Original Article

p53 as a Prognostic Marker in Carcinoma Breast in Correlation with Conventional Estrogen and Progesterone Hormone Receptors

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Keywords: *Estrogen Receptor, Progesterone Receptor, p53, Tumor Grading, Lymph Node*

ABSTRACT

Background: Carcinoma breast is the most common cancer in women worldwide. The present study was done with the objective to find expression and correlation of Estrogen Receptors(ER), Progesterone Receptors(PR) and p53 in breast carcinoma and to correlate expression of these tumor markers with histological type, grade and other parameters.

Methods: The study was conducted on 65cases of breast cancer in the department of Pathology of a tertiary health care center. Histopathological and IHC studies were done for ER, PR and p53 expression, brown nuclei were taken as positive results and IHC Scoring was done.

Results: All the cases were of Infiltrating Ductal Carcinomas(NOS) between 30-70years of age with varying tumor size1-6cm. Maximum cases were of grade III followed by grade II. Metastatic carcinomatous deposits in Lymph nodes were seen in 42cases. On the basis of ER PR positivity, tumors were separated into 4 categories with category IV having maximum number of cases. ER/PR expression in grade III cases was significantly low. With increasing grade, the expression of p53 increased which was statistically significant p=0.007. Lymph nodes showing metastatic deposits were highest in ER PR negative cases which was statistically significant(p=0.028). p53 expression was higher in category IV cases(32.3%) as compared to category I(15.4%).

Conclusion: In the present study it was observed that as the grade increases, ER PR decreases and p53 positivity increases. Thus ER PR status is inversely proportional to p53 expression and emphasizes the need to find out the prognosis, survival and line of treatment.

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Introduction

Breast cancer, second only to carcinoma lung, is the most common cancer in women worldwide. The incidence of breast cancer which was about 12% in 2012 is expected to increase by 26% by 2020 and most of these will be seen in developing countries.[1] One in every eight women stands the chance of being diagnosed with breast cancer in their lifetime.[2] Incidence of breast cancer is strongly related to age, with the highest incidence rates being in older women, supporting a link with hormonal status.[3] In Asia however, breast cancer incidence peaks among women in their forties whereas in the United States and Europe, it peaks among women in their sixties.[4] In India premenopausal women constitutes about 50% of all breast cancer patients.

Breast cancer risk in India revealed that lifetime duration of breast feeding was inversely associated with breast cancer risk among premenopausal women. [5,6] Higher education level and income are also shown to be significant reasons for an increased risk of breast cancer.[7,8] This is because economic independence may encourage women to remain single or marry late thereby increasing their risk of getting the disease.[1]

Histopathology, either excision or tru-cut biopsy, is the gold standard to confirm the breast malignancies and also to type and grade it. In recent years, for therapeutic and prognostic purposes breast cancer once diagnosed, is then subjected to immune-histochemical studies (IHC) which commonly include estrogen receptor (ER) and progesterone receptor (PR). With advent of molecular classification of breast carcinoma along with these, other ancillary cytokeratin prognostic markers (BRCA 1, BRCA 2, p53, Bcl 2 and Ki 67) have markedly revolutionized the research for breast cancer.[9] Estrogen receptors (ER) are specific proteins located mainly in the cytoplasm of cells of target tissue for estrogen action.[10] Progesterone receptor is an intracellular steroid receptor that specifically binds progesterone expressed by a single gene.[11] Recent studies also suggest that assessment of PR are equally or more valuable than those of ER in predicting the disease-free interval in patients with breast cancer. The patients expressing ER and PR positivity respond well to the hormonal treatment and have a better prognosis as well as better survival rate.[12]

p53 is the main regulator of genomic stability through regulation of the cell cycle. Over expression of p53, which is caused by TP 53 mutation, is the most frequent genetic alteration in not only breast cancers but also in various malignancies such as ovarian, esophageal and GIT.[13] Breast tumors expressing a high amount of p53 are more frequently ER-negative and PR-negative and are also associated with a high proliferation rate, high histological and nuclear grades, aneuploidy and poorer survival.[14,15]

The present study was done with the objective to find expression and correlation of ER, PR and p53 in breast carcinoma as well as to correlate expression of these tumor markers with histological type, grade and other parameters in a small cohort of north Indian female population suffering from breast cancer.

Materials and Methods

The study was conducted on 65 cases of breast cancer received as mastectomy specimens in the department of Pathology of a tertiary health care center. The patients were divided into four groups according to age. These were labeled as Group I, II, III & IV which included patients between 31-40 years of age, 41-50 Years, 51-60 Years and 61-70 Years respectively. Depending upon the tumor size, they were also classified as those having size less than 2 cm, size between 2 – 5 cm and size more than 5 cm. (Table 1)

Exclusion criteria is only cases of infiltrating ductal carcinoma NOS were included in the study. Rest of the histo-pathological variants such as medullary, tubular, etc. were not included in the study.

The tissue was formalin fixed and paraffin embedded with sections obtained and stained for Haematoxylin and Eosin for histopathological confirmation, typing and grading. In all the cases IHC studies were done for ER, PR, and p53 expression. The sections were taken on Poly-L-Lysin coated slides and antigen retrieval was done by conventional heat extraction system on pressure cooker and protein block was obtained. Protein Block was incubated for 30 min. and 2 washes in tris buffer were given for 5 minutes each. Primary antibody for ER(Monoclonal rabbit antibody RMA B001from diagnostic biosystem India), PR(Monoclonal rabbit antibody RMA B002from diagnostic biosystem India) and p53 (CME298BK from biocare medical) were employed and sections were kept for 1 hour in the moist chamber followed by 2 washes in tris buffer for 5 minutes each. The post primary block was then applied for 30 minutes, 2 buffer washings given for 5 minutes each, incubation done with Polymer for 30 minutes. Again after 2 washings with tris buffer slides were covered with DAB for 2-3 min and haematoxyllin Counterstaining was done. For ER, PR and p53, brown nuclei were taken as positive results. In all the cases positive and negative controls were run for ER, PR and p53.

Scoring of Estrogen Receptor: For number of positively staining cells less than 10%, 10-50%, 50-75% and more than 75% a score of 1, 2, 3and 4 was given respectively. Similarly a score of 1, 2and 3 was given for cells staining with low, medium and strong intensity respectively.(Fig.1)

Scoring of Progesterone Receptor: Zero score was given for no nucleus staining brown. For nuclei ranging between 1-25%, 26-50%, 51-75% and 76-100%, a percentage score (PS) of 1, 2, 3 and 4 was given. Similarly an intensity score of 0,1, 2 and 3 was given for cells showing No staining, weak, medium and high intensity staining respectively. Total score was calculated according to the formula (TS=PS+IS) Range 0-7. Low score was graded as quick score 2-3, Medium as quick score 4-5 and High as quick score 6-7. (Fig.2)

Scoring of p53: It was also done on a similar pattern with less than 5% cells getting a score of zero, 5-25% score of 1, 25-50% 2 and more than 50% being awarded a score of 3. Similarly a score of 1, 2 and 3 was awarded for mild moderate and strong staining intensity. (Fig.3)

Table 1: Showing Correlation of ER, PR & P53 with Age of Patients and Tumor Size.

	Groups According to Age				Groups According to Tumor Size		
	Group I 31-40 Yrs.	Group II 41-50 Yrs	Group III 51-60 Yrs.	Group IV 61-70 Yrs.	Group I <2cm	Group II 2-5cm	Group III >5cm.
No. of Cases	11	25	19	10	19	44	2
ER+	7	4	11	5	10	16	1
ER -	4	21	8	5	9	28	1
PR+	10	7	5	4	11	14	1
PR-	1	18	14	6	8	30	1
p53+	4	19	10	5	10	26	2
p53-	7	6	9	5	9	18	0

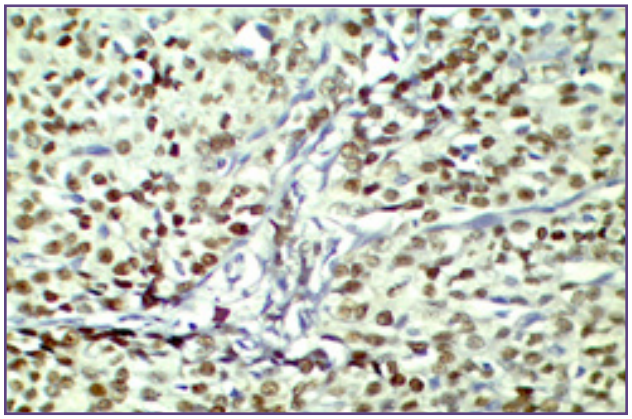

Fig. 1: ER positivity (Nuclear) – IHC.

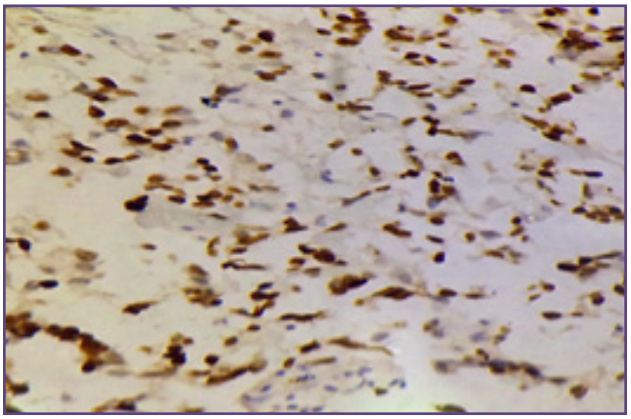

Fig. 2: PR positivity (Nuclear) – IHC.

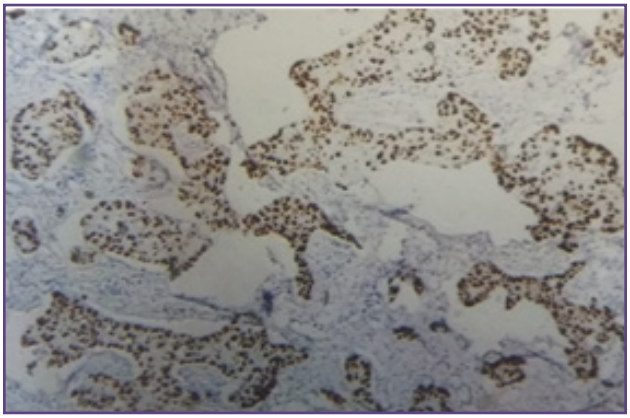

Fig. 3: p53 positivity Grade III – IHC.

Results

All the patients included in the study were females between 30-70 years of age with most of the cases in the fifth and sixth decade of life (67%). Right breasts were more commonly involved (63%) with tumor more commonly seen in upper and outer quadrant (84%). Tumor size varied from 1-6 cm with maximum number of cases ranging between 2-5 cm (44/65; 67.7%) i.e. in the T_2 stage. As per exclusion criteria of the study all the 65 cases were infiltrating ductal carcinomas (NOS).

Grading: Nottingham modification of RBB scoring when employed showed that maximum cases were of grade III (42 cases; 64.6%) followed by grade II (17 cases; 26.2%) and then grade I (6 cases; 9.2%).

Lymph Node Status: Lymph nodes were recovered in all 65 cases with metastatic carcinomatous deposits noted in 42 cases(64.6%) which included 14 cases of N1 stage (1-3 nodes), 18 cases of N2 stage (4-9 nodes) and 10 cases of N3 stage (>10 nodes). All the nodes recovered in 6 grade I tumors were reactive. Out of 17 cases of grade II carcinoma metastatic deposits were seen in 10 cases (59%) with 7 cases (41%) showing reactive hyperplasia. 32/42 (76.2%) grade III tumor cases showed metastatic carcinomatous deposits.

Immuno-Histochemical Expression: Estrogen receptor (ER) positivity was seen in 27/65 cases (41.6%) while Progesterone Receptor (PR) positivity was seen in 26/65 cases (40%). p53 expression was noted in 38/65 cases (58.5%). On the basis of ER/PR positivity, tumors were separated into 4 categories :- Category I as ER+ve PR+ve, Category II as ER+ve, PR-ve, Category III as ER-ve, PR+ve, and Category IV as ER-ve, PR-ve. It was noted that maximum number of cases were of category IV accounting for 50.7% of total cases (Table 2).

Correlation of ER, PR with Age of the patients: From the Table 1 it can be seen that maximum number of cases were in group II (25/65) followed by group III (19/65). Out of the 25 cases in group II only 4 (16%) were ER positive whereas 21 (84%) cases were ER negative. In group III, out of 19 cases 11 (57.9%) showed ER Positivity while 8 cases (42.1%) were negative for ER expression. (p=0.003; Significant). The ER positivity in group I and group IV patients was 63.7% and 50 % respectively. When we evaluated the PR expression, the group II patients showed a PR positivity of 28% as compared to group III patients where it was 26.3%. (p=0.901; Not Significant). PR positivity in group I patients was 91% and in group IV patients was 40%.

Correlation of p53 with Age of the patients: The group II cases showed a high p53 expression of 76% as compared to group III patients where the p53 expression was 52.6%. The p53 expression in group I and group IV was 36.4% and 50 % respectively.

Correlation of ER, PR with Size of the Tumor: ER positivity was 52.6% (10/19) cases with tumor size less than 2 cm while it was 36.4% (16/44) patients having a tumor size between 2-5 cm. The two cases having tumor size more than 5 cm showed an ER positivity of 50%. PR positivity for the three groups was 57.9%, 31.8% and 50% respectively.

Correlation of p53 With Size of the Tumor: Out of 38 cases showing a positive p53 expression 10 cases (26.3%) had tumor size less than 2 cm, 26 cases (68.4%) had tumor size ranging between 2-5cm and 2 cases (5.3%) had tumor size more than 5cm.

Correlation of ER, PR with Grade of Tumor: All the 6 grade I tumor cases were of category I. The 17 grade II cases expressed an ER positivity of 53% (9/17) and PR positivity of 58.8% (10/17). ER and PR expression in grade III cases was significantly low with ER positivity of 28.6 % and PR positivity of 23.8% only. (Table 3)

Correlation of p53 with Grade of Tumor: p53 immuno-expression was 38/65 (58.5%). None of the grade I tumors was positive for p53 expression. In the study conducted, with increasing grade of the tumor, the expression of p53 also increased (58.8% in grade II & 67.4% in grade III). This was statistically significant p=0.007. (Table 4)

Correlation of ER and PR with Lymph Nodes Status: In the present study, out of 19 ER, PR positive cases, 8 cases (42.1%) showed lymph nodes having metastatic carcinomatous deposits while in 11 cases (57.9%) nodes showed reactive hyperplasia. When the number of cases showing lymph nodes with metastatic deposits was calculated in ER PR negative cases, it rose to 24/33 accounting for 72.8% involvement. Only 9/33 (27.3%) cases showed reactive lymph nodes. This correlation was found to be statistically significant (p=0.028). (Table 5)

Correlation of p53 With Lymph Nodes Status: In the present study out of 38/65 p53 positive cases, 26 (68.42%) had metastatic carcinomatous deposits while 12 cases (31.6%) showed reactive lymph nodes. The percentage of cases showing metastatic deposits in 27 p53 negative cases was 59.3% whereas 40.7% of p53 negative cases did not show any metastatic deposits. The results were however were not found to be statistically significant. (Table 5)

Correlation of ER, PR and p53: p53 expression was found to be higher in category IV cases which were both ER & PR-ve (Hormonal -ve) 32.3% as compared to patients who were hormonally positive i.e. both ER & PR +ve category I (15.4%) but the correlation was not significant. (Table 6)

Table 2: Showing Combination of ER and PR Cases.

Combination of ER and PR	Number of cases (n=65)	Percentage
ER+PR+ (Category I)	19	29.3%
ER+PR- (Category II)	7	10.7%
ER-PR+ (Category III)	6	9.3%
ER-PR- (Category IV)	33	50.7%

Table 3: Showing Correlation of ER & PR with Grade of Tumor.

Grade of Tumour	ER+	PR+	ER+ PR+	ER- PR+	ER+ PR-	ER- PR-
I	6	6	6	-	-	-
II	9	10	7	3	2	5
III	12	10	7	3	4	28

Table 4: Showing Correlation of P53 with Grade of Tumor.

Grade Of Tumor	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	-	-	-	-	6	0	6	0%
II	-	3	1	6	7	10	17	58.8%
III	-	6	2	20	14	28	42	67.4%

Table 5: Showing Correlation of ER PR and P53 with Lymph Node Status.

	ER+ PR+	ER+PR-	ER- PR+	ER- PR-	P53+	P53-
No. of Cases	19	7	6	33	38	27
Metastatic Lymph Nodes	8	6	4	24	26	16
Reactive Lymph Nodes	11	1	2	9	12	11

Table 6: Showing Correlation of ER, PR and p53.

ER PR status	p53 Positive	p53 Negative	Total
Category I (ER + PR+)	10	9	19
Category II (ER + PR-)	5	02	07
Category III (ER- PR+)	02	04	06
Category IV (ER- PR-)	21	12	33

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.[16] In India though ranked second after carcinoma cervix at the moment, it is all set to overtake cervical cancer and become the most common cancer in Indian women by 2020. An ICMR release in 2011 showed an increase in incidence from 10 per 100,000 to 23 per 100,000 in just 10 years.[17] Breast cancer incidence and death rates generally increase with age. In the present study, the maximum numbers of the patients were in the age group of 41-60 years comprising 67% of the total cases. This is in accordance with work done by Kaur et al who noted a peak age of 45 - 55 years in a study done on 177 cases.[18]

In the present study, right side was more commonly involved (60% of cases) than the left side (40% of cases) which is in accordance with work done by Saleh & Abdeen on 166 patients of breast carcinoma.[19] In the present study, there was definite preponderance for the upper outer quadrant(84%). Possible explanation is that the upper outer quadrant has a relatively larger volume of breast tissue. [20,21,22] One of the most important and well established prognostic factors in carcinoma breast is tumor size.[23] In the present study, the size of the tumor varied from 1 cm to 6 cm and in 67.7% cases tumor size was between 2-5 cm constituting the largest group. This is in concurrence with results obtained by Kaur et al and Saleh & Abdeen [18,19] Where as in the western countries, Taucher et al reported that the tumors were predominantly less than 2 cm in size

which could be due to early detection programs prevalent in the western countries.[24]

Tumor grade is another well established prognostic marker in case of breast carcinoma.[23] Most of the cases in present study were of grade III (64.6%) which is in contrast to findings noted by Muhammad Hanif, who reported maximum number of tumors (59%) to be of grade II.[25] Maximum number of grade II cases were also noted in studies done in Singapore, Malaysia, Pakistan and India. [26-30] This difference in the tumor grades may be due to lack of routine mammographic screening in our population coupled with the lack of awareness. Correlating tumor grade with lymph node involvement in the present study, it was observed that grade III tumors demonstrated the highest frequency (64.8%) of lymph node involvement. The findings are in concurrence with the work done by Shokouh et al (Grade 3 tumors 73.5%).[31] Kaur and others noted that in grade III tumors lymph node metastasis was present in 57.6% cases.[18]

When correlation of tumor size with lymph node involvement was attempted, it was seen that in the present study, 6 cases (28.57%) out of a total 21 with size <2 cm showed metastatic carcinomatous lymph node deposits and this percentage increased to 70.5% (31/44 cases) when the tumor size was between 2-5 cm. This shows a direct relationship between increased tumor size and lymph node involvement. This was in concordance with the studies done by Shokouh et al and Bojić et al who showed a strong correlation between the two.[31,32] Indian literature reports estrogen receptor positivity varying between 30-50%.[33] It was also reflected in the present study with 27 cases (41.5%) showing ER positivity. Out of 38 ER negative cases in the present study, 28 cases were below the age of 55 years and 10 above the age of 55 years. Manjunath et al demonstrated that ER negative disease occurred at a younger age, at a mean of 50.2 years (SD 10.28), whereas the mean age of ER positive disease was 55.7years.[34] Progesterone receptor expression also showed a similar trend with 26 cases comprising 40% of the total cases showing PR positivity. Desai *et al.* from India have documented the prevalence of 46.1% for PR-positive breast cancers.[35] Ambroise *et al.* in their study from South India have showed 51% PR positivity.[30] Similarly, Mudduwa, in a study from Srilanka documented a prevalence of 48.3% PR-positive tumors.[36]

Maximum number of cases in the present study were in category IV (49.2%) followed by category I (30.7%) as shown in Table 2. The prevalence of hormones receptor positive breast cancer in Asian countries has been found to be lower than those in the western world [37] because of lesser use of OCP's and increased hormonal replacement therapy after menopause. The percentage of tumors expressing PR but negative for ER was 9.23% in the present study. The results are in concurrence with the study done by Patnayak et al. [38]

In the present study, when we compared the ER expression according to age in two groups with highest number of cases (Group II & III), it was seen that group II patients did not express ER in 84% of cases as compared to group III patients where this negative expression was seen in 42.1% of the cases. This correlation was found to be significant (p=0.003; Significant).

Furthermore when p53 expression was evaluated in these 29 ER negative cases, it was seen that out of 21 ER negative cases in group II, 18 cases showed a positive p53 expression. On the other hand only 3 out of 8 ER negative cases in group III exhibited a p53 positivity (37.5%). This correlation was again found to be significant (p=0.009; Significant).

Similar results were echoed by Mohammed et al who reported that patients younger than 50 years of age with Grade II and III infiltrating ductal carcinoma had significantly lower levels of estrogen receptors than patients older than 50 years of age (P <0.001).[39] Breast tumors arising in older patients have slower growth rates, are more likely to be ER-positive, and are less likely to be p53-positive.

In the present study, the ER and PR expression in patients with tumor size less than 2 cm was higher than that seen in patients with tumor size between 2-5cm (52.6% & 57.9% vs 37% & 32.6% respectively). P53 expression however demonstrated a reverse relation with patients having tumor size varying between 2-5cm showing a higher positivity (73.3%). The results however were not statistically significant. Similar results were obtained by Ahmed et al in their study done on 157 Yemeni women.[15]

In the present study ER positivity decreased from 100% to 53% to 28.6% as the grade increased from first to third, though the results were not statistically significant. Manjunath et al, Jovicić-Milentijević et al and Barnes et al also showed the same results, as the grade of the tumor increases ER positivity decreases.[34,40,41]Progesterone receptor expression also showed a similar trend that as the grade increased PR positivity decreased although the results were not statistically significant. Similar results were noted in various other studies.[37,41,42] None of grade 1 tumors in the present study showed p53 positivity. Out of 17 cases of grade II, 10 cases (58.2%) showed p53 positivity and out of 43 cases of grade III, 29 cases (67.4%) showed p53 positivity which was found to be

statistically significant (p=0.007). This shows as the grade increases p53 positivity increases. A retrospective study done by Shokouh et al from 2008 to 2014 on 566 patients of breast carcinoma also showed that higher grades had greater p53 positivity.[31]

In the present study the percentage of cases showing metastatic carcinomatous deposits was highest in ER PR negative group (category IV; 72.8%) as compared to the ER PR positive category I (42.1%) (p=0.028; Significant). However no significant correlation was noted for p53 expression where metastatic lymph node involvement was seen in 68.4% p53 positive cases and 59.3% p53 negative cases. The findings of the present study are in concurrence with the work done by Ali et al who reported that the ER PR negative cases have 2.8 times increased risk of metastasis in comparison to ER PR positive breast cancer cases.[43] Dunnwald et al in their study demonstrated the association between ER/PR status and breast cancer specific mortality with in subgroups of women defined by tumor characteristics like axillary lymph node metastasis. They observed a higher risk of lymph node involvement in ER PR negative tumors relative to ER PR positive tumors. [44]

When we attempted a correlation between ER, PR and p53 in the present study, it was seen that 21/33 (63.6%) of ER, PR negative cases showed p53 positivity. This constituted the largest group of p53 positive cases (21cases) and showed that ER PR and p53 are inversely related. An inverse association between hormones receptors and p53 has been demonstrated by Ahmed et al on Yemini women with breast cancer.[15] This was further confirmed by Sirvent et al.[45]

In the present study, it was observed that out of 42 cases of grade III tumors, 29 cases showed p53 positivity (66.7%) and 28 cases showed ER PR negativity (66.6%). In grade II tumors, out of 17 cases, 10 showed p53 positivity (58.8%) with 5 ER PR negative cases (29.4%). This shows that as the grade of the tumor increases p53 positivity increases and ER PR positivity decreases suggesting further that ER PR are inversely related to P53 status. However this difference was not statistically significant. Marc Lacroix et al showed that breast tumors expressing a high amount of p53 (as measured by IHC) are more frequently ER-negative and PR-negative. They are also associated with a high proliferation rate, high histological and nuclear grades, aneuploidy and poorer survival.[14]

Conclusion

It was concluded in the present study that ER expression was significantly low in patients younger than 50 years of age as compared to those older than 50 years. The number of lymph nodes showing metastatic deposits was more in ER PR negative cases as compared to patients showing a positive ER PR expression. It was also concluded that as the grade of the tumor increased, p53 positivity increased and ER, PR expression decreased suggesting an inverse relationship between the two. It was further observed that ER, PR positivity is high in low grade tumor and p53 positivity is associated with high grade tumors. 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.

Acknowledgements

I acknowledge the efforts of Dr. Monika Lalit in providing the technical help, statistical help and writing assistance. i state that there was no conflict of interests.

Funding

None

Competing Interests

None Declared

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