e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(11); 525-531

Original Research Article

Prevalance of Acute Leukemia in Children with Special Reference to Immunocytochemistry

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Received: 25-08-2023 / Revised: 28-09-2023 / Accepted: 30-10-2023
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Conflict of interest: Nil

Abstract:

Introduction: There are 50 to 200 cases of paediatric cancer for every million children worldwide. In developing nations, juvenile cancer accounts for 2% of all cancer cases and 0.5% of those in more industrialised nations. Children's malignancies that are common worldwide include lymphoma and leukaemia. Childhood cancers most commonly diagnosed in industrialised and developing nations include leukaemias, which are the most prevalent kind, and lymphomas. It is estimated that leukaemia makes up one-third of childhood cancer cases, with acute lymphoblastic leukaemia (ALL) being the most prevalent kind.

Material and Methods: a prospective observational research that included all patients with newly diagnosed acute leukaemia admitted to the paediatrics department at MKCGMCH. Parents were asked for their informed permission. A thorough history of fever, joint pain, weakness, bleeding areas, and edoema was obtained upon admission. A clinical examination was then performed to check for signs of hepatomegaly, splenomegaly, purpuric, petechial patches, lymphadenopathy, and moderate to high grade fever. When the patient was admitted, further factors including age and sex were noted. To search for blast cells, studies such as DC TLC, TPC, and haemoglobin with peripheral smear were conducted. Additional testing, such as uric acid

Results: Being a hospital based study; it is not strictly representive of background populations. The data of a large number of patients could not be retrieved. Only 76 patients were included as per the case availability and time constraint. In our study because of the non-availability karyotyping and cytogenetic profile as a prognostic factor was not studied which could have yielded noteworthy results. Finally, because of various treatment protocols used by physicians, the outcome, based on therapy, could not be evaluated.

Conclusion: Acute leukaemia was 0.80% common, according to the study. Males and children under the age of 10 had higher rates of the illness. The most prevalent symptoms in children were fever, bleeding, and generalised weakness. The most common symptom in all leukaemia patients was lymphadenopathy, which was followed by hepatosplenomegaly. Anaemia affected most of the patients. Two further crucial peripheral blood markers for the diagnosis of acute leukaemia are thrombocytopenia and leukocytosis. It was discovered that acute lymphoblastic leukaemia frequently occurs in children. Acute myeloid leukaemia, T cell acute lymphoblastic leukaemia, and B cell acute lymphoblastic leukaemia were the three most frequent types of leukaemia. Using bone marrow analysis and flow cytometry, the diagnosis of leukaemia is more conclusive.

Keyword: Prevalance, Acute, Leukaemia, Immunocytochemistry.

Introduction

Incidence rate of childhood cancer across the world varies between 50 and 200 per million children [1]. On an average, 2% of cancers in less developed countries and 0.5% of cancers in more developed countries are childhood cancers [2]. Leukemia and lymphoma are among the prevalent childhood

cancers globally [2]. Leukemias being the most common and lymphomas are also frequently occurring childhood cancer in most industrialized and also in developing countries [3, 4].Leukemia accounts approximately for 1/3 of cancers occurring in early childhood, with acute lymphoblastic leukemia (ALL) being the most common entity. It is biologically and clinically a distinct entity, with peak prevalence during 2-5 years of life [5, 6]. There has been insufficient and limited data available on childhood cancers in India; Chaudhuri et al [7] have reported that 39.2% of childhood cancers in the state of West Bengal, India, are acute leukemias. An increase in the incidence in childhood leukemia has been observed throughout the world and in India annually >10,000 cases have been reported [8]. Studies from India have reported that ALL accounted for 60 to 85% of all childhood leukemias [9-11]. Tyagi et al [12] reported the incidence of leukemia in Indian pediatric population as 34%, of which 25% was ALL. In India, cancer is the ninth common cause of death among children between 5 and 14 years [13] and approximately 45,000 children are diagnosed with cancer annually [14]. Remarkable progress has been made in the treatment of acute lymphoblastic leukemia (ALL, which constitute 75–80% of childhood acute leukemias) with 5-year overall survival rate reaching 90% in the high-income countries (HICs) [15]. Advances in acute myeloid leukemia (AML), while not so spectacular, have been steady with 5-year overall survival rates approaching 70% [16].

Depending upon the morphology of the immature cells, immunological and cytogenetic findings, clinical features and molecular irregularities findings, Acute Leukemia is divided into two major types: acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) where myeloblasts and lymphoblasts are dominant respectively [17]. AML is further classifiable into eight subtypes (M0–M7) based on the cell type of origin and its grade/stage of maturity, while ALL is classified into three subtypes (L1, L2, and L3) [18, 19].

The diagnosis of acute leukemia requires the identification of an expanded population of hematopoietic progenitors having the morphologic appearance of blasts [20]. In 2016 a revision of the World Health Organization (WHO) classification of leukemia resulted in disease categories that are defined by a combination of clinical, morphologic, immunophenotypic, and genetic features in an attempt to define clinically relevant, biologic entities [21]. For diagnosis Starting from peripheral blood smears and bone marrow cytomorphology, it is mandatory to further perform multiparameter flow cytometry (MFC), and metaphase cytogenetics in every case, in which acute leukemia is suspected. The latter has to be accompanied by FISH and also by PCR analysis or even screening for specific molecular markers.

Flow cytometric (FC) immuno phenotyping allow classification of acute leukemia by establishing the proliferating cell line and the degree of maturation of the neoplastic cells. It should be noted that this hierarchy is operative for clinical practice, giving the most useful data for therapeutic decision making. In terms of the diagnostic algorithm however, the first investigations remain cytomorphology and immuno phenotyping. Cytomorphology and immuno phenotyping complement each other primarily because they have as common object malignant cell phenotype as a whole (morphology, i.e. surface and intracellular marker expression) [22].

In the past, hallmark for the diagnosis of acute leukemias was morphology and cytochemistry and the accuracy of the diagnosis was 80 % [23]. In the present era, the ability of immuno phenotyping to identify myeloid versus lymphoid differentiation increased to 98% [24]. Flow cytometry immuno phenotyping is a strong and mighty technological tool that is used to recognize antigens present on the cell membrane of the immature cells [25].The identification of these antigens on leukemic cells not only helpful in classifying and sub-classifying the leukemia grade and stage but also helps in deciding the treatment protocols for the for the patients. Flow cytometry also helps in estimating the prognosis of acute leukemia patients and explore for applicable markers to detect minimal residual disease. Flow cytometry is used in the diagnosis, classification and prognostic evaluation of acute leukemias. The lineage of hematopoietic cells is defined both by the preferential expression of relevant antigens and the absence of expression of antigens associated with a different lineage.

Leukemic cells, however, may aberrantly express some antigens of another lineage or lack expression of an expected antigen. Multiparametric flow cytometry (FCM) allows the simultaneous analysis of several parameters on a large number of cells providing high sensitivity and specificity. Furthermore, FCM allows the storage of the results in list-mode files and subsequent evaluation by other observers to facilitate objective interpretation, thus circumventing the subjective bias of cytochemistry [29] Although FCM has become an essential component of the multi-step leukemia diagnostic procedure, morphology and cytochemistry are still in use as a first-line screening protocol in most of the cases. In spite of its great power, the application of FCM is still limited in countries like ours with limited resources, the major prohibitive factor being the cost involved [29].

Aims & Objective

1. To estimate the prevalence of acute leukemia cases in children under 14 years.
2. To study the clinical and laboratory parameters for diagnosis of acute leukemia.
3. To study immunophenotypic profile in patients with acute leukemia.

Materials and Method

Study Design: Cross sectional study

Study Setting: Department of Pediatrics, MKCGMCH, Berhampur

Study Period: NOV 2020- OCT 2022

Inclusion Criteria: All newly admitted patients of suspected acute leukemia in Pediatric ward of MKCGMCH.

Exclusion Criteria: Diagnosed as Lymphoma, CML, MDS.

Patients on adjuvant chemotherapy. Patients not consenting for study

Sample Size: Since acute leukemia is a relatively rare disease and the study is an institution based study, we included all available cases. A total of 76 Acute leukemia cases were diagnosed during November 2020- October 2022 in Pediatrics department, MKCG MCH and were included in the study.

Ethical Approval: This study was started after getting ethical clearance from the institutional ethical committee.

Methodology:

A prospective observational study in which all newly diagnosed acute leukemia admitted in department of Pediatrics MKCGMCH were enrolled. Informed consent was taken from parents. After admission, detailed history of fever, joint pain weakness, bleeding spots and edema was taken and clinical examination was done to look for moderate to high grade fever, purpuric, petechial spots, lymphadenopathy, hepatomegaly and splenomegaly. Other variables like age, sex was recorded at time of admission. Investigations like DC TLC, TPC, hemoglobin with peripheral smear to look for blast cells was done. Other tests like uric acid estimation, liver function test, kidney function test, sepsis screen was done. Ultrasound, chest x-ray, csf study and neuroimaging whenever required was done. Bone marrow aspiration study with and special investigation like flow cytometry was done. The required quantity of venous blood was collected in EDTA vials subsequently peripheral blood smears were prepared in such cases on glass slides and stained with Leishman's stain. Bone marrow examination was done from the posterior iliac spine.

Approximately 1 ml of bone marrow aspirate was taken separately for making smears and in EDTA vials for flow cytometry. Bone marrow biopsy was taken with Jamshidi trephine biopsy needle (No. 13). It was fixed in 10% buffered formalin. Smears were examined using May–Grunwald Giemsa and special stains using myeloperoxidase (MPO) and Periodic acid–Schiff and its findings will be correlated with immuno phenotyping (done by BD FACS Canto, based on the principle of hydrodynamic focusing) and cytogenetics for further confirmation.

All cases were evaluated morphologically based on the FAB criteria. Approximately 1 ml of blood was taken separately for making smears and in ethylenediaminetetra acetic acid (EDTA) vials for FCM. Flow cytometric analysis was performed on flow cytometer (BD FACS caliber/BD FACS Canto II) and analyzed with cell quest/FACS Diva, respectively. Results were obtained by gating the blast cells with side scatter analysis versus CD45PerCP gating. Patients were treated with chemotherapy, G CSF, IV antibiotics, blood & blood component therapy and other supportive therapies as required. A predesigned pro forma was made in which patient characteristics were entered. Medical records were studied and data were collected.

Statistical analysis:

Data were recorded and analyzed using Statistical Package for Social Sciences version 23 (SPSS version 23). Results were recorded as frequencies, percentage, means and p-values. Chi square test was used for finding dependency between two variables. For all purposes, a p-value of less than 0.05 (95% confidence level) was considered as the criteria of significance.

Results

Table 1: Prevalance of Acute Leukemia

	Frequency	Percentage
Total Admitted Cases	9431	100%
Acute Leukemia Cases	76	0.80%

Total no. of patients admitted in MKCG MCH Department of pediatrics age- 1mth - 14 yrs during november 2020- October 2022 was- 9431 Out of which 76(0.80%) were diagnosed as Acute Leukemia cases.

Table 2: Geographic Distribution of Acute Leukemia (n=76)

	No.	Percentage
Urban	17	22.3
Rural	59	77.6

Table 2 represents the demographic distribution. 59 children (77.6%) were from rural areas and 17 children (23.3%) were from urban areas.

Table 3: Age Distribution of Acute Leukemia (n=76)

Age	Frequency	Percentage
<1 Year	1	1.3
1-4 Year	27	35.5
4-6 Year	10	13
6-8 Year	14	18.4
8-10 Year	12	15.7
>10 Year	12	15.7

Table 3 shows the age distribution. Maximum number of children belongs to age group of 1-4 years of age (35.5%). 1 child (1.3%) was under 1 year of age, 10(13%) were 4-6 years, 14 (18.4%) were 6-8 years, 12(15.7%) were 8-10 years and12(15.7%) were more than 10 years of age.

Table 4: Sex Distribution of Acute Leukemia (n=76)

Sex	Frequency	Percentage
Male	48	63
Female	28	36

Table 4 shows the sex distribution of our cases. Among 76 children 48 (63%) were males and 28 children (28%) were females.The sex Ratio was 1.7:1, p-value ≤ 0.001

Table 5: Interval between Onset of Symptoms and Diagnosis of Leukemia (n=76)

Duration	No.	Percentage
0-20 Days	37	48.6
20-40 Days	19	25
40-60 Days	14	18.4
>60 Days	6	7.8

Time taken for the diagnosis of acute leukemia from onset of first symptoms wasnoted in days. Maximum cases 37 children (48.6%) presented within 20 days of onset of symptoms, 25% presented between 20-40 days, 18.4% between 40-60 days and 7.8% presented after 60 days. Mean duration was 31 days.

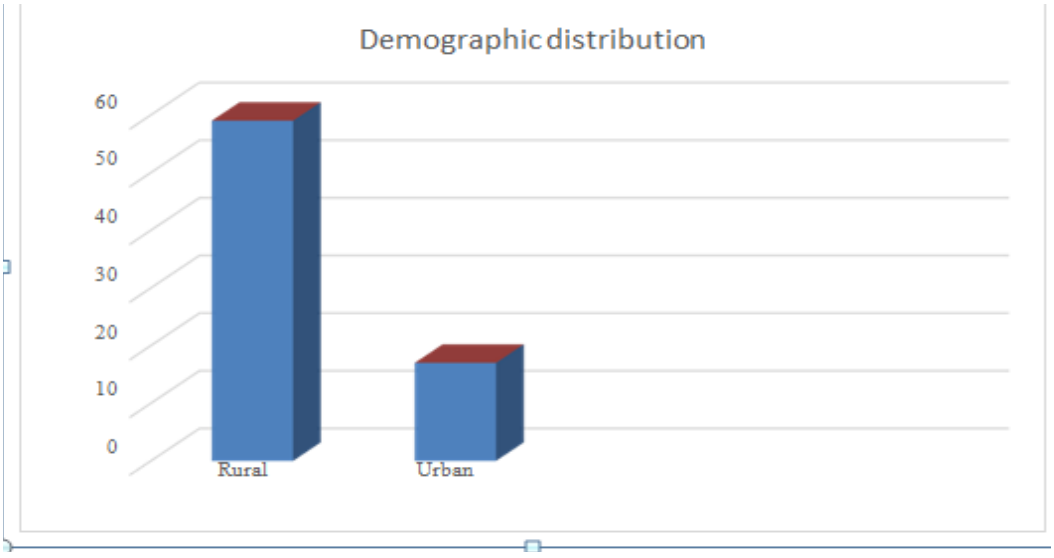

Figure 1: Demographic distribution

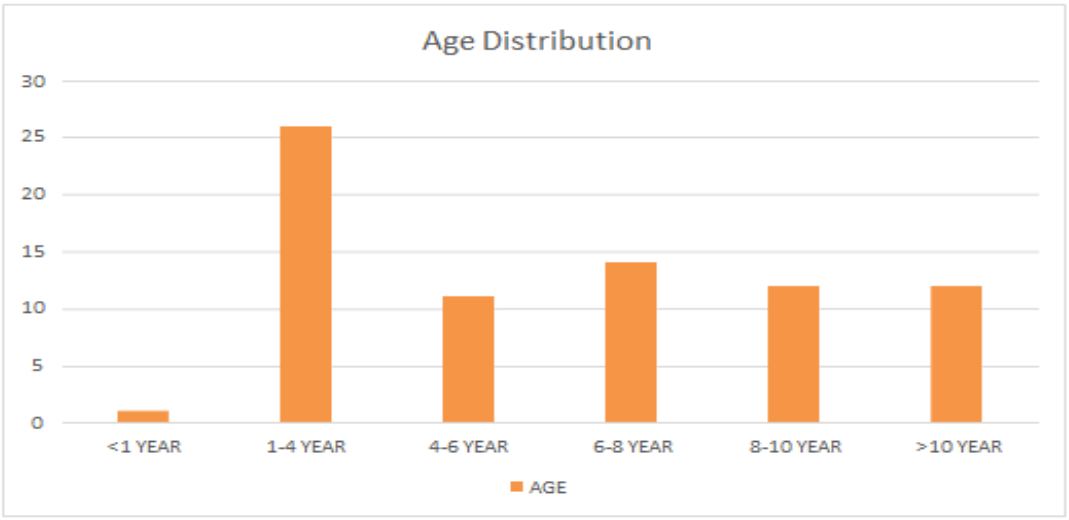

Figure 2: Age distribution

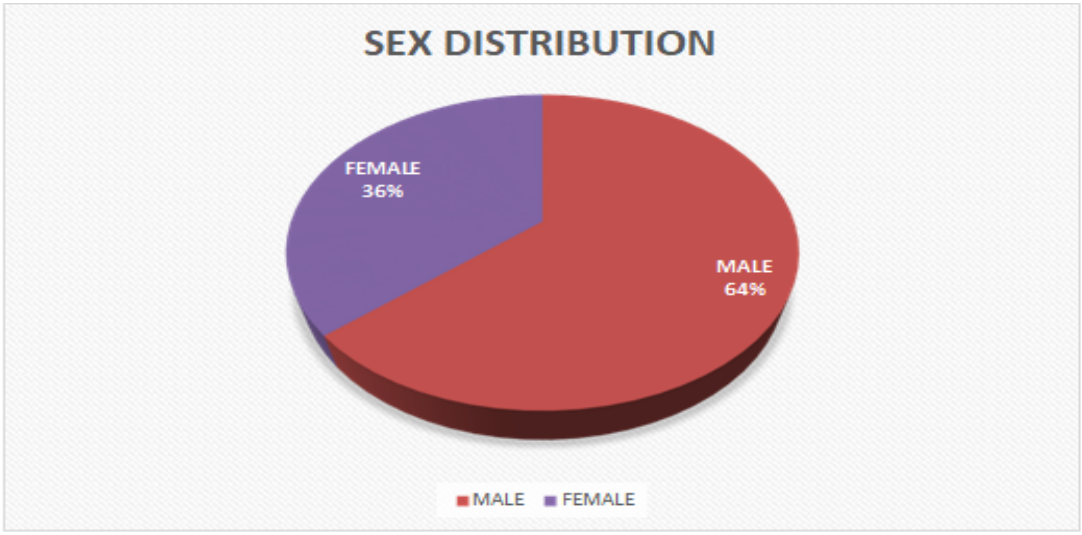

Figure 3: Sex Distribution

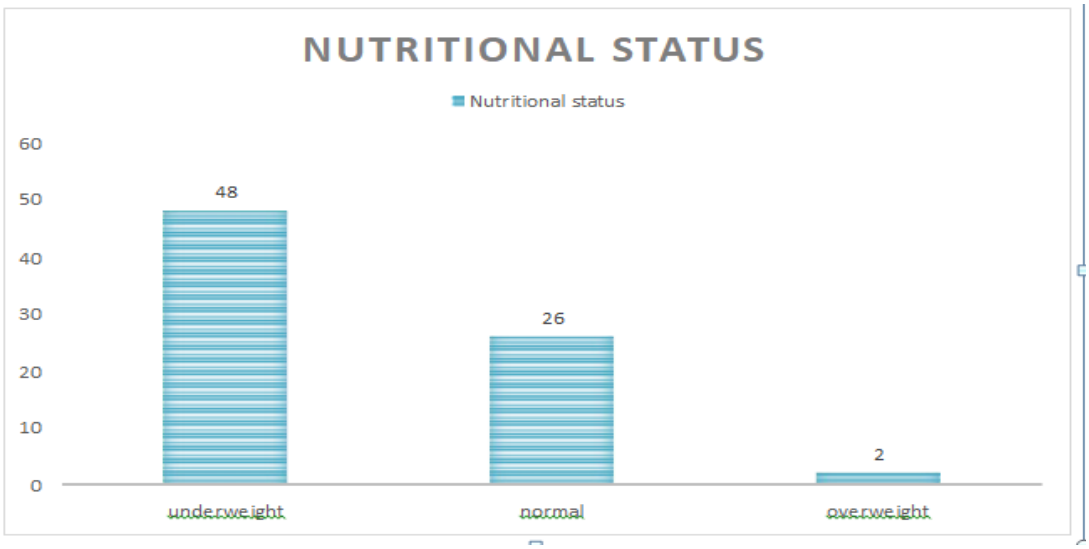

Figure 4: Nutritional status

Discussion

In our study 76 cases of newly diagnosed acute leukemia were included, from the year November 2020- November 2022 a total of 9431 no. of patients were admitted in MKCGMCH, Department of Pediatrics, of which 76 were diagnosed to be cases of acute leukemia, which account for 0.80% of total admitted cases.

In our study 17(23%) children belongs to urban and 59 (77%) belongs to rural area. In contrary to our study, a study by Rathee R et al 2014[75] shows the incidence of hematological malignancies were greater in urban as compared to rural areas. The possible explanation is that the rural and urban populations are different with regard to environmental and socioeconomic factors. The difference from our study may be due to inclusion of both pediatrics and adult patients in their study.

In our study maximum children belongs to age group of 1-4 years (n=27, 35.5%) with the minimum age of presentation being 8 months and maximum age at presentation being 14 years. 1 child is under 1 year of age and 12 children (15.7%) were above 10 years of age. 10(13%) were 4-6 years, 14 (18.4%) were 6-8 years, 12(15.7%) were 8-10 years and 12(15.7%) were more than 10 years of age. According to prognostic factors, the age group is divided into 1-10 years, <1year and >10 year of age group. 63 children (82%) belong to 1-10 years of age (p-value ≤0.001). Siddaiahgari et al conducted a study in a tertiary health center in India to look for clinical profile of ALL in children found that most common affected age group of patients was between 1-5 years (79.61%) (61].

In a study by S Khalid et al (2010) retrospective review on pediatrics patients with acute leukemia observed that twenty seven patients (58.7%) were in the age group of one to nine years [76]. Patients in the age group 1 to 9-years age group were found to have the best prognosis among all age groups [76]. In a study conducted by Sousa DW et al (2015) 76 ALL patients were under 19 years of age with patients between 1-9 years of age showing better prognosis. Similar observations were made by Guru FR et al (2018) [78] and Arya LS et al (2011) [79]. In a study by A Lingata et al (2019) [80] age group of 5-12 year was more commonly affected than age group of 1-5 year. Similar finding was reported by study done by Pandian et al [60]. The difference is may be due to their longer duration of study.

Out of 76 children included in our study, 48 (63%) were male and 28 (36%) were female. The male: female ratio was 1.7:1. Male predominance (63%, n=48) was noted in our study. Male predominance is statistically significant with p-value ≤0.05. Significant male preponderance reported can be partially explained by skewed gender selection of cases at the time of presentation rather than true gender disparity. It can also occur due to the low sex ratio in our country. In support to our study of definite male predominance, similar observations have been found in other studies. In a study by Siddaiahgari et al (2015) the gender distribution of the study subjects revealed out of 103 children, 73 (70.87%) were boys and 30 (29.13%) girls, In a study done by Sharma M et al (2020) forty-six cases were in the pediatric age group with M: F ratio of 1.23:1. Other studies also support the finding of definite male preponderance..

Time taken for the diagnosis of acute leukemia from onset of first symptoms was noted in days. In our study the duration of illness at the time of diagnosis ranged from 6 days to 90 days and mean duration was 30.82 days. Maximum cases 37 children (48.6%) presented within 20 days of onset of symptoms, 25% presented between 20-40 days, 18.4% between 40-60 days and 7.8% presented after 60 days Similar observation was found in a study by Gemuchu L et al 2019 the duration of illness at the time of presentation ranged from 1 week to 32 weeks. The mean duration of illness at presentation was 7.45 weeks and 74.5% patients presented with symptoms of 8 weeks duration or less. These data support the idea that patients in our region go to hospitals when their condition has progressed to an advance stage.

Conclusion

The study shows the prevalence of acute leukemia was 0.80%. The disorder was more common in males and children under ten years of age. Fever, bleeding and generalized weakness were most common characteristics in children. Lymphadenopathy was predominant sign in all leukemia cases followed by hepatosplenomegaly. The majority of patients had anemia. Thrombocytopenia and leukocytosis are two another important peripheral blood picture which indicates for diagnosis of acute leukemia. Acute Lymphoblastic Leukemia was found to be a frequent childhood Acute Leukemia. The most common type of leukemia was B cell-Acute Lymphoblastic Leukemia followed by T cell-Acute Lymphoblastic Leukemia and Acute Myeloid Leukemia. Diagnosis of leukemia is more definitive in flow cytometry and bone marrow study. CD19 and CD10 were the most positive marker for B cell-Acute Lymphoblastic Leukemia and CD and CD38 for T cell-Acute Lymophoblastic Leukemia. CD13, 34, 33 and CD117 were most positive for Acute Myeloid Leukemia. Flow cytometeric immuno phenotyping could precisely delineate different forms of Acute Leukemia and is especially important for confirming cytomorphologically diagnosed acute leukemia and can guide case specific mutational analysis and targeted therapy which can change the

prognosis of Leukemia.

References

1. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Canc 2010; 127:2893e917.
2. Magrath I, Steliarova-Foucher E, Epelman S, Ribeiro RC, Harif M, Li CK, Kebudi R, Macfarlane SD, Howard SC. Paediatric cancer in low-income and middle-income countries. Lancet Oncol. 2013 Mar;14(3):e104-16.
3. Dinand V, Arya LS. Epidemiology of childhood Hodgkin's disease: is it different in developing countries? Indian Pediatr. 2006; 43:141e7.
4. Yaris N, Mandiracioglu A, Buyukpamukcu M. Childhood cancer in developing countries. Pediatr Hematol Oncol 2004; 21:237e53.
5. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet 2008; 371:1030-43.
6. Pizzo PA, Poplock DG. Principle and Practice of Paediatric Oncology. 5th ed. Philadelphia: Lippincatt Williams & Wilkins; 2006. p. 539-90.
7. Chaudhuri K, Sinha A, Hati GC, Karmakar R, Banerjee A. Childhood malignancies at BS Medical College: A ten year study. Indian J Pathol Microbiol 2003; 46:194-6.
8. Marwaha RK, Kulkarni KP. Childhood acute lymphoblastic leukemia: Need of a national population based registry. Indian Pediatr 2011; 48:821.
9. Biswas S, Chakrabarti S, Chakraborty J, Paul PC, Konar A, Das S. Childhood acute leukemia in West Bengal, India with an emphasis on uncommon clinical features. Asian Pac J Cancer Prev 2009; 10:903-6.
10. Swaminathan R, Rama R, Shanta V. Childhood cancers in Chennai, India, 1990-2001: Incidence and survival. Int J Cancer 2008; 122:2607-11.
11. Arora RS, Eden TO, Kapoor G. Epidemiology of childhood cancer in India. Indian J Cancer 2009; 46:264-73.
12. Tyagi BB, Manoharan N, Raina V. Childhood Cancer Incidence in Delhi, 1996-2000. Indian J Medi Paediatr Oncol 2006; 27:13-8.
13. Summary- Report on Causes of Death: 2001-03 in India. Available from: http://censusindia.gov.in/Vital_Statistics/Summary_Report_Death_01_03.pdf. [Accessed 3 May 2016].
14. Arora RS, Eden TO, Kapoor G. Epidemiology of childhood cancer in India. Indian J Canc 2009; 46:264e73.
15. Pui CH, Yang JJ, Hunger SP, Pieters R, Schrappe M, Biondi A, et al. Childhood acute lymphoblastic leukemia: Progress through collaboration. J Clin Oncol 2015; 33:2938-48.
16. Zwaan CM, Kolb EA, Reinhardt D, Abrahamsson J, Adachi S, Aplenc R, et al. Collaborative efforts driving progress in pediatric acute myeloid leukemia. J Clin Oncol 2015; 33:2949-62.
17. Petrov I, Suntsova M, Mutorova O et al. Molecular pathway activation features of pediatric acute myeloid leukemia (AML) and acute lymphoblast leukemia (ALL) cells. Aging (Albany NY) 2016; 8:2936-2947.
18. Bennett JM, Catovsky D, Daniel MT et al. Proposals for the classification of the acute leukaemias. FrenchAmerican-British (FAB) co-operative group. Br J Haematol 1976; 33:451-458.
19. Davey FR, Castella A, Lauenstein K et al. Prognostic significance of the revised French-American-British classification for acute lymphocytic leukaemia. Clin Lab Haematol 1983; 5:343-351.
20. Swerdlow SH et al (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. IARC Press, Lyon
21. Arber DA. The 2016 WHO classification of acute myeloid leukemia: What the practicing clinician needs to know. SeminHematol 2019;56:90-5
22. Pandey G, Nichlani R, Jalaly F, Durrani N, Ali MA. Correlation of cytomorphology with flowcytometric immunophenotyping in patients of acute leukemia in tertiary care hospital. J Pathol Nep 2022; 12(1):1917-21.
23. Shrestha S, Shrestha J, Pun C.B1, Pathak T, Bastola S, Bhatta R. Immunophenotypic study of acute leukemia by flow cytometry at BPKMCH. Journal of Pathology of Nepal 2013; 3:345-350.
24. Weir EG, Borowitz MJ. Flow cytometry in the diagnosis of acute leukemia. Seminars in hematology 2001; 38:124-128.
25. Xu B, Hu C, Miao XD et al. Immuno phenotyping of 106 adult patients with acute leukemia by flow cytometry. Di Yi Jun Yi Da Xue Xue Bao 2003; 23:1043-1046.