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Evaluation of conventional karyotyping, lactate dehydrogenase levels, white blood cell count, and bone marrow blast percentage as good prognostic tests in patients with acute lymphoblastic leukemia

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ABSTRACT: Acute lymphoblastic leukemia is a specific type of hematologic neoplasm in which outcomes may be affected by many variables such as the patient's age, gender, conventional karyotyping reports, lactate dehydrogenase concentration, and white blood cell count at diagnosis. The aim of this study was to assess the prognostic significance of those factors on the overall survival of patients diagnosed with ALL. The current study includes 65 patients diagnosed with ALL who were tested for cytology, conventional karyotyping, and immunophenotyping. The results of the current study have indicated the average patient's age was 14 years old. In addition, around 47.7% of patients had chromosomal abnormalities. Furthermore, the patients with LDH levels higher than 450 IU/L, and WBC counts over 100 $X10^9$ (Cell /L) had a shorter overall survival in month compared to other groups. However, the results suggested that conventional karyotyping, LDH, and WBC count might be used as good prognostic tests in patients with ALL at diagnosis.

KEYWORDS: Acute lymphoblastic Leukemia, Conventional Karyotyping, LDH, WBC.

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a kind of hematological cancer that affects B or T cell progenitor cells and is more common in children than in adults. ALL pathophysiology is defined by chromosomal abnormalities and gene mutations that affect the development and proliferation of lymphocyte precursor cells [1]. Age and gender, white blood cell (WBC) count, immunophenotypic, cytogenetic, and molecular parameters at diagnosis are used to stratify patients into risk categories [2,3].

With several distinct chromosomal abnormalities discovered, conventional karyotyping was a clinical diagnostic technique. It is becoming increasingly important in ALL owing to its role in subclassification and its prognosis role. Currently, cytogenetic analysis results have independent prognostic relevance [4,5]. Among the cytogenetic risk factors found in ALL patients are [6,7]:

- Favorable risk factors: High hyperdiploidy, t(2;8)(p11;q24), t(8;14)(q24;q32), t(8;22)(q24;q11), t(12;21)(p13;q22), and del(21)(q22.2q22.2).
- Intermediate risk factors: t(1;19)(q23;p13.3), t(X;14)(p22;q32), t(Y;14)(p11;q32), del(X)(p22.33p22.33), del(Y)(p11.32p11.32), and Cytogenetic abnormalities not classified as favorable or adverse.
- Unfavorable risk factors: Near haploidy, low hypodiploidy, high hypodiploidy, trisomy 5, t(4;11)(q21;q23), del(5)(q32q33.3), t(5;9) (q22;q34), t(17;19)(q22;p13.3), t(5;14)(q35;q32), t(6;11)(q27;q23), del(7)(p12.2p12.2), t(7;19)(q34;p13), dic(9;20)(p13;q11), t(10;11) (p12;q14), t(11;19)(q23;p13.3), and t(14;18)(q32;q21).

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Malignancy is associated with higher serum lactate dehydrogenase (LDH) levels. The majority of ALL patients have markedly brought up LDH levels, indicating rapid cell proliferation and renewal [8].

Most cancer cells have altered LDH functions as compared to normal ones. Cancer cells used LDH to increase their aerobic metabolic pathway (glycolysis, ATP synthesis, and lactate production) even in the presence of oxygen [9].

Lactate dehydrogenase (LDH) is a biomarker that may be easily identified in peripheral blood and is commonly utilized in the diagnosis of various hematological malignancies [10]. such as multiple myeloma (MM) [11], non-Hodgkin lymphoma (NHL) [12], myelodysplastic syndrome (MDS) [13], Hodgkin lymphoma (HL) [14], acute myeloid leukemia (AML) [15,16], and acute lymphoblastic leukemia (ALL) [8].

Hyperleukocytosis, generally defined as a blast cells count of more than $100x10^9/L$ of white blood cells, has been associated with poor prognosis [17]. In contrast, a patient with hyperleukocytosis is associated with greater rates of leukostasis, disseminated intravascular coagulation (DIC), and tumor lysis syndrome (TLS) compared to those without hyperleukocytosis [18,19].

2. RESULTS

2.1. Clinical features:

A total of 79 individuals were diagnosed with *de novo* ALL during the present research period. Cytology, conventional karyotyping, and immunophenotyping features were studied on 65 individuals who met the inclusion criteria (**Figure 1)**. The patients were divided into 40 males and 25 females. However, 49 of them (75.4%) were children with an average age of 14 ± 16 years (range, 1- 69 years).

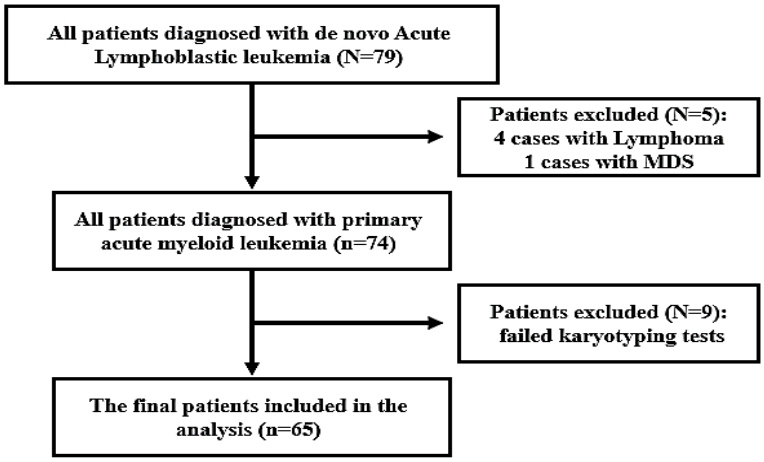

Figure 1. Diagram of patient exclusion criteria**.**

According to conventional karyotyping results, 34 patients (23 males versus 11 females) had a normal idiogram, 12 patients (6 males versus 6 females) had a favorite idiogram group risk, 6 patients (3 males versus 3 females) had an intermediate idiogram group risk, and 13 patients (8 males versus 5 females) had a poor idiogram group risk (**Figure 2)**.

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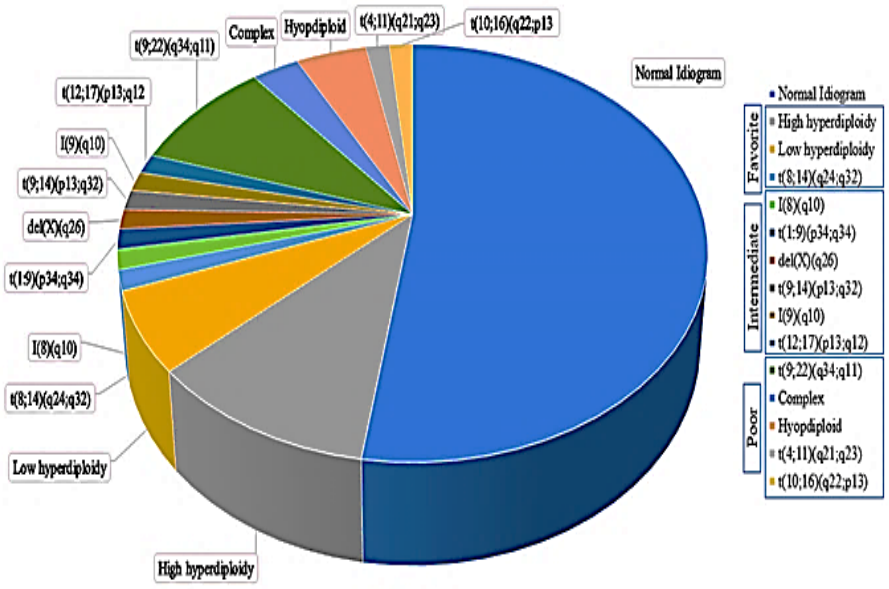

Figure 2. Patient distribution according to conversation karyotyping.

The statistical analysis showed significant differences between conventional karyotyping subgroups' risk and patients' age (p value =0.042), LDH concentration levels (p value = 0.003), and white blood cell count (p value = 0.003). However, there were no significant differences between conventional karyotyping subgroup risk and gender (p value 0.671), ALL subtypes (p value 0.342), and bone marrow blast percentage (p value 0.270) (**Table 1)**.

Table 1. Clinical characteristics of patients with Acute Lymphoblastic Leukemia according to conventional karyotyping results.

Variable	Conventional Karyotype risk group					p value
	Normal	Favorite	Intermate	Poor	Total	
Total (%)	34 (52.3%)	12 (18.5%)	6 (9.2%)	13 (20.0%)	65 (100%)	
Gender						0.671
Male	23	6	3	8	40 (61.5%)	
Female	11	6	3	5	25 (38.5%)	
Age, Year						0.042
18>	27	11	5	6	49 (75.4%)	
18<	7	1	1	7	16 (24.5%)	
ALL subtypes according to FAB classification						0.342
Pro B ALL	2	2	2	1	7 (10.8%)	
Pre B-ALL	11	2	0	4	17 (26.1%)	
B-ALL	19	8	3	8	38 (58.5%)	
T-ALL	2	0	1	0	3 (4.6%)	
LDH levels						0.003
450>	25	7	1	3	36 (55.4%)	
450<	9	5	5	10	29 (44.6%)	
Bone marrow blast (%)						0.270
50% >	22	7	2	5	36 (55.4%)	
50% <	12	5	4	8	29 (44.6%)	
WBC (X 10^9/L)						0.003
100 >	27	9	1	5	42 (64.6%)	
100 <	7	3	5	8	23 (35.4%)	

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According to serum LDH concentration, 36 patients had an LDH concentration lower than 450 U/L (22 males and 14 females) that was distributed according to conventional karyotyping subgroups risk to (25 cases with normal chromosomes, 7 cases with favorite risk, one case with intermediate risk, and 3 cases with poor risk), while 29 cases had an LDH concentration higher than 450 U/L (18 males and 11 females) that was distributed according to conventional karyotyping subgroups risk to (9 cases with normal chromosomes, 5 cases with favorite risks, 5 cases with intermediate risk, 10 cases with poor risk). Besides that, the current study found statistically significant variations between high serum LDH levels in ALL patients and the following characteristics: conventional karyotyping results (p value =0.003), WBC >100 $X10^9$/L (p value <0.000), bone marrow blast percentage >50% (p value <0.000), and age (p value =0.025). However, no significant difference was seen between LDH concentration with either acute lymphoblastic leukemia classification (p value = 0.451) or gender (p value 0.571)**.**

2.2. Survival Analysis:

From January 2019 to January 2023, 65 patients were followed-up, the median follow-up period was 27.04 ± 14.08 months (range, 28 days- 48.50 months). At the end of the time of follow-up, 47 individuals were still surviving (72.30%), whereas 18 patients died (27.7%). The Kaplan-Meier statistical analysis results showed that there were statistically significant differences between overall survival time and conventional karyotyping groups' risk, LDH concentration, bone marrow blast percentage, and white blood cells count. However, the patients with LDH concentration less than 450 U/L, WBC count less than 100 $X10^9$/L, and bone marrow blast percentage less than 50% had a longer overall survival time than those with LDH levels higher than 450 U/L (33.81 versus 18.10, p value <0.000), WBC count higher than 100 $X10^9$/L (32.98 versus 16.90, p value <0.000), and bone marrow blast cells higher than 50 % (33.06 versus 19.56, p value <0.000) **(Table 2)**.

Table 2. The correlation between overall survival times and conventional karyotyping, LDH concentration, BM blast%, and WBCs in patients with de novo ALL.

Variables		Number (%)	Overall Survival time (months) Mean ± SD	p value
Gender				0.510
Male		40 (61.5%)	27.67±13.30	
Female		25 (38.5%)	26.04±14.93	
Age, Year				0.124
	<18	49 (75.4%)	27.69±13.61	
	>18	16 (24.6%)	25.04±14.88	
ALL subtypes according to FAB classification				0.451
Pro B ALL		7 (10.8%)	29.90±14.63	
Pre B-ALL		17 (26.1%)	27.85±14.42	
B-ALL		38 (58.5%)	26.42±13.12	
T-ALL		3 (4.6%)	23.71±18.29	
Conventional karyotyping				0.024
Normal		34 (52.3%)	26.95±13.33	
Favorite		12 (18.5%)	33.72±8.81	
Intermate		6 (9.2%)	29.93±14.66	
Poor		13 (20.0%)	19.78±15.58	
Bone marrow blast (%)				0.000
50% >		36 (55.4%)	33.06±11.82	
50% <		29 (44.6%)	19.56±12.78	
LDH U/L				0.000
450 U/L >		36 (55.4%)	33.81±10.14	
450 U/L <		29 (44.6%)	18.10±13.30	
WBC (X 10^9/L)				0.000
100 >		42 (64.6%)	32.98±10.37	
100 <		23 (35.4%)	16.90±13.49	

Also, the present outcomes of this study showed that the overall survival time of patients with the favorite conventional karyotyping risk was longer than other conventional karyotyping groups' risk, there was the mean overall survival time (months) in patients with the favorite conventional karyotyping group risk

(33.72±8.81) but it was around (19.78 ± 15.28) for patients with the poor conventional karyotyping group risk (**Figure 3)**.

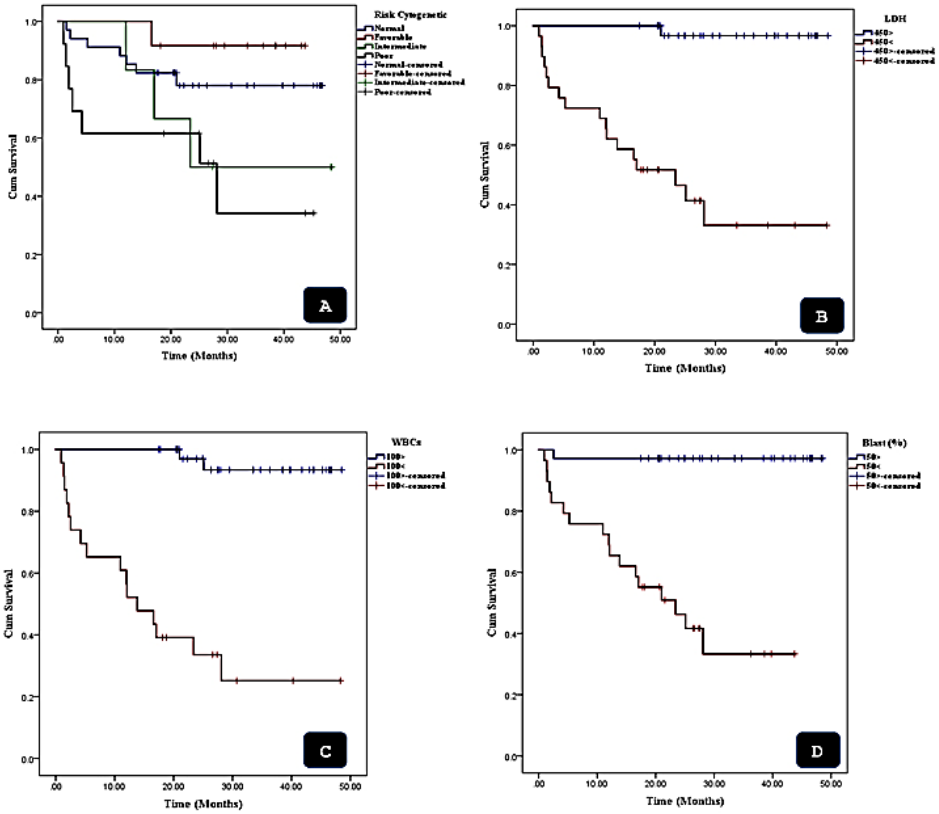

Figure 3. Relation between overall survival times and conventional karyotyping results (A), LDH concentration (B), BM blast percentage (C), and white blood cells (D) in patients with ALL.

However, A multivariate Cox regression analysis was therefore conducted on the variables with *p* value <0.05. **Table 3** shows results showed that independent adverse predictors of OS included the following factors: LDH levels higher than 450 U/L (Hazard factor [HR] = 0.031; 95% CI: 0.004-0.231; *p* value < 0.000), bone marrow blast percentage more than 50% (HR= 0.033; 95% CI: 0.004-0.248; *p* value =0.001) and WBC more than 100X10^9/L (HR= 0.040; 95% CI: 0.009-0.174; *p* value <0.000).

Table 3. A multivariate analysis that affects overall survival in de novo ALL patients.

Variables	Overall Survival Time (months)		
	***p* value**	**HR**	**(95% CI)**
LDH U/L (450> versus 450<)	0.000	0.031	(0.004-0.231)
Blast% (50%> versus 50%<)	0.001	0.033	(0.004-0.248)
WBC X10^9 (100> versus 100<)	0.000	0.040	(0.009-0.174)

2.3. Sensitivity and specificity analysis:

Receiver operating characteristic (ROC) curve analysis was used to assess the overall survival time performance of conventional karyotyping groups risk, serum LDH levels, White blood cell count, and bone marrow blast percentage that were used as prognosis tests for patients with acute lymphoblastic leukemia; the area under the ROC curve for conventional karyotyping groups risk was 0.654 (*p* value =0.043), LDH concentration was 0.845 (*p* value <0.000), WBC count was 0.870 (*p* value <0.000), and bone marrow blast cells percentage was 0.845 (*p* value <0.000). The ROC curve is shown in **Figure 4**.

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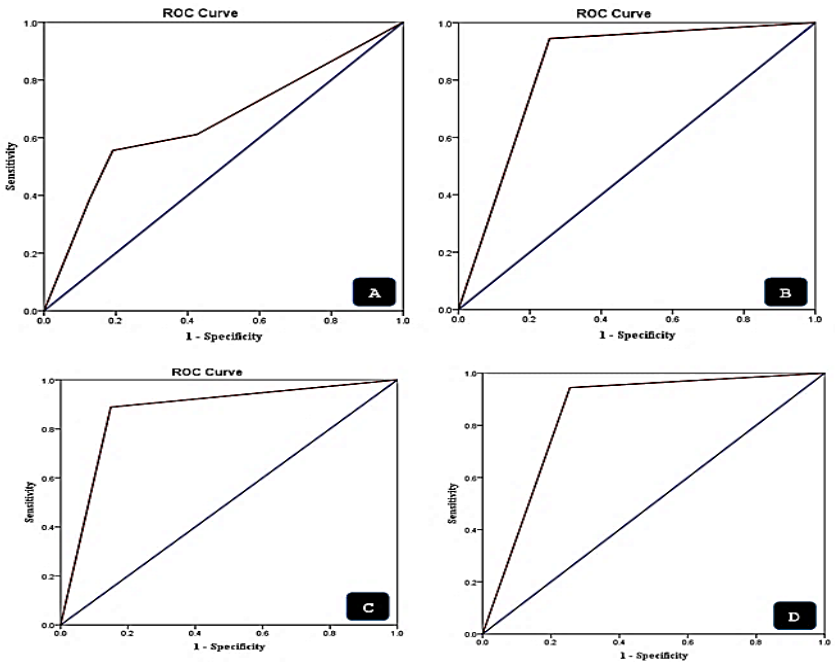

Figure 4. Receiver operating characteristic curves showed sensitivity and specificity for (A) Conventional karyotyping groups risk, (B) Lactate dehydrogenase concentration (IU/L), (C) Bone marrow blasts (%), and (D) White blood cells count X109/L.

3. DISCUSSION

Acute lymphoblastic leukemia is a disease that develops from a single hematopoietic progenitor that affects B or T cell lineage. These cells may undergo a series of genomic changes that compromise normal maturation processes, resulting in differentiation arrest and modified cell proliferation [20]. The current study aimed to evaluate the prognostic value of conventional karyotyping risk, serum LDH levels, and white blood cell count in individuals with acute lymphoblastic leukemia.

Over the last two decades, survival rates for acute lymphoblastic leukemia (ALL) have increased significantly, with overall survival (OS) rate of more than 85% in the Western community [21]. However, survival rates in underdeveloped nations continue to be lower, ranging between 30% and to 70% [22]. During the present research period, 47 of the patients (72.3%) are still alive.

In most populations, childhood malignancy represents 3% to 10% of all cancers. Hematologic malignancies account for 44% of all types of cancer diagnosed in children worldwide (33% leukemia and 11% lymphoma) [23,24]. In cases with a lymphomatous presentation, the likelihood of B-lymphoid origin is significantly higher (80%-85%) than T-cell derivation (10%-15%) [7,25]. In this study, approximately 95% of patients had leukemia with B lineage cells.

ALL is the most prevalent childhood cancer, accounting for 75-80% of all acute leukemia cases in this age range. Despite affecting children of all ages, the prevalence is highest between the ages of two and five, with a slight male prevalence [26]. In this research, about 75.4% of patients were children; these findings are similar to those reported by Sousa et al3 and Sasaki et al [27]. The current study, on the other hand, showed that males are more prevalent (61%), and the male-to-female ratio was 1.8:1. These findings are in agreement with Sousa et al [27], Claudia et al [28], Abbasi et al [29], Chennamaneni et al [30], and YI LI et al [31]. However, Matsumura et al [32] showed that the incidence of ALL in Japan was mostly female. Although it is uncertain why males are more likely than women to develop leukemia and other malignancies, some evidence suggests that sex-specific hormones such as estrogen protect cancer cells, including leukemia cells [33,34].

Conventional cytogenetics is an important technique for the identification of neoplastic or premalignant disease, as well as for providing valuable prognostic and therapeutic information. The percentage of failed karyotyping in hematological malignancies is reported between 10-20% [35,36]. In our study, 9 cases (12.8%) of ALL leukemia patients had failed to karyotype, which is similar to Jha et al [35] and Santos et al [36].

In children, ALL is the commonest malignancy accounting for approximately 25 % of childhood cancer and it has 5-year event-free survival rates ranging between 76 % and 86 % in patients receiving protocol-based therapy. ALL is less common in adults and generally carries a worse prognosis with a long-term survival probability of less than 35–40 % [37,38]. The results of the current study indicated that 31 of the patients (47.7%)

had chromosomal abnormalities, 14 cases of them has numerical chromosomal abnormalities (45.1%) and 17 cases have structural chromosomal abnormalities (54.9%), which is compatible with Amare et al [39].

According to the current study's findings, hyperdiploid ALL patients made up 16.9% of the total, which is comparable to Claudia et al [38] and Amare et al [39]. Other research, however, indicates that around (20-30%) of ALL patients have t(9;22)(q34;q11.2) [40]. Translocation t(9;22)(q34;q11.2) was detected in 9.2% of our ALL patients in our analysis, which is consistent with Claudia et al [38] and Amare et al [39] results.

Lactate dehydrogenase (LDH) is an enzyme involved in the anaerobic metabolic pathway [41]. It is found in all tissues and functions as a vital checkpoint in gluconeogenesis and deoxyribonucleic acid (DNA) metabolism [42]. Malignant cells have a specific metabolism that relies on the glycolytic sequence and the Krebs cycle for energy, causing them to deplete glucose five to ten times quicker than normal cells in order to convert it to lactate, a phenomenon known as the Warburg effect [9].

Lactate dehydrogenase levels are high in acute leukemia due to cell growth and the death of malignant cells. Elevated LDH is a poor prognostic marker for a variety of cancers, including acute leukemia [43]. The current study found that patients with ALL who had an increase in LDH concentration at diagnosis had a poorer prognosis than patients with normal LDH concentration, which is consistent with Hafiz et al [44], and Liu et al [45] that found a poor prognosis for patients with an elevated increase in LDH concentration at diagnosis.

Hyperleukocytosis in patients with ALL was known as a white blood cell count of more than 100,000/L [17-19]. In this research, hyperleukocytosis was statistically associated with a poor prognosis (p value <0.000). Also, increased bone marrow blast count (BM blast <50%) has been associated with an adverse prognosis (p value <0.000). On the other hand, some studies confirmed the poor prognostic of hyperleukocytosis in patients with ALL [17,19,46,47]. Although the causes of hyperleukocytosis are unknown, it has been established that malignant blasts may adhere to the vascular endothelium and transmigrate into tissues [48].

Receiver operating characteristic (ROC) statistical analysis was used to assess the prognostic ability of conventional karyotyping, Lactate dehydrogenase, WBC count, and bone marrow blast percentage in patients with de novo acute lymphoblastic leukemia, and ROC curves were created. The AUC for conventional karyotyping, lactate dehydrogenase, WBC count, and bone marrow blast (%) were 0.654, 0.845, 0.870, and 0.845, respectively which might be used as good prognostic tests in patients with ALL at diagnosis.

4. CONCLUSION

Acute lymphoblastic leukemia is one of the most common types of acute leukemia in children. The current study's findings revealed that conventional karyotyping, LDH, WBC, and blast percentage might be used as good prognostic markers at diagnosis in patients with ALL.

5. MATERIALS AND METHODS

5.1. Study design:

Patients in the present cohort research were newly diagnosed with ALL and were treated with standard leukemia chemotherapy between January 2019 and January 2023. To confirm the ALL diagnosis, all participants in this research had bone marrow aspiration, which was obtained using heparin and EDTA coagulation tubes.

5.2. Ethical considerations:

Our research was accepted by the Ethical Committees of Aleppo University (Registration number /34/; date 7/1/2019). The patients in our study were requested to provide written informed consent before enrollment.

5.3. Exclusion criteria:

Patients with secondary acute lymphoblastic leukemia, lymphoma, mixed phenotypic acute leukemia (MPAL), myelodysplastic syndromes (MDS), or failure of cell culture to take on chromosomes were excluded.

5.4. Data collection:

The current study included 65 patients attending to hematology department at Aleppo University Hospital and Ibn Al Rushd Hospital. The overall survival time (The time that patients with acute lymphoblastic leukemia have been alive) and patient data were collected from the admission and follow-up office.

5.5. Measurements:

5.5.1. Hematological parameters:

A cytology smear was used to evaluate the morphological characteristics of the blast cells. A bone marrow smear was made and evaluated using Giemsa stain (HIMEDIA) to identify (Nucleus shape, chromatin type, and blast cell percentage), while the Mindray BC-2800 hematology analyzer was used to determine the WBC count.

5.5.2. Measurement of LDH concentration

The serum LDH concentration was measured using a Mindray BS-300 analyzer and the lactate-to-pyruvate technique with LDH (BioSystems) kit. The normal serum LDH reference range was 250-450 IU/L.

5.5.3. Flow Cytometric Immunophenotyping:

Flow cytometry was performed to immunophenotype blast cells collected samples. Single-cell suspensions (About 10^6 cells/mL) were stained and were evaluated with four different fluorochrome-conjugated monoclonal antibodies CD45-APC, HLADr-PerCP, CD2-PerCp, CD3-FITC, CD4-PE, CD5-APC, CD7-FITC, CD8-PreCP, CD10-PE, CD19-PerCP, CD20-FITC, CD22-PerCp, CD23-APC, CD38-FITC, CD11b-FITC, CD13-PE, CD33-PerCp, CD14-PE, CD16-FITC, CD56-PE, CD34-APC, CD117-APC, CD163-FITC (Becton Dickinson Biosciences). Gently mixed, and incubated at room temperature for 30 minutes in the dark. Following that, the RBCs were lyzed with a lysis solution (0.84 ammonium chloride, 0.12 gr potassium bicarbonate, and 0.002 tetrasodium EDTA in 100 mL distilled water). After 10 minutes of incubation at room temperature in the dark, the mixture was centrifuged for 5 minutes at 1200 rpm. Following the removal of the supernatant, the cells were treated with 0.5 mL of 2% paraformaldehyde solution before being evaluated with the BD FACSCanto (two lasers, six parameters) analyzer and data were analyzed by BD FACSDiva™ software.

5.5.4. Conventional karyotyping:

As a short-term culture, bone marrow aspiration samples were cultured in a flask with 10 mL of RPMI 1460 medium supplemented with (20% fetal bovine serum (FBS), L-glutamine, sodium bicarbonate, and without mitogen). The flasks were incubated for 48 hours at (37°C, 5% CO_2) before adding 100 uL of colcemid solution (10 ug/mL) and incubating for 60 minutes at 37°C. The culture should then be transferred to centrifuge tubes and centrifuged for 10 minutes at 1000 rpm. After removing the supernatants, the cells were resuspended in 10 ml of KCL solution (0.075 M) and incubated for 15 minutes at 37 °C. After centrifuging the sample tubes at 1000 rpm for 10 minutes, the cells were resuspended in fresh ice-cold fixative solution (acetic acid and methanol 1:3 ratio). The last procedure was repeated until a leukocyte precipitate was obtained. The cells pellet should be re-suspended in 2 ml of fresh fixative solution, dropped onto a clean slide, and allowed to dry by warming. The G-banding technique was employed to stain chromosomes by immersing the slides in a 25% trypsin solution jar for 4 seconds, then in a phosphate buffer solution jar for 4 seconds, and finally in a 3% Giemsa stain (HIMEDIA) for 5 minutes. CytoVision (version 3.92) software was used to evaluate chromosomes in accordance with an international system for human cytogenomic nomenclature (2020). According to the results of CK all patients were classified into four groups (Normal idiogram, favorite risk, intermediate risk, or poor risk).

5.6. Statistical analysis

The mean ± standard deviation (SD) and the Kruskal-Wallis H test were used to compare continuous and categorical variables between groups. The Kaplan-Meier method was used to estimate survival curves, and to compare survival curves between groups. The groups were divided based on gender, age (<18 years versus >18 years), WBC count (100> $X10^9$/L versus >100 $X10^9$/L), LDH value (<450 IU/L versus >450 IU/L), BMB% (<50% versus >50%), and CK results (Normal idiogram, favorite risk, intermediate risk, or poor risk). The data were analyzed using a multivariate Cox proportional hazards regression model to identify the risk variables for OS. The receiver operating characteristic (ROC) curve was created using the predicted probability of OS in patients with acute myeloid leukemia. The region under the ROC curve (AUC) was used to properly determine the overall survival of conventional karyotyping, LDH concentration, WBC count, and bone marrow blast percentage. The IBM SPSS software (version 24) was utilized for statistical analysis. A significance level for analyses was a p-value ≤0.05.

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