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Study of Hemato-morphological Features in Neuroblastoma Infiltrating Marrow

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Abstract

Objective Neuroblastoma typically affects children within the first 5 years of life and accounts for 10% of all pediatric malignancies. Neuroblastoma at onset may manifest as a localized or metastatic illness. The aim of this study was to identify hematomorphological features in neuroblastoma infiltrating marrow as well as to ascertain the prevalence of bone marrow infiltration in neuroblastoma.

Materials and Methods This retrospective study included newly diagnosed 79 cases of neuroblastoma, which were referred for bone marrow examination for the staging of the disease. Medical records were retrieved to acquire hematomorphological findings of peripheral blood and bone marrow smears. Statistical Package for Social Sciences, IBM Inc., USA, version 21.0 was used to analyze the data.

Results The interquartile age range of neuroblastoma cases was 24.0 to 72.0 months (median = 48 months) with a male to female ratio of 2.7:1. Also, 55.6% (44/79) of cases in the study population showed evidence of marrow infiltration. The bone marrow infiltration was significantly linked to thrombocytopenia ($p = 0.043$) and nucleated red blood cells ($p = 0.003$) in peripheral blood. The bone marrow smears of cases with infiltration showed a significant shift to the left in the myeloid series ($p = 0.001$) and an increased number of erythroid cells ($p = 0.001$).

Conclusion For neuroblastoma patients, a diligent, exhaustive search for infiltrating cells in bone marrow is advised if thrombocytopenia or nucleated red blood cells are identified on a peripheral blood smear and bone marrow smears showed myeloid left shift with an increased number of erythroid cells.

Keywords
- neuroblastoma
- bone marrow
- peripheral blood
- thrombocytopenia
- nucleated red blood cells

Introduction

A pediatric tumor termed neuroblastoma (NB) develops from the peripheral sympathetic nervous system. Neuroblastoma is specifically produced from neural crest (NC)-derived cells that are undergoing defective sympathetic neuronal evolution due to genomic and epigenetic abnormalities (NB). Although it can occur everywhere that

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migrating neural crest cells (NCCs) and their progeny can reach, NB most usually occurs in the paraspinal ganglia or the adrenal medulla (AM).[1] The most prevalent solid extracranial tumor in children, accounting for 10% of pediatric malignancies and 50% of those in infants, is neuroblastoma. It is a pediatric tumor, 36% of children who are diagnosed with it are under 1 year old, 75% are under 5, and more than 90% are under 10 years old.[2–4] Patients are divided into risk categories according to many prognostic criteria, such as age at diagnosis, stage, histological characteristics, and molecular abnormalities. NB at onset may manifest as a localized or metastatic illness. NB is a major cause of death from childhood cancer, especially in high-risk children with chemo-resistant recurrence, whose survival rate is only approximately 40%.[5]

Another justification for precisely determining the state of the marrow in newly diagnosed instances is autologous bone marrow "rescue."[6] The International Neuroblastoma Staging System (INSS) classifies a pediatric tumor that arises from the developing peripheral sympathetic nervous system. Involvement of the bone marrow, which is classified as stage 4, is common in patients with advanced disease. For the purpose of evaluating the marrow infiltration in solid tumors, several studies have been performed.[7–9] Only a few studies have explored the marrow infiltration in neuroblastoma and its relationship to hematological parameters.[10–12]

Aims and Objectives

The aim of this study was to identify hematomorphological features in neuroblastoma infiltrating marrow as well as to ascertain the prevalence of bone marrow infiltration in neuroblastoma.

Materials and Methods

This study was conducted in the Department of Pathology, King George Medical University, Lucknow, a tertiary care center in the state of Uttar Pradesh, North India. The study included newly diagnosed cases of neuroblastoma presented for a bone marrow examination between January 2018 to May 2022 for the staging of the disease. The clinico-radiological characteristics, histopathology, and immunohistochemistry findings were used to make the primary diagnosis of neuroblastoma in all cases. The posterior superior iliac spine was used for the bone marrow procedure. After that, according to established procedures, aspirate smears and biopsy sections were stained with May-Grunwald Giemsa and hematoxylin and eosin stain, respectively.[13,14] The complete blood count, peripheral blood smear examination, bone marrow morphological features, as well as the presence or absence of neuroblastoma infiltration and immunohistochemistry findings were retrieved from the records of each patient's bone marrow case sheets.

Statical Analysis

The Statistical Package for Social Sciences (SPSS, IBM Inc., USA) version 21.0 was used to analyze the data. To compare categorical data, the chi-square test was utilized, while independent *t*-tests were used to analyze parametric data. A statistically significant value was defined as one with a *p*-value less than 0.05.

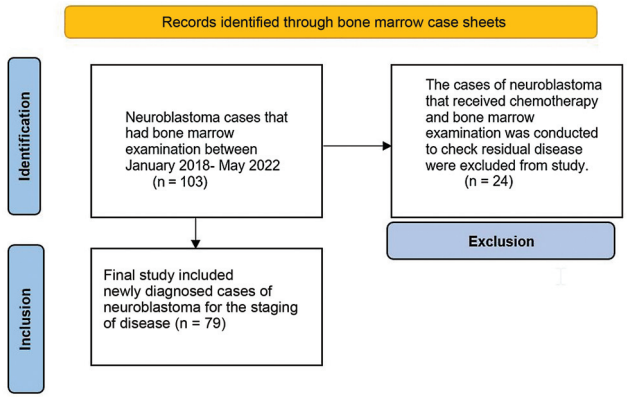

Fig. 1 PRISMA flow chart of the identification and inclusion of neuroblastoma cases.

Results

All neuroblastoma patient records that had a bone marrow examination between January 2018 and May 2022 were retrieved from bone marrow case sheets. Records of total 103 cases of neuroblastoma were retrieved. The cases that received chemotherapy (24/103) and a bone marrow examination conducted to check for any residual disease were excluded from the study because there was no information of their bone marrow condition at the time of diagnosis. The final study included 79 newly diagnosed patients with neuroblastoma for the staging of the disease (►**Fig. 1**).

The interquartile age range of neuroblastoma cases was 24.0 to 72.0 months (median = 48 months) with a male to female ratio of 2.7:1. Two bone marrow aspirates, two trephine biopsy touch imprints, a bone marrow biopsy, and a battery of immunohistochemistry marker were evaluated in each case. All findings were retrieved from the bone marrow case sheet records. Marrow infiltration was noted in 55.6% (44/79) of the neuroblastoma cases (►**Table 1**).

The complete blood count of neuroblastoma cases with marrow infiltration revealed anemia in 81.2% (36/44) of the cases with an interquartile range of hemoglobin (Hb) 6.93 to 9.80 g/dL (median = 8.80 g/dL), leukopenia in 9.09% (4/44) of cases with an interquartile range of total leucocyte count (TLC) 8.08×10^9/L to 12.7×10^9/L (median = 10.0×10^9/L) and thrombocytopenia in 29.55% (13/44) of cases with an interquartile range of platelet count (PC) 86×10^9/L to 388×10^9/L (median = 240×10^9/L) (►**Table 1**).

It was observed that bone marrow involvement in cases of neuroblastoma was significantly linked to the presence of thrombocytopenia ($p = 0.043$) and nucleated red blood cells (nRBCs) ($p = 0.003$) in peripheral blood. All remaining hematological parameters such as anemia ($p = 0.594$), leukopenia ($p = 0.189$), and bicytopenia ($p = 0.369$) were not significantly correlated with marrow infiltration in neuroblastoma cases. Pancytopenia was present in 9.09% (4/44) of

Table 1 Demographic profile and hematological parameters in patients of the neuroblastoma

Parameters		Total cases ($n = 79$)	Cases with marrow infiltration ($n = 44$)	Cases without marrow infiltration ($n = 35$)	*p*-Value
Age (mo)	Mean ± SD	50.91 ± 34.52	50.02 ± 34.48	52.03 ± 35.05	0.799
	Median	48.00	48.00	48.00	
	Interquartile Range	24.0–72.0	24.0–72	24.0–72	
Gender (*n*, %)	Male	58 (73.42%)	32 (72.73%)	26 (74.29%)	0.876
	Female	21 (26.58%)	12 (27.27%)	9 (25.71%)	
Hemoglobin (Hb)	Mean ± SD	9.04 ± 2.33	8.67 ± 2.41	9.51 ± 2.17	0.115
	Median	9.10	8.80	9.80	
	Interquartile range	7.20–10.70	6.93–9.80	7.30–11.20	
Total leucocyte count ($\times 10^9$/L)	Mean ± SD	10.61 ± 4.05	10.01 ± 3.65	11.37 ± 4.43	0.137
	Median	10.00	10.00	11.00	
	Interquartile range	8.50–12.90	8.08–12.78	8.90–13.00	
Platelet count ($\times 10^9$/L)	Mean ± SD	305 ± 181	258 ± 186	363 ± 158	0.009*
	Median	300	240	350	
	Interquartile range	180–450	86–388	270–450	

*Significant ($p < 0.05$).

Table 2 Frequency of cytopenias and nucleated red blood cells in neuroblastoma cases with and without infiltration of the bone marrow

Parameters	Total cases ($n = 79$)		Cases with marrow infiltration ($n = 44$)		Cases without marrow infiltration ($n = 35$)		*p*-Value
	n	%	*n*	%	*n*	%	
Anemia	62	78.48	36	81.82	26	74.29	0.594
Leucopenia	4	5.06	4	9.09	0	0.00	0.189
Thrombocytopenia	16	20.25	13	29.55	3	8.57	0.043*
Bicytopenia	11	13.92	8	18.18	3	8.57	0.369
Pancytopenia	4	5.06	4	9.09	0	0.00	0.189
Nucleated red blood cells	18	22.78	16	36.36	2	5.71	0.003*

*Significant ($p < 0.05$).

cases of neuroblastoma with marrow infiltration; however, none of the cases of neuroblastoma without marrow infiltration showed pancytopenia ($p = 0.189$) (►**Table 2**).

The bone marrow aspirate smears findings were retrieved from records in each case. The bone marrow smears showed a significant left shift in myeloid series ($p = 0.001$) and an increased percentage of erythroid cells ($p = 0.001$) in cases with bone marrow infiltration by neuroblastoma in comparison to cases without bone marrow involvement. The percentage of lymphocytes ($p = 0.497$) and plasma cells ($p = 0.646$) were not significantly linked with cases of neuroblastoma infiltrating marrow (►**Table 3**).

Trephine biopsy findings were also retrieved from records in each case. In 36 of the 44 positive cases, trephine biopsy and aspirate smear both revealed infiltration. In five cases, only aspirate smears and in another three cases, only trephine biopsies were positive. In five cases where infiltration was detected only by aspirate, biopsy sections primarily revealed diffuse fibrosis, and in cases where infiltration was detected only by biopsy, there was localized involvement that was missed by aspiration due to improper sampling. The most prevalent pattern of infiltration was rosette formation, which was observed in 31.8% (14/44) of cases on trephine biopsies and in 52.2% (23/44) of cases on aspirate smears (►**Fig. 2A, B**) (►**Fig. 2C, D**). The remaining cases displayed an interstitial focal, and diffuse pattern of infiltration in bone marrow biopsies. Immunohistochemistry was applied in all cases and found positive for synaptophysin and chromogranin (►**Fig. 2E, F**). In atypical cells infiltrating the bone marrow, other immunohistochemistry

Table 3 Bone marrow aspirate smear findings in cases of Neuroblastoma with and without infiltration of the bone marrow

Percentage of bone marrow nucleated cells	Total cases ($n = 79$)		Cases with marrow infiltration ($n = 44$)		Cases without marrow infiltration ($n = 35$)		*p*-Value
	Mean	± SD	Mean	± SD	Mean	± SD	
Promyelocyte	1.61	0.84	1.84	0.96	1.31	0.53	0.005*
Myelocyte	18.81	7.27	22.11	7.22	14.66	4.85	< 0.001*
Metamyelocyte	12.87	5.75	15.91	5.63	9.06	2.97	< 0.001*
Band forms + segmented neutrophils	20.25	13.46	10.57	6.63	32.43	9.28	< 0.001*
Erythroid cells	33.70	9.91	37.27	10.52	29.20	6.94	< 0.001*
Lymphocytes	11.90	6.14	11.48	6.78	12.43	5.27	0.497
Plasma cells	2.04	1.22	2.12	1.17	1.95	1.29	0.646

*Significant ($p < 0.05$).

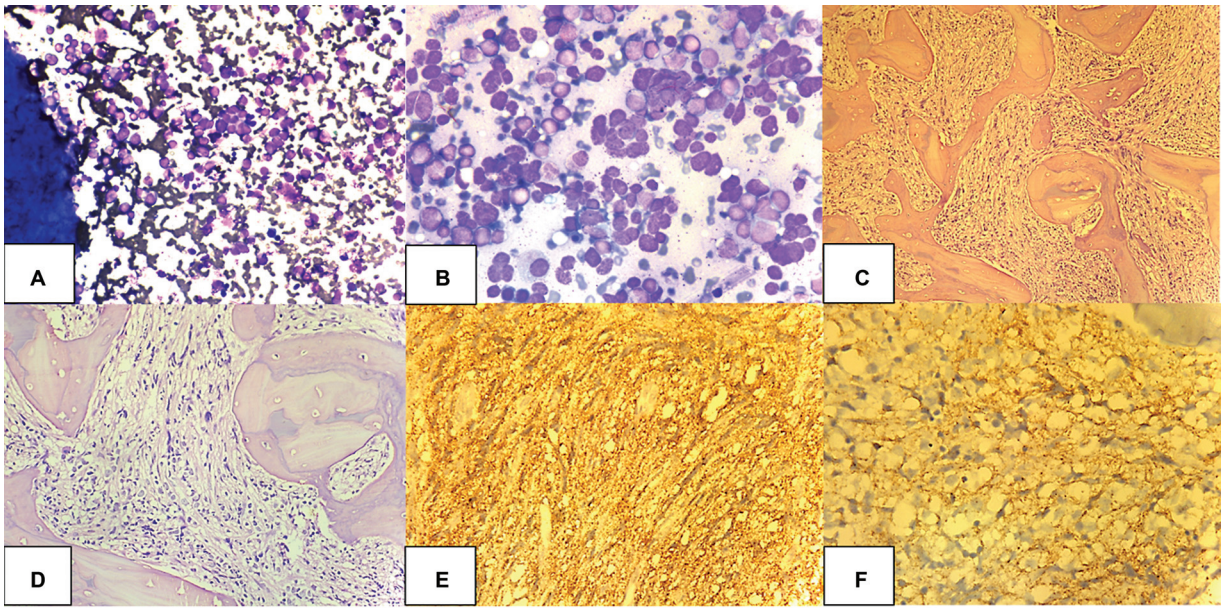

Fig. 2 Bone marrow aspirate, bone marrow biopsy and immunohistochemistry findings of bone marrow examination. (**A**) Bone marrow aspirate smear showing infiltration by neuroblastoma (May-Grunwald-Giemsa,100×) (**B**) Bone marrow aspirate smear showing infiltration by neuroblastoma with numerous rosettes (May-Grunwald-Giemsa, 400×) (**C**) Hematoxylin and eosin stained histologic section of bone marrow trephine biopsy showing infiltration by neuroblastoma (H&E, 100×) (**D**) Hematoxylin and eosin stained histologic sections showing bone marrow trephine biopsy displaying neuroblastoma infiltration with numerous rosettes (H&E, 200×) (**E**) Bone marrow biopsy displaying positive expression of chromogranin antibody in tumor cells (200×) (**F**) Bone marrow biopsy displaying positive expression of synaptophysin antibody in tumor cells (200×).

markers of round cell malignancy such as leucocyte common antigen (LCA), CD99, desmin, and myogenin were not expressed.

Discussion

Neuroblastoma is an embryonic tumor that develops from cells in the neural crest, an unstable structure made up of multipotent stem cells active in the early stages of embryonic development that arise along the borders of the closing neural tube.[15] An estimated two-thirds of primary tumors are found in the abdomen. It has the potential to spread to the local lymph nodes as well as distant locations, primarily the bone marrow, cortical bone, liver, and skin. Paraneoplastic disorders such as OMAS (opsoclonus-myoclonus-ataxia syndrome) or persistent watery diarrhea can also be symptoms of neuroblastoma.[16] All neuroblastoma patients must have their marrow examination for staging purposes; if invaded, the patient falls into a high-risk category and needs intensive chemotherapy.[7] A poor prognosis is nearly invariably associated with bone marrow metastases. The homing of tumor cells to the marrow and the ensuing bone metastases are largely the result of interactions between the tumor cells and the BM microenvironment.[17] In this study, 44 patients (55.6%) showed bone marrow infiltration by neuroblastoma. Pulkit et al[18] found similar findings and noted marrow

involvement in 54.5% of cases under the age of 5 years, with male predominance. Cozzutto et al[19] also observed similar findings in 58.3% of cases (7 out of 12 cases), and Franklin et al[20] reported a similar frequency in 48.9% of cases (24 out of 49 cases). According to Madhumati et al's study, neuroblastoma is a non-hematological tumor that affects the bone marrow most frequently (48.8%), followed by retinoblastoma (11.1%), Ewing's sarcoma/primitive neuroectodermal tumor (PNET) (8.6%), and rhabdomyosarcoma (3.2%).[21] In our study, thrombocytopenia and the presence of nucleated red blood cells (nRBCs) in patients with and without marrow invasion showed a statistically significant difference. Bone marrow involvement causes inhibition of normal hematopoiesis, which results in peripheral cytopenias. This study showed pancytopenia in 9.09% (4/44) of the cases with marrow infiltration; however, not a single case of neuroblastoma without marrow infiltration showed pancytopenia ($p = 0.189$). No significant correlation was noted for anemia in patients of neuroblastoma with and without infiltration, presumably due to the frequency of iron insufficiency in India rather than related to the illness itself. Similar findings have also been described in prior studies.[7,8] In cases of neuroblastoma with bone marrow infiltration, the aspirate smears from the bone marrow demonstrated a significant left shift in myeloid series ($p = 0.001$) and an increased percentage of erythroid cells ($p = 0.001$) when compared with cases without bone marrow infiltration. We could not compare our results due to the unavailability of such literature even after an extensive search. Patients who have metastases may benefit from therapeutic intensification because they have advanced disease stages, which indicate a poor clinical prognosis. It is advised that these individuals get routine bone marrow screening for staging and treatment. Bone marrow examination, however, is an easy, uncomplicated, and affordable method used to stage and monitor these patients[22] and is ideal in resource-constrained situations in third-world nations. The weakness of this study was not employing molecular investigations, which can help in detecting minor marrow infiltration.

Conclusion

For neuroblastoma patients, a diligent, exhaustive search for infiltrating cells in bone marrow is advised if thrombocytopenia or nucleated red blood cells are identified on a peripheral blood smear and bone marrow smears showed myeloid left shift with an increased number of erythroid cells. Bone marrow infiltration is a strong predictive feature of poor prognosis and is associated with a more advanced disease stage; hence, early diagnosis is essential. Staging and monitoring of patients in a setup with limited resources is simple and economical and is attributable to bone marrow examination.

Authors' Contributors

R.K. did study designing, conceptual analysis, data acquisition, and literature search. A.S. did literature search, and conceptual analysis and proof correction. S.R. did data analysis and designing. G.Y. did data analysis and proofreading. S.P.V. contributed to clinical studies and literature searches. U.S. Singh did study design and literature search.

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Conflict of Interest

None declared.

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