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HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF PERIPHERAL NERVE SHEATH TUMORS: A PROSPECTIVE COHORT STUDY.

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Abstract

Background

Peripheral nerve sheath tumors (PNSTs) are rare, heterogeneous soft tissue neoplasms arising from Schwann cells, fibroblasts, and histiocytic or macrophage-like cells. They include benign tumors like schwannomas and neurofibromas and the highly aggressive malignant peripheral nerve sheath tumors (MPNSTs).

Objective

To evaluate the histopathological features and immunohistochemical (IHC) profiles of different PNSTs using S-100, SOX10, and CD56 markers.

Methods

This prospective observational study was conducted from June 2022 to July 2023 at a tertiary care teaching hospital in India. Thirty patients under 65 years with benign or malignant PNSTs were included. Histological features were assessed using light microscopy, and IHC staining was performed with S100, SOX10, and CD56. Data were analyzed using SPSS software, with a significance level set at $p<0.05$.

Results

Out of 30 cases, 17 (56.7%) were neurofibromas, 12 (40%) were schwannomas, and 1 (3.3%) was an MPNST. The mean age was 38.7 years, with a male-to-female ratio of 16:14. Tumor size varied significantly between types, with MPNST and schwannomas being larger than neurofibromas (P=0.07). Schwannomas frequently exhibited Antoni A and B patterns, Verocay bodies, and hyalinized blood vessels, while neurofibromas showed spindle cells and shredded carrot-type collagen. Immunohistochemistry revealed S100 positivity in 70% of tumors, SOX10 in 86.7%, and CD56 in 43.3%. Schwannomas showed higher S100 and CD56 expression compared to neurofibromas ($p<0.05$).

Conclusions

The study highlights distinct histological and immunohistochemical features of PNST subtypes, with significant differences in marker expression aiding in differential diagnosis. Larger-scale studies are needed to further validate these findings in diverse populations.

Recommendations

The study recommends using histopathological and immunohistochemical analysis with markers (S100, SOX10, CD56) for accurate PNST diagnosis and emphasizes the need for larger-scale, follow-up studies. It also highlights the importance of routine analysis, multidisciplinary collaboration, comprehensive patient care, and enhanced training for pathologists.

Keywords: *Peripheral Nerve Sheath Tumors, Schwannomas, Neurofibromas, Malignant Peripheral Nerve Sheath Tumors, Histopathology, Immunohistochemistry, S100, SOX10, CD56.*
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Introduction

Peripheral nerve sheath tumors (PNSTs) are rare, heterogeneous soft tissue neoplasms that can develop anywhere in the peripheral nervous system, typically originating from Schwann cells, fibroblasts, and histiocytic or macrophage-like cells. Schwannomas and neurofibromas are examples of benign tumors categorized under PNSTs. Conversely, malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive. PNSTs constitute approximately 10% of all benign soft tissue neoplasms and 5-10% of all soft tissue sarcomas (1–3).

The first recorded case of what appeared to be a peripheral nerve tumor was published by Cheselden in 1741 (4). Since then, significant advancements have been made in both the treatment and epidemiological documentation of PNSTs. Schwannomas have an annual incidence of 20 cases per million people, with most diagnoses occurring between the ages of 20 and 50. Neurofibromas, typically diagnosed between the ages of 20 and 30, account for

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about 5% of all soft tissue tumors. MPNSTs are exceedingly rare, with an incidence rate of 1 case per million individuals per year in the general population. However, this rate increases to 1 case per 3,500 individuals per year for those with neurofibromatosis type 1 (NF1). The average age of onset for MPNST in the general population is 41 years, while for patients with NF1-related malignant transformation, it is 28 years (5).

While only a small proportion of benign PNSTs have the capacity to turn into malignant tumors, individuals with benign PNSTs may nonetheless experience intense pain, neurological impairment, and physical deformity, particularly in cases of genetic tumor predisposition syndromes. Despite the use of treatments such as surgical removal, further radiation therapy, and chemotherapy, malignant peripheral nerve sheath tumors (MPNSTs) have a bleak outlook, with a median survival rate of only 32 months (2,6,7).

Diagnosing and managing PNSTs is challenging and typically requires a multidisciplinary team, including surgeons, radiologists, oncologists, and pathologists. Surgical pathologists must be aware of the diverse morphological characteristics and the association with tumor-predisposing syndromes. This knowledge is crucial for providing accurate prognostic information and planning appropriate follow-up care.

The present study was conducted to evaluate the histopathological features of PNSTs and to evaluate the immunohistochemical (IHC) profile of different PNSTs using S-100, SOX10, and CD 56.

Methods

Study design

A prospective observational cohort study

Study setting

This investigation was carried out in India at a tertiary care teaching hospital's pathology department. The research was carried out between June 2022 and July 2023.

Bias

There was a chance that bias would arise when the study first started, but it was avoided by giving all participants the identical information and hiding the group allocation from the nurses who collected the data.

Procedure

Patients whose tissue specimens of PNST were received for histopathological analysis at the Department of Pathology were screened for eligibility based on specific inclusion and exclusion criteria. Eligible patients, who were all younger than 65 years with benign or malignant PNST, were invited to participate after providing informed consent. In cases where the patient could not consent, a legally authorized representative was asked to provide consent. The study excluded recurrent cases of PNST, lesions arising due to trauma, and autolytic specimens or those with autolytic changes. A total of thirty patients meeting these criteria were recruited.

After recruitment to the study, demographic details, clinical details, and laboratory results were recorded using predefined proformas. In light microscopy, slices that were 5 microns thick were obtained from the paraffin blocks and then stained with a combination of eosin and hematoxylin. The histologic characteristics, including the existence of a capsule surrounding the tumor, the arrangement of cells in whorls or fascicles, the presence of inflammatory cells, increased cell density around blood vessels, tumor invasion into blood vessels, tissue death, and cell division, were evaluated by examining whole slide sections.

The number of mitotic figures was counted in ten successive high-power fields (0.24 mm^2) to determine the amount of mitotic activity. This technique was repeated up to ten times in different tumor locations as the size allowed, with the greatest number being recorded.

Tumors exhibiting cytologic anaplasia, hypercellularity, and a maximal mitotic index higher than 6/10 high-power fields—often in conjunction with necrosis—were classified as high-grade MPNSTs. For immunohistochemistry analysis, a specific block from each case was selected. Five-micron paraffin sections were prepared and stained using standardized techniques with S100, SOX10, and CD56. Negative expression was defined as occurring in fewer than 5% of tumor cells, and diffuse positivity as occurring in 90% or more of tumor cells. Every tissue microarray core was evaluated individually and given a staining percentage value; substantial differences prompted a review and average computation.

Statistical analysis

The collected data was recorded in an MS Excel data collection sheet. "Continuous variables were reported as the mean plus or minus the standard deviation (SD) or as the median with the interquartile range (IQR) for data that did not follow a normal distribution. Categorical variables were stated as frequencies and percentages, along with a 95% confidence interval.

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). The statistical analysis involved the use of Student t-tests to compare continuous data and Chi-Squared tests to compare categorical data." A two-sided P-value of less than 0.05 was deemed to indicate statistical significance.

Ethical consideration

Institutional approval was obtained from the hospital ethics committee (EC-241; dated 15.06.2022).

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Results

This study examined 30 cases of PNSTs, with subjects having a mean age of 38.7 ± 14.85 years. Among the 30 individuals, 16 (53.3%) were male and 14 (46.7%) were female. The age range of the patients varied from 11 to 62 years old. Based on the histopathological findings 17 (56.7%) patients had neurofibroma, 12 (40%) patients had schwannoma and one patient had MPNST. The mean age of patients with neurofibromas was 39.6 (± 17.76) years and of those with schwannoma was 35.7 (± 8.43) years. The data analysis revealed no significant age difference among patients with different PNST types (p=0.283). Similarly, there was no significant association between PNST type and gender (p=0.496). The most common locations for PNSTs were the head and neck region (9, 30.0%), followed by upper limb (7, 23.3%), and lower limb (6, 20.0%). As shown in Table 1, There was no significant association between PNST type and tumor site (P = 0.588), but there was a significant association with tumor consistency (P < 0.001).

The average tumor size was 6.4 ± 3.4 cm, with neurofibromas measuring 5.1 ± 2.1 cm, and schwannomas measuring 7.9 ± 4.1 cm. There was a statistically significant difference between the sizes of the types of PNSTs with malignant variants and schwannomas being larger than neurofibromas (P = 0.07).

Out of 12 (40%) schwannomas, 1(3.4%) was an ancient schwannoma and the remaining 11 (37.9%) were classical schwannomas. Among the 17 neurofibromas, 3 (10.3%) were neurofibromas, 2 (6.9%) were plexiform neurofibroma and 12 (41.4%) were localized neurofibroma.

Table 1: Distribution and correlation of patient and tumor characteristics across various types of PNSTs.

		MPNST	Neurofibroma	Schwannoma	Total	P-value
Frequency (%)		1 (3.3%)	17 (56.7%)	12 (40.0%)	30	-
Mean Age (Std. deviation), years		60	39.6 (17.8)	35.7 (8.4)	38.7 (14.8)	0.283
Gender	**Males**	0	10 (33.3%)	6 (20%)	16 (53.3%)	0.496
	Females	1 (3.3%)	7 (23.3%)	6 (20%)	14 (46.7%)	
Site	**Abdomen**	0 (0.0%)	1 (3.3%)	1 (3.3%)	2 (6.7%)	0.588
	Back	0 (0.0%)	0 (0.0%)	2 (6.7%)	2 (6.7%)	
	Head neck	0 (0.0%)	6 (20.0%)	3 (10.0%)	9 (30.0%)	
	Lower limb	1 (3.3%)	2 (6.7%)	3 (10.0%)	6 (20.0%)	
	Multiple regions	0 (0.0%)	1 (3.3%)	1 (3.3%)	2 (6.7%)	
	Thorax	0 (0.0%)	1 (3.3%)	1 (3.3%)	2 (6.7%)	
	Upper limb	0 (0.0%)	6 (20.0%)	1 (3.3%)	7 (23.3%)	
Mean dimension (Std. deviation), cm		12	5.1 (2.1)	7.9 (4.1)	6.4 (3.4)	**0.07**
Consistency of tumor	**Firm**	0 (0.0%)	1 (3.3%)	10 (33.3%)	11 (36.7%)	**<0.001**
	Hard	1 (3.3%)	0 (0.0%)	0 (0.0%)	1 (3.3%)	
	Nodular	0 (0.0%)	0 (0.0%)	1 (3.3%)	1 (3.3%)	
	Soft	0 (0.0%)	14 (46.7%)	1 (3.3%)	15 (50.0%)	
	Soft, nodular (bag of worms)	0 (0.0%)	2 (6.7%)	0 (0.0%)	2 (6.7%)	

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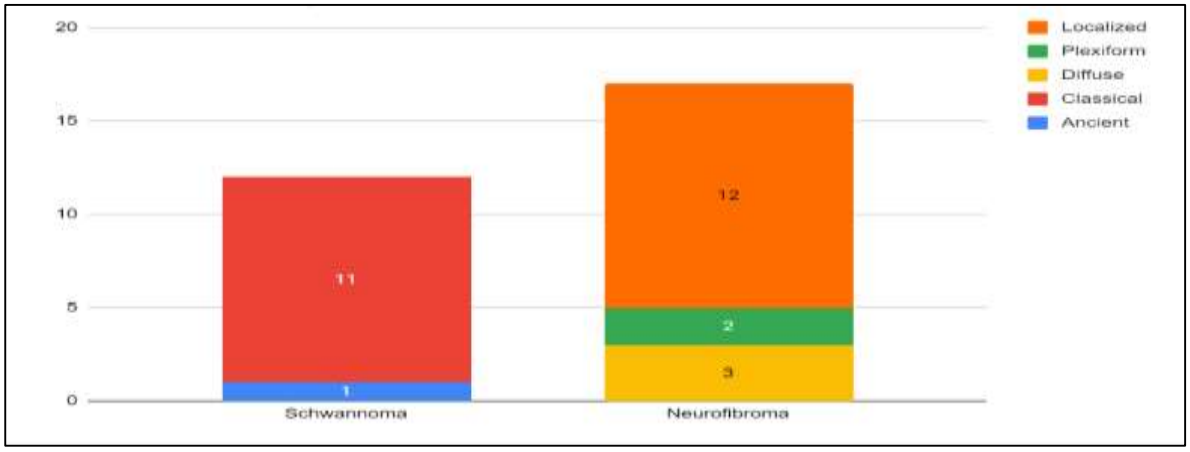

Figure 1: Histopathological Variants of Schwannomas and Neurofibromas.

Table 2: Findings on gross examination of different types of PNSTs.

Gross anatomy	**MPNST (n=1)**	**Neurofibroma (n=17)**	**Schwannoma (n=12)**	**Total (n=30)**	**P-value**
Capsule					
Absent	0 (0.0%)	14 (46.7%)	0 (0.0%)	14 (46.7%)	**<0.001**
Present	0 (0.0%)	3 (10.0%)	12 (40.0%)	15 (50.0%)	
Pseudo-capsule	1 (3.3%)	0 (0.0%)	0 (0.0%)	1 (3.3%)	
Cut Surface					
Pink	0 (0.0%)	4 (13.3%)	6 (20.0%)	10 (33.3%)	**<0.001**
Grey-white	0 (0.0%)	12 (40.0%)	0 (0.0%)	12 (40.0%)	
Tan-white with areas of necrosis and hemorrhage	1 (3.3%)	0 (0.0%)	0 (0.0%)	1 (3.3%)	
Yellow	0 (0.0%)	1 (3.3%)	6 (20.0%)	7 (23.3%)	

On gross examination, a capsule around the tumor was present in 15 (50%) of the specimens, 14 (46.7%) had no capsule whereas 1 (3.3%) had a pseudo-capsule. Out of 15 tumors without a capsule, 12 (40%) were schwannomas, and 14 (46.7%) out of 17 neurofibromas did not have any capsule. The Solitary MPNST was characterized by a pseudo-capsule. Among the tumor specimens analysed in this study, 12 (40%) had a grey-white cut surface all of which were neurofibromas, 10 (33.3%) were pink out of which 6 were schwannomas and 4 were neurofibromas,7 had a yellow cut surface of which 6 were schwannoma and 1 was a neurofibroma, and 1 (3.3%) MPNST was tan-white with areas of hemorrhage and necrosis. The presence or absence of the capsule and the difference between the cut surface appearance between different types of tumors were statistically significant as shown in Table 2.

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Table 3: Histological features of PNSTs and its subtypes.

Histological findings	MPNST	Neurofibroma	Schwannoma	Total	P-value (Chi2 test)
Antoni A	0 (0.0%)	0 (0.0%)	12 (40.0%)	12 (40.0%)	**<0.001**
Antoni B	0 (0.0%)	0 (0.0%)	12 (40.0%)	12 (40.0%)	**<0.001**
Verocay Bodies	0 (0.0%)	0 (0.0%)	8 (26.7%)	8 (26.7%)	**<0.001**
Spindle cells	1 (3.3%)	17 (56.7%)	0 (0.0%)	18 (60.0%)	**<0.001**
Shredded carrot type collagen	0 (0.0%)	17 (56.7%)	0 (0.0%)	17 (56.7%)	**<0.001**
Hyalinized blood vessels	0 (0.0%)	2 (6.7%)	12 (40.0%)	14 (46.7%)	**<0.001**
Cystic changes	0 (0.0%)	5 (16.7%)	7 (23.3%)	12 (40.0%)	0.024
Myxoid changes	1 (3.3%)	9 (30.0%)	7 (23.3%)	17 (56.7%)	0.646
Hyaline changes	0 (0.0%)	1 (3.3%)	8 (26.7%)	9 (30.0%)	0.002
Necrosis	1 (3.3%)	0 (0.0%)	1 (3.3%)	2 (6.7%)	**<0.001**
Hemorrhage	1 (3.3%)	1 (3.3%)	1 (3.3%)	3 (10.0%)	0.009
Lymphocytic infiltration	0 (0.0%)	2 (6.7%)	1 (3.3%)	3 (10.0%)	**0.902**
Pigmentation	0 (0.0%)	1 (3.3%)	0 (0.0%)	1 (3.3%)	0.673
Calcification	1 (3.3%)	0 (0.0%)	0 (0.0%)	1 (3.3%)	**<0.001**

On histopathological examination, the tumor margins were well defined in 26 (86.7%), and ill-defined in 4 (13.3%). A diagnosis of schwannoma was significantly associated with the occurrence of Antoni A and B regions with Verocay bodies, hyalinized blood vessels, and cystic and hyaline changes ($p<0.001$). The presence of spindle cells and shredded carrot-type collagen was associated with a diagnosis of neurofibroma ($p>0.001$). Necrosis and calcification were significantly linked to MPNST ($p<0.001$). The detailed description of histological features observed on light microscopy is described in Table 3.

Table 4: Immunohistochemical analysis of PNSTs using S100, SOX10 and CD56.

Tumor type	S100	SOX10	CD56
MPNST	0 (0.0%)	1 (3.3%)	1 (3.3%)
Neurofibroma	9 (30.0%)	14 (46.7%)	2 (6.6%%)
Schwannoma	12 (40.0%)	11 (36.7%)	10 (33.3%)
Total	21 (70.0%)	26 (86.7%)	13 (43.3%)
p-value (Chi2 test)	0.007	0.071	<0.001

Immunohistochemical staining with S100 was positive in 21 out of 30 tissue samples (70%). This included 12 cases of schwannomas and 9 out of 17 cases of neurofibromas. The difference in S100 positivity between the two types of tumors was statistically significant ($p=0.007$). Staining with SOX10 was positive in 26 cases of PNSTs (86.7%), with 11 out of 12 schwannomas, 14 out of 17 neurofibromas, and 1 MPNST showing positive staining. There was no statistically significant difference in SOX10 staining between different tumor types. CD56 showed a positive stain in 13 tumors (43.3%), including 10 schwannomas, 2 neurofibromas, and 1 MPNST. The

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detailed analysis of immunohistochemical staining is given in Table 4.

Discussion

In this prospective observational study, 30 patients under 65 with benign or malignant PNSTs were consecutively recruited. The main aim of the study was to examine the histopathological and immunohistochemistry patterns of PNSTs.

Among the 30 tumor samples analysed in this research, 17 (56.75) were diagnosed as neurofibromas, 12 (40%) as schwannomas, and 1 (3.3%) as MPNST. Some reports suggest that Schwannomas comprise as much as 80% of all PNSTs (6,7). They are a common type of tumor affecting soft tissues, accounting for around 8% of all such tumors with an annual incidence of 20 cases per million people (8,9). Neurofibromas rank as the second most prevalent type of benign PNST, following schwannomas. Their reported prevalence stands at 10-24% of all isolated nerve tumors, and they constitute 5% of all soft tissue tumors (10,11). The general population experiences a relatively low occurrence of MPNSTs, with an approximate incidence rate of 1/100,000 per year in the reported cases (5,12,13). However, similar to the present findings, there have been several other studies that do not confirm this epidemiological pattern. In the study neurofibromas (61.5%) were the most common type of PNSTs, followed by schwannomas (36%), and malignant PNSTs (2%) (14). Similarly, a study reported neurofibromas as the most common type of PNST in their study involving 397 patients (15). The variation of prevalence across various publications can be explained due to the study methodology, with clinical research involving symptomatic patients and histopathological diagnosis showing a lower prevalence of schwannomas, whereas research involving cadaveric diagnosis (16,17) or radiological imaging (18) shows a higher prevalence of schwannomas as many of these lesions can be asymptomatic and can be incidentally diagnosed of MRI or autopsy examination.

Schwannomas are well-circumscribed, encapsulated tumors that show Antoni A and Antoni B patterns. They often have nuclear alignment, forming Verocay bodies, and may undergo degenerative changes such as hyalinization, thrombosis, hemorrhage, lipidization, calcification, and cystic changes, with necrotic areas sometimes present due to vascular changes (19,20). In the current study, one tumor was ancient and the other 11 were classical schwannomas, all showing Antoni A and B patterns with hyalinized blood vessels. Verocay bodies were observed in 66.7% of cases. Cystic, myxoid, and hyaline changes were found in 58.3% and 66.7% of tumors, respectively, with one tumor also displaying necrosis, hemorrhage, and lymphocytic infiltration. These findings align with previous studies, such as a study which reported cystic change (85.18%), hyalinization (72.22%), necrosis (59.25%), and myxoid change (24.07%) in schwannomas, with Verocay bodies in 70.37% of cases (21). A study found cystic changes in 73%, myxoid changes in 71%, hyaline changes in 71%, and Verocay bodies in 60% of schwannomas, with necrosis in 15% and hemorrhage with lymphocytic infiltration in 17% of cases (14). A study observed Verocay bodies in one out of 12 tumors and degenerative changes in another (22).

Neurofibromas were diagnosed in 17 (56.7%) tumor specimens out of a total of 19, with a majority of them being localized variants (12, 70.6%). There were 2 (11.8%) cases of plexiform neurofibromas and 3 (16.6%) cases of diffuse neurofibromas. Neurofibromas are identified by the presence of elongated cells with indistinct cell boundaries amid a background of myxoid to light pink collagen matrix and thick collagen bundles like shredded carrots. Myxoid changes were seen in 9 (52.9%) and cystic changes were seen in 5 (29.4%) specimens. Hyalinized blood vessels and lymphocytic infiltration were observed in 2 (11.8%) cases each. Whereas hemorrhage and pigmentation were even more rare and were present in only 1 (5.9%) case each. A study (22) reported cystic changes in 25% of neurofibromas out of 88 total included in their study and myxoid changes in 46%. Lymphocytic changes were seen in 17% of patients, hemorrhage in 8%, hyaline, and necrosis in 7% each, and pigment deposition in 3.5%. They also reported calcification in one case, which was not seen in any of our samples with neurofibromas. In the research published, myxoid changes were less frequent, seen in 18.8% of cases (21).

S100 protein is a type of protein that is commonly found in neural or neural crest-derived tissues, as well as in chondrocytes, melanocytes, and Langerhans cells. Research has shown that the S100 protein is expressed more diffusely and strongly in schwannomas compared to neurofibromas. Previous studies conducted (23-25) have all reported this finding. In addition, the S100 protein has been found to have patchy and scattered positivity in MPNSTs. This unique pattern of expression can differentiate MPNSTs from other types of sarcomas. In the current study, it was observed that all of the schwannomas and 8 out of 17 (47.1%) neurofibromas showed positive staining. The solitary MPNST did not stain with S100. Statistical analysis showed a significant difference between tumor types for IHC by S100 ($p<0.05$). Unlike the current findings, a study discovered contradictory results. They reported that there was no statistically significant distinction in the expression patterns of S-100 protein between the groups of schwannomas and neurofibromas ($p=0.75$) (26).

Out of 30 samples analysed for IHC 26 were SOX10 protein positive. This included all except one specimen of schwannomas, 14 out of 17 neurofibromas, and the solitary case of MPNST as well.

During the study, it was observed that 10 out of 12 schwannomas (83.3%) expressed CD56, while only 2 out

of 17 (11.8%) of the neurofibromas did. These findings are consistent with a study by Park et al. that discovered that CD56 expression was present in 77.2% of schwannomas but only in 9.8% of neurofibromas. These results imply that CD56 might function as a sensitive schwannoma indication.

The combination of CD56 and S-100 has been shown (27) to be useful in diagnosing peripheral nerve sheath tumors. According to (25) too, CD56 is frequently present in schwannomas in comparison to neurofibromas and has slightly lower specificity than calretinin.

The present study, while conducted with sound methodology, had several limitations. The small sample size of 30 patients was insufficient for generating strong high-quality evidence. As a cross-sectional observational study, it did not include follow-up examinations or outcome observations. Sample bias was present due to it being a single-center study at a tertiary multi-specialty hospital, limiting participant diversity. Additionally, the study included only one MPNST patient, and sub-types of schwannomas and neurofibromas were underrepresented.

Conclusion

In conclusion, the histological features of each PNST subtype are distinctly different. Schwannomas have both Antoni A and Antoni B regions, while neurofibromas are characterized by spindle cells and a shredded carrot-like collagen matrix. Immunohistochemistry reveals that staining with S100 and CD56 is significantly higher for Schwannomas compared to neurofibromas. In contrast, almost all PNST tumors in our study are positive for SOX10. Further large-scale studies must be conducted in the Indian population to generate high-quality evidence regarding pathological findings in peripheral nerve sheath tumors.

Limitations

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of comparison group also poses a limitation for this study's findings.

Recommendations

The study recommends using histopathological and immunohistochemical analysis with markers (S100, SOX10, CD56) for accurate PNST diagnosis and emphasizes the need for larger-scale, follow-up studies. It also highlights the importance of routine analysis, multidisciplinary collaboration, comprehensive patient care, and enhanced training for pathologists.

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List of abbreviations

PNST: Peripheral Nerve Sheath Tumor
MPNST: Malignant Peripheral Nerve Sheath Tumor
IHC: Immunohistochemical
NF1: Neurofibromatosis Type 1
S100: S-100 protein
SOX10: SRY-Box Transcription Factor 10
CD56: Neural Cell Adhesion Molecule (NCAM)
SD: Standard Deviation
IQR: Interquartile Range
H&E: Hematoxylin and Eosin
MRI: Magnetic Resonance Imaging
MS Excel: Microsoft Excel

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Conflict of interest

The authors have no competing interests to declare.

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