

Cytochemical Study of Acute Leukemia in Special Relation to Children

Prasanta Kr. Baruah

Associate Professor of Pathology, Gauhati Medical College & Hospital, Guwahati, Assam, India and Deputy Director of Assam AIDS Control Society, Khanapara-22, Assam, India

Abstract: *Objectives:* study of childhood leukemia among the Hospital attending Patients in Gauhati Medical Colleges and Hospital. *Material and Method:* The study was conducted in Gauhati Medical College and Hospital, Guwahati, Hospital in General Medicine Deptt., Deptt. of Hematology from March (2002-Feb 2003). (as per Medical Registration Deptt.) As per record 2002 total Hospital attending cases was 2,65,972 nos. where Indoor admission was 41,448. Some of the Lab. works was done in the Deptt. Of Pathology Hospital Laboratory as well as College Hematology Lab. Total 70 No. of OPD patients were found with various groups of leukemia with various age groups. Patients were all age groups. Among them 50 was Male candidate where other 20 was females of different age groups. Out of 38 no. of were children and mostly 1st and 2nd decade of life (from 8 months to 13 yrs). Who were admitted in GMCH in the Department of Pediatrics, Hematology wards and General Medicine for Males and female wards respectively. *Results:* Total 70 no. of different types of leukemia admitted into the hospital within a period of 12 months were studied for hematological finding and cytochemical reactions were analyzed. All available and relevant literature on hematological finding on leukemia was reviewed. Cases were analyzed and studied according to Hayhoe et al 1964, Cawley and Hayhoe 1972. Acute leukemia was observed 46% while chronic leukemia was 24% respectively. *Conclusion:* Hematological cytochemistry should be routinely used as a reliable diagnostics aid for detection as well as classification and prognosis of different type of childhood leukemia.

Keywords: Leukemia, cytochemical stains, malignant neoplasm

1. Introduction

In the fascinating history of Pathology and its many magnificent personalities with their outstanding contribution in the very beginning to pay our obeisance to those great men who have laid foundation of our specialties.

Leukemia is Greek word...Leuk Leuc..Leuco..white. Haima-Blood. Bennet, Craigie and Virchow first described it probably one and half century before. Leukemia is a neoplastic proliferation haematopoietic tissue and most commonly form of childhood cancer. Through, thousands of Medical Literature available today leukemia remains a disease and unknown etiology and uncertain pathogenesis.

It is very difficult to identify the leukemic cells in conventional routine staining where the morphology of the blast cells are not very clear and sometimes few cases only and cannot be categorized to any particular type or form.

Hayhoe and Cawley, (1972), Flanssdrin and Barnard (1973); Beard and Hamilton Farily, 1979 had successfully explained the different types of acute leukemias in their research works. Sudan Black B or Peroxidase stains are essential to make a firm Schiff reaction employed to differentiate acute lymphoblastic leukemia with myeloblastic type and can be correlated between extend of PAS positivity and survival (Lawrie, 1968), frequent PAS positivity in blast cells being associated with better prognosis.

2. Discovery of Leukemia

Probably more than a century ago leukemia was discovered. In comparison of the other diseases it took a longer time to its own existence. Barth and Donne did examination leukemic blood in 1839. They noted the remarkable

predominance of mucous globules in the blood of a patient splenomegaly. But their observations were not published until 1844.

3. Leukemia Definition:

Till date nobody has yet been able to live a satisfactory definition of leukemia and different authors have given own definition at different times. In fact every definition has been becoming inadequate to cope with the fast moving development in this field. The nature of leukemic process is neoplastic in the sense that it is a purposeless, progressive, uncoordinated cellular proliferation, superfluous to the needs of the host (Scott, 1957 Mathe and Rappaport, 1975).

According to Boggs and Wintrobe (1974) leukemia can be defined as *"The disease of unknown etiology characterized by an uncontrolled, abnormal and wide spread proliferation of haemic precursor cells of the body which primarily affect the bone marrow, but secondly involved the peripheral blood and many tissues, particularly those of reticulo-endothelial system."* With rare exception, all types of leukemia are eventually fatal."

4. Material and Method

The present study was carried out in the department of pathology, Gauhati Medical College and Hospital (GMCH), Guwahati. The material for the study e.g. blood samples were collected from the patient attending in Medicine, Pediatrics, Hematology department of Gauhati Medical College and samples were examined in the pathology Lab. in the hospital and subsequently cytochemical studies were carried out in the special Hematology laboratory in the Gauhati Medical College & Hospital at hill top.

5. Design of the Study

The study was carried out as per detail pro forma of each individual case as described. Each individual cases were examined clinical Histories, physical examination, examination of peripheral blood smear, supplemented by bone marrow studies(whenever possible) in majority of cases of leukemia. Where the immature precursors were not beyond 30% in both peripheral blood and marrow leukemia was not declared.

The date of onset of the diseases was noted in acute leukemia's, whereas such attempts were not made in chronic leukemia, for the onset of the symptoms in and when he felt ill. In children the history was remembered from their parents and also questions relating to their mothers health during antenatal period was asked preferably to the mothers.

Hematological finding such as total leucocytes counts, estimation of hemoglobin percentage, total red cell count, direct comb's test and packed cell volume was estimated from oxalated blood.

Erythrocyte sedimentation rate was estimated from citrated venous blood and platelet count from the EDTA venous blood.

For differential leucocyte count and cytochemical staining were done in direct well spread, thin peripheral smears stained as guideline Dacie et al and examinations were done.

6. Methods

The total leucocyte count was estimated from oxalated venous blood by a visual method described by Dacie and Lewis (1975) and when WBC count was very high red blood corpuscles pipette was used with 1:200 dilution.

Haemoglobin level was estimated from oxalated venous blood by Acid-haematin method in Hellige's Haemometer(Dacie and Lewis,1975).RBC counts by Visual method described by Dacie and Lewis(1975).

Packed cell volume (PCV) was measured by macro method using Wintrobe tube or Wintrobe's method (Dacie and Lewis, 1975).

BONE MARROW EXAMINATION METHODOLOGY:

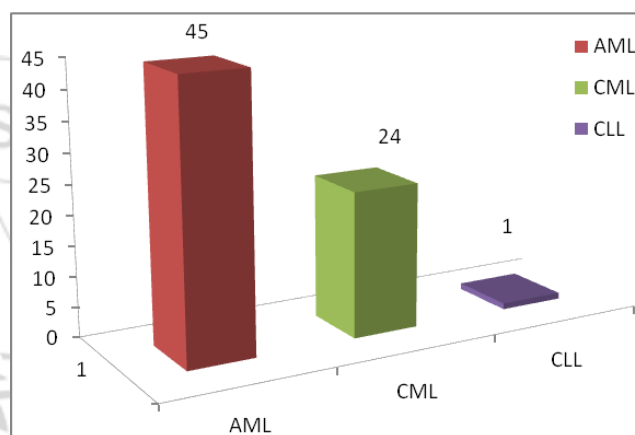
The bone marrow aspiration was done with Salah marrow puncture needle as described by Wintrobe (1947). Usually the site of puncture in order children and adult was the body of the sternum at the level of second intercostals space and in younger children or in patients having sternal pain, iliac crest puncture was done and the aspirated marrow was taken in a glass slides (previously cleaned) and allowed to settled for few seconds and the slide is then slowly inclined to draw off the mixed blood. The marrow particles were taken on the edge of the drawing slides and films were made on four to five other slides. The marrow films were stained with Leishman's staining (Dacie and Lewis 1975).Then examine the whole film under 10 nm objectives for the cellularity of the marrow and proportion of the Megakaryocytes was

assessed. These were expressed in broad limits as Normo cellular, hypercellular or hypocellular. Differential leucocytes count was done under oil-immersion objectives and at least 300 cells at different places were counted, the myeloid and erythroid ratio was determined by relating the percentage of myeloid cells to the percentage of erythroid precursors.

Incidence of Various Types of Leukemia

The relative incidence various leukemias in all ages, both sexes are shown below, incidence according to cell type:

Cell Type	No of Cases	Percentage
Acute leukemia	45	64.28
C.M.L	24	34.3
C.L.L	1	1.5
Total	70	100.0



It is seen from the table that acute leukemia were found in 64.3%,acute lymphoid leukemia were found in 34.3 % and chronic lymphocytic leukemia 1.5 % only.

Sub-type of diff. leukemia: Cell Type	No of Cases	Percentage
A.M.L	9	12.8
A.P.L	2	2.8
A.Mo.L	4	5.7
A.L.L(L1)	12	17.1
A.L.L(2)	18	25.7
Total	45	64.2

It is seen from the above table that incidence of ALL was common in case of children (42.8%) than A.M.L (12.8%).

Types of Leukemia	No of Cases	Percentage
Acute leukemia	34	48.6
Chronic leukemia	4	5.7
Total	38	54.3%

Types of Leukemia	No of Cases	Percentage
Acute leukemia	11	15.7
Chronic myl. leukemia	20	28.6
C.L.L	1	1.4
Total	32	100.0

It is observed from the above table that the incidence of acute leukemia was more common in(48.6%) than in adults (15.7%) while chronic myelocytic leukemia was common in adults(28.6%) and it is less common in children.

Showing Different Types of Acute Leukemia

Cell Type	No of Cases	Percentage
Acutelymph. Leukemia(L1)	7	10
Acutelymph. Leukemia(L2)	16	22.9
Acute myelomonocytic	02	2.0
Acutemyeloblas.leukemia	04	5.7
A.Mo.L	01	1.4
Total	30	42%

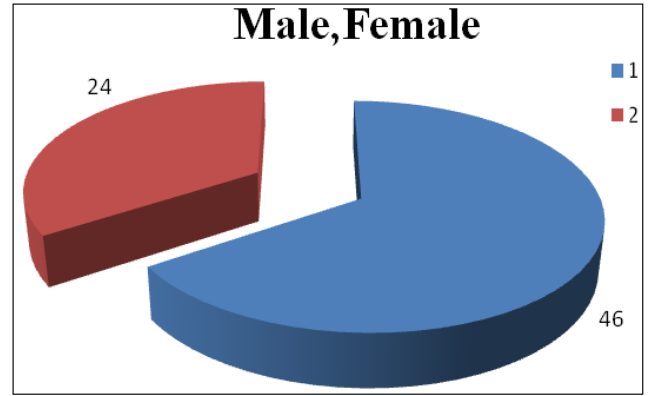
It is observed from the above Table-that the incidence of acute lymphoblastic leukemia (L₁+L₂) was commonest type of leukemia in case of children (32.9%) than acute myeloblastic leukemia(10.1%)

Age and Sex Distribution

Age in years	Acute leukemia			Chronic myeloid leukemia			Chronic lympho leukemia		
	M	F	Total	M	F	Total	M	F	Total
0-5	6	3	9	-	-	-	-	-	-
12-Jun	1	14	15	3	4	7	-	-	-
13-20	9	1	10	3	0	3	-	-	-
21-30	3	2	5	5	3	8	-	-	-
31-40	2	1	3	1	0	1	-	-	-
41-50	-	-	-	1	1	2	1	-	-
51 above 60	-	-	-	-	-	-	-	-	-
Total	33	12	45	17	7	24	1	-	-

It is observed from the above table that the peak incidence in case of children was 6-12 years while for adults was during adolescence (13-20) in case of acute leukemia and that of chronic leukemia (myelocytic) between 31-40 years ago.

There were 46 males and 24 females the Male: Female being 2.5 in case of total leukemia. In children male: female 2.1:1



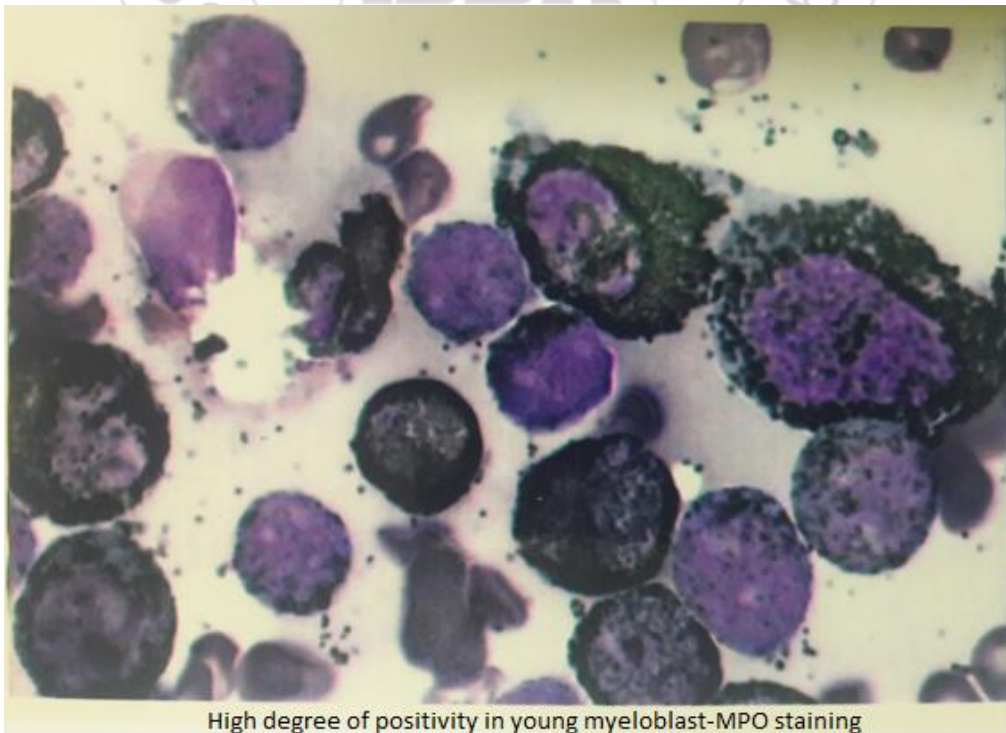
In these series the youngest patient in case of acute leukemia was 9 month old boy and oldest was 65 yrs. Female while in chronic leukemia youngest patient was five years old female and oldest patient 55 years male.

Hematological Finding: The Hematological picture of acute leukemia and chronic leukemia observed in present study were subsequently noted below.

Acute Leukemia: The total leucocytes count, it ranges from 27×10 to 120×10/L. The frequency distribution was shown in table below

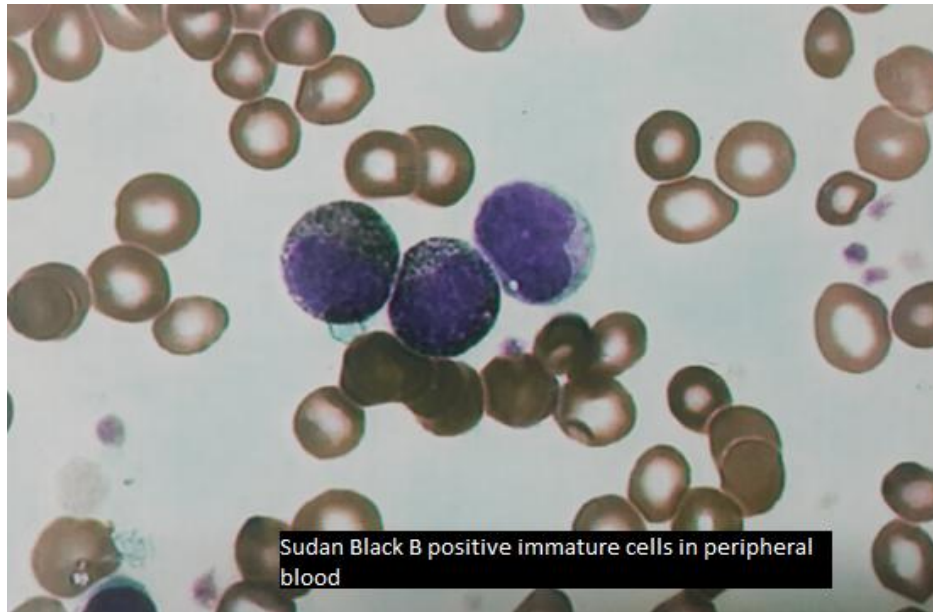
Leucocyte count/liter	No. of cases	Percentage
11×10 ⁹ /L-20×10 ⁹ /L	11	15.8
20×10 ⁹ /L-30×10 ⁹ /L	19	27.2
30×10 ⁹ /L-40×10 ⁹ /L	20	28
40×10 ⁹ /L-50×10 ⁹ /L	9	12.8
Above-60×10 ⁹ /L	11	15.8
Total	70	100.0

Showing the blasts cells in peripheral blood of acute leukemia:

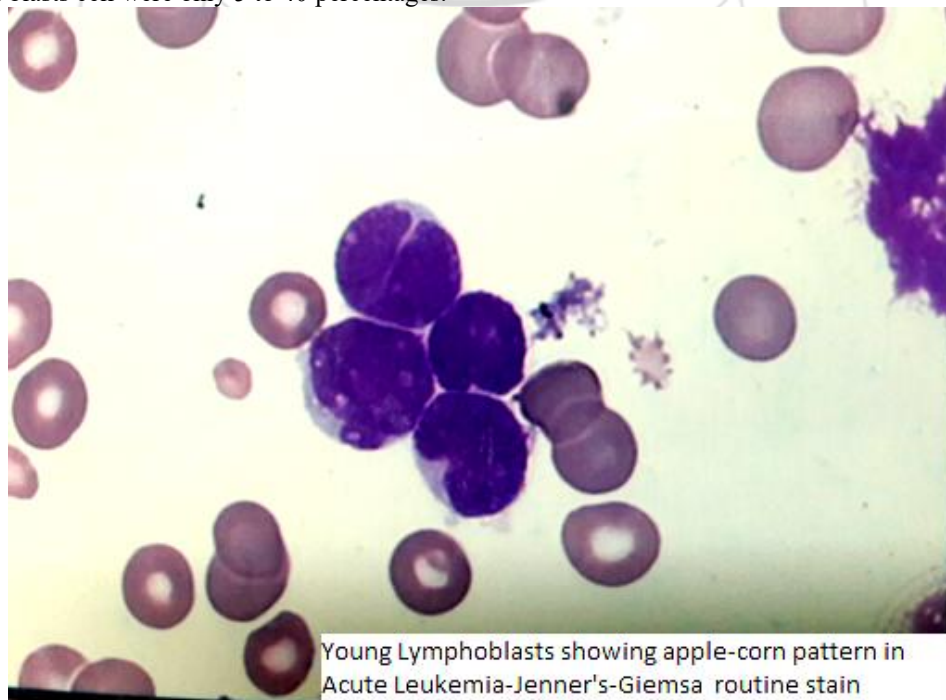


High degree of positivity in young myeloblast-MPO staining

Percentage of blasts cells	No. of cases	Percentage
5-10	11	15.7
11-30	19	27.1
31-50	20	28.6
51-70	9	12.9
70 and above	11	15.7
Total	70	100

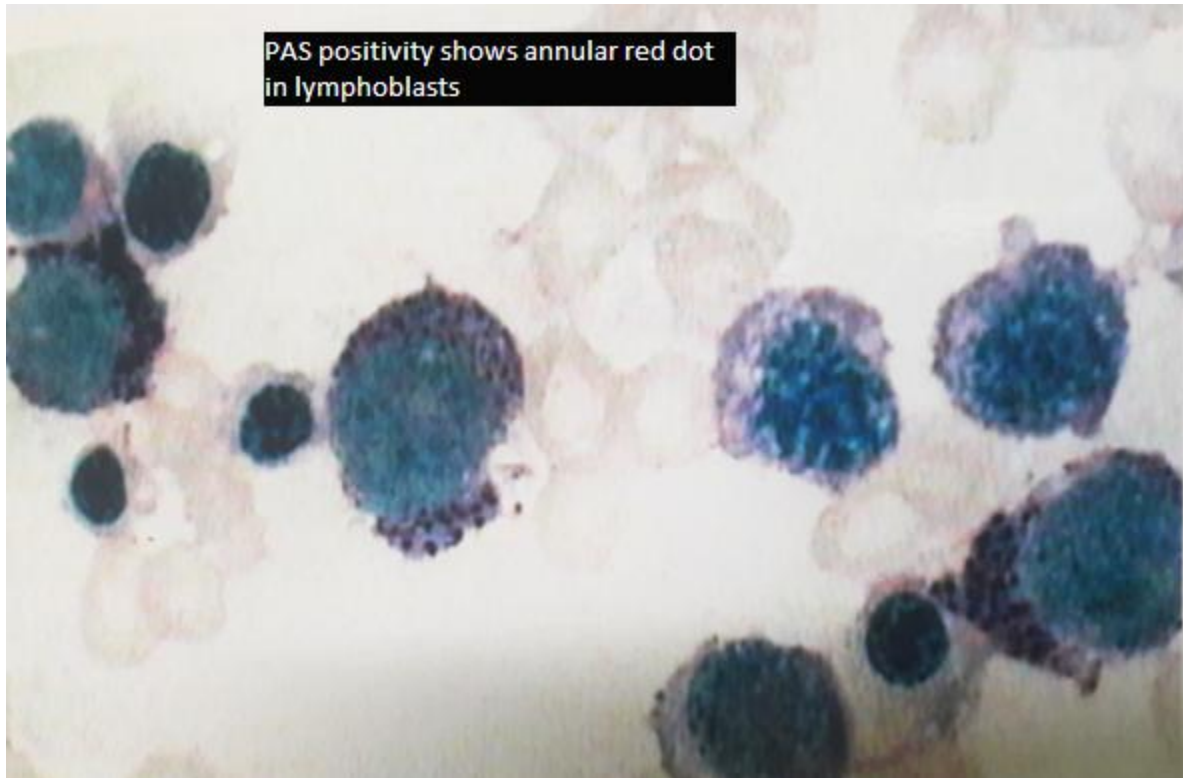


It is seen that from above table the percentage of blasts cell in peripheral blood of acute leukemia ranges from 5-99 while 25 percentage patient blasts cell were only 5 to 40 percentages.



Showing the percentage of blasts cell in peripheral blood of acute leukemia in children

<i>Percentage of blasts cells</i>	<i>No. of cases</i>	<i>Percentage</i>
5-10	1	2.6
11-30	8	21.1
31-50	20	52.7
51-70	5	13.1
70 and above	4	10.5
Total	38	100



In acute leukemia hemoglobin ranges from 3.7 to 10.9 gm/dl of blood, with median 5.7 gm/dl.

The frequency distribution were shown in the following
 Showing the hemoglobin levels in acute leukemia:
 HB% in case of children:

$50 \times 10^9 - 100 \times 10^9 / L$	29	41.4
ABOVE $100 \times 10^9 / L$	11	15.7
TOTAL	70	100.0

Hb% in children	No. of cases	Percentage
Below 5gm%	12	31.6
5.0-7.5gm%	20	52.6
Above 7.5gm%	06	15.8
Total	38	100

Showing the frequency of percentage of platelet count of acute leukemia:

Platelets/liter	No. of cases	Percentage
Less than $50 \times 10^9 / L$	30	42.9

Incidence of leukemia in general:

Leukemia is comparatively rare disease .Its contribution to the total morality is very small(Stewart and Hevitt,1959).The recent World Health Statistics Annual 1973-74(WHO-1976) included leukemia death rate for 56 countries where incidence varies from 0.5 per 100.00

Cytochemistry of blasts cells in acute leukemia:

Cytochemical reaction of various sub-types of leukemia were shown in following



Myeloperoxidase stain and Periodic Acid Schiff's (PAS) reagent

Types of leukemia	Total no of cases	peroxidase	reaction
		No of positive cases with percentage	Semiquantitative or degree of reaction in no of cases
A.M.L	9	9(100%)	10(+) 8(++) 6(+++)
A.P.L	2	2(100%)	6(+++)
A.Mo.L	4	3(100%)	4(++)
A.L.L(L1)	12	Nil	-
A.L.L(L2)	18	Nil	-

Showing the blasts cells reaction to Sudan Black –B stain

Types of acute leukemia	Total no of cases	Sudan Black-B reaction	
		No of positive cases with percentage	Semiquantitative or degree of reaction in no of cases
A.M.L	9	9(100%)	10(+) 8(++) 6(+++)
A.P.L	2	2(100%)	6(+++)
A.Mo.L	4	3(100%)	4(++)
A.L.L(L1)	12	Nil	-
A.L.L(L2)	18	Nil	-

Degree of reaction:

- Negative
- + Few positive scattered granules (in 10% of blasts cell)
- ++ Moderate no. of blasts cells.
- +++ Heavy clumps of granules (in 85-90% blasts cells)

Degree of reaction as same as Myeloperoxidase.

Showing the blasts cell reaction to periodic-Acid Schiff (PAS)



Bone marrow set used



1% Toluidine Blue and Sudan Black B reagent used in test series

Types of acute leukemia	Total no of cases	Periodic acid Schiff(PAS)	
		No of positive cases with percentage	Semiquantative or degree of reaction in no of cases
A.M.L	9	Nil sometimes few mature polymorphs show diffuse reaction	NIL
A.P.L	2	2(100%)	6(+)
A.Mo.L	4	4(100%)	8(+++) 6(++)
A.L.L(L1)	12	10(100%)	8(++) 6(+)
A.L.L(L2)	18	16(80%)	6(++) 4(++) 2(+++)

Degree of reaction:

Degree	positivity
-	Negative
+	Fine annular ring of granules
++	coarse annular ring of granules
+++	Heavy blocks of granules

Bone Marrow Examination:

Bone marrow examination was done in 35 cases. Cellularity of marrow was increased in 32 cases, almost normal in 2 cases and decreased in 1 case. The proportion of megakaryocytes was decreased in 30 no of cases, normal in 2 no of cases and increased in 3 no of cases, while myeloid hyperplasia was present in most of the cases. Erythroid hypplasia with maturation arrest was noted in five cases. In 2 cases of promyelocytic leukemia, myeloid cells in the marrow nearly 50 percentage were degenerated.

The percentage of blasts in bone marrow ranged from 20-95 percentage and frequently distribution were shown below. Marrow examination reveals the following

Percentage of blasts cells (approx)	No of cases
20 to 25	3
26 to 50	5
51 to 75	8
76-95	19

Incidence of leukemia in general:

Leukemia is comparatively a rare disease. Its contribution to the total mortality is very small (Stewart and Hevitt, 1959). The recent World health statistics Annual 1973-74 (WHO-1976) included leukemia death rate for 56 countries where incidence varies from 0.5 per 100,000.

Changing Incidence:

Sacks and Seeman (1947) reported that leukemia in the United States was rising continuously since 1900, with an accelerated rate after 1930. The persistence of this increasing trend was also noted by others in other countries (Cooke 1954, Mac Mohan and Clerk 1965, Lo Abbatt, 1958, Davies et al 1961)

The rate of increase was found equal in male and female, remarkable in age above 50 and slight below it (Sacks and Seeman 1947, Cooke 1954, Gunz and Hough 1956, Lo Abbatt 1958)

In India also (Bhatia et al 1957, Chatterjee et al 1962 and Jhala and Tilak 1967) reported problem existence of the rising incidence. Again Faraque et al (1972) and Kushwaha et al (1978) show an increase in the incidence of leukemia. But Talwakar (1961) and Boombs et al (1975) failed to detect any relative increase in number of leukemia. But whether the observed increasing incidence is true or an outcome of advances in diagnosis has been controversial and also not real due to many factors like social composition, consciousness about the disease (Dameshak and Gunz, 1958, Stewart and Hevitt 1959, Wintrobe 1974). On the other hand after about 1960 a decline in leukemia mortality rate has been observed in the United States and in Norway (Clatter, 1970)

7. Discussion

In present work, an attempt has been made to study the distribution of leukemia according to the cell types, haematological, cytochemical studies of various types of leukemia, especially in children who were admitted to pediatric (total 4872 nos in one year time) and other departments of Gauhati Medical College and Hospital from March 2002 to Sep 2003. During this period 70 no of cases of various types of leukemia were studied and the comparisons to other diseases the incidence of pediatric leukemia was 0.77 percentage.

Numbers of leukemia admitted per year to some Indian Hospital:

Reference	Years	Place	All types total	All acute leukemia	Chronic myelo leukemia	Chronic lymph leukemia
Bhatia et al	1961	Kucknow	15.6	2	13.1	0.5
Talwar et al	1961	Bombay	16.7	2.8	11.5	2.4
Chatterjee et al	1962	Calcutta	38.0	20.7	15.0	2.3
Vaswada et al	1962	Indore	12.5	1.5	10.0	0.8
Patel and Mehta	1965	Bombay	13.4	6.2	5.2	2.0
Jhala et al	1965	Ahmedabad	15.0	0.6	11.6	2.8
Swami et al	1970	Dibrugarh	10.0	2.0	7.0	1.0
Mankodi et al	1973	Ahmedamed	15.0	5.3	7.2	2.5
Bombs et al	1975	Udaigiri	19.6	7.3	9.6	2.7
Kushwaha et al	1978	Lucknow	56.0	22.4	31.4	2.2
Sangma et al	1981	Guwahati	37.2	24.0	12.6	0.6
Present Study	2002	Guwahati	36	25	10	1.0

Leukemia cases admitted to pediatric depart. Of some Indian Hospital

Reference	Year	Place	All types total	All acute leukemia	All chronic leukemia
Jain et al	1971	Ludhiana	6.6	3.3	3.3
Rajeswari et al	1980	Thanajavar	10.0	9.3	0.7
Last study	1981	Guwahati	14.6	13.3	1.3
Present study	2002	Guwahati	20	17.2	2.8

In present study the incidence of leukemia in percentage of total hospital admission was 41,550 and 38 nos of children could be diagnosed so the hospital percentage was 0.168, while in comparison to some authors reported 0.04 to 0.114

percent (Patel and Mehta 1965, Tilak and Jhala 1956 and Boomb's et al 1975, Jain et al 1971) and showed 0.53 percent of children in their series.

8. Summary

Total 70 no of cases of different types of leukemia admitted into the different departments of Gauhati Medical College hospital during the period of 12 months from March 2002 to Feb 2003 and were studied for hematological finding and cytochemical reactions. All the available and relevant literature on haematological finding and cytochemical reactions on leukemia is reviewed and those cases were analyzed and studied according to Hayhoe et al (1964), Cawley and Hayhae (1972) and Hayhoe and Quaglino (1980).

Various hematological finding and cytochemical reaction were discussed.

- 1) Of the total cases of leukemia the types of leukemia encountered were Acute Myeloblastic leukemia (9%), chronic myelocytic leukemia (24%), Acute Myelomonocytic leukemia (4%), and chronic lymphocytic leukemia (1%) of each.
- 2) The Hospital incidence was 0.168 percentage of total admission during the period of study and in case of children the incidence showed 0.77 percentages.
- 3) Ages of the patients with Acute leukemia was ranges from 9 months to 50 yrs. with peak incidence 6-12 yrs of age in children and between 13-20 yrs in adolescence, while chronic leukemia ranged from 8 yrs to 65 yrs. with a peak incidence between 11-20 yrs of age.
- 4) In children acute leukemia was more common (34%) than in adults (11%), while chronic leukemia was in between (31-40) yrs of age.
- 5) 70 no. of patients were both Male and Females with adult Male to Female ratio 2.5 and children Male to Female ratio was 2.1:1.
- 6) Total Leukocyte count in Acute leukemia was found to vary from 2.7 to $12 \times 10^9/L$. The majority of cases were being in the range 11 to $100 \times 10^9/L$ (6%) and in case of children it ranged from 11 to $100 \times 10^9/L$ in (26%) but in chronic leukemia high leukocyte count was present in all cases.
- 7) Platelet count was diminished in most of the cases of acute leukemia (42.9%) especially in children (26.1%), while in chronic leukemia was normal or increased.
- 8) Hemoglobin was lowered in all types of leukemia, but in acute leukemia Hemoglobin level was comparatively more diminished.
- 9) In cyto chemical reactions most of the myeloblast shows positive reactions to peroxidase (100%) and Sudan Black B staining (almost 100%) with varying degrees of positivity. Heavy clumps of peroxidase and Sudan Black B positive granules were observed in promyelocytes, while lymphoblasts erythroblasts showed negative staining reactions. Periodic Acid Schiff's (average 80%) staining gives block of positive granules in case of lymphoblasts and fine granules to moderate clumps in case of monoblasts and promyelocytes, while myeloblasts showed negative staining reactions.
- 10) Bone marrow examination were done in 35 no. of cases of acute leukemia and most of them, the marrow

reaction were hyperplastic with raised myeloid-erythroid ratio, increased blasts cells, decreased in erythroid precursors and megakaryocytes. Only in few cases hypoplastic marrow, erythroid hypoplasia and few other cases increased number of megakaryocytes and megaloblastic reaction of marrow noticed.

The incidence of leukemia in the present study conforms to that of published data by various research workers (Both Indian and abroad) in the recent years. It may be due to increased incidence of acute leukemia (Lymphoblastis) in children and adolescence. In the present study it was also observed that leukemia is more prevalent among males than in females. The childhood peak was in 6 to 12 yrs of age. Cytochemical study in acute leukemia helps in differentiation of various cell types and also assessment of prognosis of the disease.

It can be concluded that hematological cytochemistry should routinely be used a reliable diagnostic aid for detection, differentiation, classification, progression of the disease and prognosis of different types of leukemia.

9. Conflict interest: None

10. Declaration of Author

We declared that the study was conducted in GMCH, Guwahati and all liabilities pertaining to claims relating to the content of the article will be borne by the author.

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