EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
22-28
Year: 2017

IMMUNOPHENOTYPIC FEATURES OF T CELL- ACUTE LYMPHOBLASTIC LEUKEMIA IN SUDAN

Jadallah A. Ebrahim[1], Abanoub A. Tanious[1], Ahmed G.Mirgani[1], Esmail T.Almahi, Khabbab I. Elshareef[1], Eltayeb A. Abd Elgadir[1], Enaam M.A.A.Hussein*[1], Osama Altayeb[2] and Eman Abbass[2]

[1]JAE, AAT, AGM, ETA, KIE and EAA contributed equally to this work, Department of Hematology and Immune-haematology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan.
[2]Flow Cytometry Laboratory, Khartoum, Sudan.

***Corresponding Author: Enaam M.A.A.Hussein**
JAE, AAT, AGM, ETA, KIE and EAA contributed equally to this work, Department of Hematology and Immune-haematology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan.

Article Received on 07/05/2017 **Article Revised on 27/05/2017** **Article Accepted on 17/06/2017**

ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is the most common type of neoplastic disorder diagnosed in childhood. It is the cause of one third of all pediatric malignancies. Immunophenotyping has become extremely important not only in diagnosis and sub classification of T-ALL but also in the detection of the minimal residual disease. Immunophynotypic pattern of T-ALL in Sudanese patients have not been addressed before. This study was designed to characterize immunophenotypic patterns of T-ALL in Sudanese patients. Multiparameter flow cytometry and CD45/SSC gating were used to analyze the surface and cytoplasmic antigen expressions in 50 cases of T-ALL during the period January 2017 to April 2017 at Flowcytometry laboratory-Khartoum, Sudan. The following antigens: CD45, HLA-DR, CD34, CD1a, CD2, surface and cytoplasmic CD3, CD4, CD5, CD7 and CD8 were used. **Results:** All T-ALL blasts expressed CD45 with no significant differences between different T-ALL subtypes, whereas, CD34 showed different expressions. cCD3 (cytoplasmic) and sCD3 (surface) were studied among the blast population, having mean positivity of 77.7% and 19.2%, respectively in all T-ALL subtypes, collectively. CD1a was found to have higher positivity among cortical T-ALL, with mean positivity of 81.7%. No significant correlation between gender, and age to T-ALL subtype (P value = 3.75, P value = 5.38), respectively. CD5 and CD2 were mostly expressed in pre T-ALL with mean positivity of (92.8%) and (55.6%), respectively. CD5 and CD2 were not expressed in pro T-ALL and have a mean negativity of (20.1%), (7.3%); respectively. sCD3 was highly expressed in cases of mature T-ALL with mean (87.7%). Interestingly, our results revealed that cortical T-ALL is the most predominant subtype among the Sudanese patients (68%). **Conclusion:** In summary, we provide immunophenotype Flow cytometric pattern of T-ALL in Sudanese patients and our findings are shown to be similar to the main immunophenotypic features published in literature. In addition, the analysis of combining the patterns and intensity of antigen expression is expected to improve the diagnosis of T-ALL in Sudan.

KEYWORDS: *T subtype- acute lymphoblastic leukemia, immunophenotyping, Sudan.*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common type of neoplastic disorder diagnosed in childhood. It is the cause of one third of all pediatric malignancies. It is estimated that about 3000 children develop ALL in USA each year. White children are at higher risk compared to black ones and males have a slight superiority.[1]

ALL is a heterogeneous disorder which can occur in childhood and adolescence. The peak age-specific incidence of disease in children is between 2 and 5 and its other peak incidence is in old ages. It is characterized by abnormal production and proliferation of immature lymphoblasts in bone marrow (BM), accumulation of lymphoblasts in lymphoid tissues and peripheral blood.[2]

It seems that ALL occurs due to a genetic mutation in DNA structure producing white blood cell (WBC) stem cells. The two-hit theory has been proposed for ALL, which means that few children might be born with an inherent potential for ALL and if they are exposed to another trigger factor (e.g. environmental factors) they would develop ALL. Other possible causes of childhood ALL might be electromagnetic or nuclear radiation, infections and some chemical agents.[3]

Children with acute lymphoblastic leukemia present bone marrow destruction or extra medullary signs and symptoms. Fever, fatigue, growth retardation, petechiae, liver and spleen enlargement, and lymphadenopathy are some of the most common signs in ALL. Central nervous system and testicular involvement are rare. ALL might be suspected in children with abnormal complete blood cell count and impaired peripheral blood smear. Biochemistry or coagulation test might be helpful. Chromosome and cryptogenic studies and immunophenotyping provide data for ALL classification. Although there are not any specific imaging tests to diagnosis the ALL, chest radiography might display mediastinal mass and ultrasonography could be helpful in testicular involvement condition. Bone marrow aspiration and biopsy confirm ALL diagnosis.[4,5] It is estimated that up to 90% of pediatric ALL cases are curable. Traditional prognostic factors in ALL include: age, WBC count, cytogenesis and response to treatment.[2]

T cell–acute lymphoblastic leukemia accounts for about 15% and 25% of ALL in children and adults, respectively.[21] Patients usually have high white blood cells count and may show organomegally particularly mediastinal enlargement and CNS involvement. Flow cytometry is of great value in identification of different subtypes and can define the leukemic clone, while cytogenetic is not informative.[22]

Flowcytometric immunophenotyping of haematologic malignancies involving the blood and bone marrow is rapidly gaining prominence as an adjunct to traditional morphologic examination and cytochemistry. Multiparameter flowcytometry allows one to identify and characterize individual cells in suspension by using fluorescent-labeled antibodies to cell lineage and differentiation associated antigens expressed by these cells. It provides a rapid methodology for the assignment of cell lineage and stage of differentiation in acute leukaemia or blast crisis of chronic myeloid leukemia, this is particularly useful in those cases where the morphologic and cytochemical examinations do not clearly indicate lymphoid or myeloid lineage. The role of flowcytometry in subclassification of ALL was improved by utilization of a variety of gating strategies including the use of CD3/side scatter gating.[6]

Immunophynotypic pattern of T-cell acute lymphoid leukemia in Sudanese patients have not been explored before. This study was conducted to characterize immunophenotypic features of T-ALL presented to the Flow Cytometry unit at Flowcytometry laboratory in Khartoum, Sudan. It is one of the main referral Centre for patients with hematological malignancies and currently is the only centre running the service of flowcytometry immunophenotyping of haematological malignancy in Sudan.

MATERIALS AND METHODS

The study was conducted at Flowcytometry laboratory of Khartoum, Sudan, during a period of four months from January 2017 to April 2017. A total of 50 acute lymphoid leukemia (T-ALL) cases were immunophenotyped using 4 colors flow cytometer. The analyzed samples were either of peripheral blood (PB) or bone marrow aspirate (BMA) according to availability and presence of blast cells. Morphological examinations to all blood or B.M. slide films was first done followed by cytochemical stains.

Venous blood sample: For each patient a 3 ml of venous blood sample was collected in E.D.T.A. vacutainer (5ml). Bone marrow aspiration: 2 ml of bone marrow aspiration was collected in E.D.T.A. (3ml).

The tubes were labeled for analysis. 20 µL of monoclonal antibody (Immunostep, SL, Spain) was added into each tube. 100 µL of sample was added containing no more than 1 x 10^4 leukocytes / ml (each sample was counted by hematology analyzer – SYSMEX KX-21). Each tube was vortexed for 5 seconds. Thereafter, incubated at room temperature (18-25 °C) for 15 minutes. A volume of 1.0 ml of the "fix-and-lyse-mixture was added to the tube and vortexed immediately for three seconds. The tube was incubated at room temperature for at least 10 minutes, while protected from light. Centrifugation of the tubes was done at 150 x g for 5 minutes and the supernatant was discarded. 3.0 mL of PBS were added. All tubes were centrifuged at 150 x g for 5 minutes and the supernatant was discarded by aspiration. The pellets were re-suspended by addition of 0.5mL of 0.1% formaldehyde. All tubes were vortexed for 5 seconds. Finally, the tubes were analyzed by the flowcytometer (EPICS XL-MCL 4 colors Beckman Coulter flowcytometer, Maiami, FL, USA).

Each sample was stained by monoclonal antibodies for the following antigens: CD1a, CD2, cCD3, sCD3, CD4, CD5, CD7, CD8, HLA-DR, CD45, CD34 (Immunostep-SL, Spain).

Depending up on pilot study in the quality control results (that saved in the Q.C system II software file) of EPICS XL Flow cytometer, which adjusted the cut off points between negative and positive scale for every marker, positivity was considered when ≥ 30% of the population expressed the marker. The parentheses were also recorded for most of the markers.

Statistical analysis

Data was analysed using SPSS 23, correlation tests were done using Pearson correlation coefficient.

RESULTS

According to the demographic data of the study, age of patients ranged from 1 to 60 years (mean: 14.38 years) and the predominant age group was (6-16) years, which

approximately comprised 58% of cases. There were 36 (72%) males and 14 (28%) females (Figure.1, Figure.2).

Findings of *Complete Blood Cells count* (CBC) were as follows:
Hemoglobin (HB): The mean of Hb level for patients of T-ALL was (9.2g/dl).

TWBC: mean TWBC was 221.1x10^9/ml among subtypes of T-ALL and the lowest mean was 6.8x10^9/ml that presented by the pro T-ALL subtype.

Platelets: The highest mean was 252 X10^9/ml, found in pro T-ALL and the lowest mean was 28.6X10^9/ml showed by mature T-ALL subtype (Table.1).

Immunophenotyping markers
The results of immunophenotypic markers for T-ALL cases are shown in Table 2. According to immunophenotypic markers applied in this study, T-ALL cases were classified in to one case (2%) of pro. T cell, 35 (68%) cases of cortical subtype, 11 (24%) cases of pre.T cell subtype, and 3 (6%) cases of the mature T. cell subtype (Table. 3).

HLA-DR: HLA-DR was not expressed in all of cases
CD34: CD34 was positive in 5 (10%) cases and negative in 45 (90%) cases. Among T-ALL cases, there were 2 cases of cortical T-ALL that were strongly positive (40%) and 33 cases were negative (73.3%), 2 cases of Pre-T-ALL were positive (40%), whereas, 9 cases were negative (20%) and only one case of mature T-ALL was strongly positive (20%) and 2 cases were negative (4.4%). The only case of Pro T-ALL was also negative (2.2%) (Table. 4 & Figure. 3).

CD45: All T-ALL blasts were expressing CD45 with the mean positivity of 90.8% with low (SSC).

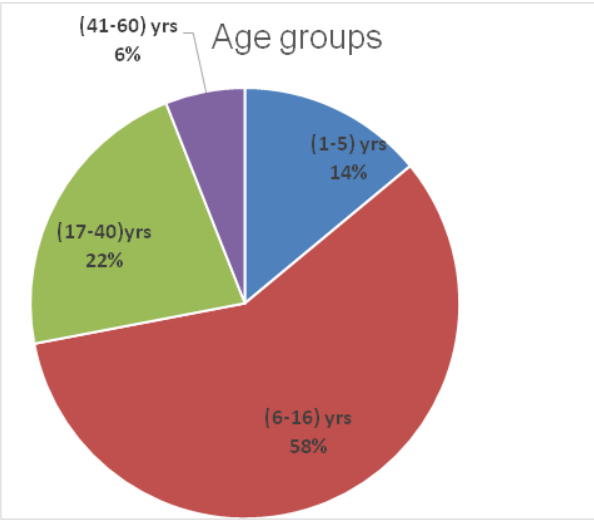

Figure 1: The age distribution among T-ALL cases.

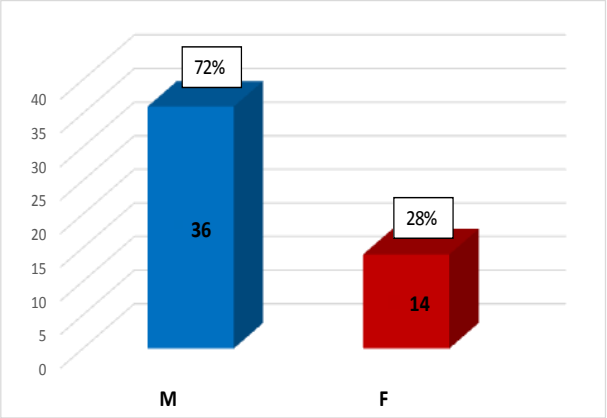

Figure 2: Distribution of gender among T-ALL cases.

CD1a: CD1a was positive in all of the 35 (70%) cases of cortical T-ALL and negative in the rest of cases 15 (30%) of other T-ALL subtypes.

CD2: CD2 was found to be positive in 30 (60%) cases and negative in 20 (40%) cases. There were 20 cases of cortical T-ALL were positive (66.7%) for CD2 and 15 cases were negative (75%). Nine cases of pre-T-ALL were positive (30%) and 3 cases were negative (15%), while only One case of Mature-T-ALL was positive (3.3%) and 2 cases were negative (10%). The pro-T-ALL case was also negative (5%).

cCD3: All T-ALL blasts were expressing cCD3 with the mean positivity of 79.25%.
sCD3: sCD3 was positive in 13 (26%) cases and negative in 37 (74%) cases. Eight cases of cortical T-ALL were positive (61.5%) and 27 cases were negative (73%). Two cases of pre-T-ALL were positive (15.3%) and 9 cases were negative (24.3%), whereas all cases of Mature-T-ALL were positive (23.1%). The pro-T-ALL case was negative (2.7%).

CD4:CD4 was found to be positive in 27 (54%) cases and negative in 23 (46%) cases, 25 cases of cortical T-ALL were positive (92.6%) and 10 cases were negative (43.5%). Only one case of pre-T-ALL was positive (3.7%), 10 cases were negative (43.5%), only One case of Mature-T-ALL was positive (3.7%) and 2 cases were negative (8.7%). The pro-T-ALL case was negative (4.3%).

CD5: CD5 was positively expressed in 48 (96%) cases and negative in 2 (4%) cases, all cases of cortical T-ALL were positive (70.8%) and all cases of pre-T-ALL were also positive (25%). Two cases of Mature-T-ALL were positive (4.2%) and only one case was negative (50%). The pro-T-ALL case was negative (50%).

CD7: CD7 was positive in 43 (86%) cases and negative in 7 (14%) cases. Among different subtypes, 31 cases of cortical T-ALL were positive (72%) and 4 cases were negative (57.1%). Nine cases of pre-T-ALL were positive (21%) and 2 cases were negative (28.6%). For

the Mature-T-ALL subtype, there were 2 cases positive (4.7%) and 1 case was negative (14.3%). The pro-T-ALL was positive (2.3%).

CD8: CD8 was positive in 15 (30%) cases and negative in 35 (70%) cases, 14 cases of cortical T-ALL were positive (93.3%) and 21 cases were negative (60%). Only one case of Pre T-ALL was positive (6.6%) while 10 cases were negative (28.6%). For the Mature T-ALL subtype, all 3 cases were negative (8.6%). The pro-T-ALL was also negative (2.9%).

Table 1: Parameters of complete Blood cells count among different subtypes of T-ALL.

Mean								
Sub Diagnosis	**TWBC x10³/µl**	**RBC x10⁶/µl**	**PLT x10³/µl**	**HB g/dl**	**Granulocytes %**	**Monocytes %**	**Lymphocytes %**	**Blasts %**
Pro. T cell	6.8	3.0	252.0	9.40	3.2	0.54	6.56	88.5
Cortical	222.5	3.4	60.9	9.21	4.2	1.73	3.32	86.9
Pre. T cell	233.5	3.3	63.8	9.18	6.1	1.19	6.30	81.9
Mature T. cell	227.2	2.6	28.7	9.23	15.2	3.09	3.62	72.9

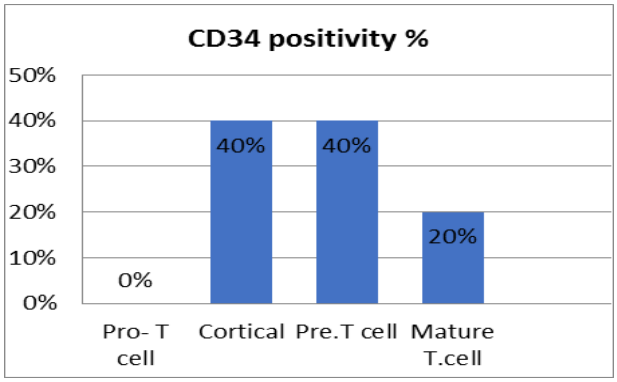

Figure 3: Frequency of CD34 positivity percentage among T-ALL subtypes.

Concerning gender and age groups, no statistical significance correlation was found in relation subtype of T-ALL (P value = 3.74, P value = 5.38, respectively (Table 5, Table 6).

Table 2: The mean percentage of immunophenotyping markers of T-ALL subtypes.

Mean %								
Sub Diagnosis	**CD1a**	**CD2**	**sCD3**	**cCD3**	**CD4**	**CD5**	**CD7**	**CD8**
Pro. T cell	0.80	7.35	3.97	87.40	1.34	20.10	91.20	2.09
Cortical	81.72	56.77	19.30	77.80	56.33	90.98	81.50	32.10
Pre. T cell	15.82	55.63	21.79	80.88	17.28	92.89	67.28	10.21
Mature T. cell	8.80	27.59	87.73	86.53	34.69	63.85	59.00	12.15

Table 3: The frequency of T-ALL sub classification.

Subtype	Frequency	Percent
Pro. T cell	1	2%
Cortical	35	68%
Pre. T cell	11	24%
Mature T. cell	3	6%
Total	50	100%

Table 4: CD34 expression in all T-ALL sub classification.

Subtypes	Frequency	Percent
Pro- T cell	0	0%
Cortical	2	40%
Pre T cell	2	40%
Mature T cell	1	20%
Total	5	100%

Table 5: Gender distribution among the cases and Sub Diagnosis subtypes (P value = 3.74).*

Sub Diagnosis		Gender		
		M	**F**	**Total**
Pro. T cell	Count % of Total	0 0.0%	1 2.0%	1 2.0%
Cortical	Count % of Total	25 48.0%	10 20.0%	35 68.0%
Pre T cell	Count % of Total	9 18.0%	2 6.0%	11 24.0%
Mature T cell	Count % of Total	3 6.0%	0 0.0%	3 6.0%
Total	Count % of Total	36 72.0%	14 28.0%	50 100.0%
*P value > 0.05; indicates insignificant correlation between gender and different T-ALL subtype.				

Table 6: The age distribution among the cases and subtypes (P value = 5.38)*

Sub Diagnosis		Age Categorized				Total
		(1 – 5) Years	(6-16) Years	(17- 40) Years	(41-60) Years	
Pro. T cell	Count	0	1	0	0	1
	% of Total	0.0%	2.0%	0.0%	0.0%	2.0%
Cortical	Count	6	20	8	1	35
	% of Total	12.0%	38.0%	16.0%	2.0%	68.0%
Pre. T cell	Count	1	7	2	1	11
	% of Total	2.0%	14.0%	4.0%	4.0%	24.0%
Mature T cell	Count	0	2	1	0	3
	% of Total	0.0%	4.0%	2.0%	0.0%	6.0%
Total	Count	7	29	11	3	50
	% of Total	14.0%	58.0%	22.0%	6.0%	100.0%
*P value > 0.05; indicates insignificant correlation between age and different T-ALL subtype.						

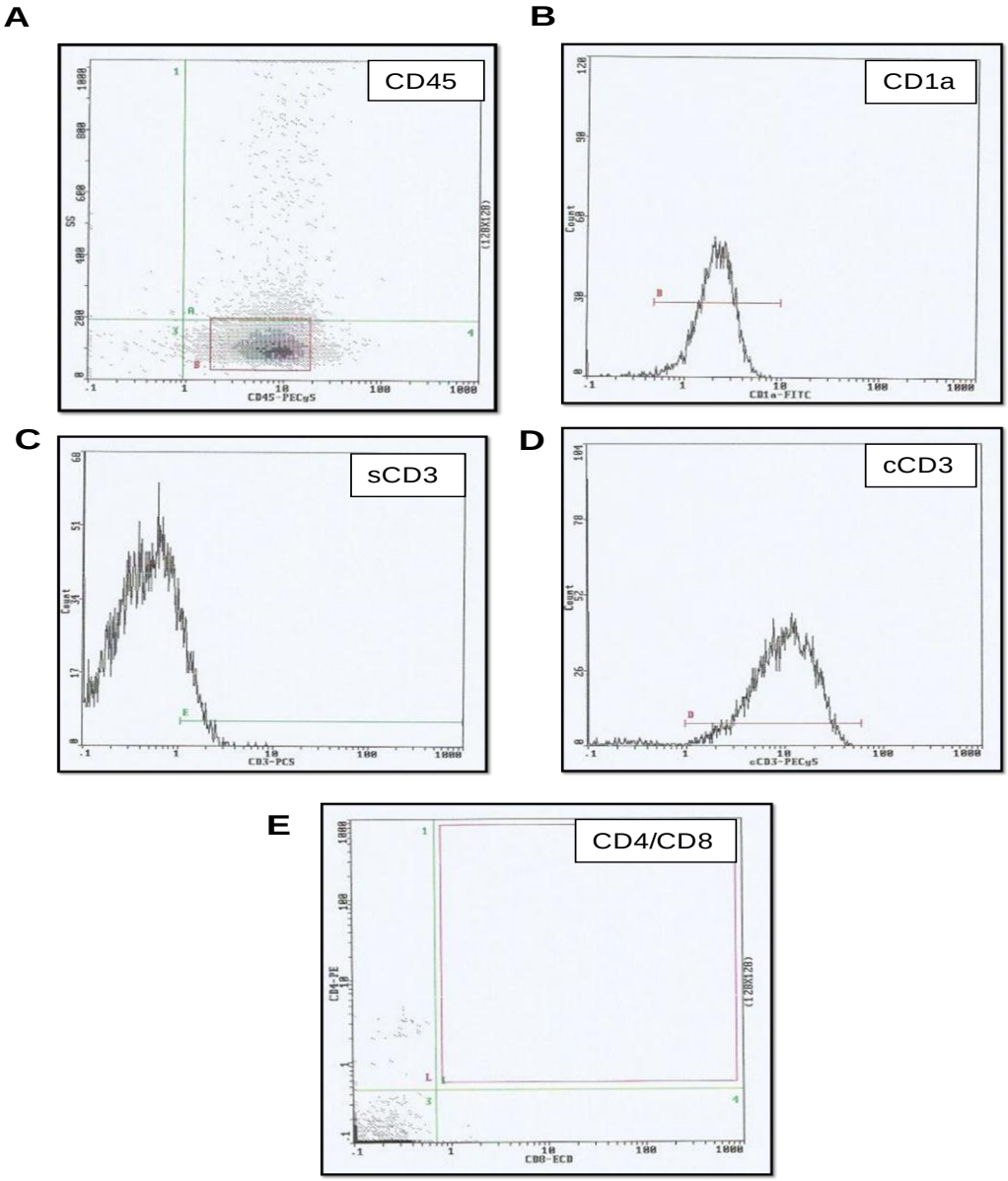

Figure 4: Immunophenotypic features of a case of 24 yrs old man presented with medistinal mass and high TWBCs and was diagnosed of T-ALL. The histograms (A - F) show results of immunophenotyping for patient's population cells. **(A):** weak expression of CD45 in the blast region, **(B):** CD1a positive expression in the blast population, **(C):** Negative expression of surface CD3 (sCD3), **(D):** Positive cells for cytoplasmic CD3, **(E):** CD4/CD8 double negative expression gated in the blast population.

DISCUSSION

We studied the immunophenotype of T-ALL in a cross sectional study in Sudan. Our findings indicate an almost similar pattern to that described in previous reports.

Acute lymphoblastic leukemia is the most common form of childhood leukemia[8] with higher incidence in males than females. T-cell ALL constitutes approximately 25% of all adult cases of ALL.[22] Our findings are in agreement, as we found that 72% of T-ALL cases were in children and 2.6 was the male to female ratio. Similarly, a study done in the United States, has found that the children ALLs represent 75% of all acute leukemia cases.

Compared to a peak incidence at 2 to 5 years of Age in a previous study, two third (58%) of our cases were at age between 6 - 16 yrs.[9,10] However, no significant association we found between the age group of the patient and the incidence of the disease.

Clinically, most of our patients suffered from fever, epistaxis, bone pain, fatigue which agrees with a previous American study in which the commonest symptoms are fever; fatigue and lethargy, bone and joint pain, and a bleeding diathesis.[13]

CBC results revealed a leukocytosis (mean = $221*10^9$/L), anemia and thrombocytopenia, which is in agree with literature. For example, a previous work has concluded that the most common laboratory abnormalities in ALL include anemia, thrombocytopenia, neutropenia, and leucopenia or Leukocytosis, with hyper leukocytosis (> 100 x 10^9/L) present in approximately 15% of the pediatric patients.[12]

According to the World Health Organization classification, acute leukemia is diagnosed by the presence of more than 30% blast cells in the peripheral blood or the bone marrow.[7] In this study, the mean percentage of blasts cells was found to be 84.8% among different subtypes of T-ALL patients. Approximately half of our patients (44%) were diagnosed with T-ALL from peripheral blood samples containing more than 30% blasts which applied as the definitive diagnosis of T-ALL.

The phenotypic heterogenecity of T-cell neoplasms is well documented and covers the range of phenotypes expressed during thymic differentiation.[16,18] Most of T-ALL cases express more than one T-lineage marker. Aberrant deletion of one or more pan T-cell antigens is common in this disease, however, this was suggested to be a helpful diagnostic finding.[19]

T-ALL is characterized by expression of T-lineage–associated antigens (CD1a, CD2, CD3 (membrane and cytoplasm), CD4, CD5, CD7, and CD8) as well as, CD34.[15,22] Immunophenotypic features of our subjects were almost similar to the pattern of T-ALL in literature. Our cases showed a small size, medium complexity on the side scatter (ss), with CD45 strong positive, cCD3 positive and HLA-DR negative cells. CD7 and CD5 were also expressed in most cells (86%) and (96%), respectively. CD2 is expressed in (60%) of cells. Therefore, our findings are in concordance in considering CD2, cCD3, CD5, CD7 of having a crucial significant role in the identification of T-lymphoblasts.

CD1a is expressed by all cortical T-ALL patient cells and negative in the other subtypes which make it the most important marker in diagnosis of cortical T-ALL. CD2 and CD5 are mostly expressed in pre T-ALL and not expressed in pro T-ALL. sCD3 was highly expressed in all T-lymphoblasts of mature T-ALL.[22]

According to our findings, Cortical T-ALL was the most predominant subtype among the Sudanese patients (68%) with T-ALL, followed by Pre T-ALL (24%). The other subtypes were minority among our cases (Table 3). When comparing our results to other series, we found that we demonstrated a higher incidence of the cortical subtype. This is clearly indicated by the presence of 46% of cortical T-ALL subtype followed by 23% of the mature T-ALL subtype.[23] Interestingly, the cortical subtype followed by the mature T-ALL have the best prognosis among other subtypes.[23]

The HLA-DR antigen is not expressed in T-cell ALL,[17] which is one of phenotypic feature that distinguishes this acute leukemia from Pre-B-cell ALL and B-cell ALL. None of our T-ALL cases of T-cell ALL was HLA-DR positive thus concurring with previous published data in literature.[20]

CONCLUSION

Flowcytometry is a powerful tool for diagnosis and classification of T-ALL, as proved by the current study which enabled us to subclassify T-ALL into proT-ALL, preT-ALL, cortical T-ALL and mature T-ALL. Our results were comparable with that of other series. In summary, we provide an immunophenotypic characterization of T-ALL for Sudanese patients with leukemia. Accordingly, Flowcytometry will be applied as an important tool to diagnose T- ALL. Further studies correlating together the flowcytometric, and genetic findings are recommended.

AKNOWLEDGMENT

Our thanks and gratitude to staff members of the Department of Hematology and Immunohematology, Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan. Our deep and great thanks will not complete without mentioning the efforts of the staff members at Flow cytometry training center- Khartoum, Sudan.

REFERENCES

1. Ronald H, Hoffman BF, Philip McGlave, Leslie ES, Sanford JS, Edward JB Jr, Helen H. Hematology: Basic Principles and Practice, 5th ed. Philadelphia: Churchill Livingstone; 2009.
2. Khalid S, Moiz B, Adil SN, et al. Retrospective review of pediatric patients with acute lymphoblastic leukemia: a single center experience. Indian J Pathol Microbiol, 2010; 53: 704-710.
3. Braoudaki M, Lambrou GI, Vougas K, et al. Protein biomarkers distinguish between high-and low-risk pediatric acute lymphoblastic leukemia in a tissue specific manner. J Hematol Oncol., 2013; 6: 52.
4. Assumpção JG, Paula FD, Xavier SG, et al. Gene rearrangement study for minimal residual disease monitoring in children with acute Lymphocytic leukemia. Rev Bras Hematol Hemoter, 2013; 35: 337-342.
5. Kiyokawa N, Iijima K, Tomita O, et al. Significance of CD66c expression in childhood acute lymphoblastic leukemia. Leuk Res., 2014; 38: 42-48.
6. Elaine M, A.L. Shaffer, Kuo-I Lin, Tracy C. Kuo, Xin Yu, Hurt, Andreas Rosenwald, Jena M. Giltnane, Liming Yang, Hong Zhao and Louis M. Staudt Plasma Cell Differentiation by Extinguishing the Mature B cell Gene Expression Program. Immunity, 2001; 17: 51-62.
7. http://www.allbloodcancers.org/leukemia?gclid=CPz7jsjahtQCFYoQ0wodEiEMkA.
8. National Cancer Institute. SEER Cancer Statistics Review, 1975–2006. Available at: http://seer.cancer.gov/csr/1975_2006/. Accessed January 23, 2009.
9. Gurney JG, Severson RK, Davis S, et al. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. Cancer, 1995; 75(8): 2186–95.
10. American Cancer Society. Cancer facts and figures 2008. Available at: ttp://www.cancer.org. Accessed January 23: 2009.
11. Pui CH. Acute lymphoblastic leukemia. In: Pui CH, editor. Childhood leukemias. New York: Cambridge University Press, 2006; 439–72.
12. Kahwash SB, Qualman SJ. Cutaneous lymphoblastic lymphoma in children: Report of six cases with precursor B-cell lineage. Pediatr Dev Pathol, 2002; 5(1): 45–53.
13. Swerdlow SH, Campo E, Harris NL, et al, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARCPress, 2008; 157–78.
14. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia, 1995; 9(10): 1783–6.
15. Dowell BL, Borowitz MJ, Boyett JM,et al. Immunologic and clinicopathologic features of common acute lymphoblastic leukemia antigen-positive childhood T cell leukemia: A Pediatric Oncology Group study. Cancer, 1987; 59: 2020-2026.
16. Feller AC, Parwaresch MR, Stein H.et al. Immunophenotypic of T-Lymphoblastic leukemia/lymphoma: Correlations with normal T cell maturation. Leuk Res., 1986; 10: 1025-1031.
17. Crist WM, Shuster JJ, Faletta J et al. Clinical features and outcome in childhood T cell leukemia – lymphoma according to stage of thymocytes differentiation: A Pediatric Oncology Study Group. Blood, 1988; 72: 1891-1897.
18. Knowles DM. The human T cell leukemias: Clinical, cytomorphologic, immunophenotypic, and genotypic characteristics. Hum Pathol, 1986; 17: 14-33.
19. Knowles DM. Immunophenotypic and antigen receptor gene rearrangement analysis in T Cell neoplasia. Am J Pathol., 1989; 134: 761-785.
20. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med, 2004; 350: 1535-48.
21. Sabina Chiaretti and Robin Foà. T-cell acute lymphoblastic leukemia. Haematologica, 2009; 94(2).
22. Sabina Chiaretti, Gina Zini and Renato Bassan. Diagnosis and Subclassification of Acute Lymphoblastic Leukemia. Mediterr J Hematol Infect Dis, 2014; 6.
23. Proleskovskaia IV, Buglova SE, Aleĭnikova OV. Immunological subtypes of childhood T-cell acute lymphoblastic leukemia and their prognostic significance. Vopr Onkol., 2002; 48(3): 322-6.