The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 2, Issue 3, 2024

UTILITY OF H3K27ME3 IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF MALIGNANT PERIPHERAL NERVE SHEATH TUMOUR: A CROSS-SECTIONAL STUDY FROM A TERTIARY CARE CENTRE IN PAKISTAN

Dr. M. Tariq Iqbal Khan[*1], Dr. Omer Rasheed Chughtai[2], Dr. Seher Bashir[3], Dr. Noreen Javaid[4], Dr. Moazzam Naqi Abbas[5]

*[*1,5]Resident Histopathologist, Department of Histopathology, Chughtai Institute of Pathology, Lahore, Pakistan*
[2,3]Consultant Histopathologist, Department of Histopathology, Chughtai Institute of Pathology, Lahore, Pakistan
[4]Fellow Consultant Histopathologist, Department of Histopathology, Chughtai Institute of Pathology, Lahore, Pakistan

[*1]medisolepro@gmail.com, [3]medisolepro@gmail.com, [4]medisolepro@gmail.com, [5]medisolepro@gmail.com

DOI: https://doi.org/10.5281/zenodo.20664479

Keywords
MPNST, H3K27me3, Immunohistochemistry, Neurofibromatosis type 1, Soft tissue sarcoma, Epigenetics.

Article History
Received: 03 November 2024
Accepted: 15 December 2024
Published: 30 December 2024

Copyright @Author
Corresponding Author: *
Dr. M. Tariq Iqbal Khan

Abstract

Objective: *To evaluate the diagnostic utility of H3K27me3 immunohistochemistry in malignant peripheral nerve sheath tumour (MPNST) and its correlation with clinicopathological features.*

Study Design: *Cross-sectional descriptive study.*

Place and Duration of Study: *Department of Histopathology, Chughtai Institute of Pathology, Lahore, Pakistan, over one year after ethical approval.*

Methodology: *A total of 40 histologically confirmed cases of MPNST were included. H3K27me3 immunostaining was performed on formalin-fixed paraffin-embedded (FFPE) tissue. Staining patterns were classified as complete loss, focal loss, mosaic loss, or retained expression. Findings were correlated with tumour grade, patient age, sex, and neurofibromatosis type 1 (NF1) status.*

Results: *Of 40 cases, 35 were evaluable for H3K27me3 expression (87.5%). Loss of expression (complete, focal, or mosaic) was observed in 21 cases (60.0%), with complete loss being the most common pattern (n = 13; 37.1%). The mean patient age was 54.9 ± 17.5 years, with male predominance (60%). The extremities were the most frequent tumour site (52.5%). Mosaic loss was observed in both NF1-associated cases in which H3K27me3 was evaluable. No statistically significant association was identified between H3K27me3 loss and tumour grade, patient age, or sex ($p > 0.05$).*

Conclusion: *H3K27me3 loss is a diagnostically useful immunohistochemical marker in MPNST, demonstrating loss in 60% of evaluable cases. Interpretation should be contextualised within the overall morphological and immunohistochemical profile.*

INTRODUCTION

Malignant peripheral nerve sheath tumor (MPNST) is a rare aggressive neural crest-derived soft tissue sarcoma that represents only 5–10% of all soft tissue malignancies. [1] Estimations from the literature suggest its occurrence in the general population is less than 0.001% but represents a relatively high clinical burden due

to aggressive behaviour, high probabilities of recurrence and poor overall prognosis. [2]

MPNST is either de novo (50% of cases), in the setting of neurofibromatosis type 1 (NF1) (40–50%), or post radiation therapy. [3] MPNST can be especially difficult to diagnose due to overlap of morphological and immunophenotypic features with a number of spindle cell neoplasms, including synovial sarcoma, fibrosarcoma, leiomyosarcoma and cellular schwannoma. Conventional immunohistochemical markers like S-100, SOX10, CD34, and EMA have low sensitivity and specificity alone but perform much better in grossly high grade tumours. [5]

Recently, the loss of H3K27 trimethylation (H3K27me3) has been identified as a novel epigenetic marker that is useful for diagnosing MPNST. The histone H3 lysine 27 trimethylation (H3K27me3) is a repressive PRC2-mediated histone modification. Global loss of H3K27me3 expression in tumour nuclei due to inactivating mutations in genes that encode subunits of polycomb repressive complex 2 (PRC2), most frequently EED or SUZ12. 6 These mutations are present in around half to two-thirds of sporadic and NF1-associated MPNSTs, and rarely detected in benign peripheral nerve sheath tumours as well as most histological mimics. [7]

Multiple studies have validated H3K27me3 loss as a sensitive and relatively specific marker for MPNST when interpreted in the appropriate clinical and morphological context. 8, 9 However, this marker has not been investigated in detail locally and local validation is warranted due to differences in tumour demographics, NF1 prevalence and institutional practice.

Objectives This study was conducted to assess the diagnostic value of H3K27me3 immunohistochemistry in histologically confirmed MPNST cases at a tertiary referral centre in Lahore, Pakistan and to correlate the mode of expression with clinicopathological parameters.

METHODOLOGY

This study is a cross sectional descriptive study conducted at the department of Histopathology Chughtai Institute of Pathology Lahore, Pakistan for one year after approval from Chughtai Institute of Pathology Institutional Review Board (IRB). Ethical aspects This study was carried out in compliance with the Declaration of Helsinki and informed written consent was given by all relevant parties.

All histologically confirmed MPNST diagnoses made during the study period in the UK, regardless of patient age, sex, tumour site or grade were included. The exclusion criteria were: MPNST cases not diagnosed, insufficient tissue for immunohistochemistry and missing clinical data. A total of 40 cases was selected was used as the cut-off number for minimum sample selection, providing a convenient sampling.

Demographic data (age, sex, tumour site and clinical history including NF1 status) were obtained on all cases. Surgical specimens and biopsy material were routinely formalin fixed and paraffin-embedded (FFPE). Tumour grading was determined using the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system, which included tumour differentiation score, mitotic count and necrosis score when applicable.

Immunohistochemistry for H3K27me3 was performed on 4 micron sections of FFPE tissue, using a validated antibody. Staining adequacy was confirmed by the presence of internal positive controls (non-neoplastic stromal cells, endothelial cells, and lymphocytes). The staining pattern was classified as; (i) complete loss - no nuclear staining in all tumour cells and intact internal controls, (ii) focal loss- loss in a minority (< 50%) of nuclei within the mutant cell population, (iii) mosaic loss - areas with preserved expression interspersed with areas of complete or focal loss within the same section (characteristic for NF1 tumours), or (iv) retained expression - preservation of nuclear staining in tumour cells.

The data was statistically analysed with SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Absolute and relative frequencies were presented for categorical variables. Statistical analysisCorrelation between the expression of H3K27me3 and clinicopathological variables (grade, age group, sex, NF1 status) was evaluated by Chi-square test or Fisher's exact test, depending on the data. A p value of < 0.05 was considered as statistically significant.

RESULTS

The study included forty histologically confirmed cases of MPNST. The average patient age was 54.9 ± 17.5 years (range, 17–83 years). Of the total cases, 24 (60.0%) male and 16 (40.0%) were female patients. The patients aged >60 years old (n = 16; 40.0%) were the most common age group, followed by the 40â€“60-year (n = 13; 32.5%) and <40-year groups (n =11; 27.5%).

The most common anatomic site was the extremities (n = 21; 52.5%) followed by trunk/chest wall (n = 10; 25%), head and neck (n = 5;12.5%) and spine, retroperitoneum or other sites (n=4;10%). Clinical history revealed NF1 in five patients (12.5%). FNCLCC grading was available in 25 cases, of which 12 high grade (Grade 3; 48.0%), eight intermediate grade (Grade 2; [32.0%) and five low grade cases (Grade 1; [20.0%). Fifteen were ungraded, mainly due to fragmented specimens or trucut biopsy material.

Table-I — Summary of clinicopathological characteristics.

Table-I: Clinicopathological Characteristics of Patients (n = 40)

Variable	Frequency (n)	Percentage (%)
Gender		
Male	24	60.0
Female	16	40.0
Age Group (years)		
< 40	11	27.5
40–60	13	32.5
> 60	16	40.0
Tumour Site		
Extremities	21	52.5
Trunk / Chest Wall	10	25.0
Head & Neck	5	12.5
Spine / Retroperitoneum / Others	4	10.0
NF1 Status		
Present	5	12.5
Absent	35	87.5
FNCLCC Grade (n = 25 graded)		
Grade 1 / Low Grade	5	20.0
Grade 2 / Intermediate Grade	8	32.0
Grade 3 / High Grade	12	48.0

Immunostaining for H3K27me3 was evaluable in 35 of the 40 (87.5%) cases. The other five cases were not evaluable for staining due to lack of appropriate internal positive controls or poor tissue preservation. Among the 35 evaluable cases, loss of H3K27me3 expression in was detected in 21 (60.0%) and retained expression was found in 14 (40.0%).

Of the 21 cases with loss, complete loss was the most dominant pattern seen in 13 (37.1%) patients. Six patients (17.1 %) had sections showing focal loss, and two of them (5.7 %) were diagnosed with mosaic loss. Mosaic loss of

H3K27me3 was observed in both NF1-associated cases where it could be evaluated, which is consistent with the established association of this pattern with NF1-related MPNST. H3K27me3 expression pattern is shown in Table-II.

Table-II: H3K27me3 Expression Patterns in Evaluable Cases (n = 35)

Expression Pattern	Frequency (n)	Percentage (%)
Complete Loss	13	37.1
Focal Loss	6	17.1
Mosaic Loss	2	5.7
Total Loss	**21**	**60.0**
Retained Expression	14	40.0
Total Evaluable	**35**	**100.0**

There was no statistically significant association between loss of H3K27me3 with either tumour grade ($p = 0.43$), patient age group ($p = 0.61$) or sex ($p = 0.72$). A formal statistical comparison of H3K27me3 loss with NF1 status was not conducted due to the limited number of evaluable NF1-associated cases (n = 3).

DISCUSSION

Despite the absence of a single highly specific immunohistochemical marker and significant morphological similarity to other spindle cell sarcomas, MPNST remains one of the more challenging soft tissue sarcomas to diagnose. In this field, some achievements have been made with the identification of epigenetically regulated diagnostic markers like H3K27me3.

In the current study, 21/35 cases of evaluable MPNSTs showed a loss of expression for H3K27me3. This rate falls within previously reported data, in which loss was observed to be between 50-70%, which varies based on population studied and technical methodology (17). The landmark study of 140 cases by Prieto-Granada et al., found a loss of H3K27me3 in 67.6% MPNSTs, and significantly lower rates in benign nerve sheath tumours and other mimics [8,10]. In our experience, this utility is also a diagnostic tool in an institutional setting of South Asia.

Complete loss (37.1%) was the most frequent pattern observed and has been reported in previous publications, and is hypothesized to be due to biallelic inactivation of PRC2 component genes (EED or SUZ12) in all tumour cells. By contrast, in our evaluable cohort, only one case had mosaic loss (mosaic with alternating zones of loss and retention) and that was a case with NF1. This mosaic pattern is now believed to be related to a genomic heterogeneity between NF1 related MPNST, and is considered to be an informative diagnostic pattern which should not be ignored as a retained finding. 12,13

The majority of the tumours were in the extremities (52.5%) and the trunk (25.0%). This anatomic distribution is consistent with published series, which have always shown the extremities as a significant site along with the paraspinal areas. The average age of 54.9 years and the predominance of males (60%) was generally similar to what was reported in the literature, with some series showing a more even sex distribution. 2,4

Overall FNCLCC grading was feasible in only 62.5% of cases (25/40) this was by far the fraction most likely due to important specimen composition being comprised of trucut biopsies or fragmented excisions where necrosis and tumour differentiation assessment was limited. More cases were graded (48.0%) than ungraded and, as expected, there was a greater proportion of high grade tumours, especially when presented with NF1 and retroperitoneal and/or deep-seated tumours. 14

Our absence of statistical correlation between H3K27me3 expression with tumour grade is in agreement with several other groups. This is in line with the hypothesis that loss of H3K27me3 most likely indicates a diagnostic rather than a histological grade or a form of tumour aggressive

behaviour, as there was dysregulation of any process other than PRC2 inactivation. Similarly, there were no age or sex associations which is not surprising, as the mutation mechanism of EED/SUZ12 is not specific.

The non-evaluable rate is interesting, with a 12.5% in our series. Technical non-evaluability is due to different reasons such as small biopsy size, over fixation in formalin and artefact caused by ischaemia in tissue. Reliable intratumoral endothelial cells and stromal fibroblasts can assist in ensuring strong internal positive controls to interpret with accuracy. It demonstrates the importance of having standardized technical protocols in testing centres for H3K27me3.

The main limitation of this study is the number of people in the sample. This is a large institutional series of 40 cases, but not sufficient to analyze in multivariate fashion by strong associations or subgroups, particularly for the NF1-associated cases. In such a study, this is not the optimal and most rigorous evaluation in an experimental setting, as might be achieved in a prospective multi-institutive design. Lastly, the findings would have been molecularly confirmed by correlation with loss of expression for EED/SUZ12 mutations. The next steps in this area should be further integration of next generation sequencing platforms with the results of immunohistochemical analysis for even further refinement of diagnostic accuracy.

CONCLUSION

In this institutional series, loss of H3K27me3 expression was shown in a growing proportion (60.0%) of evaluable MPNST cases with complete loss the predominant pattern and mosaic loss seen only in NF1-associated tumours. Immunohistochemistry for H3K27me3 is a diagnostically valuable and practically available adjunct for MPNST, particularly useful in separating it from benign peripheral nerve sheath tumours and other spindle cell mimics. Interpretation should be done in conjunction with morphological features and a full spectrum of an immunohistochemical panel. Centres that start incorporating this marker in their diagnostic workflow should standardise technical protocols and provide pattern recognition training.

CONFLICT OF INTEREST: None declared.

FUNDING SOURCE: None.

REFERENCES

Yao C, Zhou H, Dong Y, Alhaskawi A, Wang Z, Lai J, et al. Malignant peripheral nerve sheath tumors: current concepts in pathogenesis and management. Cancers (Basel). 2023;15(4):1077. doi: 10.3390/cancers15041077

Mack T, Purgina B. Malignant peripheral nerve sheath tumor: advances in diagnosis and biomarkers. Curr Oncol. 2022;29(9):6400-18. doi: 10.3390/curroncol29090504

Kim A, Stewart DR. Neurofibromatosis type 1 and associated tumors. Curr Opin Oncol. 2022;34(1):31-7. doi: 10.1097/CCO.0000000000000793

Schaefer IM, Fletcher CDM. Recent advances in the diagnosis of soft tissue tumours. Histopathology. 2022;80(1):3-19. doi: 10.1111/his.14513

Thway K, Fisher C. Immunohistochemistry in soft tissue tumours: new markers and evolving applications. Histopathology. 2021;78(1):100-18. doi: 10.1111/his.14225

Ito Y, Kohashi K, Endo M, Yoshimoto M, Ishihara S, Toda Y, et al. Clinicopathological significance and diagnostic utility of H3K27 methylation status in malignant peripheral nerve sheath tumors. Virchows Arch. 2021;479(6):1233-44. doi: 10.1007/s00428-021-03189-0

Yoshimoto M, Kobashi K, Endo M, Ishihara S, Toda Y, Oda Y, et al. Clinicopathological and prognostic significance of H3K27 methylation status in malignant peripheral nerve sheath tumors. Diagn Pathol. 2021;16(1):89. doi: 10.1186/s13000-021-01140-0

Lyskjaer I, Lindsay D, Tirabosco R, Steele CD, Lombard P, Strobl AC, et al. H3K27me3 expression and methylation status in histological variants of malignant peripheral nerve sheath tumours. J Pathol. 2020;252(2):151-64. doi: 10.1002/path.5507

Prieto-Granada CN, Wiesner T, Messina JL, Verra JC, Torres-Cabala CA, Jungbluth AA, et al. Loss of H3K27me3 expression is a highly sensitive marker for sporadic and radiation-induced MPNST. Am J Surg Pathol. 2016;40(4):479-89. doi: 10.1097/PAS.0000000000000564

Yordama W, Evelina E, Kodarah R, Nahandhita A, Kanwogbach D, Kawogochi K, et al. Cell sarcoma mimicking MPNST: analysis of H3K27me3 expression and immunohistochemistry in malignant peripheral nerve sheath tumor (MPNST) and other spindle cell sarcoma mimics from Indonesia. Asian Pac J Cancer Prev. 2020 Mar 1;21(3):699-705. doi: 10.31557/APJCP.2020.21.3.699. PMID: 32212796; PMCID: PMC7437312

Jo VY, Fletcher CDM. WHO classification updates in soft tissue and bone tumours. Semin Diagn Pathol. 2021;38(1):2-12. doi: 10.1053/j.semdp.2020.10.001

Patel RM, Downs-Kelly E, Weiss SW. Diagnostic challenges in malignant peripheral nerve sheath tumor: a clinicopathological review with emphasis on emerging ancillary markers. Arch Pathol Lab Med. 2021;145(9):1123-30. doi: 10.5858/arpa.2020-0437-RA

Zilla ML, John I, Naous R. H3K27me3 loss as a diagnostic pitfall: histological mimics of malignant peripheral nerve sheath tumor. Int J Surg Pathol. 2024;32(6):1089-94. doi: 10.1177/10668969231215819

Chmiel P, Szumera-Cieczkiewicz A, Bartnik E, Rutkowski P. FNCLCC grading and survival outcomes in malignant peripheral nerve sheath tumors: a systematic review. Curr Oncol. 2024;31(1):307-23. doi: 10.3390/curroncol31010020

Wang J, Liu L, Li Z, Chen M, Xu Z, Ma R, et al. Regulation of H3K27me3 modification and its implication in tumour diagnosis and therapy. BMC Genomics. 2024;25:225. doi: 10.1186/s12864-024-10120-9

IEER
Excellence in Education & Research