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Original Research Article

STUDY OF CARCINOMA OF BREAST WITH REFERENCE TO HISTOLOGICAL GRADING AND ITS CORRELATION WITH P53 AND KI-67 IMMUNOMARKERS

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ABSTRACT

Background: Breast cancer is the most common cancer in women and commonest cause of death from cancer in women worldwide. Breast cancer is one of the most frequent cancers among women in developing country. The combined study of p53 expression and Ki-67 in breast carcinoma is very helpful in assessing the prognosis & patient outcome.

Materials and Methods: 50 cases of breast carcinoma was carried out in the department of pathology. The haematoxylin and eosin(H&E) stained sections of the cases were made and diagnosed and selection of representative tumour paraffin blocks was done on which IHC was performed. Immunohistochemical staining is conducted and the proportion of the malignant cells staining positive for the nuclear antigen Ki-67 is evaluated in a quantitative and visual way using light microscopes. Evaluation of p53 immunostaining was also done. Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean±SD (min-max) and results on categorial measurement are presented in number (%).

Results: Majority 23 cases (45.9%) showed moderate proliferative activity, followed by 16 cases (31.9%) showing low proliferative activity. 11 cases (22.2%) showed high proliferative activity. The majority of 44 cases (88.9%) were positive for p53 expression of which maximum 18 cases (36.1%) showed>50% of p53 expression. 19 cases (38.9%) had 20-50% of p53 expression. In our study 41.6% of Grade 1 tumors showed moderate p53 expression. Maximum no of Grade II tumors showed moderate to high p53 expression. 40% of Grade III tumors showed high p53 expression. In our study IDC (NOS) showed maximum moderate to high p53 expression. In our study the maximum number of cases (23) belong to Grade 2 showing moderate Ki-67 expression, and >49% of p53 expression.

Conclusion: In breast cancer, we suggested that the over expression of Ki67 & p53 protein in the nucleus is an indicator of poor prognosis.

Key words: Breast Cancer, p53, Ki67.

INTRODUCTION

Breast cancer is the most common cancer in women and commonest cause of death from cancer in women worldwide. Breast cancer is one of the most frequent cancers among women in developing country. The prognostic and therapeutic markers of breast can be broadly grouped into three groups: classical parameters that includes variables such as histological type, nuclear grade, tumor size, lymph node status & skin invasion,[1,2] immunohistochemical parameters like Estrogen receptor (ER) & Progesterone receptor (PR) of the tumor and HER-2/neu,[3] Ki-67 (MIB-1) high proliferative index, P-53 protein over expression and molecular markers.[2-4]

The immunohistochemical classification (IHC) provides both therapeutic and prognostic information yet is relatively less expensive and can be performed routinely. Ki-67 is a nuclear protein

being associated with cellular. It can be used as a prognostic marker as well as proliferative index in breast carcinoma. Ki-67 labelling index can be measured by using IHC methods using Antibody directed against nuclear.[5,6]

The p53 gene is one of the most important tumour suppressor genes that is located on the chromosomes 17 and encodes a 53 kDa nuclear protein. This has been implicated in controlling cell cycle regulation, genomic stability, DNA repairs, apoptosis, effectiveness of chemotherapy, prognosis of the diseases.[7] The combined study of p53 expression and Ki-67 in breast carcinoma is very helpful in assessing the prognosis & patient outcome.

MATERIALS AND METHODS

50 cases of breast carcinoma were carried out in the department of pathology in co-ordination with department of surgery of Hi-Tech medical college & Hospital. The haematoxylin and eosin(H&E) stained sections of the cases were made and diagnosed and selection of representative tumour paraffin blocks was done on which IHC was performed. The representative neoplastic tissue blocks (paraffin embedded) were cut at 3.0µ on Poly-L-Lysine coated slides. One of these sections were routinely stained with H&E. The histological grading of tumour was done on H&E-stained sections according to Modified Bloom Richardson grading. The histologic typing of the tumour was done along with grading of tumor as per Modified Bloom Richardson grading and staging was also done from the H&E stain. Immunohistochemical staining is conducted and the proportion of the malignant cells staining positive for the nuclear antigen Ki-67 is evaluated in a quantitative and visual way using light microscopes. Low –power magnification (10x) to determine areas with the highest number of positive nuclei (hot-spots) within the invasive component. Nuclear immunostaining in tumour cells was accepted as positive. At least 500 cells were counted at 100x magnification. The number of Ki-67 staining reactive cells in each field was determined as a% of the total number of tumour cells counted.

Group-1 <10% (low proliferative activity)
Group-2 10%-40% (moderate proliferative activity)
Group-3 >40% (High proliferative activity)

Evaluation of p53 immunostaining

Breast carcinoma was used as a positive control. Tumour cells with nuclear staining were accepted as positive. The extent of p53 was graded semiquantitative for intensity and distribution.

1. Negative <5%
2. Positive (low) 5%-19%
3. Positive (moderate) 20%-50%
4. Positive (high) >50%

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean±SD (min-max) and results on categorial measurement are presented in number (%).

RESULTS

Age ranged from 20-80 years and the mean age was 50 yrs. Majority, 28 cases (55.6%) belong to 41-60yrs. In our study Majority 23 cases (45.9%) showed moderate proliferative activity, followed by 16 cases (31.9%) showing low proliferative activity. 11 cases (22.2%) showed high proliferative activity. The majority of 44 cases (88.9%) were positive for p53 expression of which maximum 18 cases (36.1%) showed>50% of p53 expression. 19 cases (38.9%) had 20-50% of p53 expression. In our study 41.6% of Grade 1 tumors showed moderate p53 expression. Maximum no of Grade II tumors showed moderate to high p53 expression. 40% of Grade III tumors showed high p53 expression. In our study IDC (NOS) showed maximum moderate to high p53 expression. In our study the maximum number of cases (23) belong to Grade 2 showing moderate Ki-67 expression, and >49% of p53 expression.

Table 1: Ki-67 (proliferative) Expression

Ki-67 expression	No of patients	Percentage
Low proliferative activity (<10%)	16	31.9%
Moderate proliferative activity (10-40%)	23	45.9%
High proliferative activity (>40%)	11	22.2%
Total	**50%**	**100%**

Table 2: P53 Expression

P53 expression	No of patients	Percentage
Negative <5%	6	11.1%
Positive 5-10%	7	13.9%
Positive 20-50%	19	38.9%
Positive >50%	18	36.1%
Total	**50**	**100%**

Table 3: Correlation of p53 with histological Grading (SBR)

Histological Grade (SBR)	No of patients (n=50)	P53 expression			
		<5%	5-19	20-49	>49%
Grade 1	12	2	3	5	1

Grade 2	23	2	2	9	11
Grade 3	15	2	2	5	6
Total	**50**	**6**	**7**	**19**	**18**

Table 4: Correlation of p53 with histological type

Final Diagnosis	Number of patients (n=50)	P53 expression			
		<5%	5-19%	20-49%	>49%
IDC(NOS)	46	5	7	19	15
LCA	3	0	0	1	2
METAPLASTIC	1	0	0	0	1
TOTAL	**50**	**5**	**8**	**20**	**17**

Table 5: Correlation of Ki-67 and p53 with histological grading.

Grade	No of patients	Ki-67 expression			P53 expression			
		low	moderate	High	<5%	5-19%	20-49%	>49%
Grade 1	12	10	1	1	2	3	5	1
Grade 2	23	6	14	3	2	2	9	11
Grade 3	15	1	7	7	2	1	5	6
Total	**50**	**17**	**22**	**11**	**6**	**7**	**19**	**18**

DISCUSSION

Breast carcinoma is a disease with a tremendous heterogenity in its clinical behaviour with distinct pathological and histological features and can be classified into several subtypes based on the expression of 3 receptors, ER, PR, HER-2, which are the most acceptable ones for predicting prognosis, response/resistant to treatment and in deciding the use of newer drugs.

Prospective study was conducted during the period Nov 2016-Oct2018 in which 50 cases of breast carcinoma were examined.

In our study age of presentation ranged from 20-80 years with mean age of 50 years. Similar observation was made by Joshi K et al.[8] There was no significant association between Ki-67 with lymph node involvement. Similar observations with no significant association was found in study conducted by Banu Lebe et al & concluded saying the differences may be due to heterogenous group of population, different methods for assaying Ki-67, or different cut offs to designate high or low Ki-67.

Routine assessment of cell proliferation is recommended in the pathological evaluation for all breast cancers. This has traditionally taken the form of mitotic activity scoring, which is an integral component of histologic grading and considered as an established prognostic marker in breast cancer.[9-11]

In the current study Ki-67 was significantly associated with tumor grade and the median of Ki-67 of grade III tumors was higher than that of grade II tumors. This is to be expected given that mitotic index is one of the three components of grade and Ki-67 labelling is commonly used as a complement to grading systems. Our finding coincides with study conducted by Yamashita et al.[12]

In our study we had maximum number of IDC(NOS)cases and very few numbers of other histopathologic types; hence we could not correlate Ki-67 with histologic type of other tumors. However the study conducted by Awadelkarim et al,[13] found no correlation of Ki-67 expression and different histological types.

The most important prognostic factor for breast is lymph node status, Nevertheless, numerous attempts have been made to find other parameters that will help in predicting the clinical outcome more accurately and in selecting the most appropriate therapy for each case.

In our study; there was a significant association of p53 with lymph node involvement &similar observations were made in study conducted by Ivkovic –Kapicl et al.[14]

It is also possible that the p53 protein plays an important role in, progression of malignant human tumors (Porter et al,1992).[15]

For cancer, immunohistochemical positivity is found in up to 25% of breast carcinomas, which suggests that they may occur in early stages of the cancer before it becomes infiltrating. The staining pattern of metastatic lymph node are usually similar to those of primary tumors; only very rarely does a positive stain for p53 occur in a node when the tumor is negative (Poller et al Bhargava et al 1994).[15,16]

We found a significant association between tumor grade and p53 expression. Our finding coincides with study conducted by Yamashita et al.[12]

The p53 alteration may reflect a greater degree of tumor progression and higher proliferation rate, as well as greater probability of micro metastasis. Mutation and the over expression of p53 protein are directly related to histological grade and cell proliferation fraction. Cases positive for p53 could be interpreted as those which have lost a mechanism for controlling the inhibition of cell proliferation and have gained an activator for malignancy potential.17

In our study we had maximum number of IDC(NOS) cases and very few numbers of other histopathological types; hence we could not find correlation of p53 with histological type of tumor. But study conducted by Sirvent et al,[18] showed p53 expression distribution by histological type of tumor. But study conducted by Sirvent et al,[18]

showed p53 expression distribution by histological type highlighted the absence of any preference for p53 positivity and/or negativity in the case of ductal carcinoma, negativity in lobular carcinoma and strong positivity in medullary carcinoma.

Tumor grade, a parameter although easily assessed on core biopsies, but is not sufficient to define prognosis and it cannot be assessed optimally in post neoadjuvant settings(Matsubara et al 2013).[19] Furthermore ,as more conservative surgeries and staging techniques increasingly are introduced into the management of breast carcinoma e.g, increasing use of fine needle aspiration over tissue biopsies, much useful prognostic information, including tumor size, tumor grading, vascular invasion and lymph nodes involvement, will not be available. In this setting new markers such as Ki-67, p53 can be applied on small samples and they may be of prognostic significance which will be invaluable (Bilgren et al;2002),[20]

There are studies with variable observation in comparison to our findings and the differences may be due to heterogenous group of population, different methods for assaying Ki-67 & p53, or different cut offs to designate high or low.

CONCLUSION

Prognosis and management of breast cancer are influenced by classic variables such as tumor size, histological type, grade & lymph node status. Other prognostic factors include hormonal receptors like ER, PR, and HER2/neu status, and under present study prognostic markers like Ki67 & p53. In breast cancer, we suggested that the over expression of Ki67 & p53 protein in the nucleus is an indicator of poor prognosis. We also suggest a large scale, standard multivariate studies to determine correlation between high Ki67 & p53 index with other prognostic markers in breast carcinoma patients.

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