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*c-erb*B-2 ONCOPROTEIN OVER-EXPRESSION IN BREAST CANCER AND ITS RELATIONSHIP TO HISTOLOGY AND GRADE IN A UGANDAN POPULATION
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*c-erb*B-2 ONCOPROTEIN OVER-EXPRESSION IN BREAST CANCER AND ITS RELATIONSHIP TO HISTOLOGY AND GRADE IN A UGANDAN POPULATION

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ABSTRACT

Objective: **To evaluate *c-erb*B-2 oncoprotein over expression in breast cancer patients of Kyadondo county and also relate this oncoprotein to histological type and grade of the tumour.**
Design: **Cross-sectional study.**
Setting: **Pathology Department, Faculty of Medicine, Makerere University.**
Subjects: **Seventy two breast cancer patients who were among the 174 entered in survival study had their paraffin breast tissue blocks retrieved from archives of Pathology Department and had their *c-erbB-2* determined by peroxidase-labeled streptavidin-biotin immunohistochemical method.**
Results: ***c-erb*B-2 oncoprotein was over expressed in 33.3% of the tumours and correlated positively with histological grade ($p = 0.007$). TH oncoprotein over expression was not restricted to any particular histological type and there was no association with age of patient.**
Conclusion: **The over expression of *c-erbB-2* oncoprotein observed in one third of breast cancer patients of this African population could partly explain the observed poor survival rate reported in this community.**

INTRODUCTION

Breast cancer is the second most common malignancy in women of Kyadondo county in Uganda with incidence rates continuing to rise (1). Recently it was found that the five-year survival rate of breast cancer in patients of Kyadondo county was 45% and this poor survival rate has been attributed to late presentation of patients to poorly facilitated health care system (2). However it appears the above-mentioned factors may not be solely responsible for the poor survival in this population. Reports of studies carried out on African Americans breast cancer patients in the United States also show poor prognosis when compared to the whites (3). It has been therefore postulated that intrinsic biological factors may partly contribute to the poor prognosis of breast cancer patients of African decent. Histological type and differentiation are now recognised inherited biological features used as powerful prognostic indicators. However absence of uniform classification and grading of these tumours makes comparison of findings difficult. Recent evidence suggests that over expression of the protein product of the *c-erb*B-2 oncogene is an independent indicator of poor prognosis and has greater prognostic value than most currently used prognostic factors including hormonal receptor status (4). The study to be described was therefore undertaken to determine the over expression of *c-erb*B-2 oncogene product and also relate this over expression to histological type and grade of breast cancer in that population of Kyadondo county that participated in the survival study.

MATERIALS AND METHODS

Kyadondo county comprises Kampala, the capital city of Uganda, with surrounding urban and semi urban areas. This county is covered by Kampala Cancer Registry (KCR) which is a population based registry situated in Department of Pathology, Faculty of Medicine, Makerere University. A follow-up study was carried out on 13 most common cancers, which included breast cancer reported to KCR between 1993 and 1997 and results described previously by Gondos *et al* (2). Using the Keshi and Leslie formula (5) for cross sectional study on the population of women with histologically proven breast cancer registered at the Kampala Cancer Registry who were involved in the cancer survival study, the appropriate sample size was determined as 72 cases. Seventy two paraffin tissue blocks of these patients were retrieved from the archives of Department of Pathology and sections were cut at 4 um thickness. These sections were conventionally stained with haematoxylin and eosin and the tumours were histologically typed according to WHO international classification of breast tumours (6). Tumour grades were also determined using the modified Bloom Richardson method (7).

The peroxidase labeled streptavidin biotin (LSAB) staining procedure was employed using the Dako LSAB 2 as the detection system for *c-erb*B-2 protein over expression as described below:

The four-micrometer thick sections were mounted on silanized slides and fixed by heating. These sections were transferred to the citrate buffer and heated for 40 minutes to retrieve the antigen then allowed to cool to room temperature (20-25°C) for 20 minutes. After rinsing the sections with 0.05M,Tris buffered saline, were pre-treated with 3% hydrogen peroxide for five minutes to inhibit endogenous peroxidase activity. The rabbit antihuman *c-erb*B-2 oncoprotein primary antibody pre-diluted 1:200 was then applied and incubated for 20 minutes at room temperature. The sections were rinsed with three changes of buffered solutions, placed in fresh buffer and then incubated with biotinylated antibody for 20 minutes. After rinsing gently with buffer solution, the sections were incubated at room temperature with peroxidase labeled streptavidin-biotin for 20 minutes and then rinsed again. Peroxidase was developed by incubating the sections at room temperature with freshly prepared 3,3'-diaminobenzidine (DAB) substrate-chromogen solution for 15 minutes. Finally sections were rinsed with distilled water and counter stained the nuclei with Mayer's haematoxylin for three minutes. After rinsing the sections thoroughly in distilled water, were mounted using DPX. Substituting the primary antibody with negative control reagents supplied by the manufacturer provided the negative control. Breast cancer known to be positive for *c-erb*B-2 was used as positive control.

Immunoreactivity was considered as brown cell membrane staining of the tumour cells. Cytoplasmic staining without simultaneous membranous staining was considered non-specific and not included in the assessment. *C-erb*B-2 over-expression was determined by evaluating and scoring the membranous staining intensity and pattern semi-quantitatively.

RESULTS

A total of 72 samples of breast cancer patients were studied. The age of the patients ranged from 21 to 89 years with mean age of 48.7 (SD 14.8). Figure 1 shows tumour strongly positive for *c-erb*B-2 oncoprotein and 21 of cases (29%) were strongly positive. Forty eight (67%) stained negative for this protein and two cases were weakly positive.

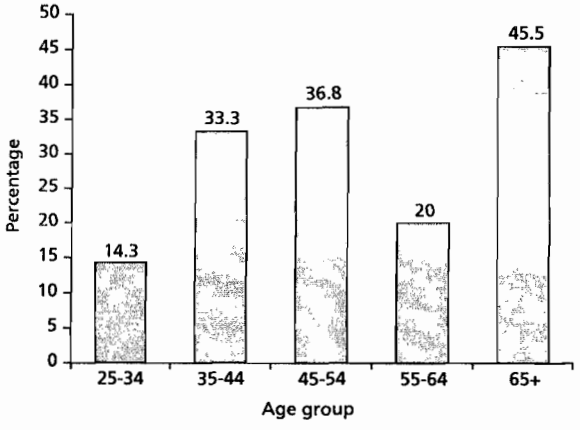
Figure 2 shows the distribution of *c-erb*B-2 positive tumours according to age. The mean age for *c-erb*B-2 positive patients were 52 years (SD 16.1) whereas that for *c-erb*B-2 negative patients was 47.2 (SD 14.1). There was therefore no correlation between age of patient and over-expression of *c-erb*B-2 oncoprotein (P = 0.191).

Figure 1

Invasive ductal carcinoma (NOS) showing strong c-erbB-2 Immunoreactivity x 400

Figure 2

Distribution of c-erbB-2 oncoprotein positive tumours with age



Forty one (57%) of the cases were invasive ductal carcinoma (IDC) not otherwise specified (NOS) and of these 14 (34%) stained positive for the *c-erb*B-2 oncoprotein. Sixteen cases were invasive lobular carcinoma and of these only three were positive for the protein. The other histological types found in this study included six cases of papillary carcinomas in which only one case was positive for the protein and nine cases of IDC with predominate ductal carcinoma *in situ* (DCIS) in which six were positive. There was no significant difference in the distribution of positive case among the various histological types (P = 0.41).

Table 1 shows the distribution of histological grades of breast cancer and their relationship to *c-erb*B-2 over expression. Most cases were either moderate or poorly differentiated adenocarcinoma and *c-erb*B-2 over expression was statistically more prevalent in poorer grade (0.007).

Table 1

Association of c-erbB-2 status and histological grade of breast carcinoma

Grade	*C-erb*B-2 over-expression					
	Positive		Negative		Total	
	No.	(%)	No.	(%)	No.	(%)
I	1	4.2	8	16.7	9	12.5
II	8	33.3	28	58.3	36	50
III	15	62.5	12	25	27	37.5
Total	24	100	48	100	72	100

(P = 0.007)

DISCUSSION

Many authors have shown that the membrane staining of *c-erb*B-2 gene product in breast carcinoma cells obtained by immunohistochemistry is a reliable marker of *c-erb*B-2 gene amplification (8,9). Studies carried out by Barnes *et al* (10) and Walter *et al* (8) showed that any degree of membrane staining is of significance since those tumours with lesser degree of staining showed correlations similar to those with greater reactivity.

Our finding that 33% of the primary breast cancer had over expression of *c-erb*B-2 oncoprotien is in agreement with the data of Slamon *et al* (11). However there have been varying reports of frequency of over expression of *c-erb*B-2 oncoprotien ranging from 10% up to 60% (12-14). This wide variation in frequency has been partly attributed to subsets of biologically aggressive tumours (15,16).

The lack of association between over expression of *c-erb*B-2 oncoprotein with age found in this study was similar to that reported by May *et al* (17) and van de Vijner *et al* (18). It has always been remarked upon in clinical series that in African populations breast cancer occurs at a much younger age and is more aggressive (19-23). However the finding in this study suggests that in Africa like in any other part of the world aggressiveness is not related to age. In fact it has now come to be known that the young age structure of African population coupled with observed flatten or even negative age-incidence curves after menopause, reflect increasing risks in successive generations of women rather than a true decline in risk with age and has no aetiological or prognostic significance (24).

The most common histological type of breast cancer in this study population was IDC and this finding is similar to that of other African populations (21,25). IDC not otherwise specified has been known to have the worst prognosis *(26)* and it has been thought that the high prevalence of IDC in Africans is partly responsible for the poor prognosis of breast cancer. In this study it was found that 34% of IDC over expressed *c-erb*B-2 oncoprotein. Borressen *et al* (27) had also found in their study 34% of IDC over expressed the protein while no over expression was found in invasive lobular carcinoma. High expression of *c-erb*B-2 has also been previously reported to occur in a significant percentage of IDC (28). It is now thought that *c-erb*B-2 may be an early

step in the development of a distinct IDC. This was well exemplified by van de Vijver *et al* (18) who found 19 of 45 ductal carcinomas-*in-situ* over expressed *c-erb*B-2 oncoprotien and these 19 cases were all large-cell comedo growth type. It is also observed in this study that although the numbers of IDC with predominantly DCIS were only three, two were positive for the protein. Other studies have however shown no correlation between *c-erb*B-2 and histological types (10,18).

There was a statistically significant association between over expression of *c-erb*B-2 oncoprotein and histological grade of tumour with poorly differentiated tumours more likely to over express the protein. This finding was similar to that of Walker *et al* (8) who found more poorly differentiated carcinomas with evidence of staining (36%) than well (17%) or moderately (14%) differentiated carcinoma. Similarly Berger *et al* (12) demonstrated a correlation between oncoprotein expression and high nuclear grade and Barnes *et al* (10) also found an association between grade of tumour and over expression of the oncoprotein. However Vijver *et al* (18) and Zhou *et al* (16) found no association between grade of tumour and *c-erb*B-2 oncoprotein over expression.

In conclusion, this study reveals that invasive ductal carcinoma is the commonest histological type and frequently over express *c-erb*B-2 protein product. This study also shows that poor histological grade is more likely to over express the *c-erb*B-2 protein. These findings suggest that inherent biological factors as determined by *c-erb*B-2 over expression may contribute to the poor survival of patients with breast cancer in the Kyadondo community.

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REFERENCES

1. Wabinga H.R., Parkin D.M., Wabwire-Mangeni F. and Nambooze S. Trends in cancer incidence in Kyadondo county, Uganda, 1960-1997. *Br. J. Cancer.* 2000; **82:** 1585-1592.
2. Gondos A., Brenner H., Wabinga H. and Parkin D.M. Cancer survival in Kampala, Uganda. *Br. J. Cancer.* 2005; **92:** 1808-1812.
3. Ries L.A.G., Miller B.A., *et al.* SEER cancer statistics review 1973-1991. NIH Publication No. 94-2789, U.S. Department of Health and Human Services Bethesda, 1994.
4. Rilker F., Colnaglin M.I., Cascinelli N., *et al.* Prognostic significance of HER-2/neu expression in breast cancer and its relationship to other prognostic factors. *Int. J. Cancer.* 1991; **49:** 44-49.
5. Kish L. Survey sampling. John Wiley & sons. 1965.
6. Hartman W.H., Ozzello L., Sobin L.H. and Stalserg N. International histological classification of tumours No. 2. Histological typing of breast tumours. Geneva Switzerland: World Health Organisation. 1981.
7. Elston C.W. and Ellis I.O. Pathological prognostic factors in breast cancer I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. *Histopathology.* 1991; **19:** 403-410.
8. Walker R.A., Gullick W.J. and Varley S.M. An evaluation of immunoreactivity for *c-erb*B-2 protein as a marker of poor short-term prognosis in breast cancer. *Br. J. Cancer.* 1989; **60:** 426-429.
9. Venter D.J., Kumar S., Tuzi N.L. and Gullick W.J. Over expression of the *c-erb*B-2 oncoprotein in human breast carcinomas immunohistological assessment correlates with gene amplification. *Lancet.* 1987; **11:** 69-72.
10. Barnes D.M., Lammie G.A., Millis R.R., *et al.* An immunohistochemical evaluation of *c-erb*B-2 expression in human breast carcinoma. *Br. J. Cancer.* 1988; **58:** 448-452.
11. Slamon D.J., Godo Iphin W., Jones L.A., *et al.* Studies of the Her -2/Neu proto-oncogene in human breast and ovarian cancer. *Science.* 1989; **244:** 707-712.
12. Berger M.S., Locher G.W., Saurer S., *et al.* Correlation of *c-erb* B2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res.* 1988; **48:** 1238-1243.

13. Bilous M., Ades C., Armes J., *et al.* Predicting the HER2 status of breast cancer from basic histopathology data: an analysis of 1500 breast cancers as part of the HER 2000 International study. *Breast.* 2003; **12:** 92-98.
14. Bartlett J.M., Going J.J., Mallon E.A., *et al.* Evaluating HER 2 amplification and over expression in breast cancer. *J. Pathol.* 2001; **195:** 422-428.
15. Clark G.M. and McGuirine W. Follow-up study of HER-2/neu amplification in primary breast cancer. *Cancer Res.* 1991; **51:** 944-948.
16. Zhou D.J., Ahnnja H. and Cline M.J. Proto-oncogene abnormalities in human breast cancer: *c-erb* B-2 amplification does not correlate with recurrence of disease. *Oncogene.* 1989; **4:**105-108.
17. May E., Mouriesse H., Maylevin F., *et al.* Human breast cancer identification of populations with a high risk of early relapse in relation to both oestrogen receptor status and *c-erb*B-2 over expression. *Br. J. Cancer.* 1990; **62:** 420-435.
18. Van de Vijver M.J., Paterse J.L., Mooi W.J., *et al.* Neu protein over-expression in breast cancer. Association with comedo type ductal carcinoma *in situ* and limited prognostic value in stage II breast cancer. N. *Engl. J. Med.* 1988; **319:** 1239-1245.
19. Kenda J.F., Chirimwami B.H. and Voyi Tit. Clinicopathologic analysis of carcinoma of the breast in an African population. *Arch. Surg.* 1988; **123:** 972-974.
20. Bjerregaard B. and Kung'u A. Breast cancer in Kenya: A histopathologic and epidemiologic study. *East Afr. Med. J.* 1992; **69:** 22-26.
21. Amir H., Kitinya J.N. and Parkin D.M. A comparative study of carcinoma of the breast in an African population. *East Afr. Med. J.* 1994; **71:** 215-218.
22. Mbonde M.P., Amir H., Mbemlati N.A., *et al.* Characterisation of benign lesions and carcinomas of the female breast in a sub-Sahara African population. *Pathol. Res. Pract.* 1998; **194:** 623-629.
23. Ikpatt O.F., Kuopio Ti., Ndome-Egba R. and Collan Y. Breast cancer in Nigeria and Finland: Epidemiological, clinical and histological comparison. *Anti-cancer Res.* 2002; **22:** 3005-3012.
24. Moolgavkar S.H., Stevens R.G. and Lee J.A. Effect of age on incidence of breast cancer in females. *J. Natl. Cancer Inst.* 1979; **62:** 493-501.
25. Amir H., Azizi M.R., Makwaya C.K. and Jescani S. TNM classification and breast cancer in African population a descriptive study. *Cent. Afr. J.* 1997; **43:** 357-359.
26 Pereira H., Punder S.E., Sibbering D.M., *et al.* Pathological prognostic factors in breast cancer IV: Should you be a type of a grader? A comparative study of two histological prognostic features in operable breast carcinoma. *Histopathology.* 1995; **27:** 219-226.
27. Borresen A.L., Ottestad L., Ganstad A., *et al.* Amplification and protein over expression of the neu/HER-2/*c-erb*B-2 proto-oncogene in human breast carcinoma: relationship to loss of gene sequences on chromosome 17, family history and prognosis. *Br. J. Cancer.* 1990; **62:** 585-590.
28. Soomro S., Shousha S., Taylor P., *et al. c-erb*B-2 expression in different histological types of invasive breast carcinoma. *Clin. Pathol.* 1991; **44:** 211-214.