



# Cellular and Molecular Biology

Original Article

## IL-15 gene mutation as a molecular risk factor in acute lymphoblastic leukemia

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### Article Info

### Abstract



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The present study investigated the relationship between single nucleotide polymorphisms in the interleukin (IL)-15 gene located (exon 8) on the chromosomal location 4q31.21 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 were 4-21.5), were enrolled at the Nanakaly Hospital for blood Disease and Oncology of Erbil Province between February 2021 and January 2022. The genotype analysis was performed using polymerase chain reaction (PCR) 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 clusters of differentiation (CD) biomarkers between both subtypes of ALL, including, CD10, CD19, CD22, CD79a, CD99, terminal deoxynucleotidyl transferase (TdT), and human leukocyte antigen DR (HLA-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 including red blood cells (RBCs), haemoglobin (g/dl), haematocrite (Hct %), red blood cell distribution width (RDW %), and platelet counts (PLT×10<sup>9</sup>/L) compared with those of healthy subjects. Finally, it was concluded that various novel mutations were recorded with different subtypes of ALL diseases, and moderate to severe anemia was observed among patients. Future studies will be towered to associate these mutations with prognosis and therapeutic response of diseases.

**Keywords:** IL-15, CD markers, Acute lymphoblastic leukaemia, SNPs, Anemia

### 1. Introduction

Acute lymphoblastic leukaemia (ALL) forms a heterogeneous 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, 2]

Interleukin (IL)-15 is a proinflammatory cytokine that plays an essential role in enhancing or inhibiting native and acquired immune responses [3-5]. 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<sup>+</sup>) and NK cells, which in turn enhance anti-cancer responses and act as novel immunomodulators for the treatment of various malignancies [6-8].

It has indicated [9] that exposure to IL-15 altered the

ability of NK cells and induced a moderate elevation in T-cell numbers (CD8<sup>+</sup>) which controlled tumor progression and provided novel perspectives for treating white blood cell malignancies. Administration of IL-15 or IL-15-releasing leukaemia cells in a leukemic mouse model led to an immune response against leukemia progression [10].

Several genotypes of IL-15 have been identified and linked with the biological aspects of leukaemia. It has been shown that SNPs rs17015014, rs17007695, rs10519613, and rs10519612 of the IL15 gene are strongly associated with the treatment response of young patients with lymphocytic leukemia [11]. In contrast to these findings, the rs17007695 and rs10519612 genes were highly correlated with the incidence of leukemia. These SNPs are significantly associated with the treatment response of infants with acute LL (ALL) [12].

It has been demonstrated [13] 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

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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.

It has further been found [14] 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 T- and B cells of subjects with ALL, respectively. Several genetic variations of IL-15 may provide important prognostic information in leukemic patients [15].

The results of Yin et al found [16] that the A>C substitution mutation of the rs2228059 polymorphism found in the IL15 receptor may be involved in esophageal carcinoma and stomach cancer [17] whereas the rs10519613 polymorphism may be an independent factor affecting the development of hepatocellular carcinoma following liver transplantation [18]. In contrast to these findings, it has been observed [19] no relationship between the presence of the two SNPs rs10519613 and rs35964658 and the incidence of acute leukemia.

Clustor differentiations 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 [20, 21] (20,21). 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 [1, 22]. Therefore, the identification of the predictive efficacy of phenotypes with abnormal expressions of CD biomarkers could be used to assist in the diagnosis and the clinical progression of leukemic patients, which may improve medical treatment options [23].

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

## 2. Materials and Methods

### 2.1 Subjects

The current investigation was based on a case-control study design. Seventy-eight (49 males and 29 females) patients with ALL (62 B-cell- 16 T-cells linages) were admitted to Nanakaly Hospital for Blood Diseases and Oncology (Erbil/ Kurdistan Region/ Iraq) for investigations and treatment between February 2021 and January 2022. Peripheral blood and bone marrow biopsies (BMB) were obtained from newly diagnosed acute lymphoblastic leukemic patients and 30 seemingly healthy individuals were 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.

### 2.2. Blood collection

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

### 2.3. Bone marrow aspiration and biopsies

The bone marrow aspiration and biopsy specimens were taken by a clinical hematologist. The specimens were sent for cellular assessment by and flow cytometry analysis.

### 2.4. 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'-CTATGCTGGTAGGCTCC-TG-3' and reverse, 5'-GTTCCATTAGAAGAGAGCT-TGC-3'.

PCR was performed using the following steps: Denaturation for (5 min at 95 °C); denaturation for 30 sec at 95 °C, annealing for 30 sec at 56 °C and elongation at 72 °C for 30 sec; and a final extension step was performed at 72 °C 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 a UV Transilluminator (UST-20M-8K; Biostep GmbH).

Following PCR, DNA extraction was performed to obtain the DNA samples to be used for the sequencing process. The latter 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>).

### 2.5. Flow cytometry analysis

All specimens were dyed and lysed using a direct immunofluorescence technique, as described by the study of Costa et al [24]. After following the manufacturer's instructions, samples were analysed using a two-laser, four-color, six-parameter BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). CellQuest Pro software (BD Biosciences) was used to collect and interpret data. BD CaliBRITE beads were used for daily calibration. The following monoclonal antibodies (MAb) were in-

cluded in the panel: CD19 (Cat. No. 345777), CD20 (Cat. No. 555677), CD10 (Cat. No. 555375), CD22 (Cat. No. 562859), cytoplasmic (c) CD79a (Cat. No. 563777), c and surface (s) IgM for B- lineage, CD1a (Cat. No. 561754), CD2 (Cat. No. 555327), cCD3 (Cat. No. 566683) and sCD3 (Cat. No. 555330), CD4 (Cat. No. 550280), CD5 (Cat. No. 563381), CD7 (Cat. No. 340581), and CD8 (Cat. No. 555369) for T- lineage, CD13 (Cat. No. 347837), CD33 (Cat. No. 561816), CD64 (Cat. No. 560970), CD15 (Cat. No. 560997), CD16 (Cat. No. 561313), CD11b (Cat. No. 550282), CD14 (Cat. No. 555398), CD117 (Cat. No. 555714), and cMPO for myeloid lineage, and CD34 (Cat. No. 553731), CD45 (Cat. No. 560975), CD36 (Cat. No. 550956), CD38 (Cat. No. 555460), HLA- DR (Cat. No. 556642), CD56 (Cat. No. ) (100 cases), and nTdT (Cat. No. 347208) as nonlineage markers. These reagents have been pre-diluted for use at the recommended Volume per Test, which typically uses  $1 \times 10^6$  cells in a 100 $\mu$ l of experimental sample (a test). In separate tubes, isotype antibodies were utilised as a negative control. All MAb were obtained from BD Bioscience.

Blast cells were detected using a side scatter (SSC) vs CD45 plot, then CD34+ blasts were identified using a CD34 vs SSC plot, and multiple plots were used to investigate the expression of the other crucial markers. The expression of the aforementioned markers was investigated in the region of blast identified by the SSC vs CD45 in samples where the blasts were CD34 negative. The EGIL consensus criteria of 10% for cytoplasmic markers and 20% for surface markers were used to classify positive blasts.

## 2.6. CBP measurements

The blood samples (n=78) were collected in the EDTA tubes and sent to the haematology laboratory of the Nana-kaly Hospital-Erbil-Iraq. Complete blood picture including total white blood cell differential counts were examined using (an automated Coulter Ac.T diff Hematology analyzer (Beckman Coulter, Inc.).

## 2.7. Statistical Analysis

GraphPad Prism 8 was used for all statistical analyses. The results were presented as mean  $\pm$  Standard Error. The Anderson-Darling, D'Agostino & Pearson, Kolmogorov-Smirnov, and Shapiro-Wilk tests were used to test for normality. For blood parameter analysis, an unpaired t-test was used, and 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.

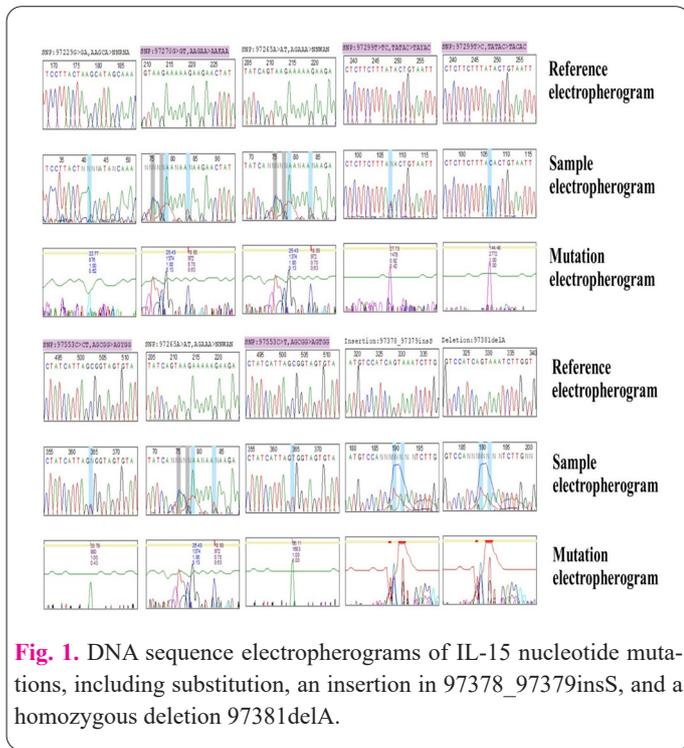
## 3. Results

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

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

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



**Fig. 1.** DNA sequence electropherograms of IL-15 nucleotide mutations, including substitution, an insertion in 97378\_97379insS, and a homozygous deletion 97381delA.

(97381delA) and one insertion (97378\_97379insS) were also noted. The homozygous variant (97299T>C) exhibited a frequency of 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, as shown in Figure 1.

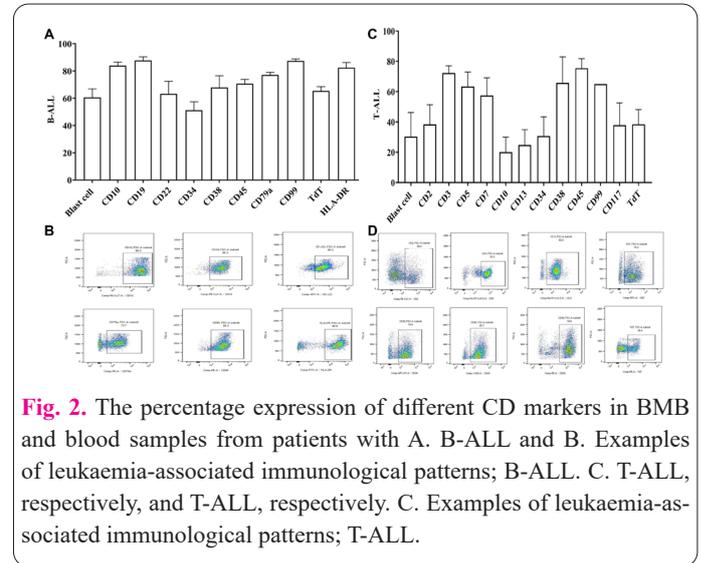
However, the remaining novel substitution, insertion and deletion variants of IL-15 were not found or recorded in the external databases and no missense mutation record was reported in the current study. All IL-15 mutations noted in patients with ALL did not alter the amino acid sequence of this cytokine.

### 3.1. Aberrant CD antigens in ALL

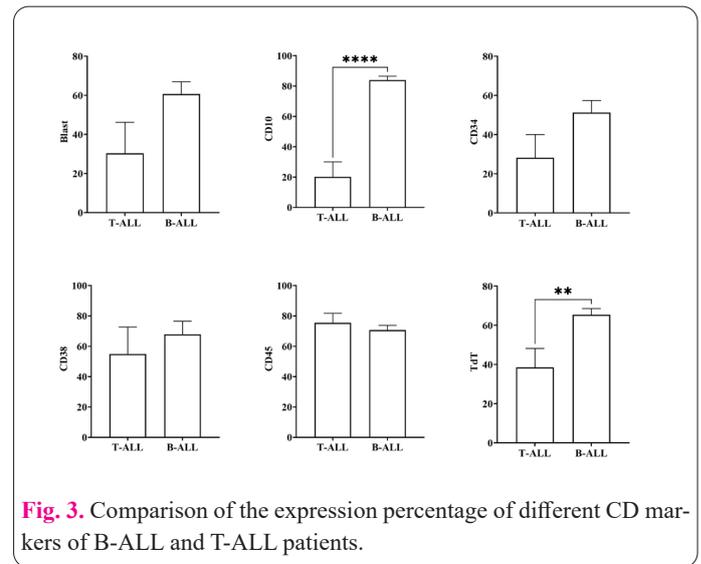
The result of aberrant CD markers expression in B-cells and T-cells ALL are shown in Figure 2, 3, and 4. The results of the flow cytometry analysis assist in the subtypes classification of newly diagnosed patients with ALL in the Iraqi population into B-cell and T-cell lineages by using some specific 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. CD2, CD3, CD5, CD7, CD13, CD34, CD38, CD45, CD99, CD117 and TdT have expressed 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 ( $\leq 20\%$ ) 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% Figure 4.

### 3.1. Hematological analysis of patients with ALL

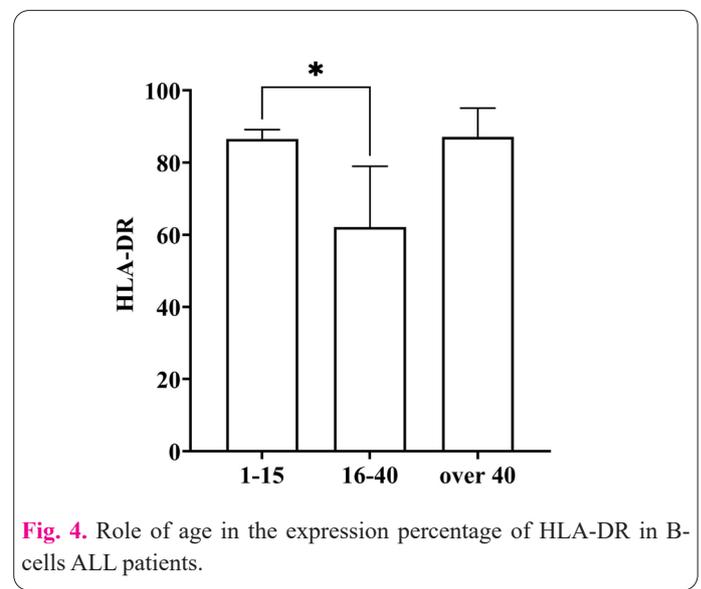
The CBC analysis investigated significant differences between the patients with ALL and the control subjects. Figure 5, 6, and 7 display a summary of the white blood count (WBC), red blood count (RBC), and platelet counts corresponding to patients with ALL. The RBC count, hemoglobin (Hb) concentration, hematocrit (Hct) %, and platelet count decreased significantly (0.0001), while the



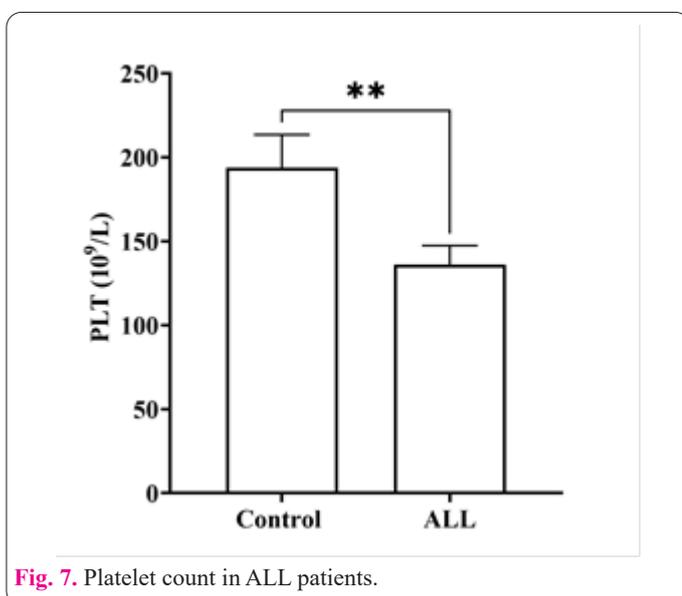
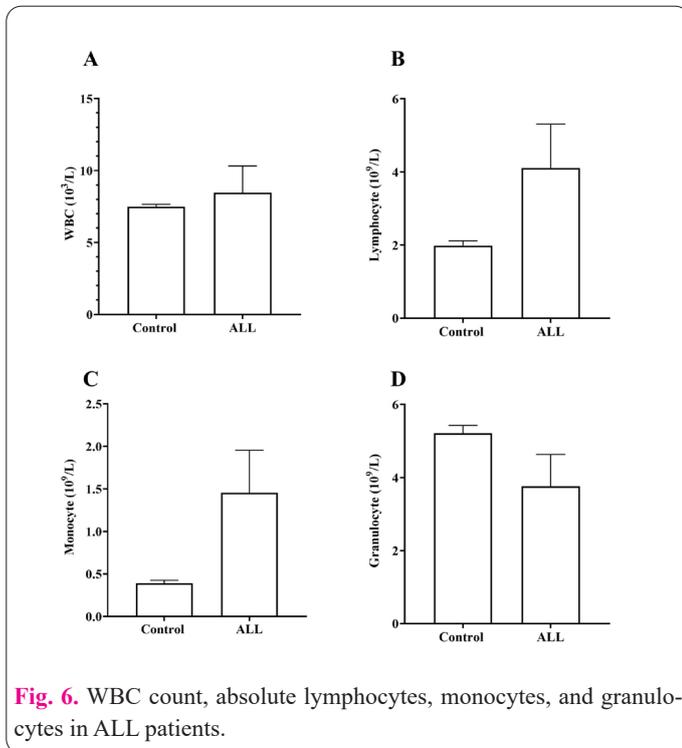
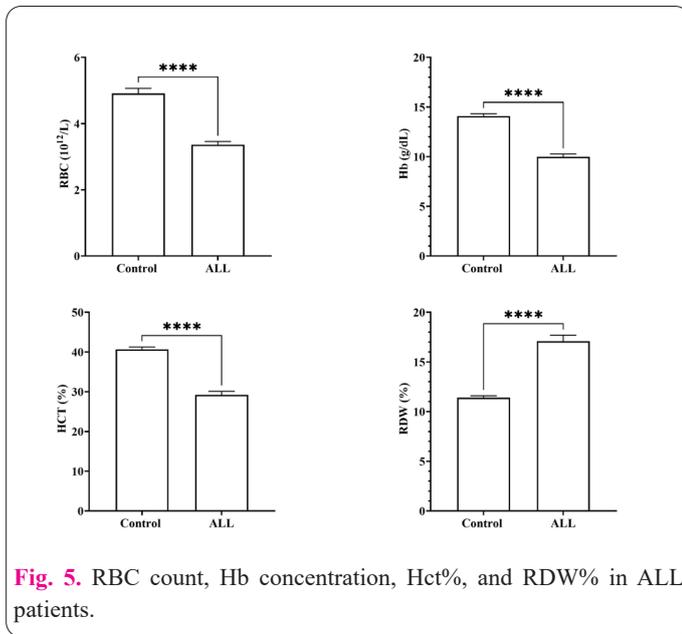
**Fig. 2.** The percentage expression of different CD markers in BMB and blood samples from patients with A. B-ALL and B. Examples of leukaemia-associated immunological patterns; B-ALL. C. T-ALL, respectively, and T-ALL, respectively. C. Examples of leukaemia-associated immunological patterns; T-ALL.



**Fig. 3.** Comparison of the expression percentage of different CD markers of B-ALL and T-ALL patients.



**Fig. 4.** Role of age in the expression percentage of HLA-DR in B-cells ALL patients.



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.

#### 4. 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, investigated the role of expression levels of CD markers in patients with ALL. These biomarkers can be used as a diagnostic immunophenotype for the definition and classification of subtypes of ALL.

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 characterized 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 contribute to the pathogenesis of diseases through their impact on 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 [12]. Lin *et al* observed [11] that the mutations of the SNPs 10519612 and 17007695 were more frequent in adult leukemic individuals than in healthy subjects. Aly *et al* [14] observed an association between the mutations of the rs10519612, and 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 [25, 12]. Rots *et al* [13] 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 studies indicated that high expression levels of the IL-15 gene were associated with the growth of leukemic cancers [26]. Polymorphisms in the IL15 gene may influence the assessment of an advanced threat assignment plan in the treatment of pediatric ALL [27]. SNPs in IL-15 receptor A (IL15RA; rs2228059) may lower the threat of developing esophageal tumors [17]. The IL-15 mutation corresponds to an enhanced risk of developing lung tumors [28].

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 is 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 [29, 30]. IL-15 signifies efficacy for treatment due

to its efficiency in enhancing the antineoplastic function of defense activator cells[31]. The current study indicated the role of specific diagnostic CD markers in improving the diagnosis of T-cell and B-cell lineage 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 leukemic patients investigated in the current study, such as CD2, CD3, CD5, CD7, CD13, CD117 and TdT. The results were confirmed by [23, 32] who demonstrated 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 previous research[33] who noted that CD19, CD20, and CD22 expressed in different ranges in acute B-LL. Kavianpour et al [22] who demonstrated a high frequency of CD13 and CD33 expression in patients with ALL and a low frequency of CD117 expression [21].

Shahrabi et al [34] 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 [35].

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 [36-39].

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 previous research [40] which 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. Moussavi *et al* [41] also demonstrated and 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 [42]. One of the limitations of this study is the restricted number of analyzed samples and SNPs used in this study which limits the evaluation of the results.

## 5. Conclusion

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, diagnosis 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 the expression percentage of different diagnostic cluster differentiation and subtypes of disease. There was many hematological changes including anemia, thrombocytopenia and leukocytosis with increased blast cells in comparison with healthy subjects. Future studies will be aimed to find a significant relationship between these variant SNPs and different CD markers with disease progression, prognosis and therapeutic response.

## Conflict of Interests

The author has no conflicts with any step of the article preparation.

## Consent for publications

The author read and approved the final manuscript for publication.

## 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.

## Availability of data and materials

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

## Authors' contributions

Darya M. Azeez, Kawa M. Hassn, Fikry A. Qader and Abass B. Salihi performed experiments. Sarbaz I. Mohammed designed the experiments, analyzed data and co-wrote the manuscript. All authors read and approved the final manuscript.

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