Int J Adv Life Sci Res. Volume 9(3) 197-207
https://doi.org/10.31632/ijalsr.2026.v09i03.015

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

Mismatch Repair Protein Expression Patterns in HER 2 Positive Breast Cancer Patients: A Clinical Pathological Correlation

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Abstract

Background: Mismatch repair (MMR) deficiency is linked to tumor genomic instability in diverse cancers. Although MMR loss has been comprehensively analyzed for the therapeutic responses in multiple malignancies, the capacity of MMR loss to impact disease severity in breast cancer (BC) and prognostic factors, including human epidermal growth factor receptor 2 (HER2) positive subtypes is still to be clarified. Aim: The aim of this study was to analyze the expression of MMR proteins (MLH1, PMS2, MSH2, and MSH6) in HER2-positive BC, as well as to check their association with ER/PR, Ki67, and tumor grade. Furthermore, researchers explored if the expression of MMR genes may reflect patient heterogeneity and contribute to developing resistance to therapy in hormone receptor-positive breast cancer subtypes. **Methods**: In this study, 63 HER2+ BC specimens, collected from 2016 to 2024, were analyzed for MMR protein expression (MLH1, PMS2, MSH2 and MSH6) via immunohistochemistry (IHC). Fluorescence in situ hybridization (FISH) was used to confirm HER2 amplification in cases of HER2 two-pluses in IHC analysis. Results were correlated with clinicopathological data, including estrogen receptor (ER), progesterone receptor (PR), Ki67 and tumor grade among others. Statistical significance was set at $p < 0.05$. **Results:** All HER2-positive breast cancer specimens demonstrated positive MMR protein expression with varying intensities. ER-positive cases exhibited a higher frequency of strong MMR expressions compared to ER-negative cases, which showed a higher proportion of weak PMS2 expression (43.2% vs. 21.1%, respectively). Most HER2-positive breast cancer patients (81%) showed Ki67 expression greater than 15%, predominantly observed in high-grade tumors. However, weak MMR expression showed no significant correlation with Ki67 or hormone receptor status. **Conclusion:** The total intact MMR with intensity alterations, especially for PMS2 in ER+ tumors, may reflect tumor heterogeneity and could contribute to therapy resistance in hormone receptor-positive BC. The young age at presentation, particularly for ER+ cases and the high value of Ki67 with the intact MMR may imply a broader, possibly genetic or molecular basis that should be addressed. MMR profiling has clinical relevance and can help take precision oncology further in BC management. **Recommendation:** Future studies should include larger cohorts and molecular analyses to clarify the role of MMR alterations in HER2-positive breast cancer. Incorporating MMR testing into routine diagnostics may improve treatment planning, and further research is needed to assess its impact on therapy response and patient outcomes.

Received on :14th March 2026; Revised version received on :11th May 2026; Accepted: 15th May 2026

Keywords: *Breast Cancer; HER2; Hormonal Receptors; Immunohistochemistry; Mismatch Repair Deficiency*

Introduction

The mismatch repair (MMR) system maintains genomic stability by correcting replication errors during DNA replication. Dysfunction of the MMR pathway leads to genomic instability and contributes to cancer development (Pećina-Šlaus *et al*., 2020). This repairing system is part of the DNA damage response (DDR), which orchestrates cellular responses such as cell cycle arrest and apoptosis to prevent the malignant transformation of cells when DNA damage is irreparable (He *et al*., 2022). In humans, the MMR system is composed of eight genes encoding its protein components. These include homologs of E. coli MutS genes (MSH2, MSH3, MSH5, and MSH6) and MutL genes (MLH1, MLH2, MLH3, MLH4, PMS1, and PMS2). Due to its role in maintaining genomic integrity, the MMR system is a key focus in cancer treatment and has a particular role in screening for Lynch syndrome, a hereditary condition associated with colorectal, endometrial, and other cancers (Piumatti *et al*., 2025).

In the Kurdistan Region, limited studies have evaluated MMR alterations in cancer patients, although altered MMR protein expression has been reported in endometrial cancer cases from Duhok City (Ali*et al.*, 2024). This regional evidence underscores the importance of investigating MMR alterations in local cancer populations. Breast cancer (BC) is the most common malignancy among women globally and the leading cause of cancer-related deaths in women, with an estimated 9 new cases diagnosed every second (Heer *et al*., 2020).

Breast cancer (BC) classification is based on hormone receptor (ER and PR) expression, HER2 status, and Ki67 index. However, many HR+ BC patients develop therapeutic resistance associated with changes in the tumor immune microenvironment and loss of MMR pathway expression (Grizzi *et al*., 2020; Huang *et al*., 2021; Wang *et al*., 2025). The frequency of MMR deficiency in BC has been reported to range from 1.7% to 17% (Fusco *et al*., 2018). Research on MMR alterations in breast cancer has gained increasing attention because of its role in hereditary cancer screening, prediction of immune checkpoint inhibitor response, and treatment resistance (Valiente *et al*., 2023; Valenza *et al*., 2023). The frequency and types of MMR mutations, as well as the patterns of MMR gene expression changes, are distinct, suggesting that HR+ BC is fundamentally different from HR– BC and other malignancies (Venetis *et al*., 2020). Numerous studies concluded that MMR deficiency is a strong predictor of BC-specific mortality, especially in HR+ patients (Cheng *et al*., 2020; Kiss *et al*., 2024). Dysfunction of the MMR system in breast adenocarcinomas consists of gene mutations as well as promoter hypermethylation, RNA downregulation, and aberrant localization of the complex protein (Cheng *et al*., 2020; Lopez *et al*., 2020).The prognostic impact of changes in MMR is unambiguous, but its predictive value in thehistology remains controversial (Klümper *et al*., 2024). Evaluation of MMR status by immunohistochemistry (IHC) for four key proteins is a reliable standard method; however, its application in breast cancer remains limited because of variable protein expression and reduced sensitivity (Sajjadi *et al*., 2021). Despite the growing clinical importance of MMR deficiency in breast cancer, studies investigating these alterations remain limited in this region. Therefore, this study aimed to determine the frequency of MMR deficiency or altered expression and evaluate its association with clinicopathological variables in HER2+ BC patients.

Materials and Methods

Patients, Study Samples, and Ethics

A total of 63 formalin-fixed paraffin-embedded BC tissue blocks (FFPEBs), containing core needle biopsies, were obtained from the archives of Vajeen Private Histopathology Laboratory in Duhok Governorate, Iraq. The study reviewed the tissue blocks along with their corresponding Hematoxylin and Eosin (H&E) slides, with block ages ranging from 2 months to 8 years (collected between 2016 and 2024). Clinicopathological data, including hormone receptor status (ER, PR), Ki67, and

HER2/neu expression, were also collected for analysis. The H&E slides were all reviewed by pathologists to reconfirm the original diagnosis and to carry out the IHC and FISH techniques. Ethical approval for the study was granted by the Ethics Committee of the Duhok Health Directorate on July 23, 2024 (reference number: 31072024-6-28).

The following inclusion criteria were established for the tissue samples:

- Cases with complete clinicopathological data in the histopathological files.
- Cases of breast cancer with sufficient core needle biopsy.
- HER2/neu-positive cases confirmed by FISH technique for questionable cases.
- Documented ER, PR and Ki67 results.

The exclusion criteria included:

- Cases with absent clinicopathological data
- Insufficient tissue in the FFPE blocks for the IHC markers
- HER2/neu-negative cases.

Fluorescent in Situ Hybridization (FISH)

For questionable cases (equivocal HER2++ status by IHC), FISH had been performed to characterize if the tumor is positive for HER2 or not; in general, a positive FISH result would indicate HER2 amplification and that the cancer cells have too many copies of the HER2 gene so that it may play a role in the therapeutic decision making.

Molecular identification of the HER2/Neu gene was conducted using FISH with the HER2 IQFISH pharmDx (Dako code: K5731), which contains all key reagents required to complete a FISH procedure for FFPEBs.

After deparaffinization and rehydration, slides were heated in a water bath with a pre-treatment solution for 15 minutes at 95-99°C. The next step involved a proteolytic digestion using ready-to-use Pepsin at 37 °C for 3-5 minutes. Following the heating and proteolytic pre-treatment steps, this kit employs a ready-to-use FISH Probe Mix based on a combination of PNA (peptide nucleic acid23) and DNA technology. This Probe Mix consists of a mixture of Texas Red-labeled DNA probes covering a 218 kb region, including the HER2 gene on chromosome 17, and a mixture of fluorescein-labeled PNA probes targeted at the centromeric region of chromosome 17 (CEN-17). The HER2/CEN-17 probe was applied to the slides, which were subsequently coverslipped and sealed. The slides were placed in a hybridizer (DAKO, code: S2451, USA) and subjected to a denaturation step at 66°C for 10 minutes, followed by hybridization at 45°C for 60 to 120 minutes. This specific hybridization to the two targets produces base pair hybridization and results in the formation of a distinct red fluorescent signal at each HER2 gene locus and a distinguishable green fluorescent signal at each chromosome 17 centromere. Specimens were mounted with fluorescence mounting medium containing DAPI and covered following a stringent washing and dehydration. Tumor cells were identified and counted under a fluorescence microscope (Nikon/ECLIPSE Ci-L PLUS) with relevant filters at a magnification of 100× (for red [HER2] and green [CEN-17] signals) and 400× (for chromatin). The HER2/CEN-17 ratio was determined using Fluorescence in Situ Hybridization (FISH). Cases with a HER2/CEN-17 ratio <1.8 were considered negative for HER2 amplification, whereas cases with a ratio >2.0 were considered positive. Ratios between 1.8 and 2.0 were classified as equivocal and were re-evaluated according to ASCO/CAP guidelines (Wolff *et al*., 2023). Under fluorescence microscopy, negative cases showed nearly equal numbers of HER2 (red) and CEN-17 (green) signals distributed evenly within the nuclei, while positive cases demonstrated increased HER2 signals relative to CEN-17 signals, often appearing as clustered or amplified signals within tumor cell nuclei (Figure 1).

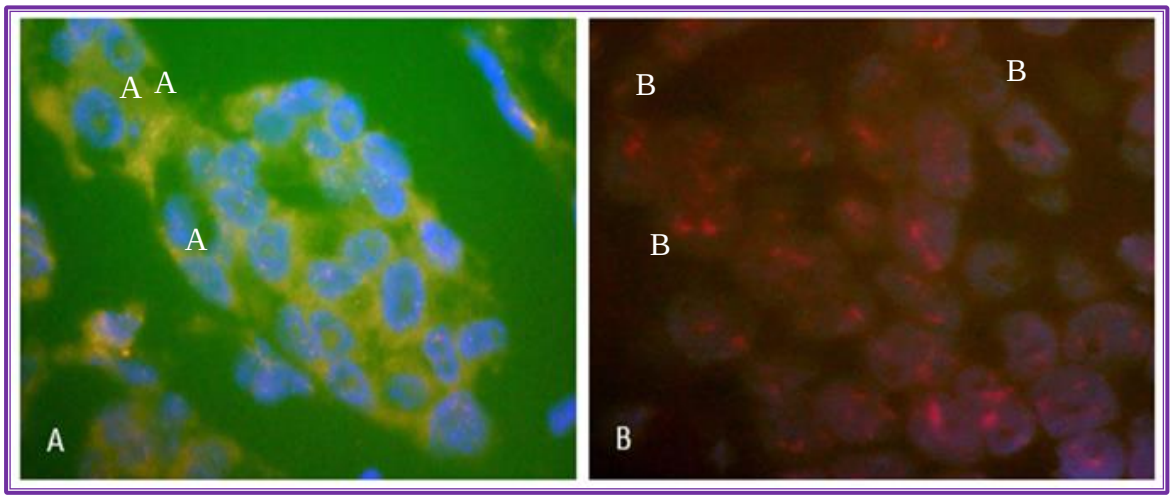

Figure 1: *Fluorescence in Situ Hybridization (FISH) Images Illustrating HER2/NEU Amplification Status. A: Negative Result (HER2/CEN-17 Ratio <1.8). B: Positive Result (HER2/CEN-17 Ratio >2.0). Cases With Ratios Between 1.8 And 2.0 Were Considered Equivocal*

Immunohistochemistry for MMR

From each tissue block, 4 sections of 3 µm thickness were sectioned and mounted on 4 charged adhesion microscope slides (Thermo Scientific HM 325 Manual Rotary Microtome (Runcorn, WA7 1TA, UK) and water bath at 45-50°C (by Bio-Optica Milano S.p.A.).

The baking step of the slides was done by vertically putting the slides into the hot air oven overnight at 65°C. For the antigen retrieval step, heat-induced epitope retrieval (HIER) was performed using a diluted EnVision FLEX Target Retrieval Solution, High pH (50x) (DM828, Code K8004); tissue sections were processed for HIER using PT Link (Dako). It included 65°C preheating of the target retrieval solution and the slides incubated at 97°C for 20 minutes. After incubation, the temperature was lowered to 65°C and the slides were immediately placed into a tank filled with EnVision FLEX Wash Buffer (20x) (DM831, Code K8007) (prepared by diluting EnVision FLEX Wash Buffer 1:20 using distilled water at room temperature).

After antigen retrieval, the staining process was carried out using the Dako automated stainer. The staining process began with blocking endogenous enzyme activity using FLEX Peroxidase Block for 5 minutes for all four biomarkers. Dako-Agilent (Glostrup, Denmark) produced DAKO FLEX ready-to-use monoclonal mouse primary antibodies were used. For MSH6 and MLH1, a 20-minute incubation was used; for MSH2 and PMS2, a 30-minute incubation was used, coupled with a Mouse LINKER (Dako) for MLH1 and MSH2, followed by a visualization reagent containing secondary antibodies, which is horseradish peroxidase (FLEX/HRP). The staining process involved the application of the chromogen DAB (3,3'-diaminobenzidine) for two consecutive 5-minute intervals, producing an insoluble brown precipitate at the antigen site. The slides were then washed twice with TBS buffer for 5 minutes each. Counterstaining was performed using EnVision FLEX Hematoxylin for 5 minutes. The slides underwent dehydration through graded alcohol and clearing with xylene and were finally mounted and covered with coverslips.

The MMR expression for core proteins MLH1, MSH2, MSH6, and PMS2 was classified according to the guidelines established by the College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO) into the following: negative or deficiency of MMR (dMMR), when there is complete loss of nuclear staining; weak but still positive MMR (pMMR), when there is pale or uneven nuclear staining in cells even if the percentage exceeds 10% as long as internal controls confirm appropriate staining; and strong positive, when there is dark nuclear staining in ten percent or more of the tumor cells (Table 1) and this status relies on comparison against positive controls within the same tissue section, such as internal lymphocytes or stromal fibroblasts (Sepulveda *et al*., 2017).

Table 1: *Scoring Categories for MMR Expression*

Expression Strength	% Tumor Cell Nuclei Stained	Interpretation
Strong	>10%	**Positive (pMMR)**
Weak but present	0-10%	Often still **Positive** (may reflect biological variation or pre-analytical factors)
Complete loss (0%)	0% in tumor cells with intact control cells	**Negative (dMMR)**

Statistical Analysis

The results of MMR expression were correlated with other clinicopathological data, including the age, grade of the tumor, ER and PR status, and Ki-67 percentage. The data were analyzed by using IBM SPSS software version 22. Chi-square (x2) test was used to test the proportions of these variables. Statistical significance was set at $p < 0.05$.

Results

Out of the 63 HER2 positive patients included in this study, 69.8% exhibited positive ER expression, while the remaining 30.2% were ER-negative. PR expression exhibited similar percentages. The Ki67 assessment revealed that most HER2-positive BC patients (81%) displayed values >15% (57.1% displayed values between 15% and 30%, and 23.8% had Ki67 expression above 30%) and only 19.1% of cases had an index of <15%. The age range of the patients varied from 28 to 82 years, with a mean age of 51.7 years. When divided into age groups, the results showed that 87.5% of patients over 65 years had ER positivity. A similar trend was observed for PR positivity. Regarding Ki67 expression, most cases in all age groups fell within the 15-30% range, though the highest proportion of cases with Ki67 expressions above 30% was observed in patients over 65 years (37.5%). Despite these variations, no statistically significant differences were found between ER, PR, and Ki67 expression across different age groups (p=0.124, p=0.257, and p=0.131, respectively). Tumor grade distribution showed that 70% of low-grade tumors, 66.7% of intermediate-grade tumors, and 72.7% of high-grade tumors were positive for both ER and PR. Ki67 expression was most prominent in high-grade tumors, with 40.9% exhibiting Ki67 expression above 30%, whereas low- and intermediate-grade tumors predominantly showed Ki67 expression between 15% and 30% (Table 2).

Table 2:*The Relation between the ER, PR, Ki67 and the Age and Grade of HER2 Positive BC Patients*

Clinicopathological Variables		**ER**		**PR**		**Ki67**			***p*-Value**
		Negative	**Positive**	**Negative**	**Positive**	**<15%**	**15-30%**	**>30%**	
Age	<50 years No=33	0.124	7 (21.2%)	19 (57.6%)	7 (21.2%)	25 (75.8%)	8 (24.2%)	25 (75.8%)	8 (24.2%)
	50-64 years No=22	0.257	5 (22.7%)	15 (68.2%)	2 (9.1%)	12 (54.5%)	10 (45.5%)	12 (54.5%)	10 (45.5%)
	>65 years No=8	0.131	3 (37.5%)	2 (25.0%)	3 (37.5%)	7 (87.5%)	1 (12.5%)	7 (87.5%)	1 (12.5%)
Grade	Low No=20	0.766	4 (20.0%)	12 (60.0%)	4 (20.0%)	14 (70.0%)	6 (30.0%)	14 (70.0%)	6 (30.0%)
	Intermediate No=21	0.910	2 (9.5%)	13 (61.9%)	6 (28.6%)	14 (66.7%)	7 (33.3%)	14 (66.7%)	7 (33.3%)
	High No=22	0.131	9 (40.9%)	11 (50.0%)	2 (9.1%)	16 (72.7%)	6 (27.3%)	16 (72.7%)	6 (27.3%)
Total		**19 (30.2%)**	**15 (23.8%)**	**36 (57.1%)**	**12 (19.0%)**	**44 (69.8%)**	**19 (30.2%)**	**44 (69.8%)**	

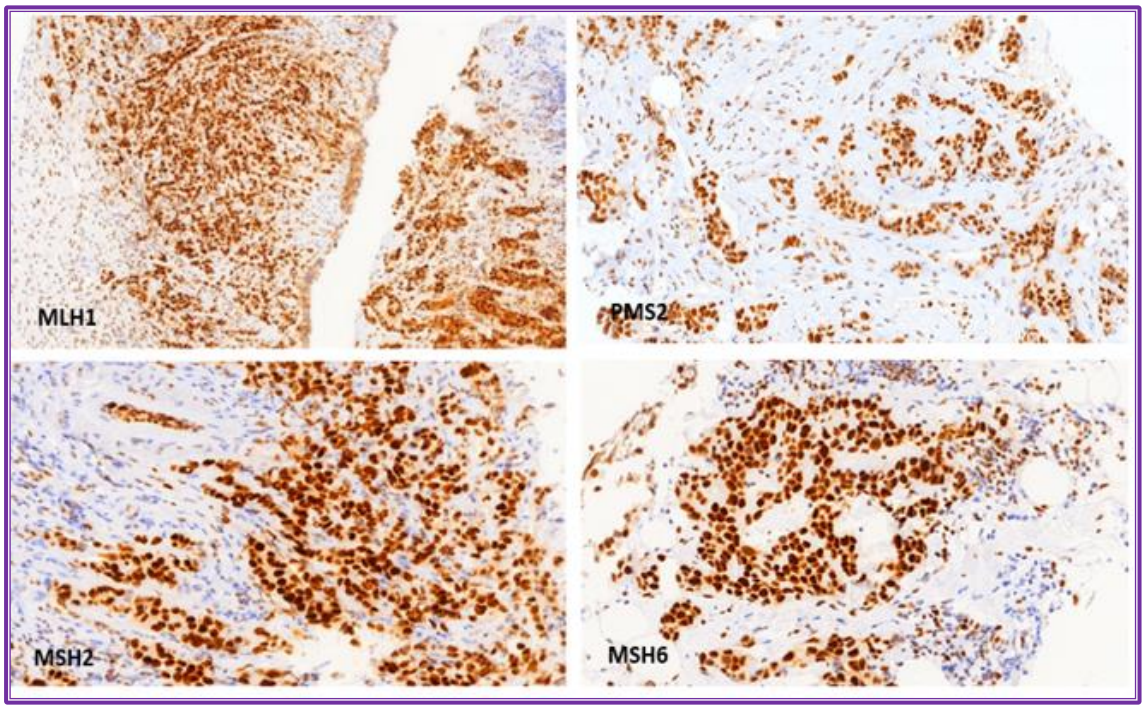

Figure 2: *Immunohistochemical Images (20×–40× Magnification) Showing Strong Expression of Mutl Homolog 1 (MLH1), Postmeiotic Segregation Increased 2 (PMS2), Muts Homolog 2 (MSH2), And Muts Homolog 6 (MSH6) Proteins in Breast Cancer (BC) Tissue*

All MMR proteins exhibited positive nuclear staining, indicating intact mismatch repair function in the analyzed BC samples with either strong (Figure 2) or weak (Figure 3) staining.

Statistically significant differences for age groups (<50, 50-64, >65 years), tumor grade (low, intermediate, high), and receptor status (ER, PR, Ki67) were found to be above the conventional significance level of 0.05 for these factors in MMR gene expression among this cohort.

However, ER-positive cases demonstrated a higher frequency of strong MMR gene expression compared to ER-negative cases, with a similar trend also observed in PR-positive tumors. (Table 3). Interestingly, PMS2 showed a higher proportion of weak expressions in ER-positive cases (43.2%) compared to ER-negative cases (21.1%). PMS2 expression showed a higher percentage of weak expression in intermediate-grade tumors (23.8%) compared to high-grade tumors (18.2%).

MSH2 and MSH6 expressions were predominantly strong in all age groups and grades, with weak expressions being relatively rare. Specifically, all patients over 65 years had strong MSH2 and MSH6 expressions, while weak expressions were only observed in younger age groups. Ki67 expression did not show a significant association with MMR gene expression levels, as weak and strong expressions were distributed similarly across different Ki67 categories.

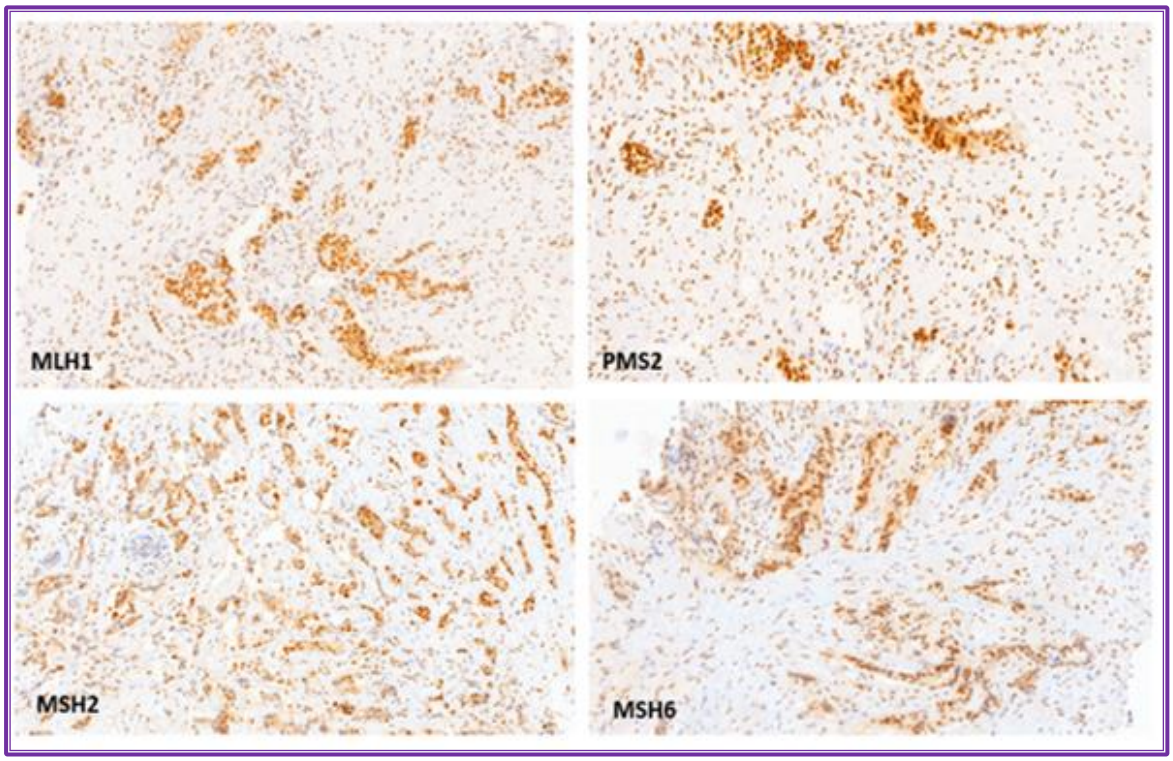

***Figure 3**: Immunohistochemical Images (20× Magnification) Showing Weak Expression of MutI Homolog 1 (MLH1), Postmeiotic Segregation Increased 2 (PMS2), Muts Homolog 2 (MSH2), And Muts Homolog 6 (MSH6) Proteins in Breast Cancer (BC) Tissue*

***Table 3:** The Relation of The MMR Gene Expression with the Different Clinicopathological Variables of HER2 Positive BC Patients*

Clinicopathological Variables		MLH1		PMS2		MSH2		MSH6		*p*-value
		Weak	Strong	Weak	Strong	Weak	Strong	Weak	Strong	
Age	<50 No=33	5 (15.2%)	28 (84.8%)	11 (33.3%)	22 (66.7%)	5 (15.2%)	28 (84.8%)	4 (12.1%)	29 (87.9%)	0.766
	50-64 No=22	6 (27.3%)	16 (72.7%)	9 (40.9%)	13 (59.1%)	1 (4.5%)	21 (95.5%)	1 (4.5%)	21 (95.5%)	0.083
	>65 No=8	1 (12.5%)	7 (87.5%)	3 (37.5%)	5 (62.5%)	0 (0.0%)	8 (100.0%)	0 (0.0%)	8 (100.0%)	0.189
Grade	Low No=20	3 (15.0%)	17 (85.0%)	6 (30.0%)	14 (70.0%)	4 (20.0%)	16 (80.0%)	3 (15.0%)	17 (85.0%)	0.124
	Intermediate No=21	5 (23.8%)	16 (76.2%)	5 (23.8%)	16 (76.2%)	2 (9.5%)	19 (90.5%)	2 (9.5%)	19 (90.5%)	0.257
	High No=22	4 (18.2%)	18 (81.8%)	12 (54.5%)	10 (45.5%)	0 (0.0%)	22 (100.0%)	0 (0.0%)	22 (100.0%)	0.124
ER	Positive No=44	8 (18.2%)	36 (81.8%)	19 (43.2%)	25 (56.8%)	4 (9.1%)	40 (90.9%)	3 (6.8%)	41 (93.2%)	0.257
	Negative No=19	4 (21.1%)	15 (78.9%)	4 (21.1%)	15 (78.9%)	2 (10.5%)	17 (89.5%)	2 (10.5%)	17 (89.5%)	0.131
PR	Positive No=44	8 (18.2%)	36 (81.8%)	19 (43.2%)	25 (56.8%)	4 (9.1%)	40 (90.9%)	3 (6.8%)	41 (93.2%)	0.910
	Negative No=19	4 (21.1%)	15 (78.9%)	4 (21.1%)	15 (78.9%)	2 (10.5%)	17 (89.5%)	2 (10.5%)	17 (89.5%)	0.131
KI67	<15% No=12	3 (25.0%)	9 (75.0%)	5 (41.7%)	7 (58.3%)	0 (0.0%)	12 (100.0%)	1 (8.3%)	11 (91.7%)	0.741
	15-30% No=36	6 (16.7%)	30 (83.3%)	13 (36.1%)	23 (63.9%)	6 (16.7%)	30 (83.3%)	4 (11.1%)	32 (88.9%)	0.684
	>30% No=15	3 (20.0%)	12 (80.0%)	5 (33.3%)	10 (66.7%)	0 (0.0%)	15 (100.0%)	0 (0.0%)	15 (100.0%)	0.549
Total		**63**	**100%**	**63**	**100%**	**63**	**100%**	**63**	**100%**	

Discussion

The findings of this study considered only the HER2-positive BC cases and their DNA repair mechanisms by evaluating the key MMR proteins' expression profiles, including MLH1, PMS2, MSH2, and MSH6. About 69.8% of the cases included were positive for ER. This rate matched well with what was found by a study that included 324 patients and reported a 67.5% ER positivity in the same city, Duhok (Yalda, 2013).

The Ki67 proliferation marker showed a substantial frequency in HER2-positive BC of this study, where 81% of cases showed elevated Ki67 expression (≥15%) and only 19.1% were expressed in a percentage below 15%. These results are slightly higher but aligned with the low percentage of low Ki-67 expression by a recent study on cases of BC females in Duhok (Mohammed *et al*., 2024). Notably, the current results showed a higher Ki67 proliferation rate than some other authors (Sandilya & K, 2024), which probably indicates a higher proliferation in malignant cells that are positive for HER 2 expressions. However, the three studies appreciated Ki67 as a clinically useful proliferation marker and this study supports the use of Ki67 as a tool in treating decision making and underlines the importance of an interpreted proliferation index in the management of HER-2 positive breast cancer on a context-dependent basis.

All these variables were correlated with some clinicopathological variables, including age. The mean age was 51.7 years, which is slightly higher than the mean age reported in this city for breast cancer patients, both with positive and negative HER2 status (Yalda, 2022; Mohammed *et al*., 2024). A higher age than decay (62 years and above) was reported in advanced countries, including the USA (Siegel *et al*., 2023). This wide difference may indicate a possible genetic underlying cause. Interestingly, 87.5% of patients over 65 years of age had positive ER, implying a strong relationship between age and ER expression. Clinically, this relationship is of importance since ER-positive tumors are more treatable by hormone therapies, i.e., tamoxifen or aromatase inhibitors. On the other hand, the highest proportion of cases with Ki67 expression above 30% was observed in patients over 65 years old and most prominent in high-grade tumors, which agreed with other authors (Mohammed *et al*., 2024).

Despite meeting the criteria for positivity of MMR expressions in all specimens, there was variation of expression intensity, including the higher frequency of weak PMS2 expression in ER+ tumors, suggesting the possibility of partial functional deficiency or regulatory suppression that might not satisfy the criteria for actual deficiency but could still have clinical significance. This aligns with the findings by other studies (Cheng *et al*., 2020; Kiss *et al*., 2024), who emphasized the impact of subtle MMR pathway disruptions on endocrine resistance and tumor behavior. However, the relevance of MMR deficiency as a biomarker of immune checkpoint inhibitor (ICI) responsiveness in BC is unclear, as this topic has been a controversial previous area of research (Ivanova *et al*., 2022). Unlike Lopez *et al*. (2020), our findings suggested that the isolated assessment of MLH1, PMS2, MSH2 and MSH6 is insufficient to incorporate into the BC prognostic assessment or aggressiveness prediction. Increased occurrence of weak PMS2 staining in ER-positive tumors may suggest a hormonal regulation axis that affects the fidelity of DNA repair and may contribute to both the progression of tumors and treatment resistance. BC, with a heterogeneous clinical response, is known for the difficulty of endocrine resistance as a major therapeutic challenge in HR-positive BC. With these resistance mechanisms, the MMR system may participate in genome stability maintenance and this is the rationale to incorporate MMR status into a wider set of molecular diagnostic panels. In addition, expression of MSH2 and MSH6 was strong within all groups, suggesting that the alteration within the MutLα spectrum (MLH1/PMS2) is selectively permeable to molecular study. This reinforces the emerging hypothesis that the MMR alterations may be in excess of classical dMMR and MSI and may indicate a modulated pathway of significance to immunological or endocrine therapeutics (Venetis *et al*., 2020). Therefore, other MMR assessments, such as using multimodal tools like microsatellite instability (MSI) testing and next-generation sequencing (NGS) and transcriptomic profiling, may reveal other latent dysfunctions that IHC alone cannot detect (Hacking *et al*., 2022). Longitudinal, multicenter datasets encompassing treatment outcomes, survival metrics, and even MMR protein

expression and its effects on BC would be useful for establishing MMR protein expression as a predictive or prognostic marker in BC.

Future Scope & Limitations

Based on the findings of this study, the following recommendations are proposed:

Expand Sample Size and Molecular Research: Future large-scale studies should confirm these findings and explore partial MMR alterations through genomic, epigenomic, and transcriptomic analyses using MSI-PCR and NGS.

Routine MMR Evaluation in HER2-positive BC:Although complete MMR loss was not observed, reduced expression—especially of PMS2—may have clinical relevance. Therefore, MMR IHC profiling should be considered in HER2-positive, hormone receptor–positive cases.

Population-Specific Stratification:The younger age of onset and biomarker distribution suggest regional variation; thus, locally tailored screening and treatment models should be developed.

Combination Therapy Consideration:Given the frequent co-expression of ER and HER2 and elevated Ki67 levels, combined HER2-targeted and endocrine therapy (including CDK4/6 inhibitors) should be further evaluated in clinical trials.

Multicenter and Longitudinal Studies:Larger, multicenter studies with long-term follow-up are recommended to assess clinical outcomes such as recurrence, treatment response, and survival in relation to MMR status.

Conclusion

The intact MMR function in all HER2-positive breast cancer patients, along with their relatively young age at diagnosis and the high percentage of positive estrogen receptors despite the elevated Ki67 values in most patients, suggests a potential regional genetic predisposition that warrants further study. The specific classes of MMR protein arrest observed in this study, in particular the downregulation of PMS2 in ER-positive tumors, point to the possible involvement of hormonal signaling and DNA repair. Heterogeneity of MMR protein expression among several clinicopathological subgroups underlines the complexity of the molecular part of BC biology. These results suggest that MMR profiling should be incorporated into molecular frameworks of BC patients, in particular for HER2-positive cases. Further investigations involving genomic, epigenomic and transcriptomic analyses are needed in future studies to improve individualized BC management.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgement

Authors are thankful to University of Duhok, Kurdistan Region for academic support.

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