

Immunohistochemical Evaluation of Mismatch Repair Deficiency in Prostate Cancer

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ABSTRACT

Introduction: Prostate cancer is a leading malignancy among men worldwide, with rising incidence in South Asia. Traditional prognostic parameters such as Gleason score and PSA are being complemented by molecular biomarkers, including mismatch repair (MMR) deficiency, which has gained importance due to its association with microsatellite instability and therapeutic responsiveness. This study aimed to evaluate the frequency and patterns of MMR protein loss in prostate carcinoma using immunohistochemistry (IHC).

Methodology: This cross-sectional study was conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, between June 2023 and July 2024. Seventy-two histologically confirmed cases of prostate carcinoma were analyzed. Formalin-fixed, paraffin-embedded tissue blocks underwent IHC staining for MLH1, MSH2, MSH6, and PMS2. Nuclear staining loss in tumor cells, with intact internal controls, was interpreted as MMR deficiency. Data were statistically analyzed using SPSS version 26.0, with $p < 0.05$ considered significant.

Results: The mean patient age was 71.4 ± 8.2 years. Acinar adenocarcinoma was the predominant histological subtype (81.9%). MMR deficiency was identified in 16 cases (22.2%), with isolated losses observed for PMS-2 (13.9%) and MSH-6 (16.7%) being most frequent. No significant associations were found between MMR deficiency and clinicopathological parameters such as age, histological subtype, or tumor stage.

Conclusion: A substantial proportion of prostate carcinoma cases demonstrated MMR protein loss, predominantly involving PMS-2 and MSH-6. Routine IHC evaluation for MMR proteins may provide clinically relevant insights, supporting personalized treatment strategies and genetic counseling in prostate cancer.

Keywords: Prostate cancer, mismatch repair deficiency, immunohistochemistry, PMS-2, MSH-6.

INTRODUCTION

The most common type of oral surgery procedures permeate the world, with the prevalence of impaction being estimated to be between 20 to 30% within the general population.^{1,2} In spite of being a usual operation, this type of surgery is said to be accompanied by high-intraoperative pain and post-operative morbidity, with this phenomenon affiliated to a high degree with variations in anesthetic responsiveness and action related to the intricate nature of mandible innervation.³ Deep anesthesia is therefore a significant variable that predetermines the outcome of a surgery and patient satisfaction. Historically, inferior alveolar nerve block (IANB) can be described as a techniqueology which turned out to be a direct-backbone of anesthesia practice when performing surgery on the mandibular deformity on the third molar. However, when the failure of which has been documented as 15 to 20 percent because of variation in anatomy and accessory innervation coupled with technique limitation, it indicates that adjunctive measures are needed to enhance the analgesic effect.

The incidence habit of patients experiencing pain throughout surgery on third molar not only affects patient comfort but it also exudes the performance of the surgery, which results to longer duration of operation, stress assumption, and in few instances, there is need to use extra pathetic injections. It has been argued that accessory sensory innervation especially that of the mylohyoid, buccal, auriculotemporal, and greater auricular nerve might cause incomplete anesthesia after traditional IANB. Although additional blocks addressing the buccal nerve and lingual nerve have been thoroughly conducted, they are even less effective, and patients frequently complain of breakthrough pain.^{4,5} These constraints demonstrate the need to consider alternative or supplementary methods that go beyond the main mandibular nerve distribution.^{6,7}

The parotid region, angle of the mandible, and some sections of the ear are sensitized by a cervical plexus (C23; greater auricular

nerve), which serves the parotid region,⁸ and angle of the mandible region.⁹ Its course anatomy and sensory distribution congruence with that of the mandibular territory imply that it can be involved in providing accessory innervation in case of third molar surgery.¹⁰ Greater auricular nerve infiltration (GANI) is used with success in otolaryngology and head-and-neck surgery and specifically in postoperative analgesia during parotidectomy and with the microsurgery of the ear.¹¹ Nonetheless, little effort has been on its use in dental anesthesia. The role of GANI in oral surgery is an innovative and pertinent research focus, because the use of GANI in dentistry supports the anatomical feasibility of the procedure and is supported by reverse engineering, given the close connection to related surgical specialties.

The measurement of pain in surgery is expressed most frequently in validated scale, i.e., the Visual Analogue Scale (VAS), which gives a strong indication of subjective pain severity.^{12,13} In the context of oral and maxillofacial surgery, efficient pain management during the third molar removal procedure is directly associated with decreased perioperative stress levels, enhanced cooperation, and patient satisfaction in general.^{14,15} Further, reportedly poor anesthesia during surgery is a potential risk factor in relation to the increase in postoperative pain, which stresses the significance of comprehensive approaches to analgesics during operations.^{16,17}

In addition to intraosseous anesthesia, akinosi or Gow-Gates nerve blocks, several adjunctive methods have been also suggested in order to improve the effectiveness of IANB.¹⁸ Although such methods have proved up to differing degrees effective, none of them fully help to resolve the problem of accessory innervation. GANI provides a theoretically more comprehensive range of anesthetics by acting upon the greater auricular nerve, with the potential to surmount a setback of mandibular blocks and narcotics used alone. Notably, its application does not require any specific devices or require a significant time extension to the work process of the operations, which will become a relevant add-on in everyday oral surgery facilities.

It is based on this background that the current study was developed to examine the immunohistochemical expression of MMR proteins (MLH1, MSH2, MSH6, PMS2) in the cases of prostate carcinoma diagnosed through a tertiary care center in Pakistan. This study will help to use MMR protein loss frequency and pattern to add to existing literature on the molecular heterogeneity of prostate cancer, which may make a contribution to diagnosis, prognosis as well as treatment stratification in clinical practice.

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METHODOLOGY

This was a cross-sectional study that was done in the Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, between June 2023 and July 2024. A sample population involving 72 prostatic carcinoma cases were enrolled. Muscular biopsy, histology and demographic data were obtained through institutional databases. Only patients who were transurethral resection of the prostate (TURP) or radical prostatectomy was taken, but those with insufficient tissue blocks, neoadjuvants, inconclusive pathological results were not included to guarantee the quality of samples and diagnostics.

Tissue blocks that have been fixed in formaldehyde and embedded with paraffin were cut into 4 mm thick pieces and immunohistochemically (IHC) stained with the monoclonal antibodies against MLH-1, MSH-2, MSH-6, and PMS-2. The epitope retrieval was punched using a pressure chamber using heat treatment in the Albepure buffer (pH 6.0) and emission was subsequently performed on the tubes with the primary antibodies at optimized dilution standards. AHRQ Detection was done using a polymer-based horseradish peroxidase (HRP) system using diaminobenzidine (DAB) as a chromogen, and hematoxylin was used as a counterstain. Staining reliability was also verified using internal positive controls, typically lymphocytes and stromal cells, whereas negative controls were carried out, without primary antibodies and in parallel.

The analysis of IHC was undertaken by two senior histopathologists independent of each other and blinded to clinical outcome. Nuclear expression was regarded as intact, and full lack of nuclear enterprises in tumor cells, in favor of the presence of positive internal controls, were regarded as protein loss the mismatch repair deficiency (dMMR). Review of discordant cases were done together to achieve consensus.

To conduct statistical analysis relevant patient characteristics such as age, histological subtype, and IHC-stained expression profiles were put into SPSS version 26.0. Continuous variables were presented in terms of standard deviation plus mean whereas in terms of frequency and percentages or frequencies reasonably represented for categorical variables. The Chi-square test was used to identify associations between clinicopathological parameters and MMR deficiency and the p-value of less than 0.05 was considered significant.

RESULTS

A total of 72 patients with histologically confirmed prostatic carcinoma were included in the study. The mean age at presentation was 71.4 ± 8.2 years (range: 52–88 years). The majority of cases were diagnosed as acinar adenocarcinoma (59; 81.9%), followed by ductal adenocarcinoma (7; 9.7%) and other rare variants (6; 8.3%). Most patients presented with advanced-stage disease (≥pT2: 46; 63.9%).

Loss of immunohistochemical expression of at least one MMR protein was observed in 16 patients (22.2%), while intact expression was retained in 56 cases (77.8%). Among deficient cases, 1 (1.4%) demonstrated simultaneous loss of all four proteins, 2 (2.8%) showed isolated loss of MLH-1, 3 (4.2%) showed isolated loss of MSH-2, 10 (13.9%) exhibited PMS-2 loss, and 12 (16.7%) demonstrated MSH-6 loss.

No statistically significant association was observed between age, histological subtype, or tumor stage and the presence of MMR deficiency (p > 0.05).

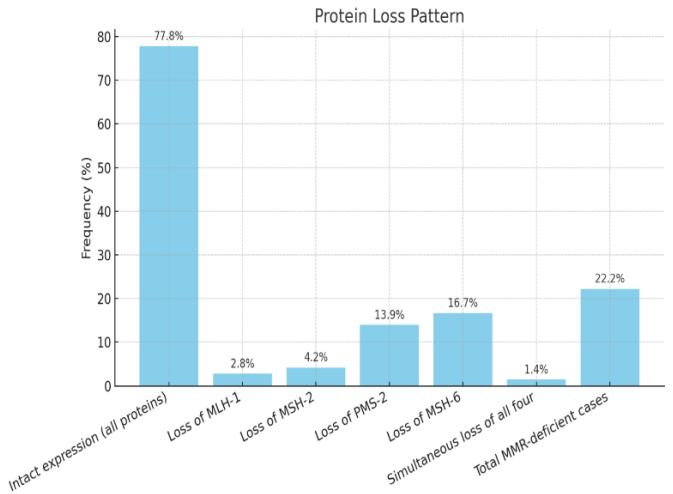
Table 1: Baseline Demographic and Clinical Characteristics of Patients (n = 72)

Variable		Frequency (%) / Mean ± SD
Mean age (years)		71.4 ± 8.2
Age range (years)		52–88
Histological subtype	Acinar adenocarcinoma	59 (81.9%)
	Ductal adenocarcinoma	7 (9.7%)
	Others (rare variants)	6 (8.3%)
Tumor stage (pathological)	pT1	26 (36.1%)
	≥pT2	46 (63.9%)

Table 2: Immunohistochemical Expression of MMR Proteins (n = 72)

Protein Loss Pattern	Frequency (%)
Intact expression (all proteins)	56 (77.8%)
Loss of MLH-1	2 (2.8%)
Loss of MSH-2	3 (4.2%)
Loss of PMS-2	10 (13.9%)
Loss of MSH-6	12 (16.7%)
Simultaneous loss of all four	1 (1.4%)
Total MMR-deficient cases	16 (22.2%)

Figure 1



DISCUSSION

The current research determined the incidence of mismatch repair (MMR) deficiency among patients with prostate carcinoma by immun-histochemistry (IHC) detection of MLH-1, MSH-2, MSH-6 and PMS-2 proteins. Out of 72 patients recruited, a baseline of one-quarter of the patients (22.2) shown to have lost at least one of the MMR proteins, the loss of PMS-2 and MSH-6 were the most common ones. The results offer valuable ideas on the demonstration of the molecular heterogeneity of prostate cancer and the emphasis on the newest feature of the MMR status as a possible biomarker in precision oncology.

There is previous evidence of wide variation of MMR deficiency by analyzing the effect on prostate cancer with ranges of 5% to 30% depending on the size of the cohort, methodology, and patient selection.¹⁹ Our prevalence of 22.2% is closer to the upper end of reported frequencies indicating that MMR deficiency is possibly a relatively higher prevalence of South Asian populations. These discrepancies may in part be attributed to variations in the genetic predisposition on geographic and ethnic factors, and to differences in tumor biology. Interestingly, PMS-2 and MSH-6 loss, which contributed to the preponderance of deficiencies in our series, have also been characterized as a mark of occurrence of an early and frequent event in DNA repair failure, whereas the MLH-1 loss was less common in prostate carcinoma than in colorectal and endometrial malignancy.

Clinically, characterisation of MMR deficiency in prostate cancer has immense therapeutic consequences. Tumors that repair DNA defectively have a high mutational burden, which results into enhanced neoantigen presentation and immunogenicity. This supports the clinical utility of immune checkpoint inhibitors, including pembrolizumab, in MMR-deficient or microsatellite instability-high (MSI-H) tumours of various cancer types. Although prostate cancer is not traditionally addressed with immunotherapy, there are recent positive clinical trials that have shown successful outcome in a small group of patients that either have MMR deficiency or are reported to have DNA repair defects. Therefore, a regular check of MMR status in advanced/high-risks would allow finding those that may receive immunotherapy, which would be translated into more individualized care plans.

Surprisingly our research did not consider any significant relationship between age, histological subtype, as well as, pathological stage, and MMR deficiency. This implies that loss of MMR can independently impact without traditional clinicopathological parameters, which backs up the fact that it is a discrete molecular group of prostate cancer. The same has been concluded in other reports, where MMR lack was not always related to the Gleason score or tumor stage, though some researchers concluded it to be associated with aggressive clinical behavior and low prognosis. Future research should be done using larger multicenter studies to confirm these associations and elucidate the prognostic role of MMR deficiency in prostate cancer.

Our strengths are based on standardized IHC protocols, assessment by senior pathologists in blind mode, and inclusion of in house controls so as to get the assessment accurate when it comes to protein expression. But there are some limitations that should be admitted. First, the sample size is rather small and can restrict the extrapolation of our results. Second, although IHC is a highly affordable and easily accessible method, it does not measure the microsatellite instability or underlying germline mutations directly. Molecular testing (PCR-based MSI assays or next-generation sequencing NGS) would give a more detailed assessment. Lastly, the absence of survival data more than two months follow-up makes it impossible to evaluate MMR deficiency prognostic value in our study.

Overall, our research work has shedding light on the fact that a significant percentage of patients with prostate carcinoma in our area are affected with MMR deficiency especially in the case of PMS-2 and MSH-6 membranes. The results suggest the importance of MMR status as a molecular target and therapy. Including regular screening of MMR in the diagnostic process of prostate cancer could help inform treatment choices as well as inform the beginnings of genetic counseling and family risk assessment. The future studies are based on the increased sample size, organising molecular analyses and investigating the response

to immune checkpoint-inhibitors in model of grotesque prostate cancer with MMR deficiency.

CONCLUSION

This study demonstrates that the adjunctive use of Greater Auricular Nerve Infiltration (GANI) with conventional IANB provides superior intraoperative analgesia in mandibular third molar surgery. Patients receiving GANI reported significantly lower pain scores and a higher proportion achieved pain-free procedures compared to those managed with IANB alone. Importantly, the addition of GANI did not increase operative duration, making it a practical enhancement to standard practice. Given the limitations of IANB and the anatomical contribution of accessory innervation, GANI emerges as a valuable adjunctive strategy for improving patient comfort, reducing breakthrough pain, and facilitating smoother surgical performance. Future multicenter randomized controlled trials are warranted to validate these findings and assess long-term outcomes, but the present evidence strongly supports the integration of GANI into routine oral surgery anesthesia protocols.

Authors' Contribution

RM: Concept & study design, data collection and drafting of the manuscript.

SM: Data entry, statistical analysis, interpretation of the results.

HP: Manuscript editing and critical revision of the manuscript.

All authors approved the final version of the manuscript to be published.

Conflict of Interest

The authors declared no conflict of interest.

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Data Availability

Data is available with authors and can be provided on request.

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