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Cross-Sectional Analysis of Immunohistochemical Expression of Mismatch Repair Proteins in Prostate Cancer

Muhammad Atif Khalil, Nadeem Zafar, Hassan Tariq*, Wajahat Ahmed Khan, Moniba Zafar, Muhammad Owais Qurni, Siyab Ahmed**

Department of Histopathology, Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS), Rawalpindi Pakistan, *Department of Histopathology, Combined Military Hospital Malir/National University of Medical Sciences (NUMS) Pakistan, **Department of Histopathology, Combined Military Hospital Multan/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To determine the immunohistochemistry-based frequency of mismatch repair protein (MSH-2, MSH-6, MLH-1 & PMS-2) deficiency in prostate cancer.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jan 2021 to Jul 2022.

Methodology: The characteristics and clinical data of 82 prostatic cancer patients who had transurethral resection of the prostate and surgical resections were examined. MLH-1, MSH-2, PMS-2, and MSH-6 antibodies were utilized during immune-histochemical staining. The status of each patient's mismatch repair deficit was established once the results were assessed.

Results: In the study, mean age of presentation was 70.90±8.36 years. Most common histological type was acinar adenocarcinoma, 67(81.70%). Out of 82 patients with prostatic carcinoma, 17(20.73%) patients had lost their immunohistochemistry staining for MMR proteins. Nonetheless, no mismatch repair deficit was found in the 65(79.27%) remaining instances. One patient had a simultaneous loss of all four antibodies, two had loss of MLH-1, three had loss of MSH-2, eleven had loss of PMS-2, and twelve had loss of MSH-6.

Conclusion: The frequency of mismatch repair deficiency in our community's prostate cancer patients was reported to be 34.1%, which may be utilized as a base for genetic testing to track the effectiveness of treatment and forecast the overall survival rate for patients with prostate cancer. Studies are also needed to explain the MMR protein's genetic role in cancer formation, together with the signaling pathway.

Keywords: Immunohistochemical Expression, Microsatellite Instability (MSI), Mismatch Repair (MMR) Deficiency, MSH-2, MSH-6, MLH-1, PMS-2, Prostatic Carcinoma.

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INTRODUCTION

In 2020, prostate cancer accounted for approximately 191,930 new cases and 33,330 fatalities in the United States, making it the most commonly diagnosed malignancy among males.[1] Together, prostate, lung, and colorectal carcinoma account for 42% among all cases recorded in males, while prostate cancer accounts for one out of every five occurrences.[2] Prostate cancer is the second-highest common malignancy in males among Pakistani males, as indicated by the annual cancer registry data issued by Shaukat Khanum Memorial Cancer Hospital and Research Centre.[3] Pakistan has a comparatively low age-adjusted occurrence of prostate cancer in comparison to other Asian nations (5.3 per 100,000); however, more instances are being recorded.[4]

Correspondence: Dr Muhammad Atif Khalil, Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan
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While radical prostatectomy for localized prostate cancer frequently results in a cure, tumor recurrence following the operation still poses a significant therapeutic issue. It's significant because the tumor's Gleason grade and pre-operative blood levels of prostate-specific antigen (PSA), can reliably determine the course of illness.[5] Likewise, multi-genomics techniques have discovered possible molecular indicators that might be beneficial for the early recognition of localized prostate cancer and making decisions on its management.[6]

The highly conserved biological system that is called DNA mismatch repair (MMR) primarily maintains genomic integrity. MMR's specificity is mostly derived from base-base mismatches and insertion/deletion mispairs created during DNA replication and recombination. It corrects errors caused by DNA replication and acts as a DNA damage sensor. The most popular complexes include MLH-1

and PMS-2, which, along with MSH-2 and MSH-6, respectively, create MutS and MutL.[7] Moreover, recent research has linked the risk of developing prostate cancer to MMR insufficiency, specifically abnormalities in the MSH-2 or MSH-6 gene.[8] Programmed death ligand 1 (PD-L1), a PD-1 ligand, exhibits immunoreactivity or MMR deficiency have both been linked to favorable responses to anti-programmed cell death protein 1 (PD-1) therapy as well as to PD-L1 protein expression in tumors, suggesting that MMR deficiency may serve as a biomarker for the immune checkpoint inhibitor.[9] The newest endorsement of Pembrolizumab, an immunotherapy-based PD-1 inhibitor therapeutic, through the U.S. Food and Drug Administration (FDA) for individuals with MSI or MMR deficiency who have advanced or incurable solid neoplasms, may have made this research area particularly pertinent.[10]

The primary objective of the current study was to conduct a thorough evaluation of the immunohistochemical (IHC) expression levels of four key mismatch repair (MMR) proteins—MSH2, MSH6, MLH1, and PMS2—in prostate cancer tissues. This analysis aims to assess the potential relationships between these protein expressions and various pathological features of prostate cancer, such as tumor grade and stage, as well as their implications for tumorigenesis and treatment responses. By understanding the expression patterns and functional roles of these MMR proteins, the study aims to provide valuable insights into the molecular mechanisms underlying prostate cancer progression. It may offer guidance for personalized therapeutic strategies.

METHODOLOGY

This cross-sectional study was conducted from January 2021 to June 2022 at the Histopathology Department of the Armed Forces Institute of Pathology (AFIP) in Rawalpindi, Pakistan. Employing the WHO sample size tool, the sample size was established. Keeping probability of mismatch repair of 32% in previous study, confidence interval of 95% and margin of error 5%.[11]

There were 82 participants in the actual sample. A convenience sampling approach was used for selection of study participants. Radical prostatectomy, core biopsy, and transurethral resection of the prostate (TURP) were used to obtain specimens. Age at onset, gender, family history of illness, and smoking habits (smokers versus non-smokers) were all contributing variables documented in the institutional tumor registry portion.

Inclusion Criteria: All diagnosed cases of prostate cancer with age ranging from 55–85 years were chosen.

Exclusion Criteria: Patients with insufficient prostate cancer diagnostic records were eliminated.

For 82 patients, formalin-fixed paraffin-embedded tissue sections of the neoplasms and the corresponding normal tissues were prepared. Utilizing representative tissue blocks, immunohistochemical staining was performed using the Dakoen Vision process with antibodies against MLH-1, MSH-2, PMS-2, and MSH-6. For immunohistological staining in each instance, a specific representative block with a conserved tumor morphology was selected. The four MMR proteins, MLH-1 (monoclonal mouse anti-human antibody, clone ES05), PMS-2 (monoclonal rabbit anti-human antibody, clone EP51), and MSH-2 (monoclonal mouse anti-human antibody, clone FE11), were stained (all supplied by Agilent Dako, Glostrup, Denmark). The obtained slides were evaluated by two pathologists-in-consultation after the immunohisto-chemical method was used under the established procedure (Dako Auto stainer Link 48, Detection Kit K8002, Agilent Dako).

A positive internal control includes the nuclear positivity of the respective MMR protein within the benign prostatic epithelium, lymphocytes, stromal cells, and endothelial cells. Each batch also contained a positive external control involving healthy colonic tissue. Nuclear reactivity was divided into two groups: expression loss and expression retention. A decrease in expression was thought to have occurred if there was no nuclear reactivity (negative in all tumor cells). Comparatively, tumor cells were regarded to have preserved expression if each antibody stained the nucleus at least 1% of the time (positive in tumor cells). MMR deficiency was defined as the loss of one or all MMR proteins, whereas MMR proficiency was defined as the presence of retained MMR proteins. Records were kept of patient information, as well as pertinent information and biopsy numbers.

The status of each patient's mismatch repair deficit was established once the results were analyzed. The patients were split into four groups: individuals with a positive mismatch repair deficiency status (lack of expression in at least one MMR gene), coupled loss of expression, loss of expression of each of the four antibodies, and those lacking absence of expression (mismatch repair deficiency status negative). The

factor of age is a quantitative variable that was analyzed using mean and standard deviation, whereas baseline categorical variables like gender and expression of MSI markers were analyzed descriptively using frequencies and percentages.

RESULTS

In this study, out of 82 patients, the mean age was calculated and found to be 70.90±8.36 years. Most common histological type was acinar adenocarcinoma in 67(81.7%) of the patients, followed by ductal adenocarcinoma in 11(13.4%) and 2(2.4%) cases with neuroendocrine differentiation, and 1(1.22%) case of mixed ductal and acinar adenocarcinoma and acinic cell adenocarcinoma each. The most typical Gleason score was 7, which was observed in 40(48.8%) of the instances, followed by Gleason scores of 9 in 19(23.2%) and 8 in 13(15.9%). Among these 82 cases, 21(25.6%) were in grade Group-2, followed by 19(23.2%) cases of grade Group-3 and 5 each and 13(15.9%) cases were grade Group-4.

In 17(20.73%) of the 82 patients, there was a loss of immunohistochemical staining for MMR proteins. Loss of immunohistochemical expression of individual markers was observed in the following cases: MSH-6 in 12(14.6%), MSH-2 in 3(3.7%), PMS-2 in 11(13.4%), and MLH-1 in 2(2.4%) cases. One patient (1.22%) experienced the simultaneous loss of all four antibodies, two (2.43%) had the paired loss of MLH-1 and PMS-2, and three (3.67%) had the paired loss of MSH-2 and MSH-6. Yet, in 65(79.27%) instances, there was no loss of MMR protein immunohistochemical staining. These findings were tallied and displayed in Table-I.

Table-I: Frequency of Loss of MMR Proteins (MLH-1, PMS-2, MSH-2, and MSH-6) in Prostate Cancer Cases (n=82)

Patterns	Frequency of Loss of MMR Proteins
Combined Loss of all 4 MMR Proteins	1(1.22%)
Combined Loss of PMS-2 & MLH-1	2(2.43%)
Combined Loss of MSH-2 & MSH-6	3(3.67%)
MLH-1	2(2.43%)
PMS-2	11(13.4%)
MSH-2	3(3.67%)
MSH-6	12(14.6%)
No Loss of MMR Immunohistochemical Staining	65(79.27%)

**MMR – Mismatch Repair*
PMS – Post Meiotic Segregation
MLH - MutL Homolog
MSH - Melanocyte-Stimulating Hormone

DISCUSSION

In this study, 15(18.2%) out of the 82 patients had lost immunohistochemistry staining for MMR proteins. Out of 82 cases, only one patient had the loss of all four antibodies, while the combined loss of MLH-1 and PMS-2 was found in two patients, the combined loss of MSH-2 and MSH-6 in three cases, and the loss of MLH-1 alone in two patients. However, in 65 cases, there was retained expression of MMR proteins on immunohistochemistry.

Defects in MMR proteins have recently been linked in males with prostatic cancer associated with Lynch syndrome and spontaneous instances. Immunohistochemistry is frequently used in colorectal, endometrial, and even prostatic cancer around the world to detect MMR proteins. Yet there isn't any regular assessment for MMR proteins found in prostatic adenocarcinoma in Pakistan.[9]

Prostatic cancer and MMR protein deficiency are related, according to Pakistani research. In a study by Javeed *et al.*, the MSH-2, MSH-6, MLH-1, and PMS-2 proteins' immunohistochemical expression in terms of their deficit was assessed either individually or collectively, in prostatic cancer. This study found that MSH-2, MSH-6, and PMS-2 loss occurs at low frequencies (12.20%, 2.70%, and 12.20%, respectively); they are not statistically significant. MLH-1 did not suffer a loss.[12] This nearby concentration in our populace had equivalent outcomes, about a joint loss of MSI proteins in 28.1% though in our review consolidated loss of MSI in 30% cases.

Table-II: Comparison of Current and Previous Studies on Mismatch Repair Deficiency and Patterns of Antibody Expression Loss

Author	Year	Frequency of MSI	Loss of Antibody Expression	Combined and Isolated Loss of Antibody Expression
Javeed et al.[12]	2022	28.1%	MLH-1, PMS-2, MSH-2 & MSH-6 0%, 12.20%, 12.20% & 2.70% respectively	MSH-2 & MSH-6 in 1%
Sharma et al.[13]	2020	32.7%	MLH-1, PMS-2, MSH-2 & MSH-6 0.9%, 12.3%, 2.7% & 16.8% respectively	All four MMR proteins in 0.9% cases
Albero-González et al.[14]	2019	57.1%	MLH-1, PMS-2, MSH-2 & MSH-6 5%, 2%, 8% & 42.1% respectively	-

**MSI - Microsatellite Instability*
MMR – Mismatch Repair
PMS – Post Meiotic Segregation
MLH - MutL Homolog
MSH - Melanocyte-Stimulating Hormone

A global investigation conducted by Sharma *et al.*, examined the relationship between the loss of expression of certain proteins and various clinico-pathological characteristics in tissue microarrays consisting of 220 radical prostatectomy specimens. The researchers used immunohistochemical staining to

assess the presence of MLH-1, MSH-2, MSH-6, and PMS-2. The results revealed a loss of these proteins in the following percentages of prostate tumors: 2(0.9%) had a loss of MLH-1, 6(2.7%) had a loss of MSH-2, 37(16.8%) had a loss of MSH-6, and 27(12.3%) had a loss of PMS-2. Overall, there was a loss of at least one mismatch repair (MMR) protein in 50(22.7%) of the cases, as summarized in Table-II.[13] The reasons for focusing on a specific segment of the Pakistani population are similar to those observed in international studies.

Another worldwide investigation, Albero-González *et al.*, examined 200 prostate cancer patients' immunohistochemistry expression of MSH-2, MSH-6, MLH-1, and PMS-2. MSH-2 loss of nuclear expression was seen in 8% of cases, MLH-1 loss in 5%, PMS-2 loss in only 2%, and MLH-1 loss in 30% of cases. In 42.1% of the instances, MSH-6 expression was higher compared to baseline levels, as shown in Table-II.[14] Mismatch repair deficiency was not associated with any factors in past international research; however, This study does not support that conclusion.

The function of anti-PD-1 and PD-L1 for therapy purposes in the case of colorectal carcinoma, as well as other cancers linked to MSI, is well documented and acknowledged by the US Food and Drug Administration.[15] The function of anti-PD-1 and PD-L1, on the other hand, is yet to be demonstrated, mainly due to a lack of proof in the case of prostate cancer.[16]

The role of targeting PD-1 and PD-L1 in therapy objectives is notable and recognized, especially in colorectal cancer and other MSI-related malignancies, by the US Food and Drug Administration. In fact, for prostate cancer, the role of anti-PD-1 and PD-L1 remains unclear, mainly due to a lack of data.[17] Clinical trials examining PD-1/PD-L1 inhibitors should be encouraged in patients with prostate cancer to support further research.[18]

The purpose of this study was not to assess mismatch repair protein immunohistochemistry, which is a standard test used to determine microsatellite instability (MSI) status in many pathology laboratories for prostate cancer. Additionally, our validation collection does not include data on responses to pembrolizumab or other clinical outcomes. Since the samples are not part of an ongoing prospective series, the design of our study does not allow for the evaluation of either positive or negative predictive values. Finally, we did not assess the total mutational burden, which is a newly identified biomarker associated with MMR gene mutations and susceptibility to immune checkpoint inhibitors.

CONCLUSION

The frequency of mismatch repair deficiency among individuals with prostate cancer in the study population was found to be 34.1%, which may be utilized as a base for genetic testing to track the effectiveness of treatment and forecast the overall survival rate for prostate cancer patients.

Conflict of Interest: None.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MAK & NZ: Data acquisition, data analysis, critical review, approval of the final version to be published.

HT & WAK: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MZ, MOQ & SA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69(1): 7-34. https://doi:10.3322/caac.21551
2. 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 Cancer J Clin 2018; 68(6): 394-424. https://doi:10.3322/caac.21492
3. Bugoye FC, Torrorey-Sawe R, Biegon R, Dharsee N, Mafumiko FMS, Patel K, et al. Mutational spectrum of DNA damage and mismatch repair genes in prostate cancer. Front Genet 2023; 14: 1231536. https://doi:10.3389/fgene.2023.1231536
4. Mahmood S, Qasmi G, Ahmed A, Kokab F, Zahid MF, Afridi MI, et al. Lifestyle factors associated with the risk of prostate cancer among Pakistani men. J Ayub Med Coll Abbottabad 2012; 24(2): 111-115.
5. VVan den Broeck T, van den Bergh RCN, Arfi N, Gross T, Moris L, Briers E, et al. Prognostic Value of Biochemical Recurrence Following Treatment with Curative Intent for Prostate Cancer: A Systematic Review. Eur Urol 2019; 75(6): 967-987. https://doi:10.1016/j.eururo.2018.10.011
6. Ferro M, de Cobelli O, Vartolomei MD, Lucarelli G, Crocetto F, Barone B, et al. Prostate Cancer Radiogenomics-From Imaging to Molecular Characterization. Int J Mol Sci 2021; 22(18): 9971. https://doi:10.3390/ijms22189971
7. Pritchard CC, Morrissey C, Kumar A, Zhang X, Smith C, Coleman I, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun 2014; 5: 4988. https://doi:10.1038/ncomms5988

8. Raymond VM, Mukherjee B, Wang F, Huang SC, Stoffel EM, Kastrinos F, et al. Elevated risk of prostate cancer among men with Lynch syndrome. J Clin Oncol 2013; 31(14): 1713-1718. https://doi:10.1200/JCO.2012.44.1238
9. Rodrigues DN, Rescigno P, Liu D, Yuan W, Carreira S, Lambros MB, et al. Immunogenomic analyses associate immunological alterations with mismatch repair defects in prostate cancer. J Clin Invest 2018; 128(10): 4441-4453. https://doi.org/10.1172/jci121924
10. Lord CJ, Ashworth A. The DNA damage response and cancer therapy. Nature 2012; 481(7381): 287-294. https://doi:10.1038/nature10760
11. Wilczak W, Rashed S, Hube-Magg C, Kluth M, Simon R, Büscheck F, et al. Up-regulation of mismatch repair genes MSH6, PMS2 and MLH1 parallels development of genetic instability and is linked to tumor aggressiveness and early PSA recurrence in prostate cancer. Carcinogenesis 2017; 38(1): 19-27. https://doi:10.1093/carcin/bgw116
12. Javeed S, Chughtai A, Zafar G, Khalid F, Batool A, Chughtai AS. An Evaluation of the Immunohistochemical Expression of Mismatch Repair Proteins (MSH2, MSH6, MLH1, and PMS2) in Prostate Adenocarcinoma. Cureus 2022; 14(7): e27448. https://doi:10.7759/cureus.27448
13. Sharma M, Yang Z, Miyamoto H. Loss of DNA mismatch repair proteins in prostate cancer. Medicine 2020; 99(19): e20124. https://doi:10.1097/MD.0000000000020124
14. Albero-González R, Hernández-Llodrà S, Juanpere N, Lorenzo M, Lloret A, Segalés L, et al. Immunohistochemical expression of mismatch repair proteins (MSH2, MSH6, MLH1, and PMS2) in prostate cancer: correlation with grade groups (WHO 2016) and ERG and PTEN status. Virchows Arch 2019; 475(2): 223-231. https://doi:10.1007/s00428-019-02591-z
15. Goldberg KB, Blumenthal GM, Pazdur R. The First Year of the Food and Drug Administration Oncology Center of Excellence: Landmark Approvals in a Dynamic Regulatory Environment. Cancer J 2018;24(3):131-135. https://doi:10.1097/PPO.0000000000000316.
16. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 2015; 372(26): 2509-2520. https://doi:10.1056/NEJMoa1500596
17. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations. Hum Vaccin Immunother 2019; 15(5): 1111-1122. https://doi:10.1080/21645515.2019.1571892
18. Isaacsson Velho P, Antonarakis ES. PD-1/PD-L1 pathway inhibitors in advanced prostate cancer. Expert Rev Clin Pharmacol 2018; 11(5): 475-486. https://doi:10.1080/17512433.2018.1464388

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