

FREQUENCY OF BONE INVASION AND METASTASIS IN ROUND CELL TUMOURS OF PAEDIATRIC AGE GROUPS

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In this study a total of 14 patients of paediatric age group were included with round cell tumours of Retinoblastoma, Neuroblastoma, Ewings's sarcoma and Rhabdomyosarcoma. The frequency of metastatic involvement of the bone marrow was determined by aspiration and biopsy from posterior iliac crest. Routine investigations like haemoglobin, total leukocyte count and, platelets count were also carried out in all cases. Results show that the neuroblastoma was most common to metastasize to bone marrow. It was also noted that, aspirate and biopsy were found complementary in diagnosis of metastasis. However further work is needed to find out the frequency of metastatic involvement of bone marrow in a larger series of cases.

Tumour cells frequently contaminate autologous stem cell products in a variety of malignancies, but their clinical significance remains controversial¹. The extension of a cancer is a major prognostic factor which determines the therapeutic strategy. The occurrence of metastatic relapses in patients with initially localized tumours, despite a good local control, gives evidence for the possibility of spreading of occult tumour cells. The recent improvements of immunohistochemistry and molecular biology methods enable to detect tumour cells in various sites such as lymph nodes, bone marrow and blood with a considerably increased sensitivity as compared to conventional approaches².

Bone marrow metastases from small round cell tumours can present diagnostic difficulties. Neuroblastoma, along with Rhabdomyosarcoma, Ewing's sarcoma, and acute lymphoblastic leukaemia/lymphoma are a group of small, round-cell tumours of childhood. All of these malignancies show a propensity to metastasize to bone marrow. Occasionally when the clinical picture is unclear and the tumour is particularly anaplastic, it can be difficult to arrive at a diagnosis by conventional histological and biochemical procedures. It is found that an unusual clinical presentation of diffuse bone marrow involvement is the sole manifestation of the disease and poses a difficult diagnostic challenge.^{3,4}

Bone marrow aspirations and biopsies are standard staging procedures for neuroblastoma because the tumour frequently metastasizes to the bone marrow. The presence of bone marrow metastases indicates stage 4 or 4S neuroblastoma by International Neuroblastoma Staging System

criteria; these stages are also associated with other metastatic sites of disease.⁵ It is found that neuroblastoma cells activate osteoclasts to form osteolytic lesions. Activation occurs via the receptor activator of NF kappaB ligand (RANKL) or in the absence of RANKL via activation of bone marrow mesenchymal stem cells and stimulation by these cells of the expression of IL-6, a potent osteoclast activating factor.⁷ Ewing's sarcoma was diagnosed in three men, one aged 22 and two aged 30. The disease was diagnosed by biopsy and chromosome investigations. Ewing's sarcoma is a musculoskeletal malignancy that occurs in children and adolescents but also in young adults. It generally manifests itself as a painful swelling originating in bone or soft tissue. There are often accompanying symptoms such as weight loss and fever. In 20-25% of cases there are already metastases (to the lungs, bone and bone marrow) by the time of diagnosis.⁸ The presence of metastasis is a major prognostic factor in Ewing tumour.⁹ The purpose of this study was to obtain the frequency of bone marrow metastasis in different round cell tumours, and to study the significance of bone marrow aspirate and biopsy.

MATERIALS AND METHODS

A total of 14 patients with stages III and IV disease were included in this study with established malignant disorders. Patients were taken from different hospitals of Lahore but majority of them belonged to Institute of Nuclear Medicine and Oncology (INMOL), Lahore. Information on sex, age at diagnosis, presenting signs, spread of tumour, treatment modality, survival rate, and family history were collected.

Methodology

Bone marrow examination:

Collection of aspirate:

Bone marrow infiltration was estimated by obtaining concurrent aspiration and trephine biopsy drawn from right and left posterior iliac crests using Illinois and Islam needles. Aspirates were stained by giemsa stain and the histological sections of trephines by H&E stain.

Haematological parameters:

Collection of blood:

About 2.5 ml of blood was drawn from the vein.

DISCUSSION

The presence of isolated tumour cells in the bone marrow at the time of primary diagnosis indicates an increased risk for subsequent development of distant metastases in various solid tumours. The bone is the third most common site of cancer metastasis. To invade the bone, tumour cells produce osteoclast-activating factors that increase non resorbtion by osteoclasts⁷.

There were 6 cases of retinoblastoma in this study, I case (16.6%) showed infiltration, whereas 5 cases (83.4%) did not show infiltration. This is not in agreement with a study that showed a high turn over of 52.17%. Reason may be attributed to

Table 1: Blood parameters in different cancers of male and female patients.

Type of cancer	Female			Male		
	Hb (g%)	TLC	Platelets	Hb (g%)	TLC	Platelets
Retinoblastoma (6)	11.4 ± 1.1	9.8 × 10 ⁹	182 ± 1 × 10 ⁹	10.8 ± 2.1	7.2 × 10 ⁹	174 ± 98 × 10 ⁹
Neuroblastoma (3)	8.02 ± 1.0	8.8 × 10 ⁹	162 ± 68 × 10 ⁹	9.8 ± 2.4	8.7 × 10 ⁹	185 ± 88 × 10 ⁹
Ewings Sarcoma (2)	11.56 ± 1.4	10.1 × 10 ⁹	150 ± 40 × 10 ⁹	10.6 ± 2.4	8.8 × 10 ⁹	182 ± 98 × 10 ⁹
Rhabdomyosarcoma (3)	12.56 ± 1.4	9.9 × 10 ⁹	190 ± 90 × 10 ⁹	11.56 ± 1.2	8.9 × 10 ⁹	192 ± 90 × 10 ⁹

Ethylenediamine tetra acetic acid (EDTA) in the ratio of 1.2 mg per ml of blood is used for determination of blood parameters like Haemoglobin, total leukocyte count and platelets. These tests were performed on Sysmex K-1000 Autoanalyzer.

RESULTS

Table 1 shows blood parameters in different cancers of male and female patients. It was observed that the level of haemoglobin and platelets were moderately decreased in majority of cases of both sexes.

Table 2 shows the frequency of positive cases of bone marrow infiltration in different round cell tumors. Incase of retinoblastoma only 16.5% metastasized to marrow, in neuroblastoma all the patients showed bone marrow infiltration i.e. 100%. In case of Ewing’s sarcoma 50% cases had infiltration of marrow whereas rhabdomyosarcoma showed infiltration in 66% cases. On the other hand the frequency of negative cases of bone marrow infiltration in retinoblastoma was 83.4%, in neuroblastoma 0% in Ewing's sarcoma was 50% and in rhabdomyosarcoma was 33%.

Table 2: Frequency of positive cases of bone marrow infiltration.

Type of Tumour	Total No. of cases	Positive Cases	Negative cases
Retinoblastoma (6)	6	1 (16.5%)	5 (83.5%)
Neuroblastoma (3)	3	3 (100%)	Nil
Ewings Sarcoma (2)	2	1 (50%)	1 (50%)
Rhabdomyosarcoma (3)	3	2 (66%)	1 (33%)
Total	14	7 (50%)	7 (50%)

small no of cases of retinoblastoma in our study. Large number of cases may yield a different result. Another study also shows that despite intensive therapy, approximately 60-80% of children diagnosed with metastatic neuroblastoma succumb to the disease and it preferentially metastasizes to the bone marrow.¹⁰

Present study shows that 16.5% cases of bone marrow infiltration with retinoblastoma and in neuroblastoma all the patients showed bone marrow infiltration in 100%. In case of Ewing's sarcoma 50% cases had infiltrated the marrow whereas rhabdomyosarcoma showed infiltration in 66%

cases. According to a study,¹¹ bone marrow involvement was highest in neuroblastoma (9/14), retinoblastoma (3/7), Ewing's sarcoma (14/47) and in rhabdomyosarcoma (5/20). The study showed that bone marrow aspiration smears were adequate in paediatric tumours (neuroblastoma, retinoblastoma, rhabdomyosarcoma) while bone marrow biopsies were more useful in patients with Ewing's sarcoma. They concluded that bone marrow is an easy investigation in the diagnosis and staging of nonhaematological cancers. A comparison of these data with those obtained from the studies of rhabdomyosarcoma and Ewing's sarcoma cell lines and tissues suggests that when high levels of tumour cells are present in the marrow, it is possible to obtain a confident diagnosis of either neuroblastoma or acute lymphoblastic leukaemia. In addition, the immunocytological identification of neuroblasts in bone marrow enables accurate staging without histological examination.^{3,12} However another group showed that bone marrow studies did not contribute data that changed the stage of patients who had surgically resectable tumours and no evidence of metastatic spread on imaging studies. When present, metastatic spread to the marrow was associated with advanced local tumours or other sites of metastatic disease.⁵ It is reported that human neuroblastoma cells that form osteolytic lesions *in vivo* do not produce osteoclast-activating factors but rather stimulate osteoclast activity in the presence of human bone marrow mesenchymal stem cells. This alternative pathway of osteoclast activation involves a nonadhesive interaction between neuroblastoma cells and bone marrow mesenchymal stem cells. Stimulated bone marrow mesenchymal stem cells express markedly increased levels of interleukin-6, which is then responsible for osteoclast activation.⁶

In our study we also examined the peripheral blood smears and found that the level of haemoglobin and platelet were moderately decreased in majority of the cases of both sexes. The present study is in agreement with another study¹³ in which they suggested that peripheral blood smears should be examined cautiously to detect occasional circulating retinoblastoma cells when the bone marrow is massively involved. Collectively, these model systems provide important tools for investigation of the biology of neuroblastoma and evaluation of mechanisms that mediate the regression of these tumours in response to novel therapeutic agents, including cytokines such as interleukin-12.¹⁴

In present study we found that aspirate and biopsy are complementary as 3 cases had aspirates positive while their biopsies were negative. It was also found that bilateral sampling increased the

rate of detection by 33%. However more research is needed to find out the frequency of metastatic involvement of bone marrow in large number of cases.

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