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Analysis of H3k27me3 Expression in *Malignant Peripheral Nerve Sheath Tumor* (MPNST) and Other Spindle Cell Sarcoma Mimicking MPNST

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ABSTRACT

Malignant Peripheral Nerve Sheath Tumor (MPNST) is a type of spindle cell sarcoma with approximately 5% of all sarcomas. Its diagnosis is challenging due to the absence of specific immunohistochemical markers. Recently, H3K27me3 was discovered as a potential specific immunohistochemical marker to differentiate MPNST from other sarcomas and distinguish between low and high-grade MPNST. Therefore, this research aims to investigate the use of the H3K27me3 as a potential specific marker for Malignant Peripheral Nerve Sheath Tumor (MPNST). A cross-sectional analysis was conducted on 50 cases of sarcomas, including 13 MPNST, 14 synovial sarcomas, 13 dermatofibrosarcoma protuberans (DFSP), and 10 leiomyosarcomas originating from the Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital (FMUI-CMH) from January 2013 to December 2021. H3K27me3 images were obtained and categorized as complete loss when more than 95% of the tumor cells showed loss of nuclear staining. The results found in MPNST showed ~~a~~ loss of H3K27me3 expression, which was statistically significant compared to other sarcomas mimicking MPNST (p=0.021), indicating its potential as a diagnostic marker. There was a difference in the expression of H3K27me3 between the high and low-grade MPNST but it was not statistically significant (p=0.105). This showed that H3K27me3 loss of expression can be used to diagnose MPNST, especially high-grade MPNST, and differentiate it from other sarcomas mimicking MPNST.

Keywords: MPNST, non-MPNST, H3K27me3

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INTRODUCTION

Malignant Peripheral Nerve Sheath Tumor (MPNST) is a malignant tumor of the peripheral nerve sheath that originates from benign nerve sheath tumors or in patients suffering from type 1 neurofibromatosis. It can occur sporadically, in association with radiotherapy or neurofibromatosis type 1. The symptoms of MPNST are in form of a mass, which may be accompanied by pain, especially in cases related to NF1. Other possible symptoms include paresthesia, motor weakness, and radicular pain in cases originating from the nerves. These tumors can grow more than 5 cm and have a gray-white, fleshy cross-section with areas of necrosis and bleeding.[1,2]

MPNST is a rare type of tumor that constitutes between 3% to 10% of total cases of soft tissue sarcoma. The microscopic appearance of MPNST varies, presenting spindle-shaped cells that arrange to form long pathways with alternating hypercellular areas. There are still features that resemble other spindle cell tumors, such as synonial sarcoma or DFSP. The features such as blood vessel structures like hemangiopericytoma. However, due to the absence of a characteristic histopathological picture and specific molecular markers for MPNST, the diagnosis of this tumor is established by eliminating the differential diagnosis.[1-3]

According to Mito et al[4], MPNST is related to mutations in the Polycomb Repressive Complex 2 (PRC2) gene complex, which causes loss of H3K27me3 protein expression. The expression pattern of H3K27me3 in MPNST is different from other tumors with a similar histopathological appearance. Pekmezci et al[5] and Asano et al[7] stated that there were differences in the expression of H3K27me3 in various grades of MPNST malignancy and other tumors with histopathological features mimicking MPNST. The relatively lower expression of H3K27me3 in MPNST compared to other similar tumors is thought to contribute to the tumorigenesis processes in MPNST.[4-6]

The inactivation of the PRC2 complex by mutations in the PRC2 subunit embryonic ectoderm development (EED) or suppressor of zeste 12 (SUZ12) will cause the loss of trimethylation on lysine 27 on histone H3 (H3K27me3). The catalytic process of the PRC2 complex results in the formation of H3k27me3, which is essential for regulating tumorigenesis processes. However, when there is a mutation in the PRC2 complex, H3K27me3 cannot form and it will be replaced by H3K27ac, causing an increase in tumorigenesis processes. In the case of MPNST, mutations in the PRC2 complex are suspected, leading to loss of H3K27me3 expression that helps in establishing the diagnosis of MPNST.[4,5]

H3K27me3 has the potential as a specific marker for MPNST. Lyskjaer et al[7] discovered that H3K27me3 expression is significantly influenced by the tumor morphology, where the loss of expression is more frequently found in high-grade MPNST compared to low grade MPNST. H3K27me3 expression has not been used in diagnosing MPNST in Indonesia due to the varying results. Therefore, this research aims to determine the relationship between H3K27me3 expression and the malignancy level of MPNST, as well as its differences in other tumors with similar histological features.[4,7]

Diagnosis of MPNST remains a challenge due to its non-specific histological features and the absence of specific molecular markers. Although the loss of H3K27me3 expression is suspected to be related to MPNST, various research still shows different results. Some investigations suggested that the loss of H3K27me3 expression is related to the malignancy level of MPNST. However, this result needs further investigation to confirm whether H3K27me3 can be used as a diagnostic marker for MPNST.[1,2]

MATERIAL AND PROCEDURE

This is analytical research with a cross-sectional design. The samples used were secondary data obtained from the archive of the Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital for a period of 9 years, ranging from January 1st, 2013 to December 31st, 2021. The histopathology specimens that met the inclusion and exclusion criteria were analyzed based on their immunohistochemistry staining. The inclusion criteria were cases with histopathological diagnosis of MPNST and spindle cell sarcoma mimicking MPNST that passed through immunohistochemical examination, as well as cases of synovial

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sarcoma, leiomyosarcoma, and DFSP. The exclusion criteria were cases with inadequate/damaged or not found paraffin blocks.

All 50 cases that met the inclusion and exclusion criteria and were suitable for analysis were sampled using total sampling. The evaluated histopathology immunohisto-chemistry specimens were stained with hematoxylin-eosin and H3K27me3 markers, respectively.

The histopathology and immuno-histochemistry specimens were reviewed by WIL and EVE to confirm the diagnosis of the samples. Subsequently, H3K27me3 staining was performed on all samples semi-quantitatively, referring to Mito et al[4], based on the percentage of stained cells, assessed with the categories of complete loss, partial loss, and retained. The complete loss was defined as the loss of expression in more than 95% of tumor cells, the partial loss was defined as the loss of expression in 5%-95% of tumor cells, and retained was defined as the loss of expression in less than 5%. For data analysis, the complete loss category was one group, while the partial loss and retained categories were grouped.

The H3K27me3 expression was assessed blindly by WIL and EVE. The screening was performed on the entire tumor area and continued with photographing 5 representative fields of view using an Olympus CX23 microscope equipped with a camera. The percentage of tumor cells that did not express H3K27me3 (loss of expression) was calculated relative to all tumor cells in the 5 fields of view, using a minimum criterion of 500 cells. Endothelial and lymphoid cells were used as the internal positive control for H3k27me3 and these cells were positively stained in the assessed specimens. The negative control was an MPNST case that did not receive a primary antibody, while a spindle cell sarcoma mimicking MPNST (synovial sarcoma) was used as the positive control.

The data were analyzed using SPSS 20.0, and comparative tests were performed with the Chi-square test. When the requirements for the Chi-square test were not met, the analysis was performed using Fisher's exact test and the data were considered significant when the p-value was <0.05.

RESULT

The data used were obtained from the Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital from January 2013 to December 2021. The 50 cases of sarcomas identified included 17 MPNST, 35 synovial sarcomas, 29 DFSP, and 25 leiomyosarcomas. Their diagnosis was confirmed based on histopathological and immunohistochemical examinations. A total of 50 cases, consisting of 13 cases of MPNST and 37 cases of non-MPNST (14 synovial sarcomas, 13 DFSP, and 10 leiomyosarcomas), fulfilled the inclusion and exclusion criteria. The data on the characteristics of the sample are presented in Table 1.

Table 1. The characteristic data of the research sample.

Demographic data	MPNST (n=13)	Non-MPNST (n=37)		
		Synovial sarcoma (n=14)	DFSP (n=13)	Leiomyo sarcoma (n=10)
Gender				
Male	8 (61.5%)	8 (57.14%)	9 (69.2%)	3 (30%)
Female	5 (38.5%)	6 (42.86%)	4 (30.8%)	7 (70%)
Age (year)				
$\bar{X}$ ± standard deviation	41.00±20.30	24.50 ± 8.50	48.54±16.65	44.50±9.71
Tumor location				
Upper extremities	2 (15.4%)	3 (20%)	3 (23.1%)	1 (10%)
Lower extremities	5 (38.5%)	8 (57.14%)	2 (15.4%)	2 (20%)
Trunk	3 (23%)	2 (13.3%)	5 (38.4%)	5 (50%)
Head and neck	2 (15.4%)	1 (6.7%)	3 (23.1%)	0 (0%)
Other areas	1 (7.7%)	0 (0%)	0 (0%)	2(2%)

In this study, most of the low-grade MPNST cases had high cellularity. A total of 3 cases had high cellularity, 1 case showed moderate cellularity, while all 9 cases of high-grade MPNST had high cellularity. Among the non-MPNST cases, 8 cases had moderate cellularity and 29 cases had high cellularity. The fascicular pattern was found in all cases of MPNST, where one high-grade MPNST case showed a herringbone pattern, and 2 low-grade

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MPNST cases showed similar patterns. Among non-MPNST cases, a herringbone pattern was found in 2 cases of synovial sarcoma and 3 cases of DFSP (fibrosarcomatous variant). Furthermore, a lobular pattern was found in 1 case of synovial sarcoma and 1 case of leiomyosarcoma. A haphazard pattern was found in 3 cases of leiomyosarcoma. A storiform pattern was found in 2 cases of high-grade MPNST, 1 case of low-grade MPNST, and 2 cases of synovial sarcoma.

Marble-like patterns were observed in 2 cases of high-grade MPNST and 2 cases of synovial sarcoma, while all cases of MPNST exhibited spindle-shaped cells. Wavy-shaped nuclei were observed in 1 case of high-grade MPNST and 2 cases of low-grade MPNST. Leiomyosarcoma showed spindle-shaped cells with cigar-shaped nuclei in all 4 cases. Eosinophilic cytoplasm was found in all samples, but 6 cases of synovial sarcomas exhibited tumor cells with little cytoplasm.

In high-grade MPNST, 8 cases had mitosis ≥10/10 HPF and 1 case had mitosis <10/10 HPF, while all cases of low-grade MPNST exhibited mitosis <10/10 HPF. Among the 16 non-MPNST cases, mitosis was <10/10 HPF, and out of the 21 non-MPNST cases, mitosis was ≥10/10 HPF. Necrotic areas were found in all high-grade MPNST cases and 25 non-MPNST cases. Hemangiopericytoma-like patterns of blood vessels were observed in 2 cases of high-grade MPNST, 1 case of low-grade MPNST, and 3 cases of non-MPNST, including 2 synovial sarcoma and 1 DFSP. Information regarding the histopathological features of the subjects is presented in Table 2.

Table 2. Histopathological features of research samples.

	MPNST		Non-MPNST		
	High grade (n=9)	Low grade (n=4)	Synovial sarcoma (n=14)	DFSP (n=13)	LMS (n=10)
Tumor cellularity					
Low	0	0	0	0	0
Average	0	1	2	0	6
High	9	3	12	13	4
Growth pattern					
Fascicular	9	4	13	3	7
Herringbone pattern	1	2	2	3	0
Solid	0	0	1	0	1
Haphazard	0	0	0	0	3
Storiform	2	1	0	13	2
Marble-like pattern	2	0	2	0	0
Tumor cell					
Shape					
Spindel	9	4	11	13	9
Round/oval	6	1	10	5	4
Nuclei					
Spindle	9	4	0	0	0
Oval	0	0	14	13	10
Cigar	0	0	0	0	4
Wavy	1	2	0	0	0
Nuclei Pleomorphism					
Mild	0	0	0	5	0
Moderate	6	4	14	8	3
Bizzare	3	0	0	0	7
Cytoplasm					
Eosinophilic	9	4	14	13	10
Clear	0	0	0	0	0
Rare	0	0	6	0	0
Mitotic activity					
3-9/10 HPF	1	4	7	7	2
>10/10 HPF	8	0	7	6	8
Necrosis	9	0	12	4	9
Blood vessels hemangio pericytoma-like pattern	2	1	2	1	0

The loss of expression of H3K27me3 was evaluated by the percentage of tumor cells that showed loss of nuclear staining, along with the positively stained internal controls. A positive control was used from tumor tissue of synovial sarcoma that was positively stained in the tumor cell nucleus. The expression of H3K27me3 was classified into 3 categories,

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namely complete loss (loss of expression in more than 95% of tumor cells), partial loss (loss of expression in 5% to 95% of tumor cells), and retained (loss of expression in less than 5% of tumor cells), as shown in Figure 1 and 2. For data analysis, the complete loss category was one group, while the partial loss and retained categories were grouped.

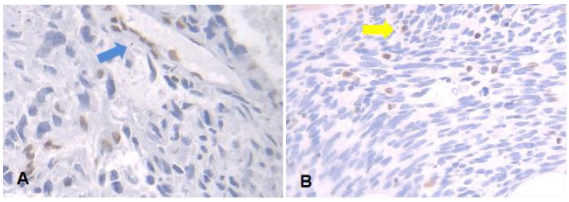

Figure 1. Staining of H3K27me3 positive in internal control. A. Positive staining in blood vessel endothelium (blue arrow) (400 times). B. Positive staining in lymphocyte cell (yellow arrow) (400 times).

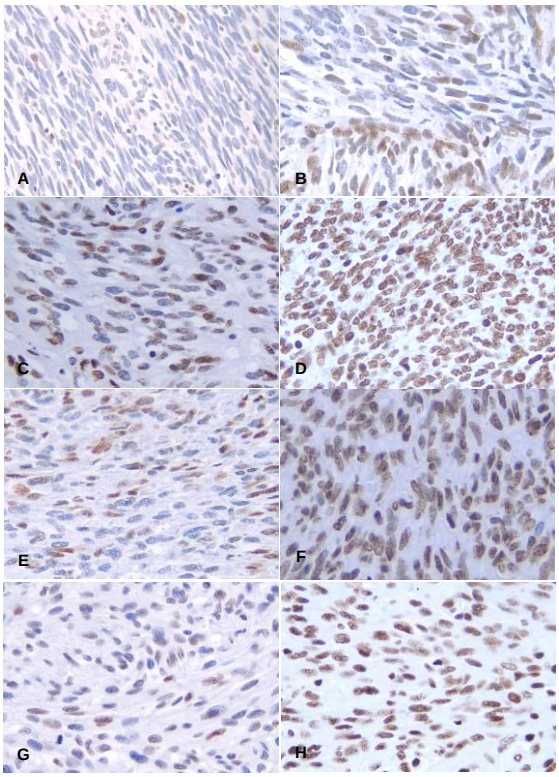

Figure 2. H3K27me3 expression in MPNST and non-MPNST. A. MPNST: complete loss (less than 5% of tumor cells retained) (400 times). B. MPNST: partial loss (5-95% of tumor cells retained) (400 times). C. Synovial sarcoma: partial loss (400 times). D. Synovial sarcoma: retained (400 times). E. DFSP: partial loss (400 times). F. DFSP: retained (400 times). G. Leiomyosarcoma: partial loss (400 times). H. Leiomyosarcoma: retained (400 times).

Table 3 shows the results of statistical testing, which indicated a significant difference in the proportion of H3K27me3 protein expression between the histopathological status of MPNST and non-MPNST (X2 test, p=0.021).

Based on further analysis by differentiating the non-MPNST group, the results showed a non-significant difference in the proportion of H3K27me3 expression between MPNST and the synovial sarcoma (X2 test, p=0.209). However, there was a significant difference in the proportion of H3K27me3 expression between the histopathological status of MPNST and DFSP (X2 test, p=0.039). In the MPNST and leiomyosarcoma groups, there were non-significant results between the proportion of H3K27me3 expression in the MPNST and the leiomyosarcoma groups (X2 test, p=0.179).

There were 13 cases of MPNST, consisting of 4 low-grade and 9 high-grade MPNST. A non-significant result was found between the proportion of H3K27me3 expression in the high-and low-grade MPNST groups (X2 test, p=0.105). The results of H3K27me3 staining based on the grade of MPNST malignancy are presented in Table 4.

A total of 3 non-MPNST cases experienced complete loss, making them more consistent with MPNST. In the first case, non-specific S100 staining, positive CD99, and TLE1 staining were found, initially diagnosed as synovial sarcoma, but H3K27me3 staining revealed complete loss. In the second case, S100 was negative and CD99 was positive, initially diagnosed as synovial sarcoma, while H3K27me3 staining showed a complete loss. In the third case, S100 and desmin were negative, SMA was partially positive, and CD34 was only stained in blood vessels, initially diagnosed as leiomyosarcoma, while H3K27me3 staining revealed complete loss. The diagnoses of these three cases were more indicative of MPNST.

A diagnostic test was also performed in this research to assess the use of the H3K27me3 immunohistochemical expression test as a predictor of the histopathological status of MPNST and non-MPNST. The results of a proportion test analysis of the 2 methods showed no significant difference (McNemar test, p=0.227), as presented in Table 5. Furthermore, the conformity analysis between the 2 methods showed a kappa value of 0.347.

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Table 3. H3K27me3 staining of MPNST and other sarcomas that have a histological appearance mimicking MPNST.

	Histopathology		Total	*P score* (x^2*test)*
	MPNST	Non-MPNST		
H3K27me3 complete loss	5 (62.5%)	3 (37.5%)	8	0.021
H3K27me3 retained	8 (19.05%)	34 (80.95%)	42	

Table 4. H3K27me3 staining on MPNST based on the grade of MPNST malignancy.

	H3K27me3		Total	*P score* (x^2*test)*
	Complete loss	Retained		
High-grade MPNST	5 (66.67%)	4 (33.33%)	9	0.105
Low-grade MPNST	0 (0%)	4 (100%)	4	
Total	5	8	13	

Tabel 5. McNemar test.

	Histopathology		Total	*P score (McNemar test)*
	MPNST	Non-MPNST		
H3K27me3 complete loss	5 (62.5%)	3 (37.5%)	8	0.227
H3K27me3 retained	8 (19.05%)	34 (80.95%)	42	

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) analyses are listed in Table 6. The results indicated that H3k27me3 was more useful for diagnosing non-MPNST based on the obtained high specificity (91.9%) and high negative predictive value (NPV) (80.9%).

Table 6. Diagnostic test.

	H3K27me3
Sensitivity	38.5% (12-65)
Specificity	91.9% (83-100)
Positive Predictive Value (PPV)	62.5% (29-96)
Negative Predictive Value (NPV)	80.9% (69-93)

DISCUSSION

Data on the sample characteristics of MPNST based on gender revealed a total of 13 cases consisting of 8 males and 5 females. Some literature stated that the male and female populations with MPNST are equal, while others reported a high prevalence in males. In this research, males were higher than females with MPNST.[1,2,4,7-9]

The sample characteristics data of non-MPNST based on gender showed 20 cases in males and 14 cases in females. The non-MPNST cases consisted of synovial sarcoma, DFSP, and leiomyosarcoma. In synovial sarcoma cases, 8 males and 6 females were found. Some literature suggested that the population of synovial sarcoma was slightly more common in males.[2,10] In DFSP cases, 9 males and 4 females were found, which occurred more frequently in males.[2,11] Meanwhile, there were 3 males and 7 females in leiomyosarcoma, as discovered in previous research where females were more likely to suffer from this condition.[2,12]

The mean ages for MPNST, synovial sarcoma, DFSP, and leiomyosarcoma were 41, 24.5, 48.54, and 44.5 years, respectively, which were not significantly different from the existing results.[1,2,5,10-12]

On the immunohistochemistry staining of H3K27me3, for MPNST cases, data on tumor location showed 2 cases in the upper extremities, 5 in the lower extremities, 3 in the trunk, 2 in the head and neck area, and 1 in other areas. According to the literature, MPNST is more commonly found in the upper and lower extremities as well as the trunk, which is related to the location of major nerves that are often affected, such as the sciatic nerve, brachial plexus, and sacral plexus.[1,2]

The data on tumor location for synovial sarcoma cases showed 3 cases in the upper extremities, 8 in the lower extremities, 2 in the trunk, as well as 1 in the head and neck area. According to the literature, synovial sarcoma can occur anywhere, with the most common location being in the lower extremities.[2,10]

For DFSP cases, data on tumor location showed 3 cases in the upper extremities, 2 in the lower extremities, 5 in the trunk, and 3 in the head and neck area. Based on previous research, DFSP can occur in various locations, but mostly at the trunk and proximal extremities.[2,11]

For leiomyosarcoma cases, data on tumor location showed 1 case in the upper extremities, 2 in the lower extremities, 5 in the trunk, and 2 in other areas. According to the literature, leiomyosarcoma is often found in

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areas with large blood vessels, and in the extremities.[2,12]

In MPNST, 5 cases of complete loss and 8 cases of partial loss were found. In synovial sarcoma, there were 2 cases of complete loss, 7 cases of partial loss, and 5 cases of retention. In DFSP, there were 9 cases of partial loss and 4 cases of retention. In leiomyosarcoma, there was 1 case of complete loss, 7 cases of partial loss, and 2 cases of retention. This research found a significant difference in the proportion of H3K27me3 expression between histopathological diagnoses of MPNST and non-MPNST. Further analysis of the proportion of H3K27me3 protein between MPNST and DFSP also showed the same results. However, different values were obtained for the proportion of H3K27me3 expression between MPNST and synovial sarcoma, as well as MPNST and leiomyosarcoma, where there were no significant differences in the proportion of H3K27me3 protein. This condition can be caused by the limited sample size.

This research grouped MPNST into 2 categories, namely low-grade and high-grade, consisting of 4 and 9 cases, respectively. All cases of low-grade MPNST consisted of partial loss, while there were 5 and 4 cases of complete loss and partial loss (retained group), respectively, in the high-grade MPNST. However, there was no significant difference in the proportion of H3K27me3 expression between the high-grade and low-grade MPNST groups.

Among all MPNST cases, partial loss of H3K27me3 was observed in 4 cases of low-grade MPNST showed a partial loss and 4 high-grade MPNST. Meanwhile, a complete loss was found in 5 cases of high-grade MPNST. In the non-MPNST cases, 3 cases were initially classified as non-MPNST, including 2 cases of synovial sarcoma and 1 leiomyosarcoma. After H3K27me3 staining, they were more likely to be classified as MPNST because more than 95% of tumor cells did not express H3K27me3 (complete loss group).

The loss of H3K27me3 expression in MPNST can vary based on the grade of the tumor's malignancy. In high-grade MPNST, more loss of H3K27me3 expression was observed than in low-grade MPNST. Schaefer et al[13] reported that 25 (83%) of 30 cases of high-grade MPNST exhibited a complete absence of staining for H3k27me3. This was also observed in 17 (59%) of 29 cases of intermediate-grade MPNST, and 9 cases (29%) of 31 cases of low-grade MPNST, which were in the negative H3K27me3 group. Among 200 non-MPNST cases examined, 4 cases also showed a complete absence of staining for H3K27me3, while the others expressed H3K27me3, which were the positive and heterogeneous H3K27me3 group.

Mustapar et al[14] discovered 9 cases of high-grade MPNST with a score of 0, indicating no tumor cells expressing H3K27me3, with 4 cases exhibiting a score of 1+ (1-24% of tumor cells expressing H3K27me3), and 1 case with a score of 3+ (more than 50% of tumor cells expressing H3K27me3). There were also 2 cases of intermediate-grade MPNST with a score of 0 and 2 cases of low-grade MPNST with a score of 2+ (25-49% of tumor cells expressing H3K27me3). Out of the 41 non-MPNST cases, a total of 3, 8, 13, and 17 cases had a score of 0, 1+, 2+, and 3+, respectively.

According to Schaefer et al[13] and Mustapar et al[14], high-grade MPNST experienced more loss of H3K27me3 expression compared to low-grade. This was consistent with this research, where all low-grade MPNST cases experienced partial loss. In high-grade MPNST cases, 4 cases had a partial loss and 5 cases had a complete loss. Therefore, further examination is needed for cases of high-grade MPNST that fall into the partial loss group for diagnostic purposes.

In MPNST, there were no specific histopathological features. The marble-like pattern, as one of the characteristics of MPNST, was also observed in cases of synovial sarcoma. The herringbone pattern was found in cases of both low-grade and high-grade MPNST, as well as in non-MPNST cases such as DFSP (fibrosarcomatous DFSP variant) and synovial sarcoma.

A total of 3 non-MPNST cases had a complete loss, which leaned towards the MPNST group. In the first case, histopathological features such as a fascicular growth pattern accompanied by a herringbone pattern and marble-like pattern were found. Mitotic activity was <10/10 HPF and necrotic areas were also presented in this case, as shown in MPNST cases. Immunohistochemical staining showed non-specific S100 staining, while CD99 and TLE1 staining were positive, leading to an initial diagnosis of synovial sarcoma. However, only a few tumor cells were stained with H3K27me3 (complete loss). Synovial sarcoma

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is one of the tumors with histopathological features similar to MPNST. According to Shaikh M, CD99 staining can be positive in MPNST. Additional investigations such as TLE1 immunohistochemical staining can differentiate synovial sarcoma from MPNST, but in some MPNST cases, TLE1 can also be positive. Therefore, further examination is needed to confirm the SS18-SSX1/2 gene fusion marker that is definitive for synovial sarcoma.[4,10,15]

In the second case, the histopathological findings showed a fascicular growth pattern with a marble-like pattern. Mitotic activity was ≥10/10LPF and a necrotic area was also found in this case, which is commonly observed in MPNST. Immunohistochemistry staining showed negative S100 and positive CD99, leading to the initial diagnosis of synovial sarcoma, but H3K27me3 staining showed very few tumor cells that were positive (complete loss), indicating the prevalence of MPNST. Further examination is also needed to determine the existence of SS18-SSX1/2 gene fusion, as a definitive marker of synovial sarcoma.[4,10]

The histopathological findings in the third case showed a fascicular growth pattern with a storiform area. Mitotic activity of <10/10LPF and a necrotic area, which were characteristics of MPNST, were also in this case. Immunohistochemistry staining showed negative S100 and desmin expression, partly positive SMA, and CD34 only positive in blood vessels. This led to the initial diagnosis of leiomyosarcoma but H3K27me3 staining showed very few positive tumor cells (complete loss), which is more suggestive of MPNST. Moreover, a diagnosis of leiomyosarcoma is more convincing when 2 smooth muscle markers are positive. However, in this case, only SMA staining was positive in some tumor cells, while desmin staining gave a negative result.[1,2,12]

In this research, the diagnosis of MPNST was established based on histopathological evaluation and immunohistochemistry to exclude other types of sarcoma. The limitations of this research included the small number of MPNST and non-MPNST samples that did not meet the minimum sample size. Additionally, FISH or RT-PCR testing for confirmation of gene translocation had not been performed in the non-MPNST group. The diagnosis of MPNST was established by excluding other sarcomas that mimicking MPNST, while the diagnosis of non-MPNST was based on immunohistochemical examination.

CONCLUSION

Based on the analysis of the research results and discussions that have been carried out, it can be concluded there is ~~a~~ loss of H3K27me3 expression in MPNST compared to other sarcomas mimicking MPNST, which is statistically significant. There is a difference in the expression of H3K27me3 between the high and low-grade MPNST but the result is not statistically significant. Expression of H3K27me3 can be used as one indicator to eliminate the possibility of MPNST. H3K27me3 staining can be used to assist in confirming MPNST diagnosis but it should be accompanied by other immunohistochemistry staining.

Further research with a larger sample size is needed to obtain more valid results. Further molecular study or chromosomal translocation is also required to establish a diagnosis in other sarcomas mimicking MPNST.

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