

Studies on the morphology of leukaemic blast cells in relation to haematological parameters

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Abstract

A combination of haematological parameters with morphological evaluation of peripheral blood and bone marrow blast cells is crucial for leukaemia diagnosis. FAB (French–American–British) classification is a simple and powerful diagnostic tool for leukaemia in developing countries like India. Differentiation block in the early stages of haematopoiesis and morphological characteristics of leukemic blast cells are directly related to haematological parameters. The present study is an approach to increase understanding of the simple morphological FAB classification of leukaemia in relation to haematological parameters. The present study revealed that Chronic Myeloid Leukaemia (CML) was the most common type of leukaemia, followed by Acute Myeloid Leukaemia, Acute Lymphoid Leukaemia (ALL), and Chronic Lymphoid Leukaemia (CLL) in Nagpur. Most of the cases of Acute Leukaemia had severe anaemia and thrombocytopenia. Highest variation was found in Total WBCs count of different types of leukaemia, particularly in different subtypes of AML. The present study also suggested that FAB classification is not outdated, but it does require continuous revalidation and other procedures for refinement.

Keywords: Blast cell, Bone marrow, FAB classification, Haematopoiesis, Leukaemia

INTRODUCTION

Leukaemia is a malignant neoplasm of haematopoietic cells. Specific genetic events related to cellular differentiation and proliferation of haematopoietic cell contributes to malignant transformation of cells and their progeny forming clones of leukaemia cells. In leukaemia, differentiation block occurs in the early stages of haematopoiesis, thus resulting in undifferentiated cells are named as blast cells. In a few cases, cells other than blast cells are counted that are known as blast equivalent (Singh, 2018). Leukemic blast cell and other

blast equivalent demonstrate extra-ordinary biological, morphological, and clinical heterogeneity (Baviskar, 2016). The perspective of the classification and characterization of leukemic blast cell is imperative for the treatment of different subtypes of leukaemia because in the leukemic form, by definition the bone marrow must contain at least 20% blast cell and study of blast cell morphology is the first step in the diagnostic pathway (Sabina *et al.*, 2014). The present study was carried out with the aim to know the morphological characteristics of blast cells of peripheral blood and bone marrow in relation to different haemato-

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logical parameters in newly diagnosed cases of leukaemia.

MATERIALS AND METHODS

The study was conducted at the Animal Physiology Laboratory, Department of Zoology RTM Nagpur University, Nagpur, and all samples were collected from the Ganju Hematology Clinic & Hospital Nagpur and Kane Hematology and Oncology Laboratory Nagpur. In this study total numbers of 89 subjects were selected in which 10 were normal healthy individuals and 79 were leukemic patients who were divided into four subgroups: 31 were diagnosed as CML (Chronic Lymphoid Leukaemia), 25 as AML (Acute Myeloid Leukaemia), 21 as ALL (Acute Lymphoid Leukaemia) and 2 were as Chronic Lymphoid Leukaemia (CLL). The case selection was based on clinical features. Informed consent was taken from all the patients and the study was ethically approved. Bone marrow aspirate and peripheral blood were collected from Ganju Hematology Clinic & Hospital Nagpur and Kane Hematology and Oncology Laboratory Nagpur.

For haematological variables, samples were collected in EDTA coated purple-top test tube. Haematological parameters R.B.Cs, W.B.Cs, haemoglobin, and platelets were estimated by an Automated Haematological Analyzer (Swelab Alfa analyzer SN18766), and blast cells were counted by Hemocytometer.

The study comprised of peripheral blood smears and bone marrow aspirate blood smears. Minor modification in standard Leishman's stain was done by either incubating slide or buffer solution or both (Sareen *et al.*, 2018). Leishman's stain was modified using phenol crystals and liquefied phenol (Fasakin *et al.*, 2014).

RESULTS AND DISCUSSION

In the present study, out of 79 leukemic peripheral blood samples of the patients, 31 (39.240%) were diagnosed as CML, 25 (30.656%), as AML 21 (26.582%), as ALL and 2 (2.532%) were as CLL (Table 1). In our study, among all cases, CML had the highest, and CLL had the lowest incidence

Table 1. Type of leukaemia in patients.

Leukaemia Type	AML	ALL	CML	CLL
No. of Patients	25	21	31	2

Table 2. Haemoglobin level in leukaemia patients.

Haemoglobin level	No. of Patients			
	AML	ALL	CML	CLL
< 7gm%	20	15	04	00
7 – 10 gm%	04	03	18	02
> 10 gm %	01	03	09	00
Total	25	21	31	02

Haemoglobin range values in normal persons: Male; 14 gm % -18 gm%, Female; 12-15 gm%

rate. The incidence of CML was noted the highest (45.3%) and that was the lowest of CLL (5.7%) in Delhi (Vasavada *et al.*, 1962, Rani *et al.*,1982). Similar observations were noted in Chandigarh and other metro cities of India like Mumbai and Calcutta (Chatterjea *et al.*, 1962, Radha *et al.*, 2014, Ahirwar *et al.*, 2018). In the present study, AML had the second-highest incidence rate followed by ALL among all classes of leukaemia . The incidence rate of ALL was higher than AML in South India (Prakash *et al.*, 1981, Varghese *et al.*, 1984). In our study, the cases of ALL were the third predominant. In the present study most frequent FAB subtype of AML was M2 which comprised of 9 (36%) patients, whereas M0, M1, M3, M4, M5, M6, M7 comprised of 1 (4%), 2 (8%), 5 (20%), 1 (4%) , 2 (8%) , 3 (12%), 2 (8%) respectively. Tata Memorial Institute reported AML-M2 as the most common FAB subtype of AML (Ghosh *et al.*, 2003). Similarly, In the L1 subtype of ALL comprised of 15 (71.4%) patients, whereas L2 and L3 comprised 4 (19.04%) and 2 (9.52%) patients respectively. It has been seen that the spectrum of leukaemia varies in different geographical regions according to varying lifestyles, economic conditions, and poverty rates (Gunz., 1977). But, in Nagpur, the incidence of different types of leukaemia does not differ markedly with other metro cities of India. CML was a more common type of leukaemia followed by AML, ALL, and CLL. Anaemia is a constant feature in all classes of leukaemia . In the majority of cases, it is due to bone marrow infiltration leading to decreased production, decreased red cell life span, and autoimmune destruction. (Farzana *et al.*, 2016).

In the present study, 80.00% of cases of AML and 71.42% of cases of ALL had less than 7 gm% Hb. In CML 12.90% of cases had less than 7 gm% Hb. In CLL both patients had mild anaemia with 9.02 and 8.92 gm% Hb Count (Table 2). The mean haemoglobin count of all classes of leukaemia

Table 3. Total WBCs count in Leukaemia Patients.

WBCs counts	No. of Patients			
	AML	ALL	CML	CLL
4×10 ³ /µl – 10×10 ³ /µl	06	06	–	–
10×10 ³ /µl – 100×10 ³ /µl	15	14	04	–
100×10 ³ /µl – 160×10 ³ /µl	03	01	24	02
>160×10 ³ /µl	01	–	03	–

Total WBCs range values in normal persons: 4.8 X10³/µl-10.8 X10³/µl

Table 4. Total Platelet counts in leukaemia patients.

Platelet Counts	No. of Patients.			
	AML	ALL	CML	CLL
40×10 ³ /µl – 100×10 ³ /µl	21	20	–	–
100×10 ³ /µl – 150×10 ³ /µl	02	00	01	–
150×10 ³ /µl – 350×10 ³ /µl	–	01	01	02
350×10 ³ /µl – 550×10 ³ /µl	02	–	29	–

Total Platelet range values in normal persons: 175 X10³/µl-450 X10³/µl

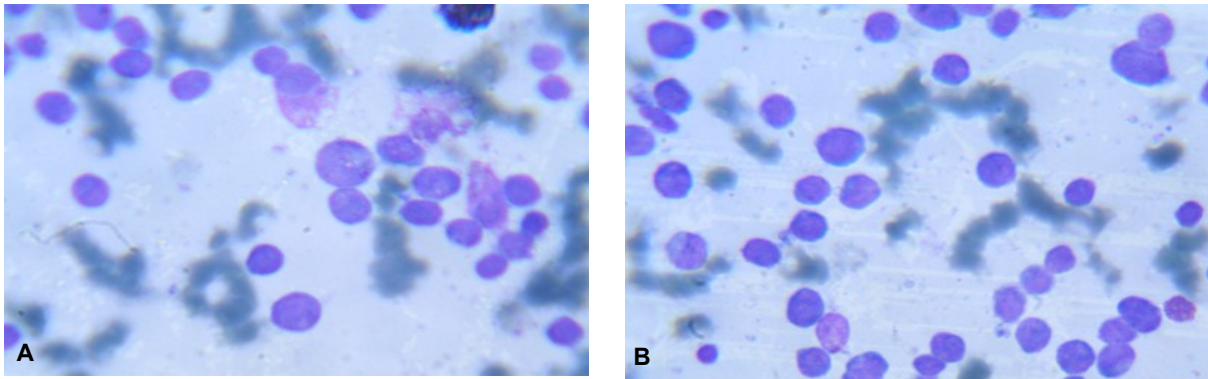


Fig. 1. AML-M0 sub-type; A and B. Bone marrow aspirate smear showing large and uniform blast cells, scanty cytoplasm, nucleus with open chromatin.

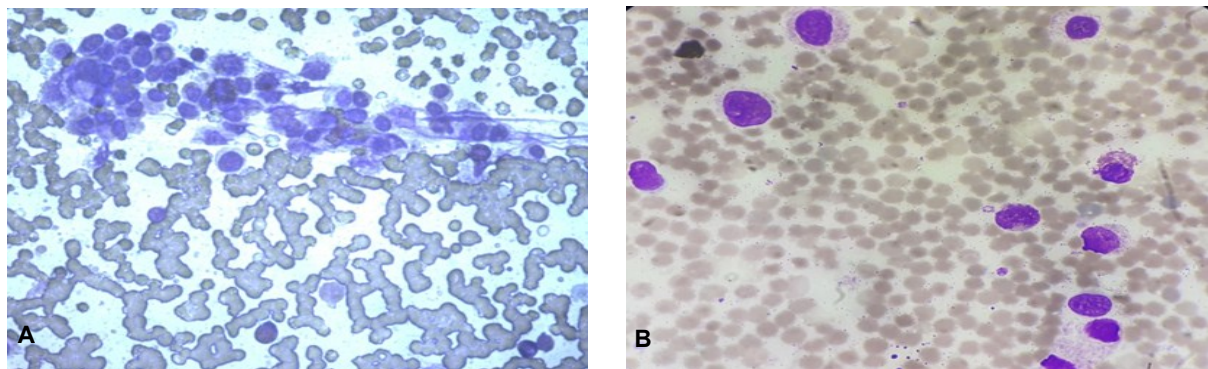


Fig. 2. AML-M1 sub-type; A- Bone marrow aspirate smear, B- peripheral blood smear; showing large blast cells with variable nucleocytoplasmic ratio, rare blast cell demonstrating Auer rods.

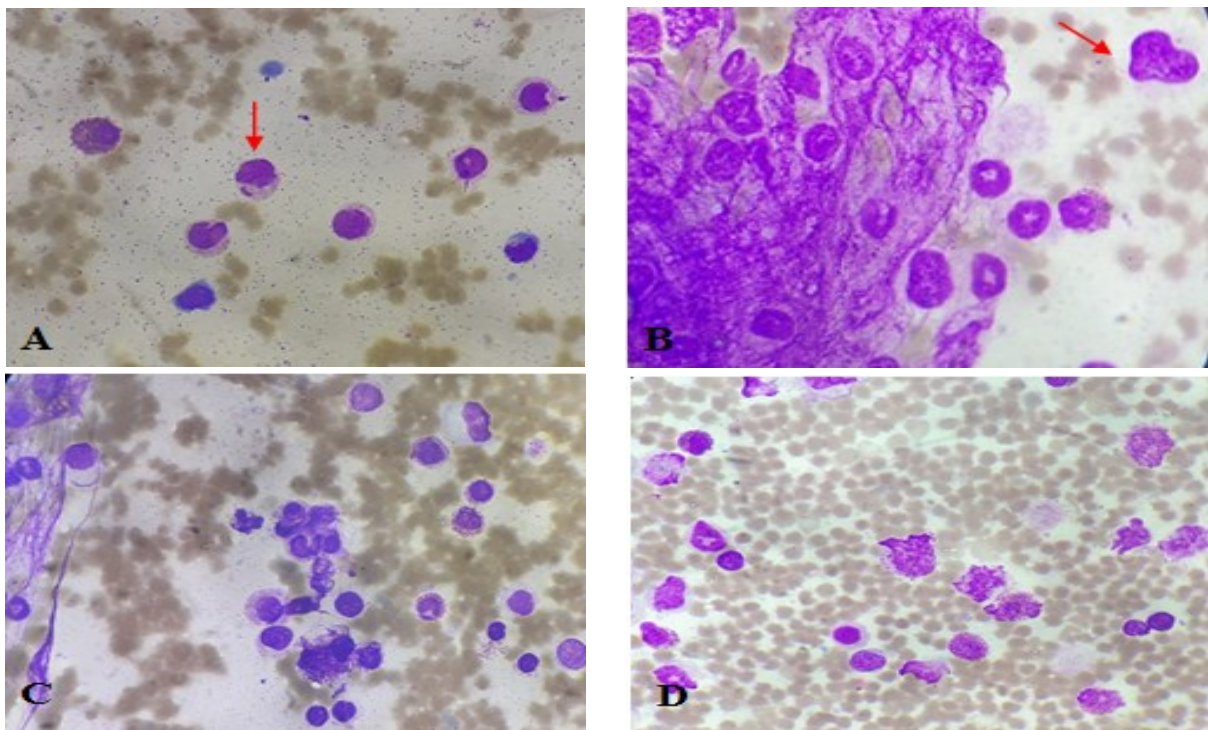


Fig. 3. AML-M2 sub-type; (Bone marrow aspirate smear (A,B,C and D); showing large blast with oval and slightly indented nuclei and moderate amount of granulophilic cytoplasm. A-one of the blast showing an Auer rod (red arrow), B-one of the blast cell shows distinctive heart shape nucleus. C and D- promyelocyte, myelocytes and metamyelocytes and neutrophils. C and D showing highest number of eosinophilic myelocytes.

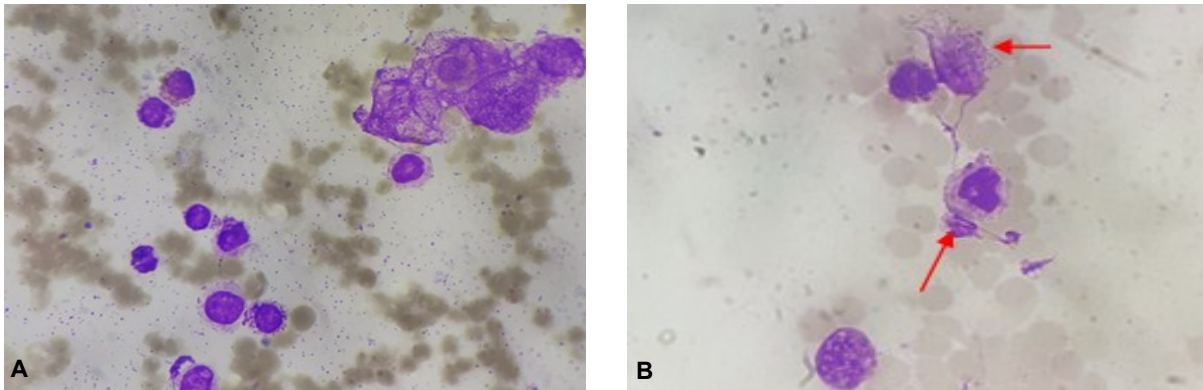


Fig. 4. AML-M3 sub-type; Bone marrow aspirate smear (A, and B) showing medium size Promyelocytes with densely packed coarse purple granular cytoplasm, Some cells showing auer rods in the form of bundle (Faggot cells)(red arrows), presence of Phi bodies in some cells (green arrow).

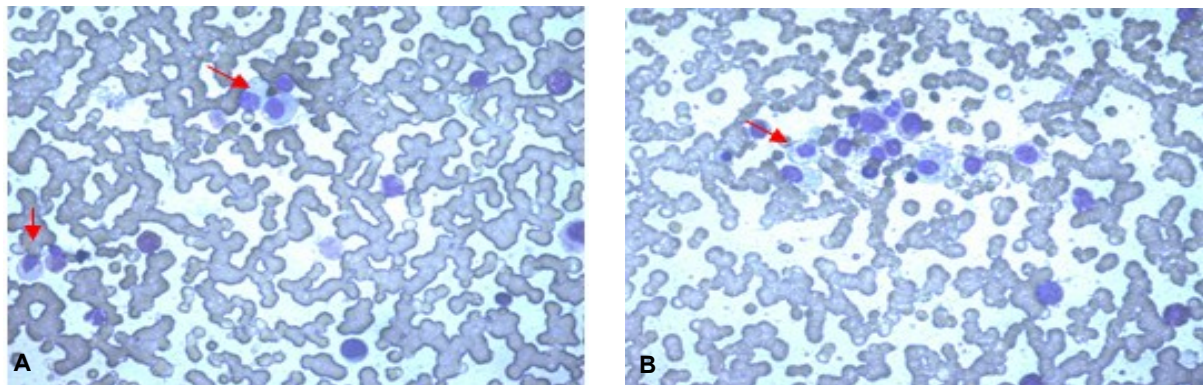


Fig. 5. AML-M4 sub-type. Peripheral blood smear (A, and B), showing medium size Myeloblast and Monoblast with lower nuclear to cytoplasmic ratio and agranular cytoplasm.

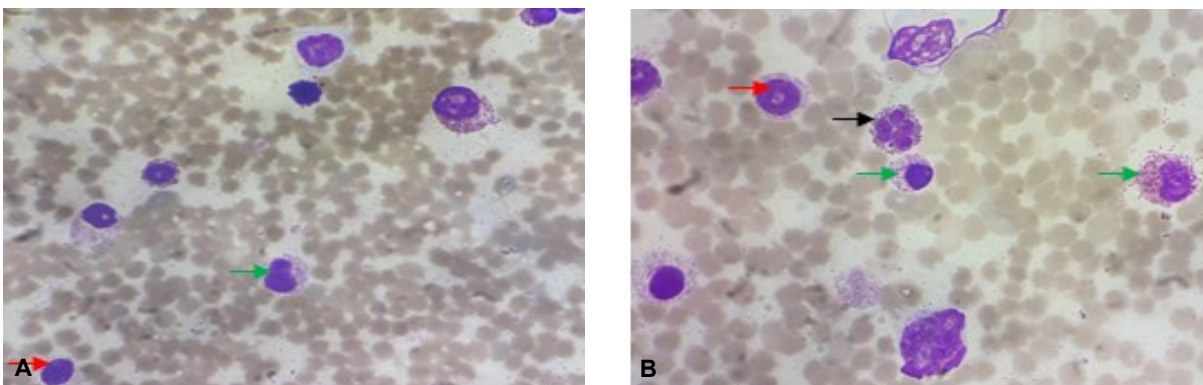


Fig. 6. AML-M5 sub-type. Peripheral blood smear (A, and B), showing blast cell (red arrow) and Promonocytes (green arrow) with lower N/C ratio, vacuolated cytoplasm. One cell showing apoptosis (black arrow).

was quite low, among them the most of the cases of acute leukaemia had severe anaemia. Other studies done by various authors also reported similar haemoglobin profiles of acute and chronic leukaemia patients in Hamidia Hospital, Bhopal (Ahirwar *et al.*, 2015).

Leukocytosis is the common finding associated with all classes of leukaemia. In the present study, the total WBCs count of 77.41% cases of CML showed hyperleukocytosis (Table 3). Hyperleukocytosis is defined as a total peripheral WBC above $100 \times 10^9/L$. Hyperleukocytosis increases the blood

viscosity and is associated with the aggregation of leukemic cells in the microcirculation (Farzana *et al.*, 2016). Thrombocytopenia is a well-known expression of acute leukaemias. In the present study, most of the cases of acute leukaemia had severe thrombocytopenia, whereas chronic leukaemia most of the cases had normal to moderate thrombocytopenia (Table 4). The association of bleeding with platelet count has been well documented in the literature by Gaydos *et al.* (1962) and was the first to document this finding in patients with acute leukaemia. They demonstrated

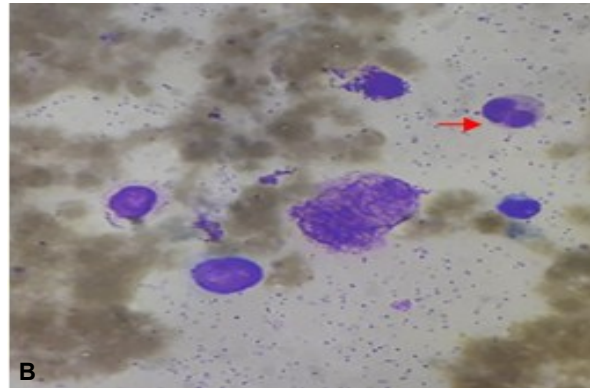
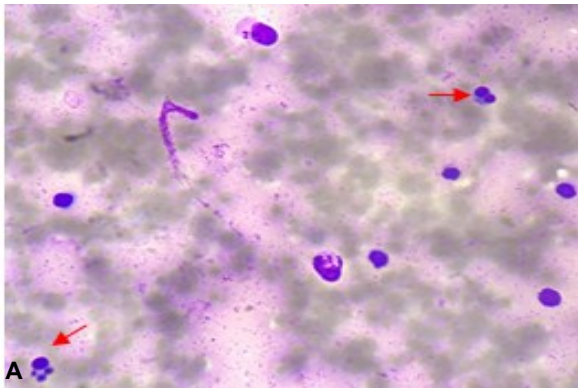


Fig. 7. AML-M6 sub-type; Peripheral blood smear (A, and B), showing blast cells with basophilic agranular cytoplasm. Some cells with two or more than two nuclei (red arrow).

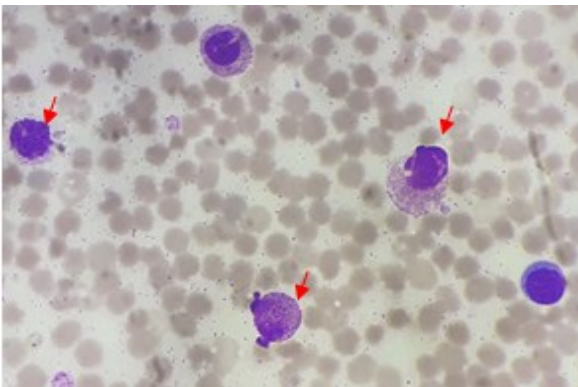


Fig. 8. AML-M7 sub-type; Peripheral blood smear A, showing Megakaryoblast with membrane budding (red arrow).

correlated the morphological feature of acute megakaryoblastic leukaemia (AML-M7) with thrombocytopenia. (Gaydos *et al.*, 1962, Quazi, *et al.*, 2002). These morphological features like platelet budding help in correctly diagnosing acute megakaryoblastic leukaemia (Sharma *et al.*, 2009).

Morphological evaluation of peripheral blood smear and bone marrow cells is crucial for diagnosis and for the follow up in leukaemia. Therefore, microscopy with the combination of haematological parameters still remains a very important tool for diagnosis in leukaemia. In acute leukaemia, the classification now most widely used is the FAB classification proposed in 1976, which is based on morphology. In addition to FAB Classification, several new classification systems have been proposed for the diagnosis of leukaemia.

FAB Classification stills an important and simple tool for leukaemia diagnosis in developing countries like India. But this classification requires continuous evaluation and refinement. The present study is an approach to understand how morphological characters concerning haematological parameters are important for the diagnostic classification of leukaemia. The FAB cooperative working group classified ALL into L1, L2, and L3 on the

that there was a linear relationship between bleeding and low platelet count. The severity of thrombocytopenia in patients with leukaemia varies according to the type and stage of the disease. Patients with CML tend to have an elevated platelet count during the chronic phase and thrombocytopenia develops as the disease progresses from chronic to blastic phase. Thrombocytopenia is very common in the presentation in patients with acute leukaemia (AML and ALL). Many authors

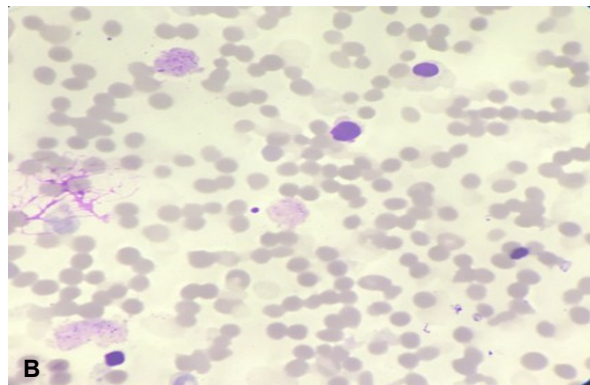
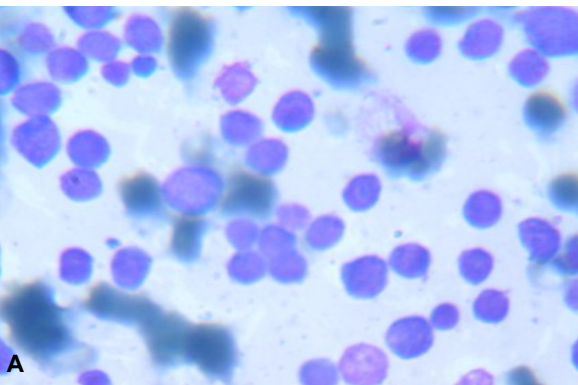


Fig. 9. ALL-L1 sub-type. A- Bone marrow aspirate smear, B- peripheral blood smear showing blast cells are small, uniform size, scanty cytoplasm, Nuclei have coarsely clumped chromatin, Inconspicuous 1-2 small Nucleoli.

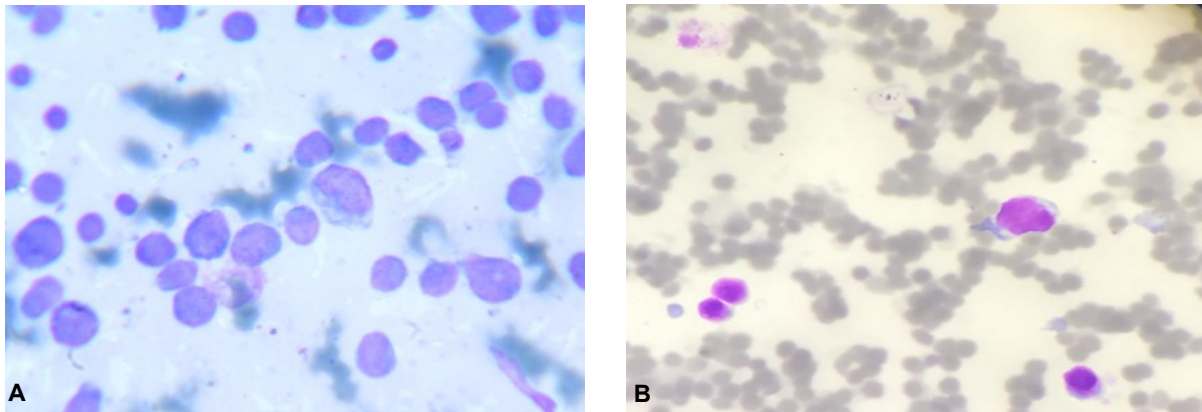


Fig.10. ALL-L2 sub-type; A- Bone marrow aspirate smear, B- peripheral blood smear showing blast cells are medium to large size, nuclei are variable in shape and size, 1-2 nucleoli, clumped chromatin, irregular nuclear membrane, moderate amount of basophilic cytoplasm.

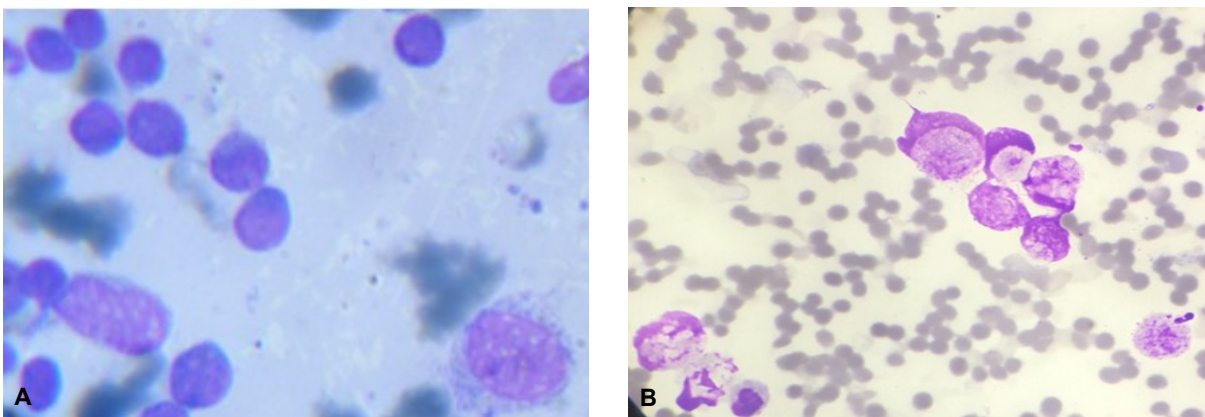


Fig. 11. ALL-L3 sub-type. A- Bone marrow aspirate smear, B- peripheral blood smear showing blast cells are intermediate to large size with clumped chromatin, prominent vacuoles, deeply basophilic cytoplasm with multiple vacuoles which also overlie the nucleus, prominent nucleolus.

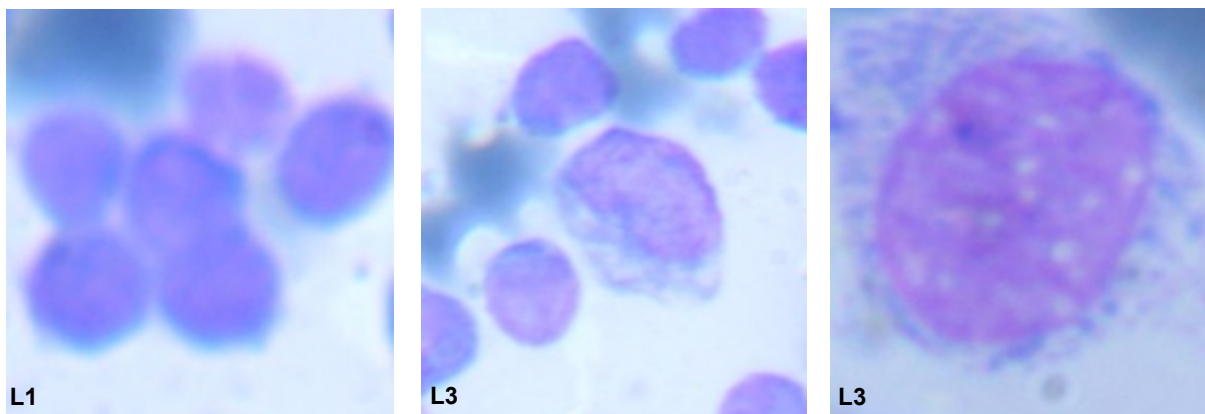


Fig. 12. Showing comparative morphology of blast cell in sub-types of ALL.

basis of four morphological characteristics: N: C ratio, presences of nucleoli, characteristics of the nuclear membrane, and cell size (Bennett\ *et al.*, 1976).

The morphological characteristics of leukemic blast cells are given in Figs. 1-9. In our study, it was indicated that L1 lymphoblasts were small, uniform, and characterized by a high N: C ratio (Fig. 9). L2 lymphoblasts were medium to large

size, heterogeneous, and lower N: C ratio (Fig. 10), while L3 lymphoblasts were heterogeneous and showed prominent vacuolation with deeply basophilic cytoplasm (Fig. 11). The study confirmed the work of authors who have also reported similar morphological characters in ALL patients (Conter *et al.*, 2004, Sabina *et al.*, 2014, Ladines *et al.*, 2016).

Bone marrow aspirate smear of CLL patient

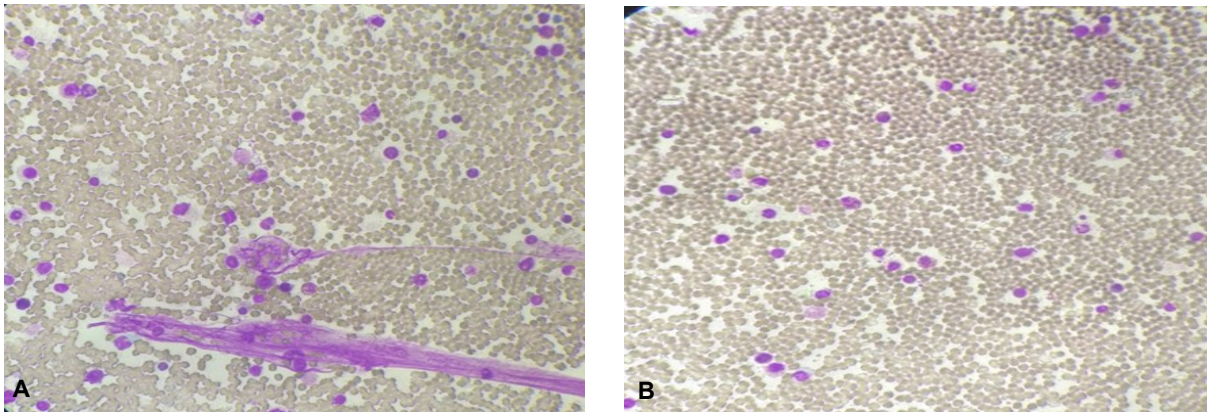


Fig. 13. CML, A and B, Bone marrow aspirate smear showing low power view showing leukocytosis, with all stages of myeloid cells from blast cell to neutrophils.

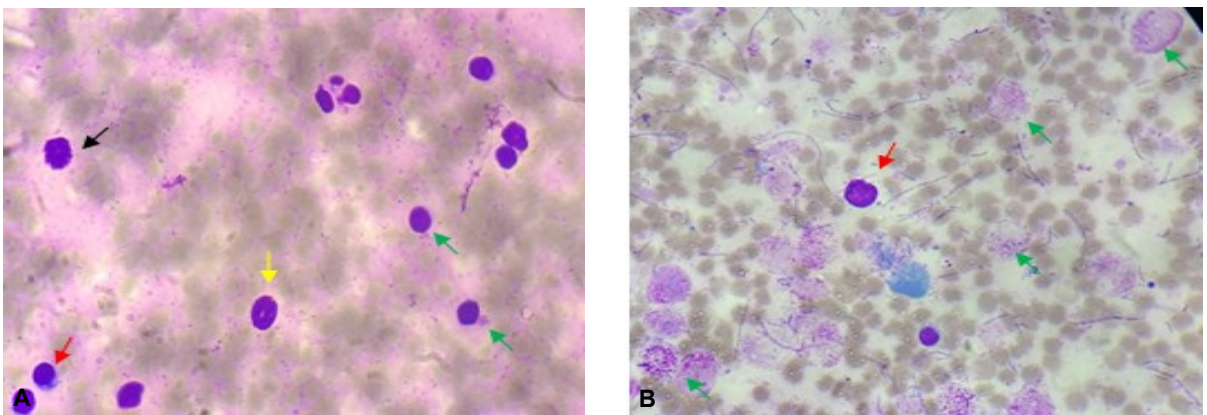


Fig. 14. CML A: Peripheral blood smear showing low power view showing leukocytosis with all stages of myeloid cells- myeloblast (green arrow), promyelocytes (red arrow) and myelocytes (yellow arrow), basophile made out by their coarse granules (black arrow), hyper granular basophiles. B: Peripheral blood smear showing large number of smudge cells (green arrow) and myelocyte bulge (red arrow).

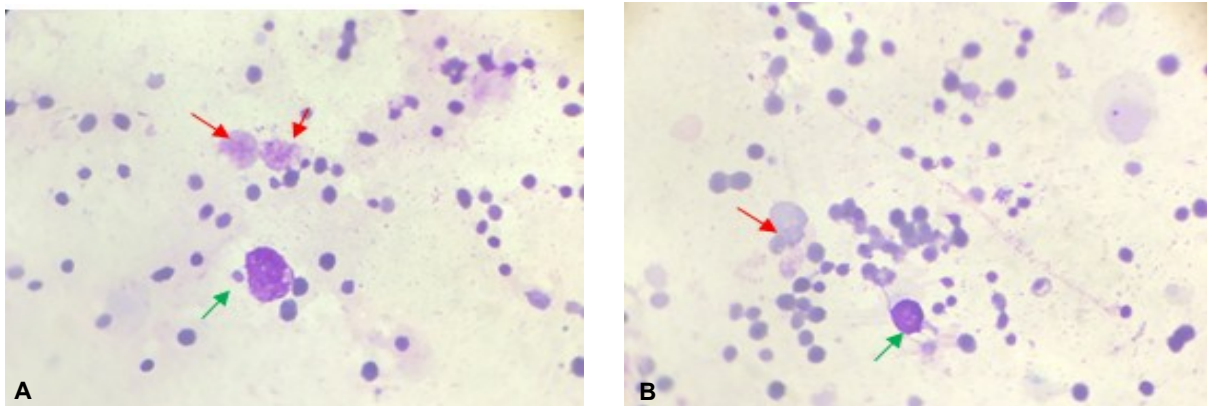


Fig. 15. CLL A and B, Bone marrow aspirate smear low power view showing lymphocytosis, smudge cells (red arrow) and megakaryocyte (green arrow).

showed smudge cells and megakaryocytes (Fig. 15-17). Smudge cells were remnant cells that lacked any identifiable cytoplasmic membrane or nuclear structure. These smudge cells are also called basket cells (David *et al.*, 2016). The peripheral blood smear of CML patient showed hyperleukocytosis with all stages of myeloid cells (Fig. 13-14). The present study confirmed the

work of authors who have also reported similar hyperleukocytosis in CML patients (Karl *et al.*, 2000, Danielle *et al.*, 2008,) AML-M0 blast is difficult to diagnose the morphological point of view. In our study, AML-M0 myeloblasts were medium to large size with scanty cytoplasm and open chromatin nucleus (Fig. 1). AML-M1 myeloblasts were larger compared to the M0 blast and showed

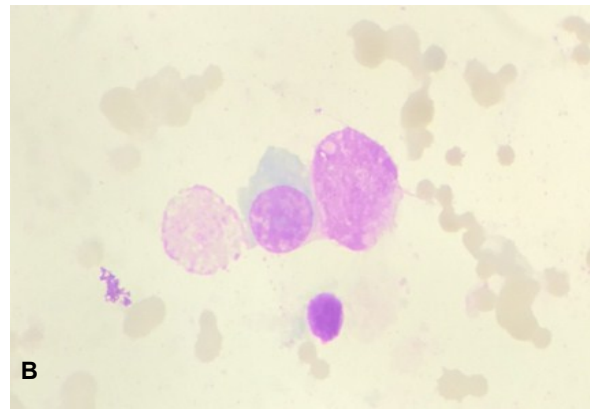
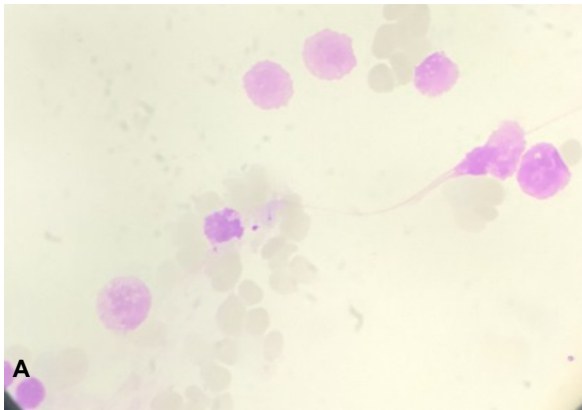


Fig. 16. CLL. (A and B, Bone marrow aspirate smear) showing smudge cells. Smudge cells are remnants of cells that lack any identifiable cytoplasmic membrane or nuclear structure. Smudge cells also called basket cells.

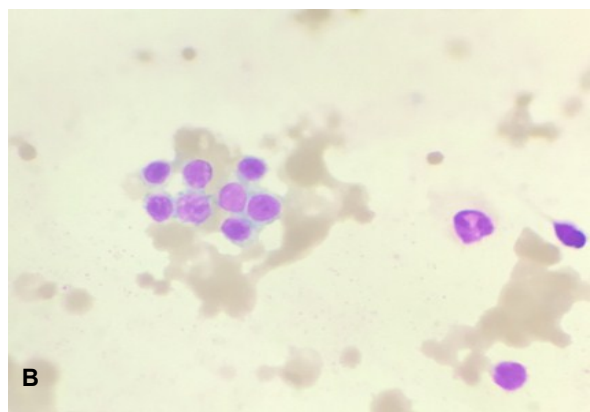
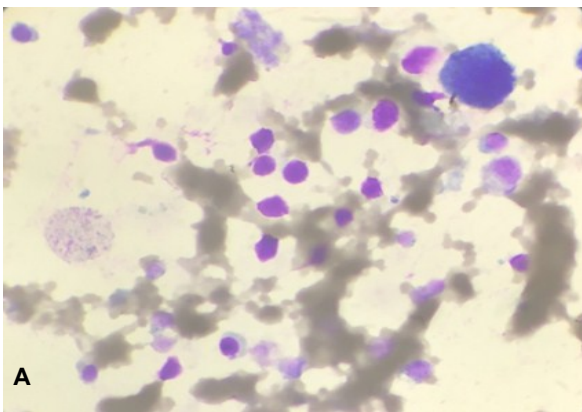


Fig. 17. CLL. A and B, Bone marrow aspirate smear showing leukemic Lymphocytes are uniform with round nucleus and cytoplasmic outline. Nuclear chromatin is coarsely clumped with no nucleoli. Large no. of smudge cells and some megakaryocyte. **Abbreviations:** AML: Acute myeloid leukaemia ; ALL: Acute lymphoblastic leukaemia ; CML: Chronic myeloid leukaemia ; CLL: Chronic lymphocytic leukaemia

variable nucleocytoplasmic ratio (Fig. 2). AML-M2 myeloblasts were large in size, oval in shape and contained a moderate amount of granulophilic cytoplasm. In our study, we found a distinct heart shape nuclear morphology in some AML-M2 blast (Fig. 3). In AML-M3 some promyelocytes show auer rods in the form of a bundle and Phi body (Fig. 4). AML-M4 myeloblast and monoblast were medium in size, low N: C ratio and contain agranular cytoplasm (Fig. 5). AML-M5 myeloblasts were characterized by peculiar vacuolated cytoplasm and some cells with apoptotic bodies (Fig. 6). Some AML-M6 blast contains more than two nuclei (Fig. 7). AML-M7 megakaryoblast showed membrane budding (Fig. 8). The most of our morphological characteristics of different subclasses of AML, except for AML-M2 and AML-M7, confirmed the finding of Ladines *et al.* (2016) in distinct heart shape nuclear morphology of AML-M2 blast and membrane budding of AML-M7 megakaryoblast have not been reported yet and supposed to be a new morphological feature.

Conclusion

The diagnosis is the first and most important step in the treatment of any disease. The present study is an approach to increase understanding of the simple morphological diagnostic FAB classification of leukaemia in relation to haematological parameters. The FAB classification is replaced by WHO. Still, FAB classification is a simple and powerful diagnostic tool in developing countries like India. The present study revealed that CML was the most common type of leukaemia, followed by AML, ALL, and CLL. In Nagpur, the incidence of different types of leukaemia does not differ markedly with other metro cities of India. Most of the cases of Acute Leukaemia had severe anaemia and thrombocytopenia. The highest variation found in Total WBCs count of different types of leukaemia, particularly in different subtypes of AML. The study concluded that FAB classification is a good guide for clinicians and hematologists. It is not outdated but it does require continuous revalidation and other procedures for refine-

ment. Based on FAB morphology, a computer-aided method is required for the diagnosis of specific subclasses of leukaemia. It is also required to correlate this abnormal morphological feature with prognostic approaches.

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