Original Article

Pattern of hematological malignancies diagnosed by peripheral smear examination

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Abstract

Background: Leukemia is a malignant neoplasm of the hematopoietic stem cells. Examination of the peripheral blood smear is an inexpensive but powerful diagnostic tool in both children and adults suffering from leukemia because it provides rapid, reliable access to information about a variety of hematologic disorders.

Objectives: To study the various patterns of leukemia, clinicoepidemiological profile and hematological features of leukemia

Materials and Methods: This is a cross sectional study conducted in the Hematology section of Department of Pathology of a tertiary care hospital. This study included all consecutive cases of leukemia diagnosed by peripheral blood smear examination from 1st June 2013 to 30th May 2014. The demographic indices were noted in a proforma. Investigations including haemoglobin estimation, total leucocyte count and platelet count were done for the study of hematological features. The morphological sub-typing was done according to the FAB classification system for leukemia.

Results: Out of total 52 cases, majority of cases were of acute leukemia (65.38%), followed by chronic leukemia (26.92%) and lymphoma spill/ acute leukemia (7.69%). The age range was 2 to 90 years. Mean age was 37.6 year. Majority were male. Mean hemoglobin count for AML and ALL was 6.8 and 5.3 gm/dl respectively.

Conclusion: The finding of this study reflects the pattern of leukemia at BPKIHS. Majority of acute leukemia constituted of acute myeloid leukemia (36.53%) cases and majority of chronic leukemia constituted of chronic myeloid leukemia (17.30%) cases.

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Introduction

Hematological malignancy (leukemia) is a malignant neoplasm of the hematopoietic stem cells characterized by diffuse replacement of the bone marrow and/or peripheral blood by neoplastic cells. It was identified as a separate malignancy in 1889.[1]

Leukemia is part of a broader group of neoplasms which affect the blood, bone marrow and lymphoid system, known as tumors of the hematopoietic and lymphoid tissues.[2,3]

Examination of the peripheral blood smear is an inexpensive but powerful diagnostic tool in both children and adults suffering from Leukemia. It provides rapid, reliable access to information about a variety of hematologic disorders.[4] The role of the blood smear in the diagnosis of leukemia and lymphoma is to suggest a likely diagnosis or range of diagnoses, to indicate which additional tests should be performed and to provide a morphologic context without which immune-phenotyping and other sophisticated investigations cannot be interpreted.[4]

Peripheral blood analysis by complete blood count and thin smear analysis are first steps to detect most hematologic malignancies which have emerged as a major cause of morbidity and mortality.[4]

The diagnosis involves a multiparameter approach including morphologic examination and phenotypic or genotypic studies.[5] However; the smear offers a window into the functional status of the bone marrow, the factory producing all blood elements. Review of the smear is an important adjunct to other clinical data. In some cases, the peripheral smear alone is sufficient to establish a diagnosis.[4]

This study has been done to find out the pattern of leukemia, its clinic-epidemiological profile and hematological features.

Materials and Methods

This study was conducted in the Hematology section of Department of Pathology. The study period was of one year. Ethical clearance was obtained from the institutional ethical review board. This study included all consecutive cases of Leukemia diagnosed during a study period by peripheral blood smear examination.

The haematological malignancies diagnosed from 1st June 2013 to 30th May 2014 were included. The demographic indices and the clinical details provided by the various departments were noted in a proforma. Investigation in all cases of leukemia including haemoglobin estimation, total leucocyte count and platelet count were done. After staining at least 2 well made smears by Jenner's Giemsa stain, the peripheral blood smears were analyzed by the Pathologists. When peripheral smear is not sufficient for the diagnosis, a cytochemical stains were performed. Peripheral smears were analyzed considering the type of leukemia, age, sex. The morphological sub-typing

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was done according to the FAB classification system for leukemia using morphologic and cytochemical criteria to characterize the blast cells, wherever possible.

The entire samples positive for malignancy in peripheral smears were included in the study till the total sample size is achieved.

All collected data were entered in Microsoft Excel 2010 spread sheet and converted into SPSS (Statistical Package for Social Sciences) version 17 program for statistical analysis. For descriptive statistical analysis; mean, standard deviation, proportion, percentage, median inter quartile range were calculated and tabular and graphical presentation were made.

Results

Out of total 52 cases (as shown in Table 1), 34 (65.38%) cases were of acute leukemia (AL) and 14 (26.92%) cases were of chronic leukemia (CL). Among total cases of acute leukemia, acute myeloid leukemia (AML) was found to be the frequently diagnosed AL comprising of 19 (36.53%) cases followed by 11 (21.15%) cases of AL only because of morphologic overlap and 4 (7.69%) cases of acute lymphoblastic leukemia (ALL). Among total cases of CL, chronic myeloid leukemia (CML) was the commonest type of CL comprised of 9 (17.30%) cases followed by 4 (7.69%) cases of chronic lymphocytic leukemia/ prolymphocytic

leukemia (CLL/PLL) and only 1 (1.92%) case of chronic lymphocytic leukemia (CLL). Rest 4 (7.69%) cases of acute leukemia/ lymphoma spill (AL/LS) could not be further categorized due to morphological overlap and non specific staining pattern of cytochemical stains. Biopsy of lymph nodes and bone marrow aspiration was advised by our side to confirm the diagnosis but patient lost for follow up.

Peripheral smear (PS) examination of AML revealed more than 20% myeloblasts having enlarged nuclei, opened up chromatin, irregular nuclear membrane and 2-3 prominent nucleoli (Figure 1). Some of them contain auer rod and faggots as well.

Table 1. Hematological pattern of leukemia at BPKIHS

Type of Leukemia	Total number	Percentage
Acute Leukemia		
AML	19	36.53
AL	11	21.15
ALL	4	7.69
TOTAL	**34**	**65.38**
Chronic Leukemia		
CML	9	17.30
CLL/PLL	4	7.69
CLL	1	1.92
TOTAL	**14**	**26.92**
AL/LS	**4**	**7.69**
TOTAL	**52**	**100**

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Diagnosis of acute leukemia only could be possible in 11 cases because of non specific staining pattern of cytochemical stains and patient immediately being referred to cancer hospital. PS showed blasts having morphologic overlap between AML and ALL. Based only on morphology of blast cells diagnosis of acute leukemia was made. PS of CML revealed leucocytosis with left shift of white blood cells (WBC) along with
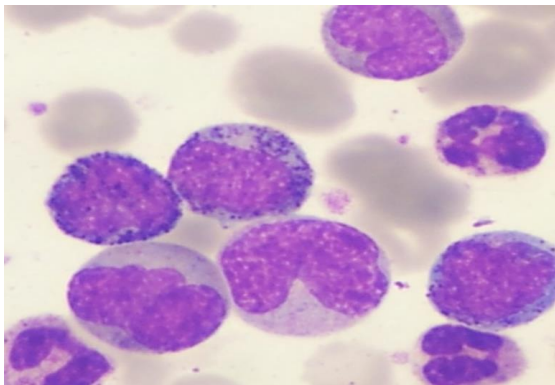
presence of blasts and basophilia (Figure 2). PS of ALL revealed more than 20% of lymphoblasts with condensed nuclei, inconspicuous to single nucleoli, irregular nuclear membrane and scant amount of cytoplasm (Figure 3). PS of CLL/PLL showed mature appearing lymphocytes and few larger cells having central prominent nucleoli and scant amount of basophilic cytoplasm (Figure 4)

Figure 1: AML revealing myeloblasts having enlarged nuclei, opened up chromatin, irregular nuclear membrane and 2-3 prominent nucleoli (JG, 100X)
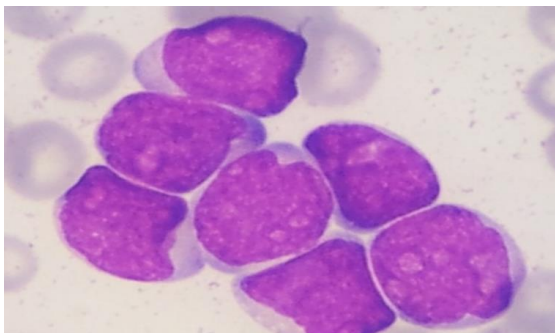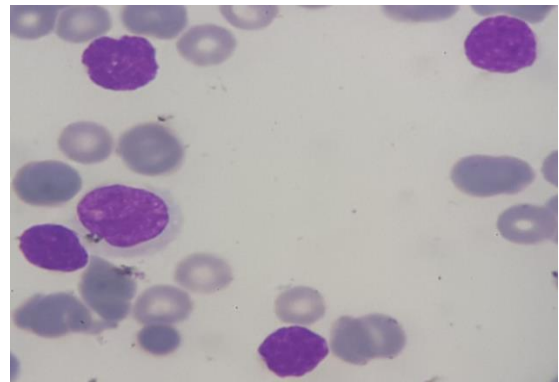

Figure 3: ALL revealing lymphoblasts with condensed chromatin, inconspicuous to single nucleoli, irregular nuclear membrane and scant amount of cytoplasm (JG, 100X)

Figure 2: CML revealing leucocytosis and left shift of WBC along with presence of blasts and basophilia (JG, 100X)
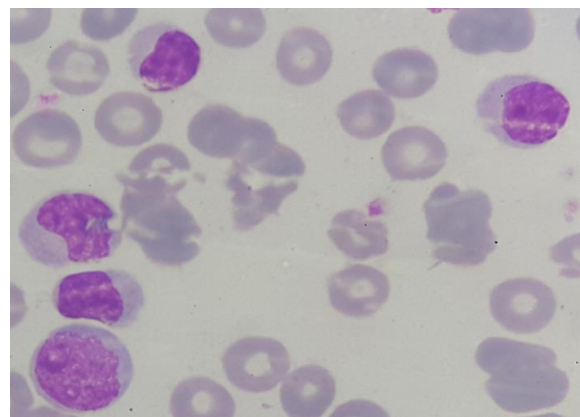

Figure 4: CLL/PLL revealing mature appearing lymphocytes and few larger cells having central prominent nucleoli and scant amount of basophilic cytoplasm (shown by arrow) (JG, 100X)

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Overall the age range for all the hematological malignancies was from 2-90 years. Mean age was 37.6 year. Majority of Leukemia were seen in two peaks. First peak was from 1-10 years of age and 2nd peak was from 31-40 years of age. Out of total cases, 54% were males and 46% were females. Fever (83%), weakness (83%) and pallor (63%) were the most frequently observed clinical features. Organomegaly was found in 40.4% of cases. Out of 40.4% of organomegaly, liver and spleen was found to be the predominant organ to be enlarged. Hemoglobin count range from 2.4 to 11.0 gm/dl and the mean was 7.1 gm/dl. Total leucocyte count ranged from 2000 to 128000/mm^3 and the mean was 31,054/mm^3. Platelet count ranged from 10000 to 4000000/mcl and the mean was 1, 50,326 /mcl.

Hematological features and age group distribution of AML, ALL, acute leukemia, CML and CLL/PLL are shown in table 2.

Table 2. Hematological features and age group distribution in AML, ALL, acute leukemia, CML and CLL/PLL

Type of leukemia	Values	Age (in year)	HB (gm/dl)	TLC (/ mm^3)	Platelets (/mcl)
AML	Min	6.5	4	2000	10000
	Max	64.0	10	44000	236000
	Mean	30.5	6.85	22178.95	54315.79
ALL	Min	3	2.6	10000	10000
	Max	4	10.0	46000	140000
	Mean	3.5	5.3	27500.00	59500
Acute leukemia	Min	2	2.4	2000	12000
	Max	85	11.0	93600	154000
	Mean	36.45	6.555	31296.36	63000
CML	Min	32	6	19000	20000
	Max	70	11	128000	40000
	Mean	49.78	8.844	62777.78	146222.22
CLL/PLL	Min	61	9	10000	60000
	Max	75	11	37000	150000
	Mean				119200

Hb: hemoglobin; TLC: total leucocyte count; Min: minimum; Max: maximum

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Discussion

Laboratory diagnosis of acute leukemia in modern hematology practice is increasingly relying on guidelines that require the availability of relatively expensive machines.[5] Therefore, where these expensive machines are not available, the laboratory diagnosis would mostly depend on more basic laboratory techniques that should at least include complete blood count (CBC) and peripheral blood morphology, followed by cytochemistry.[5,6]

For the diagnosis of AML/ALL, FAB classification of acute leukemia should be applied in under-resourced laboratories where the only available routine techniques for diagnosis are morphology and cytochemical stains.[5,6]

In the WHO classification of acute leukemia, the diagnosis is based on an arbitrary cut-off point of 20% blasts as a percentage of bone marrow total or non-erythroid cells or as a percentage of peripheral blood cells.[7] This exact percent is also applied nowadays in under-resourced laboratories where the FAB classification is used.[8]

This 20% blasts cut-off point seems to be universally accepted and for the time being, it represents the best known tool for defining acute leukemia.[9]

CML is often suspected on the basis of a complete blood count which shows increased granulocytes of all types, typically including mature myeloid cells. Basophils and eosinophils are almost universally increased.[10]

The diagnosis of CML is basically based on the pathologic findings of peripheral blood and Philadelphia chromosome in bone marrow cells.[11]

CLL is usually first suspected by the presence of a lymphocytosis, an increase in one type of white blood cell, on a complete blood count (CBC) test. This feature is an incidental finding on a routine outpatient department visit. Most often the lymphocyte count is greater than 4000 cells per microliter of blood, but can be much higher. The presence of a lymphocytosis in an elderly individual should raise strong suspicion for CLL.[12,13]

This study includes those cases which were diagnosed on the basis of peripheral smear.

Patterns of leukemia

In this study, out of total 52 cases studied, based on morphology and cytochemistry, acute leukemia comprised of 34 cases and 14 cases were of chronic leukemia. Diagnosis of AML and ALL was made in 19 and 4 cases respectively. Diagnosis of CML and CLL/PLL could be possible in 9 and 4 cases respectively. Only acute leukemia (AL) was made in 11 cases. A study conducted by Ghartimagar et al among 123 cases of leukemia in a span of 11 years, 96 cases were acute leukemia

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which included 80 cases of AML and 16 cases of All, 27 were diagnosed as CML and 7 cases of CLL.[14] Another study conducted by Weldetsadik AT et al for 4 years; out of total 67 patients, CML comprised of 17/67, 13/67 CLL and 15/67 AML.[15]

Of the 198 cases diagnosed in five years by Kulshrestha R at this hospital in 2003, 121 cases were of acute leukemia and 75 of chronic leukemia. CML constituted the single largest group comprising of 69/198 followed by AML constituting 56/198 cases.[16]

Similarly, another study conducted by Idrish M et al where 60 patients with hematological malignancies were studied, showed that about 35.9% had AML, while 19.15% patients had ALL. Non Hodgkin's lymphoma was seen in 15.39% cases. Among chronic leukemia, CLL outnumbered CML.[17]

Pattern of hematological malignancies has been compared with various studies within Nepal and across the globe (Table 3)

Table 3. Patterns of hematological malignancy in Nepal and across the globe

Country Duration (D)	AML (%)	CML (%)	ALL (%)	CLL (%)
Current study, Nepal D: 1 year	36.53	17.30	7.69	1.92 CLL/PLL:7.69
Kulshrestha R et al. Nepal[16] (BPKIHS, 2003) D: 6 years	28.57	35.20	19.90	3.06
Hamal P, Nepal[22] (TUTH, 1993) D: 8 years	33	29.5	55.5	0
D' Costa G et al. India[18] (1989) D: 10 years	21.9	38.4	35.95	2.89
Rani S et al. India[23] (1982) D: 10 years	29.7	45.4	19.3	5.71
Hassan K. Pakistan[24] (1997) D: 8 years	AL- 62.8	CL- 37.2	-	-
Al-Bahar S et al. Kuwait[25] (1994) D: 10 years	32.4	14.8	44.2	8.6
Khan MQ et al. Riyadh[26] (1991) D: 1 year	37.54	19.11	24.23	18.77
William CK et al. Africa[27] (1984) D: 4 years	28.7	30.13	29.42	21.23
Hansen NE et al. Denmark[28] (1983) D:34 years	AL- 40	20		40
Kwiatkowski A, Poland[29] (1994) D:23 years	AL- 50	15	UL- 10	25

UL: undifferentiated leukemia; AL: acute leukemia; CL: chronic leukemia

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Sub-typing of Acute Myeloid Leukemia

Sub-typing of AML could be possible in only 8/19 cases in this study. Out of 8 cases, 5 were sub-typed as AML, M2 and 3 were sub-typed as AML, M3. According to the FAB classification of AML, there were 11/52 cases which could not be categorized as AML/ALL and was diagnosed as Acute Leukemia only, this could be due to non specific staining pattern of cytochemical stain and morphological overlap between AML/ALL.

Among FAB subtypes of AML; the commonest was M2, followed by M1, M4 and M3 in the study conducted by Ghartimagar D et al.[14] The other study conducted by D' Costa G et al. also showed M1 and M2 to be comprised of maximum cases, followed by M3 and M4.[18]

Age range in all types of leukemia

The overall age range for all the hematological malignancy was from 2-90 years and mean age was 37.6 year in this study. Similarly in the study done by Kulshrestha R et al., the age range was from 11 day old to 81 years old.[16] In the study done by Weldestsadik AT et al., the mean age for the same was 42 years.[15] Similarly, in the study conducted by D' Costa G et al., the youngest patient of the series was three weeks aged female and

the oldest patient was eighty years old.[18] These findings are consistent with our study.

Male: Female ratio in all types of leukemia

In this study, 54% were males and 46% were females. Similarly in the study conducted by Kulshrestha R et al., male preponderance was observed, with M: F ratio of 1.8:1.[16] Males were affected more by leukemia than females in a ratio of 1.2:1.[15] Male to female ratio was 1.4:1 in the study conducted by Idrish M et al.[17] In the study by D'costa G et al. also, the overall M: F ratio was 2.7:1.[18] These findings are consistent with our study.

Age range and mean age of Acute Myeloid Leukemia

The incidence of AML increases with age; the median age at diagnosis is 63 years. AML accounts for about 90% of all acute leukemias in adults, but is rare in children. AML is slightly more common in men, with a male: female ratio of 1.3:1.[15] The age of patients with AML ranged from 2-82 years with a mean of 38 years in a study conducted by Ghartimagar D et al.[14] This finding is consistent with our study.

Hematological features of acute leukemia

In this study, hemoglobin (Hb) count ranged from 2.4gm/dl to 11gm/dl, TLC ranged from 2000 to 93600/mm^3 and platelet count ranged from 10000 to

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236000/mcl in case of acute leukemia. In the study by D' Costa G, Hb < 5 gm/dl was found in 25% cases, while others had Hb 5-10 gm/dl. The incidence of moderate and severe anemia was equal in ALL and AML.[18]

AML patients (n= 80) showed a wide variation in Hb, TLC and platelets. 69 patients had anemia (Hb< 10 gm/dl) and 65 patients had low platelets (< 10^4/cmm). Interestingly, 48 patients had normal or low TLC.[15]

Age distribution of CML, phases and its hematological features

CML is often divided into three phases based on clinical characteristics and laboratory findings.[18] In this study, out of 9/52 CML cases, 6 were in chronic phase, 2 in accelerated phase and single case in blast crisis. In the study by Ghartimagar D et al, out of 20 CML cases, 16 cases were in chronic phase, 4 in accelerated phase and they did not encounter any case in blast crisis.[14]

Various studies showed that CML is common in males than in females.[15,16,18] In the study done by Provan D et al., the male to female ratio was 1.4:1 and appears more common in the elderly with a median age at diagnosis of 65 years.[19] However, in the study done by D' Costa G et al. the oldest patient diagnosed with CML was 80 years old.[18]

Mean age of CML in this study was 49 years and male predominance was seen, as observed in other studies.[14-16,18]

Organomegaly was the most common presenting clinical feature. Most patients are diagnosed during the chronic phase which is most often asymptomatic. These findings are consistent with the study done by Besa EC et al.[20]

Mean hemoglobin, TLC and platelet count was found to be 8.8 gm/dl, 62,777 /mm^3 and 1,46,222/mcl respectively in this study in a case of CML. Similarly, the total count of more than 1,00,000/mm^3 was a more frequent finding in CML in the study done by D' Costa G.[18]

All patients with CML had high WBC and majority had anemia and high platelets in one more study.[14]

Chronic Lymphocytic Leukemia

CLL is a disease of adults. Most (> 75%) people newly diagnosed with CLL are over the age of 50, and the majority are men.[12,13] This finding is similar to the finding seen in our study. However, in rare cases, it can occur in teenagers and occasionally in children (inherited). Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count, but, as it advances, CLL results in swollen lymph nodes, enlarged spleen, and liver, and eventually anemia and

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infections.[12,13,21] In this study also, the most common clinical manifestation was lymphadenopathy.

In this study; most of the cases were clinically diagnosed as anemia, fever, organomegaly and pancytopenia. However, only 13 cases were clinically diagnosed as hematological malignancy and in 8 cases, clinical diagnosis was not provided.

Thus, peripheral smear in under resourced laboratory like ours has a significant role in the diagnosis of hematological malignancies even when clinically the diagnosis could not be made.

Conclusion

This is a small study conducted on a hospital based sample at BPKIHS, a tertiary level hospital. All 52 hematological malignancies were diagnosed with the help of peripheral smear examination during a period of one year and acute leukemias were confirmed with cytochemistry.

AL comprises of 65.38% whereas CL comprises of 26.92% and 7.69% cases were of LS/AL. AML was the commonest type of hematological malignancy among all ALs and CML among all CLs.

Overall, the age range for all the hematological malignancies was from 2-90 years. Mean age was 37.6 year. Out of total cases, 54% were males and 46% were

females. Hemoglobin count ranged from 2.4 to 11.0 gm/dl and the mean was 7.1 gm/dl. Total leucocyte count range from 2000 to 1,28,000/mm^3 and the mean was 31,054 /mm^3. Platelet count range from 10,000/mcl to 40,00000/mcl and the mean was 1,50,326/mcl.

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