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Expression of Mismatch Repair Proteins in Triple Negative Breast Carcinoma Patients and its Correlation with the Clinicopathological Parameters

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ABSTRACT

Background: Triple negative breast carcinoma (TNBC) is an aggressive disease, with a lack of response to targeted therapies, leaving a limited list of effective treatments, including chemotherapy and radiotherapy. Recent studies have shown that certain types of TNBC are immunogenic tumors, that may be suitable targets for immunotherapies. On the other hand, mismatch repair (MMR) expression status is considered an effective biomarker for assessing susceptibility to immunotherapies in multiple solid tumors.
Objectives: To assess the expression status of the MMR proteins and their correlation with the clinicopathological parameters in TNBC patients.
Materials and methods: Fifty-three formalin-fixed paraffin-embedded histopathological blocks of diagnosed triple negative invasive ductal breast carcinoma cases were retrieved from the archives department of the histopathological laboratories in Medical City campus hospitals. These blocks were sectioned and stained with monoclonal antibodies, targeting four MMR proteins (MSH2, MSH6, MLH1, and PMS2) to assess the expression status of these proteins in the tumor cells.
Results: Of 53 cases, there were 10 (18.9%) deficient in the expression of one or more of the MMR proteins, the MSH2 protein was the least frequently missed protein (n = 3, 5.7%). While PMS2 was the most frequently missed one (n = 10, 18.9%). An insignificant correlation (P-value > 0.05) was found between MMR status and the clinicopathological parameters except for age (P-value < 0.05).
Conclusion: Deficient expression status of the MMR proteins was common and showed an insignificant relation with the clinicopathological parameters.
Keywords: Breast carcinoma; Mismatch repair proteins; Triple negative breast carcinoma.

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INTRODUCTION

Breast carcinoma, considered one of the most prevalent malignancies affecting women worldwide, is the second leading cause of cancer-related death in women [1]. Globally, according to the World Health Organization (WHO) registration in 2022, a staggering 2.3 million women were diagnosed with breast carcinoma, and 670,000 deaths [1]. According to the Iraqi Cancer Registry, in 2023, breast carcinoma in Iraq is considered the most common carcinoma in females, and representing 34.8% of all carcinoma cases diagnosed among females. The age-specific incidence rate (ASR) of this carcinoma is 65.5 per 100,000 for the Iraqi female population. It is also the most frequently occurring cause of carcinoma-related death among Iraqi females [1].

Breast carcinoma is classified molecularly, based on the expression status of the hormonal receptors (estrogen and progesterone) and human epidermal growth factor receptor 2 (HER2). This helps classify breast carcinoma into four groups: Luminal A, Luminal B, HER2-enriched, and triple negative breast carcinoma (TNBC) [2]. TNBC is distinguishable by the lack of expression of the hormonal receptors (estrogen and progesterone) and the absence of HER2 receptor expression [3]. TNBC is regarded as an aggressive disease with high recurrence rates and metastasis associated with poor response to targeted therapies (anti-hormonal and anti-HER2

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therapies) [4], offering a narrow range of effective treatments, including chemotherapy and radiotherapy [4]. TNBC represents 15%-20% of all breast carcinomas [5].

Certain types of TNBC are distinguished by inducing a strong immune response and increasing the number of tumor-infiltrating lymphocytes, making it an immunogenic carcinoma, and enabling it to respond to a new treatment line known as immunotherapies. Immunotherapies impact by modulating the immune response in the tumor cell environment, either through stimulation of the anti-tumor activity of immune cells or by removing the blockade of immune checkpoint regulators developed during immunoediting by the tumor cells in their attempts to escape destruction by the immune cells [6].

Mismatch repair deficiency (dMMR) is one of the biomarkers that predicts the benefits from immunotherapies [7]. The MMR system controls the integrity of the human genome during deoxynucleic acid (DNA) replication. Errors such as single-base mismatch, and short insertions or deletions, that may occur during DNA replication, and would normally be rectified by the MMR proteins [8]. The MMR pathway is composed of four proteins: MutS Homologues 2 (MSH2), MutS Homologues 6 (MSH6), MutL Homologues 1 (MLH1), and Post meiotic Segregation increased 2 (PMS2). These proteins are found in a complex together, forming 2 pairs (MSH2 and MSH6) and (MLH1 and PMS2). The MSH2 and MLH1 are the principle proteins expressed in the cells by forming other heterodimers with other cellular proteins, while the MSH6 and PMS2 are present only when the MSH2 and MLH1 are intact and expressed in cells [9]. Each complex has a special function. The MutS complex recognizes and attaches to the abnormal DNA, while the MutL complex enhances recognition and facilitates the formation of the repair complex [10].

When defects occur in the MMR pathway, whether as somatic mutation, inherited germline mutation, or hypermethylation of the promoter region of the MLH1 gene, the genes that encode the MMR proteins are affected. This, in turn, affects the expression of the MMR proteins, resulting in the deficiency of one or more of these MMR proteins, whether the deficiency is partial or complete [11]. Defects in the MMR pathway inhibit the ability to repair replicative errors, resulting in the accumulation of mutations and, thus, increasing the tumor mutational burden. These mutations result in the transcription and translation of abnormal peptides and proteins called neoantigens. The immune T-cells recognize these neoantigens as non-self-antigens, causing the induction of an immune response and an increment in infiltration of tumor infiltrating lymphocytes, and raising the susceptibility of these tumors to immunotherapies [12]. Therefore, this study aims to assess the expression status of the MMR proteins in breast carcinoma to determine their potential use in identifying the patients who might benefit from immunotherapy and to test their correlation with the clinicopathological parameters.

MATERIALS AND METHODS

Patient selection

A retrospective study was conducted from October 2024 to April 2025. A total of 53 cases of TNBC in females were included in the study; 25 of them were diagnostic tru-cut biopsies, and 28 were mastectomy samples. All the cases were diagnosed as triple-negative invasive ductal breast carcinoma [not otherwise specified (NOS)]. The clinical data includes age, tumor size, tumor grade, pathological tumor (T) and nodes (N) stages (which were applied only for the mastectomy samples), and menopausal status drawn from the archived of histopathological laboratories files at the Medical City campus. The current study included newly diagnosed TNBC patients, while those who had received chemotherapy or radiotherapy were excluded. Male patients were also excluded. The formalin-fixed paraffin-embedded blocks were retrieved, and sections of 4-microns in thickness were taken. These sections were prepared and stained with monoclonal antibodies targeting the four MMR proteins. Sections from normal colonic mucosa were processed and stained in parallel with the same monoclonal antibodies, serving as positive control slides. Negative control slides were prepared using the same protocol, omitting the primary antibody. The required sample size was calculated using an online sample size calculator, namely, the following formula:

Unlimited population:

$$n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2}$$

Finite population:

$$\acute{n} = \frac{n}{1 + \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2 \times N}}$$

Where: z is the score, ε is the margin of error, N is the population size, $\hat{p}$ is the population proportion.

$$n = \frac{(1.645)^2 \times 0.15(1-0.15)}{(0.05)^2} = 138$$

$$\acute{n} = \frac{138}{1 + \frac{(1.645)^2 \times 0.15(1-0.15)}{(0.05)^2 \times 175}} = 77.18$$

The number of collected cases was lower than the estimated sample size due to the unavailability of histopathological blocks, as many had been retrieved by patients themselves. Because of the small size of the sample studied, and the wide range of distributed age (24-81years), the cases were categorized over large age categories (<35, 35-54, 55-74, and $\geq$ 75 years).

Staining procedure

After the tissue blocks were sectioned, the deparaffinization process was performed. Next, the slides were placed in a high pH retrieval solution. Then, the endogenous peroxidase activity was blocked by adding hydrogen peroxide to the slides. The next step was to add the primary antibodies to the slides (monoclonal anti-human antibodies targeting the four MMR proteins (MSH2, MSH6, MLH1, and PMS2), with code numbers (IR085, IR086, IR079, and IR087, respectively), (Agilent Technologies, Dako, Denmark) and incubate for 30 minutes. Then, the Rabbit linker and Mouse linker were applied (the Mouse linker for MSH2 and MLH1, and the Rabbit linker for MSH6 and PMS2) for 20 minutes. In the next stage, the secondary antibody (HRP) was applied for 20 minutes. Then, the DAB substrate chromogen solution was added to the slides, which were left for 10 minutes. After this, the hematoxylin counter stain was applied to the background for just one minute. Finally, the dehydration process was performed.

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MMR proteins staining assessment

The MMR proteins are assessed by examining the nuclear staining of the MMR protein antibodies. When the MMR proteins are expressed and intact, the nucleus is stained homogeneously with brown. On the contrary, the absence of nuclear staining is associated with MMR protein deficiency [13].

Ethical approval

This study received ethical approval from the Research Ethics Committee of the Department of Pathology and Forensic Medicine, College of Medicine, University of Baghdad. Registered under the code 133B (15^{th} October 2024). All the procedures followed were in accordance with the institutional guidelines. Informed consent from participants was waived due to the study's retrospective nature.

Statistical analysis

Statistical analyses were performed using the statistical package for the social sciences (SPSS) version 20.0 for Windows (IBM, Chicago, IL, USA). The Shapiro-Wilk test was used to test the normality of the continuous variable. The data were presented using descriptive statistical measures, including median, interquartile range (IQR), and range (minimum and maximum) for qualitative variables, while the quantitative variables were represented as frequency and percentage. The chi-square test was used to test the relation between the study groups. The Mann-Whitney U test was used to test the difference between two means. A P-value of < 0.05 was considered significant.

RESULTS

The median age was 51 years with an IQR 19.5, with a range of 24-81 years. Most cases were distributed in the 35-54 years age group (n=25, 47.17%), as shown in Table 1.

The median tumor size in the mastectomy samples was 4.5 cm and IQR 3, with a range of 1-16 cm. About half the cases were in grade II. Most mastectomy samples were in the T2 stage category, 57.1%, while the N0 stage and Nx (no information available regarding regional lymph node status) stages showed the most cases around 25% in each category, as shown in Table 2.

In this study, 43 out of 53 cases showed intact expression of MMR proteins, while 10 out of 53 revealed a deficient status. The MSH2 protein was the least frequently missing protein, being missed in three cases only; however, the PMS2 protein was the most frequently missing protein, and was lost in 10 cases, as illustrated in Table 3.

The expression status of MMR proteins is shown in Figures 1 and 2.

Table 1. Distribution of 53 triple negative breast carcinoma cases according to age at time of diagnosis.

Age per years	Frequency	Percentage
< 35	6	11.32
35–54	25	47.17
55–74	21	39.62
> 74	1	1.89

Table 2. Distribution of 53 triple-negative breast carcinoma cases according to clinicopathological parameters.*

Variable	Count	Percentage
Grade		
I	6	11.4
II	27	50.9
III	20	37.7
pT stage (n = 28) *		
T1	3	10.8
T2	16	57.1
T3	6	21.4
T4	3	10.7
pN stage (n = 28)		
N0	7	25
N1	5	17.9
N2	3	10.7
N3	6	21.4
Nx	7	25
Menopause status		
Pre menopause	23	43.4
Post menopause	30	56.6

* Regarding the tumor stage, it was applied only for mastectomy samples.

Table 3. Distribution of 53 triple negative breast carcinoma cases according to mismatch repair (MMR) status and individual MMR proteins.*

Variable	Number	Percentage
MMR status		
pMMR	43	81.1
dMMR	10	18.9
MSH2		
Positive	50	94.3
Negative	3	5.7
MSH6		
Positive	49	92.5
Negative	4	7.5
MLH1		
Positive	45	84.9
Negative	8	15.1
PMS2		
Positive	43	81.1
Negative	10	18.9

* pMMR: Proficient (intact) MMR status, dMMR: deficient MMR status.

The current study showed a significant correlation between MMR expression status and patient age (P-value = 0.044), while it was insignificant with the other clinicopathological parameters (P-value > 0.05), as shown in Table 4.

DISCUSSION

TNBC is an aggressive malignancy with a poor prognosis and associated with a high mutational burden; unfortunately, only a limited number of effective medications are available [14]. Immunotherapy has emerged as a promising strategy by

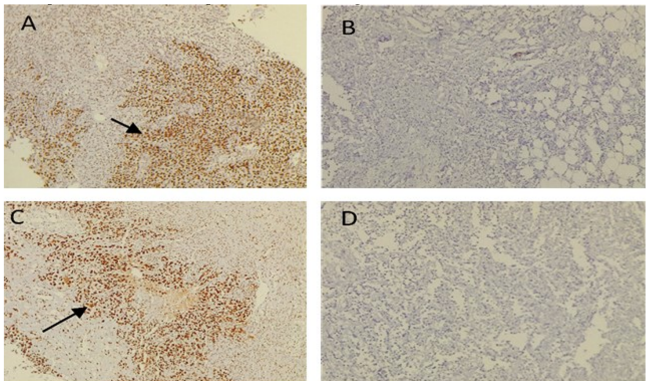

Figure 1. Microphotograph showing the expression status of MSH2 and MSH6 proteins, 10×. A and C: Showing the positive nuclear expression (brown color) of MSH2 and MSH6 proteins, respectively. B and D: Showing the negative nuclear expression of MSH2 and MSH6 proteins, respectively.

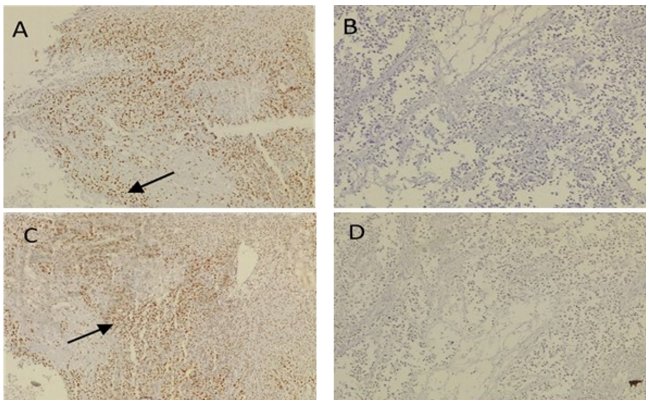

Figure 2. Microphotograph showing the nuclear expression status of MLH1 and PMS2 proteins, 10×. A and C: Showing the positive nuclear expression (brown color) of MLH1 and PMS2, respectively. B and D: Showing the negative nuclear expression of MLH1 and PMS2, respectively.

modulating the immune response within the tumor microenvironment. The MMR pathway repairs the replicative errors that arise during DNA replication, when defects affecting the MMR system occur, resulting in the accumulation of mutations, thereby increasing the mutational burden of the tumor, and potentially tumor immunogenicity, rendering such tumors more responsive to immunotherapies [11, 12]. The assessment of the expression status of the MMR proteins is an established predictive biomarker for immunotherapy eligibility in multiple solid tumors, such as colorectal and endometrial carcinoma [14]. However, the reported frequency of MMR protein deficiency (dMMR) in breast carcinoma varies considerably across studies. In one study, it was found in the range of 1.7%-3.8% [12]; in other studies, the range was around 9.2% and 12% [15, 16].

The current study revealed that 18.9% of the population studied demonstrated loss of expression of one or more of the MMR proteins, suggesting that evaluating of MMR status may have a clinical advantage in identifying TNBC patients who could benefit from immunotherapy. The results observed

Table 4. Correlation of the mismatch repair (MMR) with demographic, clinical, and pathological characteristics*.

Variables	MMR status		*P-value
	pMMR§ (n=43)	dMMR¶ (n=10)	
Age per years			
Median	49.5	62	0.044
IQR	20	10.75	
Range	24-81	35-73	
Tumor size			
Median	4	4.85	0.354
IQR	3.25	6.88	
Range	1-16	2-16	
Grade n(%)			
GI	4 (9.3)	2 (20)	
GII	23 (53.5)	4 (40)	0.569
GIII	16 (37.2)	4 (40)	
pT stage n(%)			
T1	3 (15)	0 (0)	
T2	12 (60)	4 (50)	0.443
T3	3 (15)	3 (37.5)	
T4	2 (10)	1 (12.5)	
pN stage n(%)			
N0	5 (25)	2 (25)	
N1	5 (25)	0 (0)	
N2	2 (10)	1 (12.5)	0.263
N3	5 (25)	1 (12.5)	
Nx	3 (15)	4 (50)	
Menopausal status n(%)			
Pre-menopause	21 (48.8)	2 (20)	0.159
Post-menopause	22 (51.2)	8 (80)	

* Mann Whitney U test was used to test the continuous variables; the chi-square test was used to test the categorical variables. §: Proficient MMR, ¶: Deficient MMR.

in the current study are generally consistent with previously published data. The median age reported by Özcan et al. study (2021) was 49 years, with a range of 31-68 years [17], The Rammal et al. study (2024) reported a higher result, as the median age was 64 years, while the range was 27-100 years [18]. Li et al. study (2013) reported the highest frequency in the category of 35-55 years [19]. In their work, Li et al. (2013) and Troschel et al. (2020) reported that most cases were in the grade II category, with percentages 42.2% and 89.4%, respectively [19, 20]. Further, Özcan et al. (2021) and Burstein et al. (2015) reported that the majority of cases were in the T2 stage, with percentages 43% and 71%, respectively [17, 21], while in the Rammal et al. (2024) and the Burstein et al. (2015), the N0 stage was found to be the most frequently reported one, with percentages of 83% and 49% respectively [18, 21]. The majority of cases were post-menopausal in the work of Burstein et al. (2015) and the Troschel et al. (2020), with percentages of 56% and 57,9%, respectively [20, 21].

Other studies reported results comparable to those in the current work, as in the study by Özcan et al. (2021), which reported that around 11% of cases had dMMR status, while 39% of cases revealed retained protein expression pMMR status, and 50% of the cases showed heterogeneous expression (partial loss). This study also reported that the PMS2 pro-

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tein was the most frequently lost, while the MSH2 protein was the least missed [17]. In their work, Cheng et al. (2019), also reported that the MSH2 protein was the least frequently missed protein, while PMS2 was the most frequently missed [10]. Variability in reported dMMR frequencies across studies may be attributable to differences in sample size, genetic background, antibody clones, and staining protocols.

In the current study, grade II and grade III were more predominant among the dMMR cases; the pT2 stage predominated in both the pMMR and dMMR groups. The postmenopausal status was prevalent in both pMMR and dMMR groups. However, an insignificant correlation was found between MMR status and clinicopathological parameters except for age; the dMMR cases tend to be older in age. Comparable findings were found by Cheng et al. (2019) and Maraqa et al. (2024), as they reported an insignificant correlation between the MMR status and clinicopathological parameters [10, 15]. The differences in results between the present work and other studies can be explained by differences in sample size, different clones and staining techniques, and the underlying genetic background.

The present study was subjected to several limitations. The sample size was relatively small due to the unavailability of paraffin blocks, as some specimens had been retrieved by the patients. Additionally, the tumor under study is relatively uncommon, further restricting the number of available cases. Suboptimal preparation of some blocks compromised sample quality, resulting in inadequate findings and poor results, necessitating the exclusion of those cases. Further, missing data from some patients was another reason to exclude them from the study. Finally, the study was performed within a predefined timeframe, and associated time constraints may have further impacted case inclusion and data collection and analysis.

CONCLUSION

MMR protein deficiency was identified in 18.9% of cases. These findings suggest that the dMMR status can serve as a useful biomarker to identify which patients with TNBC will be best suited to receive immunotherapies. Among the individual MMR proteins evaluated, PMS2 had the highest frequency of loss, whereas the MSH2 protein showed the lowest frequency of loss. A statistically significant association was found between age and MMR status, whereas there was insignificant correlation between the expression status of the MMR proteins and the other clinicopathological data of the studied cases. However, the small sample size limits the generalizability of these findings. Further studies involving larger cohorts are recommended to validate the results of this study. It is recommended to compare the MMR expression status between different subtypes of breast carcinoma. Cases exhibiting MMR deficiency should also be evaluated for microsatellite instability using polymerase chain reaction (PCR)- based assays.

ETHICAL DECLARATIONS

Acknowledgments

None.

Ethics Approval and Consent to Participate

This study received ethical approval from the research ethics committee of the Department of Pathology and Forensic Medicine, College of Medicine, University of Baghdad. Registered under the code 133B, dated October 15, 2024. All procedures followed were in accordance with institutional guidelines. Informed consent from participants was waived due to the study's retrospective nature.

Consent for Publication

Not applicable, as no personal information or data are included in the manuscript.

Availability of Data and Material

The data generated and analyzed during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Use of Artificial Intelligence

Artificial intelligence has been used to correct spelling and grammar.

Authors' Contributions

Study concept and design: Abdul Ghafour KH. Literature search Al-Saffar HA. Data acquisition: Al-Saffar HA. Data analysis and interpretation: Al-Saffar HA and Abdul Ghafour KH. Manuscript preparation and writing: Al-Saffar HA. Manuscript revision and editing: Abdul Ghafour KH and Al-Saffar HA. Both authors reviewed and endorsed the final version of the manuscript.

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