IL-15 gene mutation as a molecular risk factor in lymphoid leukemia

Sarbaz I. Mohammed[1]*, Kawa Muhammed-amin Hasan, Darya Mohammed Aziz[1], Fikry Ali Qadir[1], Abbas Burhan Salih[1]
[1] Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq
[2] Ministry of Health, Kurdistan Region, Iraq

ABSTRACT

The present study investigated the relationship between polymorphisms in the interleukin (IL)-15 gene located on exon 8 and acute lymphoblastic leukaemia (ALL) risk in Iraqi patients. A total of 78 (49 male -29 female) primary ALL (62B-cell, 16 T-cells lineages cases and 30 healthy control subjects (median age 11, age range 4-21.5), were enrolled at the Nanakaly Hospital of Erbil Province between March 2020 and January 2021. The genotype analysis was performed using polymerase chain reaction and Sanger DNA sequencing. The IL15 homozygous rs10833 (100%) and rs2291596 (63.6%) genotypes indicated high frequencies and were associated with a risk of developing ALL, while the remaining 16 novel mutations indicated in low frequency (9.1%) except for the 97270G>GT genotype (18.2%). High expression levels were noted for different cluster of differentiation (CD) biomarkers between both subtypes of ALL, including , CD10, CD19, CD22, CD79a, CD99, , terminal deoxynucleotidyl transferase, and human leukocyte antigen DR isotype in B-cells lineages, while, CD2, CD3, CD5, CD7, CD13, CD117 and TdT are more specific to T-cells lineages. On the other hand, significant changes were noted in certain hematological parameters compared with those of healthy subjects. Finally, it was concluded that various novel mutations recorded with different subtypes of ALL diseases. Future studies will be towered to associate of these mutations with prognosis and therapeutic response of diseases.

Keywords: IL-15, CD markers, ALL, SNPs, Anemia

Introduction

Acute lymphoblastic leukaemia (ALL) forms a heterogenous group of life-threatening hematopoietic stem cell neoplasm of B or T lymphoblasts which is characterized by bone marrow malignant cell infiltration. This process impacts normal blood cell formation and affects the expression levels of a set of clusters of differentiation (CD) biomarkers. It is the most common childhood and adult malignancy (1-3).

Interleukin (IL)-15 play an essential role in enhancing or inhibiting native and acquired immune responses (4-6). IL15 is a member of the IL2 cytokine group located on chromosome 4. It is released by different immune cells and has been related to the inflammatory process during the development of malignancy. IL15 has a vital function in natural killer (NK) cell homeostasis. It can stimulate T-cells ($CD8^+$) and NK cells, which in turn enhance anti-cancer responses and act as a novel immunomodulators for the treatment of various malignancies (7-10).

Waldmann et al, (11) indicated that exposure to IL-15 altered the ability of NK cells and induced a moderate elevation in T-cells numbers ($CD8^+$) which controlled tumors progression and provided novel perspectives for treating white blood cells malignancies. Administration of IL-15 or IL-15 releasing leukaemia cells in a leukemic mouse model led to an immune response against leukemia progression (12, 13).

Several genotypes of IL-15 have been identified and linked with the biological aspects of leukaemia. It has been shown that the single nucleotide polymorphisms (SNPs) rs17015014, rs17007695, rs10519613, and rs10519612 of the IL15 gene are strongly associated with the treatment response of young patients with lymphocytic leukemia (14). In contrast to these findings, the rs17007695 and rs10519612 genes were highly correlated

with the incidence of leukemia. These SNPs have shown to be significantly associated with the treatment response of infants with acute LL (ALL) (15).

(16) demonstrated that the presence of the rs10519612 and rs10519613 genotypes containing the A and C substitutions significantly increased the risk of developing early age B cell lymphocytic leukemia following two doses of treatments. The rs10519612 and rs17007695 genotypes contained the A and T substitutions, respectively and the rs17007695, rs10519613, and rs10519612 genotypes contained (A, C, and T substitutions, respectively. These genotypes exert a protective effect on the development of acute leukemia following low and high doses of treatment.

Aly et al (17) further found that several IL-15 variants contribute to the development of ALL in Egyptian subjects, such as the rs10519612 and the rs17007695 polymorphisms detected in the in the T- and B cells of subjects with ALL, respectively. Several genetic variations of IL-15 may provide important prognostic information in leukemic patients (18).

The findings of (19) domenstrated that the A>C substation of the rs2228059 polymorphism found in the IL15 receptor may be involved in the esophageal carcinoma, and stomach cancer (20) whereas the rs10519613 polymorphism may be an independent factor affecting the development of hepatocellular carcinoma following a liver transplantation (21). In contrast to these findings, (22) observed no relationship between the presence of the two SNPs rs10519613 and rs35964658 and the incidence of acute leukemia.

CDs are surface markers with a particular function depending on the cell type, and they can be independently expressed in response to extracellular and intracellular physiological stimuli. The expression levels of different CD biomarkers have been detected on T and B-lymphocytes [CD2, 3, 5, 7, 8, 10,

13, 19, 20, 33, 34, 45, 64, 79a, 117, myeloperoxidase (MPO) and (TdT) (23) (24) (25) (26). A previous study revealed that concurrent evaluation of CD45, CD56 and CD3 in addition to CD19, CD271 and CD2 could be used as a diagnostic method of malignancy in children (27) (1). Therefore, the identification of the predictive efficacy of phenotypes with abnormal expressions of CD biomarkers could be used to assist the diagnosis and the clinical progression of leukemic patients, which may improve medical treatment options (28).

The current study aimed to determine the association between IL15 genotype mutations and the development and progression of childhood and adult ALL in Iraqi patients.

2. Materials and Methods

Patient subject

The current investigation was based on a case-control study design. Seventy-eight (49 males and 29 females) Seventy-eight (49 males and 29 females) patients with ALL (62 B-cell- 16 T-cells linages) who were admitted to the Oncology Department of the Nanakaly Hospital (Erbil/ Kurdistan Region/ Iraq) for examination and diagnoses between February 2021 and December 2021. Peripheral blood and bone marrow biopsies (BMB) were obtained from newly diagnosed acute leukemic patients and 30 healthy individuals used as a control. Their median age was 11 years (age range 4-21.5) years, divided into three groups (less than 15≤, 16-40, and more than <40 years). The patients with ALL were preferably diagnosed by the following clinical screening tests; medical history, physical examination, Complete blood pictures(CBP), bone marrow aspiration & biopsy analysis, immunophenotyping analysis and cytogenetic studies.

Blood collection

The blood samples were collected by phlebotomy in an EDTA-tube at the Oncology Department, Nanakaly Hospital, in Erbil/Iraq. Subsequently, the peripheral blood samples were divided into three parts as follows:- One tube was used for routine CBC test examination, one for immunophenotyping analysis and one for DNA extraction and sequencing. The latter was directly transferred to the Immunogen Center, Erbil, Iraq.

Bone marrow aspiration and biopsies

The bone marrow aspiration and biopsy specimens were taken from newly diagnosed ALL followed at the Oncology Department, Nanakaly Hospital, in Erbil/Iraq. The specimens for cellular assessment and flow cytometry analysis.

Genotype determination

The current study analyzed the genotype of IL-15(gene exon 8) on the chromosomal location 4q31.21 (Immunogene Center, Erbil-Kurdistan region - Iraq). According to the manufacturer's operating instructions, the DNA was initially extracted to detect polymorphic variants from blood cells of patients with ALL who were diagnosed early, using the AddPrep (Genomic DNA Extraction Kit Add Bio, KOREA). A NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc, USA) was used to quantify the DNA. The polymerase chain reaction (PCR) amplification was performed using the AddPrep kit according to the manufacturer’s protocol with 35 amplification cycles (AB Applied Biosystems). The following primers were used: IL-15 exon eight

forward, 5'-CTATGCTGGTAGGCTCCTG-3` and reverse, 5`-GTTCCATTAGAAGAGAGCTTGC-3`.

PCR was performed using the following steps: Denaturation for (5 min at 95℃); denaturation for 30 sec at 95℃, annealing for 30 sec at 56℃ and elongation at 72℃ for 30 sec; and a final extension step was performed at 72℃ for 5 min. The DNA products were isolated by electrophoresis in 3% agarose gels and with a reference 50 bp DNA ladder in one lane. Subsequently, they were stained with Safe DNA Gel Stain dye (Add Bio, Inc.). The stained DNA bands were visualized using a UV light source using UV Transilluminator (UST-20M-8K; Biostep GmbH).

Following PCR, DNA extraction was performed so as to obtain the DNA samples to be used for the sequencing process. The later was performed using the automatic gene analyzer 3130 Genetic Analyzer (Applied Biosystems). 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) (https://www.ncbi.nlm.nih.gov/genbank)

Flow cytometry analysis

The collected bone marrow biopsy and fresh peripheral blood samples of the newly diagnosed acute leukemic patients were maintained at room temperature and subsequently processed for the detection of specific several clusters of differentiation CD markers to T and B cells, the standard antibody panel and a FACSCanto II flow cytometer device (BD Biosciences, San Jose, California, USA were used. A positive result was obtained when expression > 20% of CD markers.

CBP measurements

The blood fluids (n=78) were collected in the EDTA tubes and sent to the haematology laboratory of the Nanakaly Hospital-Erbil-Iraq. Total blood cell count and differential white blood cells counts were examined using (an automated Coulter Ac.T diff Hematology analyzer (Beckman Coulter, Inc.)

Statistical Analysis

All statistical analyses were performed using GraphPad prism 8. The values were expressed as mean ± Standard Error and median. Normality was tested using the Anderson-Darling test, D'Agostino & Pearson test, Kolmogorov-Smirnov, and Shapiro-Wilk test. . For blood parameter analysis, an unpaired t-test was used, and a one-way ANOVA with Tukey's multiple comparisons test was used to assess statistically significant differences in HLA-DR among different ages. $P<0.05$ was considered to be a statistically significant difference.

Results

The result indicate that the severity of the diseases is higher in childhood (61.6%) than in adult (38.4%) and cells linage of diseases B-cells (79.365%) and T-cells (20.635%).

Genomic polymorphism of IL-15: The IL-15 (exon 8) gene mutations were analyzed using Mutation Surveyor 5 (Table 1).

A total of 18 variations were obtained in exon 8 of the IL-15 gene at chromosome 4q31.21. The sequencing results of IL-15 resulted in the identification of different types of genomic variations: Specifically, a total of 16 nucleotide substitutions were observed in the IL-15 mutation genotypes with the following frequencies: Two G>GA, three G>GT, one A>AC, two

A>AT, three T>TC, one T>C, two T>TA, one C>CA, one C>T. One deletion (97381delA) and one insertion (97378_97379insS) were also noted. The homozygous variant (97299T>C) exhibited a frequency 9.1% and the heterozygous variant (97229G>GA) a frequency of 100% on chromosome location 4:142654547; the homozygous variant (97553C>T) was found on chromosome location 4:142654801 and was previously registered in the external databases as reference SNPs 10833 and dbSNP:2291596 (63.6%) respectively. Although the heterozygous variants (97270G>GT) were found on position 4:142654518, its mutation percentage was 18.2% in the patients with ALL investigated in the current study.

However, the remaining novel substitution, insertion and deletion variants of IL-15 were not found or recorded in the external databases and no missense mutation record was reported in the current study. All IL-15 mutations noted in patients with LL did not alter the amino acid sequence of this cytokine.

Aberrant CD antigens in ALL

The result of aberrant CD markers expression in B-cells and T-cells ALL are shown in Fig. 1, 2, 3 and 4 . The results of the flow cytometry analysis assist in the subtypes classification of newly diagnosed patients with ALL in Iraqi population into B-cells and T-cells lineages by using some specific CD markers. The result indicated that aberrant CD10, CD19, CD22, CD34, CD38, CD45, CD79a, CD99, TdT and human leukocyte antigen DR isotype (HLA-DR) were expressed at a high percentage in all age group patients with B-cells ALL. While CD2, CD3, CD5, CD7, CD13, CD34, CD38, CD45, CD99, CD117 and TdT have expressed in a different rate of percentage in all age levels of patients with subtype T-ALL lineage. Whereas, CD1a, CD11b, CD11c, CD14, CD15, CD20, CD21, CD23, CD36, CD64, CD200, MPO,

BCL2, Kappa and Lambda recorded as negative results (≤20%) in both subtypes of ALL. Finally, in the present study, the age groups had no effect on percentile expressions of CD markers except in HLA-DR% Fig. 4.

Hematological analysis of patients with ALL

The complete blood count analysis investigated significant differences between the patients with ALL and the healthy subjects. Fig. 5,6, & 7 display a summary of the white blood count (WBC), red blood count (RBC), and platelets counts corresponding to patients with ALL. The RBC count, hemoglobin (Hb) concentration, hematocrit (Hct) %, and platelet count decreased significantly (0.0001), while the red cell distribution width (RDW%) was significantly increased (0.0001) compared with the corresponding values noted in healthy subjects. By contrast, leukemic blood analysis indicated that the WBC count, absolute lymphocyte count, and number of monocytes were increased; however, no significant differences were observed. Finally, the absolute granulocyte count indicated a nonsignificant reduction in leukemic patients compared with that noted in healthy subjects.

Discussion

In the current study, different SNPs of the IL15 gene have been recorded to be related to different stages of both subtype diseases. Also, invistgated the role of expression levels of CD markers in patients with LL. These biomarkers can be used as a diagnostic immunophenotype for the definition and classification subtypes of acute of leukemia.

The examination of the sequencing result of the IL-15 gene indicated that 18 genomic mutations were identified in exon 8s of a patient with both

subtypes of ALL. Two SNPs have been previously charachterized in NCBI as rs10833 (frequency 100%) and rs2291596 (63.6%), and the remaining variations represent new SNPs that have not been previously reported in the external database. These SNPs could be contributed to the pathogenesis of diseases through they impact IL15 expression. Polymorphisms in the exon 8 regions of the IL-15 gene may influence cancer development by altering the expression levels of IL-15 in the serum.

Previous studies have demonstrated the presence of several mutations in IL15 that are significantly associated with the therapeutic response of patients with childhood leukemia (15). (14) observed that the mutations of the SNPs 10519612 and 17007695 were more frequent in adult leukemic individuals than in healthy subjects. (17) observed an association between the mutations of the rs10519612, rs17007695 genotypes and the threat of progressing leukemia. The rs10519613 and rs17007695 genotypes were linked mainly with minimal residual disease at the late and early access treatments, respectively (15)(29). Rots *et al* (16) revealed a relation between the presence of SNPs (17007695, 10519613, and 10519612) and the threat of growing adult and early age acute LL while these SNPs did not affect the treatment response.

Previous study indicated that high expression levels of the IL-15 gene were associated with the growth of leukemic cancers (30)(14). Polymorphisms in the IL15 gene may influence the assessment of an advanced threat assignment plan in the treatment of pediatric ALL (31). SNPs in IL-15 receptor A (IL15RA; rs2228059) may lower the threat of developing esophageal tumors (20). The IL-15 mutation corresponds to an enhanced risk of developing lung tumors (32).

Previous studies investigated the effects of the variation of the IL15 genotypes in response to treatment for cancer inflammation. Different mechanisms were reported, such as the ability of IL15 SNPs to promote NK, B-cell and CD8+ T-cell functions. High serum levels of IL-15 and IL-15RA have been reported in certain patients with tumors; this considered to be an immune escape mechanism. Previous results have also reflected an excellent immune response against the induction of inflammation in subjects with IL15 polymorphisms, which could aid in the control of the disease (33) (34). lL-15 signifies efficacy for treatment due to its efficiency in enhancing the antineoplastic function of defense activator cells (35). Zhang et al (36) suggested that regular assessments of IL15 polymorphisms should be performed in combination with cancer antibodies for the treatment patients at the last stages of neoplastic disease.

The current study indicated the role of specific diagnostic CD markers in improving the diagnosis of T-cell and B-cell linage of ALL, including the following: CD99, CD10, CD19, CD45, CD79a and b, TdT, and HLA-DR; these markers were expressed at a high percentage in B-cells lineage. Moreover, other prognostic CD markers were highly expressed in T-cell lineage acute the leukemic patients investigated in the current study, such as CD2, CD3, CD5, CD7, CD13, CD117 and TdT. The results were confirmed by (28, 37) who domenstrated the role of analyzing CD marker expression as a diagnostic tool (e.g. CD19, CD22, and CD79a) and as a monitoring marker for assessing the prognosis of patients with B-cells acute lymphocytic leukemia (B-ALL); this method was also improved by (38) who noted that CD19, CD20, and CD22 expressed in different ranges in acute B-LL. (27) who demonstrated a high frequency of CD13 and CD33 expression in patients with ALL and a low frequency of CD117 expression (26).

Shahrabi et al (39) highlighted that the evaluation of the mutations of CD markers (CD33, CD44, and CD38) could be used to validate the primary diagnosis of hematological malignancies, their prognosis and the patient response to treatment. The CD13, CD14, CD15, and CD33 immunophenotyping examination of ALL is adequate for detecting and categorizing cases of leukemia (40).

The detection of CD200 marker expression appears to be helpful in the diagnosis of certain cancer types, such as leukemia and their corresponding treatment. Patients who present with CLL, ALL and low CD200 marker expression are associated with a shorter treatment time than patients with CLL, ALL, and a higher expression of CD200 (41) (42) (43) (44).

The results indicated that the majority of the individuals with newly diagnosed B-cells and T-cells ALL diseases had certain common CBC abnormalities. Moreover, RBC, Hb levels, the Hct percentage, and the platelet count were lower (0.0001) while RDW was significantly increased (0.0001) in patients with ALL compared with those noted in healthy subjects. These findings were confirmed by (45) who revealed that anemia and thrombocytopenia were associated with the majority of patients with leukemia. Considerable diversity was noted in the analysis of CBC results in leukemic subjects; this heterogeneity resulted from a period of diagnosis, the presence of variable mutations and SNPs involved in developing the disease, and other biological factors influencing these parameters. Furthermore, leukocytosis was not frequently repeated compared with leukopenia in children with malignancies. (46) also demonstrated that investigated that anemia and platelet deficiency were most frequent in leukemia due to abnormal hematopoiesis or infiltration of the bone marrow with malignant

stem cells (47). One of the limitations of this study is the restricted number of analyzed samples and SNPs used in this study that limits the evaluation of the results.

Finally, in the present study, different novel mutations were observed in the interleukin-15 gene associated with both subtypes of acute lymphocytic leukemia. indicated a significant association between several novel polymorphisms of the IL15 SNPs may be essential in the development, diagnoses and disease prediction of lymphocytic leukemia in Iraqi patients. The data revealed a relationship between rs2291596, rs10833 and 16 other novel mutations with ALL disease in Iraqi patients. A significant relation between expression percentage of different diagnostic cluster differentiation and subtypes of disease. Also, some hematological parameters with the disease in compression with healthy subjects. Future studies will be aimed to find a significant relationship of these variant SNPs and different CD markers with disease progression, prognosis and therapeutic response..

Acknowledgements

The authors would like to express special thanks to all physicians, staff and with LL patients of 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.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DMA, KMH and FAQ performed experiments. SIM designed the experiments, analyzed data and co-wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was authorized and approved by the Human Ethics Committee of Salahaddin University-Erbil, Erbil, Iraq. Reference No: 7/54/589; date, 4/2/2021; Erbil-Iraq.

Patient consent for publication

All patients provided written informed consent for the publication of data in the present study.

Competing interests

The authors declare that they have no competing interests.

References

1. Ferreira-Facio CdS, Botafogo V, Ferrão PM, et al.: Flow Cytometry Immunophenotyping for Diagnostic Orientation and Classification of Pediatric Cancer Based on the EuroFlow Solid Tumor Orientation Tube (STOT). Cancers 13: 4945, 2021.
2. Puckett Y and Chan O: Acute Lymphocytic Leukemia. In: StatPearls [Internet] StatPearls Publishing2021.
3. Puckett Y and Chan O: Acute Lymphocytic Leukemia. In: StatPearls StatPearls Publishing

Copyright © 2022, StatPearls Publishing LLC., Treasure Island (FL)2022.
4. Waldmann TA: The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. Nature Reviews Immunology 6: 595-601, 2006.
5. Patidar M, Yadav N and Dalai SK: Interleukin 15: A key cytokine for immunotherapy. Cytokine & growth factor reviews 31: 49-59, 2016.
6. Allard-Chamard H, Mishra HK, Nandi M, et al.: Interleukin-15 in autoimmunity. Cytokine 136: 155258, 2020.
7. Morris JC, Ramlogan-Steel CA, Yu P, et al.: Vaccination with tumor cells expressing IL-15 and IL-15Rα inhibits murine breast and prostate cancer. Gene therapy 21: 393-401, 2014.
8. Raeber ME, Zurbuchen Y, Impellizzieri D and Boyman O: The role of cytokines in T-cell memory in health and disease. Immunological reviews 283: 176-193, 2018.

9. Waldmann TA: Cytokines in cancer immunotherapy. Cold Spring Harbor perspectives in biology 10: a028472, 2018.
10. Waldmann TA, Miljkovic MD and Conlon KC: Interleukin-15 (dys) regulation of lymphoid homeostasis: Implications for therapy of autoimmunity and cancer. Journal of Experimental Medicine 2172020.
11. Waldmann TA, Dubois S, Miljkovic MD and Conlon KC: IL-15 in the Combination Immunotherapy of Cancer. Frontiers in Immunology 11: 868, 2020.
12. Felices M, Lenvik AJ, McElmurry R, et al.: Continuous treatment with IL-15 exhausts human NK cells via a metabolic defect. JCI insight 32018.
13. Berger A, Colpitts SJ, Seabrook MS, et al.: Interleukin-15 in cancer immunotherapy: IL-15 receptor complex versus soluble IL-15 in a cancer cell-delivered murine leukemia model. Journal for ImmunoTherapy of Cancer 7: 1-13, 2019.
14. Lin D, Liu C, Xue M, et al.: The role of interleukin-15 polymorphisms in adult acute lymphoblastic leukemia. PLoS One 5: e13626, 2010.
15. Yang JJ, Cheng C, Yang W, et al.: Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. Jama 301: 393-403, 2009.
16. Rots D, Kreile M, Nikulshin S, Kovalova Z and Gailite L: Influence of IL15 gene variations on the clinical features, treatment response and risk of developing childhood acute lymphoblastic leukemia in Latvian population. Pediatric Hematology and Oncology 35: 37-44, 2018.
17. Aly RM, Taalab MM and Ghazy HF: Influence of interleukin-15 polymorphism on the survival of adult patients with acute lymphoblastic leukemia in Egypt. Leukemia & Lymphoma 56: 151-156, 2015.
18. Xiong Y, Bensoussan D and Decot V: IL-15 as a potential target in leukemia. Blood and lymphatic cancer: targets and therapy 5: 55—63, 2015.
19. Yin J, Wang L, Shi Y, et al.: IL-15 receptor alpha rs2228059 A> C polymorphism was associated with a decreased risk of esophageal cancer in a Chinese population. Molecular biology reports 41: 1951-1957, 2014.
20. Yin J, Liu C, Wang X, et al.: Interleukin 15 receptor alpha rs2228059 A> C polymorphism decreased risk of gastric cardiac adenocarcinoma in a Chinese population. Tumor Biology 35: 6593-6600, 2014.
21. Zhang T, Liu Y, Peng X, Fan J and Peng Z: Association between recipient IL-15 genetic variant and the prognosis of HBV-related hepatocellular carcinoma after liver transplantation. Disease Markers 20172017.
22. El-Ghobashy Y, Azmy R, Dawood A, Kelany A, Shaltot E and El-Daly M: ASSESSMENT OF INTERLEUKIN 15 (IL-15) GENE POLYMORPHISM IN ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA. Egyptian Journal of Biochemistry & Molecular Biology 30: 117-136, 2012.
23. Mazher N, Malik N, Imran AK and Chughtai O: Aberrant Expression of CD Markers in Acute Leukemia. 2013.
24. Gorczyca W: Flow Cytometry in neoplastic hematology: morphologic-Immunophenotypic correlation. CRC Press2017.
25. Cuéllar-Mendoza ME, Chávez-Sánchez FR, Dorantes-Acosta E, Arsuaga-Jiménez BM and Zapata-Tarrés M: Aberrant immunophenotypes in acute lymphoblastic leukemia. Boletín médico del Hospital Infantil de México 77: 287-292, 2020.
26. Shahni A, Saud M, Siddiqui S and Mukry SN: Expression of aberrant antigens in hematological malignancies: A single center experience. Pakistan journal of medical sciences 34: 457, 2018.

27. Kavianpour M, Ketabchi N and Saki N: Prognostic significance of aberrant expression of CD markers in acute lymphoblastic leukemia. memo-Magazine of European Medical Oncology 10: 164-169, 2017.
28. Vosoughi T, Bagheri M, Hosseinzadeh M, Ehsanpour A, Davari N and Saki N: CD markers variations in chronic lymphocytic leukemia: new insights into prognosis. Journal of Cellular Physiology 234: 19420-19439, 2019.
29. Dawidowska M, Kosmalska M, Sędek Ł, et al.: Association of germline genetic variants in RFC, IL15 and VDR genes with minimal residual disease in pediatric B-cell precursor ALL. Scientific reports 6: 1-15, 2016.
30. Wu S, Fischer L, Gökbuget N, et al.: Expression of interleukin 15 in primary adult acute lymphoblastic leukemia. Cancer: Interdisciplinary International Journal of the American Cancer Society 116: 387-392, 2010.
31. Lu Y, Kham SKY, Ariffin H, et al.: Host genetic variants of ABCB1 and IL15 influence treatment outcome in paediatric acute lymphoblastic leukaemia. British Journal of Cancer 110: 1673-1680, 2014.
32. Van Dyke AL, Cote ML, Wenzlaff AS, et al.: Cytokine and cytokine receptor single-nucleotide polymorphisms predict risk for non-small cell lung cancer among women. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 18: 1829-1840, 2009.
33. Gupta R, Li W, Yan XJ, et al.: Mechanism for IL-15–Driven B Cell Chronic Lymphocytic Leukemia Cycling: Roles for AKT and STAT5 in Modulating Cyclin D2 and DNA Damage Response Proteins. The Journal of Immunology 202: 2924-2944, 2019.
34. Fiore PF, Di Matteo S, Tumino N, et al.: Interleukin-15 and cancer: some solved and many unsolved questions. J Immunother Cancer 82020.
35. Isvoranu G, Surcel M, Munteanu AN, et al.: Therapeutic potential of interleukin-15 in cancer (Review). Exp Ther Med 22: 675, 2021.
36. Zhang M, Wen B, Anton OM, et al.: IL-15 enhanced antibody-dependent cellular cytotoxicity mediated by NK cells and macrophages. Proceedings of the National Academy of Sciences 115: E10915-E10924, 2018.
37. Yu X, Li P, Feng H, et al.: CD22 Gating for Flow Cytometric Analysis in Relapsed/Refractory B-Cell Acute Lymphoblastic Leukemia after CD19 CAR-T Therapy. Blood 136: 13-13, 2020.
38. Fousek K, Watanabe J, Joseph SK, et al.: CAR T-cells that target acute B-lineage leukemia irrespective of CD19 expression. Leukemia 35: 75-89, 2021.
39. Shahrabi S, Ghanavat M, Behzad MM, Purrahman D and Saki N: CD markers polymorphisms as prognostic biomarkers in hematological malignancies. Oncology reviews 14: 466, 2020.
40. Hamed EO and El-Deen AF: Flow Cytometric Diagnosis of Acute Leukemia and Aberrant Antigen: Sohag University Experience. Open Journal of Blood Diseases Vol.08No.02: 12, 2018.
41. Miao MH, Ji XQ, Zhang H, Xu J, Zhu H and Shao XJ: miR-590 promotes cell proliferation and invasion in T-cell acute lymphoblastic leukaemia by inhibiting RB1. Oncotarget 7: 39527-39534, 2016.
42. Arlindo EM, Marcondes NA, Fernandes FB and Faulhaber GAM: Quantitative flow cytometric evaluation of CD200, CD123, CD43 and CD52 as a tool for the differential diagnosis of mature B-cell neoplasms. Revista brasileira de hematologia e hemoterapia 39: 252-258, 2017.
43. D'Arena G, Vitale C, Rossi G, et al.: CD200 included in a 4-marker modified Matutes score provides optimal sensitivity and specificity for the diagnosis of chronic lymphocytic leukaemia. Hematological Oncology 36: 543-546, 2018.

44. Zahedi M, Khorasani SSK, Nasirzadeh A, et al.: Promising Role of CD200 Expression in Diagnosis and Prognosis of Chronic Lymphocytic Leukemia: A Review. International Journal of Medical Investigation 8: 16-22, 2019.
45. Jaime-Pérez JC, García-Arellano G, Herrera-Garza JL, Marfil-Rivera LJ and Gómez-Almaguer D: Revisiting the complete blood count and clinical findings at diagnosis of childhood acute lymphoblastic leukemia: 10-year experience at a single center. Hematology, transfusion and cell therapy 41: 57-61, 2019.
46. Moussavi F, Hosseini S, Saket S and Derakhshanfar H: THE FIRST CBC IN DIAGNOSIS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA. International Journal of Medical Investigation 3: 0-0, 2014.
47. Abdullah NF, Abo- El Wafa HA, Allam AA and Mohamed AM: Aberrant Expression of CD Markers in Cases with Acute Leukemia in Sohag University Hospital. Sohag Medical Journal 22: 287-295, 2018.

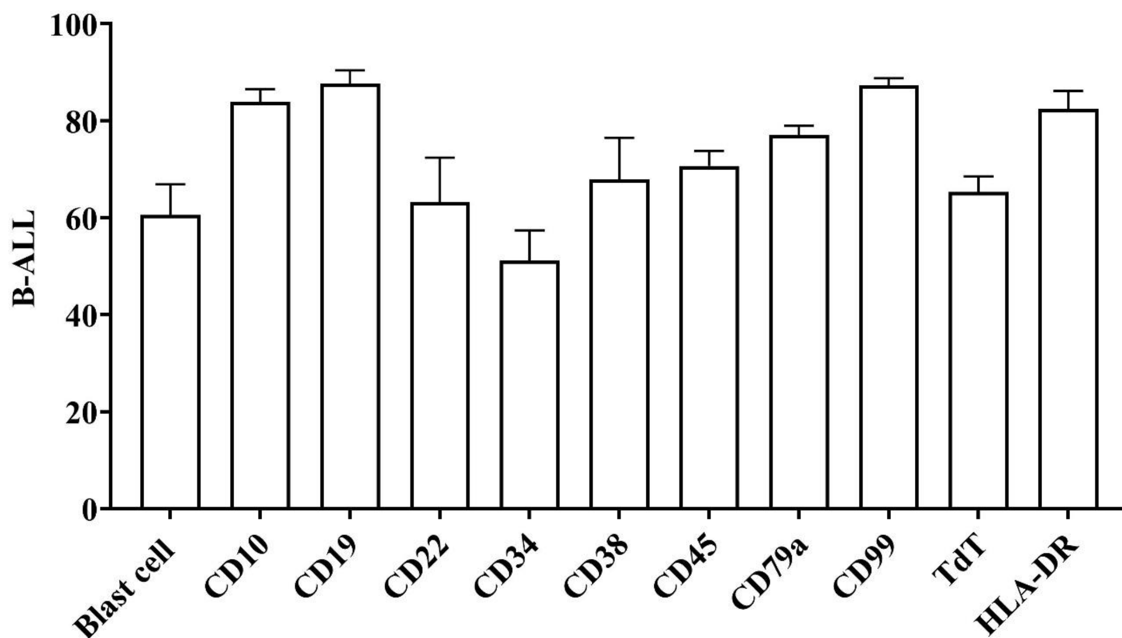

Figure 1: Expression percentage of different diagnostic cluster differentiation (CD) in BMB and blood of B-cells acute lymphoblastic leukemia patients.

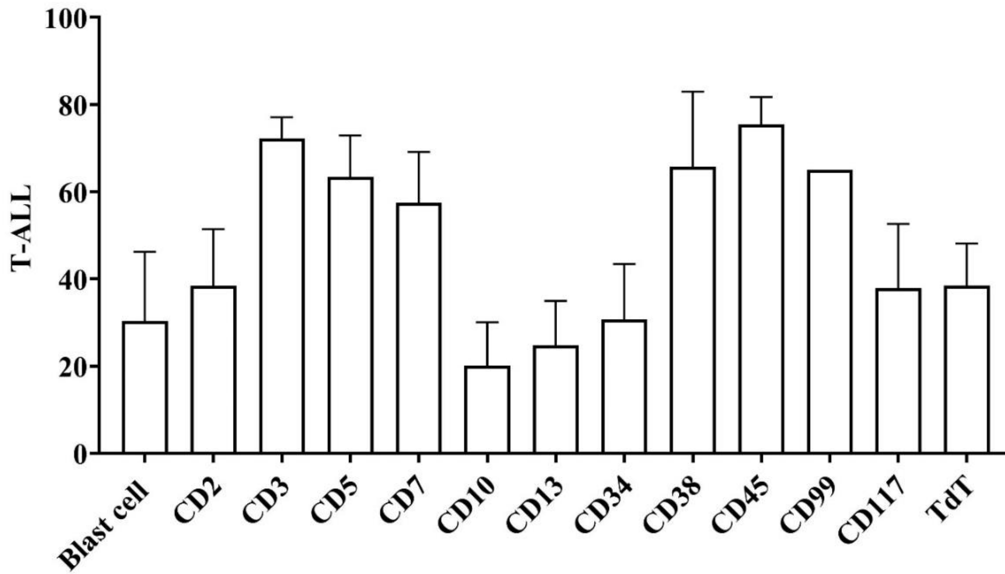

Figure 2: Expression percentage of different diagnostic cluster differentiation (CD) in BMB and blood of T-cells acute lymphoblastic leukemia patients.

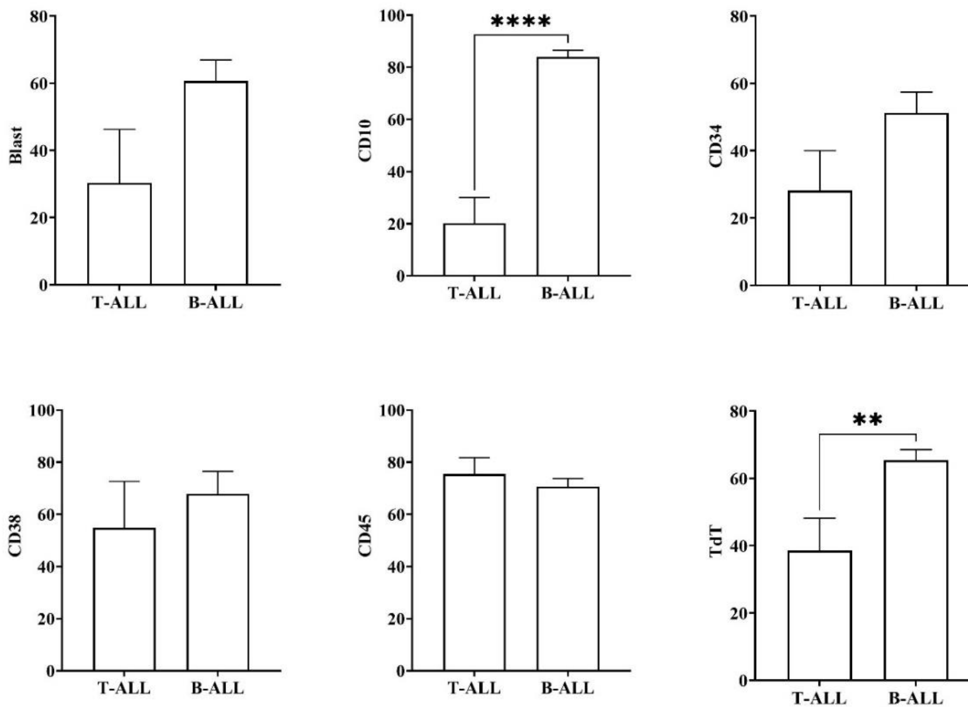

Figure 3: Expression percentage of different diagnostic cluster differentiation (CD) between subtypes of acute lymphoblastic leukemia patients.

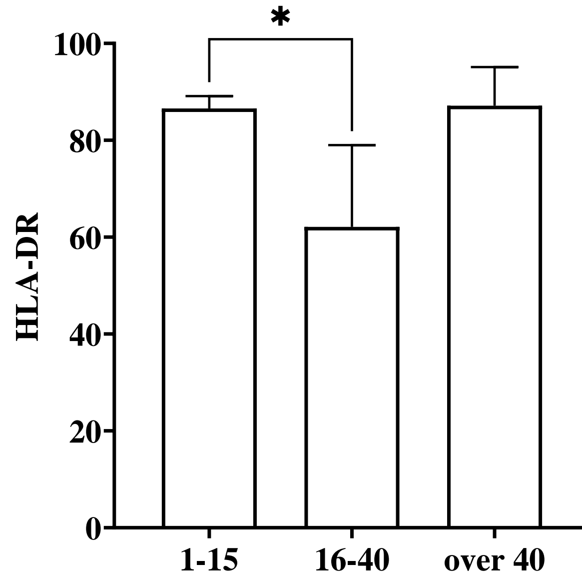

Figure 4: Role of age in the expression percentage of HLA-DR in B-cells acute lymphoblastic leukemia patients.

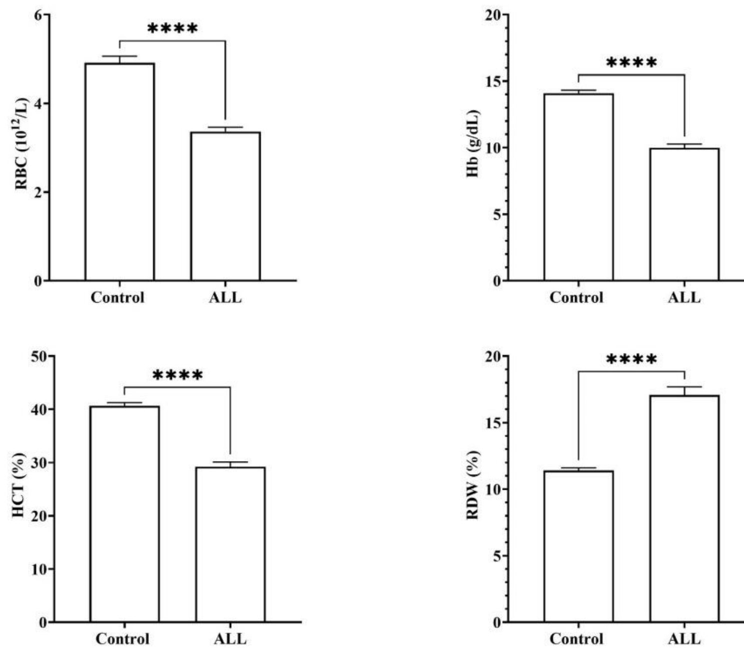

Figure 5. RBC count, Hb concentration, Hct%, and RDW% in acute lymphoblastic leukemia patients.

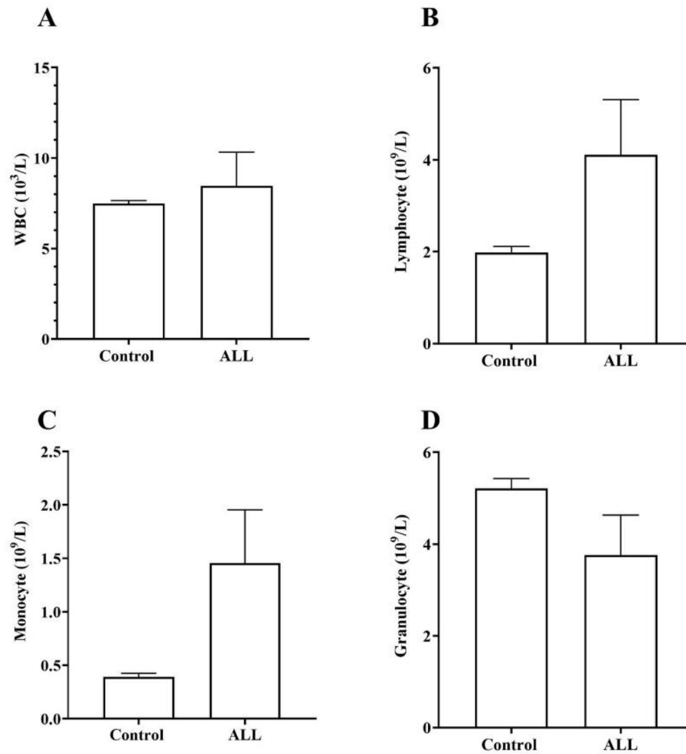

Figure 6. WBC count, absolute lymphocytes, monocytes, and granulocytes in acute lymphoblastic leukemia patients.

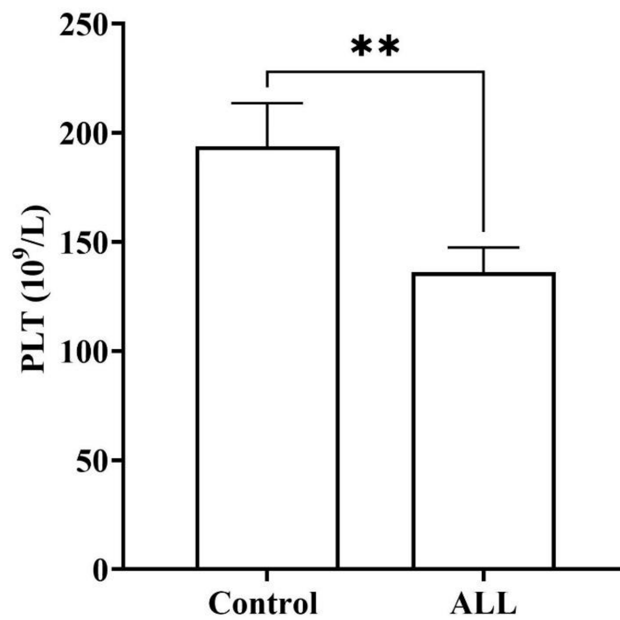

Figure 7. Platelet count in acute lymphoblastic leukemia patients.

Table 1. IL-15 exon eight gene mutation and variants in lymphoblastic leukemia

	Chromosome	Mutations	Mutation genotype	Heterozygous	Variants	Variant Percentage	External Database
	Location			Homozygous			
1	4:142654547	Substitution	G>GA	Heterozygous	97229G>GA	9.10%	dbSNP:10833
2	4:142654511	Substitution	A>AC	Heterozygous	97263A>AC	9.10%	Not found
3	4:142654513	Substitution	A>AT	Heterozygous	97265A>AT	9.10%	Not found
4	4:142654514	Substitution	A>AT	Heterozygous	97266A>AT	9.10%	Not found
5	4:142654518	Substitution	G>GT	Heterozygous	97270G>GT	18.20%	Not found
6	4:142654547	Substitution	T>C	Homozygous	97299T>C	100.00%	dbSNP:10833
7	4:142654604	Substitution	T>TC	Heterozygous	97356T>TC	9.10%	Not found
8	4:142654626_4:142654627	Insertion	insS	--	97378_97379insS	9.10%	Not found
9	4:142654629	Deletion	delA	--	97381delA	9.10%	Not found
10	4:142654775	Substitution	G>GA	Heterozygous	97527G>GA	9.10%	Not found
11	4:142654800	Substitution	G>GT	Heterozygous	97552G>GT	9.10%	Not found
12	4:142654801	Substitution	C>T	Homozygous	97553C>T	63.60%	dbSNP:2291596
13	4:142654853	Substitution	C>CA	Heterozygous	97605C>CA	9.10%	Not found
14	4:142654863	Substitution	T>TA	Heterozygous	97615T>TA	9.10%	Not found
15	4:142654869	Substitution	G>GT	Heterozygous	97621G>GT	9.10%	Not found
16	4:142654925	Substitution	T>TC	Heterozygous	97677T>TC	9.10%	Not found
17	4:142654968	Substitution	T>TA	Heterozygous	97720T>TA	9.10%	Not found
18	4:142654995	Substitution	T>TC	Heterozygous	97747T>TC	9.10%	Not found