Evaluation of Expression and Correlation of ER, PR and Ki 67 Tumor Markers in Breast Carcinoma

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ABSTRACT

Introduction: Tumor markers are used for the detection of risk, population screening, diagnosis, staging and prognosis. The present study was commenced out to find correlation of ER, PR and Ki 67 tumor markers with histopathological grading.

Material and methods: The study was conducted on 50 operable cases of breast cancer. The histopathological grading of the breast carcinoma was done according to the Nottingham modification of the Bloom Richardson grading system. All the cases underwent immunohistochemistry for ER, PR and Ki 67 expression. The results were compiled and analyzed statistically using SPSS-16 and were expressed as number and percentage.

Results: ER positivity decreased as the grade of the tumor increased. PR positivity decreased as the grade of the tumor increased. Ki 67 positivity was seen in 84% cases. Ki 67 value was directly related to the grade of the tumor(p value = 0.007). Majority of grade II tumors were ER and PR positive whereas grade III tumors were ER,PR negative and Ki 67 positive. Ki 67 score also increased as the mitotic count increased but the association was not statistically significant. No correlation was found between Ki 67 and lymphovascular invasion.

Conclusion: The present study concludes that ER, PR reveals inverse relationship and Ki 67 showed direct relationship with the grade of the tumor; and is also inversely correlated with ER, PR positivity. As the patients who show increased Ki 67 expression have also got increased mitotic activity and less expression of ER,PR; thus carrying a poor prognosis and requires the treatment accordingly.

Keywords: Breast Cancer; Estrogen receptor; Progesterone receptor; Ki 67; Tumor Markers

INTRODUCTION

Tumor markers are used for the detection of risk, population screening, diagnosis, staging and prognosis. It can also predict the response to therapy, monitor treatment, detect the presence of occult metastatic disease and monitor the course of the disease.[1] Immunohistochemical expression of tumour markers like estrogen and progesterone receptors, Ki-67, p53, PCNA (Proliferating Cell Nuclear Antigen), Her-2/neu, cytokeratins, BRCA 1, BRCA 2 etc help to assess the tumor status.[2]

Estrogen has effect on target tissues by binding to fractions of cells called estrogen receptors and thus play an important role in the growth and development.[3] Estrogen causes tumorigenesis by binding to the estrogen receptor (ER) which causes proliferation of mammary cells leading to increase in cell division and DNA replication, resulting in mutations. The result of both processes is disruption of cell cycle, apoptosis and DNA repair and therefore, tumour formation.[4] ER positive breast cancers generally have a better prognosis and are often responsive to hormone therapy.[5]

Progesterone receptor (PR) is an intracellular steroid that specifically binds progesterone expressed by a single gene [Chr 11 q 22]. Estrogen is necessary to induce progesterone receptors. PR along with ER is expressed in various histological types of breast cancer.[6] PR positivity is around 46% in Indian population which is much lower than those reported in the Western literature reported as around 79%.[7,8]

Ki67 is a proliferative marker which is studied extensively in the recent past. It is non histone nuclear protein that is expressed in G1 through M phase of cell cycle and is not detected in resting phase of cells i.e.G0.[9] Ki-67 increases as cells prepare to divide into new cells. The more positive cells there are, the more quickly they are dividing and forming new cells.[10] The present study was commenced out to find correlation of ER, PR and Ki 67 tumor markers with histopathological grading.

MATERIAL AND METHODS

The study was conducted on 50 cases of breast cancer received as lumpectomy or mastectomy specimens in the Department of Pathology, Sri Guru Ram Das institute of medical sciences, Sri Amritsar. Ethical approval from the institution and informed consent from the patient was taken before the initiation of the study. Detailed history was taken. The tissue so obtained was formalin fixed and paraffin embedded. For histopathological typing and grading, the tissue was stained for Haematoxylin and Eosin. The Nottingham modification of the Bloom Richardson grading system[2] was used for histopathological grading of the breast carcinoma. All the cases were subjected to immunohistochemistry for ER, PR and Ki 67 expression. Interpretation of the IHC scoring was carried as given below:

Positive and negative controls were run with every batch of the IHC.

For estrogen and progestrone receptor, brown nuclei were taken as positive. Cytoplasmic brownish colouration was ignored.

Quick score method of assessment was used to assess the range of immunostaining.

Total Score (TS) = PS + IS (Range 0-7)

Negative	–	quick score 0
Low	-	quick score 2-3
Medium	-	quick score 4-5
High	-	quick score 6-7

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Ki-67 expression status was assessed according to the estimate proportion of nuclear stainig of tumor cells that were positively stained.

None = 0
<1/100 = 1
1/100-1/10 = 2
1/10-1/2 = 3
>1/2 = 4

Tumors with a score of 2 or greater for Ki-67 were considered to be positive for Ki-67 expression. Intensity of staining whether strong, moderate or weakly positive was also be noted

The results were compiled and analyzed statistically using SPSS-16 and were expressed as number and percentage. Chi-square test was used, considering p=0.05 as significant value.

RESULTS

Table 2 shows immunohistochemistry results for ER, PR and Ki 67 expression. Estrogen receptor positivity was seen in 18 cases comprising 36% of the total cases. Percentage of positive cells varied from 22 to 90 % with mild, moderate and strong intensity. Progesterone receptor positivity was seen in 18 cases comprising 36% of the total cases. Percentage of positive cells varied from 1 to 90% with mild, moderate and strong intensity. Ki 67 positivity was seen in 42 cases comprising 84% of the total cases. Percentage of positive cells varied from 2 % to 84% with mild, moderate and strong intensity.

Table 3 demonstrates correlation of tumor markers with grade of tumor. In case of estrogen receptors, out of 12 cases,6 were positive in grade II, whereas in grade III, of the 38 cases, only 12 were positive, thus it was found that as the grade increased, the positivity decreased. In case of progesterone receptors, there was 66% positivity whereas in grade III, the positivity decreased to 26%, thus showing as grade increased, the positivity decreased.

The Ki 67 score increased as the grade of tumor increased. In grade II, there were 5 cases of each with Ki 67 score 2 an 3 and none of the cases had score 4. But in grade III, 3 cases had score 2, 19 had Ki 67 score 3 and 10 had score 4. Ki 67 positivity and its score were directly proportional to the grade of the tumor. This correlation was found to be highly significant with the p value = 0.007.

Combination of ER and PR

According to ER PR positivity tumors were separated into four categories: ER+PR+, ER+PR-, ER-PR+ and ER-PR-. 52% of cases were combined ER and PR negative followed by ER and PR positive cases constituting 26% cases (table 4). Thus, there was a significant association between ER and PR receptor positivity (p= 0.001)

Ki 67 positivity with mitotic count

The Ki 67 score increases as the mitotic count increased. Out of the 6 cases with mitotic count < 5, 4 were Ki 67 positive and 2 were Ki 67 negative and out of the 42 cases with mitotic count more than 5, 38 cases had Ki67 positive and 6 were Ki 67 negative. So it was found that as the Ki7 score increased, there was increase in the mitotic count also, but it was not statistically significant

Ki 67 with lymph node status (table 5)

Out of 50 cases, in 48 cases lymph nodes were recovered. In 30 cases, metastatic deposits were found. Ki 67 did not have any direct correlation with lymph node status as its positivity was seen in reactive lymph nodes as well.

Ki 67 with vascular invasion

Vascular invasion was present in 37 cases. Out of the 37 cases,

Scoring of estrogen receptor			
Percentage positivity	**Score**	**Staining intensity**	**Score**
<10 %	1	Low	1
10–50 %	2	Moderate	2
50–75 %	3	Strong	3
>75 %	4		
Scoring of progestrone receptor			
Percentage positivity	**Score (PS)**	**Staining Intensity**	**Score (IS)**
No staining	0	Negative	0
1-25%	1	Weak	1
26-50%	2	Medium	2
51-75%	3	High	3
76-100%	4		

Table-1: Scoring criteria

Tumor marker	**No of cases**	**%age**
Estrogen receptor		
Positive	18	36%
Negative	32	64%
Progesterone receptor		
Positive	18	36%
Negative	32	64%
Ki 67 Receptor		
Positive	42	84%
Negative	08	16%

Table-2: Immunohistochemistry demonstration for ER, PR and Ki 67 expression

Grade of tumor	**ER Positive**	**ER Negative**	**Total**
Grade I	0	0	0
Grade II	06	06	12
Grade III	12	26	38
Grade of tumor	**PR Positive**	**PR Negative**	**Total**
Grade I	0	0	0
Grade II	08	04	12
Grade III	10	28	38
Total	18	32	50
Grade of tumor	**Ki 67 Score**		
	1	**2**	**3**
II	0	5	5
III	0	3	19
Total	0	8	24

Table-3: Correlation of tumor markers with grade of tumor

Combination of ER and PR	**Number of cases (n=50)**	**Percentage**
ER+PR+	12	24%
ER+PR-	6	12%
ER-PR+	6	12%
ER-PR-	26	52%

Table-4: Combination of ER and PR cases

30 were reported as Ki 67+ve whereas 7 cases were Ki67 negative. Vascular invasion was absent in 13 cases. Out of the 13 cases, 1 case was Ki 67 negative and 12 were reported as Ki 67 positive.

Correlation of ER, PR and Ki 67

Out of 26 ER PR negative cases, Ki 67 was positive in 22 cases and negative in 4 cases. Out of 11 ER and PR positive cases, 11 cases were Ki 67 positive and 1 cases was Ki 67 negative (table 6).

Thus it was observed that as the grade of tumor increased, ER and PR positivity decreased and as the ki67 showed direct correlation with grade showing that as the grade increased, the Ki67 positivity also increased with a significant p value. No definite correlation of Ki 67 with mitotic count, lymph node status and lymphovascular invasion was seen.

DISCUSSION

Breast cancer is most common cancer in women worldwide and accounts for 20% of all cancers in women.[11] Over 100,000 new breast cancer patients are estimated to be diagnosed annually in India. It is expected to increase by 26% by 2020 in developing countries. It is the second most common carcinoma in rural areas with only a narrow figure separating it from the top placed malignancy of cervix, in terms of both incidence as well as mortality but it is considered to overtake the cervical malignancy to reach the top by 2020. However, in urban India it is the commonest cancer among women, where it constitutes >30% of all cancers in females.[12,13] A number of well established prognostic factors, such as clinical stage, histological grade, hormone receptors and tumor markers of cell proliferation have been postulated to predict the clinical course of breast cancer.[14]

In the present study, estrogen receptor positivity was found in 18 cases comprising 36% of the total cases. This is in concordance with various Indian studies conducted by Rashmi K et al[15] in northern India reporting it as 34.5% and Desai SB et al[7] reporting it as 32.6 %. However the western authors reported it to be 79.1%, 73% and 74% respectively[8,16,17] In white women, a higher incidence of hormone receptor positive breast cancer was reported as compared to black and Asians, which may be attributed to different inherent biology and low incidence in Indian population may be due to decreased Hormone Replacement Therapy.[18]

In the present study, out of 50 cases, 36% were reported positive for progesterone. Rashmi K et al,[15] Desai SB et al,[7] Ghosh J et al,[19] Haroon S et al[20] also reported almost the same incidence as 36.4%, 46.1%, 51.2% and 38.1% respectively. While the western studies reported PR positivity to be around 79% done in USA.[8]

In the present study, maximum number of cases (52%) reported ER and PR negative followed by ER and PR positive cases (24%) and this was in contrast to the western study conducted by Dunnwald LK et al.[21] This was possibly due to the reason that HRT is still not prevalent in India and at the same time, many people report late for the diagnosis and the treatment, thus increasing the tumor size and the grade of the tumor at the time of reporting.[18] Table 7 shows comparative evaluation of combination of ER and PR cases reported in various studies.

Out of the 50 cases, 84% were Ki 67 positive. 8 cases had Ki 67 positivity between 1 -10 %, 24 cases had Ki 67 positivity between 10 – 50 % and 10 cases with positivity > 50%. Pandyeah M et al[23] reported Ki 67 positivity in 90% of the patients with 62% cases having Ki 67 > 10% and 28% with Ki 67 <10%. With the increasing mitotic count, the Ki 67 score increased, as stated by many studies.[24,25] Similar results were obsereved in the present study although it was not statistically significant. Various studies have reported direct correlation between Ki 67 positivity and lymphovascular invasion.[25,26] However no such correlation was found in the present study.

Regarding the ER status, it was observed that ER expression decreased with increasing grade of tumor but this correlation was not statistically significant, which can be attributed to the small sample size. Antoniades K et al[27] found 100% ER positivity in grade I tumors, 61.5% in grade II and 20% in grade III tumors. Shet T et al[28] also found that all grade I tumors, 93% of grade II tumors and 39.5% of grade III tumors expressed estrogen receptors.

Similarly regarding PR status, it was observed that the grade of the tumor and PR expression were inversely related to one another. Fatima N et al[29] observed similar results in their study. Shet T et al[28] also observed that only 39.5% of grade III tumors expressed progesterone receptors.

Regarding Ki 67 receptor status, significant correlation was found between the grade of tumor and Ki 67 score. Ki 67 score increased with increasing grade of the tumor. This was statistically significant with p value being 0.007. Similarly a significant association between Ki 67 and tumor grade was observed by Haroon S et al[20] and Inwald EC et al.[30] Thus it was

Ki 67	Not recovered	N0 reactive	N_x Metastatic	Total
Negative	0	2	6	8
Positive	2	16	24	42
Total	2	18	30	50

Table-5: Ki 67 with lymph node status

ER PR status	Ki 67 Positive	Ki 67 Negative	Total
ER + PR-	04	02	06
ER- PR+	05	01	06
ER + PR +	11	1	12
ER- PR-	22	4	26

Table-6: Correlation of ER, PR and Ki 67

Author	Combination of ER and PR			
	ER+PR+	ER+PR-	ER-PR+	ER-PR-
Desai SB et al[7]	25%	7.4%	21.1%	46.5%
Mehta RR et al[22]	30.3%	20.9%	7.7%	41.2%
Dunnwald LK et al[21]	63%	13%	3%	21%
Present study	24%	12%	12%	52%

Table-7: Comparison of ER and PR cases reported in various studies

evident that as the grade of the tumor increased, ER and PR positivity decreased while Ki67 positivity increased.

Higher positivity levels of Ki 67 have been reported in triple negative tumors with lower status of Ki 67 in ER and PR positive cases.[31,32] In the present study, no such correlation was found as even ER, PR negative cases showed high Ki 67 positivity. However, it could be due to the small sample size in the present study. The expression of ER, PR is associated with lower grade and better prognosis.[33] But Ki-67 being a proliferative marker, its expression is associated with higher grade and poor prognosis.[26] Thus it is evident that the breast carcinoma patients should be subjected to ER, PR and Ki 67 status as ER,PR positivity will define the line of treatment as these tumors respond well to hormone therapy and have got a good prognosis whereas Ki 67 when increased especially in ER, PR negative cases, carries a poor prognosis. Thus it can be used as a prognostic marker.

CONCLUSION

The present study concludes that ER, PR reveals inverse relationship with the grade of the tumor as the grade of the tumor increased, ER,PR expression decreased. The Ki 67 show direct relationship with the grade as it increases with increasing grade and is also inversely correlated with ER,PR positivity as its expression was seen more in grade III carcinoma as compared to grade II. This shows that the patients who show increased Ki 67 expression have also got increased mitotic activity and less expression of ER,PR; thus carrying a poor prognosis and requires the treatment accordingly.

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