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ORIGINAL ARTICLE

EVALUATION OF CORRELATION OF TUMOR MARKERS WITH TUMOR GRADING IN BREAST CARCINOMA PATIENTS

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ABSTRACT:

Introduction: Histopathology is the main diagnostic procedure to determine the malignancy. Breast cancer once diagnosed, is then subjected to IHC studies. The present study was planned to find correlation of ER, PR and p53 in breast carcinoma among Indian population. **Material and Methods:** The present study was conducted among 50 cases of breast cancer. Haematoxylin and Eosin stained sections were used for histopathological typing and grading. All the cases were subjected to immunohistochemistry for ER, PR and p53 expression. Data so obtained was analyzed using the SPSS Version 17 software and was arranged according to characteristics and represented as a number and percentage. **Results:** ER positivity decreased as the grade of the tumor increased. PR positivity decreased as the grade of the tumor increased. p53 value was directly related to the grade of the tumor although not statistically significant. As the grade of the tumor increased p53 positivity was increased and ER PR positivity decreased suggesting further that ER and PR are inversely related to P53 status. **Conclusion:** The present study observed that ER PR positivity was present in low grade tumors and p53 positivity was more seen in high grade tumors. Thus, inverse relationship was found between ER PR positivity p53 with grade of tumor and emphasizes the need to find out the prognosis, survival and line of treatment.

Keywords: Breast Carcinoma; Estrogen receptors; Progesterone receptors; p53; Women

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INTRODUCTION:

The outcome for women with breast cancer varies widely. It has been well established that the progression in some breast cancers is partially dependent on the interaction of various hormones and growth factors on the tumor cells themselves.[1]Histopathology is the main diagnostic procedure to determine the malignancy.[2]Breast cancer once diagnosed, is then subjected to IHC studies including ER, PR, Her 2 neu, BRCA 1, BRCA 2, p53, Bcl 2 and Ki 67.[3]Estrogen receptors are specific proteins located mainly in the cytoplasm of cells of target tissue for estrogen action.[4]Progesterone receptor is an intracellular steroid receptor that specifically binds progesterone expressed by a single gene.[5]Recent studies also suggest that assessment of progesterone receptor are equally or more valuable than those of ER in predicting the disease-free interval in patients with breast cancer. Western data showed progesterone receptor positivity of 57.74% but in Indian literature the positivity is reported to be 41.5%.[6,7] p53 is the main regulator of genomic stability through regulation of the cell cycle. Overexpression of p53, which is caused by TP 53 mutation, is the most frequent genetic alteration in breast cancer.[8] p53 over-expression has been observed in 20-50% of primary breast tumours.[9] In view of the available literature, the present study was planned to find correlation of ER, PR and p53 in breast carcinoma among Indian population.

MATERIAL AND METHODS

The present study was conducted among 50 cases of breast cancer. The ethical clearance was obtained from the

institutional ethical committee. The tissue was formalin fixed and paraffin embedded and was stained for Haematoxylin and Eosin for histopathological typing and grading. All the cases were subjected to immunohistochemistry for ER, PR and p53 expression.3–5 µm sections were cut and mounted on poly–l–lysine coated slides. Slides were dried overnight at 37°C and dewaxed in xylene and hydrated. For antigen retrieval, 1500 mL of citrate buffer solution was heated, pH 6.0, unless until boiled in a stainless steel pressure cooker. Covered but lid was not locked.Slides were positioned into metal staining racks (slides were not placed closed together as uneven staining might occur) and lowered into pressure cooker ensuring slides were completely immersed in unmasking solution. Lid was locked.When the pressure cooker reached the operating temperature and pressure (after about 5 minutes),1 minute timer was started.When the timer rang, pressure cooker was removed from heat source and was run under cold water with lid on. Endogenous peroxidise was neutralised using Peroxidase Block for 5 minutes.Two washings in Phosphate Buffer Saline/ Tris buffer saline were given each for 5 minutes.Protein Block was incubated for 5 minutes.Then 2 washes in tris buffer were gven for 5 minutes each.The primary antibody was put on the sections and sections were kept for 1 hour in the moist chamber.This was followed by 2 washes in tris buffer for 5 minutes each.The post primary block was then applied for 30 minutes at room temperature.Again 2 washings of tris buffer were given for 5 minutes each.Incubation with Polymer was done for 30 minutes.Again 2 washings were given with tris buffer for 5 minutes each with gentle rocking.Slides were then covered with DAB for 2-3 minutes. All the time slides were kept in a moist chamber.Sections were washed in deionised water for 5 minutes.Haematoxylin counterstaining was done for 2-5 minutes and sections were washed under running tap water.Dehydration and clearing of the sections was done in propanol and xylene respectively. Breast carcinoma cases reported as positive for p53.Endometrium was taken as positive control for ER. Breast carcinoma cases reported as positive for PR was taken as positive control section for PR. Breast carcinoma cases reported as positive for p53 was taken as positive control section for p53. Negative control section was provided by omission of primary antibody. Target antigen retrieval will be done by heat induced epitope retrieval technique. Antigen retrieval will be followed by avidin biotin method of immunostaining.Data so obtained was analyzed using the SPSS Version 17 software and was arranged according to characteristics and represented as a number and percentage.

RESULTS

In the present study, 6 cases out of 12 cases of grade II were ER positive and 18 cases out of 38 cases of grade III were ER positive(26 cases were ER negative). This showed that as the grade increased ER positivity was decreased(table1, graph 1). Out of 12 grade II cases, 8 were PR positive and out of 38 grade III cases 28 cases were PR negative(10 were PR positive). This means that as the grade of the tumour increased PR positivity was decreased (table 2, graph 2).

Table 1: Correlation of grade with ER positivity

Grade of tumor	ER positive	ER negative	Total cases	% positivity
I	-	-		-
II	06	06	12	50%
III	12	26	38	31%

Graph 1: Correlation of PR with grade of tumor

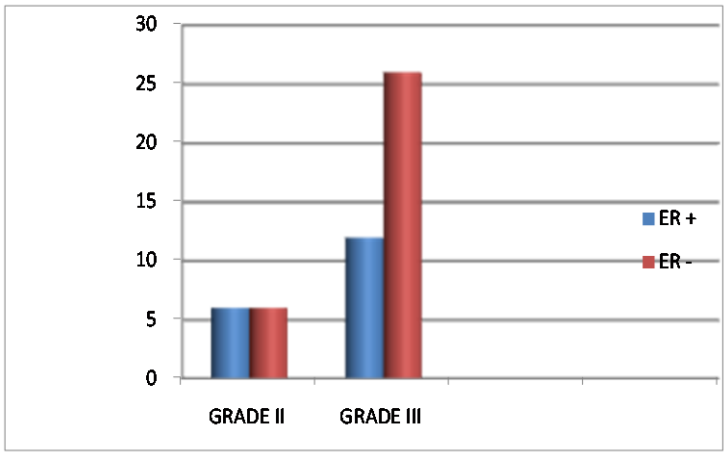

Table 2: Correlation of grade of tumor with PR positivity

Grade of tumor	PR positive	PR negative	Total cases	% positivity
I	-	-		-
II	08	04	12	66%
III	10	28	38	26%

Graph 2: Correlation of grade of tumor with PR positivity

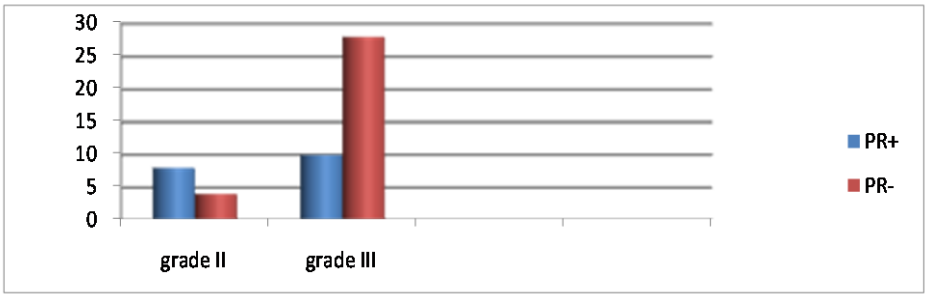

Table 3: Correlation of p53 with grade of tumour

Grade of tumour	p53 positive Score 0	p53 positive Score 1	p53 positive score 2	p53 positive score 3	p53 negative	Total no. of positive cases	Total no. of cases	% of positivity
I	-	-	-	-	-	-	-	-
II	-	3	1	3	5	7	12	58%
III	-	6	2	16	14	24	38	63%

Graph 3: Correlation of p53 with grade of tumour

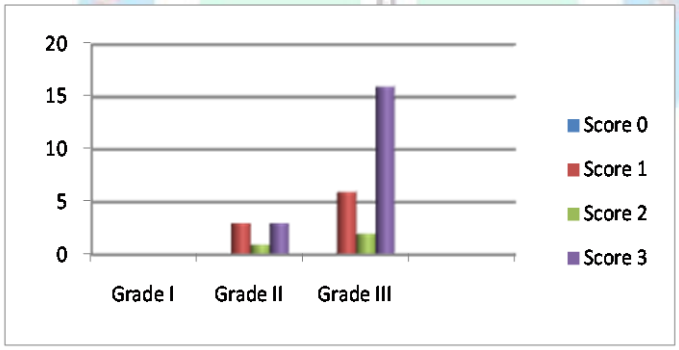

Table 4: Correlation of ER, PR and p53 with grade of tumor

Grade	ER+	PR+	ER+ PR+	ER- PR+	ER+ PR-	ER- PR-	P53 +
I	-	-	-	-	-	-	-
II	6	08	5	3	1	3	7
III	12	10	7	3	5	23	24

Out of 12 cases of grade II, 7 cases were positive for p53 (58% positivity) and out of 38 cases of grade III, 24 cases were positive for p53 (63% positivity) which means that as the grade of the tumor increased p53 positivity was increased. 16 cases showed score 3 positivity in grade III further suggesting that score was also increased with grade of the tumor (table 3).

It was seen that 24 cases in grade III tumor were p53 positive and 23 cases in grade III tumours were ER PR negative. This showed that as the grade of the tumour increased p53 positivity was increased and ER PR positivity decreased suggesting further that ER PR are inversely related to P53 status (table 4).

The present study observed that ER PR positivity was present in low grade tumors and p53 positivity was more seen in high grade tumors. Thus, inverse relationship was found between ER PR positivityp53 with grade of tumor.

DISCUSSION

Carcinoma breast is the most frequent cancer in females throughout the world with 1.6 million cases diagnosed and 4,25,000 deaths reported in 2010. At this rate, new cases and deaths in next 25 years will be 41 million and 10.6 million respectively.[10]

Immunohistochemistry (IHC) is an excellent technique for identifying cellular or tissue constituents by means of antigen antibody interactions, and is used to detect various tissue antigens causing cancer and are helpful in management and predicting the prognosis.[11]

In the present study, 18 cases showed estrogen receptor positivity comprising 36% of the total cases. In the Western and Indian literature Estrogen Receptor positivity varies between 50-70% and 30-50% respectively.[12]In the present study, 22 cases with age <55 years showed ER negativity and 10 cases with age >55 showed ER negativity. Manjunath et al demonstrated that ER negative disease occurred at a younger age, at a mean of 50.2 years, whereas the mean age of ER positive disease was 55.7 years.[13]

50% of the grade II cases showed ER positivity and 31% of the grade III cases showed ER positivity. It was seen as the grade increased ER positivity decreased but the results were not statistically significant. Jovicic-Milentijevic M et al,[14]Manjunath S et al[13]and Barnes NL et al[15] also found the similar results, i.e. as the grade of the tumour increased ER positivity decreasesdParise CA et al[16] showed that all of the ER positive subtypes had better survival than the ER negative subtypes.

Progesterone receptor positivity was seen in 18 cases comprising 36% of the total cases. When accurately measured, PR status is an independent predictive factor for benefit from adjuvant endocrine therapy with tamoxifen.[17] 50% of the grade II cases showed PR positivity and 31 % of the grade III cases showed PR positivity. It was seen as the grade increased PR positivity decreased although the results were not statistically significant. Stierer M et al also reported similar.[18]Similarly, Desai SBet al,[19] Ambroise et al[20]also documented the prevalence of 46.1% and 51% for PRpositivty in breast cancers in Indian patients, respectively. Similarly, Mudduwa LK, [21] in a study from Srilanka documented a prevalence of 48.3% PRpositive tumours.[90] Western studies have reported increased 60-70% PR expression in the cases of invasive ductal carcinoma.[12]

Maximum number of cases were combined ER and PR negative constituting 52% followed by ER and PR positive cases (24%). This is because of the reason that the grade III cases were more in the study and also ER PR is positive in lower grades and its expression markedly decreases as the grade increases.The prevalence of hormones receptor positive breast cancer in Asian countries has been found to be lower than those in the western world.[22]

p53 positivity was seen in 31 cases which constitutes 62% and negativity was seen in 19 (38%) of the cases. 16 cases (51%) of breast cancer have age <50 yrs and 15 cases(49%) cases of breast cancer have age >50yrs. Shokouh TZ et al[23] found an inverse correlation between age and p53 mutation, but this correlation was not statistically significant. Out of 12 cases of grade II, 7 cases (58%) showed p53 positivity and out of 38 cases of grade III, 24 cases (63%) showed p53 positivity. This shows as the grade increases p53 positivity increases. Yang P et al[24]documented high p53 expression with advanced TNM stage with multiple organ involvement and the median disease free survival was 10 months for p53-positive patients and 25 months for p53-negative patients.

61% of ER, PR negative cases showed p53 positivity constituting maximum number of p53 positive cases which shows that the ER PR and p53 are inversely related.The inverse association between hormones receptors and p53 was also revealedby Sirvent JJ et al.[25]

In the present study, out of grade III tumors, 66.6% cases showed p53 positivity and 60% cases showed ER PR negativity. In grade II tumors, 58% cases showed p53 positivity with 3 ER PR negative cases (25%). This showed that as the grade of the tumor increased, p53 positivity increased and ERPR positivity decreased suggesting further that ERPR are inversely related to P53 status. However this difference was not statistically significant.Lacroix M et al[26]found that breast tumors expressing a high amount of p53 were more frequently ER negative and PR negative. They were also associated with a high proliferation rate, high histological and nuclear grades, aneuploidy and poorer survival.Significant correlation between p53 expression, grade III disease, oestrogen or progesterone receptor negativity was also observed by Jacquemierl J et al.[27]Al-Moundhri M et al[28]and Climent MA et al[29] also considered the negative association of ER and/ or PR expression with p53 over-expression.Varna M et al[30] demonstrated that breast tumors with positive immunostaining for p53 are usually ER and PR negative. This is often associated with a high rate of proliferation, a high histological grade, aneuploidy, and a poor prognosis.

It is known that breast feeding reduces a woman's lifetime exposure to hormones like estrogen, which promotes breast cancer cell growth.[31] As, Breast-feeding is a common practice In India, the risk was found to be more among nulliparous because of lack of breast-feeding . Breast cancer risk in India revealed that lifetime duration of breast feeding was inversely associated with breast cancer risk among premenopausal women.[32,33] Higher education level and income are also shown to be significant reasons for an increased risk of breast cancer.[34,35] This is because economic independence may encourage women to remain single or marry late thereby increasing their risk of getting the disease.[36]

CONCLUSION

ER positivity decreased as the grade of the tumor increased. PR positivity decreased as the grade of the tumor increased. p53 value was directly related to the grade of the

tumor although not statistically significant. Thus it shows that ER and PR status are inversely proportional to p53 expression and emphasizes the need to find out the prognosis, survival and line of treatment.

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