Original Article

OPEN ACCESS

Infiltration of Round Blue Cell Tumors in Bone Marrow

Zahra Tasleem[1], Hamid Saeed Malik[2], Saima Bashir[3], Hassan Shaukat[4], Halima Babar [5], Kehkashan Hassan[6]

[1,5,6]Registrar Department of hematology Armed Forces Institute of Pathology CMH, Rawalpindi
[2]Hematology, Head of Department, Armed Forces Institute of Pathology CMH, Rawalpindi
[3]Lecturer, Department Hematology, Armed Forces Institute of Pathology CMH, Rawalpindi
[4]Consultant, Department of Medicine, Armed Forces Institute of Pathology CMH, Rawalpindi

Authors Contribution

[1,3,6]Substantial contributions to the conception or design of the work; or the acquisition, [4,5]Drafting the work or revising it critically for important intellectual content, [2]Final approval of the version to be published

Funding Source: None
Conflict of Interest: None

Received: May 21, 2023
Accepted: Oct 30, 2023

Address of Correspondent
Dr Zahra Tasleem
Registrar hematology Armed Forces Institute of Pathology CMH, Rawalpindi
khanzt4@gmail.com

ABSTRACT

Objective: To identify different types of round blue cell tumors on bone marrow biopsy and their pattern of marrow infiltration.

Methodology: This cross-sectional descriptive study was conducted at the Department of Hematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from July 2021 to December 2021. A total of 60 diagnosed cases of round blue cell tumors were included. Patients with acute lymphoblastic leukemia (ALL) and those receiving treatment for round blue cell tumors were excluded. Bone marrow aspiration and biopsy were performed. Peripheral film results, clinical observations, and patterns of marrow infiltration of round blue cell malignancies were analyzed using Statistical Package for the Social Sciences (SPSS) version 23.

Results: The study revealed a male to female ratio of 2:1, with 40 (66.6%) males and 20 (33.3%) females. The overall average age was 5.69 ± 5.0 years. Ewing sarcoma and rhabdomyosarcoma patients did not exhibit infiltration, while three instances of retinoblastoma (9.37%) and two cases of neuroblastoma (10.52%) did. Immunohistochemistry (IHC) results for synaptophysin and chromogranin were positive for retinoblastoma and neuroblastoma, while S100 results were positive for neuroblastoma infiltration and negative for retinoblastoma. Both retinoblastoma and neuroblastoma showed diffuse, focal, and interstitial patterns of infiltration on trephine biopsy.

Conclusion: Small round blue cell tumors were noted in bone marrow infiltration in 5 (8.33%) cases. Early detection of malignant cancers necessitates appropriate medical care, and in more severe cases, the need for neoadjuvant chemotherapy.

Keywords: Small round blue cell tumors, neuroblastoma, retinoblastoma.

Cite this article as: Tasleem Z, Malik HS, Bashir S, Shaukat H, Babar H, Hassan K. Infiltration of Round Blue Cell Tumors in Bone Marrow. Ann Pak Inst Med Sci. 2024; 19(4):30-34. doi. 10.48036/apims.v20i1.799.

Introduction

Round blue cell tumors include entities such as Ewing's sarcoma, Retinoblastoma, Nephroblastoma, Peripheral Neuroectodermal tumors, Small Cell Osteosarcoma, Rhabdomyosarcoma**,** and Mesenchymal Chondrosarcoma. These are malignant small round to oval cells usually double the size of normal red blood cell in air dried smear.[1]

As the name indicates these are group of highly aggressive tumors comprising of monotonous, undifferentiated cells with increased nuclear-cytoplasmic ratio, basophilic cytoplasm with hyper chromatic nuclei, evenly distributed chromatin and small nucleoli.[2] These tumors occur at any age however, incidence is high in children and adolescent. The most frequent intraocular cancer in children, retinoblastoma makes for 5 to 24 percent of all malignancies diagnosed in the first year of life.[3]The degree of the disease and the presence of an extra ocular extension are used to stage patients, and treatment varies depending on the stage of the disease.[4] Among children soft tissue sarcoma. Rhabdomyosarcoma are most frequent that arise from skeletal muscle progenitors and classified into Alveolar Rhabdomyosarcoma (ARMS), Embryonal Rhabdomyosarcoma (ERMS), and Pleomorphic

Rhabdomyosarcoma (PRMS) subtypes.[5] PNET and Ewing's sarcoma are both tiny round cell tumors with varied degrees of neuroectodermal differentiation.[6]

Neuroblastoma is the one of the most common, solid, malignant tumors of infancy and childhood. These tumors primarily affect individuals under the age of 10, with a minor male preponderance. Better prognosis occurs regardless of tumor stage in infants. Some of these cancers might even regress spontaneously. Despite intensive treatment, around 60% of >1-year children with this tumor present metastatic disease at diagnosis with poor outcome.[7] The retro peritoneum is affected by Neuroblastoma in about 70% of cases, and the adrenal gland is most frequently involved.[8] Majority of cases included in our study were of Retinoblastoma followed by Neuroblastoma and Ewing's Sarcoma. Only a few patients of Nephroblastoma and Medulloblastoma were included in our study. Due to the undifferentiated and primitive nature of the cells, it is challenging to discern between different types of small round blue cell tumors.[9] Only tumors with slightly differentiated cells could be easily diagnosed. When diagnosing the primary focus, the pathologists face difficulties due to the detection of metastasis in random biopsies, necessitating a thorough workup.[10]

Fine Needle Aspirate Cytology (FNAC) is considered an important tool for diagnosis. But there are several other modalities for identification and diagnosis of these tumors. Other important tests to classify these tumors include Fluorescence in Situ hybridization (FISH), Immunophenotyping by flow cytometry, Immunohistochemistry (IHC) and Reverse transcriptase (RT-PCR). Recent advancements in Immunohistochemistry and molecular biology techniques make it possible to detect tumor cells in a different location including the lymph node, blood and BM with much higher level of sensitivity than was previously possible.[11]

Rationale

Bone marrow biopsy is essential for the staging of disease and this will provide a guideline for the treatment decision. This study aims to determine the prevalence of small round blue cell tumors within our population.

Methodology

This cross-sectional descriptive study was conducted in the Department of Hematology, Armed Forces institute of Pathology, Rawalpindi from July 2021-Dec 2021. Ethical approval was obtained from institutional review board (IRB) FC-HEM20-5/READ-IRB/22/1296. After a thorough literature search, we calculated sample size of 60 using WHO calculator, by keeping a 5% margin of error, 95% confidence interval (CI). Sampling was done by non-probability convenient sampling technique**.**

Patients with newly diagnosed cases of round blue cell tumors were included and Acute lymphoblastic leukemia patients and patient of round blue cell tumors on treatment were excluded from the study. total 60 diagnosed cases of round blue cell tumors, referred for bone marrow examination were included in this study. All patients' ages, genders, clinical findings, and results of investigations (including X-rays, computer tomography scans, magnetic resonance imaging, and ultrasound) were recorded. For complete blood picture and peripheral blood film, ethylenediaminetetraacetic acid samples were obtained. Leishman stain was used for staining peripheral blood samples. Using a 16G lumbar puncture needle, bone marrow was aspirated and stained with Giemsa. Under local anaesthesia, Jamshidi needles were used to take bone marrow samples from the posterior superior iliac spine. Adequate length biopsies (1.5-2 cm), fixed in 10% formalin solution and then decalcified by using 10% formic acid for 4-6hrs followed by routine processing and paraffin embedding. 4-6 um serial sections were cut and stained by hemotoxylin and eosin(H&E) and immune stains were applied where required. On peripheral blood film RBC morphology and any atypical cells were noted. Detailed examination of bone marrow smears were done especially for presence of any abnormal cell or infiltrate. Bone marrow biopsy with at least seven well preserved marrow spaces were studied for normal hematopoietic element, infiltration by round blue cell tumors and reticulin fibrosis. Descriptive statistics were expressed in terms of mean± standard deviation (SD). Chi square test was applied and p value $\leq$ 0.05 was considered significant. Results were recorded in SPSS version 23.

Results

Between July 2021 and December 2021, round cell tumors involvement in BMA (n = 60), imprints BMA (n = 60), and BMBx (n = 60) was investigated. There was a male majority with male: female 2:1. Sixty cases were examined, and 5 (8.33%) of them had evidence of BM involvement (BMI). Erythroid hyperplasia was seen in 8/60 (13.33%), reactive marrow in 15/60 (25%) and

doi. 10.48036/apims.v19i4.799

Table I: Crosstab of age with type of tumor.

Age	Type of tumor				Total	p-value
	Retinoblastoma	Neuroblastoma	Ewing sarcoma	Rhabdomyo Sarcoma		
0-10 Years	30(63.8%)	16(34.0%)	1(2.1%)	0(0.0%)	47(100%)	<0.001
11-18 Years	2(15.4%)	3(23.1%)	7(53.8%)	1(7.7%)	13(100%)	

Table II: Type of tumor infiltration.

Type of tumor	Infiltration seen	Infiltration not seen
Retinoblastoma	03 (5%)	29(48.3%)
Neuroblastoma	02 (3.3%)	17(28.3%)
Ewing sarcoma	0	08(13.3%)
Rhabdomyosarcoma	0	01(1.6%)
Total	**05(8.3%)**	**55(91.6%)**

Table-III: Patient's characteristics.

Patient characteristics	Mean± SD/ n(%)
Age	5.7±5.0years
Gender	
Male	38(63.3%)
Female	22(36.6%)
Fever	20(33.3%)
Weight loss	10(16.7%)
Generalized weakness	21(35%)
Hemoglobin	9.93±2.0
Total leukocyte count	7.75±3.25
Platelet	376±128.7
Tumor type	
Retinoblastoma	32(53.3%)
Neuroblastoma	19(31.7%)
Ewing sarcoma	8(13.3%)
Rhabdomyosarcoma	1(1.7%)

marrow within normal limits in 37/60 (61.6%). Out of 60 cases, leucoerythroblastic picture was seen in 5 (8.33%) cases on peripheral blood examination. Among 5 cases which showed infiltration, there was no case positive on

bone marrow aspiration, in one case, tumor was picked up in the imprint, while other cases were positive on biopsy. Of the 5 positive cases in bone marrow biopsy, 2 cases were Neuroblastoma (10.52%) and 3 cases of Retinoblastoma (9.37%). In majority 47(78.3%) of cases patients were less than 10 years of age and patient between 11-18 years were 13(21.6%) as shown in table I.

Retinoblastoma accounted for 32 (53.3%) of the 60 cases, followed by Neuroblastoma (19.7%), Ewing sarcoma (8.3%), and Rhabdomyosarcoma (1.7%), in that order. Out of the 5 cases, 3 (9.37%) Retinoblastoma cases and 2 (10.52%) Neuroblastoma cases demonstrated marrow infiltration, whereas Ewing sarcoma and Rhabdomyosarcoma did not. (Table II)

Synaptophysin and Chromgranin were positive for Retinoblastoma and Neuroblastoma on IHC, while S100 was positive for Neuroblastoma infiltration and negative for Retinoblastoma. Diffuse, focal and interstitial pattern of infiltration was observed in cases of Retinoblastoma and Neuroblastoma on trephine biopsy. Changes in hematological parameters, patients sign and symptoms were also noted as shown in table III.

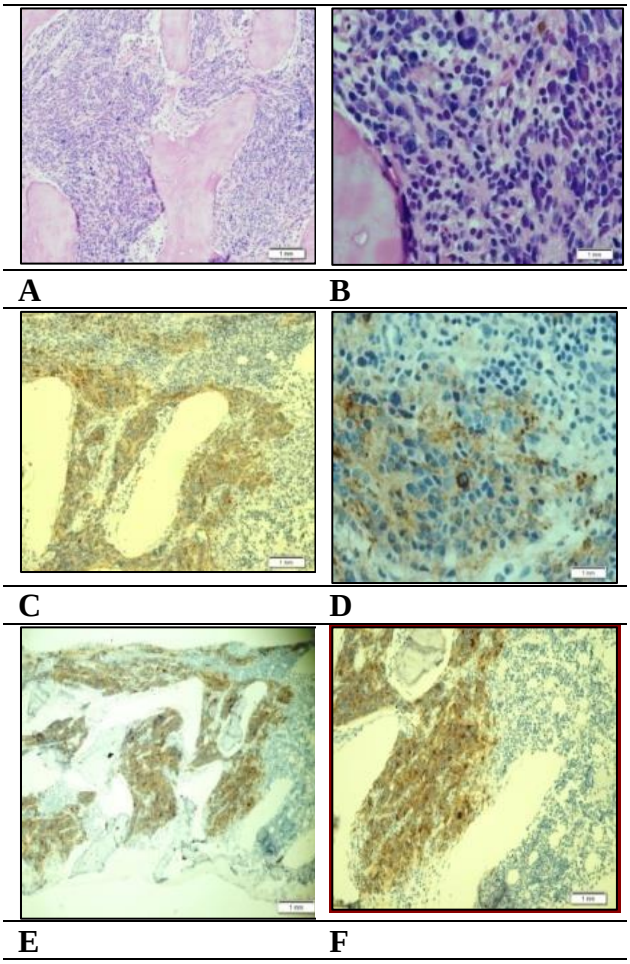

Figure 1: A case of Retinoblastoma showing bone marrow infiltration. (A and B) trephine imprint showing atypical round blue cells at (10X & 40X), respectively. (C and D): IHC with Synaptophysin showing positivity for round blue cells at(10X and 40X), respectively. (E and F):IHC with Chromgranin showing positivity for round blue cells at (10X and 40X),respectively.

Discussion

Four of the pediatric population's most common cancers are small round blue cell tumors and accurate diagnosis is essential for managing these patients. At the time of diagnosis, involvement of the bone marrow would enhance the chance of subsequent levels of distant metastasis.

Round blue cell tumor infiltration of the bone marrow was 8.33% frequent in our investigation. While study done by Asif N et al[12] in Pakistan showed that the frequency of RBCTs infiltrating marrow was 19.5%(n=82) and, while study done by neighboring country Trivia et al[13] showed 17.7%. Another study done by Rafiqueet al[14] at King Edward medical university showed infiltration in 50% of cases. In our study maximum infiltration was seen by Neuroblastoma 10.52% followed by Retinoblastoma 9.37% which had similar findings with studies done by Naghmi et al and Kumar et al[15] where Neuroblastoma showed maximum infiltration. In contrast to our study where Retinoblastoma showed increased infiltration second to Neuroblastoma, other studies such as Trivia et al and Kumar et al showed 11.2% and 29.7% infiltration by Ewing's Sarcoma respectively in Bone marrow following Neuroblastoma. Mishra et al[16] study revealed, Neuroblastoma and Ewing's sarcoma, each with a 40% prevalence, were the most frequent pediatric tumors to metastasis to bone marrow.

In our study on blood complete picture examination of many patients showed findings of anemia and thrombocytopenia irrespective of bone marrow infiltration. In addition, peripheral smear and bone marrow iron stores revealed that it was hypo chromic microcytic anemia with absent iron stores; this fact may be due to increase prevalence of Iron deficiency anemia in our country. Similarly, studies done by Asif et al and Humera et al. found decrease hemoglobin and platelets levels. No abnormal cell found in the peripheral blood examination of the patients in which bone marrow trephine biopsy show infiltration, however, blood smears should be examined attentively to detect abnormal like circulating retinoblastoma cells when bone marrow is massively involved.[17] Other abnormalities such as pattern of infiltration were also noted in our study. We found that among 5 cases with bone marrow infiltration, cases of Retinoblastoma and Neuroblastoma both showed diffuse, focal and interstitial pattern of infiltration.

In our study there was no case positive on Bone Marrow aspiration however infiltrate was found on trephine biopsy which is in contrast with Asif et al and Humera et al who showed 2 and 3 positive cases on aspiration respectively while their biopsies were negative. Bone marrow aspirate and biopsy are thus considered complementary. As RBCTs mimic acute leukemia it is however necessary to differentiate them from leukaemias. Leukocyte common antigen (CD45 negativity and negativity of lymphoid and myeloid surface antigen) helps in exclusion of acute leukaemia.[18] Bilateral aspirate and trephine will aid in increase the rate of detection of infiltration in RBCTS. A much larger volume of the marrow can be examined through multiple sections of biopsy which aids to recognize infiltration[19]. IHC should be employed to elucidate the primary site in addition to the morphological findings in the bone marrow. In addition to IHC, molecular studies of bone marrow sample significantly help in detection of minimal disseminated disease at the time of diagnosis.[20]

Limitation of our study was small sample size due to limited time duration. Multicenter studies with the large sample size can give better idea about prevalence.

Conclusion

Bone marrow biopsy is highly effective tool in staging of disease and provides information about haemopoietic activity of bone marrow and guiding treating physician about selecting appropriate treatment options for the patients.

References

1. Pathak K, Nayak P, Karadwal A, Nayak S, Tekade S. Round cell tumors-classification & overview. Indian Journal of Forensic Medicine & Toxicology. 2021 May 17;15(3):228-39.
2. Sajjan J. *Cytology of Cutaneous Tumors and Tumorlike Lesions* (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
3. Vempuluru VS, Kaliki S. Screening for retinoblastoma: a systematic review of current strategies. The Asia-Pacific Journal of Ophthalmology. 2021 Mar 1;10(2):192-9. *DOI:* 10.1097/APO.0000000000000378
4. Yousef YA, Qaddoumi I, Al-Nawaiseh I, Mohammad M, AlRimawi D, Toro MD, Zweifel S, Rejdak R, Nazzal R, Mehyar M, Jaradat I. A proposal for future modifications on clinical TNM staging system of retinoblastoma based on the American joint committee on cancer staging manual, 7th and 8th editions. Journal of Cancer. 2022;13(4):1336. doi: 10.7150/jca.61005
5. Zarrabi A, Perrin D, Kavoosi M, Sommer M, Sezen S, Mehrbod P, Bhushan B, Machaj F, Rosik J, Kawalec P, Afifi S. Rhabdomyosarcoma: current therapy, challenges, and future approaches to treatment strategies. Cancers. 2023 Nov 2;15(21):5269. https://doi.org/10.3390/cancers15215269
6. Kilpatrick SE, Reith JD, Rubin B. Ewing sarcoma and the history of similar and possibly related small round cell tumors: from whence have we come and where are we going?. Advances in Anatomic Pathology. 2018 Sep 1;25(5):314-26. *DOI:* 10.1097/PAP.0000000000000203
7. Abbas AA, Samkari A. High-Risk Neuroblastoma: Poor outcomes despite aggressive multimodal therapy. Current Cancer Therapy Reviews. 2022 Feb 1;18(1):14-40.

DOI: https://doi.org/10.2174/1573394717666210805114226

8. Telecan T, Andras I, Bungardean MR, Muntean D, Militaru C, Perciuleac I, Maga A, Crisan N. Adrenal Gland Primary Neuroblastoma in an Adult Patient: A Case Report and Literature Review. Medicina. 2022 Dec 23;59(1):33. https://doi.org/10.3390/medicina59010033
9. Sbaraglia M, Bellan E, Dei Tos AP. The 2020 WHO classification of soft tissue tumours: news and perspectives. Pathologica. 2021 Apr;113(2):70. doi: 10.32074/1591-951X-213
10. Compton C, Compton C. Fundamentals of Cancer Diagnosis and Assessment. Cancer: The Enemy from Within: A Comprehensive Textbook of Cancer's Causes, Complexities and Consequences. 2020:161-95. https://doi.org/10.1007/978-3-030-40651-6_7
11. Magaki S, Hojat SA, Wei B, So A, Yong WH. An introduction to the performance of immunohistochemistry. Biobanking: methods and protocols. 2019:289-98. https://doi.org/10.1007/978-1-4939-8935-5_25
12. Asif N, Hassan K, Yasmeen N. Bone marrow infiltration in small blue cell tumours. J Islamabad Med Dent Coll (JIMDC). 2013;2:3-8.
13. Bhopal T, Hussain A, Mallikarjun S, Lakshmi S, Sinha S. Bone marrow infiltration by nonhematopoetic small round cell tumors: A clinicopathological study from a tertiary care centre in South India. Indian Journal of Medical and Paediatric Oncology. 2019 Jul;40(S 01):S1-5.https://doi.org/10.4103/ijmpo_218_17
14. Rafiq H, Khurshid RK, Hayee AH. Frequency of bone invasion and metastasis in round cell tumours of paediatric age groups. Biomedica. 2017 May 18;23(1):8-11.
15. Kumar L, Majhi U, Shanta V. Frequency of bone marrow involvement in non-haematological malignancies. The Journal of the Association of Physicians of India. 1990 Aug 1;38(8):553-5.
16. Mishra P, Das S, Kar R, Jacob SE, Basu D. Non-haematopoietic malignancies metastasing to the bone marrow: A 5 year record-based descriptive study from a tertiary care centre in South India. Indian Journal of Cancer. 2014 Jan 1;51(1):S30-34. https://doi.org/10.4103/0019-509X.134614
17. Hanke CA, Roessler J, Stegmaier S, Koscielniak E, Niemeyer CM, Kontny U. Alveolar rhabdomyosarcoma mimicking lymphoma with bone marrow involvement. European journal of pediatrics. 2007 May;166:505-6. https://doi.org/10.1007/s00431-006-0269-y
18. Jani P, Charles CY. Massive bone marrow involvement by clear cell variant of rhabdomyosarcoma. The Indian Journal of Pediatrics. 2009 Feb;76:224-8. https://doi.org/10.1007/s12098-008-0230-3
19. Basu D, Singh T, Shinghal RN. Micrometastasis in bone marrow in breast cancer. Indian Journal of Pathology & Microbiology. 1994 Apr 1;37(2):159-64.
20. Bhopal T, Hussain A, Mallikarjun S, Lakshmi S, Sinha S. Bone marrow infiltration by nonhematopoetic small round cell tumors: A clinicopathological study from a tertiary care centre in South India. Indian Journal of Medical and Paediatric Oncology. 2019 Jul;40(S 01):S1-5. https://doi.org/10.4103/ijmpo_218_17