ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 7, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

HISTOMORPHOLOGICAL SPECTRUM AND IMMUNOHISTOCHEMICAL EXPRESSION OF S100 AND CD56 AMONG TUMORS OF PERIPHERAL NERVES-A CROSS-SECTIONAL STUDY

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Received: 02 April 2025, Revised and Accepted: 19 May 2025

ABSTRACT

Objectives: Peripheral nerve sheath tumors (PNSTs) encompass a range of distinct clinicopathological entities, from benign tumors such as schwannoma to high-grade malignant neoplasms known as malignant PNST (MPNST). Despite having classic, recognizable microscopic features, these neoplasms can occasionally be difficult to diagnose. Immunohistochemistry (IHC) using a panel of markers like S100 and CD56 is helpful in arriving at the correct diagnosis.

Methods: This was a cross-sectional study based on laboratory records. All the cases of peripheral nerve sheath tumors reported between January 2021 and December 2021 were retrieved and IHC using S100 and CD56 was carried out. The results were tabulated by scoring the intensity and extent of IHC staining.

Results: Neurofibromas and schwannomas did not differ significantly in terms of patient age or the anatomical sites of these tumors. CD56 was positive in the majority of neurofibromas (90%) compared to schwannomas (80%), whereas S100 was positive in all cases of schwannomas (100%) in comparison to neurofibromas (95%). There was a statistically significant difference in the staining intensity of CD56 more for neurofibromas (p=0.015) and that of S100 more for schwannomas (p=0.04).

Conclusion: CD56 and S100 IHC analysis will be a helpful supplementary tool for the histopathologist to distinguish peripheral nerve sheath tumors from other soft tissue tumors.

Keywords: Neurofibroma, Schwannoma, Malignant peripheral nerve sheath tumors, Immunohistochemistry.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i7.54673. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The peripheral nerves are complex structures distributed throughout the body providing somatic and autonomic innervations. They are composed of bundles of axons ensheathed by Schwann cells held together by connective tissue organized into three specialized layers namely epineurium, perineurium, and endoneurium. The tumors of the peripheral nerves show differentiation toward its various components including axons, Schwann cells, perineurial cells, and supporting mesenchymal connective tissue [1]. Thus, these tumors exhibit a wide spectrum of histomorphological variants with varied biological behavior ranging from benign to malignant tumors. The common benign tumors are schwannoma, neurofibroma, and Perineurioma including hybrid nerve sheath tumors, whereas malignant peripheral nerve sheath tumor (MPNST) and malignant melanotic nerve sheath tumor are the common malignant peripheral nerve tumors [1].

Current methods for diagnosing and treating peripheral nerve sheath tumors include core needle biopsy analysis for the initial diagnosis followed by neoadjuvant chemotherapy in case of malignant lesions (sarcomas) and shrinking the tumor before administering chemotherapy. Core needle biopsies have the inherent drawback of only obtaining a small sample of tissue, which may not be representative of the entire lesion due to its heterogeneous nature (e.g., alternating hypercellular and hypocellular lesions, varying degrees of nuclear atypia in different parts of the tumor) [2].

Despite the fact that schwannomas and neurofibromas can usually be distinguished from one another using normal light microscopy, there are a few instances in which there may be significant morphologic overlap. Some schwannomas may have sparse cellularity, large myxomatous areas, and only hypocellular (Antoni B) areas, which may make them resemble a neurofibroma. Cellular schwannomas may resemble leiomyosarcoma and low-grade MPNST. Differentiating MPNST from cellular schwannoma is crucial because they have different prognostic and therapeutic consequences [3]. Plexiform schwannomas are primarily made up of cellular Antoni A regions and can resemble MPNST when they show increased cellularity and mitotic features [2-5]. Neurofibromas exhibiting degenerative atypia and increased cellularity can be mistaken for MPNST [6]. Plexiform neurofibromas can also progress to MPNST, which can have morphological overlap with fibrosarcoma, leiomyosarcoma, dedifferentiated liposarcoma, and monophasic synovial sarcoma. Therefore, morphological features alone may not be sufficient (especially in core needle biopsies) to provide a definitive diagnosis in soft tissue tumors, especially peripheral nerve sheath tumors (PNST) and immunohistochemical (IHC) analysis using a panel of markers have been found to be crucial in resolving these diagnostic problems [7-12].

In addition, most neurofibromas involve the nerve as a component of the mass, requiring nerve grafting to retain and restore function after the mass is removed whereas schwannomas can be removed without harming the nerve [3]. Furthermore, compared to schwannomas, neurofibromas exhibit a significantly higher correlation with hereditary syndromes like neurofibromatosis type 1 and have a slight but non-negligible malignant potential [13]. The ability to distinguish between these two entities had been investigated using a number of IHC markers, including Glut-1, S-100 protein, glial fibrillary acidic protein, epithelial membrane antigen, factor XIIIa, Leu-7, calretinin, SOX10, and

myelin basic protein. These markers had demonstrated varying degrees of sensitivity and specificity [4,14]. It is found that the spectrum of peripheral nerve sheath tumors exhibits varied expressions of distinct IHC markers [15-17].

Among the various IHC markers, S100 is the commonly used marker for PNST and shows varied intensity of positivity among the several peripheral nerve tumors [14]. Due to the lack of specificity of S100 for neural tumors, it is often used in combination with another IHC marker for confirming the diagnosis. There are limited studies carried out on the utility of the neural cell adhesion molecule namely CD56 in the diagnosis of peripheral nerve sheath tumors.

In the present study, we have evaluated the histomorphological features and compared the IHC expression profiles of S100 and CD56 in PNSTs.

METHODS

This cross-sectional study was carried out in the department of pathology of a tertiary care hospital after obtaining approval from the Institutional Ethical Committee (IEC Ref. No: KIMS/F/2022/11). Relevant clinical and pathological data of all the peripheral nerve sheath tumors reported in histopathology during the study period from January 2021 to December 2021 were retrieved from the laboratory records based on the inclusion and exclusion criteria.

Inclusion criteria

- Patients of all age groups and genders diagnosed with peripheral nerve tumors.
- All kinds of biopsy samples including incision biopsy, excision biopsy, and resection specimens of peripheral nerve tumors.

Exclusion criteria

- Poorly fixed/autolyzed samples.
- Soft tissue tumor specimens other than neural tumors.

Hematoxylin and Eosin (H&E) stained slides of all the peripheral nerve tumors were reviewed. IHC analysis using the markers S100 and CD56 was carried out and the findings were tabulated. The extent of IHC staining of S100 and CD56 within tumor cells was graded as 1+ (<25% cells stained), 2+ (25–75% cells stained), and 3+ (>75% cells stained), whereas the intensity of IHC staining was graded as 1+ (weak), 2+ (moderate) and 3+ (strong) for both markers [18].

Statistical analysis

The data was entered in a Microsoft Excel spreadsheet and analyzed using Statistical Packages for the Social Sciences version 23 software. Pearson Chi-square test was used for comparing the IHC expression of the two markers among neurofibromas and schwannomas. p-value <0.05 was taken as statistically significant.

RESULTS

In this study, a total of 41 PNSTs were encountered during the study period, among which 20 cases were diagnosed as neurofibroma and its variants, 20 cases as schwannoma and its variants, whereas one case as MPNST morphologically. The mean age of the patients was 40.81 ± 18.56 years with the majority (40%) of schwannomas encountered in the 41–60 years age group while the majority (35%) of neurofibromas occurring in the 21–40 years age group and the single case of MPNST occurring in more than 60 years age group. There was slight female predominance for peripheral nerve sheath tumors in this study with female: male ratio of 1.4:1. The site distribution revealed that the majority of peripheral nerve sheath tumors occurred in the lower extremities with 40% of schwannomas and 30% of neurofibromas occurring in that site while the single case of MPNST occurred in the trunk region in the current study (Table 1).

Among the twenty cases of schwannomas in this study, classical schwannoma (55%) was the common histological type, whereas localized neurofibroma (70%) was the common histological type among the 20 cases of neurofibromas in this study (Table 2) (Figs. 1a and 2a).

In the current study, IHC analysis revealed that S100 was positive in 95% of cases (Fig. 2b) and CD56 was positive in 90% of cases of neurofibromas. The staining intensity of S100 and CD56 was moderate (2+) in the majority of neurofibromas and its variants (Fig. 2b and c). Among the histological variants of neurofibromas, both the cases of myxoid neurofibroma were negative for CD56, whereas S100 was positive in one case (Table 3).

IHC analysis of all the 20 cases of schwannomas in the present study revealed that S100 was positive in 100% of cases, whereas CD56 was positive in 80% of cases (Fig. 1b and c). Around 40% of schwannomas showed strong staining intensity (3+) for S100, whereas 30% of schwannomas showed strong IHC intensity (3+) for the CD56 marker. Among the histological subtypes of schwannomas, epithelioid schwannoma (100%), microcystic/reticular schwannoma (100%), and two cases of ancient schwannomas (67%) were negative for CD56 (Table 4).

In the current study, it was observed that CD56 was positive in the majority of neurofibromas (90%) compared to schwannomas (80%), whereas S100 was positive in all cases of schwannomas (100%) in comparison to neurofibromas (95%). Although the IHC intensity score varied among the types of neurofibromas and schwannomas, there was a statistically significant difference in the staining intensity of CD56 more for neurofibromas (p=0.015) and that of S100 more for schwannomas (p=0.04) (Table 5; Figs. 1 and 2).

Table 1: Descriptive statistics of the study variables

Study variables	Schwannoma and its variants (n=20) (%)	Neurofibroma and its variants (n=20) (%)
Gender distribution		
Males	09 (45)	7 (35)
Females	11 (55)	13 (65)
Age distribution		
≤20 years	2 (10)	6 (30)
21–40 years	5 (25)	7 (35)
41–60 years	8 (40)	5 (25)
>60 years	5 (25)	2 (10)
Site distribution		
Upper extremities	7 (35)	5 (25)
Lower extremities	8 (40)	6 (30)
Head and Neck	4 (20)	5 (25)
Trunk	1 (5)	4 (20)

Mean age: 40.81 years. Standard deviation: 18.56 years

Table 2: Histopathological distribution of peripheral nerve sheath tumors

S. No.	Schwannoma and its variants (n=20)		Neurofibroma and its variants (n=20)	
	Histopathological type	n (%)	Histopathological type	n (%)
1.	Classical schwannoma	11 (55)	Localized neurofibroma	14 (70)
2.	Ancient schwannoma	3 (15)	Diffuse neurofibroma	2 (10)
3.	Plexiform schwannoma	2 (10)	Plexiform neurofibroma	2 (10)
4.	Cellular schwannoma	2 (10)	Myxoid neurofibroma	2 (10)
5.	Epithelioid schwannoma	1 (05)	-	-
6.	Microcystic/Reticular schwannoma	1 (05)	-	-

Table 3: IHC expression of S100 and CD56 in neurofibromas

S. No.	Neurofibroma and its variants (n=20)	S100 expression		CD56 expression	
		Extent (%)	Intensity (%)	Extent (%)	Intensity (%)
1	Localised neurofibroma (14)	2+ (100)	2+ (93) 3+ (7)	2+ (100)	1+ (57) 2+ (43)
2	Diffuse neurofibroma (2)	2+ (100)	2+ (100)	2+ (100)	2+ (100)
3	Plexiform neurofibroma (2)	2+ (100)	2+ (100)	2+ (100)	2+ (100)
4	Myxoid neurofibroma (2)	2+ (50)	1+ (50)	-	-

IHC: Immunohistochemistry

Table 4: IHC expression of S100 and CD56 in schwannomas

S. No.	Schwannomas and its variants (n=20)	S100 expression		CD56 expression	
		Extent (%)	Intensity (%)	Extent (%)	Intensity (%)
1	Classical schwannoma (11)	3+ (100)	2+ (27) 3+ (73)	+ (100)	2+ (45) 3+ (55)
2	Ancient schwannoma (3)	3+ (100)	2+ (100)	+ (33)	2+ (33)
3	Plexiform schwannoma (2)	3+ (100)	2+ (100)	+ (100)	1+ (50) 2+ (50)
4	Cellular schwannoma (2)	3+ (100)	2+ (100)	+ (100)	1+ (50) 2+ (50)
5	Epithelioid schwannoma (1)	3+ (100)	2+ (100)	-	-
6	Microcystic/reticular schwannoma (1)	3+ (100)	2+ (100)	-	-

IHC: Immunohistochemistry

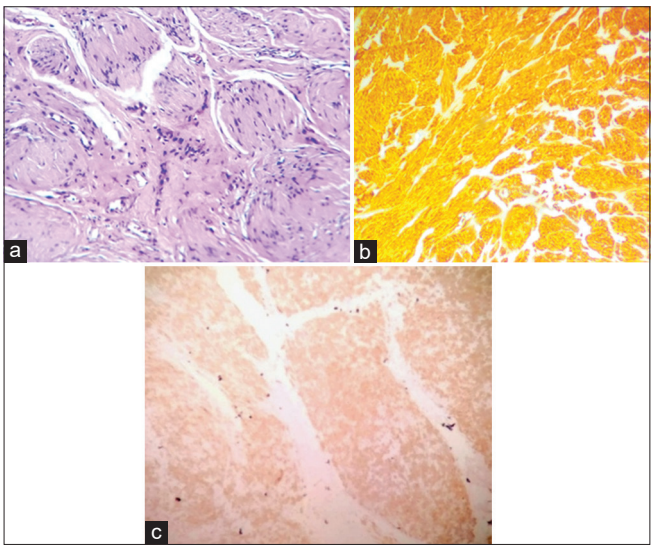

Fig. 1: (a) Plexiform schwannoma showing plexiform bundles of spindle cells with nuclear palisading (H&E stain ×40) (b) Diffuse strong staining intensity (3+) of S100 in plexiform schwannoma (IHC ×10) (c) Diffuse moderate staining intensity (2+) of CD56 in Plexiform schwannoma (IHC ×40). IHC: Immunohistochemistry, H&E: Hematoxylin and Eosin

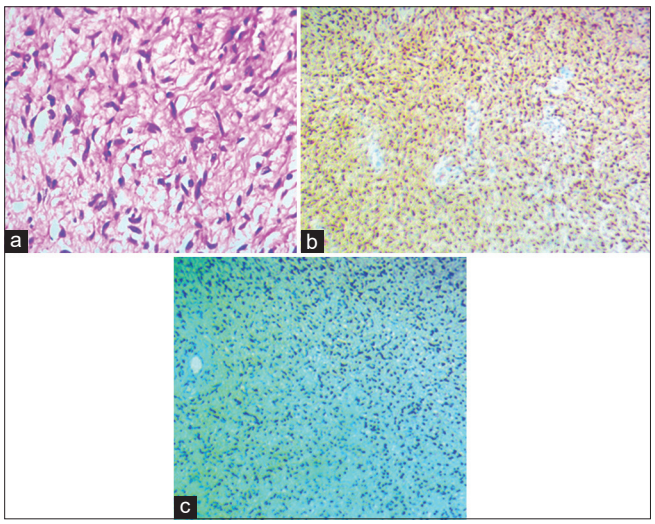

Fig. 2: (a) Neurofibroma showing short fascicles of spindle cells with wavy nuclei along with fibroblasts (H&E stain ×40) (b) Diffuse moderate staining intensity (2+) of S100 in neurofibroma (IHC ×10) (c) Patchy moderate staining intensity (2+) of CD56 in neurofibroma (IHC ×10). IHC: Immunohistochemistry, H&E: Hematoxylin and Eosin

The single case of MPNST in this study showed moderate intensity IHC positivity (2+) for S100 and weak intensity IHC positivity (1+) for the CD56 marker.

DISCUSSION

Clinical parameters

Out of the 41 PNSTs encountered during the study period, 20 cases were neurofibroma and its variants, 20 cases were schwannoma and its variants while one case was MPNST morphologically. This was in correlation with most of the previous studies done by Ghosh *et al.* [12], Jaiswal *et al.* [18], Park *et al.* [19], and Gabhane *et al.* [20] (Table 6).

Ghosh *et al.* [12] and Gabhane *et al.* [20] reported that benign PNSTs were more common than MPNSTs as in the present study (Table 7).

There was no significant difference in the mean age distribution of schwannomas (42.6 years) and neurofibromas (42.2 years) in this study which was in concordance with the findings of Rodriguez *et al.* [21]. The average age of 127 cases of neurofibromas encountered in the study by Oliveira *et al.* [22] was 37.8 years in contrast to the current study. Park *et al.* [19] reported that the incidence of schwannomas peaked in the third decade of life with the mean age of occurrence being 46.8 years. In another study by Patil and Chatura [23], classic schwannomas were more commonly encountered in the 2nd–4th decade of life.

In the current study, gender distribution revealed a slight female preponderance among both neurofibromas (1:1.8) and schwannomas (1:1.2) which was consistent with earlier research by Oliveira *et al.* [22] who reported male to female ratio of 1:1.8 in their study. Furthermore, Ghosh *et al.* [12] evaluated 114 cases of PNSTs and concluded that females were more commonly affected than males.

In the studies by Ghosh *et al.* [12] (12 cases in extremities, 9 cases in Head and Neck [H&N]), Jaiswal *et al.* [18] (11 cases in extremities, 4 cases in H&N) and Park *et al.* [19] (19 cases in extremities and

Table 5: Comparison of S100 and CD 56 IHC expression in neurofibromas and schwannomas

IHC marker	IHC score	Neurofibroma and its variants (n=20) (%)	Schwannoma and its variants (n=20) (%)	Pearson Chi-square test
S100	Negative	1 (5)	0	p=0.04
	1+	1 (5)	0	
	2+	17 (85)	12 (60)	
	3+	1 (5)	8 (40)	
CD56	Negative	2 (10)	4 (20)	p=0.015
	1+	8 (40)	2 (10)	
	2+	10 (50)	8 (40)	
	3+	0	6 (30)	

IHC: Immunohistochemistry

Table 6: Comparision of distribution of peripheral nerve tumors with other studies

Comparative studies	Schwannoma and its variants n (%)	Neurofibroma and its variants n (%)	Total cases (n)
Ghosh *et al*. [12]	39 (34.21)	51 (44.74)	114
Jaiswal *et al*. [18]	18 (40)	27 (60)	45
Park *et al*. [19]	101 (49.5)	103 (50.5)	204
Gabhane *et al*. [20]	64 (50.8)	54 (42.85)	126
Current study	20 (48.8)	20 (48.8)	41

Table 7: Comparative studies on the distribution of benign and malignant PNSTs

Comparative studies	Benign PNSTs n (%)	Malignant PNSTs n (%)
Ghosh *et al*. [12]	106 (93)	8 (7)
Gabhane *et al*. [20]	118 ((93.65)	8 (6.34)
Current study	40 (97.56)	1 (2.44)

PNSTs: Peripheral nerve sheath tumors

14 cases in H&N), schwannomas most frequently occurred in the upper and lower extremities followed by the H&N region [24] in concordance to the present study. However, in the study by Patil and Chatura [23] and Kumari *et al*. [24], the majority of conventional schwannomas were located in the H&N region (13/31 cases) followed by extremities (9/31 instances). This difference could be attributed to geographical variations, syndromic association of PNSTs, and a smaller sample size.

The histopathological distribution revealed that localized neurofibroma accounted for 14 cases followed by 11 cases of classical schwannoma in the current study. The uncommon variants encountered were microcystic/reticular and epithelioid schwannomas. These findings were similar to the studies by Jaiswal *et al*. [18] and Ghosh *et al*. [12]. However, in the studies by Patil and Chatura [23] and Gabhane *et al*. [20], ancient, cellular, and plexiform schwannomas were more prevalent subtypes, unlike the current study.

S100 expression

S100 is a protein expressed by chondrocytes, melanocytes, Langerhans cells, and neural or neural crest-derived tissues. Studies by Mahmood *et al*. [5], Guo *et al*. [7] and Park *et al.* [19] revealed that S100 was positive in both neurofibromas and schwannomas. They also observed that S100 was expressed more strongly and diffusely in schwannomas than in neurofibromas while MPNSTs showed patchy S100 positivity. S100 was positive in 100% of schwannomas and 95% of neurofibromas in the current study which was consistent with the observations by Jaiswal *et al*. [18], Park *et al*. [19] and Oliveira *et al*. [22]. Jaiswal *et al*. [18] observed diffuse strong intensity (3+) of S100 positivity in all the schwannomas (100%) and most of the neurofibromas. In contrast, only 40% of schwannomas exhibited strong (3+) staining intensity, whereas the majority of neurofibromas exhibited only moderate intensity (2+) staining pattern in the present study (Figs. 1b and 2b). Since S100 is a protein expressed by Schwann cells, it is observed that its expression is high in all cases of schwannomas compared to neurofibromas. Neurofibroma has a mixed cell population that includes fibroblasts, Schwann cells, and perineurial-like cells which explains the slightly lower staining intensity of S100 among neurofibromas [19]. Thus, there was a statistically significant difference in the staining intensity of S100 which was more for schwannomas (p=0.04) in the present study.

One case of MPNST in the current study displayed moderate staining intensity (2+) for S100. Karamchandani *et al*. [8] reported that S100 had 40% sensitivity for the detection of MPNST with slight downregulation of S100 in MPNST compared to benign PNSTs.

CD 56 (neural cell adhesion molecule) expression

Guo *et al*. [7] observed a preferential CD56 expression in schwannomas compared to neurofibromas. Around 90% of neurofibromas and 80% of schwannomas were positive for CD56 with variable staining intensity for CD56 in the current study (Figs. 1c and 2c) similar to the findings of Jaiswal *et al*. [18]. The current study showed a statistically significant difference (p=0.015) in the staining intensity of CD 56 more for neurofibromas compared to schwannomas. Park *et al*. [19] and Guedes-Corrêa and Cardoso [25] emphasized that CD56 could be used in combination with S100 in the diagnosis of PNSTs.

Limitations

The histological subtypes of PNSTs were not uniformly distributed in the present study. Further research by expanding the sample size and including equal representations of all the histological subtypes of PNSTs including those cases with overlapping hybrid features will provide better insights into the intensity of IHC expression of CD56 and S100 among all the histological subtypes of PNSTs. Furthermore, to understand the prognosis and aggressiveness of PNSTs, clinical data including patient follow-up, particularly recurrence and malignant transformation, may be helpful.

CONCLUSION

Precise diagnosis of PNSTs is crucial, particularly in the case of core-needle biopsies that might not fully depict the tumor. On the basis of histopathology alone, it may be difficult to diagnose and differentiate certain histological variants of neurofibromas and schwannomas from low-grade MPNST. In these situations, IHC has demonstrated its ability to aid in the accurate diagnosis, which may be crucial in determining the course of treatment, including the type of surgery (which may involve nerve-sparing methods). IHC analysis employing CD56 along with S100 will be a useful supplementary tool for the histopathologist in differentiating peripheral nerve sheath tumors from other soft tissue tumors, particularly in the setting of core needle/small biopsies and in cases where histological overlapping features, including variants, exist.

ACKNOWLEDGMENT

The authors would like to take this opportunity to express sincere gratitude to the Management, Dean, Our colleagues, and lab technicians for their support and encouragement. We also thank the patients for giving us consent to do the research.

AUTHORS' CONTRIBUTION

Concept and Study design were carried out by Dr. Karthik Sigamani. Dr. Nayana Chandran was responsible for data acquisition, data analysis and interpretation. Dr. Shobana B was responsible for drafting the manuscript and performing statistical analysis. Critical revision of manuscript and overall supervision were provided by Dr. Karthik Sigamani and Dr. Shobana B. All authors have read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

AUTHOR FUNDING

None.

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