Original Research Article

Morphologic spectrum of Peripheral Neuroblastic Tumors- A 5 year retrospective study at a tertiary care centre in South India

Archana Rajan[1*], Prema N.S[2]

[1]Assistant Professor, [2]Additional Professor, [1]Dept. of Transfusion Medicine, [2]Dept. of Pathology, [1]Government Medical College, Kozhikode, Kerala, [2]Government Medical College, Kollam, Kerala, India

Abstract

Introduction: This study conducted at the department of Pathology, Govt. Medical College, Thiruvananthapuram aimed at studying the morphologic spectrum of Peripheral Neuroblastic tumors based on the International Neuroblastoma Pathology Committee (INPC) criteria.

Materials and Methods: The study included all cases of Peripheral Neuroblastic tumors received in the Department of Pathology, Government Medical College, Thiruvananthapuram during the period January 2004 to December 2008. Resected specimens and Incisional biopsy specimen's/ lymph nodes of size more than 2x2 cm were included as per INPC criteria. Specimens were grossly and histopathologically studied and classified. Immunohistochemical markers were used only for undifferentiated neuroblastoma.

Results: Of 56 cases received, one incisional biopsy was rejected. Of the remaining 55, 37 were Neuroblastomas (67%), 8 Ganglioneuroblastomas (15%) and 10 Ganglioneuromas (18%). The predominant type of Neuroblastoma received was Differentiated - 26 cases (70%). Among Ganglioneuroblastomas 50% were of the Nodular classical type and in Ganglioneuromas 100% were of the mature type.

Conclusion: The morphological spectrum of Peripheral Neuroblastic tumors were analyzed in 55 cases which included 37 cases of Neuroblastomas, 8 Ganglioneuroblastomas and 10 Ganglioneuromas. Further subtyping based on the I.N.P.C classification showed Neuroblastomas- 26 Differentiating, 9 Poorly Differentiated and 2 Undifferentiated. Among the Ganglioneuroblastomas- 4 were Nodular Classical, 2 Variant and 2 Intermixed. All 10 Ganglioneuromas were mature.

Introduction

Morphologically, Peripheral neuroblastic tumors can range from undifferentiated truly malignant Neuroblastomas (NB), via Ganglioneuroblastomas (GNB) to well differentiated benign Ganglioneuromas (GN). As a family, neuroblastic tumors demonstrate certain characteristic features such as spontaneous or therapy-induced differentiation of primitive neuroblasts into mature elements, spontaneous tumor regression, and a wide range of clinical behavior and prognosis which often mirror the extent of histologic differentiation.[1-4]

This heterogeneous group of tumors do not lend themselves to a simple, readily reproducible morphologic classification of nosologic and prognostic significance.[2,5] This could be because they do not have a widely accepted and widely used uniform terminology and criteria for designation of types and subtypes; and are often diagnosed on the basis of an incisional biopsy which maybe non-representative. Furthermore the pathologist also has to take into consideration the well-established genetic prognostic markers.[6]

To date several classifications have been put forth for this group of tumors. The latest accepted pathologic classification is that based on the recommendations of the International Neuroblastoma Pathology Committee (INPC), 1999 and partly revised in 2003.[7,8]

The present study conducted at the Department of Pathology, Medical College, Thiruvananthapuram aims at studying the morphologic spectrum of Peripheral Neuroblastic tumors based on the INPC criteria.[7,8]

Materials and Methods

This study included all cases of Peripheral Neuroblastic tumors received in the Department of Pathology, Government Medical College, Thiruvananthapuram during the 5year period January 2004 to December 2008.

Specimens were categorized as
1. Resected specimens
2. Incisional Biopsy specimens / Lymph nodes

Biopsy specimens of less than 2x2cm size were not included in the study as per the I.N.P.C guidelines.[7,8]

All the specimens received were fixed in 10% formalin.

Gross features of resected specimens were noted, lymph nodes- both adherent/ non-adherent to capsule were looked into.

For resected specimens, sections were taken from grossly heterogeneous areas, any normal tissue if present, and from lymph nodes. For biopsy specimens the tissue was all embedded.

The tissues were processed, paraffin embedding was done and sections were stained with Haematoxylin and Eosin. Multiple serial sections were also taken and studied.Based on the size of the specimen, the number of slides studied varied from One (with 2-3 sections per slide) in cases of incisional biopsy to Ten (with 2-3 sections per slide) in case of resection specimens.

Histopathological findings were categorized accordingly as per the I.N.P.C criteria[7,8] (Table 1)

Table 1: International Neuroblastoma Pathology Committee Classification

Neuroblastoma (NB)	1.	Undifferentiated
	2.	Poorly Differentiated
	3.	Differentiating
Ganglioneuroblastoma (GNB)	1.	Nodular Classical
	2.	Variant [± macroscopically visible nodule(s)]
	3.	Intermixed
Ganglioneuroma (GN)	1.	Maturing
	2.	Mature

The diagnosis of Undifferentiated Neuroblastoma was confirmed by a panel of immunohistochemical markers that included Neuron Specific Enolase, Desmin and Leucocyte Common Antigen.

Results

During the study period of 5years, we received a total of 56 cases of Peripheral Neuroblastic tumors. Of these 45 cases were resection specimens and 11 were incision biopsy specimens. Among the biopsy specimens 1 case was less than 2x2cms, the criteria for inclusion as per I.N.P.C criteria and was hence excluded.

The study was hence carried forth on a total of 55 cases which included 45 resection specimens and 10 incision biopsies.

The results are as follows-

All the cases received were divided into the three classes of Peripheral Neuroblastic tumors-

Neuroblastoma (NB)- 37 cases, Ganglioneuroblastoma (GNB)- 8 cases and Ganglioneuroma (GN)- 10 cases.

The relative proportion of cases are depicted in Fig. 1.
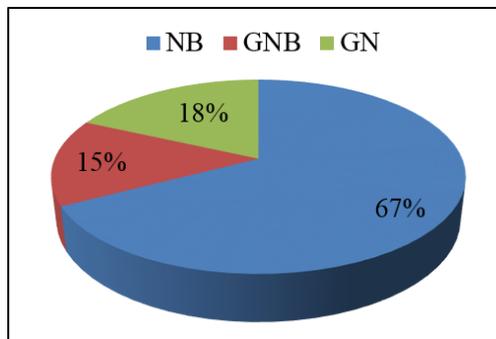
Fig. 1: Case distribution

Pre-operative chemotherapy in Neuroblastomas

All the 27 [73%] resection specimens of neuroblastoma that were received had history of pre-operative chemotherapy. The remaining 10 cases [27%] of incision biopsy specimens had no pre- operative chemotherapy.

Gross Specimen Findings

We received 27 resection specimens of neuroblastoma post-chemotherapy. The weight of the specimens ranged from 10gms to 300gms with majority [10 cases] having weights between 20-40gms.

The sizes of the specimens ranged from 15cms in greatest diameter to 5cms. Only those incision biopsy specimens that measured > 2x2cms were included.

All but one specimen were well circumscribed with the typical appearance of grey-white lobulated growth with variable calcification, cystic change, hemorrhage and necrosis. The other showed infiltration into adjacent fat. The most common features seen on gross examination were hemorrhage [26 cases] and necrosis [17 cases] (Fig.2,3). None of the cases had adherent lymph nodes. Eleven cases had associated non-adherent lymph node enlargement.
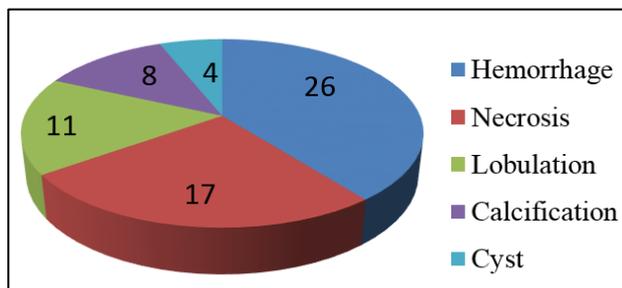

Fig. 2: Gross features of Neuroblastoma in 27 resection post chemotherapy specimens

Of the eight Ganglioneuroblastomas received the most common feature was nodularity [5 cases].Associated features of hemorrhage necrosis and calcification were seen and was correlated with the histology for the final diagnosis. (Fig. 3)

All ten cases of Ganglioneuromas had a grey-white firm cut-surface with whorling seen at least focally. (Fig. 3)
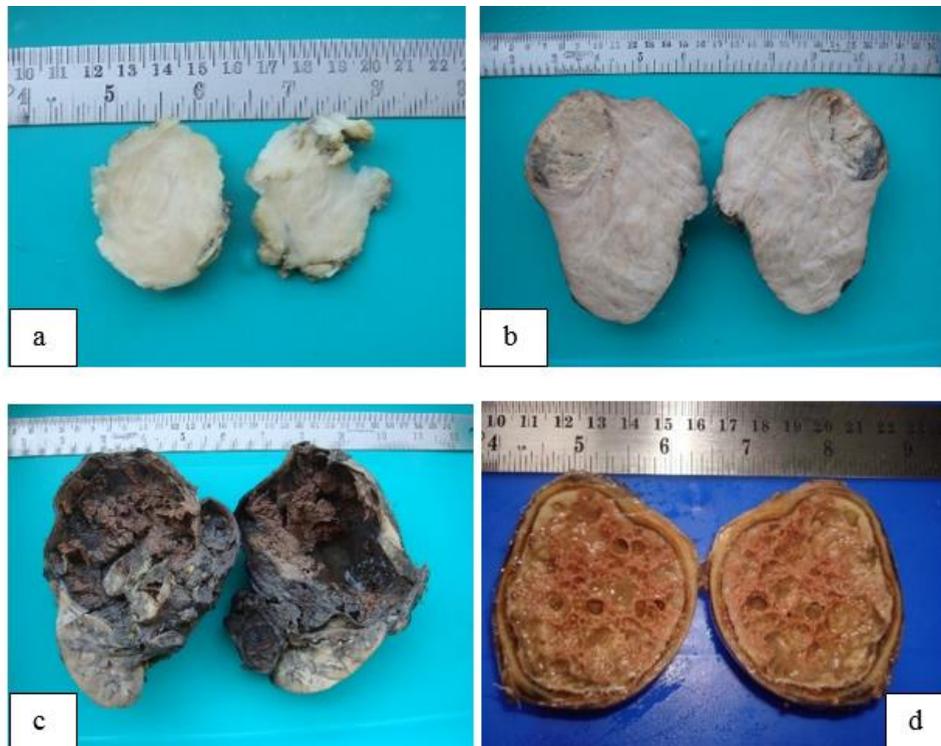
Fig. 3: Cut Surface of Gross Specimens of Peripheral Neuroblastic Tumors; **a:** Ganglioneuroma with whorled appearance (Histology GN Mature); **b:** Ganglioneuroblastoma with whorled Ganglioneuromatous area and circumscribed Neuroblastomatous area (Histology GNB Nodular); **c:** Neuroblastoma with cystic change, hemorrhage and necrosis. Compressed Kidney seen below. (Histology NB Poorly differentiated); **d:** Neuroblastoma with cystic change (Histology NB Well differentiated)
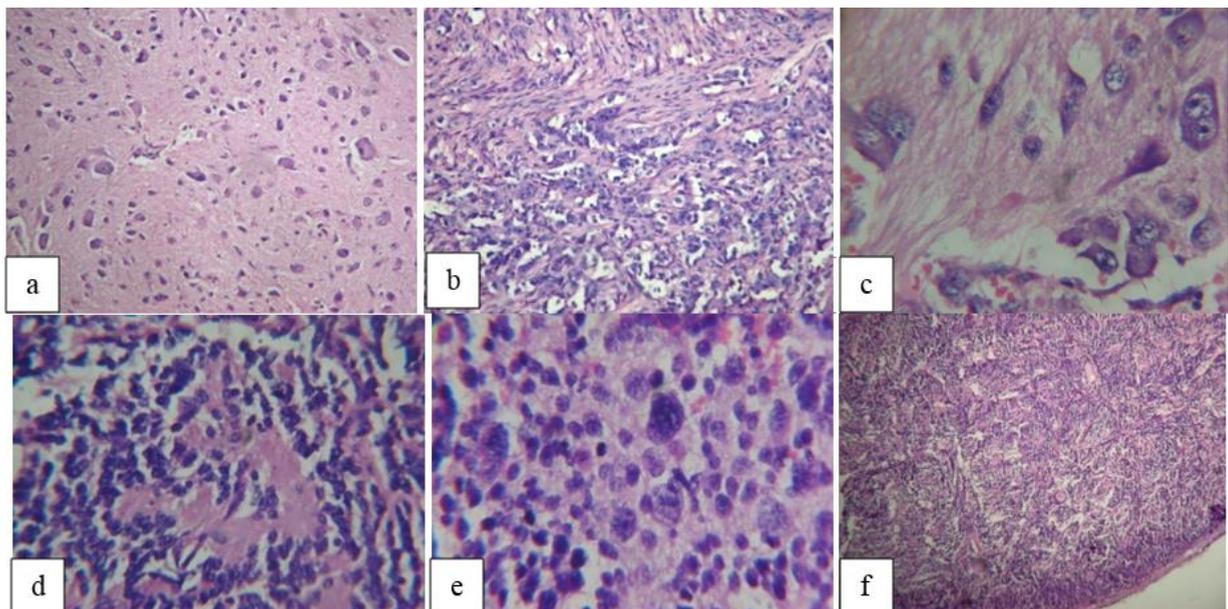
Fig. 6: Photomicrographs showing histology of **a:** Ganglioneuroma Mature (10x); **b:** Ganglioneuroblastoma with mature ganglion cells, schwannian stroma and differentiating neuroblasts (10x) **c:** Mature Ganglion cells (40x); **d:** Neuroblastoma with rosette (40x); **e:** Bizzare cells in Post chemotherapy Neuroblastoma (40x); **f:** Lymph node metastasis(4x)

Microscopy

The classification of the tumors was done as per the I.N.P.C criteria.[7,8]

The Neuroblastoma cases that we received belonged predominantly to the differentiating category- 26 cases [70%] with 5-50% of the neuroblasts showing ganglionic differentiation in a background of neuropil. Most showed areas of hemorrhage, necrosis and /or calcification. (Fig. 4)

One case showed infiltration of surrounding fat. Adherent lymph nodes were identified in eleven cases of which seven showed metastasis.

The poorly differentiated cases, which were 9 in number (24%) showed only <5% differentiation towards ganglion cells. Background showed neuropil. Areas of calcification were seen in some. One case also showed bizarre cells.

The undifferentiated group of 2 cases (6%) showed sheets of small round cells with frequent mitosis and apoptotic bodies. No neural differentiation was seen light microscopically. Confirmation was by Immunohistochemistry using Neuron Specific Enolase, Desmin and Leucocyte Common Antigen.
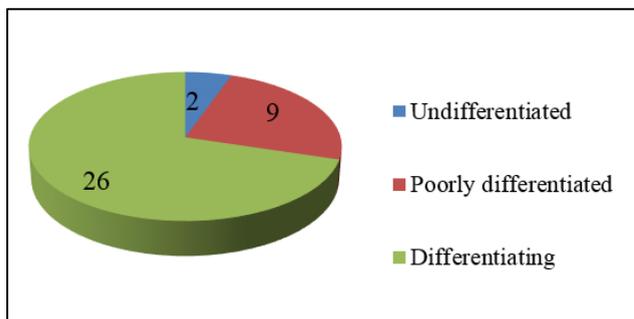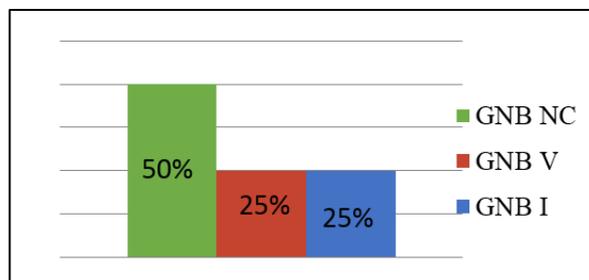

Fig. 4: Neuroblastoma subtypes

In both pre and post chemotherapy cases the predominant group was Differentiated Neuroblastoma. (Table 2). The post chemotherapy cases showed extensive areas of fibrosis and hyalinization. Areas of necrosis were also noted.

Table 2: Neuroblastoma- association with chemotherapy

	U.NB	P.NB	D.NB
Chemotherapy	0	9	18
No Chemotherapy	2	0	8

The Ganglioneuroblastoma cases (Fig. 5) received were mostly of the Nodular Classical type- 4 cases [50%], showing grossly visible neuroblastomatous nodule and ganglioneuromatous component. Microscopically the neuroblastomatous elements in all the four cases were of the poorly differentiated type. There were two cases each of variant and intermixed types. The variant cases showed predominantly neuroblastomatous element with rimming by ganglioneuromatous element. The intermixed type showed predominantly ganglioneuromatous component with small nodules of neuroblastomatous component.

Fig. 5: Ganglioneuroblastoma subtypes

All ten cases of Ganglioneuromas received were of the mature type (100%) showing mature ganglion cells, neuritic processes and mature fibrous tissue.

Discussion

During the study period of five years from January 2004 to December 2008, we received a total of 56 cases of Peripheral Neuroblastic tumors which included both incisional biopsy and resection specimens. One case was less than 2x2cms, the criteria for inclusion as per I.N.P.C criteria and was hence excluded. Among the remaining 55 cases, 37 cases (67%) were Neuroblastomas, 8 (15%) were Ganglioneuroblastomas and 10 (18%) were Ganglioneuromas.

Comparison with other studies is provided in Table 3

Table 3

S. No	Study	NB (%)	GNB (%)	GN (%)
1.	Hiroyuki Shimada et al[9]	84%	14%	2%
2.	Shoko Goto et al[10]	85%	13%	2%
3.	Samuel Navarro et al[11]	69%	25%	Unclassifiable
4.	Present study	67%	15%	18%

In all the three studies, maximum incidence was in the Neuroblastoma subgroup. The incidence of neuroblastomas can also be seen to be comparable with the study by Samuel Navarro et al.[11] A higher incidence is noted in the studies by Hiroyuki Shimada et al[9] and Shoko Goto et al.[10] The present study showed a higher incidence of ganglioneuromas than ganglioneuroblastomas (18%:15%). In the studies by Hiroyuki Shimada et al and Shoko Goto et al a reverse proportion is seen.

Gross Features

All the resection specimens of Neuroblastoma received were post-Chemotherapy cases. The commonest features seen were Hemorrhage (96.3%) and Necrosis (63%). The other features were Lobulation (40.7%), Calcification (29.6%) and Cystic change (14.8%).

The common features of neuroblastoma includes variegation with hemorrhage, calcification, necrosis, cystic change and grey white areas. Dense fibrosis with or without calcification has been mentioned in some with pre-operative chemotherapy.[12]

Microscopy

70% of the Neuroblastoma cases that we received were of the Differentiating type followed by poorly differentiated in 24% and Undifferentiated in 6% of the cases.

In the study by Hiroyuki Shimada et al[9] Neuroblastomas were Poorly differentiated in 85%, Differentiating in 13% and Undifferentiated in 2%. The study by Samuel Navarro et al[11] also showed a preponderance of the Poorly differentiated subtype in 97% the remainder being Differentiating. Undifferentiated cases were not analyzed.

50% of Ganglioneuroblastomas were Nodular classical, 25% Variant and 25% Intermixed. The study by Hiroyuki Shimada et al[9] also showed predominance of the Nodular Ganglioneuroblastoma (71.7%). These were not further subtyped.

All the cases of Ganglioneuromas that we received were of the mature subtype. All the cases in the study by Hiroyuki Shimada et al[9] were of the Mature type too.

Conclusion

The morphological spectrum of Peripheral Neuroblastic tumors was analyzed in 55 cases and each of the major subgroup was further subtyped based on the I.N.P.C classification.

During the five year study period the Peripheral neuroblastic tumors received showed maximum incidence of the Malignant Neuroblastomas-67%, followed by the Benign Ganglioneuromas -18%, with the Intermediate Ganglioneuroblastomas showing an incidence of 15%.

Among Neuroblastomas the commonest histolological subtype was Differentiated (70%) followed by Poorly Differentiated (24%) and Undifferentiated (6%). 50% of Ganglioneuroblastomas were Nodular Classical, 25% Variant and 25% Intermixed. All Ganglioneuromas (100%) were Mature.

Similar to previous studies, this study also showed a greater incidence of Neuroblastomas among all Peripheral neuroblastic tumors. But the incidence of Ganglioneuromas is higher in the present study compared to Ganglioneuroblastomas, unlike similar studies where the reverse incidence was seen.

Conflict of Interest: None.

Source of Funding: None.

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