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THE ROLE OF C erb-B2 PROTO-ONCOGENE OVER-EXPRESSION IN ENDOMETRIAL HYPERPLASIA AND ADENOCARCINOMA

S. M. Hasan[1], Fauzia Imtiaz[2], S. Q. H. Naqvi[3] and S. A. H. Zaidi[1]

[1]*Hamdard College of Medicine and Dentistry, Karachi, Pakistan*
[2]*The Aga Khan University Hospital, Karachi, Pakistan*
[3]*Peoples Medical College, Nawabshah, Pakistan*

ABSTRACT

A retrospective study was carried out on formalin fixed paraffin embedded blocks of 50 out of 392 diagnosed cases of hyperplasia and adenocarcinoma. The aim of this study was to compare the incidence of C erb-B2 Proto-oncogene over-expression in hyperplasia and adenocarcinoma of endometrium. The 5µ thick sections made and stained with H & E for review and diagnostic typing and grading of the cases. The immunohistochemical staining was done on sections using polycolonal rabbit anti erb-B2 ZYMED USA, and ZYMED 2nd Generation Immunodetection system to observe oncoprotein over expression. The grades were distributed of erb-B2 over expression in selected cases of adenocarcinoma was 66%, 66.66% and 75% for grade I, II and grade III respectively.

Key-words: C erb-B2 Proto-oncogen, adenocarcinoma, immunohistochemical staining, oncoprotein.

INTRODUCTION

The word oncogene literally means cancer-producing gene, which was derived from proto-oncogene, a cellular gene that promote normal growth identification (Shih *et al*., 1981). The neu oncogene was first derived from fibroblast cells lines with rat neuroblastoma of neonatal rats (Scherchter *et al*., 1984; Semba *et al*., 1985). The human homologous of this cellular oncogene erb-B2 neu was independently identified by three groups (Semba *et al*., 1985; Coussens *et al*., 1985). When cloned and sequenced was found to be related human epidermal growth factor (HEGF) neoplasm erb-B2 (Cotran *et al*., 1991). The c-erb-B2 gene code for a transmembrane 185 KD Class 1 tyrosine kinase receptor protein is considered as a member of closely related epidermal growth factor receptor (EGFR) group of trans-membrane tyrosine kinase receptor (Burchuck *et al*., 1991).

The mechanism of transformation is unknown but it is postulated to constitute tyrosine kinase activity of proto-oncogene and it is located in female genital tract cancers including cervix, endometrium and ovarian cancers (Costa and Wall, 1996). The identification of proto-oncogene amplification and over-expression of their gene product has been associated with poor prognosis of same tumor type (Neilsen and Nyholm, 1999).

The role of erb-B2 has been studied widely in the breast cancers. It was observed that over expression of this oncoprotein was related to poor clinical outcome in 20-30% of cases (Scherlue *et al*., 2000). Increasing importance of the erb-B2 oncogene and oncoprotein in clinical management of patients with breast cancers the accurate and consistent evaluation of its status is critical (Wang *et al*., 2000). There are also therapeutic implications and applications of this oncoprotein in the treatment of breast cancer (Naqvi *et al*., 2002). The drug Herceptin produces anticancer effects by blocking the erb-B2 receptor and by initiating the immune cells mediated cell toxicity (Dillman *et al*., 1999).

MATERIALS AND METHODS

This study was performed on formalin fixed paraffin embedded blocks of diagnosed cases of cystic hyperplasia, adenomatous hyperplasia and endometrial adenocarcinoma, were retrieved from the record of Department of Pathology, Basic Medical Sciences Institute, JPMC, Karachi, received during 1st January 1996 to 31st December 2003. This consists of three hundred and ninety two cases of hyperplasia and adenocarcinoma. Fifty diagnosed cases were selected, out of which 26 (52%) cases comprising of adenocarcinoma of endometrium, 18 (36%) of hyperplasia, 2 (4%) of atypical hyperplasia and 4 (8%) were found normal cyclic endometrium. All slides were reviewed critically for typing and grading of cancers according to WHO classification.

During the selection of cases, special care was taken that the selections do not contain too much fibrosis, necrosis, degenerative areas and hemorrhage. Prompt care was also taken that the selected section contained adequate material. The 5µ thick sections were retrieved for haematoxylin and Eosin (H & E) staining and grading of adenocarcinoma was done according to WHO classification (Prat 1996). Immunohistochemistry is the identification of tissue antigen *in situ* by antigen antibody reaction visualized by a label. Historically, specific demonstration of

antigens has been done with fluorescent or enzymatic secondary antibody conjugates PAP and ABC. Currently, the LAB-SA method also known as the Streptavidin Peroxidase (SP) and Streptavidin Alkaline phosphatase (SAP) methods, is preferred in the immunohistochemistry laboratory due to its higher sensitivity, less background staining and shorter protocol time. Staining was strongly associated with the plasma membrane or with some diffused cytoplasmic staining. The immunostaining intensity reflects the effects of tissue preparation as well as antigen concentration. An intense immuno-stain indicates a relatively higher concentration of erb-B2 while lighter immunostaining is indicative of a lower concentration.

The interpretation of staining or its absence was complemented by positive as well as negative controls. The erb-B2 staining was considered positive when majority of tumor cells showed intense circumferential cell membrane staining which was easily identified with a 10X or 40X objectives and cytoplasmic staining without cell membrane staining was scored as negative (Costa and Wall, 1996; Jacob *et al*., 2000).

The computer package MS EXCEL was used for data feeding and EPI-INFOR (Pittsburgh, USA) was used for statistical analysis. To compare proportion and percentage between groups (hyperplasia and adenocarcinoma) Chi-square test and for mean standard deviation between groups (hyperplasia and adenocarcinoma) Student t-test was used. In all statistical analysis only p-value less than 0.05 were considered significant.

RESULTS

In this study, a cohort of 392 cases received during the duration of 1996-2003 diagnosed as Hyperplasia (cystic and adenomatous) and adenocarcinoma of endometrium. The cases included curetting and abdominal hysterectomies. 186 (467.4%) cases were cystic hyperplasia, 166 (42.3%) of adenomatous hyperplasia and remaining 40 (10.2%) of adenocarcinoma (Table 1). The distribution of cystic hyperplasia is shown in Table 2. The data reveals that the maximum number of cases present in the age limit 21-30 years with 91 (48.9%) out of 186 cases. The distribution of adenomatous hyperplasia is shown in Table 3. The data depicts that 31-45 with 75 out of total 166 cases of the said variety of hyperplasia. The distribution of adenocarcinoma of endometrial origin is shown is Table 4. A total of 40 cases received during 1996 to 2003 time period. 21 cases out of 40 were present in 45-60 years of age group. And 10 cases were observed in >60 years. Tables 5 showed the frequency of erb-B2 over expression in 50 selected cases of cystic hyperplasia, adenomatous hyperplasia and adenocarcinoma of endometrium with 40 %, 53.3 % and 68 % respectively. Grade-wise distribution of erb-B2 over expression in 25 selected cases of endometrial adenocarcinoma (Table 6). The data reveal 3 out of 4 cases over expressed for the Oncoprotein over expression.

Table 1. Frequency of hyperplasia and adenocarcinoma.

TYPE OF LESION	1996	1997	1998	1999	2000	2001	2002	2003	TOTAL
Cystic Hyperplasia	45	28	17	9	20	17	34	16	186
Adenomatous Hyperplasia	54	5	32	20	9	4	20	22	166
Adenocarcinoma	3	2	6	2	20	4	1	2	40
TOTAL	102	35	55	31	49	25	55	40	**392**

Table 2. Distribution of cystic hyperplasia according to age.

AGE (YEARS)	1996	1997	1998	1999	2000	2001	2002	2003	TOTAL
20-30	10	6	12	12	8	1	22	10	81
31-45	12	0	14	18	8	17	12	10	91
46-60	01	1	03	02	0	1	2	3	13
> 60	0	0	0	0	0	0	0	1	1
TOTAL	23	7	29	32	16	19	36	24	**186**

Table 3. Distribution of adenomatous hyperplasia according to age

AGE (YEARS)	1996	1997	1998	1999	2000	2001	2002	2003	TOTAL
20-30	24	0	12	10	0	1	6	4	57
31-45	26	5	11	6	7	3	9	8	75
46-60	2	00	8	4	1	0	6	5	26
> 60	2	0	1	0	1	0	2	2	8
TOTAL	54	5	32	20	9	4	23	9	**166**

Table 4. Distribution of adenocarcinoma endometrium according to age.

AGE (YEARS)	1996	1997	1998	1999	2000	2001	2002	2003	TOTAL
20-30	-	-	-	-	-	-	-	-	0
31-45	-	1	2	1	4	-	1	-	9
46-60	3	1	3	2	7	2	1	2	21
> 60	-	-	1	1	4	2	-	2	10
TOTAL	3	2	6	4	15	4	2	4	**40**

Table 5. Frequency of erb-b2 over-expression in hyperplasia and adenocarcinoma in 50 selected cases.

LESION TYPE	TOTAL CASES	OVER-EXPRESSION	%
Cystic Hyperplasia	10	04	40
Adenomatous Hyperplasia	15	08	53.3
Adenocarcinoma	25	17	68

Table 6. Grade wise distribution of erb-b2 in 25 selected cases of adenocarcinoma.

GRADE	TOTAL CASES	OVER-EXPRESSION	%
I	15	10	66.0
II	06	04	66.6
III	04	03	75.0

DISCUSSION

Endometrial hyperplasia is one of the important causes of abnormal uterine bleeding that differs from typical anovulation by degree of epithelial alteration in endometerium (Jacob *et al*., 2000). Endometrial hyperplasia

deserves special attention because of its relationship to endometrial carcinoma (Crum, 1999). Hertig and Sommers (1979) proposed a progression of endometrial changes from hyperplasia through a spectrum of atypical changes leading in some cases to endometrial adenocarcinoma. Numerous studies have confirmed the malignant potential of certain endometrial hyperplasia and concept of a continuum of glandular atypia culminating in some cases in carcinomas (Kurman, 1985). Endometrial hyperplasia is related to an abnormally high prolonged stimulation of estrogen with diminished or absence of progesterone activity. Therefore, hyperplasia occurs most commonly around menopause or in association with persistent anovulation in young female. The type of hyperplasia includes low grade / simple / Cystic, complex / adenomatous and high grade with atypia (Crum, 1999). The endometrial adenocarcinoma is the third most common cancer of the female genital tract and accounts for 7% of all female genital tract malignancies (Brinton *et al*., 1992). The cancer of endometrium is uncommon in female <40 years. The peak incidence is in the 55-60 years old female. The higher frequency of this form of neoplasm is seen with obesity, diabetes mellitus, hypertension, infertility (Silverberg and Kurma, 1991), and prolonged estrogen stimulation (Nazeer *et al*., 1995), the mechanism most commonly implicated in the pathogenesis of cancer is the amplification and over-expression of protooncogene. The over expression is associated with poor prognostic value, independent of other factors (Luy *et al*., 1996). They also studied the survival of the patients with amplification of erb-B2 on 103 cases of hyperplasia and adenocarcinoma using Polymerase Chain Reaction (PCR) and immunohistochemistry.

Berchuck *et al*. (1991) were the first, who examined the erb-B2 amplification in the year 1991, in the normal and adenocarcinomatous endometrial tissue using monoclonal antibody specific reaction with the external domain of erb-B2 to localize it immunohistochemically in frozen section. The amplified erb-B2 was significantly higher in complex and atypical hyperplasia verses simple hyperplasia and correlated well with histological grade of endometrial carcinoma, lymphatic and vascular spread.

Prat (1996), studied the relationship between FIGO stage and erb-B2 expression in cancers. It was concluded that in FIGO stage A, the over expression was 8% for I-B it was 5% and in stage IV-B distinct metastases of erb-B2 was 42%. In contrast, our work was in the light of Grading of cancer. It was found that in grade I, II and III the over expression was 66.6, 66 and 75% respectively.

CONCLUSION

Therapeutic implications and applications of this oncoprotein in the treatment of breast cancer have been widely studied worldwide. The drug Herceptin produces its anticancer effects by blocking the C erb-B2 receptor and by initiating the immune cells mediated cell toxicity in breast neoplasia. It is concluded that the same line of action be followed in the treatment of endometrial adenocarcinoma to improve the overall survival and prognosis of the patients.

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