Clinicopathological and molecular characteristics of Ku 70/80 expression in Nigerian breast cancer and its potential therapeutic implications

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ABSTRACT

Introduction

Ku 70/80, a regulator of the Non-Homologous End Joining (NHEJ) has been shown to have clinicopathological and prognostic significance in breast cancer (BC) from Caucasian populations. However, its significance in the Nigerian BC population, which is characterized by a higher rate of triple-negativity, and basal phenotype with high p53 mutation rate and BRCA1 deficiency, is yet to be investigated. We hypothesized that Ku70/80 expression would show adverse clinicopathological and survival characteristics in Nigerian and also, likely to have therapeutic implication on Black BC management.

Aim

To investigate the biological, clinicopathological and prognostic significance of Ku 70/80 expression in a BC cohort from the Nigerian population

Materials & Methods

189 well-characterised BC cases were included in the study. Ku 70/80 expression was sought in formalin-fixed, paraffin-embedded (FFPE) BC samples from these patients using Tissue Microarray and Immunohistochemistry. Ku 70/80 expression was correlated with the clinicopathological, molecular and prognostic characteristics of patients.

Results

Ku 70/80 was expressed in 113 (60.1%) of tumours and positively associated with metastatic disease, triple-negativity, basal phenotype, BRCA1 down regulators (MTA-1 and ID4) p-cadherin, PI3KCA, p53 expression and inversely correlated with BRCA1, BRCA2, BARD1 and

p27. Ku 70/80 was predictive of breast cancer-specific survival (BCSS) survival in multivariate analysis, but not of disease-free interval (DFI).

Conclusion

Ku 70/80 expression is associated with metastatic disease, down-regulation of the Homologous Recombination pathway of DNA repair, loss of the G1-S phase checkpoint, high EMT potential and poor prognosis.

Keywords: Nigerian breast cancer, Ku 70/80, clinicopathological, biological, prognosis

INTRODUCTION

The Non-Homologous End Joining (NHEJ) is an important cellular DNA double-stranded break (DSB) repair mechanism which is undertaken by a complex of proteins including the Ku heterodimer, Ku 70/80 [1-4]. The specific functions of the Ku heterodimer include recognition of and binding to the DSBs, recruitment of DNA protein kinase c (DNA-PKc) to the ends of the DSB. Once bound to the DSB ends the DNA-PKcs undergoes auto-phosphorylation, disengages from the damaged ends and allows downstream repair factors, such as Artemis, polynucleotide kinase 3'-phosphatase (PNKP), DNA polymerases, the MRN complex, XLF and XRCC4/DNA Ligase IV, to access the damaged site [1-4]. Ku 70/80 therefore acts as a regulator of the NHEJ, and its expression can be used as a marker for the NHEJ [4].

The NHEJ is involved in the repair of DSBs induced by radiation and some chemotherapeutic agents such as cyclophosphamide and topoisomerase poisons [1, 5]. In addition, the NHEJ process itself is error-prone and therefore induces additional mutations and genomic instability in tumour cells [6-11]. Recent studies show that Ku 70/80 is over expressed in tumour more than in normal cells [6, 10]. Furthermore, Ku 70/80 protects cancer cells from apoptosis [11]. Ku 70/80 can therefore be regarded as an oncogene in cancer cells as it promotes tumour progression.

The prognostic significance of Ku 70/80 expression in relation to clinical, pathological and survival indices are organ-specific. In advanced head and neck carcinoma, patients whose tumours had higher Ku 70/80 expression show better response to chemotherapy with 5-fluorouracil and cisplatin [12]. In gastrointestinal malignancies, high Ku 70/80 expression is associated with higher gastric cancer clinical stage; with depth of invasion, pathological stage, histopathological grade and prognosis in advance colorectal carcinoma. Furthermore, in rectal carcinoma its relationship with pathological stage, histopathological grade, radio-sensitivity and disease-free interval has been reported [6, 13, 14]. Conversely, in endometrial carcinoma, disease-free interval is longer in patients whose tumour show low expression of Ku 70/80. However, there was no association between clinicopathological features and other survival characteristics [15].

A study of a Caucasian BC cohort showed that nuclear expression of Ku 70/80 is associated with higher histological grade, lympho-vascular invasion, negative estrogen receptor (ER) expression,

basal-like phenotype, p53 and checkpoint kinase1 (CHK1) positivity. Ku 70/80 expression also showed an association with disease-free interval in univariate but not in multivariate analysis [16].

In this study we sought to determine the expression of Ku 70/80 in a well-characterised Nigerian BC cohort using Tissue Microarray and immunohistochemistry; and compared its expression with the clinicopathological and survival characteristics of the patients.

MATERIALS AND METHODS

Patients' characteristics

The patients included a cohort of Nigerian BC women with adequate data on clinicopathological features and survival characteristics, as used in previous studies [17]. Briefly, 189 of formalin-fixed paraffin embedded (FFPE) breast cases from women presenting at the Olabisi Onabanjo University Teaching Hospital, Sagamu, and Histopathology Specialist laboratory, Idi-Araba Lagos, Nigeria from January 2002 to December 2008 were included. Clinical history and tumour characteristics including age, menopausal status, tumour type, histological grade, tumour size, lymph node status and vascular invasion were assessed in a standardised manner for all the patients.

Patient outcome and treatment data were retrieved from the patient's records. All patients were treated with combination of classical chemotherapy cyclophosphamide, methotrexate and 5FU and hormonal therapy (tamoxifen). Eighty five out of the patients (about 45%) received radiotherapy. Patients were followed up for at least 60 months (260 weeks). During this follow-up period; 83 patients (43.9%) died, while 106 patients either remained alive (n= 13, 6.9%) or were lost to follow-up (n=93, 49.2%). Forty (40) patients (21.2%) had recurrence within the maximum disease-free interval of 15 months (60weeks).

The Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) criteria, recommended by McShane et al [18] were followed. This study was approved by the Medical Advisory Committee, Olabisi Onabanjo University Teaching Hospital.

Western blotting

The KU 70/80 antibody was first validated using Western blotting as described in [16]. Briefly, protein extraction was accomplished using RIPA buffer containing protease and phosphatase inhibitors. About 50µg of protein was used to perform a protein gel electrophoresis. The resolved

proteins were blotted onto a nitrocellulose membrane. Membrane blocking was accomplished with a solution of 5% non-fat dried milk in PBS-Tween-20. Primary incubation was performed in 1:1000 dilution of KU 70/80 mouse monoclonal antibody for 1hour at room temperature. Beta-actin was used as control. Detection of proteins was performed by chemiluminescence.

Immunohistochemistry (IHC) staining

TMAs were constructed as previously described [17]. The expression of markers was determined using IHC in 4um TMA sections. The standard strept Avidin–Biotin complex method as described in Agboola et al was used for the detection of tissue markers [17]. In each IHC staining both positive and negative controls were included. The negative controls were performed by omitting the primary antibodies. The sources of primary antibodies, antibodies, positive controls, and dilution methods used in this study are shown in Table 1.

Immunohistochemistry scoring

The scoring of immunoreacitivty for Ku 70/80 was performed by determining the percentage of invasive malignant cells within showing positive staining. All samples were scored by one observer (JA) and a further observer (T-AF) countered scored a proportion. The cases were scored without knowledge of the patient outcome. The whole tissue mounts and TMA samples were scored twice. The mean of the scores were calculated to reach a final score. The median score of 74% was used to dichotomise immunoreactivity into high/low groups for subsequent analysis. All the other markers used in this study were scored as shown in Table 1

Statistical Analyses

Statistical analysis was performed using SPSS 16.0 statistical software. Chi-squared analyses were used for inter-relationships between the Ku 70/80 expression, clinicopathological parameters and other biomarkers. The Kaplan–Meier survival method and the log-rank test were used for survival curves. Multivariate analyses using Cox proportional hazard regression models were performed and from the model both the risk factor and 95% confidence intervals were generated. A two-sided p-value of <0.05 was considered significant.

RESULTS

Western blot confirmed the specificity of Ku 70/80 antibody (Ab3108 Abcam) used in this study. The nuclear Ku 70/80 biomarker expression was dichotomised according to the frequency histogram distributions using the median of the percentage of the nuclear staining. Zero to seventy-four percent (0-73%) staining was considered negative/low expression while 74% and above was regarded as positive/ high expression. A total of 113 (60.1%) cases were considered positive and 76 (39.9%) were negative/low for Ku 70/80.

Clinicopathological features of Ku 70/80 expression in Nigerian breast cancer

Table 2 shows the relationship between the clinicopathological parameters and Ku 70/80 protein expression, where the majority of the tumours that expressed Ku 70/80 showed positive vascular invasion (p=0.009). There were no other significant associations between Ku 70/80 and other clinicopathological characteristics.

Ku 70/80 expression shows associations with hormone receptors, molecular subtypes, cell cycle and DNA repair markers

The relationship between the Ku 70/80 and other biomarkers expression (Table 3) revealed an inverse correlation between oestrogen receptor (ER) and progesterone reactor (PgR), BRCA1-associated RING domain-1(BARD1), p27 (all $p<0.001$), HER-2 ($p=0.01$), Breast cancer associated genes 1 (BRCA-1, $p=0.004$) and (BRCA-2, $p=0.006$), Conversely, there was a positive correlation of Ku 70/80 expression with cytokeratins: CK5/6 and CK14, BRCA1 down-regulators (metastasis antigen 1 (MTA1) and Helix-loop –helix protein of differentiation 4 (ID4))

protein inhibitor of activated start protein gamma (PIASɣ), Ubiquitin conjugating enzyme 9 (UBC9), Phosphoinositide 3-kinases (PI3KCA), p53, Epithelia growth factor receptor 1 (EGFR), Triple-negative tumour and Basal-like phenotype (all $p<0.001$)., cyclin B ($p=0.006$) and Placental (P)-cadherin ($p=0.04$), There was no significant association between Ku 70/80 with ataxia telangiectasia and Rad 3 related (ATR), Epithelia (E)-cadherin or protein inhibitor of activated start protein 1 (PIAS1).

Prognostic significance of Ku 70/80 expression in the Nigerian breast cancer cases

Multivariate analysis shows that tumours which were positive for Ku70/80 protein expression had a significantly poorer BCSS compared to tumours with negative/low Ku70/80 protein expression, independent of tumour grade, tumour size and lymph node involvement (Table 4). There was no significant association with disease-free interval, DFI.

DISCUSSION

Ku 70/80 is one of the key determinants in the DNA damage response [1-4]. The roles of this biomarker in relation to clinicopathological parameters, biological behaviour and patients' outcome in BC, particularly in women from an indigenous Black African population, had hitherto not been studied. In line with its role in tumour progression, this study found a high expression of the NHEJ marker, Ku 70/80, in more than 60% of BC from a Nigerian population [6-9, 12-16]. This finding agrees with the rate of expression of Ku 70/80 in clinical cancers generally [10, 13, 14, 19].

In concurrence with previous studies, the expression of Ku 70/80 was associated with adverse clinicopathological and survival characteristics: vascular invasion, triple-negative and basal-like subtypes of BC, as well as the molecular markers of poor prognosis [12-15]. In particular, these findings are similar to the results that were obtained from a UK study [16].

Ku 70/80 expression was a predictor of poor clinical outcome in Nigerian BC patients, independent of tumour grade, size and lymph node involvement. This is the first study to report Ku 70/80 association with survival in Nigerian BC.

The expression patterns of BRCA1, BRCA2, BARD1, p53, MTA, ID4 and Ku 70/80 in this and our previous studies imply a down-regulation of the Homologous Recombination pathway (HR) in the majority of Nigerian BC patients and the tumours may be depending on the NHEJ pathway, a pathway which is adept at repairing cyclophosphamide- and radiotherapy-induced DSBs [1, 5, 20, 21].While BRCA1, BRCA2 and BARD1 function in the HR pathway, MTA and ID4 are known down-regulators of BRCA1. UBC9 and PIASγ stabilize Ku 70 to enable the DNA repair by NHEJ mechanisms [1, 5, 22-25].

In this study, Ku 70/80 expression is associated with the epithelial-mesenchymal transition (EMT) markers; p-cadherin and PIK3CA [26, 27]. The EMT enables tumour cells to undergo invasion and metastasis [26, 27]. This is in line with our finding that Ku 70/80-positive tumours had high vascular invasive activities. Loss of p53 function is associated with uninhibited cell cycle G1 to S phase transition regulated by the cyclin-cyclin-dependent kinase complexes [28]. Therefore, the high expression of cyclin B found in this study may be in keeping with the loss of p53 function, i.e. p53 overexpression, seen in Ku 70/80-positive tumours. Furthermore, the p27 loss, which denotes, in conjunction with p53 loss of function, the absence of the G1 to S phase checkpoint, in these Ku 70/80 tumours may be due to increased activity of the PIK3CA pathway in our study, since the PIK3CA pathway has been shown to down-regulate p27 [29], this might probably induced aneuploidy cells capable of enhancing tumours metastasis.

With respect to the therapeutic significance of Ku 70/80 expression in our BC cohort, it is noteworthy that cyclophosphamide-based chemotherapy, hormonal therapy and radiotherapy were offered to many patients in the Nigerian series, and yet the majority of them died within 5 years of diagnosis [30]. It is plausible to assume that the poor prognosis observed in our BC cohort is partly due to the adeptness of tumour cells at repairing therapy-induced DSBs using NHEJ pathway, and also, probably subjected them to high resistant rate to chemotherapy and radiotherapy [1, 5]. Going further, it might be arguable that there is probably a need to change from the current “one-size-fits-all” approach to therapy, where all patients are treated with cyclophosphamide-based chemotherapy, to a biomarker-assisted selection of patients for

chemotherapy and radiotherapy. For example, a study has shown that patients whose tumours with higher expression levels of Ku 70/80 respond better to cisplatin and 5-fluorouracil [12] Also, low expression of Ku 70/80 was shown to predict good response to radiotherapy in early BC [31]. More recently, patients with deficient BRCA1 expression have been shown to respond better to cisplatin than patients with proficient BRCA1 expression [32, 33]. Biomarker-assisted chemo- and radiotherapy may therefore be a superior strategy for clinical cancer therapy than the current approach. A further potential therapeutic implication of the findings in this study is that the Ku 70/80 expression pattern in our cohort opens up a possibility of targeting the NHEJ pathway in black BC This is particularly important in the triple-negative BC subtype which currently has paucity of targeted therapy and commonly occurred among the black women. For example, inhibition of the NHEJ pathway such as with DNA-PK inhibitor, Nu7026 was reported to sensitize cells to topoisomerase II poisons [34]. Considering that most tumours which show high expression of Ku 70/80 in our study are also deficient in BRCA 1 and BRCA 2 (i.e. the HR pathway), it becomes even more attractive to target the NHEJ pathway in Nigerian BC cases. In many preclinical studies, inhibition of both the HR and NHEJ pathways has been demonstrated to enhance therapeutic sensitivity to radiotherapy and chemotherapy [35-38].

In conclusion, this study has for the first time shown that Ku 70/80 protein expression is high in Nigerian BC cases, has adverse clinicopathological significance, defective HR pathway of DNA repair, loss of G1-S checkpoint, high EMT potential and also confers poor prognosis. In addition, it has revealed a possibility for targeting the DNA repair pathways to improve patients' outcome among the Black BC.

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TABLES

Table 1: Sources, dilution, distribution, cut-offs point and pre-treatment used for revalidation

Antibody	Clone	Source	Dilution	Distribution	Scoring System	Cut-offs	Pre-treatment	Positive control	Negative control
ATM	Ab32420	Abcam	1:25	Nuclear	% of positive cells	≥1% (positive)	Antigen retrieval Microwave	Known breast carcinoma	Omitting the antibody

BARD 1	NBP1-19636	Novus Biologicals	1:50	Cytoplasm	% of positive cells	≥1% (positive)	Antigen retrieval microwave	Normal breast acini	Omitting the antibody
BRCA 1	Ab-1 (MS110)	Calbiochem	1:150	Nuclear	% of positive cells	<25% (negative)	Antigen retrieval Microwave	MCF 7 cells (human breast adenocarcinoma cell line)	Omitting the antibody
BRCA 2	Ab110967	Abcam	1:100	Nuclear	% of positive cells	>25% (positive)	Antigen retrieval Microwave	Human brain cancer	Omitting the antibody
Ck5/6	M7237	Dako-cytomation	1:60	Cytoplasm	% of positive cells	≥10% (positive)	Antigen retrieval Microwave	Known case of CK56 BC	Omitting the antibody
Ck14	LL002	Novocastra	1:40	Cytoplasm	% of positive cells	≥10% (positive)	Antigen retrieval Microwave	Known case of CK14 breast cancer	Omitting the antibody
Cyclin B1	Ab 32053	Abcam	1:2000	Nuclear	% of positive cells	≥60% (positive)	Antigen retrieval Microwave	Human skin carcinoma	Omitting the antibody
E – cadherin	NCH-38	Dako-Cytomation	1:100	Cytoplasm and membrane	% of positive cells	≥100 H score (positive)	Antigen retrieval Microwave	Normal gastric mucosa	Omitting the antibody
EGFR	31G7	Novocastra	1:30	Membrane	% of positive cells	≥10% (positive)	Not required	Myoepithelial cells of normal duct in normal mammary gland	Omitting the antibody
erbB2	Polyclonal	Dako-Cytomation	1:100	Membrane			Not required	Known case of erbB2 strong BC expression	Omitting the antibody
ER	1D5	Dako-Cytomation	1:200	Nuclear	% of positive cells	≥0(positive)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
ID4	Ab77345	Abcam	1:100	Nuclear	% of positive cells	≥50% (positive)	Antigen retrieval Microwave	Human colon cancer	Omitting the antibody
KU70/80	Ab3108	Abcam	1:2500	Nuclear	% of positive cells	>74% (positive)	Antigen retrieval microwave	Tonsil tissue	Omitting the antibody
MTA 1	Ab84136	Abcam	1:100	Nuclear	% of positive cells	≥1% (positive)	Antigen retrieval microwave	Human gastric adenocarcinoma	Omitting the antibody

P - cadherin	NCL-P-cad	Novocastra	1:200	Cytoplasm	% of positive cells	≥5% (positive)	Antigen retrieval Microwave	Known case of P-cadherin strong BC expression	Omitting the antibody
PgR	PgR	Dako-Cytomation	1:150	Nuclear	% of positive cells	≥0 (positive)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
PIASγ	NBP1-31215	Novus Biologicals	1:100	Nuclear	% of positive cells	≥65% (positive	Antigen retrieval Microwave	Human breast carcinoma	Omitting the antibody
P13KCA	HPA00 09985	Sigma	1:50	Nuclear	% of positive cells	>60% (positive)	Antigen retrieval microwave	Human breast carcinoma	Omitting the antibody
p27	SX53 G8	Dako-Cytomation	1:10	Nuclear	% of positive cells	≥10% (positive)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
p53	DO7	Novocastra	1:50	Nuclear	% of positive cells	>10% (negative)	Antigen retrieval Microwave	Normal breast acini	Omitting the antibody
UBC9	Ep293 8Y	Novus Biologicals	1:100	Nuclear	% of positive cells	>70% (positive)	Antigen retrieval microwave	Human brain tissue	Omitting the antibody

Table 2: Relationship between KU70/80 expression and clinicopathological parameters in Nigerian breast cancer

Variables	KU70/80

	Negative/low (%)	Positive (%)	χ2 value	p-value
Age (years)				
≤ 50	48 (64.0)	73 (64.6)	0.007	0.93
> 50	27 (36.0)	40 (35.4)		
Lymphnode involvement				
Negative				
Positive	5 (6.7)	10 (8.8)	0.29	0.58
	70 (93.3)	103 (91.2)		
Menopausal				
Pre	47 (62.7)	80 (70.8)	1.35	0.24
Post	28 (37.3)	33 (29.2)		
Mitotic figure				
Low	48 (64)	64 (56.6)	1.43	0.49
Medium	17 (22.7)	27 (23.9)		
High	10 (13.3)	22 (19.5)		
Nuclear pleomorphism				
Small uniform cells	0 (0.0)	0(0.0)		
Moderate increase in size	29 (38.7)	29 (25.7)	3.57	0.06
Marked variation	46 (61.3)	84 (74.3)		
Size (cm)				
≤2.0	4 (5.3)	12 (10.6)	1.61	0.20
>2.0	71 (94.7)	101 (89.4)		
Tubule formation				
> 75 %	2 (2.7)	1 (0.9)		
10 -75 %	1 (1.3)	7 (6.2)	3.45	0.17
< 10 %	72 (96.0)	105 (92.9)		
Tumour grade				
1	3 (4.0)	1 (0.9)		0.27
2	45 (60.0)	64 (56.6)	2.61	
3	27 (36.0)	48 (42.5)		
Tumour type				
Typical medullary	1 (1.3)	0 (0.0)	6.30	0.50
Atypical medullary	1 (1.3)	0 (0.0)		
Tubular	0 (0.0)	1 (0.9)		
Lobular	0 (0.0)	2 (1.8)		
Ductal NST	67 (89.3)	105 (92.9)		
Mucinous	1 (1.3)	1 (0.9)		
Tubulolobular	0 (0.0)	0 (0.0)		
Lobular mixed	2 (2.7)	1 (0.9)		
Tubular mixed	3 (4.0)	3 (2.7)		
Mixed NST	0 (0.0)	0 (0.0)		
Others	0 (0.0)	0 (0.0)		
Vascular invasion				
Negative	11 (14.7)	32 (28.3)	4.76	**0.009**
Positive	64 (85.3)	81 (71.7)		

Table 3: Relationship between KU70/80 expression and other biomarkers in Nigeria BC women

Variables	KU 70/80			
	Negative/low (%)	**Positive (%)**	**χ2 value**	**p-value**
Basal cytokeratin				
Ck5/6 Negative Positve	56 (88.9) 7 (11.1)	23 (22.5) 79 (77.5)	**68.68**	**<0.001**
CK14 Negative Positive	39 (76.5) 12 (23.5)	35 (39.8) 53 (60.2)	**17.46**	**<0.001**
BRCA1 down regulators				
MTA1 Negative Positive	53 (75.7) 17 (24.3)	27 (24.5) 83 (75.5)	**45.36**	**<0.001**
ID4 Negative Positive	39 (63.9) 22 (36.1)	6 (5.5) 104 (94.5)	**69.20**	**<0.001**
E-cadherin Negative Positive	35 (64.8) 19 (35.2)	56 (65.9) 29 (34.1)	0.01	0.897
P_cadherin Negative Positive	27 (50.9) 26 (49.1)	29 (33.3) 58 (66.7)	4.25	**0.04**
CYCLINB1 Negative Positive	39 (65.0) 21 (35.0)	42 (42.4) 57 (57.6)	7.61	**0.006**
P27 Negative Positive	58 (84.1) 11 (15.9)	60 (60.0) 40 (40.0)	**11.21**	**<0.001**
P53 Negative Positive	27 (61.4) 17 (38.6)	13 (12.7) 89 (87.3)	**36.52**	**<0.001**
ATM Negative Positive	70 (93.3) 5 (6.7)	100 (88.5) 13 (11.5)	1.21	0.27
BARD1 Negative Positive	68 (97.1) 2 (2.9)	84 (77.1) 25 (22.9)	**13.41**	**<0.001**
BRCA1 Negative Positive	43 (75.4) 14 (24.6)	85 (92.4) 7 (7.6)	**8.35**	**0.004**

BRCA2 Negative Positive	40 (67.8) 19 (32.2)	78 (86.7) 12 (13.3)	**7.70**	**0.006**
EGFR Negative Positive	45 (83.3) 9 (16.7)	49 (49.5) 50 (50.5)	**16.88**	**<0.001**
HER_2 Negative Positive	45 (72.6) 17 (27.4)	90 (87.4) 13 (12.6)	5.69	**0.01**
P13KCA Negative Positive	38 (54.3) 32 (45.7)	28 (25.2) 83 (74.8)	**15.64**	**<0.001**
Steroid hormone receptors				
ER Negative Positive	41(59.4) 28 (40.6)	96 (87.3) 14 (12.7)	**18.31**	**<0.001**
PgR Negative Positive	29 (56.9) 22 (43.1)	90 (90.9) 9 (9.1)	**23.79**	**< 0.001**
PIAS1 **Negative** **Positive**	27 (54.0) 23 (46.0)	49 (62.8) 29 (37.2)	0.98	0.32
PIASγ **Negative** **Positive**	40 (69.0) 18 (31.0)	12 (11.7) 91 (88.3)	**55.74**	**<0.001**
UBC9 **Negative** **Positive**	42 (79.2) 11 (20.8)	8 (7.8) 95 (92.2)	**82.09**	**<0.001**
Triple negative Negative Positive	39 (78.0) 11 (22.0)	27 (27.0) 73 (73.0)	**35.18**	**< 0.001**
Nielsen classification Basal HER-2 Luminal A Luminal B	0 (0.0) 9 (12.0) 18 (24.0) 7 (9.3)	67 (59.3) 9 (8.0) 10 (8.8) 1 (0.9)	**73.64**	**< 0.001**

Table 4: Cox Regression Analysis

Variables	p-value	Hazard ratio	95.0% CI	
			Lower	Upper
KU70_80	**0.004**	0.503	0.316	0.800
grade	**<0.001**	2.534	1.618	3.969
size	0.694	1.200	0.483	2.984
lymph_node	0.633	1.239	0.515	2.979

FIGURES

Figure 1: Ku 70/80 immunoreactivity positivity (A) and negativity (B) in Nigerian breast cancer. Mag x20

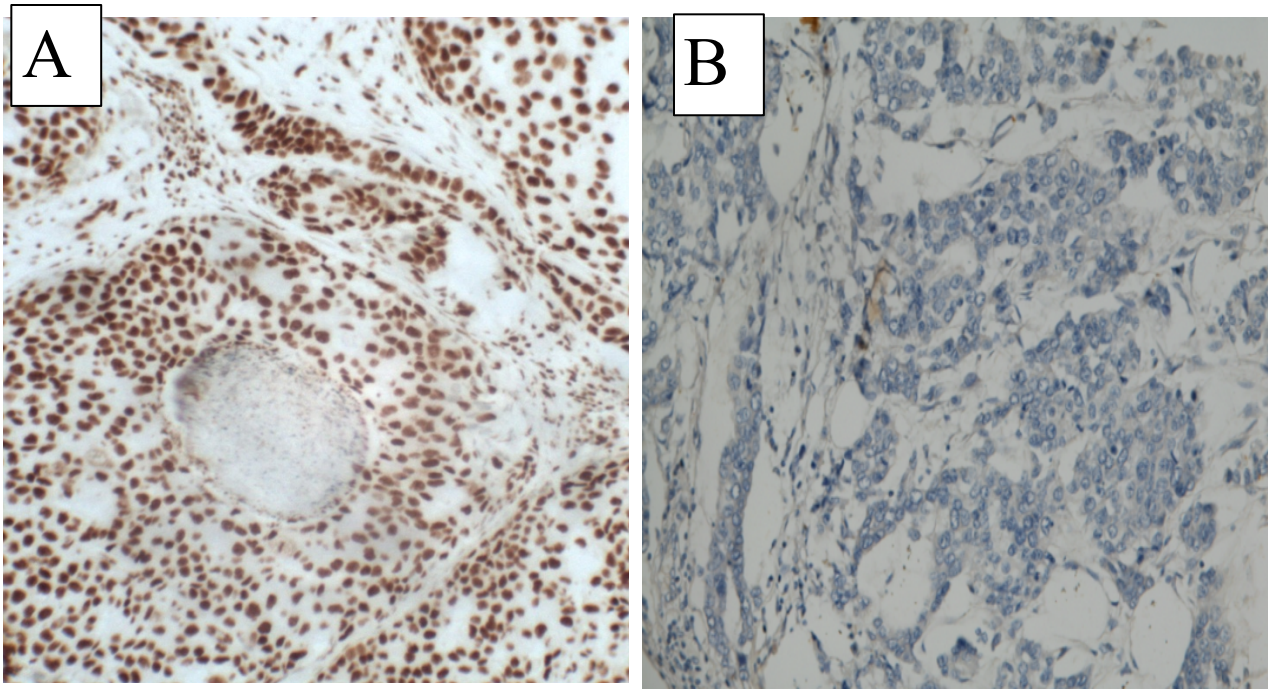
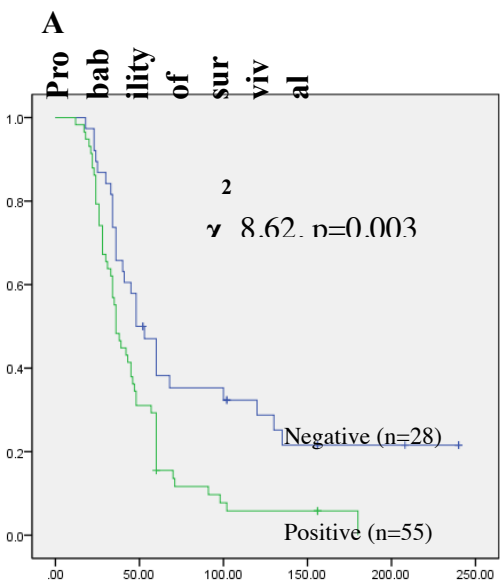
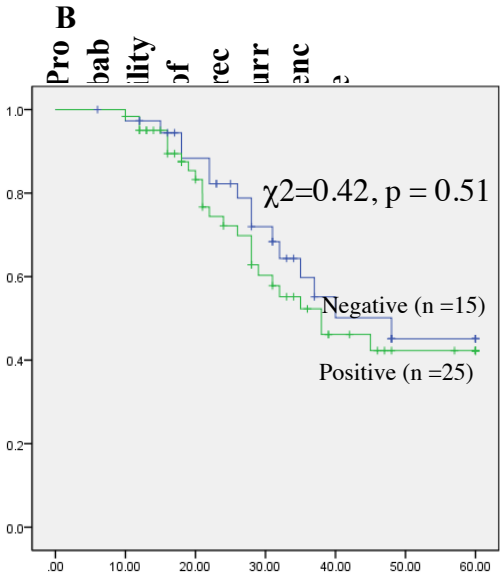


Figure 2 (A) and (B) show Ku 70/80 tumours probability of survival (A) and recurrence (B)



Survival time in weeks



DFI in weeks