The Prevalence Overexpression Of C-Erbb-2 Oncoprotein In Carcinoma Of The Prostate-Mulago Hospital

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Background: Over expression of C-erbB-2 a Human epidermal growth factor has been reported in several human carcinomas including prostate cancer. In prostate cancer studies have for it to have a prognostic role and to predict likelihood of resistance in hormonal therapy. The oncoprotein receptors are now being looked at as possibility of prognostic predictor at the same time as a target for therapy in cancers.
Objective: To determine the prevalence of over expression of C-erbB-2 oncoprotein receptor using Immunohistochemistry in Mulago Hospital.
Material and Methods: Biopsy samples were taken from patients suspected to have prostate cancer convectional histology (Hand E) done. The tumours in the Confirmed slides were then graded as well differentiated, moderately differentiated and poorly differentiated. Immunohistochemistry staining was done using avidin-biotin method. To standardize the staining, the manufacturer (DAKO) supplied both positive and negative control. A well defined scoring system based upon the number of C-erbB-2 on the cell surface was applied. The score ranges from score 0 to +3, over expression is defined as score equal or greater than +2.
Results: over expression was seen in 18 out of 40 cases. Stastistically there was no association between histological grades and over expression. But most of the patients that over expressed C-erbB-2 were either moderately differentiated or poorly differentiate 14 of the 18 positive cases.
Conclusion and Recommendation: Immunohistochemistry which is cheap and easy to use can be used in our setting to analyse the level of C-erbB-2.
Its important that long term follow up of the patients with over expression is needed to further ascertain if this outcome is deemed significant.

Introduction

C-erbB-2 Oncoprotein is a 185-KDa glycoprotein, often simply called C-erbB-2oncoprotein receptor. The C-erbB-2 oncoprotein is encoded by the human epidermal growth factor receptor -2 (HER-2 orC-erbB-2) proto-oncogene C-erbB-2 is one of the best studied factor receptor systems. The erbB or type 1-tyrosine kinase factor family compromises of four homologous epidermal growth factor receptors: erbB-2, erbB-3 and erbB-4.1 The erbB family plays an important role in regulating cell growth, survival and differentiation in a complex manner. The erbB-2 is the preferred heterodimerisation partner within the family and can be stabilised and transactivated in heterodimers by ligands for the partner erbB monomer, such as erbB-1or erbB-2 and erbB-4.2 This heterodimerisation between erbB-2 and other receptor of the family allows for its participation in signal transduction even in the absence of a cognate ligand. Infarct erbB-2 tends to show particularly high signalling potency which may explain its significant role in oncogenic phenotype[3,4].

In vitro animal studies have indicated that the erbB-2 gene amplification and oncoprotein over expression play a pivotal role in oncogenic transformation, tumorigenesis and metastasis.5 The normal epithelial cell possess two copies of C-erbB-2 gene and expresses low level of C-erbB-2 oncoprotein receptors on the cell surface this is equivalent to some tens of thousands of receptors per cell. With oncogenic transformation C-erbB-2 gene amplification generates more than the normal gene copies leading to 10-100 fold increase in erbB-2 monomers on the cell surface on the cell surface[6]. The expression of the C-erbB-2 oncogene and presence of the epidermal growth factor are becoming increasingly important as prognostic parameters in addition to standard histological and clinical

evaluation of patients with carcinomas. Over expression of the C-erbB-2 oncogene products has been identified in adenocarcinomas of breast, ovaries, colon, and lung and it's linked to poor prognosis in a subset of patients with cancer of the breast and correlates with shorter survivor but not with disease free intervals[7].

C-erbB-2 and prostate cancer: Oncoprotein amplification are frequently found in solid tumours and often associated with aggressive growth. This combined with the recent advance in the early detection of prostate cancer has stimulated the interest in the development of techniques for determining metastatic potential and prognostic factors. The over expression of C-erbB-2 is present in 70%to 80% of prostate cancer patients[8].

Koeppen et al[9] in a retrospective survey of C-erbB-2 over expression in solid tumours by standardised immunohistochemical assay, found over expression of C-erbB-2 oncoprotein in 15 of 61(24.6%) of prostate cancer cases. The prognostic role of C-erbB-2 oncoprotein has been noted in many studies. Visakorpi at el al[10.] found variable immunoreactivity in prostate cancer tissues. The patients with over expression had locally more advanced disease, higher histological grade, and a worse 10-year survival than those with C-erbB-2 negative carcinomas. The tumours with C-erbB-2positivily had two to three times higher S-phase fractions suggesting that C-erbB-2over expression confers proliferative advantage, by including secondary changes which are responsible for the acquisition of tumorigenic and metastatic prostate tissue. Sadasivan et al[11] using a monoclonal antibody and IHC method, nine out of 25 case of adenocarcinomas showed over expression on flow cytometric analysis corrected with higher histological grade, higher stage of disease and higher phase and aneuploidy, suggesting C-erbB-2 to be a prognostic marker.

Kuhn et al[12] in a prospective study with 53patients with carcinoma of the prostate using IHC found definite positive staining in 18 of 53 (34%) cases of prostate cancer. There was significant association between C-erbB-2 oncoprotein status and histological grades with(P=0.03). On following the patients for three years he found positive staining as the disease progressed in those who were previously negative.

A retrospective study using paraffin embedded specimens on 124 localised prostate cancer patients who had no involvement of seminal vesicle or lymph node was done by Veltri et al patients were divided as a progressors and non-progressors using PSA level as an indication of recurrence (mean follow up of =8.6+/-1.8 years). The status of C-erbB-2 was significant in detecting progression (P=0.0015)[13].

Meanwhile Morote et al[14] using Immunohistochemistry, studied over expression of C-erbB-2 in primary prostatic tissues of 70 patients with metastatic disease. Positive staining was present in 64.3%. No signicant relation was observed between histological grade and C-erbB-2 over expression or severity of the disease based on extent of metastasis. But the average specific survival in patients with C-erbB-2 over expression was less than in those withC-erbB-2 negativity with (P=0.034).from the results they suggested that over expression of C-erbB-2 oncoprotein would be considered as an independent prognostic factor of metastatic prostate cancer[14].

Development of androgen therapy resistant prostate cancer in many patients, for who therapeutic options remains limited, has led researchers to focus attention on understanding the molecular genetics of prostate cancer. Such analysis may lead to identification of relevant new prognostic and therapeutic indicators. The recent demonstration that a monoclonal anti C_erbB-2 anti bodies (Herceptine) used in combination with chemotherapy is effective as first line treatment for women who have C-erbB-2 positive metastatic breast cancer. This has prompted investigators to evaluate

combination of chemotherapy and Herceptine in hormone refractory prostate cancer[15]. The aim of this study was to determine the status of C-erbB-2 oncoprotein among patients with carcinoma of the prostate attending Mulago Hospital using Immunohistochemistry.

Materials and Methods

The avidin-biotin methods were used to stain for C-erbB-2. Sections were treated with antigen retrieval solution PH 6.1 (51700) in micro over for 10 minutes to improve antigenicity. The primary antibody was rabbit polyclonal antibodies (DAKO A485) at diluting of 1:20. The primary antibodies was incubated at 370C for one hour and subsequent incubations were done at room temperature After gentle washes with three changes of TBS, the sections were incubated with goat anti-rabbit polyclonal antibodies as secondary antibody at diluting of 1:200 for 30minutes. Sections were washed again in TBS and then incubated with subsliate chromogen solution for 2-8minutes.

IHC scoring was based on DAKO Hercep test score, the score ranges from 0 to 3+.
Scores equal or greater than 2+ was considered as over expression of C-erbB-2 oncoprotein.
Score O, negative; No staining is observed or membrane staining is observed in less than 10%of tumour cell.
Score 1+, negative; Afaint or barely perceptible membrane staining is observed in more than 10%of the tumour cells. The cells are only stained in part of their membrane.
Score 2+weak positive; weak to moderate complete membrane staining is observed in More than 10% of tumour cells.
Score 3+strong positive; strong complete membrane staining is observed in more than 10% of the tumour cells.

Results

Histological appraisal of the slides was done 50% of the patients had poorly differentiated carcinoma and only 20% of this had over expression of C-erbB-2 oncoprotein receptors. The age of the patients ranged from 50 to 86 years. Close to half of the patients 45% showed over expression of C-erbB-2 oncoprotein receptors Majority of the patients that had over expression had higher histology grades. Data is summarized in the table below

Table 1. C-erbB-2Status distribution by Histological grade of prostate cancer

		C-erbB-2 status	
Histology		**Negative**	**Positive**
	Well differentiated	16(80)	4(20%)
	Moderate differentiated	3(38.4 %)	10(61.5 %)
	Poorly differentiated	3(42.9 %)	4(57.1%)
	Total	22(55%)	18(45%)

X2 =4.41 Df =2
P = 0.110

No statistical significance was observed in the relation between histological grades and C-erbB-2 oncoprotein over expression.

Discussion

Its generally agreed documented that higher histological grades is associated with over expression of C-erbB-2 oncoprotein further studies showed an association with poor prognosis. The frequency of over expression has been shown to vary from 0-100% some of this has been attributed to difference in the material, reagents, and scoring system. This trend could be aided by development of certified reference materials for common IHC stains, development of reference methods, validation of commercial staining instruments and test systems.

In this study the results are in accordance with those of Kuhn[12] who studied 53 cases positive staining was in 6 out of 27(22%) well differentiated carcinoma, 8 out of

20(40%) moderately differentiated carcinoma, and 4 of 6(66%) of poorly differentiated carcinoma of the prostate (P = 0.03, chi-square test for trend).16 Although in our study the result was statistically insignificant most of the patients 14 (77.8%) that were positive for over expression had higher histological grades Giving an indication that over expression could be associated with more aggressive disease.

Conclusion and recommendation

Immunohistochemistry which is cheap and easy to use can be used in our setting to analyse the level of C-erbB-2. Its important that long term follow up of the patients with over expression is needed to further ascertain if this outcome is deemed significant.

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