Original Article

DNA in Carcinoma of Breast

Detection of DNA Mismatch Repair System in Carcinoma of Breast

Hadia Rafique Rana[1], Shamaila Razzaq[1], Amina Qayyum[1], Sakina Jamil[2] and Arooj Sattar[1]

ABSTRACT

Objective: To correlate the immunohistochemical expression of MLH1 and PMS2 antibodies in carcinoma of breast.

Study Design: Descriptive study.

Place and Duration of Study: This study was conducted at the Department of Morbid Anatomy and Histopathology, Central Park Medical College Lahore from 1st July 2016 to 31st March 2017.

Materials and Methods: Ninety blocks of already diagnosed breast cancers were collected. The histological grading was done on conventional H&E, according to Nottingham grading system. For MLH1 and PMS2 labeling was done according to nuclear and cytoplasm staining.

Results: All the cases were of ductal invasive carcinoma.MLH 1 was found strongly positive in 51(56.7%) cases, weakly positive in 4 (4.4%) and negative in 35 (38.9%) of the cases and PMS2 was found strongly positive in 55 (61.1%) cases, while, weakly positive in 5 (5.5%) and negative in 30 (33.3%) of the cases.

Conclusion: Loss of expression of MLH1 and PMS2 reveals that microsatellite instability (MSI) has a role in the development of breast carcinoma.

Key Words: Breast carcinoma, Invasive ductal carcinoma, DNA mismatch repair system (MMR), Microsatellite instability (MSI), MLH1, PMS2.

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INTRODUCTION

According to World Health Organization, in 2015, cancer is the leading cause of death before the age of 70 years. Low and middle income countries are mostly involved in these deaths. A total figure of 2,088,849 cases was reported for breast carcinoma (11.6% of all the cancer cases) with mortality of 626,679 cases. Breast carcinoma is the most common in the developing countries including Pakistan. Almost 180,000 breast carcinoma cases present in the United States annually.[1,2]The transmission of genetic information to the subsequent generations in a very accurate way is Necessary for the survival of a cell and it depends on the Proper functioning of protein factors involved in the regulation of cell cycle. If these protein factors do not work well it will lead to the development of mutation, instability in genetic makeup and chromosome breakage thereby leading towards transformation of cancer cells.[3]

[1.] Department of Pathology, CPMC, Lahore.

[4.] Department of Pathology, Gujranwala, Medical College Gujranwala.

Correspondence: Dr. Hadia Rafique Rana, Senior Demonstrator of Pathology, CPMC, Lahore.
Contact No: 0335 4690706
Email: hadiarana123@yahoo.com

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During DNA replication certain miss-incorporations, insertions and deletions of the bases occur that are recognized and repaired by DNA mismatch repair (MMR) system.[4] It also repairs some forms of DNA damage and has a vital role in the development of genetic stability. In the humans, seven MMR proteins work in a specific orders to initialize the repair of DNA mismatches, these are MLH1, MLH3, MSH2, MSH3, MSH6, PMS1 and PMS2.[5] The cancers with the DNA repair deficiency have epigenetic alterations that reduce the DNA repair gene expression. In colorectal cancers, about 13% are having DNA mismatch repair mechanism deficiency, most commonly due to the loss of MLH1 and sometimes PMS2, MSH2 or MSH6.[6,7]To recognize and repair mismatches is important for cells and failure to do so, results in microsatellite instability (MSI) resulting in increased mutation rate. MMR-deficient (MSI) cancers have high frequency of mutations.[8] with the help of immunohistochemical expression of MMR proteins we can find out the involvement of MMR gene. Some studies have worked on the positive expression and loss of expression of MMR by immune histological staining.

In this study we will check the expression of two antinuclear antibodies MLH1 and PMS2 in breast carcinoma (invasive ductal carcinoma) with the help of immunohistochemistry. To our knowledge no such study has been carried out so far in Pakistan.

MATERIALS AND METHODS

This study was conducted in the Department of Morbid Anatomy and Histopathology Department of Morbid Anatomy and Histopathology, Central Park Medical

College Lahore from 1st July 2016 to 31st March 2017. Ninety samples were collected through non-random convenient sampling. Female patients with breast carcinoma and above 18 years of age were included in this study. Clinical parameters of these patients were recorded. Paraffin embedded blocks were collected and labeled appropriately. A specific lab number was issued. These blocks were processed and three sections were taken from each i.e. one section was taken on a frosted microscope slide for staining with conventional H&E stain and other two sections were taken on poly-L-lysine coated slides and were stained with antibodies to MLH1 and PMS2 by indirect immunohistochemical method.

The histological diagnosis, tumor sub-typing and grading was done on conventional H&E, according to Nottingham grading system.[9] For MLH1 and PMS2, labeling was done according to nuclear and cytoplasm staining.[10] (Table 1). The data was entered and analyzed using SPSS version 23. A p-value <0.05 was considered as statistically significant**.**

RESULTS

This study was carried out on ninety mastectomy specimen from the females of 32 to 68 years with an average age of 52.4±9.0 years. Mean age of menarche was 13.1±0.7 years. Among these 81 (90.0%) were married, 74 (82.2%) had history of pregnancy or lactation, 17 (18.9%) had positive family history and 22 (24.4%) used contraceptive pills ever.

All the ninety cases were of invasive ductal carcinoma. The histological grading and scoring was done according to Nottingham Grading Score. There were 50 (55.6%) assigned as Grade-I, 33 (36.7%) as Grade-II and 7 (7.8%) as Grade-III. 12.0% Grade I, 48.5% grade-II and 42.9% grade III tumors were having areas of necrosis. The presence of area of necrosis was significantly different among three grades with p-value 0.001. There were 5 (10%) cases in grade-I who involved nipple, while there were 24.2% in grade-II and 42.9% in grade-III. This difference among three groups was very close to significant with p-value 0.060. The skin was involved in 12 (13.3%) cases. The distribution of these cases was not significantly different for the three grades of tumor with p-value 0.228.

Table No. 1: MLH1 and PMS2 staining

Strong Positive	Strong, diffuse, brown nuclear staining with positive labeling of cytoplasm in >10% of tumor cells
Weak/focal positive	Strong, diffuse, brown nuclear staining with positive labeling of cytoplasm in <10% of tumor cells
Negative	Complete absence of nuclear staining with or without positive labeling of cytoplasm in tumor cells

Table No. 2: DNA Mismatch repair antibodies (MLH1) in relation to Nottingham Histological Grades

Msh2	Nottingham Histological Grades							
	Grade I		Grade II		Grade III		Total	
	No.	%	No.	%	No.	%	No.	%
Strongly Positive	33	66.0	16	48.5	2	28.6	51	56.7
Focal / Weakly Positive	2	4.0	2	6.1	0	0.0	4	4.4
Negative	15	30.0	15	45.5	5	71.4	35	38.9
Total	50	100.0	33	100.0	7	100.0	90	100.0

P-value = 0.001

Table No 3: DNA Mismatch repair antibodies (PMS2) in relation to Nottingham Histological Grades

Msh2	Nottingham Histological Grades							
	Grade I		Grade II		Grade III		Total	
	No.	%	No.	%	No.	%	No.	%
Strongly Positive	39	78.0	14	42.4	2	28.6	55	61.1
Focal / Weakly Positive	1	2.0	2	6.1	2	28.6	5	5.6
Negative	10	20.0	17	51.5	3	42.9	30	33.3
Total	50	100.0	33	100.0	7	100.0	90	100.0

P-value = 0.002

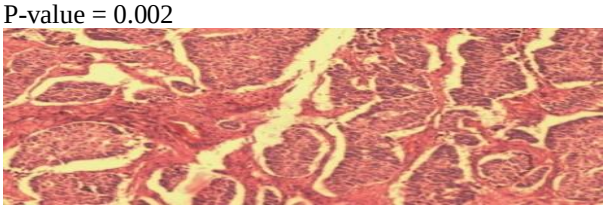

Figure No.1: Solid tumor pattern in high grade tumor

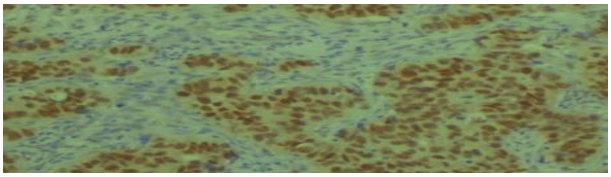

Figure No.2: Positive nuclear staining of MLH1 expression

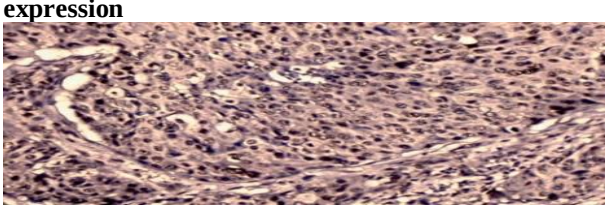

Figure No.3: Positive nuclear staining of PMS2 expression

The MLH 1 was found strongly positive in 51 (56.7%) cases, weakly positive in 4 (4.4%) and negative in 35 (38.9%) of the cases. The negative rate for MLH 1 was highest among the four observed. When distributed among three grades, it was found that the negative rate

had an increasing trend with 30.0%, 45.5% and 71.4% in grade-I, II and III respectively. So the difference among three grades for MLH 1 was not found significantly different with p-value 0.190 (Table 2).

The PMS 2 was found strongly positive in 55 (61.1%) cases, while, weakly positive in 5 (5.5%) and negative in 30 (33.3%) of the cases. When distributed among three grades, it was found that the negative rate was 20.0%, 51.5% and 42.9% in grade-I, II and III respectively. Still the difference among three grades was highly significant with p-value 0.002 (Table 3).

DISCUSSION

Breast carcinoma is the most common malignancy in the women of any race, area or ethnicity worldwide. According to the Glob can cancer statistics, breast carcinomas comprise of 11.6% of all the cancer cases having mortality rate of 30%.[1] In Pakistan it is the most prevalent cancer in females. In this study, average age of the female was 52.4±9.0 years and range was 32-68 years. Khokher et al[11] and Mahmood et al[12] stated the mean age of 47±12 and 47.57±12.02 years respectively and range of 16 to 100 years and 18 to 90 years in their studies respectively. Out of ninety females eighty one were married, 74% were having history of pregnancy and 22.4% have used oral contraceptive pills.

All the cases were diagnosed as invasive ductal carcinoma. Grading was done according to the percentage of tubular formation, severity of pleomorphism and atypical mitosis (Nottingham Grading System). 50(55.6%) of tumors labeled as Grade-I, 33(36.7%) were as Grade-II and 7(7.8%) were as Grade-III category. Only 12% of cases have involvement of skin. Previous study reveals 20% of cases having skin involvement which almost favors this study.[13]

Earlier studies reveal evidence of malfunctioning of DHA mismatch repair (MMR) genes in hereditary non-polyposis colon cancer, prostatic carcinoma, endometrial carcinoma and gastric carcinoma. MMR proteins work in specific orders to initiate the repair of DNA mismatches. We checked expression of MLH1 and PMS2 with the help of immunohistochemistry.

In this study, 51(56.7%) cases shows strong positive nuclear staining of MLH1, 4 (4.4%) of cases show weak positive nuclear staining and 35 (38.9%) of the cases have negative nuclear staining. One study reveals the loss of expression of MLH1 in 26 (31.1%) cases out of 83 cases. In India, similar kind of study documents loss of expression of MLH1 in 43.5% cases. Another study reveals 46% loss of expression of MLH1 in sporadic breast cancer.[14-17]

In this study 55(61.1%) cases show strong positive nuclear staining for PMS2, 5(5.5%) cases show weak positive nuclear staining and 30(33.3%) of the cases express negative nuclear staining. These results favor the studies conducted by Schrader et al[18], Wen et al[19] and Roberts et al[20] which document the roles of DNA mismatch repair proteins in development of breast carcinoma.

Paulson et al[21] stated that microsatellite instability (MSI) in the breast cancer can lead to more aggressive carcinoma and poor prognosis. They observed eleven patients with MSI out of which seven developed metastasis. They also observed twenty five patients which were MSI negative and only four patients developed metastasis. They also stated that the tumors having more microsatellite instability are more prone to the metastasis. On the other hand some studies reveal very low or no loss of MSI. Shia et al[22] worked on 66 samples of breast cancer but not found MSI in any case. Clades et al[23] revealed MSI in 6 cases out of 88 cases of breast cancer. Anbazhagen et al[24] observed no MSI in 267 cases of breast cancer.

CONCLUSION

This study revealed the loss of expressions of MLH1 and PMS2 in the breast carcinoma. Loss of expression reveals that deficiency of DNA mismatch proteins has a role in the development of breast carcinoma.

Author's Contribution:

Concept & Design of Study:	Hadia Rafique Rana
Drafting:	Shamaila Razzaq Amina Qayyum
Data Analysis:	Sakina Jamil, Arooj Sattar
Revisiting Critically:	Hadia Rafique Rana Shamaila Razzaq
Final Approval of version:	Hadia Rafique Rana

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer J Clin 2018;68(6):394-424.
2. Siddiqui MS, Kayani N, Sulaiman S, Hussainy AS, Shah SH, Muzaffar S. Breast carcinoma in Pakistani females: a morphological study of 572 breast specimens. JPMA 2000;50(6):174-7.
3. Her C, Vo AT, Wu X. Evidence for a direct association of hMRE11 with the human mismatch repair protein hMLH1. DNA Repair 2002;1(9): 719-29.
4. Iyer RR, Pluciennik A, Burdett V, Modrich PL. DNA mismatch repair: functions and mechanisms. Chem Rev 2006;106(2):302-23.
5. Pal T, Permuth-Wey J, Sellers TA. A review of the clinical relevance of mismatch-repair deficiency in

ovarian cancer. Cancer: Interdisciplinary Int J Am Cancer Soc 2008; 113(4):733-42.
6. Bernstein C, Bernstein H. Epigenetic reduction of DNA repair in progression to gastrointestinal cancer. World J Gastrointestinal Oncol 2015; 7(5):30.
7. Truninger K, Menigatti M, Luz J, Russell A, Haider R, Gebbers JO, et al. Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. Gastroenterol 2005;128(5): 1160-71.
8. Tuna M, Amos CI. Genomic sequencing in cancer. Cancer Letters 2013;340(2):161-70.
9. Her C, Vo AT, Wu X. Evidence for a direct association of hMRE11 with the human mismatch repair protein hMLH1. DNA repair 2002;1(9): 719-29.
10. Rakha EA, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, Blamey RW, Ellis IO. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol 2008;26(19):3153-8.
11. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: part I. The utility of immunohistochemistry. J Mol Diagn 2008; 10(4): 293-300.
12. Khokher S, Qureshi MU, Riaz M, Akhtar N, Saleem A. Clinicopathologic profile of breast cancer patients in Pakistan: ten years data of a local cancer hospital. Asian Pacific J Cancer Prev 2012;13(2):693-8.
13. Mahmood H, Faheem M, Mahmood S, Sadiq M, Irfan J. Impact of age, tumor size, lymph node metastasis, stage, receptor status and menopausal status on overall survival of breast cancer patients in Pakistan. Asian Pac J Cancer Prev 2015; 16(3):1019-24.
14. Ho CM, Mak CK, Lau Y, Cheung WY, Chan MC, Hung WK. Skin involvement in invasive breast carcinoma: safety of skin-sparing mastectomy. Ann Surg Oncol 2003; 10(2):102-7.
15. Murata H, Khattar NH, Gu L, Li GM. Roles of mismatch repair proteins hMSH2 and hMLH1 in the development of sporadic breast cancer. Cancer Letters 2005;223(1):143-50
16. Martin L, Coffey M, Lawler M, Hollywood D, Marignol L. DNA mismatch repair and the transition to hormone independence in breast and prostate cancer. Cancer Letters 2010;291(2):142-9.
17. Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D, Sinnett D. Frequent loss of heterozygosity at the DNA mismatch-repair loci hMLH1 and hMSH3 in sporadic breast cancer. Br J Cancer 1999; 79(7):1012.
18. Schrader CE, Vardo J, Stavnezer J. Role for mismatch repair proteins Msh2, Mlh1, and Pms2 in immunoglobulin class switching shown by sequence analysis of recombination junctions. J Exp Med 2002;195(3):367-73.
19. Wen YH, Brogi E, Zeng Z, Akram M, Catalano J, Paty PB, Norton L, Shia J. Dna Mismatch Repair Deficiency in Breast Carcinoma: A Pilot Study of Triple-negative and Nonâ€“triple-negative Tumors. The Am J Surg Pathol 2012;36(11):1700-8.
20. Roberts ME, Jackson SA, Susswein LR, Zeinomar N, Ma X, Marshall ML, Stettner AR, Milewski B, Xu Z, Solomon BD, Terry MB. MSH6 and PMS2 germ-line pathogenic variants implicated in Lynch syndrome are associated with breast cancer. Genetics Med 2018; 20(10): 1167-74.
21. Paulson TG, Wright FA, Parker BA, Russack V, Wahl GM. Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. Cancer Res 1996;56(17):4021-6.
22. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: part I. The utility of immunohistochemistry. J Mol Diagn 2008;10(4): 293-300.
23. Caldes T, Perez-Segura P, Tosar A, De La Hoya M, Diaz-Rubio E. Low frequency of microsatellite instability in sporadic breast cancer. Int J Oncol 2000;16(6):1235-77.
24. Anbazhagan R, Fujii H, Gabrielson E. Microsatellite instability is uncommon in breast cancer. Clin Cancer Res 1999;5(4):839-44.