



Morphological study of leukemia and its correlation with Siemens Advia 2120i cytogram and Immunophenotyping

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Abstract

Introduction: Morphological evaluation of peripheral blood (PB) and bone marrow (BM) cells through microscopic examination of properly stained smears remains crucial in haematological diagnosis of leukemias. Morphology is crucial for the diagnosis and follow-up particularly of myeloid neoplasms, and above all myelodysplastic syndromes: therefore the microscope still remains a very important tool in the integrated diagnostic process of haematological diseases.

The objectives of the present study were morphological diagnosis of leukemias and correlate them with data of Siemens autoanalyser and immunophenotyping of leukemia patients.

Material and Methods: The present study was done in the haematology, a section of central pathology laboratory, Department of Pathology of SMS Medical College, Jaipur. The investigations (full blood count with differential and morphological assessments) for each case and bone marrow examination and immunophenotyping data whenever possible were requested by the treating physician as part of the diagnostic work up.

Result: 192 blood and bone marrow aspirate samples from leukemic cases were studied in the Department of Pathology. Out of total 192 cases (100%) of leukemia, 86 cases (44.7%) were acute leukemia and 102 cases (53.1%) were chronic leukemia. 116 cases (60.4%) were male and 76 cases (39.5%). In present study the age wise distribution showed that ALL is more common in children whereas AML is more common in adult age group. The accuracy of the Advia cytograms to classify leukaemia was only 61.4 % and the accuracy of morphology was 79.6%.

Conclusion: CML (74.4%) was highest among all leukemia followed by AML. ALL was common in childhood as compared to AML which is more common in adults. Chronic leukemia were more prevalent in adult and more common in male compared to female. The analysis of WBC cytograms in conjunction with morphology can improve the overall assessment of leukaemic patient.

Keywords: acute leukemia, ALL, AML, CML, CLL.

Introduction

Leukaemia is characterized by the uncontrolled proliferation of haematopoietic cells and

displacement of normal precursors in the bone marrow. Leukaemia is the most common cause of childhood cancers representing approximately 19-

31% of all cancers worldwide. Despite advances in therapeutic technology, mortality associated with leukaemia remains high. The significant mortality rate has been attributed in part to delayed diagnosis which is the direct result of the lack of screening and diagnostic services in many areas in India⁽³⁾, in particular rural healthcare facilities. Early detection and classification of leukaemia allows the clinician to promptly initiate the most appropriate treatment plan, as the prompt initiation of chemotherapy has been shown to confer a better prognosis.

The Full Blood Count (FBC), differential count and review of peripheral blood smear usually are the first line investigations that alert the clinician to a possible underlying leukaemia, but further investigations are required to confirm the diagnosis and classify the leukaemia before appropriate therapy can be commenced. A quick and simple method that can be used to confirm and classify leukaemia could therefore be lifesaving. The aim of this study was to determine if the FBC, differential count and white blood cell (WBC) cytogram assessment from an automated haematology analyser was as reliable as morphological assessment in the classification of leukaemia when compared to flow cytometry

Material and Methods

The present study was done in the haematology, a section of central pathology Laboratory, Department of Pathology Of SMS medical college jaipur . Peripheral blood sample collected in an Etheylediamine tetra acetic acid (EDTA) anticoagulated tube and the routine FBC and differential count were analysed on the ADVIA 2120 (Siemens healthcare diagnostics, Illinois, USA). A printout of the result, including the WBC cytograms, for each case was collected. BONE MARROW smears are collected from the patients. Samples for flow cytometry were collected in lithium heparin tubes and submitted for analysis. The peripheral smears and bone marrow aspiration were stained by Leishman's stain and examined using oil immersion lens a light

microscope (Nikon, Tokyo, Japan) was used to analyse the peripheral smears. The slides were stained with the May-Grünwald-Giemsa stain and a manual count was performed on 100 WBCs at the 50x magnification (38). If $\geq 20\%$ blasts were seen, a diagnosis of acute leukaemia was made. The absence of blasts or a blast count of less than 20% indicate chronic leukemia. . The morphology was then reviewed in conjunction with the Siemens advia cytograms and a provisional diagnosis made.

Advia WBC cytogram analysis The Advia 2120 has two independent channels which are used to generate a WBC and differential count using peroxidase and Baso reagents. The Basophil method generates the total WBC and the peroxidase method provides the differential count and a secondary WBC. Myeloperoxidase activity was assessed from the peroxidase chamber using the patterns described by G. Onofrio(32). Cells that scattered near the y-axis have no myeloperoxidase while cells that scattered further away from the y-axis have increasing myeloperoxidase activity. The results of the Advia peroxidase cytogram were compared to the myeloperoxidase result obtained by flow cytometry and/or cytochemical staining. The basophil cytogram was used to assess the pattern of the mononuclear cell cluster

The final diagnosis obtained from analysis of the WBC cytograms, morphology and the combination of both analyses were compared to flow cytometry.

Result

During the our study, 192 blood and bone marrow aspirate samples from leukemic cases were studied in the Department of Pathology. Out of total 192 cases (100%) of leukemia, 86 cases (44.7%) were acute leukemia and 102 cases (53.1%) were chronic leukemia. The observations are as follows:

Table -1 Age Wise Distribution of Leukemia

age	AML	ALL	CML	CLL	OTHERS	TOTAL	percentage
0-10	0	4				4	2.1
11-20	4	10	2	0	2	18	9.3
21-30	6	2	2			10	5.2
31-40	12	0	24	0		36	18.7
41-50	14	4	18	10		46	23.9
51-60	18	0	22	10	2	52	27
61-70	10	0	4	8		22	11.4
71-80				2		2	1.1
TOTAL	64	22	72	30	4	192	100

In present study (2014) total 192 cases (100%) of leukemia were studied, 116 cases (60.4%) were male and 76 cases (39.5%) were female. Male – female ratio was 1.52:1. Out of 86 cases of acute leukemia, 64 cases (74.4%) were Acute Myeloid Leukemia (AML) and 22 cases (25.6%) were Acute Lymphoblastic Leukemia (ALL). Amongst AML group, 42 patients were males (65.6%) and 22 patients were females (34.4%) while in ALL group 14 cases (67.57%) were male and 08 cases (32.43%) were female

In present study the age wise distribution revealed that 04 cases(2.1 %) belonged to 0-10 years of age group followed by 18 cases (9.3%)

which comprising highest number of cases belonged to 11-20 years of age group, 10 cases (5.2%) belonged to 21-30 years of age group, 36 cases (18.7 %) belonged to 31-40 and 46 cases (23.9%) belonged to 41-50 years of age group. and 52 cases (27%) belonged to 51-60 years of age group which comprising of highest number of cases. While 22 cases (11.4%) were of 61-70 years of age group and only 02 cases (1.1%) were of 71-80 years of age group.

In present study the age wise distribution showed that ALL is more common in children whereas AML is more common in adult age group.

Table -2 Sex Wise Distribution of Leukemia

SEX	AML	ALL	CML	CLL	OTHERS	TOTAL
MALE	42	14	40	20	0	116
FEMALE	22	8	32	10	4	76
total	64	22	72	30	4	192

Table-3 Comparison of morphological assessment , Siemens Advia 2120 cytogram and final diagnosis by immunophenotypic analysis

TYPE OF LEUKEMIA	TOTAL	MORPHOLOGY	SIEMENS ADVIA
AML	64	42(65.6)	36(56.2)
ALL	22	16(57.1)	12(54.4)
CML	72	68(94.4)	56(77.7)
CLL	30	26(86.6)	14(46.6)
OTHERS	04	1(25)	0(0)
total	192	153(79.6)	118(61.4)

The accuracy of the Advia cytograms to classify leukaemia was only 61.4 % and the accuracy of morphology was 79.6%. The accuracy of the Advia cytograms to diagnose AML and ALL leukaemia was only 56.2 and 54.4 % and the accuracy of morphology was 65.6 % and 57.1 % respectively. The accuracy of the Advia cytograms to diagnose CML and CLL leukaemia was only 77.7 and 46.6 % and the accuracy of

morphology was 94.4 % and 86.6 % respectively. Accuracy of Siemens advia cytogram was higher in chronic leukemia (68.6) as compared to acute leukemia (55.8)

Discussion

Present study was conducted Department of Pathology and total 192 leukemias had been studied. Present study showed that prevalence of

CML (74.4%) was highest among all leukemia followed by AML. Among acute leukemia prevalence of AML (74.4%) was higher as compared to that of ALL (23.6%). In case of chronic leukemia prevalence of CML was higher than CLL

These finding was in concordance with the studies carried out by Vasavada et al.⁽¹⁾, Kushawaha et al⁽²⁾, Chatterji et al.⁽³⁾, Rani S et al.⁽⁴⁾, Shome et al⁽⁵⁾ and Rathee et al⁽⁶⁾.

Acute Leukemias were mainly classified into AML and ALL, on the basis of clinical course of disease, laboratory data by autoanalyser Siemens avia 2120 and morphological features of leukemic cells. Of total 86 cases (100%) of acute leukemia, 64 cases (74.4%) were AML and 22 cases (24.6%) were ALL. 102 CASE OF CHRONIC leukemia diagnosed ,72 cases were CML(70.5%) and 30 cases were CLL(29.5%).

In our study males were more affected with leukaemia than females, and this finding concurs with the reported increased incidence of leukaemia in males⁽⁷⁾. The majority of the samples In Our Study were from the adult population and possible reasons for this finding include transformation to an acute leukaemia from underlying chronic leukaemia, concomitant HIV infection, increased chronic leukaemias in adults and increasing genetic mutations with increasing age.

In present study, it was found that acute leukaemias were more common in male (65.11%) as compared to female (34.89%), male to female ratio was 1.76:1. In ALL, male to female ratio was 1.75:1 and in AML, male to female ratio was 1.9:1. These findings were comparable to the studies conducted by Gupta Rajat et al (2015)⁽⁷⁾. they reported male to female ratio - 1.94:1, 2.05:1 and 1.82:1 in acute leukemia, ALL and in AML respectively in their study.

In Present study, AML was more prevalent and 46 cases (62.14%) of AML out of total 83 cases of acute leukemia had been found. Findings of present study is similar to that reported by Laishram R S et al (2011)(8) as 61.36%.

In present study (2014), on age wise distribution of leukaemia patients it was found that the majority of patient (45.4 %) of ALL were belonged to age group of 11-20 years followed by (18.1%) cases in age group of 0-10 years. Findings of our study are not exactly comparable to study conducted by Gupta Rajat et al (2015)⁽⁷⁾. They reported majority of cases (35%) in age group of 0 -10 years, followed by (21%) cases in age group of 11-20 years and (19%) cases in age group of 21-30 years. In both studies (present study and Gupta Rajat et al), it was found that acute Lymphoblastic leukemia is more common in patients less than 30 years of age.

The accuracy of the Advia cytograms to classify leukaemia was only 61.4% and the accuracy of morphology was 79.6%.accuracy was higher in cases of CML and CLL both for morphology and cytogram. In our study, the accuracy of Siemens advia 2120 was nearly similar to that obtained by Gibbs (77.8%)⁽⁹⁾ and G. Onofrio (91.1%)(10).in previous studies. Gibbs correctly classified 100% of the ALL and CLL cases on cytogram pattern recognition which improved his overall accuracy. The cases that were diagnosed on morphology alone included those with features in keeping with chronic myeloproliferative and lymphoproliferative disorders overall favouring CML and CLL respectively, APL, typical L3 morphology favouring a Non-Hodgkin's lymphoma, Auer rods present confirming the presence of myeloblasts in AML and a case with plasma cell leukaemia. However when the Advia printout was used in combination with the morphology findings, there was a significant improvement in being able to make a provisional diagnosis with 90% of cases being correctly diagnosed.

Conclusion

Present study showed that prevalence of CML (74.4%) was highest among all leukemia followed by AML In our study AML was more common as compared to ALL and acute leukaemia was more prevalent in males than in females and ALL was common in childhood as compared to AML which

is more common in adults .Chronic leukemia were more more prevalent in adult and more common in male compared to female

The timeous identification and classification of leukaemia is crucial for patient survival, and is essential to prompt further investigations to confirm the diagnosis and to initiate treatment as soon as possible. . In this study it has been shown that the analysis of WBC cytograms in conjunction with morphology can improve the overall assessment of leukaemic cases by providing a provisional classification to the clinicians. The findings suggest that in order to save time and reduce laboratory costs assessment of Siemens advia cytogram can be used as a screening tool

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