al did not include CK14 and the authors experienced difficulties with CK17 staining which was difficult to score, while Livasy and colleagues only used CK5/6. It has been demonstrated that basal-like tumours tend to be infiltrating ductal carcinomas [26] and this correlates with the high number of positive cases obtained in this study for this tumour type. Another, far much earlier study had reported higher results using only CK5/6 (56%) as a basal marker from 72 samples which did not express ER and HER-2 [50], which makes CK5/6 a more reliable basal marker. The proportion observed is similar to that reported in the studies of the Ashkenazi Jewish women in which 64 – 77% had a basal-like phenotype [50], [51], [52]. This could possibly indicate that BRCA1 germline mutations may have a similar occurrence in indigenous Zambian women.

Compared to nearby regions, our results show a higher distribution frequency than those obtained from studies carried out in Kenya [53] and Uganda [54] where the prevalence was found to be 23% and 34% respectively. The study in Kenya had used CK5/6 and/or EGFR whereas that in Uganda used CK5/6 and /or p-cadherin as well as triple negative status as markers of basaloid differentiation. The use of only one basal cytokeratin marker as opposed to the three in our study could be the reason for this difference. Studies conducted in Tanzania using hormone receptor status for ER and PR but not HER-2 showed rates of negative expression exceeding 50% above [55], [56] and basing on the fact that triple negative status can be likened to basal-like breast cancer, this may infer that there is an increased proportion of breast cancer of the basal phenotype in this neighbouring region. It is difficult to compare findings between studies directly because of differences in antibodies used (clone and source), biomarker profile, processing procedures and criteria of evaluation. For instance the present study considered any clear cytoplasmic or membrane staining in cancer cells as positive whereas with some previous studies rather different cut-off points had been used, such as ≥5% cells staining [57] or ≥10% cells staining [54]. The high frequency of CK5/6 expression in the present study is a significant finding because of its clinically aggressive nature and poorly characterised molecular pathogenesis. And more importantly so, is that patients in this category especially those with a triple negative status, have been found to have a higher risk for death than those with histologically morphological basal-like features or the molecular basal-like subtype [58]. Liu and colleagues [59] also reported that patients with a triple negative status whose samples were positive for either CK5/6 or CK17 were associated with worse disease-free survival and those positive for CK5/6 or both CK5/6 and CK17 were associated with worse overall survival.

It is believed that genetic, ethnic and racial factors influence breast carcinoma molecular subtypes, possibly by determining intrinsic differences in tumour biology [9]. This is supported by studies which have demonstrated that basal-like breast carcinomas are more frequent in African Americans (26.5%) and in African women (34%) than Non-African Americans (16.0%) [9], [54]. This assumption is consistent with the high frequency of CK5/6 positive cases in the present study.

Age and expression of basal cytokeratins:

Earlier studies had reported a significant association between basal marker expression and younger patients, supporting the findings that breast cancer in younger women is more aggressive, with greater proliferative potential and a worse prognosis than in older women [28], [43], [45], [60]. Basing on our criteria of defining BLBC, we could not identify an association between younger age and this subtype of breast cancer with any of the basal cytokeratins, contrary to previous studies. The results obtained in our study are consistent with those reported in Uganda [54], Sudan [57], Japan [61] and Brazil[62]. The lack of significance for the difference in age for our series could be due to fact that the mean age at diagnosis was very minor between the CK+ group (48.3) and the CK- group (49.8), reflecting that age may not be a factor for this phenotype of breast cancer in this series. However expression of CK5/6 and CK14 was more frequent in samples from patients aged tween 41 and 60 years of age. This could reflect that expression of these two markers may be associated with older age.

Grade and expression of basal cytokeratins:

With regard to grade, a number of reports indicate a direct relationship between high histological grade and positive expression of basal cytokeratin markers either as singles or combined expression. In investigating the characteristics of basal cytokeratin expression in in breast cancer [63], it was found that positive expression of either CK5, CK5/6, CK14 or CK17 was strongly associated with a higher histological grade demonstrating that positive basal marker expression was a negative prognostic indicator. This is echoed in reports by Abd El-Rehim et al. [43] in which CK5/6 and CK14 showed positive correlation with histological grade, Kuroda et al. [61] where basal-like carcinomas included a high ratio of grade 3 tumours thereby distinguishing them from non basal-like carcinoma cases, while in another study, cases of invasive ductal carcinoma showed an association between CK5/6 or CK17 immunostaining with high histological grade [59]. In our study, expression of all the basal CKs as single markers was not associated with higher histological grade. Our explanation for these contradictory results could be related to the differences in numbers and composition of the cohort and negative cases. The other factor could be the impaired use of grade due to inherent subjectivity associated with its assessment/concordance between pathologists that ranges from 50% - 85% [64] and the large number (30%-60%) of tumours classified as intermediate grade. These tumours are known to have features of both low-grade and high-grade tumours [65]. However combined basal CK+ status, was weakly associated with higher grade. This can be attributed to the fact that malignant neoplasms constituting the basal-like breast cancer subtype are not biologically homogeneous. This is supported in a study by Rakha and colleagues [66], who divided the BLBCs into those showing >50 cells positive staining for CK5/6 and 14 and those displaying <50% of cells positive for CK5/6 and 14 and the subsets demonstrated differences in grade and other clinicopathological characteristics.

Tumour size and expression of basal cytokeratins:

In this study we observed an association between expression of basal markers and tumour size. Expression of CK5/6 and CK14 was directly related to larger tumour size supporting the finding that breast cancer with basal marker expression is more aggressive compared to those which are negative [43], [63]. Awadelkarim and colleagues [57], had however reported conflicting findings where the mean tumour size for the basal CK+ group was less than the CK- group and this was the case in our study for CK17 expression, possibly due to the small number of positive cases.

5. CONCLUSION

This study found that invasive ductal carcinoma was the most frequent histological type of invasive breast cancer with a prevalence of 90% and that the most frequently expressed basal cytokeratin was CK5/6 with a prevalence of 44%. When combined, the frequency of basal status (CK5/6+ and/or CK14+ and/or CK17+) was 64%. Basal cytokeratin expression was associated with increased tumour size and higher histological grade.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Health with the logistical help from Ndola College of Biomedical Sciences and Ndola Central Hospital without which this work could not have been possible and to them I owe my gratitude. I wish to extend my gratitude to the University of Zambia, School of Medicine through the Office of the Assistant Dean Postgraduate Studies, the Department of Pathology and Microbiology and the University Teaching Hospital Management for allowing this research to be undertaken at their institution.

REFERENCES

[1] Rakha EA, Ellis IO. (2009). Triple-negative/basal-like Breast Cancer: Review. Pathology. 41(1):40–7.

[2] Cleator S, Heller W, Coombes RC. (2007). Triple-negative breast cancer: Therapeutic options. Lancet Oncol. 8:235–244.

[3] Garcia M, Jemal A, Ward EM, et al. (2007). Global Cancer Facts & Figures. Atlanta, GA: American Cancer Society; 2007.

[4] Stuckey A. 2011. Breast cancer: epidemiology and risk factors. Clinical Obstetrics and Gynecology. 54(1): 96–102.

[5] Ferlay J, Shin H. R. Bray F, Forman D, Mathers C, Parkin D. M. (2010). "GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide. IARC Cancer base No, 10," International Agency for Research on Cancer, Lyon, France.

[6] Ferlay J, Soerjomataram I, Ervik M et al. (2013). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. International Agency for Research on Cancer, Lyon, France.

[7] Zyambo C, Nzala S.H., Babaniyi O, Songolo P, Funkhouser E, and Suziya S. (2013). Distribution of cancers inZambia: Evidence from the Zambia National Cancer Registry (1990-2009). J Public Health Epidemiol. 5 (2): 95-100.

[8] Jemal A, Siegel R, Ward E, et al. (2008). Cancer Statistics. CA Cancer J Clin. 58(2): 71–96.

[9] Carey LA, Perou CM, Livasy CA, et al. (2006). Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 295(21):2492–502.

[10] Dookeran K, Gao X, Wang Y, et al. (2007). Basal subtype predicts poorer prognosis in younger, but not older, African-American women with breast cancer. AACR Meeting Abstracts. 2007; 2007(1_Annual_Meeting):2510.

[11] Li CI, Uribe DJ, Daling JR. (2005). Clinical characteristics of different histologic types of breast cancer. Br J Cancer. 93(9):1046–1052.

[12] Weigelt B, Geyer FC, Reis-Filho JS. (2010). Histological types of breast cancer: how special are they? Mol Oncol. 4(3):192–208.

[13] Cleator S, Ashworth A(2004) Molecular profiling of breast cancer: Clinical implications. Br J Cancer. 90:1120-4

[14] Zepeda-Castilla EJ, Recinos-Money E, Cuellar-Hubbe M, Robles-Vidal CD, Maafs-Molina E. (2008). Molecular classification of breast cancer. Cir. 76:87-93.

[15] Perou CM, Sorlie T, Eisen MB, et al. (2000). Molecular portraits of human breast tumours. Nature. 406: 747- 52.

[16] Sorlie T, Perou C. M, Tibshirani R, et al. (2001). "Gene expression patterns of breast carcinomas distinguish tumour subclasses with clinical implications," Proceedings of the National Academy of Sciences of the United States of America. 98 (19): 10869-10874.

[17] Sorlie T, Tibshirani R, Parker J, et al. (2003). Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA. 100:8418- 23.

[18] Rouzier R, Perou CM, Symmans WF et al. (2005). Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res. 11: 5678–5685.

[19] Kesson EM, Allardice GM, George WD, Burns GJH, Morrison DS. (2012). Effects of multidisciplinary team working on breast cancer survival: retrospective, comparative, interventional cohort study of 13,733 women. BMJ. 344:e2718 doi: 10.1136/bmj.e2718.

[20] Chen S, Parmigiani G. (2007). Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol. 25:1329-33

[21] Wang Q, Greene M.I. (2007). The development of targeted therapy in the ErbB system. Am Soc Clin Oncol. Ed Book:79-84.

[22] Reis-Filho JS, Tutt ANJ. (2008). Triple negative tumours: a critical review. Histopathology. 52:108-118.

[23] Rakha EA, Elsheikh SE, Aleskandarany MA, et al. (2009). Triple-negative breast cancer: distinguishing between basal and nonbasal subtypes. Clin Cancer Res. 15:2302–2310.

[24] Gusterson BA, Ross DT, Heath VJ, Stein T. (2005). Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer. Breast Cancer Res. 7(4):143-148.

[25] Grigoriadis A, Mackay A, Reis-Filho JS, et al. (2006). Establishment of the epithelial-specific transcriptome of normal and malignant human breast cells based on MPSS and array expression data. Breast Cancer Res. 8:R56.

[26] Livasy CA, Karaca G, Nanda R, et al. (2006). Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol. 19:264-271.

[27] Nielsen T.O., Hsu F.D., Jensen K, Cheang M, Karaka G, Hu Z et al. (2004). Immunohistochemical and clinical characterisation of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 10: 5367-5374.

[28] Cheang MC, Voduc D, Bajdik C, et al. (2008). Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res. 14:1368-1376.

[29] Lakhani SR, Reis-Filho JS, Fulford L, et al. (2005). Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res. 11:5175–5180.

[30] Bryan BB, Schnitt SJ, Collins LC. (2006). Ductal carcinoma in situ with basal-like phenotype: a possible precursor to invasive basal-like breast cancer. Mod Pathol. 19:617–621.

[31] Banerjee S, Reis-Filho JS, Ashley S, et al. (2006). Basal-like breast carcinomas: Clinical outcome and response to chemotherapy. J Clin Pathol. 59:729–735.

[32] Fulford LG, Easton DF, Reis-Filho JS, et al. (2006) Specific morphological features predictive for the basalphenotype in grade 3 invasive ductal carcinoma of breast. Histopathology. 49: 22–3.

[33] Altundag K, Harputluoglu H, Aksoy S et al. (2007). Potential chemotherapy options in the triple negative subtypeof breast cancer. J Clin Oncol. 25: 1294–1295; author reply 1295–1296.

[34] Haffty BG, Yang Q, Reiss M, et al. (2006). Locoregional relapse and distant metastasis in conservatively managedtriple negative early-stage breast cancer. J Clin Oncol. 24:5652–5657.

[35] Liedtke C, Mazouni C, Hess KR, et al. (2008). Response to neoadjuvant therapy and long-term survival in patientswith triple-negative breast cancer. J Clin Oncol. 26:1275–1281.

[36] Rakha EA, Ellis IO, Reis-Filho JS. (2008). Immunohistochemical heterogeneity of breast carcinomas negative forestrogen receptors, progesterone receptors and Her2/neu (basal-like breast carcinomas). Mod Pathol. 21:1060–1061; author reply 1061–1062.

[37] Brenton JD, Carey LA, Ahmed AA, et al. (2005). Molecular classification and molecular forecasting of breastcancer: ready for clinical application? J Clin Oncol. 23:7350–7360.

[38] Carey LA, Dees EC, Sawyer L, et al. (2007). The triple negative paradox: primary tumor chemosensitivity of breastcancer subtypes. Clin Cancer Res. 13: 2329–2334.

[39] Hu Z, Fan C, Oh DS, et al. (2006). The molecular portraits of breast tumors are conserved across microarrayplatforms. BMC Genomics. 7:96.

[40] Kennecke H, Yerushalmi R, Woods R, et al. (2010). Metastatic behavior of breast cancer subtypes. J Clin Oncol. 28:1684–1691.

[41] Thike AA, Cheok PY, Jara-Lazaro AR, et al. (2010). Triple negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. Mod Pathol. 23:123–133.

[42] Thike AA, Iqbal J, Cheok PY, et al. (2010) Triple negative breast cancer: outcome correlation with immunohistochemical detection of basal markers. Am J Surg Pathol. 34:956–964.

[43] Abd El-Rehim DM, Pinder SE, Paish CE, et al. (2004). Expression of luminal and basal cytokeratin in humanbreast carcinoma. J Pathol. 203: 661-671.

[44] Bertucci F, Finetti P, Cervera N, et al. (2008). How basal are triple-negative breast cancers? Int J Cancer. 123:236–240.

[45] Rakha, E, El-Sayed, M.; Reis-Filho, J.; Ellis, I. (2009). Patho-biological aspects of basal-like breast cancer. Breast Cancer Res. Treat. 113: 411–422.

[46] Luck AA, Evans AJ, Green AR, Rakhay EA, Paishy C, Ellis IO. (2008). The influence of basal phenotype on themetastatic pattern of breast cancer. Clinical Oncology. 20: 40 – 45.

[47] Rosen P.P. 2001. Rosen's Breast Pathology, second ed. Lippincott Williams & Wilkins, Philadelphia.

[48] Louwman MW, Vriezen M, van Beek MW, NoltheniusPuylaert MC, van der Sangen MJ, Roumen R. (2007). Uncommon breast tumours in perspective: incidence, treatment and survival in the Netherlands. Int. J. Cancer. 121: 127e135.

[49] Awadelkarim K D, Arizzi C, Elamin E O M, Hamad H M A, De Blasio P, Mekki S O. (2008). Pathological, clinicaland prognostic characteristics of breast cancer in Central Sudan versus Northern Italy: implications for breastcancer in Africa. Histopathology. 52: 445–456.

[50] Foulkes WD, Stefansson IM, Chappuis PO, Begin LR, Goffin JR, Wong N et al. (2003). Germline BRCA1mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst. 95: 1482-5.

[51] Foulkes WD, Brunet JS, Stefansson IM et al. (2004). The prognostic implication of the basal-like (cyclin E high/p27low/ p53+/glomeruloid-microvascular-proliferation+) phenotype of BRCA-related breast cancer. Cancer Res. 64: 830-5.

[52] Arnes JB, Brunet JS, Stefansson IM et al. (2005). Placental cadherin and the basal epithelial phenotype of BRCA1-related breast cancer. Clin Cancer Res. 11: 4003-11.

[53] Gakinya S, Sayed S, Chauhan R, Sayed P. (2010). Breast cancer molecular subtypes and their clinicopathological characteristics amongst patients at the Aga Khan University hospital (Nairobi). Ann Afr Surg. 5: 1983-8.

[54] Nalwoga H, Arnes J. B, Wabinga H, Akslen L. A. (2007). Frequency of the basal-like phenotype in African breastcancer. Acta Pathologica, Microbiologica et Immunologica Scandinavica. 115 (12): 1391–1399.

[55] Mbonde M, Amir H, Schwartz-Albiez R, Akslen L, Kitinya J. (2000). Expression of estrogen and progesterone receptors in carcinomas of the female breast in Tanzania. Oncol Rep. 7:277–83.

[56] Burson AM, Soliman AS, Ngoma TA, et al. (2010). Clinical and epidemiologic profile of breast cancer in Tanzania. Breast Disease. 31:33–41.

[57] Awadelkarim DK, Arizzi C, Elamin EOM et al. (2011). Basal-like phenotype in a breast carcinoma case series from Sudan: Prevalence and clinical/pathological correlations. Pathology Research International. Article ID 806831, 10 pages. Doi: 10.4061.

[58] Gazinska P, Grigoriadis A, Brown JP et al. (2013). Comparison of basal-like triple-negative breast cancer defined by morphology, immunohistochemistry and transcriptional profiles. Modern Pathology. 26: 955–966.

[59] Liu ZB, Wu J, Ping B et al. (2009). Basal cytokeratin expression in relation to immunohistochemical and clinical characterisation in breast cancer patients with triple-negative phenotype. Tumori. 95: 53-62.

[60] Cakir A, Gonul II, Uluoglu O. (2012). A comprehensive morphological study for basal-like breast carcinomas withcomparison to nonbasal-like carcinomas. Diagnostic Pathology. 7:145.

[61] Kuroda H, Ishida F, Nakai M, Ohnisi K, Itoyama S. (2008). Basal cytokeratin expression in relation to biological factors in breast cancer. Human Pathology. 39: 1744-1750.

[62] Carvalho FM, Bacchi LM, Santos PPC, Bacchi CE. (2010). Triple-negative breast carcinomas are a heterogeneous entity that differs between young and old patients. Clinics. 65 (10): 1033-1036.

[63] Alshareeda AT, Soria D, Garibaldi JM et al. (2013). Characteristics of basal cytokeratin expression in breast cancer. Breast Cancer Res Treat. 139(1): 23-37.

[64] Robbins P, Pinder S, de Klerk N, et al. (1995). Histological grading of breast carcinomas: a study of inter-observeragreement. Hum Pathol. 26(8): 873-9.

[65] Sotiriou C, Wirapati P, Loi S, et al. (2006). Gene expression profiling in breast cancer: understanding the molecularbasis of histologic grade to improve prognosis. J Natl Cancer Inst. 98(4): 262-72.

[66] Rakha EA, Putti TC, Abd El-Rehim DM et al. (2006). Morphological and immunophenotypic analysis of breastcarcinomas with basal and myoepithelial differentiation. J Pathol. 208: 496-506.