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Haematology

HEMATOLOGICAL EVALUATION OF ACUTE LEUKEMIAS IN A TERTIARY CARE HOSPITAL: A TWO YEAR CROSS - SECTIONAL STUDY

Dr Deep Jyoti Kalita MD Pathology Department of Laboratory Medicine, Health City Hospital,guwahati

Dr Sailendra Kumar Thakuria* MD Pathology,gnrc Good Health Hospital,guwahati,assam.*Corresponding Author

ABSTRACT **Objective:** Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) are clinically and biologically diverse hematological neoplasms. Current study objectives were to diagnose and classify acute leukemias based on morphology, immunophenotypic as well as cytogenetics findings according to WHO 2016 classification and also to study their hematological profiles. **Materials and Method:** This cross-sectional study included 45 patients diagnosed with Acute leukaemias. Diagnosis was based on peripheral blood smear findings, bone marrow aspiration findings supplemented by flowcytometry and cytogenetics and molecular studies. Results: Fourty five cases of Acute leukaemias were diagnosed in a period of 2 years, where 32 cases were AML and 13 cases were ALL .According to subclassification of 2016 ,13 cases were of AML with recurrent cytogenetic abnormalities,17cases were of AML NOS and 2 cases were of AML ,MRC. Normal karyotype was found in 28(62.2%) cases. In subcategorization of AML RCA,APML with PML RARA and t(8;21) RUNX1T1 were 4/16 each. There were 2 complex karyotype cases. In AML NOS subcategory AML M4 was more prevalent than other subtypes with 6/14 subtypes. In subcategory of ALL,B ALL was more common than T ALL. B ALL NOS was more common than B ALL with RGA. **Conclusion:** The application of WHO 2016 classification confers uniformity in reporting acute leukemia cases that aids in the treatment by using targeted therapies and helps in the prediction of prognosis. This classification is very objective, therapy oriented and the need of the hour.

KEYWORDS : Acute myeloid leukemia, WHO 2016 classification, ALL.

INTRODUCTION:

Malignant proliferation of hematopoetic cells i.e. leukemias constitute major portion of hematopoetic neoplasms worldwide. Leukemias are classified into myeloid and lymphoid subtypes. Typing of leukemia is essential for effective therapy because prognosis and survival rates are different for each type and sub type.

Acute myeloid leukemia (AML) and Acute lymphoblastic leukemia (ALL) are the two main types of Acute leukemias (AL) with a global prevalence of 3-4 and 0.4-2 per 1,00,000 individuals for AML and ALL respectively. [1,2,3] ALL is more prevalent in children and AML in adults.[4] The age-old FAB classification of these diseases had their own set of drawbacks as it was based on morphology alone and led to subjective errors [5]. This was eliminated by WHO classification system which was evolved to improve the objectivity and reproducibility by adding the cytogenetic, molecular, cytochemical and immunologic characteristics for an integral diagnosis [6,7]. This change has helped the pathologists in the determination of differential diagnosis as well as prognosis of leukemia, thus facilitates the targeted therapy as per their pathologic behavior. This includes management of recognizable genetic lesions, stem cell transplantation as well as immunotherapy such as treatment directed at specific cluster differentiation (CD) markers [8].

The aim of the present study was to diagnose and classify AL cases according to the WHO classification of tumours of hematopoetic and lymphoid tissues,2016,revised 4th edition and study their hematological profiles[6].

MATERIALS AND METHODS

This cross sectional, observational study was conducted at the department of Laboratory Medicine in a 170 bedded tertiary care super speciality hospital of North east India from November 2019 to October 2021.

All 45 patients diagnosed with AL (AML and ALL) as per the WHO classification of tumours of hematopoetic and lymphoid tissues 2016,revised 4th edition criteria ,irrespective of age and gender were recruited into the study . AL cases without immunophenotyping, cytogenetic studies or advanced ancillary techniques were excluded from the study.

Hematological investigation

This included estimation of hemoglobin(Hb) levels, total leukocyte count(TLC),differential count(DLC) and platelet count using Beckmen DXH 800 cell counter. Peripheral venous blood samples were collected in EDTA vacutainers. Peripheral smears were made, stained with Leishman stain and studied in details for morphology. A provisional diagnosis was made and ancillary studies were performed. Immunophenotyping and ancillary studies

All cases underwent flow cytometric analysis using BD FACS Canto 6 color flowcytometer ,molecular studies using RT PCR and cytogenetic studies using fluorescence in situ hybridization(FISH).

Bone Marrow Aspiration Study

This was performed on all patients under aseptic conditions and local infiltration anesthesia with 3 cc of 2% lignocaine. Bone marrow aspiration was performed from the posterior superior iliac spine using Salah's aspiration needle. Smears were prepared, air dried and stained with Giemsa stain. The smears were meticulously examined for cellularity, predominant series, myeloid:erythroid ratio, erythroid series cells, myeloid series, lymphocytes, plasma cells, megakaryocytes, blast cells, atypical cells and parasites. At least 500 cells were counted to obtain the myelogram.

Bone Marrow Trephine biopsy

It was obtained from the posterior iliac spine using Jamshidi needle under all aseptic conditions and local infiltration anesthesia with 2% lignocaine. An optimal 1-2 cm long biopsy sample was obtained in its maximum diameter ,placed in 10% formalin fixative, decalcified in EDTA solution, submitted for routine paraffin processing and stained with hematoxylin and eosin for further examination.

Statistical Analysis

The data was collected, compiled and analyzed using Graph pad prism and Microsoft excel sheets. Categorial variables were expressed in terms of frequencies and percentages.

Results

The present study includes 45 cases of AL, of which 32(71.1%) cases were diagnosed with AML and 13(28.8%) were diagnosed with ALL. Age of the individuals ranged from 6-78 years, the mean age of the included participants was 45.39 years with a slight male preponderance(1.1:1). The average age for AML was 57.08+/-18.06 years and 12.22+/-6.74 years for the ALL cases. While AML was more common in male(19/32), ALL was more common in females(9/13). Among hematological characteristics, anemia was seen in all the cases. Thrombocytopenia was seen in 42/45(93.3%) cases. Similarly 43/45(95.5%) cases showed leukocytosis. All except one case showed a low neutrophil count with increased blast percentage(>20%). Average blast percentage was 68.9%. One case was diagnosed as

aleukemic leukemia with 5% blasts in peripheral blood with 22% blasts in bone marrow by morphology.

Table 1 shows percentage of acute leukemias according to WHO 2016 classification.

Table 2 shows hematological parameters of various leukemias.
Table 3 shows frequency distribution of leukemias according to prognosis.

Flowcytometry: It was done on all 45 cases . The following antigens were the most commonly expressed:

Myeloid markers CD 13, CD 33, CD 14, CD 64 and MPO were expressed in majority of the cases. These were followed by HLA DR and CD34. Aberrant expression of CD 7 which is a T cell marker was found in one case of AML NOS.

Lymphoid markers:CD19,CD 20 and Common ALL antigen CD10(5/10 B ALL) were the most common lymphoid markers. T ALL expressed cyt CD 3 ,CD5,CD7 markers of T lymphoid lineage. One case of Megakaryoblastic leukemia expressed CD41/42 which are of megakaryoblastic lineage.

Discussion:

The present study was conducted to diagnose and classify AL according to WHO 2016 classification. 45 cases were studied over a period of 2 years. AML(71.1%) was more common than ALL(29.8%). Similar findings were found in another Indian study by Patel GN [9] et al who found AML in 63.24% cases and 36.76% ALL cases. Similar data were obtained from other worldwide studies too[10,11]. ALL was more common in children(8/13) and AML in adults(27/32). This was in concordance with the findings of Dores et al., and others[6, 12-17]. There was increased number of peripheral blasts (77.8% in ALL and 60.18% in AML). Findings were similar to findings of Kumar et al., (85% in ALL) and Ghosh et al., (57.6 in AML) [8].

Acute lymphoblastic leukemia

B cell ALL (B -ALL) was found to be more common(77%) than T-ALL (23%). Kumar et al. found similar findings with 63% of B –ALL [11]. Among B –ALL, the most common immune phenotyping markers were CD 10 and CD19. This was similar to the finding of Patel et al. 2 out of 13(15%) ALL cases showed the presence of a cytogenetic abnormality of TEL AML1 (ETV6 RUNX1). CSF involvement was found in 5/13 cases of ALL. One case of Adult B -ALL showed presence of BCR-ABL fusion with presence of Major fusion protein(210kda).This patient showed presence of basophilia and myelocyte bulge like in Chronic myeloid leukemia. The patient was started on Tyrosine kinase inhibitor Imatinib along with standard ALL treatment. Despite this mortality was recorded within a month. Rest all 7 cases belonged to B-ALL NOS category .There were 3 cases of T-ALL. Immunophenotyping showed positivity for CD3, CD5 and CD7 in these cases. Blasts showed bright CD 45 positivity, were myeloperoxidase negative. Blasts percentage was as high as 90%. Cells were medium to large cells, having high N: C ratio, clumped chromatin with 1-2 prominent nucleoli. Prognosis of T-ALL was poor and patient died within 2 months. Similar findings were found in studies of Patel GN et al. and others.[17].

Acute myeloid leukemia

In our present study AML NOS (53.12%) was more common than AML RCA(40.6%). This finding was similar with findings of Patel GN et al., who found AML NOS to be more common[17]. Rahul R et al did a 3 years study in Ahmedabad, Gujarat and also found 39.6% cases with cytogenetical abnormalities[18].This was in contrast to the findings of Nunes et al who found AML-RGA to be more prevalent[19].

Acute promyelocytic leukemia which is now categorized as APL with PML RARA under AML RGA was found in 4/13 case of AML RGA. Prognostication was done as per Sanz criteria. 2 out of 4 cases died within a period of 7 days even after starting ATRA based therapy. All 2 cases fell into high risk category as per Sanz criteria[20]. ATRA syndrome was confirmed by radiological signs in all of them.

AML RGA with t (8; 21) (RUN X1-RUNX1T1) was also seen in 4 out of 13cases with RGA. In FAB classification these cases were categorized in M2 with basophilic cytoplasm in blasts and abundant azurophilic granules. Categorically they fell in the favourable category of classification. One rare case of acute megakaryoblastic leukemia (M7) was diagnosed. The child presented as pyrexia of unknown origin and only 2-3% blasts were found in the peripheral blood film. Bone marrow was difficult to aspirate and hemodiluted aspirate showed presence of dog ear appearance of megakaryoblasts. Diagnosis was confirmed by flowcytometry. 1 case had a karyotype of t (6; 9),however basophilia was not seen as described by other authors in this category. Prognosis was bad and patient didn't respond to standard dose of 3+7 regimen.

Among AML NOS M5 was more common(6/17) .This was in contrast to studies of Faleh AA et al (M1) and Bashrat M and Ghosh S et al(M2)[21,22,14].

Chromosomal abnormalities were found in 40% cases which was similar to study by Rahul R et al at Gujarat cancer centre. 4 cases each of t(15;17) and t(8;21) were found . This finding(30.7%) was higher than other Indian studies of Rahul R et al who found 13.27% cases with t(15;17). Whereas Faleh et al(7%) and Cheng Y(14.3) et al also reported much lesser prevalence of t(15;17) than our study[18,21,23].

Complex karyotype was found in 2 AML cases, one of them was categorised as AML, with MDS related changes. Karyotype of patient was 50,XX,+4,del(5),+8,del(9),del (13)(18-21).

Conclusion: In our study we found that WHO classification 2016 can be practically used for diagnosing various types of acute leukemia. Uniformity in reporting of acute leukemia cases aids in treatment by using targeted therapy and helps in prediction of prognosis. Categorization into favorable and unfavorable prognosis groups tells us about the future outcome of cases and findings of this study show that the unfavorable prognosis group has a dismal prognosis. Cytogenetics is one of the important diagnostics parameters. The cytogenetic based classification system permits the use of immunotherapy such as specific targeting of CD expression especially in patients failing induction therapies. Lately monoclonal antibody therapy has become a significant component of the treatment protocol, for example the use of Gemtuzumab for CD 33 positive AML.

This study has its limitations in being a single centre, cross-sectional study with limited sample size. Multicentric, prospective studies with larger sample size and longer follow up periods are encouraged to validate the results.

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Table 1: Percentagewise distribution of different types of acute leukemias

SL. No	Hematologicalparameters	AML	ALL
1	Hemoglobin (gm/dL	8.22+-2.17	7.72+-2.20
2	Total leucocyte count (/mm3)	66,333+-68,291	65,506+-62,055
3	Blast (%)	60.18+-27.28	77.8+-16.06
4	Platelet count (mm3)	43,300+-34,243	62,500+-63,665
5	Neutrophils (%)	15.25+-13.26	12.2+-11.76
6	Lymphocytes (%)	11.85+-14.86	12+-15.76

Table 2: Various hematological parameters with their mean values in cases of AML, ALL and AL

Types of acute leukemia	No. of cases	Percentage (%)
Acute lymphoblastic leukaemia(ALL)	13	28.8
1) T-ALL	3	23
1a) T-ALL Not otherwise specified	3	
1b) T-ALL NK		
2) B-ALL	10	77
2a) B-ALL Not otherwise specified	7	
2b) B-ALL Recurrent genetic abnormalities	3	
i)B-ALL with t(12;21) (p13;q22)-TEL AML1(ETV6 RUNX1)	2	

ii)B-ALL with t(9;22)(q34;q11.2)-BCR ABL1	1	
Acute myeloid leukemia (AML)	32	71.1
1.AML with recurrent genetic abnormalities	13	40.62
1a) AML with t(8;21)(q22;q22.1) RUNX1 RUNX1T1	4	
1b) APL with PML RARA	4	
1c) AML with mutated NPM1	1	
1d) AML with biallelic mutations of CEBPA	3	
1e) AML with t(6;9)	1	
2. AML-Myelodysplasia-related changes	2	6.25
3.AML-Not otherwise specified	17	53.12
3a)AML with minimal differentiation	1	
3 b) AML without maturation	3	
3 c) AML with maturation	4	
3d) Acute myelomonocytic leukemia	6	
3e) Acute monoblastic leukemia	2	
3 f) Pure erythroid leukaemia	0	
3g) Acute megakaryoblastic leukemia	1	

Table 3: Frequency distribution of acute leukemia cases according to prognosis

Type of Acute leukaemia	No of cases with favourable prognosis	No of cases with unfavourable prognosis.
ALL		
1) T ALL		
1 a) T-ALL Not otherwise specified		3
1 b) T-ALL NK		
2) B-ALL		
2a) B-ALL Not otherwise specified	2	5
2b) B-ALL Recurrent genetic abnormalities		
i)B-ALL with t(12;21)(p13;q22)-TEL AML1(ETV6 RUNX1)	1	1
ii)B-ALL with t(9;22)(q34;q11.2)-BCR ABL1		1
AML		
1) AML-Recurrent genetic abnormalities		
1a) AML with t(8;21)(q22;q22.1) RUNX1 RUNX1T1	4	
1b) APL with PML RARA	2	2
1c) AML with mutated NPM1	1	
1d) AML with biallelic mutations of CEBPA	2	1
1 e)AML with t(6;9)		1
2) AML-Myelodysplasia-related changes		2
3) AML-Not otherwise specified		
3a)AML with minimal differentiation		1
3b) AML without maturation	1	
3c) AML with maturation	2	1
3d) Acute myelomonocytic leukemia	5	1
3e) Acute monoblastic leukemia	2	
3 f) Pure erythroid leukemia	0	
3g) Acute megakaryoblastic leukemia		1

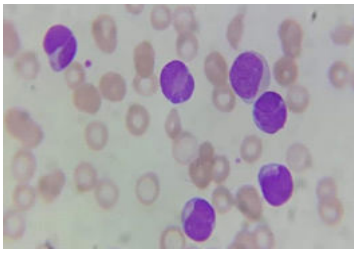

Fig 1: Myeloblasts in AML with cup like morphology, PBS 100x.

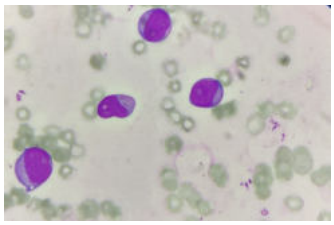

Fig 2: Auer rods in myeloblasts in a case of AML,PBS 100x.

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