Volume : 1 | Issue : 7 | Dec 2012 • ISSN No 2277 - 8179

Research Paper

Medical Science

KEYWORDS : Thrombocytopenia, Bone Marrow, Itp, Megaloblastic Anaemia

Bone Marrow Aspiration Study in Thrombocytopenia

Dr. Moumita Sengupta	MD, (Pathology). Department Of Pathology, Ipgme & R, Kolkata.
Dr. Kaushik Saha	MD, (Pathology). Department Of Pathology, Ipgme & R, Kolkata.
Dr.chetna J. Mistry	MD, Diploma In Clinopathology,Green Cross Pathology Laboratory,Ahmedabad.

ABSTRACT

Thrombocytopenia is commonly encountered in a wide range of haematological and non-haematological disorders. Bone marrow aspiration plays a major role in diagnosis. The present study was conducted to analyse the causes of thrombocytopenia, its spectrum and to interpret the bone marrow aspiration findings. This cross-sectional study was carried out in the department of pathology of our institutions. Bone marrow aspiration smears were stained with Leishman stain and examined under light microscope. Eighty five cases fulfilled the criteria of thrombocytopenia. Mean age of patients was 27.3 years. Maximum number of cases was seen in age group of below 15 years. Idiopathic thrombocytopenic purpura (ITP)was the commonest cause followed by acute myeloid leukaemia, acute lymphoblastic leukaemia, megaloblastic anaemia, and hypoplastic marrow respectively.It can be concluded that bone marrow aspiration is an important diagnostic modality to determine the aetiology of thrombocytopenia.

INTRODUCTION:

Despite the number and diversity of disorders that may be associated aetiologically, thrombocytopenia results from only four processes- artifactual thrombocytopenia, deficient platelet production, accelerated platelet destruction, and abnormal distribution or pooling of the platelets within the body (Levine, 2003).The more frequent indications of bone marrow examination were unexplained thrombocytopenia and pancytopenia. Common clinical manifestations are pallor, fatigue, splenomegaly, lymphadenopathy, fever, bleeding, weight loss, and hepatomegaly (Tariq et al., 2010).Detail clinical examination and peripheral blood smear findings help to achieve the diagnosis. However bone marrow examination is necessary for confirmation. Bone marrow aspiration is invasive but safe procedure which is done routinely in hospitals to know the aetiology in case of thrombocytopenia (Egesie et al., 2009). Risk of bleeding is very little that's why bone marrow aspiration can be carried out in case of severe thrombocytopenia (Kibria et al., 2010).The present study was conducted to evaluate the role of bone marrow aspiration in case of thrombocytopenia.

MATERIALS AND METHODS:

The present cross-sectional study was conducted for a period of two years in the department of pathology of our institutions. Total 85 cases of thrombocytopenia being referred from the departments of General Medicine, Paediatric and Gynaecology & Obstetric departments were included in this study. Clinical parameters, peripheral blood smear findings and bone marrow aspiration results were noted in detail.

Bone marrow aspiration smears were stained with Leishman stain and examined under light microscope for aetiology of thrombocytopenia which was defined as platelet count less than 1,50,000/mm3 (Battinelli et al., 2007). The complete blood counts (CBC) were determined using automated cell counter (KX-21, Sysmex Corporation, Japan). Platelet counts were cross-checked by microscopic examination of two well stained peripheral smears.

The cases with inadequate bone marrow aspirate or dry tap, patients taking drugs commonly causing thrombocytopenia, patients having recent history of receiving chemotherapy and radiotherapy, presence of specified condition that may be accounted for thrombocytopenia were excluded from the study.

Bone marrow aspirations were done from posterior superior iliac spine using Salah's needle following standard technique. 0.25 to 0.5 ml of bone marrow particles was aspirated. Slides were prepared and stained with Leishman stain to examine under light microscope. Leukaemia cases were further categorised into acute myeloid leukaemia and acute lymphoid leukaemia by cytochemistry (Myeloperoxidase and periodic acid Schiff stain).

The number of megakaryocytes were categorised as normal (one megakaryocytes/ one to three low power field), increased (more than two megakaryocytes/ low power field), decreased (one megakaryocytes/ five to ten low power field) (Houwerzijl et al., 2004)

Regarding morphological alterations of megakaryocytes, the variables were young form and dysplastic form. Immature megakaryocytes were defined as young form of megakaryocytes with scant bluish cytoplasm and lacking lobulation of the nucleus which occupied most of the cell. Those having single or multiple separate nuclei were considered as dysplastic megakaryocytes (McKenzie, 1996).

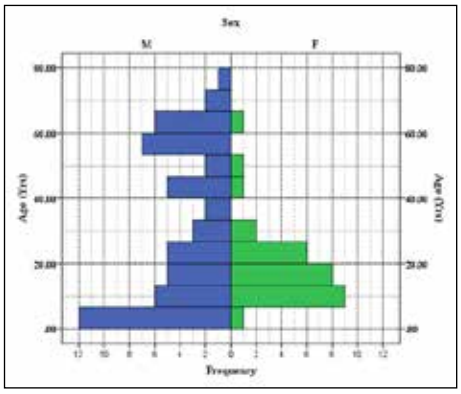
RESULTS:

Eighty five cases of thrombocytopenia fulfilling the inclusion criteria were included in the present study. Patients were found to be aged between 7 months and 75 years. The mean age was 27.3 years. Most common age group (Table 1) of the patients was below 15 years (35.3%; 30 out of 85 patients).Fifty six patients (65.9%) were male and twenty- nine patients (34.1%) were female with male female ratio 1.9:1. In the first decade thrombocytopenia was conspicuously more common in male

TABLE 1: AGE DISTRIBUTION OF THE PATIENTS

Age Group	No. of patients	Percentage (%)
<15 years	30	35.3
15-30 years	25	29.4
31-45 years	10	11.8
>45years	20	23.5
Total	85	100.0

Fig. 1] Population pyramid diagram showing the age and sex distribution of the patients.



Volume : 1 | Issue : 7 | Dec 2012 • ISSN No 2277 - 8179

As per as clinical features of the patients were concerned, pallor (78.8%) was the commonest presentation followed by bleeding manifestation (60.0%) (Table 2)

TABLE 2: CLINICAL PROFILE OF THE PATIENTS

Clinical feature	No. of patients	Percentage (%)
Fever	37	43.5
Bleeding manifestation	51	60.0
Pallor	67	78.8
Hepatomegaly	29	34.1
Splenomegaly	30	35.3
Lymphadenopathy	16	18.8
Sternal tenderness	10	11.8
Others	20	23.5

Blood counts and peripheral blood smear findings(Table 3) of all 85 patients of thrombocytopenia revealed mean platelet count of 55, 435 per mm3, mean total leucocyte count of 17,036 per mm3 and normocytic normochromic RBC (83.5%) morphology in most of the cases.

TABLE 3: HEMATOLOGICAL PROFILE OF THE PATIENTS

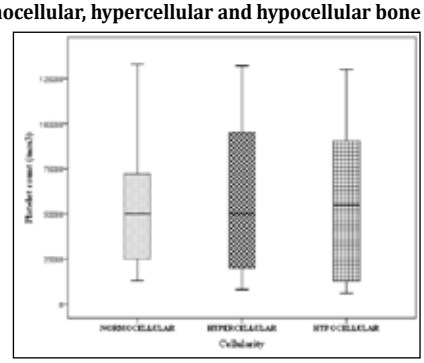
Continuous variables	Mean ± Standard deviation
Hb (gm/dl)	7.40 ± 2.78
TLC (mm^3)	17,036 ± 30,585
Absolute neutrophil count (mm^3)	5,353 ± 9,677
Platelet count (mm^3)	55,435 ± 40,403
Reticulocyte count (%)	1.20 ± 0.74
Categorical variables	No. of patients (%)
RBC morphology	Normocytic normochromic – 71 (83.5%)
	Microcytic hypochromic – 6 (7.1%)
	Macrocytic – 8 (9.4%)
Abnormal cell in PBF	24 (28.2%)

Regarding the cellularity of bone marrow, hypercellular marrow was detected in 48.2% cases. We did also come across the normocellular marrows in 24.8% cases as well as hypocellular marrow in 27.1% cases(Table 4). Fig.2] Box-Whisker plot showing distribution of platelet counts in normocellular, hypercellular and hypocellular bone marrowclearly depicts the distribution of platelet counts in normocellular, hypercellular and hypocellular bone marrow aspirates.

TABLE 4: CELLULARITY OF BONE MARROW OF THE PATIENTS

Cellularity	No. of patients	Percentage (%)
Normocellular	21	24.8
Hypercellular	41	48.2
Hypocellular	23	27.1
Total	85	100.0

Fig.2] Box-Whisker plot showing distribution of platelet counts in normocellular, hypercellular and hypocellular bone marrow



The commonest bone marrow aspiration finding was ITP (17 cases, 20%). The second most common cause was AML (12 cases, 14.1%) which was followed by ALL (10 cases, 11.8%). Megaloblastic anaemia (10.6%), Hypoplastic marrow (8.2%), Myelodysplastic syndrome (5.9%), Plasma cell dyscrasias (5.9%), Infection associated thrombocytopenia (3.5%) were other aspiration findings (Table 5, Fig. 3 to 10).

TABLE 5: NUMBER OF MEGAKARYOCYTES IN DIFFERENT BONE MARROW DIAGNOSES OF THE PATIENTS

Diagnosis	Megakaryocyte No.				Total	Percentage (%)
	Absent	Adequate	Decreased	Increased		
ALL	8	0	2	0	10	11.8
AML	10	0	2	0	12	14.1
Blast crisis of CML	0	0	1	0	1	1.2
CLPD	0	0	2	0	2	2.4
Granuloma	0	0	2	0	2	2.4
Hemophagocytic syndrome	0	0	1	0	1	1.2
Hypersplenism	0	2	0	0	2	2.4
Hypoplastic marrow	5	0	2	0	7	8.2
IAT	0	1	2	0	3	3.5
ITP	0	0	0	17	17	20.0
Juvenile CML	0	0	1	0	1	1.2
MDS	0	2	2	1	5	5.9
Megaloblastic anaemia	0	1	7	1	9	10.6
Metastatic deposit	0	0	6	0	6	7.1
Myelofibrosis	0	0	1	0	1	1.2
NHL	0	0	1	0	1	1.2
Plasma cell dyscrasias	0	2	3	0	5	5.9
Total	23	8	35	19	85	100.0

Fig. 3] Photomicrograph of Idiopathic Thrombocytopenic Purpura (Leishman stain, 100×)

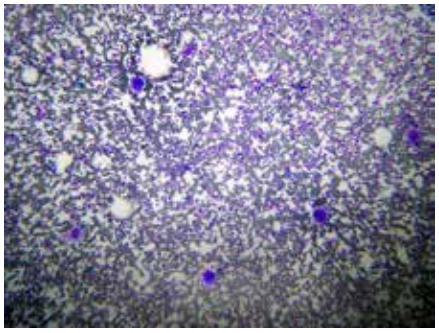


Fig. 4] Photomicrograph of Acute Myeloid Leukaemia showing M5 (FAB) morphology (Leishman stain, 1000×)

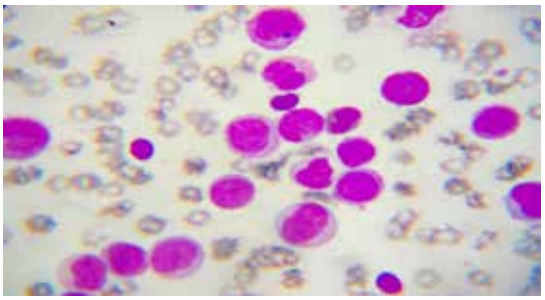


Fig. 5] Photomicrograph of Acute Lymphoblastic Leukaemia (Leishman stain, 1000×)

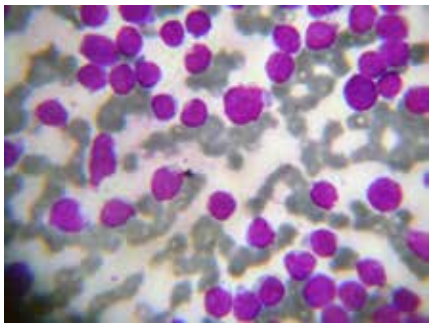


Fig. 6] Photomicrograph of Megaloblastic Anaemia (Leishman stain, 400×)

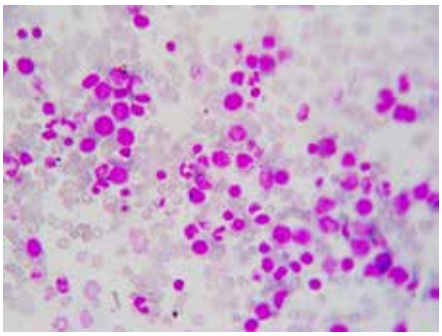


Fig. 7] Photomicrograph of Hypoplastic Bone marrow (Leishman stain, 400×)

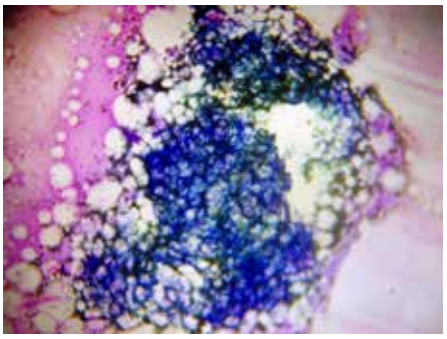


Fig. 8] Photomicrograph of Metastatic deposit of Adenocarcinoma (Leishman stain, 1000×)

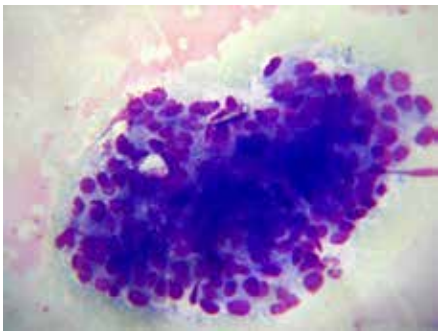


TABLE 6: CROSS TABULATION BETWEEN MEGAKARYOCYTES NUMBER AND MORPHOLOGY

Morphology	Number				Total
	Absent	Adequate	Decreased	Increased	
Absent	23	0	0	0	23
Dysplastic form	0	1	1	1	3
Normal morphology	0	7	34	0	41
Young form	0	0	0	18	18
Total	23	8	35	19	85

The number and morphology of megakaryocytes in bone marrow aspirates were noted and presented in relation to different diseases (Table 5 and Table 6). All cases of ITP revealed increased megakaryocytes and young forms or immature megakaryocytes. Dysplastic forms were found in all cases of MDS.

Plasma cells were increased in 19 cases (22.4%). Out of 19 cases, plasma cell dyscrasia was diagnosed in 5 cases (5.9 %) based on clinico-radiological and bone marrow aspiration findings.

Among 3 cases (3.5%) of Infection associated thrombocytopenia, Leishmania Donovani (LD) bodies were identified in 2 cases (2.4%) and microfilaria in one case (1.2%).

DISCUSSION:

Thrombocytopenia is commonly encountered in various haematological disorders including myelodysplastic as well as various non-myelodysplastic haematological conditions (McKenzie, 1996). Some of the haematological conditions present with isolated thrombocytopenia and others present with bi or pancytopenia. Bone marrow examination is safe and useful test in reaching the final diagnosis (Kibria et al., 2010).

In our study the most common age group was below 15 years. In a study done by Muhury et al. (2009), the majorityof the patients were below 10 years. The age of the patients ranged from 7 months and 75years with the mean age of 27.3 years in the present study. Fifty six (65.9%) weremales and twenty nine (34.1%) were females with male female ratio 1.9:1. In Muhury et al. (2009) study thrombocytopenia was commoner in male than female in the first decade. Age and sex distribution was compared with other studies as shown in Table 7.

TABLE 7: COMPARISON OF AGE AND SEX DISTRIBUTION IN DIFFERENT STUDIES

Study	age	Sex ratio
Pudasainiet al. (2002)	9 months-75 years	1:1.1
Niazi et al. (2004)	1-75years	1.7:1
Jha et al. (2008)	1-79	1.5:1
Egesie et al. (2009)	3-80	1.5:1
Gayathri et al. (2011)	2-80	1.2:1
Kibria et al. (2010)	3.5-80	1:0.59
Present study	7 months-75 years	1.9:1

In the present study of 85 cases of thrombocytopenia, 9.4 % cases were indicated for pancytopenia. In a study conducted by Jha etal. (2008),Bashawri et al. (2002), and Pudasaini et al. (2002) the frequency of bone marrow examination for evaluation of pancytopenia was 17.34% , 11.9% and 50% respectively.

ITP was the most common diagnosis seen in 17 cases (20%). Other studies showed 6.21%, 14.5%, 6.8% and 5% cases of ITP respectively in their studies (Kibria et al., 2010; Ahmad et al., 2011; Pudasaini et al., 2002;Knodke et al., 2001). The second most common finding was acute myeloid leukaemia (14.1%; 12 out of 85 cases). Out of 12 cases of AML, the commonest type was AMLM2 (9 cases), followed by AML M5 (2 cases). Acute leukaemia constituted third most common cause of pancytopenia in the study of Savage et al. (1999) and similar finding was seen in study of Varma and Dash (1992).Other series (Egesie et al., 2009; Gayathri et al., 2011; Jha et al., 2008; Kibria et al., 2010) also showed that acute leukaemia is the commonest haematological malignancy and AML is more common than ALL.

Megaloblastic anaemia was diagnosed in 9 cases (10.6%). It was the third most common cause in present study. Niazi et al. (2004),Jha et al. (2008)and Gayathri et al. (2011)found Megaloblastic anaemia as second most common cause.

All cases of Hypoplastic anaemia was presented with pancytopenia (8.2%; 7 cases out of 85cases). Diagnosis was done by bone marrow aspiration study and confirmed by bone marrow biopsy. Compared to our study 19%, 29% and 14% cases of hypoplastic anaemia were seen in other studies (Gayathri et al., 2011;Knodke et al., 2001; Niazi et al., 2004).

We also came across plasma cell dyscrasia (5.9%) and MDS (5.9%). Other series (Kibria et al., 2010; Laishram et al., 2008) showed the incidence of multiple myeloma ranging from 0.94% to 4.1%.andshowed incidence of MDS ranging from 2% to 7.9%.

Leishmaniasis was seen in two cases (2.4%) out of 3 cases of infection associated thrombocytopenia. 2.82%, 1.2%, 0.67% of Leishmaniasiswas detected in other studies (Kibria et al., 2010; Niazi et al., 2004)butthe maximum number of cases (14%) was seen in a studydone by Khodke et al. (2001).

Regarding number and morphology of megakaryocytes, increase numbers and young forms were seen in all cases of ITP. George et al. (1994) and Levine et al. (1999)also observed same findings.

CONCLUSION:

Bone marrow examination is an important step to arrive at the confirmatory diagnosis of wide varieties of haematological disorders in cases of thrombocytopenia. Idiopathic thrombocytopenic purpura was the most common cause of thrombocytopenia in our study. Correlations with clinical, radiological and peripheral blood smear findings provide valuable insight in aetiology of thrombocytopenia.

REFERENCE

1. Ahmad, S. Q., Khan, O. U., Zafar, N. (2011). Utility of Bone Marrow Examination in a Secondary Care Hospital.JRMC, 15, 40-41. | 2. Bashawri, L. A. (2002). Bone marrow examination.Indications and diagnostic value. Saudi Med J, 23(2), 191-196. | 3. Battinelli, E. M., Hartwig, J. H., Italiano, J. E. Jr. (2007). Delivering new insight into the biology of megakaryopoiesis and thrombopoiesis. CurrOpinHematol, 14(5), 419-426. | 4. Egesie, O. J., & Joseph, D. E., Egesie, U. G., Ewuga, O. J. (2009). Epidemiology of anaemia necessitating bone marrow aspiration cytology in Jos.Niger Med J, 50, 61-63. | 5. Gayathri, B. N., Rao, K. S. (2011). Pancytopenia: A clinico hematological study. J Lab Physicians, 3, 15-20. | 6. George J. N., El-Harake, M. A., Raskob, G. E. (1994). Chronic idiopathic thrombocytopenic purpura.N Engl J Med, 331, 1207-1211. | 7. Houwerzijl, E. J., Blom, N. R., van der Want, J. J., Esselink, M. T., Koornstra, J. J., Smit, J. W., Louwes, H., Vellenga, E., de Wolf, J. T. (2004). Ultrastructural study shows morphologic features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. Blood, 103(2), 500-506. | 8. Jha, A., Sayami, G., Adhikari, R. C., Panta, D., Jha, R. (2008). Bone marrow examination in cases of pancytopenia. J Nepal Med Assoc, 47, 12-17. | 9. Kibria, S. G., Islam, M. D. U., Chowdhury, A. S. M. J. et al. (2010). Prevalence of Hematological Disorder: A Bone Marrow Study of 177 Cases In a Private Hospital at Faridpur. Faridpur Med. Coll. J, 5, 11-13. | 10. Knodke, K., Marwah, S., Buxi, G., Yadav, R. B., Chaturvedi, N. K. (2001). Bone marrow examination in cases of pancytopenia.J Academy Clin Med, 2, 55–59. | 11. Laishram, S., Shimray, R., Sharma, A. B., Pukhrambam, G., Sharma, L. D. C. (2008). Neoplastic lesions in the bone marrow: a 10 year study in a teaching hospital. J Indian AcadClin Med, 9(3),175-178. | 12. Levine, F. C. (1999). "Idiopathic" thrombocytopenia.Arch Intern Med, 88, 701-728. | 13. Levine, S. P. (2003). Thrombocytopenia: pathophysiology and classification. In Greer JP, Foerster J, Lukens JN, et al. (Eds.), Wintrobe's clinical hematology 11th ed. (vol. 2, pp. 1530-1532). Philadelphia: Lippincott Williams & Wilkins. | 14. McKenzie S. B. (Ed.). (1996). Textbook of hematology. 2nd ed. Pennsylvania: Willaims& Wilkins. | 15. Muhury, M., Mathai, A. M., Rai, S., Naik, R., Pai, M. R., Sinha, R. (2009). Megakaryocytic alterations in thrombocytopenia: A bone marrow aspiration study. Indian J PatholMicrobiol, 52, 490-494. | 16. Niazi, M., Raziq, F. I. (2004). The incidence of underlying pathology in pancytopenia- an experience of 89 cases.JPMI, 18, 76-79. | 17. Pathak, R., Jha, A., Sayami, G. (2012). Evaluation of bone marrow in patients with pancytopenia. Journal of Pathology of Nepal, 2, 265-271. | 18. Pudasaini, S., Prasad, K. B. R., Rauniyar, S. K., Shrestha, R., Gautam, K., Pathak, R., Koirala, S., Manandhar, U., Shrestha, B.(2002). Interpretation of bone marrow aspiration in hematological disorder.Journal of Pathology of Nepal, 2, 309-312. | 19. Savage, D. G., Allen, R. H., Gangaidzo, I. T., Levy, L. M., Gwanzura, C.,Moya, A., et al. (1999). Pancytopenia in Zimbabwe.Am J Med Sci, 317, 22-32. | 20. Tariq, M., Khan, N., Basri, R., Amin. S. (2010).Aetiology of pancytopenia. Professional Med J, 17(2), 252-256. | 21. Varma, N., Dash, S. (1992). A reappraisal of underlying pathology in adult patients presenting with pancytopenia.Trop Georg Med, 44,322-327