Original Article

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Pathology Section

Clinico-Haematological and Cytochemical Study of Acute Myeloid Leukaemia

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ABSTRACT

Introduction: Acute Myeloid Leukaemia (AML) presents one of the common problems in the field of haematology affecting primarily adults, peaking incidence between the ages of 15-39 years. Incidence and patterns of AML vary in different parts of the world. The most commonly used classification was proposed by the French American British (FAB) co-operative group in 1976.

Aim: To study the clinical features and presentation of patients of AML and to categorise those into different subtypes based on their haematological and cytochemical features using various cytochemical stains.

Materials and Methods: All the patients diagnosed as AML in the Department of Pathology, Gajra Raja Medical College and Jayarogya group of Hospital, Gwalior from 1st December 2012 to 30th November 2013 were enrolled in this descriptive cross-sectional study. Clinical histories of all cases were retrieved subsequently and complete blood count with detailed peripheral smear and bone marrow smear examination was done. AML subtyping was done based on FAB Criteria. Cytochemical stains like Periodic Acid Schiff Stain (PAS), Non Specific Esterase (NSE) stain, Myelo-Peroxidase (MPO) stain and Sudan Black B (SBB) stain were used. MPO stain and SBB stain shows positivity in myeloid series of cells and NSE stain shows positivity in monocytic cells whereas PAS stain shows negative staining in myeloid cells.

Results: AML M2 was more prevalent than other subtypes constituting 69.44% of all cases (25 out of 36 cases), followed by AML M1 constituting 16.66% (6 out of 36 cases). All types of AML were more commonly seen in males than females, ratio being 2:1. Majority of patients of AML presented with pallor, fever, leucocytosis and thrombocytopenia.

Conclusion: Microscopic examination of peripheral blood and bone marrow along with cytochemical staining remains foundation in the diagnosis as well as subtyping of acute Leukaemia, especially in the areas where immunophenotyping and flowcytometry are not readily available.

Keywords: Bone marrow examination, Hyperleucocytosis, Myeloblast

INTRODUCTION

Leukaemia is a neoplasm of haematopoietic cells characterised by uncontrolled, abnormal and widespread proliferation of cancerous cell infiltrating the bone marrow and peripheral blood and associated with the appearance of immature abnormal leucocytes in the peripheral blood and bone marrow [1]. AML is the most common acute Leukaemia in adults, accounting for ~80% of cases in this group. AML affects mainly patients between 15-39 years of age. AML is quite heterogeneous reflecting the complexities of myeloid cell differentiation [2]. Incidence and patterns of AML vary in different parts of the world. The most commonly used classification was proposed by the FAB co-operative group in 1976 [3]. The latest World Health Organisation (WHO) classification 2008 revised in 2016 of the acute Leukaemia differs from the FAB classification in that greater than or equal to 20% blasts are used for diagnosis of acute Leukaemia [4]. AML patients may present with signs and symptoms related to pancytopenia, which include infections, fever, weakness, fatigue and haemorrhagic findings like petechiae, menorrhagia and epistaxis. This is because the proliferation of malignant cells gradually takes over the normal blood cell in bone marrow. Occasionally, there is sternal discomfort or tenderness and pain in lower extremities. There may be cutaneous or gingival infiltration by leukaemic cells. Physical examination may reveal pallor, lymph node enlargement, hepatomegaly and splenomegaly [5]. Hyperleukocytosis is defined as Total Leukocyte Count (TLC) above 100×109/L. Hyperleucocytosis leads to increased viscosity of blood and associated with aggregation of leukaemic cells in the microcirculation. The pathogenesis of AML is uncertain but chromosome abnormalities are usually present in most patients. Common pathway in leukemogenesis is cytogenetic translocations which lead to formation of fusion proteins [6]. The main objective of this study was to know the prevalence of different kind of AML in this particular area and to know the importance of cytochemical staining in categorising myeloid Leukaemias into different subtypes of FAB classification.

MATERIALS AND METHODS

The present descriptive cross-sectional study was conducted after taking approval of Ethical Committee (IEC No.GRMC/Gwl/50/151012). The study was carried out from 1st December 2012 to 30th November 2013 for one year and enrolling total 36 new patients diagnosed as AML on peripheral blood examinations in the Department of Pathology, Gajra Raja Medical College and Jayarogya group of Hospital, Gwalior. Subsequently relevant clinical findings were retrieved and thereafter bone marrow aspiration was performed after obtaining informed consent from the patients and guardian of patients. Complete blood count (by Mindray BC 5000) was performed. Using various cytochemical stains like SBB, MPO, PAS, and NSE, AML subtyping was done based on FAB Criteria [3]. The positive MPO stain and SBB stain revealed myeloid lineage. The demonstration of monocytic lineage was assessed by positive NSE and morphological feature of monocytic cells. In this study we included all new cases of AML and exclusion of cases in the blast crisis of chronic myeloid leukaemia and previously treated and relapsed cases of AML. Total 36 cases of AML were recorded and analysed. All 36 cases (100%) showed MPO stain and SBB stain positivity and 03 cases (8.33%) showed NSE stain positivity during cytochemical study.

RESULTS

Results were analysed using simple calculator for frequency analysis. Morphological typing and subtyping was based on peripheral blood

smear and bone marrow examination, automated cell counter results, with cytochemical stain findings. In almost all patients of AML the presenting and common clinical feature was pallor (34/36, 94.44%). Other features including fever in 31 patients (86.11%), generalised weakness in 19 patients (52.77%), splenomegaly in 18 patients (50%), loss of appetite in 16 patients (44.44%), joint pain in 15 patients (41.66%), hepatomegaly in 13 patients (36.11%) lymphadenopathy in 10 patients (27.77%) and sign of haemorrhage in 4 patients (11.11%) were present [Table/Fig-1].

S. no.	Clinical Symptoms	Patients of AML (%)
1	Pallor	34 (94.44)
2	Fever	31 (86.11)
3	Generalised weakness	19 (52.77)
4	Joint pain	15 (41.66)
5	Loss of appetite	16 (44.44)
6	Signs of haemorrhage	04 (11.11)
7	Splenomegaly	18 (50)
8	Lymphadenopathy	10 (27.77)
9	Hepatomegaly	13 (36.11)

[Table/Fig-1]: Clinical feature of patients of AML (n=36).

In this study, the age of patients ranged from 2-70 years and mean age was 35 years [Table/Fig-2]. Out of 36 patients, 24 were male and 12 were female patients. Male to female ratio was 2:1.

Complete blood count demonstrated haemoglobin count less than 6 gm% in 15 patients (41.66%) while 16 patients (44.44%) showed 6-9 gm%, 05 patients (13.88%) had 9-12 gm% haemoglobin and none of AML patients presented above 12 gm% of haemoglobin [Table/Fig-2].

	Patients of AML	Number	Percentage %
Age in years	02-15	4	11.11
	16-30	14	38.88
	31-45	11	30.55
	>45	7	19.44
Sex	Male	24	66.7
	Female	12	33.3
	Range	**Number**	**%**
Hb (gm %)	<6	15	41.66
	6.1-9.0	16	44.44
	9.1-12	05	13.88
	Range	**Number**	**%**
Total WBC count (×10^9/L)	<4	01	2.77
	4-10.99	01	2.77
	11-49.99	08	22.22
	50-99.99	12	33.33
	100-200	11	30.55
	>200	03	8.33
	Range	**Number**	**%**
Platelet count (×10^9/L)	<50	19	52.77
	50-100	14	38.88
	100-150	02	05.55
	>150	01	2.77
Total		36	100

[Table/Fig-2]: Showing age wise distribution, haemoglobin. Total Leucocyte count (×10^9/L) and Platelet count (×10^9/L) in patients of AML (n=36)

Total 12 patients (33.33%) showed TLC between 50×10^9/L to 99.99×10^9/L followed by 11 patients (30.55%) that showed TLC between 100×10^9/L to 200×10^9/L, 08 patients showed TLC between 11×10^9/L to 49.99×10^9/L, 03 patients (8.33%) showed TLC more than 200×10^9/L,01 patient (2.77%) showed TLC within normal range and 01 patient (2.77%) presented with leucopenia [Table/Fig-2]. Thrombocytopenia was noticed in most of the patients of AML. Nineteen patients (52%) had severe degree of thrombocytopenia (count below 50×10^9/L) and 14 patients (38.88%) had moderate degree of thrombocytopenia (count between 50-100×10^9/L) and 02 patients (5.55%) had account between 100-150×10^9/L, and only 01 (2.77%) patient presented with in normal limit of platelet count [Table/Fig-2].

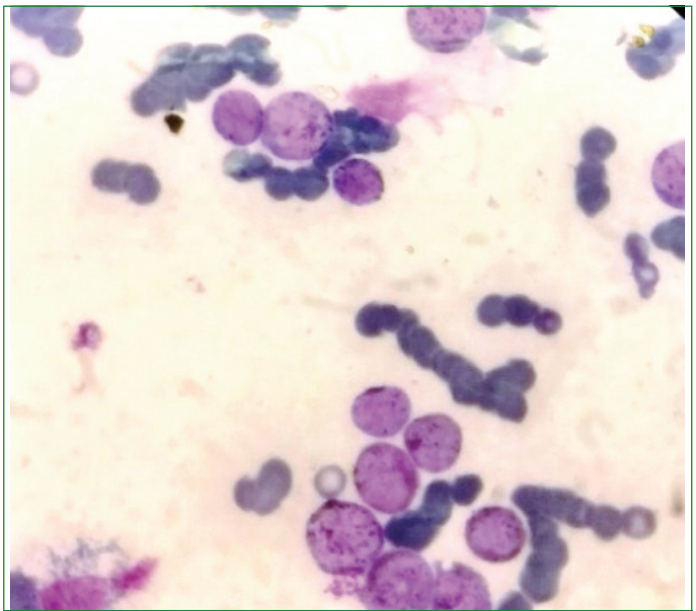
The following subtypes of AML were observed; 06 cases (16.66%) in AML M1, 25 cases (69.44%) of AML M2, 02 cases (5.55%) of AML M3, 03 cases (8.33%) of AML M4 and no patient of AML M0, AML M5, AML M6, AML M7 were not found in present study [Table/Fig-3]. The [Table/Fig-4] depicts cytochemical stains used in all cases of AML, 36 (100%) cases showed MPO/SBB positivity [Table/Fig-5,6], 03 cases (8.33%) of AML M4 showed NSE positivity [Table/Fig-7] whereas all cases of AML were negative for PAS.

S. no.	Subtypes	Male	Female	Total number of cases	Percentage %
		Number	Number		
1	M0	0	0	00	00
2	M1	5	01	06	16.66
3	M2	15	10	25	69.44
4	M3	01	01	02	5.55
5	M4	03	0	03	8.33
6	M5	0	0	00	00
7	M6	0	0	00	00
8	M7	0	0	00	00
Total		24	12	36	100

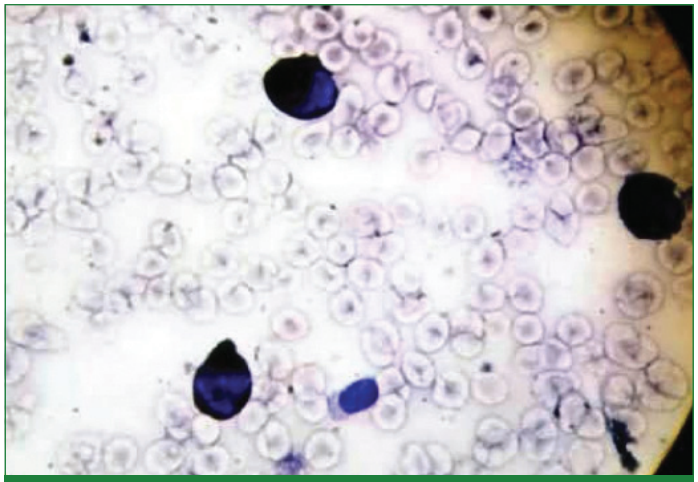
[Table/Fig-3]: Subtyping of AML (n=36).

S. no.	Subtypes	MPO stain	SBB stain	NSE stain	PAS stain
1	M0	00	00	00	00
2	M1	06	06	00	00
3	M2	25	25	00	00
4	M3	02	02	00	00
5	M4	03	03	03	00
6	M5	0	0	00	00
7	M6	0	0	00	00
8	M7	0	0	00	00
Total		36	36	03	00

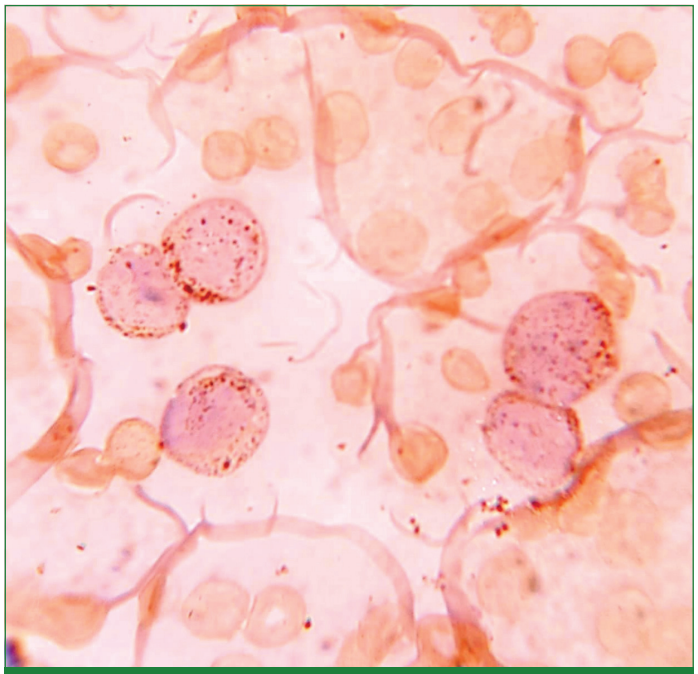
[Table/Fig-4]: Cytochemical stains used in cases of AML (n=36).



[Table/Fig-5]: Positive result (Purple Black Colour) of Myelo-Peroxidase Staining (MPO) in the cytoplasm of blasts and promyelocytes (100X).



[Table/Fig-6]: Positive result (Black Colour) of Sudan Black B (SBB) staining in the cytoplasm of blasts and promyelocytes (100X).



[Table/Fig-7]: Positive result (brown-diffuse fine granular cytoplasmic staining) of Non Specific Esterase Staining (NSE) in blasts, promonocyte and monocyte (100X).

DISCUSSION

Cytochemical stains are special stains, which are enzymatic colorimetric reaction that occurs in the cells of interest, are necessary to differentiate and confirm myeloid and lymphoid lineage of haemopoitic neoplasm. AML is a heterogeneous clonal disorder caused by maturational arrest of myeloid cell in the initial stages of development mostly by familial syndromes, chromosomal mutations and environmental factors and characterised by immature myeloid cell proliferation and bone marrow failure [7]. It is widespread in its distribution unlike the solid tumours, hence exact staging and prognosis depends on its subtypes and associated clinical and haematological parameters. Most AML subtypes are separated from other related Leukaemias by the presence of more than 20% myeloblast/monoblasts/blast equivalent in the blood and bone marrow.

In this study, the main presenting clinical symptom was pallor owing to decreased production of erythroid precursor cells in the bone, also contributing to generalised weakness. Fever being second most common clinical feature after pallor is due to decreased mature and normal myeloid precursor cells in the bone marrow. Decreased precursor cells in the bone marrow are due to marrow infiltration by abnormal cancerous cells leading to anemia, neutropenia and thrombocytopenia. Joint pain, splenomegaly and lymphadenopathy are present due to accumulation of abnormal cancerous cells in these tissues. In present study, males were more commonly involved than females with male to female ratio being 2:1 which is probably due to lifestyle changes. Similar findings were observed by Preethi CR and Tyagi SP et al., [8,9]. The mean age of various types of AML patients also correlated with the study conducted by Omer SH et al., [10]. In the present study, 10 patients (27.77%) of AML presented with lymphadenopathy while Chang F et al., observed lymphadenopathy in 33% cases and Advani SH et al., observed lymphadenopathy in only 4% cases in his study [11,12]. Difference in the percentage of lymphadenopathy in the different studies may be due the fact that there are many reasons of lymphadenopathy like infections, lymphomas and different frequency of subtypes of AML in different studies. Hyperleukocytosis and thrombocytopenia are the distinguished features of AML. Out of 36 patients, 38.88% and 97.22% presented with hyperleukocytosis and thrombocytopenia, respectively which is in concordance with findings of Shome DK et al., [13]. Applying FAB classification, AML M2 was found to be the commonest subtype in 25 cases (69.44%) in the present study that was commensurable to the observations made in studies conducted by Chaudhry MT et al., and Ghose S et al., [14,15] whereas, AML M2 was second most common subtype in studies conducted by Omer SH et al., and Hassan K et al., [10,16]. MPO and SBB were positive in all cases confirming there myeloid lineage. few cases showing NSE positivity suggest additional monocytic differentiation putting those cases into the myelomonocytic category of AML M4. All the cases were negative for PAS stain as it suggests lymphoid differentiation. Similar results were also reported by few studies [Table/Fig-8] [10,14-17]. It was found out that by examination of peripheral blood and bone marrow and with the proper use of cytochemical stains, exact subtyping of AML cases can be done precisely aiding to further prognosis and treatment of the patients specially those who sometimes cannot afford to have expensive diagnostic tests or do not have that facility at all.

Author name, Reference number	Year/Place	Subtyping data	Total number of cases
Present study	2020/India	M2>M1>M4>M3	36
Chaudhry MT et al., [14]	1993/Pakistan	M2>M4>M1>M3	54
Hassan K et al., [16]	1993/Pakistan	M4>M2>M1>M5	81
Ghosh S et al., [15]	2003/India	M2>M5>M1>M3	260
Omer SH et al., [10]	2017/Sudan	M3>M2>M4>M0	140
Choudhury R et al., [17]	2017/India	M5>M4>M3>M6	63

[Table/Fig-8]: Comparison of distribution of FAB subtype of AML in various studies [10,14-17].

Limitation(s)

The major limitation of present study was the small sample size and ancillary investigations like immunological markers, cytogenetics and molecular genetics were not available in the present setup.

CONCLUSION(S)

Microscopic examination of peripheral blood smear along with cytochemical stains still remains the foundation in the diagnosis of acute Leukaemias, especially in the areas where immunophenotyping, flow cytometry, cytogenetics and molecular genetics are not readily available. Exact subtyping is must for accurate treatment and also for prognostic purpose.

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