EVALUATION OF P53 AND KI67 PROLIFERATIVE INDEX IN TRIPLE NEGATIVE BREAST CARCINOMA

Farhan Akhtar, Mohammad Hassan Mohammad Ali, Muhammad Asif, Rabia Ahmed, Madeeha Anwar, Muhammad Tahir Khadim

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To evaluate expression of p53 and Ki67 proliferative index in triple negative breast carcinoma.
Study Design: Cross-sectional analytical study.
Place and Duration of Study: Department of Histopathology, AFIP Rawalpindi, from Jan to Jul 2018.
Methodology: Fifty-two cases of triple negative female breast carcinoma were included in this study regardless of age of patient, histological type and grade of the tumor. Tumors such as malignant tumors with positive immunohistochemical staining for ER, PR and Her 2 neu, stromal tumors, skin squamous cell carcinomas, lymphomas, poorly fixed specimens and specimens with scanty tumor tissue were excluded.
Results: The mean age of patients was 53.28 ± 7.69 years (range 40-71 years). Most of the patients were in the age group 41–50 years 21 (40.4%) and 51-60 years 21 (40.4%), followed by age group more than 60 years 10 (9.2%). High Ki67 index was seen in 34 (65.4%) of cases. In the rest of the cases 18 (34.6%) the proliferative index was low. Out of the 52 cases of triple-negative breast cancer 44 cases (84.6%) showed nuclear positivity for P53, the rest of 8 cases (15.4%) were negative for p53.
Conclusion: Most cases occurred in the 5th and 6th decades of life. The study showed strong correlation between p53 expression and high Ki67 proliferative index. This finding can help in the management of patients of TNBC and predicting the prognosis of the disease.

Keywords: Ki67, p53, Triple negative breast cancer.

INTRODUCTION

Breast cancer is the leading cause of cancer related deaths in women worldwide, with estimated 1.7 million cases diagnosed and 521,900 deaths per year. This represents about 12% of all new cancer cases (14.1 million)[1]. Breast canceris a heterogeneous neoplasm with different histological types and biological behavior. This heterogeneity necessitates a classification of this neoplasm. There are many classifications of breast cancer including histological, biological and molecular classifications. The histological classification is worldwide agreed upon. It is based on certain morphological features. According to this classification about 80% of tumors arediagnosed as invasive ductal carcinoma and invasive lobular carcinoma, the rest of the tumors are of special type such as tubular, cribriform and adenoid-cystic carcinomas. Generally the drawback of this classification, it's limited utility in prognosis and prediction of the biological behavior of the tumor. Another classification was designated, the biological classification. This classification is beneficial in the selection of appropriate therapy. It depends on expression of estrogen, progesterone and Her 2 neu receptors. Estrogen receptors are of two types: ERα and ER β. The pathway of these receptors is very important in the pathogenesis of the hormone dependant breast carcinomas. Expression of PR receptors indicates that the ER pathway is intact, even if the tumoris ER-negative. Her 2 neu receptors aretrans-membrane tyrosine kinase proteins belong to the epidermal growth factor receptor (EGFR) family. Over expression of these proteins or amplification of their genes in breast cancer are associated with poor prognosis. The introduction of anti-Her 2 neu therapy has changed the clinical outcome of

Correspondence: Dr Farhan Akhtar Consultant Histopathologist, Army Medical College, Rawalpindi Pakistan
Email: fakhtar690@yahoo.com
Received: 04 Sep 2018; revised received: 31 Oct 2019; accepted: 31 Oct 2019

these tumors. Clinically and according to the response of tumorsto endocrine and anti-Her 2 neu therapy, there are three types of breast tumors in this classification: Endocrine and anti-Her 2 neu responsive tumors, those neither endocrine nor anti-Her 2 neu responsive tumors and tumors with uncertain responsiveness. According to the ASCO/CAP guideline, breast cancer is considered positive for ER and PR receptors if 1% or more of the tumor cells have positive nuclear staining of any intensity, and positive for HER-2 if 10% or more of tumor cells exhibit strong uniform membrane staining[2,3]. Still this classification has some limitations in prognosis, prediction of biological behavior and it's inability to show the diversity of breast cancer. Molecular classification is a new classification, it was first proposed by Perou, *et al* in 2000. This classification has been proved to have a good correlation with breast cancer in the settings of prognosis and prediction of response to chemotherapy, in this classification the breast cancer is divided into luminal A, luminal B, HER 2-enriched, and basal-like. Application of ER, PR and HER 2 neu immunohistochemical stains reveal an overlap, although not complete, between molecular classes and their immunohistochemical stains. In luminal A both ER and PR are positive, the Her 2 neu receptors are negative, in luminal B according to Her 2 neu expression there are two types: Luminal B, HER 2 neu negative type, it is characterized by ER positive and either negative or weak positive PR receptors, luminal B Her 2 neu positivetype, itis characterized by positive ER regardless of PR status. HER2-enriched type has over expressed Her 2 neu protein and negative endocrine receptors. Basal like breast cancer showing negative ER, PR and Her 2 neu receptors. Further molecular studies has shown that the basal like cancer is a heterogeneous group and accordingly it can be divided into basal like subtype, mesenchymal subtype, immune enriched subtype and luminal AR subtype[4,5].

There are many modalities for treatment of breast cancer which includes surgery, chemotherapy, hormonal therapy, neoadjuvant therapy and target therapy. Selection of appropriate treatment depends on the stage of tumor which in turn based on size of tumor, lymph nodes status and distant metastasis. Status of hormone receptors and Ki67 index are important factorsfor neoadjuvant therapy[6].

The so called triple negative breast carcinoma (TNBC) can be diagnosed by it's negative staining for ER, PR and Her 2 neu receptors. This group of breast cancer has a very aggressive biological behavior with evidence of distant metastasis at the time of diagnosis. Compared too ther types of breast cancer, this group has a high Ki 67 proliferating index[6].

Ki67 is a non-histonenuclear protein; it is activated during active cell cycle. It reflects the biological activity of the DNA during the cell cycle. High Ki67 levels indicate high proliferative capacity of the tumor. This protein can be helpful infuther classification of breast cancer. It has been found that luminal A breast cancer shows low Ki67 proliferative index compared to luminal B breast cancer which shows high Ki67 proliferartive index[7].

P53 is a gene located on chromosome 17q13. It is concerned with repair of damaged DNA. Mutations in this gene are associated with the development of malignant tumors. Mutated gene can be detected by FISH or through detection of the accumulated non-functioning p53 protein by using immunohistochemical techniques. It has been implicated that p53 has an important role in the development of TNBC, where it has been observed in 80% of cases[8].

TNBC is extensively studied worldwide. In Pakistan there are few studies regarding this type of breast cancer. The rationale of this study was to evaluate the expression of Ki67 and P53 along with the relation between the two proteins in TNBC.

METHODOLOGY

This cross-sectional analytical study was conducted in department of histopathology at Armed Forces Institute of Pathology (AFIP),

Rawalpindi, from Jan 2018 to July 2018 after approval by the Institutional Review Board. Fifty-two specimens of breast cancer were collected by non-probability consecutive sampling. The sample size was calculated using WHO sample size calculator with confidence level at 95%. The samples included in this study were trucut biopsies and surgical mastectomies from female patients of all ages, diagnosed on routine histopathology and ER, PR & Her 2 neu immuno-histochemical stains as triple negative breast carcinomas. Specimen with other tumor such as phyllodes, lymphoma, skin squamous cell carcinoma involving the breast along with tumors with positive stains for ER, PR and Her 2 neu, poorly fixed tissues and specimen with scanty tumor were all excluded from this study. Tissue of 3 µm thickness was prepared by microtome from the selected blocks, then it is deparaffinized in xylene and rehydrated with decreasing concentration of ethanol. The epitopes were retrieved by using heat method in Tris/ EDTA buffer at pH 9.0. Immuno-histochemical staining for Ki67 and p53 was performed through antibodies prepared by Dako Company. Age and immunohistochemical patterns were noted and analyzed by using computer software program SPSS version 16. Mean ± SD were calculated for continuous variables. Frequency and percentages were obtained for qualitative variables.

Forki 67 the hottest area was studied by power x40. No nuclear staining was interpreted as negative, weak, less than 10% as low proliferative index and more than 10% as high proliferative index.

In P53 no nuclear staining was considered as negative and any nuclear staining was considered as positive.

RESULTS

In this study a total of fifty two cases of triple negative breast cancer were analyzed. The mean age of patients was 53.28 ± 7.69 years (range 40-71 years). Most of the patients were in the age groups 41-50 years and 51-60 years 21 (40.4%) for each group, followed byage group more than 60 years 10 (19.2%).

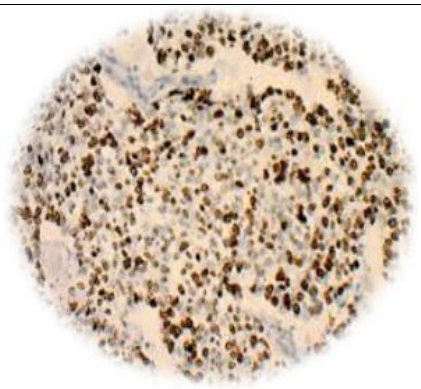

Figure-1: Ki67 in TNBC.

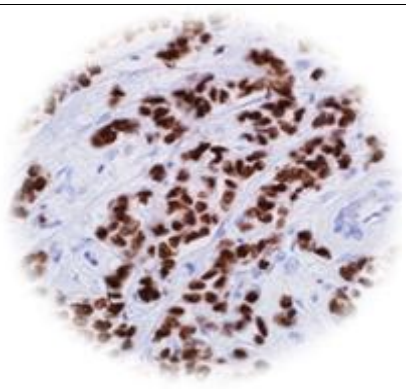

Figure-2: P53 in TNBC.

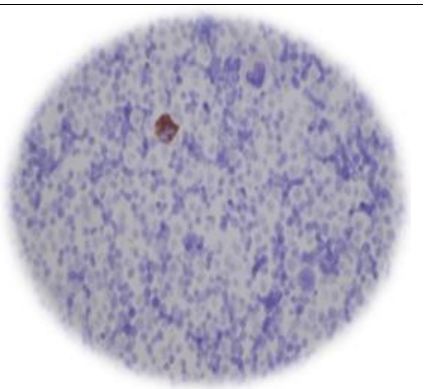

Figure-3: Triple negative breast carcinoma.

Table: Expressionof Ki67 and p53 in triple negative breast carcinoma.

Parameter	No. of Cases	Percentage
P53 expression		
Positive cases	44	84.6
Negative cases	8	15.4
Ki67 Proliferative Index		
High index	34	65.4
Low index	18	34.6

The results of immunohistochemical staining patterns of p53 and ki67 which shows significant correlation (table).

DISCUSSION

Breast carcinoma is the highest and most lethal female cancer in Pakistan and Asia. The incidence of the disease is approximately 90,000

females affected each year and almost 40,000 die of the disease every year[9]. Triple-negative breast cancer (TNBC) is a newly described subtype. It was first time mentioned in 2005 and it constitutes 15% of breast cancer. Treatment of this group of breast cancer is a real challenge. Due to negative ER, PR & Her 2 neu receptors of this tumor, it is insensitive to hormonal therapy. The high recurrence rate also creates additional problem in treatment[10].

St Gallen guidelines for treatment of breast cancer give some solutions for the treatment of this group. Due to negativity of receptors and high Ki67 proliferative index, St Gallen 2013 guidelines recommend chemotherapy as the treatment of choice for TNBC[6].

A part from its contribution in the selection of treatment, Ki67 is alsoused to evaluate chemotherapeutic response. It is also considered as an independent prognostic factor. High Ki67 index is associated with poor prognosis and high recurrence rates[11,12]. Generally tumors with high Ki67 are sensitive to chemotherapy. It has been found that TNBC usually develops resistance to chemotherapy, this in part may be due to expression of mutated p53 protein[13]. Mutations of this gene is thought to be a major factor of tumor relapse, metastatic potential and aggressive biological behavior of TNBC[14]. Due to resistance of TNBC to hormonal therapy and negative effects of p53 mutation on tumor response to chemotherapy, these phenomena make scientists to look for target therapy, in this regard anti Ki67 and anti p53 target therapy CP-31398 and STIMA-1 are promising drugs[15].

The results of this study showed that most cases were in the fourth and fifth decades of life (42, 80.8%), with mean age of 53.3 ± 7.69 years. This finding was in concordance with local studies conducted by Ahmed *et al* and Hashmi *et al*, in which the mean age was 48.9 years in Ahmed *et al* study and 48.4 years in Hashmi *et al* study. More over In Hashmi *et al* study they reported that around 60% of patients were diagnosed at less than 50 years of age, this finding can be compared to that of our study[16,17].

The analysis of immunohistochemistry in this study showed strong correlation between the high Ki67 proliferative index and P53 protein expression. This finding correlates with Hamaid *et al* study in India and Pan y study in China where they found a correlation between these proteins[18,19].

This study also showed that the Ki67 proliferative index was high in 34 (65% of cases). This finding coincided with Haitaoli *et al* study in china in which high Ki67 index was expressed in 83% of cases[20]. It is also coincided with Moazed V study in Iran in which high Ki67 index was seen in 54% of cases[21].

Regarding P53, the study showed positive nuclear staining in 44 (84.6%) of cases; this result was in concordance with Jasar *et al* study in Macedonia in which p53 was expressed in 79% of cases and also with Silvia Darab study in Germany which showed a percentage of 74.8%[22,23].

ACKNOWLEDGMENT

We would like to acknowledge all the studied patients and laboratorytechnicians for their cooperation. We also like to pay special thanks and gratitude to our families, colleagues and friends for their continuous support and motivation.

CONCLUSION

There was a strong and significant correlation between p53 and high Ki67 proliferative index in TNBC. Both Ki67 and P53 are significantly expressed. This finding can help in the management of patients of TNBC and predicting the prognosis of the disease.

Due to the prognostic and therapeutic effects of mutated p53 in triple negative breast cancer, confirmation of this mutation is very important. In wildly mutated p53 gene, the IHC for p53 may be negative. In such cases we recommend confirmation of p53 gene status by FISH technique.

Also, due to significantly higher values of Ki67 and p53 in this study we are expecting these patients to be benefitted from anti Ki67 and anti-p53 therapy, so further studies in TNBC including these drugs are recommended.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. Cancer J Clin 2015; 65(2): 87-108.
2. Nounou MI, El-Amrawy F, Ahmed N, Abdelraouf K, Goda S, Syed-Sha-Qhattal H. Breast cancer: conventional diagnosis and treatment modalities and recent patents and technologies. Breast cancer: Basic Clin Res 2015; 9(2): 17-34.
3. Viale G. The current state of breast cancer classification. Annal Oncol 2012; 23(Suppl-10): 207-10.
4. Ahn SG, Kim SJ, Kim C, Jeong J. Molecular classification of triple-negative breast cancer. J Breast Cancer 2016; 19(3): 223-30.
5. Dai X, Xiang L, Li T, Bai Z. Cancer hallmarks, biomarkers and breast cancer molecular subtypes. J Cancer 2016; 7(10): 1281-94.
6. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the st gallen international expert consensus on the primary therapy of early breast cancer. Ann Oncol 2013; 24(9): 2206-23.
7. Payandeh M, Malayeri R, Sadeghi M, Sadeghi E, Gholami F. Expression of p53 and Ki67 in the patients with triple negative breast cancer and invasive ductal carcinoma. Am J Cancer Prev 2015; 3(3): 58-61.
8. George P. p53 how crucial is its role in cancer. Int J Curr Pharm Res 2011; 3(2): 19-25.
9. Sajid MT, Ahmed M, Azhar M, Mustafa Q, Shukr I, Kamal Z. Age-related frequency of triple negative breast cancer in women. J Coll Physician Surg Pak 2014; 24(6): 400-03.
10. Ahmed R, Din HU, Afzal S, Muhammad I, Hashmi SN, Hamdani NR. Clinicopathological characteristics of triple negative breat cancer. Pak Armed Forces Med J 2017; 1(5): 838-42.
11. Kilickap S, Kaya Y, Yucel B, Tuncer E, Babacan NA, Elagoz S. Higher Ki67 expression is associates with unfavorable prognostic factors and shorter survival in breast cancer. Asian Pac J Cancer Prev 2014; 15(3): 1381-5.
12. Nishimiya H, Kosaka Y, Yamashita K, Minatani N, Kikuchi M, Ema A, et al. Prognostic significance of Ki-67 in chemotherapy-naive breast cancer patients with 10-year follow-up. Anticancer Res 2014; 34(1): 259-68.
13. Hientz K, Mohr A, Bhakta-Guha D, Efferth T. The role of p53 in cancer drug resistance and targeted chemotherapy. Oncotarget 2017; 8(5): 8921.
14. di Gennaro A, Damiano V, Brisotto G, Armellin M, Perin T, Zucchetto A, et al. A p53/mir-30a/ZEB2 axis controls triple negative breast cancer aggressiveness. Cell Death Differ 2018; 25(12): 2165-80.
15. Parrales A, Iwakuma T. Targeting oncogenic mutant p53 for cancer therapy. Front Oncol 2015; 5(1): 288-91.
16. Ahmed R, Ud Din H, Akhtar F, Afzal S, Muhammad I, Hashmi SN. Immunohistochemical expression of epidermal growth factot receptor and C-KIT in triple negative breast cancer. J Coll Physician Surg Pak 2016; 26(7): 570-72.
17. Hashmi AA, Edhi MM, Naqvi H, Faridi N, Khurshid A, Khan M. Clinicopathologic features of triple negative breast cancers: an experience from Pakistan. Diagn Pathol 2014; 9(1): 43-6.
18. Madani SH, Payandeh M, Sadeghi M, Motamed H, Sadeghi E. The correlation between Ki67 with other prognostic factors in breast cancer: A study in Iranian patients. Indian J Med Paediatr Oncol 2016; 37(2): 95-99.
19. Pan Y, Yuan Y, Liu G, Wei Y. P53 and Ki-67 as prognostic markers in triple-negative breast cancer patients. Public Library Sci One 2017; 12(2): e0172324.
20. Li H, Han X, Liu Y, Liu G, Dong G. Ki67 as a predictor of poor prognosis in patients with triple-negative breast cancer. Oncol Lett 2015; 9(1): 149-52.
21. Moazed V, Jafari E, Khandani BK, Nemati A, Roozdar A, Razavi SA. Prognostic significance of Reduction in Ki67 index after neoadjuvant chemotherapy in patients with breast cancer in kerman between 2009 and 2014. Iran J Pathol 2018; 13(1): 71-77.
22. Jasar D, Smichkoska S, Kubelka K, Filipovski V, Petrushevska G. Expression of p53 Protein Product in Triple Negative Breast Cancers and Relation with Clinical and Histopathological Parameters. Pril (Makedon Akad Nauk Umet Odd Med Nauki) 2015; 36(1): 69-79.
23. Darb-Esfahani S, Denkert C, Stenzinger A, Salat C, Sinn B. Role of TP53 mutations in triple negative and HER2-positive breast cancer treated with neoadjuvant anthracycline/taxane-based chemotherapy. Oncotarget 2016; 7(42): 67686.