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ORIGINAL ARTICLE

BONE MARROW INFILTRATION IN NEUROBLASTOMA AND ASSOCIATED HEMATOLOGICAL FEATURES

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ABSTRACT

Background and Objectives: The second commonest solid tumor in children is neuroblastoma and it accounts for about 8–10% of cancers in children in Europe & USA .Bone marrow infiltration in neuroblastoma occurs in advanced disease therefore accurately assessing bone marrow infiltration in newly diagnosed cases is of immense importance. Objective of this study is to determine the frequency and pattern of bone marrow infiltration in neuroblastoma and its correlation with hematological parameters in children.

Methodology: This study included 83 newly diagnosed cases of neuroblastoma from January 2016 to December 2017. Clinicohematological parameters and bone marrow involvement for metastasis were ana-lyzed using SPSS16, cases on chemotherapy were excluded from the study.

Results: Anemia was equally common in patients with or without marrow involvement while leucopenia & thrombocytopenia were significantly (p value 0.009 & 0.002) associated with marrow involvement. Marrow infiltration was present in 42/83 cases (50.6%) in 20/42 cases (47.6%) in whom both aspirate and trephine biopsies were involved. There were 28 positive marrow aspirates 05 showing focal infiltration and diffuse infiltrates were seen in 18 cases and 07 had both focal and diffuse infiltrates while rosetting was observed in 8 cases. Infiltration in 34 trephines was seen with focal infiltrates in 20 cases & diffuse in 7 and both focal and diffuse infiltration in another 7 cases, while fibrosis was present in seven cases. Bilateral infiltration was present in 6 trephine biopsies.

Conclusion: Marrow infiltration was detected in 50.6% cases, outcome of high risk disease is usually poor, with long-term survival being less than 50% stressing thereby necessity of early detection of disease.

Keywords: Neuroblastoma, Bone marrow.

INTRODUCTION

The second commonest solid tumor in children is neuroblastoma, It originates from neuroepithelial cells that migrate from neural crest and form sympathetic nervous system.[1] Mostly (70%) arising retroperitoneally and involve adrenal medulla and may invade surrounding tissues or have distant metastasis in lymph nodes, liver, bone marrow, orbit, skull, ovaries, paratesticular region, testis and central nervous system.[2]

Infiltration of marrow in neuroblastoma indicates advanced disease. Autologous bone marrow "rescue", is an another reason to accurately assess marrow status in newly diagnosed cases.[3] With advanced disease patients often have bone marrow infiltration which is categorised as stage 4 and 4S of the International Neuroblastoma Staging System(INSS). Marrow involvement is considered intermediate and high-risk disease category for children over 1 year and infants.[4]

Various studies have been done to determine marrow involvement in solid tumours [5,6] whereas only few studies have analysed marrow involvement in neuroblastoma and its correlation with hematological profile.[7–9] This study aimed at determining the frequency and pattern of marrow involvement in neuroblastoma in pediatric cases at initial presentation, and its correlation with hematological parameters.

MATERIAL AND METHODS

This descriptive cross sectional study was conducted in Hematology & Transfusion medicine Department of a tertiary care pediatric hospital. Diagnosed cases of neuroblastoma on hematoxylin and eosin stain along with immunohistochemistry for round blue cell tumour panel using Synaptophysin, Neuron specific Enolase, Desmin, Myogenin, CD45 & WT1 and referred for marrow biopsy from January 2016 to December 2017 were selected for this study.

Patients clinical parameters along with peripheral counts including, hemoglobin, total leukocyte & platelet count and peripheral blood smear morphology were

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evaluated. Bone marrow aspiration and core biopsies were performed from posterior superior iliac spine and in children under 18 months of age only bone marrow aspirates were obtained from tibia. Bone marrow aspirate smears were stained with May-Grunwald Giemsa stain and biopsy sections were stained with hematoxylin and eosin as per standard protocols.[10,11] Bone marrow aspirates were examined for presence or absence of infiltration and the pattern of infiltration was also noted. In case of core biopsies presence of fibrosis and necrosis was also noted. Data regarding age, sex, clinical presentation and investigations including blood counts and bone marrow biopsies was analysed using SPSS 16, chi square test was applied for statistical analysis.

RESULTS

Total of 83 newly diagnosed cases of neuroblastoma referred to our department for bone marrow biopsy were included in this study. Age of patients ranged from one month to fifteen years with average age was 3.8years with male predominance & male: female ratio was 1.9:1. Ten (10) out of 83 patients (12%) were under one year of age while 63 (76%) of patients were under 5 years of age.

Hepatomegaly was present in 9 patients (10.7%) while splenomegaly & lymphadenopathy each were seen in 6 patients (7.3%).

Primary sites involved were adrenals, pelvis, retroperitonium, posterior mediastinum and paraspinal regions. There were two cases of stage 1V S infantile neuroblastoma with infiltration of liver, bone marrow and skin.

Values of mean hemoglobin was 8g/dl, mean total leucocyte count was $11.9\times10^3/\mu l$ & platelet count $336\times10^3/\mu l$. Anemia was almost equally prevalent in patients with and without marrow infiltration while leucopenia , thrombocytopenia & pancytopenia, were more frequent in cases with bone marrow infiltration (Table 1).

Bone marrow infiltration was detected in 42 (50.6%) cases. In 20/42 (47.6%) both marrow aspirate and marrow trephine biopsies were positive. Isolated Bone Marrow Aspirates being positive in 08 cases while isolated bone marrow core biopsies were positive in 14 cases. Overall 28 Bone Marrow Aspirates were positive with 5 showing focal infiltration and diffuse infiltrates in 8 cases, 7 cases had both focal and diffuse infiltrates while rosetting was seen in 8cases. Bone marrow Trephine biopsies were infiltrated in 34 cases with focal infiltrates seen in 22 cases, diffuse in 12 while fibrosis on trephine biopsies was present in 7 cases & necrosis was seen in 4 cases. Bilateral marrow infiltration was present on trephine biopsies in 06 patients and in these cases aspirate smears showed infiltration bilaterally in one case but were negative in four cases while one marrow aspirate was hemodiluted.

Table 1: *Cytopenias in Patients with and Without Bone Marrow Neuroblastoma Infiltration.*

	Cases with Marrow Infiltration (N = 42)	*Cases without Marrow Infiltration (N = 41)*	*P Value*
Anemia	38 (45.7%)	37 (44.5%)	0.630
Leucopenia	11 (26.1%)	03 (7.3%)	0.009
Thrombocytopenia	14 (33.3%)	03 (7.3%)	0.002

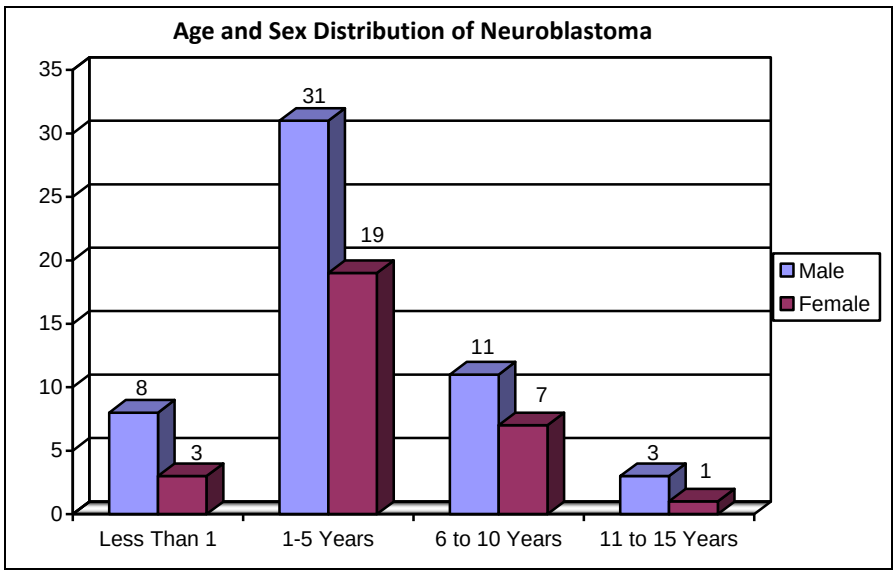

Fig. 1: *Age and Sex Distribution of Neuroblastoma Cases.*

DISCUSSION

Neuroblastoma is the commonest solid extracranial tumor in children, comprising about 8-10% of cancers in children, and 50% of cancers in infants. It is a tumor of early childhood, 36% of children diagnosed are under 1 year age, while 75% under 5 years of age and more than 90% cases under 10 years of age.[12-14] In this study 50 cases(60%) were under 5 years with male predominance in all age groups.

The status of marrow involvement has to be evaluated in all patients with neuroblastoma for staging and if found to be infiltrated, then it is categorised as high-

risk group that requires aggressive chemotherapy.[15] Bone Marrow metastases almost always confers a poor prognosis. Interactions between the bone and the BM microenvironment and tumour cells plays a central role in the homing of tumor cells to marrow with consequent bone metastasis.[16-18] Bone marrow infiltration was detected in 42 (50.6%) children in our study with 20/42 cases (47.6%) showing infiltration both on aspirate and trephine biopsy, isolated marrow aspirates were positive in 08 cases while isolated marrow core biopsies were positive in 14 cases.

Pulkit et al[19] have reported similar findings with marrow infiltration in 54.5% cases, in patients under 5 years of age with males predominating, both marrow aspirate and trephines were infiltrated in 16 out of 24 positive cases with only aspirate positivity in 4 (9%) cases and isolated trephine positivity in another 4 (9%) cases, similarly another study conducted by Franklin et al[20] reported bone marrow infiltration in 48.9% cases. Madhumati et al found neuroblastoma to be the commonest (48.8%) non-hematopoietic tumor to involve the marrow, followed by (11.1%) retinoblastoma, (8.6%) Ewing's sarcoma/PNET and (3.2%) rhabdomyosarcoma,[21,20] Another study that included 208 bone marrow samples from children diagnosed with neuroblastoma also reported trephines to be superior to marrow aspirates in detecting infiltration.

Bilateral marrow trephine infiltration was present in 06 patients in our study while the aspirate smears in these showed infiltration bilaterally in one case but were negative in four cases while in one case the marrow aspirate was hemodiluted.

Rastogi et al reported rosette formation in 40.7% aspirates and 22.2% on trephines & both diffuse and interstitial infiltration in remaining, while Franklin et al reported tumour rosettes in only 2% aspirates, this difference might be the result of smaller number of cases studied. While rosetting was detected in 8/28 (28%) aspirates by current study.

Myelofibrosis was a predominant feature of neuroblastoma metastasis in a study by Mills et al[8] we observed it in 7/34 (20%) cases while it was reported in 4/14 (28%.5) cases studied for marrow infiltration by small round blue cell tumors in children & adolescents.[22]

A 'special' stage, Neuroblastoma Stage 4s (NB4s) has a good prognosis despite disease dissemination, accounting for 7-10% of all NB cases & refers to small primary tumour with infiltration limilted to the skin, liver, and/or marrow.[23] The International Neuroblastoma Staging System restricts this diagnosis to children under 1 year at the time of diagnosis and bone marrow involvement less than 10%, despite the presence of large tumor burden, hallmark of this stage is the possibility of spontaneous regression.[24-26] In this study there were two cases of NB4s aged 2 months & 4 months respectively, both had infiltration of skin, liver and bone marrow.

Bone marrow involvement causes suppression of normal hematopoiesis and consequent peripheral cytopenias, in our study there was a statistically significant difference in frequency of leucopenia and thrombocytopenia in cases with & without marrow infiltration (table 1) pancytopenia was found in 7 cases (16.6%) with marrow infiltrates in contrast to 1 (2.4%) case with uninvolved marrow. Similar findings have been reported in earlier studies[5,27] however anemia, was equally common in patients both with and without infiltration possibly because of the prevalence of iron deficiency in our country rather than due to disease itself.

The presence of metastases is associated with advanced stage of disease which portends poor clinical outcome, these patients might benefit from therapy intensification. Routine bone marrow examination for staging and management of these patients is recommended. Performing Immunohistochemistry on core biopsies and molecular studies of bone marrows can increase the sensitivity to detect minimal disseminated disease. More sensitive techniques to assess bone marrow involvement are also being used like nuclear scans, magnetic resonance imaging (MRI), reverse transcription-polymerase chain reaction (RT-PCR) but these are expensive modalities.[28] However, marrow biopsy is simple, easy and cost effective technique used for staging and monitoring these patients[29] and thus suitable in a limited resource setting in third world countries.

It is ***concluded*** that Bone marrow infiltration was detected in 50.6% cases, outcome of high risk disease is usually poor, positing thereby necessity of early detection of disease, Marrow metastasis may not be evident on only H&E staining, limitation of our study was not using ancilliary techniques like immunohistochemistry and molecular studies which can aid in detecting minimal marrow invasion.

Authors Contribution

FSK: conceived, designed and did statistical analysis, manuscript writing &editing of manuscript. AA: review and final approval of manuscript.

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The authors have no conflict of interest to declare & the study has been approved by institutional review board.

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