Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
C M B Association Publisher

Investigating the interplay of hematological parameters, CD markers, genetic polymorphisms, and database mutations in the IL15 gene in acute myeloid leukemia patients

Darya M. Azeez[1], Sarbaz I. Mohammed[1], Kawa M. Hassan[2,3], Abbas Salihi[1,4*]

[1] Department of Biology, College of Science, Salahaddin University-Erbil, Erbil, Kurdistan Region 44001, Kurdistan region, Iraq
[2] Department of Medicine, College of Medicine, Hawler Medical University, Erbil, Iraq
[3] Nanakaly Hospital for Blood Diseases and Oncology, Erbil Directorate of Health, Erbil, Iraq
[4] Center of Research and Strategic Studies, Lebanese French University, Erbil, Kurdistan Region 44002, Iraq

ARTICLE INFO

Original paper

Article history:
Received: March 16, 2023
Accepted: July 04, 2023
Published: September 30, 2023

Keywords:

AML, IL15, CD markers, Single nucleotide polymorphisms

ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by the clonal expansion of myeloid precursor cells in the bone marrow. In this study, we investigated the interplay of hematological parameters, CD markers, genetic polymorphisms, and database mutations in the interleukin 15 (*IL15*) gene in AML patients. We enrolled 59 newly diagnosed AML patients and analyzed their bone marrow specimens using flow cytometry and molecular techniques. The hematological parameters of the AML patients revealed a significant increase in platelet count and RBC, Hb, and HCT levels compared to healthy individuals. CD marker expression analysis revealed upregulation of CD33, CD45, CD13, CD117, CD38, HLA-DR, CD15, CD64, MPO, CD34, and CD11c in AML patients. Molecular analysis showed 15 mutations in different positions of exon 8 of the *IL15* gene, with the most frequent mutation being a homozygous mutation resulting from a nucleotide substitution. Additionally, 10 novel heterozygous mutations were identified in different locations of chromosome 4, with a low variant rate. Finally, database analysis of gnomAD and Mutagene revealed a high number of potential driver mutations in the *IL15* gene in leukemia patients. These results provide valuable insights into the genetic and immunophenotypic characteristics of AML patients and highlight the potential role of *IL15* in AML pathogenesis.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.9.5
Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

Acute myeloid leukemia (AML) is one of the most aggressive and common types of hematopoietic cancer. In addition, AML most commonly affects adults and therefore its incidence increases with age (1-3). Leukemia is the third most common type of cancer in the Erbil and Duhok governorates of Iraq's Kurdistan region (4). Morphologically AML cancer cells are divided into eight subtypes from M0 to M8 or divided into several groups according to the WHO system depending on gene mutation, immunophenotypically and chromosomal abnormality (5, 6).

Blood chemistry and hematology are useful in the diagnosis and treatment of patients with acute leukemia (7). Acute leukemia was the most common cause of pancytopenia in both children and adults, and AML accounted for the majority of cases (8). However, haematological values alone cannot explain the clinical manifestations of myeloid leukemia, and complex biologic factors are most likely also involved (9). Despite this, the (10) study found that light microscopic features of peripheral smear and bone marrow continue to be the mainstay in the diagnosis of acute leukemias, while immunotyping and cytogenetics are complementary procedures performed at specialized centers.

Interleukin 15, a member of the IL-15 cytokine family, is involved in cell growth, proliferation and survival, and the activation of several lymphocyte lineages. It mainly acts by employing a wide range of pathways to transfer signals. This cytokine consists of a 14-15 kDa glycoprotein encoded by a 34-kb sequence located on chromosome 4q31 (11, 12) and is involved in innate and acquired immunity. Additionally, it has been reported that IL-15 acts through the β and ϒc chains of the IL-2 receptor and the specific IL-15Rα chain (13, 14). IL-15 plays an essential role in the growth and balance of natural killer cells and memory CD8+ T lymphocytes (15). More specifically, IL-15 enhances the antitumor CD8+-mediated immune responses and the efficiency of clinical medications such as chemotherapy (16). Additionally, it has been reported that IL-15 is involved in the synthesis of polypeptides inside the cells, while it attenuates neutrophil apoptosis via unclear pathways. Human neutrophils express three different types of IL-15 receptors (IL-15R), namely IL-2/15Rβ (CD122), ϒc (CD132) and IL15Rα (17, 18).

To date, several techniques have been developed to evaluate the clinical and biological characteristics and behavior of leukemic cells, including the detection of novel chromosomal abnormalities and gene variations, and their response to chemotherapy. Several studies have opened

* Corresponding author. Email: abbas.salihi@su.edu.krd

the path for identifying novel single nucleotide polymorphisms (SNPs), while genetic studies have provided novel insights into the understanding of AML genetics (19). The cluster of differentiation antigen is a surface antigen expression on myeloid tumer cells and it is useful for diagnosis, prognosis, and classification of acute myeloid leukemia (20-23). Flow cytometry analysis is a critical tool in subclassifying leukemia (24-26). Although several genetic abnormalities such as gene translocations have been identified in AML, other novel cryptic abnormalities and changes have been recently detected using modern techniques, including high-density SNP arrays (27, 28). Therefore, the present study aimed to investigate the association between *IL15* polymorphisms in exon 8 of chromosome 4 and the risk of AML.

Materials and Methods

Patients

The current study included 59 newly diagnosed AML patients with a median age of 33 years (range, 15 to 60 years) who attended Nanakaly Hospital (Erbil, Kurdistan Region, Iraq) for examination and diagnosis between January 2021 and November 2021, as well as 40 healthy subjects. The present study was approved by the Human Ethics Committee of Salahaddin University Erbil's College of Science (approval no. 7/54/591; date, 4/2/2021; Erbil, Iraq) and was carried out in accordance with the Helsinki Declaration principles. Prior to enrollment, all patients provided written informed consent. AML was diagnosed using WHO guidelines, which included a physical examination, clinical history, complete blood picture (CBP), bone marrow examination, and cytogenetic and immunophenotypic criteria. The bone marrow and blood samples were collected prior to any treatment or chemotherapy.

Peripheral blood analysis

On the day of the bone marrow biopsy, peripheral blood samples were collected and analyzed with an automated Coulter Ac.T diff Hematology analyzer (Beckman Coulter, Inc.).

Bone marrow biopsies

The bone marrow specimens were taken from newly diagnosed AML patients and sent to the Oncology Department at Nanakaly Hospital in Erbil, Iraq, for cellular assessment and flow cytometry analysis. The FACSCanto II flow cytometer was used for immunophenotypic analyses (BD Biosciences, San Jose, California, USA). CD7, CD11b, CD11c, CD13, CD14, CD15, CD 33, CD34, CD36, CD38, CD 41, CD 45, CD 61, CD64, CD117, MPO, TdT, and HALA-DR are the most common myeloid markers used to diagnose acute myeloid leukemia. For most CD markers, a positive value was obtained when the expression rate was greater than 20%. Giemsa stains were used to stain blood smears (Table 1).

Molecular analysis and detection of polymorphisms

Genomic DNA was extracted from whole blood using the Add Prep Genomic DNA Extraction Kit (Add Bio, Inc.), according to the manufacturer's instructions. DNA extraction was carried out at the Immunogen Center (Erbil, Kurdistan region, Iraq). The primer sequences used to detect the *IL15* gene were the following: Forward, '5-CTATGCTGGTAGGCTCCTG-3', and reverse '5-GTTCCATTAGAAGAGAGCTTGC-3'. The PCR mixture was prepared according to the standard procedure described in the Add Prep Genomic DNA Extraction Kit (Add Bio, Inc.). PCR was performed on a programmable thermocycler (AB Applied Biosystems). The thermocycling conditions used were the following: 35 cycles at 95°C for 30 sec for denaturation, 60°C for 30 sec for annealing, followed by extension at 72°C for 30 sec. The PCR was performed in a total volume of 30-µl. The DNA bands were visualized under UV light at a wavelength of approximately 240-366 nm. The mutations in exon 8 of the *IL15* gene were detected by direct Sanger sequencing (Sambrook and Russel, 2001).

The final PCR products were sequenced in both directions on the 3130 Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Known or novel mutations were identified and named by analyzing the Sanger sequencing data with Mutation Surveyor software (SoftGenetics, State College, PA, USA). Sanger sequencing was analyzed using Mutation Surveyor software package 5.1 (Soft Genetics, LLC) to detect known and unknown mutations by comparison with the GenBank database sequence reference genes (Chromosome 4 - NC_000004.12).

Mutation retrieval from databases

The complete reported mutations of the IL15 gene from all cancer types were obtained from gnomAD v2.1.1 and v3.1.2 (https://gnomad.broadinstitute.org/), and the data shown is from ClinVar's (released January 4, 2022). While the whole reported mutations of the selected leukemia gene were obtained from Mutagene (developed at Panchenko Research Group, NCBI) (https://www.ncbi.nlm.nih.gov/research/mutagene/gene).

Statistical Analysis

All statistical analyses were carried out using GraphPad Prism 9.0 Software (GraphPad Software, Inc.). Normality was tested using the D'Agostino & Pearson test, the Anderson-Darling test, Shapiro-Wilk test and the Kolmogorov-Smirnov test. An unpaired t-test was used for blood

Table 1. Classification of AML Subtypes.

Gender	AML	%
Female N=28 (47.45%)	M0	3 (10.71)
	M1	4 (14.29)
	M2	4 (14.29)
	M3	8 (28.57)
	M4	3 (10.71)
	M5	3 (10.71)
	M7	3 (10.71)
Male N=31 (52.55%)	M0	4 (12.9)
	M1	3 (9.68)
	M2	3 (9.68)
	M3	6 (19.35)
	M4	2 (6.45)
	M5	6 (19.35)
	M6	1 (3.23)
	M7	6 (19.35)

parameters analysis.

Results

Hematological parameters

The total white blood cell (WBC) count in patients with AML compared with that in the control group is shown in Figure 1. No significant changes in total WBC, lymphocyte, monocyte and granulocyte counts were observed between patients with AML and healthy individuals. Platelet (PLT) count is shown in Figure 2. The PLT number was significantly increased in patients with AML compared with healthy individuals. The total red blood cell (RBC) count, hemoglobin (Hb) levels, hematocrit (HCT) values and red blood cell distribution width (RDW) is shown in Figure 3. The results showed that RBC, Hb and HCT levels were notably increased in all patients with AML. However, RDW was not significantly altered in patients with AML compared with the control group, as shown in Table 2.

CD marker expression

The expression levels (%) of each CD marker in patients with AML are shown in Figure 4. The flow cytometry results demonstrated that CD33, CD45, CD13 CD117, CD38 and HLA-DR were all upregulated in AML. Additionally, the expression levels of CD15, CD64, MPO, CD34 and CD11c were also increased, but to a lesser extent. Finally, the expression levels of the remaining CD markers were not significantly altered in patients with AML (expression rate, <20%).

Genetic polymorphisms in the *IL15* gene

The genetic variations in the *IL15* gene of patients with AML are listed in Table 3. A total of 15 mutations in different positions of exon 8 in chromosome 4 were identified in patients with AML compared with NCBI. Among them, 14 were heterozygous and one homozygous.

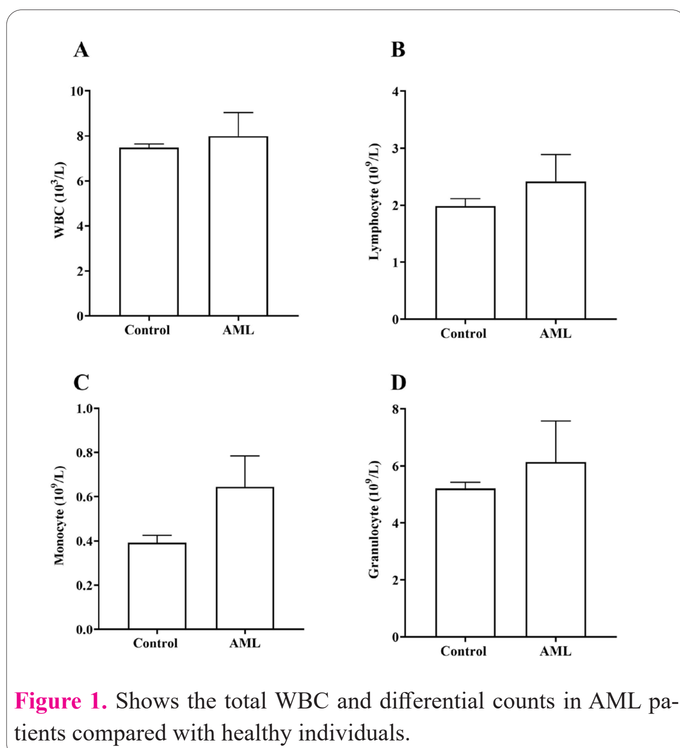

Figure 1. Shows the total WBC and differential counts in AML patients compared with healthy individuals.

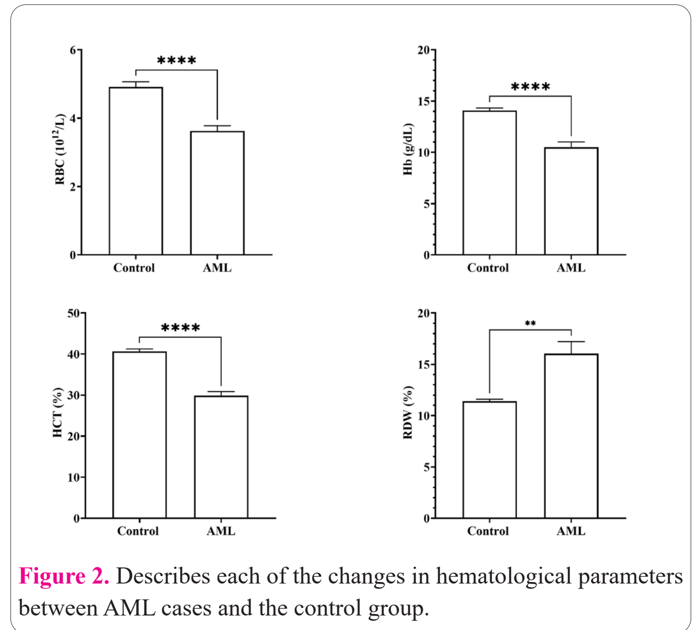

Figure 2. Describes each of the changes in hematological parameters between AML cases and the control group.

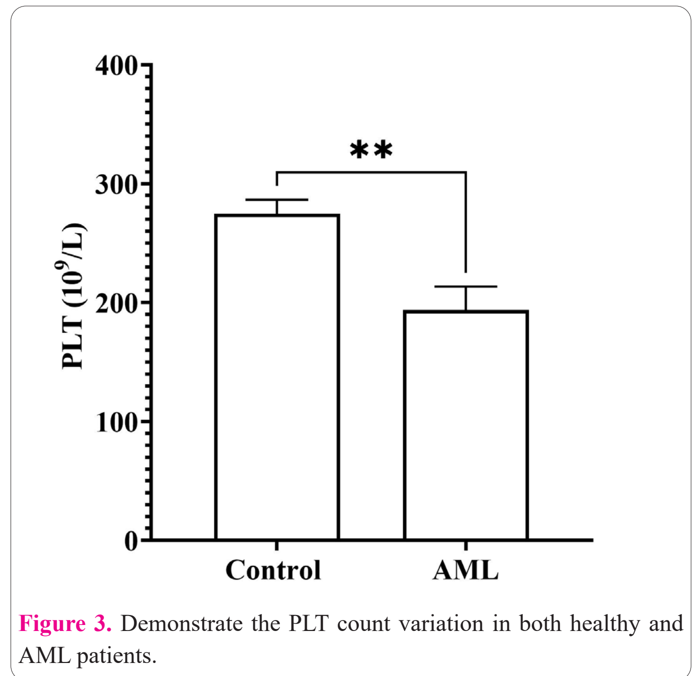

Figure 3. Demonstrate the PLT count variation in both healthy and AML patients.

The most commonly detected polymorphism in AML was an already known homozygous mutation (NCBI; dbSNP, 10833) resulting from a nucleotide substitution (97299T>C) at chromosome 4:142654547. The variant rate of this mutation was 100.00% in all cases. The second most frequently identified SNP was a known heterozygous mutation (NCBI; dbSNP, 2291596), also resulting from a nucleotide substitution, at the chromosomal locus 4:142654801. The variant rate of the above mutation was 62.50%. However, two novel heterozygous mutations, namely 7212A>AT and 97210T>TC, resulting from nucleotide substitution were also identified on the same chromosome (4:142654460 and 4:142654458 locus, respectively). The variant rate for 7212A>AT was 50.00%, while that for 97210T>TC was 33.30%. A less common and already known variation (NCBI; dbSNP, 9282743) was also detected (nucleotide substitution; 97270G>GT) in patients with AML at the 4:142654518 locus. The variant rate of this mutation was 28.60%. Furthermore, the following 10 novel heterozygous mutations were also identified in different locations of chromosome 4: 97371G>GA,

Table 2. Hematological Parameters in AML Patients: WBC, Lymphocyte, Monocyte, Granulocyte, RBC, Hb, HCT, RDW, PLT Count.

Hematological parameters	Control	AML	P-value
WBC count (10^3/L)	7.48±0.167	7.991±1.048	0.625
Lymphocyte (10^9/L)	1.984±0.131	2.417±0.473	0.521
Monocyte (10^9/L)	0.398±0.034	0.6450.14	0.194
Granulocyte (10^9/L)	5.209±0.217	6.129±1.449	0.654
RBC count (10^{12}/L)	4.914±0.149	3.626±0.153	0.001
Hb (g/dL)	14.09±0.223	10.5±0.515	0.001
HCT (%)	40.65±0.607	29.87±0.978	0.001
RDW (%)	11.41±0.19	16.04±1.169	0.01
PLT count (10^9/L)	274.7±11.93	193.7±19.77	0.01

Table 3. Variants identified in the IL-15 gene in AML patients were analyzed using DNA mutation survey.

	Chromosome Position	Mutation	Mutation genotype	Heterozygous/ Homozygous	Variants	Variant Percentage	External Database
1	4:142654458	Substitution	T>TC	Heterozygous	97210T>TC	33.30%	Not found
2	4:142654460	Substitution	A>AT	Heterozygous	97212A>AT	50.00%	Not found
3	4:142654518	Substitution	G>GT	Heterozygous	97270G>GT	28.60%	dbSNP:9282743
4	4:142654547	Substitution	T>C	Homozygous	97299T>C	100.00%	dbSNP:10833
5	4:142654619	Substitution	G>GA	Heterozygous	97371G>GA	12.50%	Not found
6	4:142654636	Substitution	G>GA	Heterozygous	97388G>GA	12.50%	Not found
7	4:142654644	Substitution	T>TG	Heterozygous	97396T>TG	12.50%	Not found
8	4:142654665	Substitution	G>GT	Heterozygous	97417G>GT	12.50%	Not found
9	4:142654679	Substitution	T>TA	Heterozygous	97431T>TA	12.50%	Not found
10	4:142654715	Substitution	T>TG	Heterozygous	97467T>TG	12.50%	Not found
11	4:142654725	Substitution	G>GA	Heterozygous	97477G>GA	12.50%	Not found
12	4:142654801	Substitution	C>CT	Heterozygous	97553C>CT	62.50%	dbSNP:2291596
13	4:142654818	Substitution	T>TC	Heterozygous	97570T>TC	12.50%	Not found
14	4:142655037	Substitution	T>TC	Heterozygous	97789T>TC	12.50%	Not found
15	4:142655058	Substitution	T>TC	Heterozygous	97810T>TC	12.50%	Not found

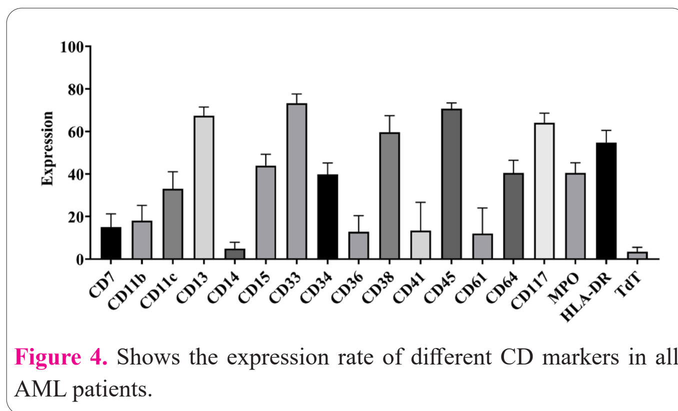

Figure 4. Shows the expression rate of different CD markers in all AML patients.

97388G>GA, 97396T>TG, 97417G>GT, 97431T>TA, 97467T>TG, 97477G>GA, 97570T>TC, 97789T>TC and 97810T>TC. The rate of the above variants was 12.50% and was rarely detected in patients with AML.

Database mutations

The total reported mutations of all cancer types acquired by gnomAD revealed that IL15 has 335 mutations, as shown in supplementary Table 1. The documented gene mutations in the Mutagene database in leukemia patients were further analyzed for the selected gene, as shown in supplementary Table 2. The most common type of mutations in the Mutagene database are missense mutations, followed by silent mutations, and the majority of them are potential driver mutations, followed by driver mutations.

Discussion

AML is caused by genetic aberrations and impaired myelocyte differentiation and proliferation at the stem cell stage. It has been reported that the abnormal secretion of cytokines or diminished cytokine receptor activation may also result in the onset of AML (29, 30). Therefore, investigating the pathogenesis and risk factors associated with the development of AML is of significant importance.

AML is characterized by a wide range of clinical features and changes in hematological parameters (7). The results showed that the total WBC and lymphocyte, monocyte and granulocyte counts were not significantly altered in patients with AML compared with healthy individuals. The total WBC count was directly decreased after treatment and reached the levels of individuals in the control group. This finding was consistent with that reported by Chang et al. in AML (31). Herein, PLT and total RBC counts, Hb and HCT levels, and RDW were notably enhanced in patients with AML compared with the healthy control group. These differences in hematological parame-

ters could be due to the alteration of megakaryocytes and erythroid precursor cells in the bone marrow of patients with leukemia (32).

The expression levels of various cytokines in AML were assessed in the present study using flow cytometry. All patients underwent flow cytometric immunophenotypic analysis. CD11c, CD15, CD34, CD64, MPO, and HLA-DR had expression levels between 20 and 60% in all cases, CD13, CD33, CD38, CD45, and CD117 had expression levels between 20 and 60%, and the remaining CD markers had expression levels of less than 20% in all cases. These results were in line with those of other researchers who studied acute myeloid leukemia (20, 22, 33). They observed that CD14 and CD15 are monocytic markers, CD13, CD33, CD41, CD42, CD61, CD117, and MPO are myeloid markers, and CD34, CD38, CD45, and HLA-DR are common progenitor markers used for AML diagnosis and classification (34-36).

In the present study, several substitution mutations were identified in different locations of chromosome 4 in patients with AML and the Mutagene database. Therefore, one homozygous mutation (97299T>C) with a variant rate of 100% and two heterozygous variations, namely 97553C>CT and 97212A>AT, with a variant rate of 62.50 and 50.00%, respectively, were identified in patients with AML. The above mutations were the most frequent ones, thus suggesting that they could be associated with AML progression. These findings were consistent with those reported by Aly et al. demonstrating that frequent genotype variations were also associated with the risk of acute lymphoblastic leukemia (ALL) (19). The results of the current study also revealed that a novel heterozygous mutation, namely 97210T>TC, with a variant rate of 33.00%, was also associated with the development of AML. Additionally, the variant rate of the 97270G>GT mutation (NCBI, dbSNP, 9282743) was 28.60%. The remaining detected mutations exhibited a lower variant rate (<20%) compared with the above ones and were associated with the onset of AML to a lesser extent. Yang et al. (37) were the first to evaluate the association between SNPs in the *IL15* gene and its expression in ALL lymphoblasts. Additionally, using genome-wide sequencing, the above study identified five SNPs in the *IL15* gene closely associated with the response of children with ALL to therapy. Furthermore, Wu et al. (38) demonstrated that *IL15* gene expression was strongly associated with the immunophenotype of leukemic cells.

Conclusion

In conclusion, the current study's findings suggested that various novel genetic variations observed in the *IL15* gene could have a significant impact on the development and pathogenicity of AML. These variants could be the result of changes in expression mechanisms or receptor expression regulation. These findings could lead to new insights into the genetic and molecular mechanisms of AML maturation, as well as a new treatment approach for AML.

Acknowledgements

The authors would like to express special thanks to all physicians, staff and AML patients of the Oncology Department of the Nanakaly Hospital-Erbil-Iraq and Salahaddin University-Erbil, Iraq, for helping and providing us with all facilities to achieve the present study.

Declarations Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Decot V, Xiong Y, Bensoussan D. IL-15 as a potential target in leukemia. Blood and Lymphatic Cancer: TargetsTher 2015; 5(55-63. doi: 10.2147/blctt.s78347.
2. Rausch C, Hiddemann W, von Bergwelt-Baildon M, Spiekermann K, Herold T. Acute Myeloid Leukemia - Update 2022. Dtsch Med Wochenschr 2022; 147(17): 1108-1114. doi: 10.1055/a-1758-2452.
3. Shimony S, Stahl M, Stone RM. Acute myeloid leukemia: 2023 update on diagnosis, risk-stratification, and management. Am J Hematol 2023; 98(3): 502-526. doi: 10.1002/ajh.26822.
4. M-Amen K, Abdullah OS, Amin AMS, Mohamed ZA, Hasan B, Shekha M et al. Cancer Incidence in the Kurdistan Region of Iraq: Results of a Seven-Year Cancer Registration in Erbil and Duhok Governorates. Asian Pacific J Cancer Prev 2022; 23(2): 601-615. doi: 10.31557/apjcp.2022.23.2.601.
5. Liu K, Hu J. Classification of acute myeloid leukemia M1 and M2 subtypes using machine learning. Comput Biol Med 2022; 147(105741. doi: 10.1016/j.compbiomed.2022.105741.
6. Jayavelu AK, Wolf S, Buettner F, Alexe G, Haupl B, Comoglio F et al. The proteogenomic subtypes of acute myeloid leukemia. Cancer Cell 2022; 40(3): 301-317 e312. doi: 10.1016/j.ccell.2022.02.006.
7. Rasheed A, Iqtidar A, Khan S. Hematological and biochemical changes in acute leukemic patients after chemotherapy. Zhongguo Yao Li Xue Bao 1996; 17(3): 207-208. doi.
8. Jalaeikhoo H, Kashfi SMH, Azimzadeh P, Narimani A, Gouhari Moghadam K, Rajaienejad M et al. Acute Myeloid Leukemia as the Main Cause of Pancytopenia in Iranian Population. Iran J Pathol 2017; 12(3): 265-271. doi: 10.30699/ijp.2017.25647.
9. Othieno-Abinya NA, Mwanda WO, Maina MD, Odhiambo AO, Mwanzi SA, Oyiro PO. Haematological parameters in chronic myeloid leukaemia as determinants of clinical manifestations in this disease. Journal of Clinical Oncology 2016; 34(15_suppl): e18529-e18529. doi: 10.1200/JCO.2016.34.15_suppl.e18529.
10. C P. Clinico-hematological study of acutemyeloid leukemias. J Clin Diagn Res 2014; 8(4): Fc14-17. doi: 10.7860/jcdr/2014/7854.4298.
11. Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. Blood 2001; 97(1): 14-32. doi: 10.1182/blood.v97.1.14.
12. Zhou Y, Husman T, Cen X, Tsao T, Brown J, Bajpai A et al. Interleukin 15 in Cell-Based Cancer Immunotherapy. Int J Mol Sci 2022; 23(13). doi: 10.3390/ijms23137311.
13. Giron-Michel J, Giuliani M, Fogli M, Brouty-Boyé D, Ferrini S, Baychelier F et al. Membrane-bound and soluble IL-15/IL-15Ralpha complexes display differential signaling and functions on human hematopoietic progenitors. Blood 2005; 106(7): 2302-2310. doi: 10.1182/blood-2005-01-0064.
14. Ma A, Koka R, Burkett P. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. Annu Rev Immunol 2006; 24(657-679. doi: 10.1146/annurev.immunol.24.021605.090727.
15. Bobbala D, Kandhi R, Chen X, Mayhue M, Bouchard E, Yan J et al. Interleukin-15 deficiency promotes the development of T-cell acute lymphoblastic leukemia in non-obese diabetes mice with severe combined immunodeficiency. Leukemia 2016; 30(8): 1749-1752. doi: 10.1038/leu.2016.28.
16. Steel JC, Waldmann TA, Morris JC. Interleukin-15 biology and

its therapeutic implications in cancer. Trends Pharmacol Sci 2012; 33(1): 35-41. doi: 10.1016/j.tips.2011.09.004.

17. Girard D, Boiani N, Beaulieu AD. Human neutrophils express the interleukin-15 receptor α chain (IL-15Rα) but not the IL-9Rα component. Clinical Immunology and immunopathology 1998; 88(3): 232-240. doi: 10.1006/clin.1998.4576.
18. Meghnem D, Morisseau S, Frutoso M, Trillet K, Maillasson M, Barbieux I et al. Cutting Edge: Differential Fine-Tuning of IL-2- and IL-15-Dependent Functions by Targeting Their Common IL-2/15Rbeta/gammac Receptor. J Immunol 2017; 198(12): 4563-4568. doi: 10.4049/jimmunol.1700046.
19. Aly RM, Taalab MM, Ghazy HF. Influence of interleukin-15 polymorphism on the survival of adult patients with acute lymphoblastic leukemia in Egypt. Leuk Lymphoma 2015; 56(1): 151-156. doi: 10.3109/10428194.2014.910659.
20. Rohnert MAA, von Bonin M, Kramer M, Ensel P, Holtschke N, Rollig C et al. Identification of Prognostic Immunophenotypes at First Diagnosis in Patients with Acute Myeloid Leukemia (AML) By a Standardized Multicolor Flow Cytometry (MFC) Panel Originally Designed to Detect Measurable Residual Disease (MRD) at Follow-up. Blood 2020; 136(Supplement 1): 35-35. doi: 10.1182/blood-2020-138683.
21. Aanei CM, Veyrat-Masson R, Selicean C, Marian M, Rigollet L, Trifa AP et al. Database-Guided Analysis for Immunophenotypic Diagnosis and Follow-Up of Acute Myeloid Leukemia With Recurrent Genetic Abnormalities. Front Oncol 2021; 11(746951. doi: 10.3389/fonc.2021.746951.
22. Piñero P, Morillas M, Gutierrez N, Barragán E, Such E, Breña J et al. Identification of Leukemia-Associated Immunophenotypes by Databaseguided Flow Cytometry Provides a Highly Sensitive and Reproducible Strategy for the Study of Measurable Residual Disease in Acute Myeloblastic Leukemia. Cancers 2022; 14(16): 4010. doi: 10.3390/cancers14164010.
23. Rasheed HM, Donia HM, Nadwan EA, Mourad ZI, Farahat N. Identifying Leukemia-associated Immunophenotypes in Acute Myeloid Leukemia Patients Using Multiparameter Flow Cytometry. Oman Med J 2021; 36(6): e323. doi: 10.5001/omj.2021.108.
24. Sanz MA, Sempere A. Immunophenotyping of AML and MDS and detection of residual disease. Baillieres Clin Haematol 1996; 9(1): 35-55. doi: 10.1016/s0950-3536(96)80036-9.
25. Oliveira GHdM, Júnior LSDAS, Silva AEMOE, Lima JPA, Soares Vl, Freitas RV et al. Clinical Utility of Flow Cytometry Immunophenotyping in Acute Myeloid Leukemia. Blood 2019; 134(Supplement_1): 5191-5191. doi: 10.1182/blood-2019-127099.
26. Galera PK, Jiang C, Braylan R. Immunophenotyping of Acute Myeloid Leukemia. Methods Mol Biol 2019; 2032(281-296. doi: 10.1007/978-1-4939-9650-6_15.
27. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007; 446(7137): 758-764. doi: 10.1038/nature05690.
28. Wu S, Gessner R, von Stackelberg A, Kirchner R, Henze G, Seeger K. Cytokine/cytokine receptor gene expression in childhood acute lymphoblastic leukemia: correlation of expression and clinical outcome at first disease recurrence. Cancer 2005; 103(5): 1054-1063. doi: 10.1002/cncr.20869.
29. Stone RM, O'Donnell MR, Sekeres MA. Acute myeloid leukemia. Hematology Am Soc Hematol Educ Program 2004; doi:10.1182/asheducation-2004.1.98: 98-117. doi: 10.1182/asheducation-2004.1.98.
30. Luciano M, Krenn PW, Horejs-Hoeck J. The cytokine network in acute myeloid leukemia. Front Immunol 2022; 13(1000996. doi: 10.3389/fimmu.2022.1000996.
31. Chang F, Shamsi TS, Waryah AM. Clinical and Hematological Profile of Acute Myeloid Leukemia (AML) Patients of Sindh. J Hematol Thromb Dis 2016; 4(1-5. doi.
32. Naeem R, Naeem S, Sharif A, Rafique H, Naveed A. Acute myeloid leukemia; demographic features and frequency of various subtypes in adult age group. Professional Med J 2017; 24(09): 1302-1305. doi: https://doi.org/10.29309/TPMJ/2017.24.09.820.
33. Matarraz S, Leoz P, Calvo X, García Alonso L, Ayala Bueno R, Sánchez-Gallego JI et al. Altered Immunophenotypes on Leukemic and/or Monocytic Cells from Acute Myeloid Leukemia Highly Predict for Nucleophosmin Gene Mutation. Blood 2019; 134(Supplement_1): 2687-2687. doi: 10.1182/blood-2019-131733.
34. Singh DK, Pathak V, Singh N, Singh RK, Kaif M, Yadav K. C1 lateral mass reduction screws for treating atlantoaxial dislocations: Bringing ease by modification. J Craniovertebr Junction Spine 2022; 13(2): 140-145. doi: 10.4103/jcvjs.jcvjs_8_22.
35. Grønningsæter IS, Reikvam H, Aasebø E, Bartaula-Brevik S, Tvedt TH, Bruserud Ø et al. Targeting Cellular Metabolism in Acute Myeloid Leukemia and the Role of Patient Heterogeneity. Cells 2020; 9(5): 1155. doi: 10.3390/cells9051155.
36. Mason EF, Hasserjian RP, Aggarwal N, Seegmiller AC, Pozdnyakova O. Blast phenotype and comutations in acute myeloid leukemia with mutated NPM1 influence disease biology and outcome. Blood Adv 2019; 3(21): 3322-3332. doi: 10.1182/bloodadvances.2019000328.
37. Yang JJ, Cheng C, Yang W, Pei D, Cao X, Fan Y et al. Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. JAMA 2009; 301(4): 393-403. doi: 10.1001/jama.2009.7.
38. Wu S, Fischer L, Gokbuget N, Schwartz S, Burmeister T, Notter M et al. Expression of interleukin 15 in primary adult acute lymphoblastic leukemia. Cancer 2010; 116(2): 387-392. doi: 10.1002/cncr.24729.