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In-Silico and Biological Analysis of B-Cell Lymphoma-2 Gene and Genetic Mutation as Diagnostic Marker in Childhood Sudanese Acute Lymphoblastic Leukemia Patients, Gezira State, Sudan (2018)

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Abstract

Objective: To analyze the *BCL2* genetic as diagnostic marker among acute lymphoblastic leukemia in childhood Sudanese patients, to detect genetic polymorphism in *BCL2* in childhood acute lymphoblastic leukemia patients using Polymerase chain reaction (PCR) and DNA sequencing technique to confirm the harmfulness of the detected mutation using in silico analysis. **Material and Methods:** Venous blood was drained by means of clean venipuncture into a labeled 5 ml K-EDTA tubes. Subsequent mixing of blood with anticoagulant was followed and the tubes were stored at −20˚C, 40 blood samples were carried out using Sysmex XP-300 automated hematology analyzer, DNA extraction using innuPREP blood DNA mini extraction kit, quality of the purified DNA was evaluated by electrophoresis in 0.25 g agarose gel & DNA amplification using polymerase chain reaction. **Results:** In this study, sequencing showed that harmful mutation of a homozygous AA allele in one case of pre B-ALL and a heterozygous mutation AC allele in one control, *BCL2* promoter region polymorphism is more reliable gene promoter polymorphism in ALL.

Subject Areas

Geriatrics, Pathology

Keywords

ALL, *BCL2*, DNA

1. Introduction

Leukemia is a gathering of cancers that for the most part start in the bone marrow and result in high quantities of unusual white blood cells (atypical leukocytes). Classifying intense acute leukemia (AL) is to separate acute lymphoblastic leukemia (ALL) from acute myeloid leukemia (AML). This should as a rule be possible by assessing cell morphology and translating cytochemical results about. Acute lymphoblastic leukemia is a kind of cancer of the blood and bone marrow, which is the most common childhood cancer [1]. In a child with ALL, too many undeveloped cells progress toward becoming lymphoblast, B lymphocytes, or T lymphocytes. The observed incidence of acute lymphoblastic leukemia in the Nordic countries was approximately 4.0 cases per 100,000 child-years [2], accounting for approximately 25% of all childhood cancers and about 77% of childhood leukemias. An estimated 6070 new cases, 3350 male patients and 2720 female, were newly diagnosed in 2013 in the United States. The incidence of ALL increased across the last 3 decades 3.4, 3.5, and 3.7 per 100,000 populations, respectively, especially for the 1 - 4 years age group 6.3, 6.9, and 7.4 per 100,000 populations, respectively. In subgroups of children aged 0 - 14, the incidences were 3.6, 3.7, and 4.1, respectively, for boys and 3.1, 3.1 and 3.3 respectively, for girls per 100,000 population over the last 3 decades; the incidences were lower in poor areas than in affluent areas for the first 2 decades, but between 2001 and 2010, the incidence in high-poverty counties increased to 4.3 per 100,000 population higher than that in low and medium poverty counties, which reversed the previous trend. The high incidences in white children kept rising to 4.1 per 100,000 populations in the last decade, which accounted for most of the overall increase in incidence among children. In contrast, the incidence among black children remained low, 1.9 per 100,000 populations, across the last 3 decades [3]. Before the 1960s, a patient diagnosed with acute leukemia could expect to die within a few months. With new treatment modalities, remission rates for ALL (T- and/or B-ALL) have improved dramatically. Approximately 80% of children treated for ALL can be expected to enter a prolonged remission with an indefinite period of survival. The prognosis of ALL in adults is not as good as in children. Only 10% - 25% has achieved a 5-year survival. A significant number of acute leukemias express inappropriate combinations of antigens making diagnosis challenging. Treatment protocols and prognosis are proving to be more effective and accurate when the leukemic cell lineage is immunologically classified correctly. In addition, the detection of residual leukemic cells and minimal residual disease (MRD) enables to assess the therapeutic response using immunophenotyping and genetic testing [4]. B-Cell Lymphoma protein-2 (*BCL2*) is

also known as apoptosis regulator.

The point of the present examination was to research whether *BCL2* polymorphism can impact the susceptibility of ALL and to assess the prognostic importance of *BCL2* polymorphism, in which discoveries in regards to lineage-dependent *BCL2* expression in ALL demonstrated that blasts from pediatric patients with T-ALL communicated bring down *BCL2* protein when contrasted with patients with B-ALL [5]. *BCL2* expression in neoplastic cells from patients with antecedent B-ALL, typical ALL and atypical ALL was observed to be aberrant in 84%, 77%, and 75% of the cases, separately, reliable with a various expression of *BCL2* in the different kinds of ALL as indicated by the phase of B cell development. [6]. At the end of the day, abnormal *BCL2* gene expression appears influence the survival limit of B-cell progenitors and contribute to leukemogenesis [7].

Table 1 shows the WHO criteria for the subtypes of ALL. Table 2 shows the WHO classification of ALL using chromosomal and molecular aberration. Table 3 lists the prognostic factors associated with favorable and unfavorable outcomes. Cytogenetic changes toward risk category can be found in Table 4. Prognosis based on bone marrow cytogenetics can be found in Table 5. Figure 1 shows the Cytogenetic band and Figure 2 shows the structure of *BCL2*.

1.1. Problem Identification and Justification

Acute lymphoblastic leukemia is the most frequently diagnosed malignancy among childhood Sudanese patients. *BCL2* is a regulatory protein that regulates the cell apoptosis by stimulating the pro-apoptotic protein or inhibiting the anti-apoptotic protein. This gene encodes for the essential outer mitochondrial membrane protein that stops the self-death program of lymphocyte resulting in increased number of lymphocytes in blood. There are no sufficient studies or

Table 1. WHO criteria for the subtypes of ALL.

Morphological feature	ALL	Burkitt type of ALL
Cell size	Small or large, heterogeneous.	Large
Nuclear chromatin	Ranges from fine or clumped to variable among cells within a single case.	Fine and homogeneous
Nuclear shape	Occasional clefting or indentation common	Regular, oval to round
Nucleoli	Range from not visible or small and inconspicuous to large and prominent	Prominent, one or more
Cytoplasm amount	Variable from scant to abundant	Moderately abundant
Basophilia	Variable	Very deep
Vacuolation	Variable	Often prominent

(Pinto *et al.*, 2005) [8].

Table 2. WHO classification of ALL using chromosomal and molecular aberration.

Cytogenetic abnormality		Genetic alteration
Precursor B-cell ALL	t (9; 22) (q34: q11)	BCR/ABL
	t (11; v) (11q23; var)	MLL/rearranged
	t (1; 19) (q23: p13)	E2A/PBX1
	t (12; 21) (p12: q22)	TEL/AML1
	T (17; 19)	E2A/HLF
Precursor T-cell ALL	T (1; 14)	MYC/TCR
	t (11; 14) (p15: q11)	LMO/TCRα/δ
	t (11; 14) (p13: q11)	LMO2/TCRα/δ

V = various (Harrison, 2001) [9].

Table 3. Prognostic factors associated with favorable and unfavorable outcome.

Factor	Unfavorable	Favorable
Age	<2 or >10 years	3 - 5 years
Sex	Male	Female
Race	Black	Caucasian
Organomegaly	Present	Absent
Mediastinal mass	Present	Absent
CVS involvement	Present	Absent
Leukocyte count	B-ALL > 30,000 mm^3 T-ALL > 100,000 mm^3	Low
Hemoglobin concentration	>10 g/dl	<10 g/dl
Cell type	Non Lymphoid	Lymphoid
Cell lineage	Pre B cell + T-ALL (children)	Early Pre B cell
Karyotype	Translocation	Hyperdiploidy
Response to treatment	Slow > 1 week to clear blasts from blood	Rapid < 1 week to clear blasts from blood
Time to remission	>4 weeks	<4 weeks
Minimal residual disease	Positive at 3 - 6 months	Negative at 1 month (children) or 3 months (adults

Table 4. Cytogenetic changes toward risk category.

Cytogenetic change	Risk category
Philadelphia chromosome	Poor prognosis
t (4; 11) (q21; q23)	Poor prognosis
t (8; 14) (q24.1; q32)	Poor prognosis

Continued

Complex karyotype (more than four abnormalities)	Poor prognosis
Low hypodiploidy or near triploidy	Poor prognosis
Deletion of chromosome 7	Poor prognosis
Trisomy 8	Poor prognosis
High hyperdiploidy (trisomy 4, 10, 17)	Good prognosis
del (9p)	Good prognosis

(Seiter, 2014) [10].

Table 5. Prognosis based on bone marrow cytogenetics.

Prognosis	Cytogenetic findings
Favorable	Hyperdiploidy > 50; t (12; 21)
Intermediate	Hyperdiploidy 47 - 50; Normal (diploidy); del (6q); Rearrangements of 8q24
Unfavorable	Hypodiploidy-near haploidy; Near tetraploidy; del (17p); t (9;22); t (11q23)

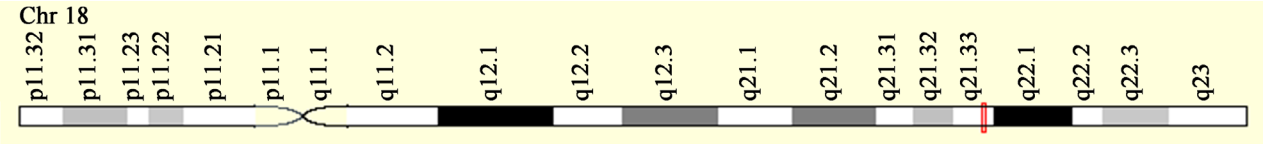

Figure 1. *Cytogenetic band:* 18q21.33 by Entrez Gene.18q21.33 by HGNC.18q21.33 by Ensembl.

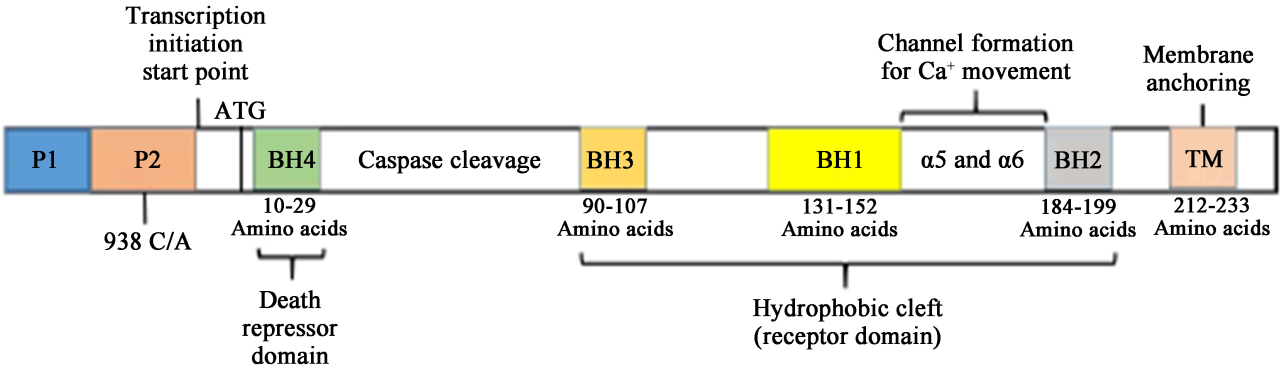

Figure 2. Structure of *BCL2* (Huma Butt *et al.*, 2017) [2].

information concerning *BCL2* gene mutation as associated genetic factors in acute lymphoblastic leukemia in childhood Sudanese patients. ALL is worldwide problem and its diagnosis so difficult to detect it in all countries in which the Sudan is one of them.

1.2. Objectives

1.2.1. General Objective

To analyze the *BCL2* genetic as diagnostic marker among acute lymphoblastic leukemia in childhood Sudanese patients.

1.2.2. Specific Objectives

1) To detect genetic polymorphism in *BCL2* in childhood acute lymphoblastic leukemia patients using Polymerase chain reaction (PCR) and DNA sequencing technique.

2) To confirm the harmfulness of the detected mutation using in silico analysis.

3) To correlate this polymorphism with age and gender.

2. Methodology

2.1. Study Design

This is a prospective hospital based case control study.

2.2. Study Area

This study was conducted in the National Cancer Institute and the Medical Laboratory the reference Laboratory in Gezira State, Pathology Department, Gezira University**.** They were chosen due to their medical and reference importance and their location to all Gezira State.

2.3. Study Duration

The Study was conducted during the period from November 2017 to September 2018.

2.4. Study Population

Patients attending the National Cancer Institute seeking the medical care referred from other Hospitals, Departments and Clinics.

2.5. Inclusion Criteria

Patients attending the National Cancer Institute less than 18 years of age diagnosed as Acute Lymphoblastic Leukemia.

2.6. Exclusion Criteria

Patients more than 18 years, diagnosed with malignancy other than ALL.

Patients receiving recent blood transfusion within the last three months.

2.7. Sample Size

This study included 20 patients as cases and 20 healthy as control subjects.

2.8. Ethical Consideration

Ethical clearance was obtained from ethical committee of the Gezira University and National Cancer Institute.

The permission to conduct this study was obtained from the Ministry of Health Gezira State.

Informed questioner consents were obtained from each patient.

Research approval was obtained from research board Faculty of Medical La-

boratory Sciences, University of Gezira.

2.9. Data Collection

Structured tested questionnaire was used to collect demographical and clinical data from each patients and controls.

2.10. Statistical Analysis

This study was analyzed by the use of package for social sciences (SPSS) software (The T test was used to determine the differences in frequency distribution of CBC and its association with the different variables. An association between Cases and control A p-value < 0.05 was considered as statistically significant).

2.11. Sample Collection

Venous blood was drained by means of clean venipuncture into a labeled 5 ml K-EDTA tubes. Subsequent mixing of blood with anticoagulant was followed and the tubes were stored at −20˚C.

2.12. CBC Methodology

The 40 blood samples were carried out using Sysmex XP-300 automated hematology analyzer. Whole blood sample was aspirated using whole blood mode then the result was obtained after 60 seconds through output source.

2.13. DNA Extraction Procedure

Use innuPREP blood DNA mini extraction kit by following these steps:

1) 400 ul of whole blood sample was pipetted into 2 ml reaction tube (Eppendorf tube).

2) 30 ul of proteinase K and 400 ul of Lysis solution SLS was added into sample tube and mix vigorously by pulsed vortexing for 10 seconds and was incubated at 60˚C for 10 min (note: for complete lysis, the lysate was mixed 3 or 4 times during incubation by shaking the sample perfectly).

3) Briefly the 1.5 ml tube was centrifuged to remove drop from inside the rid. Tube was inverted upside down to see if there is clot. When the clot was founded the supernatant was removed into new 1.5 ml tube and the clot was leaved.

4) 700 µl of binding solution BL was added to the lysed sample. Mix carefully by pipetting up and down several times (3 - 4 times).

5) 750 µl of the sample was applied to a spin filter (red) located in a 2.0 ml receiver tube and centrifuged at 12.000 rpm for 1 min.

6) The residual sample was applied to the spin filter and centrifuged at 12.000 rpm for 1 min. The filtrate was discarded and the spin filter was placed into anew 2.0 ml receiver tube.

7) 400 µl of washing solution C was added to the spin filter and centrifuge at 12.000 rpm for 1 min. The filtrate was discarded and the spin filter was placed into anew 2.0 ml receiver tube.

8) 600 µl of washing solution BS was added to the spin filter and centrifuge at 12.000 rpm for 1 min. The filtrate was discarded and the spin filter was placed into anew 2.0 ml receiver tube.

9) 600 µl of washing solution BS was added to the spin filter and centrifuge at 12.000 rpm for 1 min. The filtrate was discarded and the spin filter was placed into anew 2.0 ml receiver tube.

10) Spin column was centrifuged at max speed for 3 min to remove all trace of ethanol. The 2.0 ml receiver tube was discarded.

11) The flow-through was discarded and the spin column was removed into Eppendorf tube.

12) The elution buffer was pre-warmed in 60˚C, and then 100 µl of elution buffer was added in filter. Incubated at room temperature for 2 min and centrifuged for 1 min at 12000 rpm, and then 100 µl of elution buffer was added and centrifuged for 1 min at 12,000 rpm to complete volume 200 µl.

13) The spin filter was removed and Eppendorf tube was closed and then was stored the DNA at −20˚C.

2.14. Quantification of DNA

The quality of the purified DNA was evaluated by electrophoresis in 0.25 g agarose gel, stained using 5µl RedSafe™ nucleic acid staining solution and visualized by UV light. Table 6 shows the primer used in the PCR for *BCL2* gene promoter region (P2).

2.15. Preparation of Master Mix

Table 7 shows the preparation of master mix.

2.16. DNA Amplification Using Polymerase Chain Reaction (PCR)

The PCR reactions were performed to replicate *BCL2* gene using purified genomic DNA as template. This PCR reaction was done by using Taq PCR Master

Table 6. Primer used in the PCR for *BCL2* gene promoter region (P2): Primer ordered from Humanizing Genomics, Marcogen Company (Seoul, Korea).

Product Size	Sequence	GC%
366	Forward sequence (F) (20) 5'- 5-GCGTCCTGCCTTCATTTATC-3	50%
	Reverse sequence (R) (20) 5'- 5-TTCCAGATCGATTCCCAGAC-3	50%

Table 7. Preparation of master mix.

Component in	20 µl reaction
i-Taq™ DNA polymerase (5 U/µl).	0.2 µl
10 mM DNTP mixture.	2 µl
Reaction buffer (10×).	2 µl

Mix. The PCR reaction is consisting of three steps: denaturation, annealing and extension, with different temperature for each one. The first step of PCR is the denaturation in which the DNA sample is heated in 94˚C to separates the double strands. The high temperature breaks down the hydrogen bond between the nucleotides that form the DNA code. The second step is annealing in which the two primer (forward and reverse) bind to appropriate complementary strand. The temperature of this step various depending on the size of the primer and its homology to target DNA (the appropriate temperature for *BCL2* promoter region is 58˚C). Finally, DNA polymerase extend the primers by its polymerase activity, this is done in a temperature optimal for the Taq polymerase which is (72˚C). These steps are repeated for (35) times. In sterile 0.2 ml microcentrifuge tubes the PCR ingredients were added in the ratio shown in Table 8.

The conditions for the PCR were as follows (Table 9).

This step was followed by 35 cycles of the three stages.

Gel electrophoresis:

1) Preparation of agarose gel:

a) 2 g of agarose powder was measured (2%) by sensitive balance.

b) Agarose powder was mixed with 100 ml TBE buffer X (500 ml of DW to ml TBE) in a microwavable flask.

c) Then was microwaved for 1-2 min until the agarose is completely dissolved.

d) Agarose solution was lifted to cool down to about 50˚C.

e) 15 ul of the Ethidium Bromide was added to final concentration.

f) The agarose was poured into a gel tray with the well comb in place.

g) Newly poured gel was placed at room temperature for 20 - 30 mins until it

Table 8. PCR ingredients and concentration used in the reactions.

Ingredient	1X Volume
Forward Primer	1 µl
Reverse Primer	1 µl
Ready Master Mix	8 µl
DW	12 µl
DNA	3 µl
Total	25 µl

Table 9. Stages, temperature and time used for PCR for *BCL2* promoter region.

Initiation denaturation	94˚C	5 min
Denaturation	94˚C	30 sec
Annealing	58˚C	30 sec
Elongation	72˚C	30 sec
Final elongation	72˚C	10 min
Refrigerator	4˚C	Infinity

has completely solidified.

2) Loading samples and running an agarose gel:

a) The running buffer was prepared by add ml of 500 ml of DW to ml of TBE (X) buffer to prepare TBE 1X buffer.

b) Once solidified, the agarose gel was placed into the gel box (electrophoreses unit).

c) Gel box was filled with 1X TBE until the gel is covered.

d) ul of each PCR product carefully was loaded into the additional wells of gel.

e) The gel was ruined at 120 voltages until the dye line is approximately 75% - 80% of the way down the gel.

f) The power was turned off, the electrodes were disconnected from the power source and then the gel was removed carefully from the gel box.

g) By using UV transilluminator to the DNA fragments was visualized.

2.17. DNA Sequencing

Normal sequencing is a process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases adenine, guanine, cytosine and thymine in a strand of DNA. In this study, the DNA sequencing was used for scanning *BCL*2 promoter region as mutation detection method. Normal sequencing was carried out for 3 samples of cases and 3 samples of controls by Macrgene Company (Seoul, Korea) using Sanger technique.

2.18. Data Analysis

Data was analyzed using Microsoft excel sheet (20) and statistical package of social science (SPSS).

2.19. Bioinformatics Tools

2.19.1. Finch TV

Bioinformatics programs use to view and edit DNA sequence chromatogram data. Also, it displays quality values, when available, and can adjust the scale in both vertical and horizontal directions in both single and multipane views. In a chromatogram file, the signal intensities are presented in a graph with the four bases, each is identified by different color. Like many sequence analysis programs, Finch TV uses green for adenine, red for thymine, black for guanine, and blue for cytosine.

2.19.2. Bioedit

It is most common program used in molecular biology studies. It was developed initially as biological sequence alignment editor written for windows only. It contains many features for sequence alignments modes of easy hand alignment, split window view, user defined colour, and information based shading and auto integration with other programs such as Clustal W and Blast [11].

2.19.3. Blast

Blast is an abbreviation for Basic Local Alignment Tool which is an online bioinformatics program. The online bioinformatics program is an algorithm for comparing primary biological sequence information such as the amino acid sequence of proteins or the nucleotides of DNA sequences.

3. Results

3.1. Characteristics of Study Population

As illustrated in Figure 3, from the 40 individuals included in this study, (12, 60%) were males and were (8, 40%) females. Figure 4 shows the age distribution. Table 10 lists the frequencies and percentage of the ALL types among cases.

3.2. Hematological Parameters among Cases and Controls

Tables 11-15 show the results of hematological parameters among cases and controls.

3.3. Hematological Parameters among Types of ALL

Tables 16-20 show the results of hematological parameters among types of ALL.

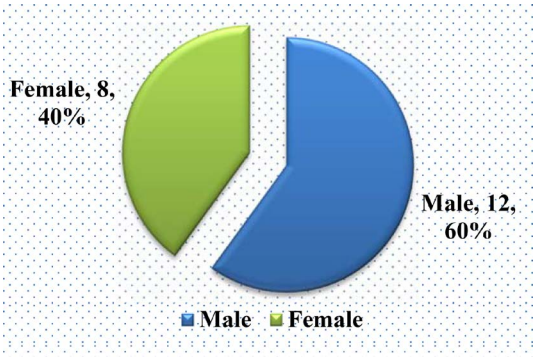

Figure 3. Distribution of the study subjects according to gender.

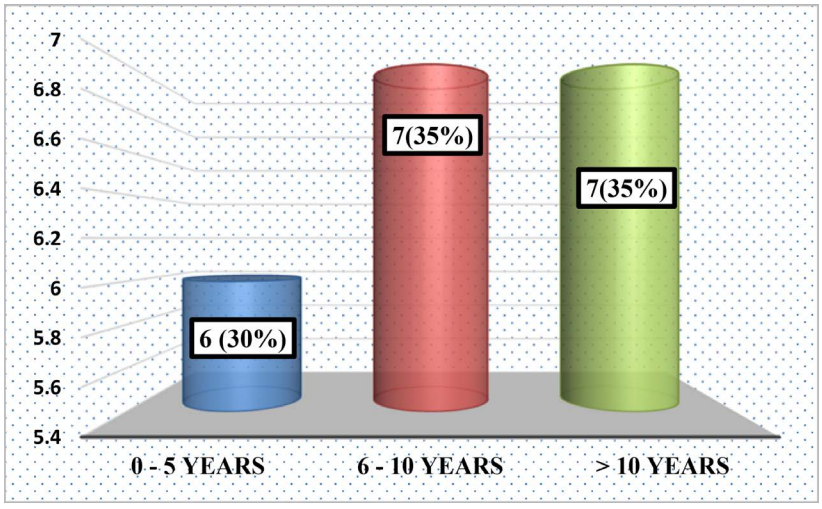

Figure 4. Distribution of the study subjects according to age.

Table 10. Frequencies and percentage of the ALL types among cases.

Types of ALL	Frequency	Percentage
L1	6	30
Ĺ2	2	10
Ĺ3	1	5
PRE-B ALL	10	50
T ALL	1	5
Total	20	100

Table 11. TWBCs in cases and controls.

Cases Control	N	Mean	Std. Deviation	P. Value
Cases	20	48.185	53.0701	0.000
Control	20	7.235	2.1514	

Table 12. RBCs in cases and control.

Cases Control	N	Mean	Std. Deviation	P. Value
Cases	20	3.0745	0.91369	0.068
Control	20	4.851	0.55035	

Table 13. Hemoglobin level in cases and control.

Cases Control	N	Mean	Std. Deviation	P. Value
Cases	20	8.92	2.9318	0.273
Control	20	11.97	2.0846	

Table 14. RDW-SD in cases and control.

Cases Control	N	Mean	Std. Deviation	P. Value
Cases	20	49.16	4.4751	0.194
Control	20	41.415	3.1543	

Table 15. Platelet counts in cases and control

Cases Control	N	Mean	Std. Deviation	P. Value
Cases	20	40.95	71.074	0.006*
Control	20	372.1	122.415	

*Mann-Whitney Test.

Table 16. TWBCs in different type of ALL.

ALL type	N	Mean	Std. Deviation	P. Value
L1	6	10.033	2.9884	0.001
Pre-B ALL	10	48.99	38.979	

3.4. Molecular Techniques

Figure 5 shows the electro photogram of amplified DNA *BCL2* promoter region (3 ul).

3.5. Sequence Analysis and Bioinformatics Tools

Figures 6-9 show the results of sequence analysis and bioinformatics tools.

Table 17. RBCs in different type of ALL.

ALL type	N	Mean	Std. Deviation	P. Value
L1	6	2.57	1.19029	0.122
Pre-B ALL	10	3.226	0.5549	

Table 18. Hemoglobin level in different type of ALL.

ALL type	N	Mean	Std. Deviation	P. Value
L1	6	7.3	3.8668	0.076
Pre-B ALL	10	9.4	1.7764	

Table 19. RDW-SD different type of ALL.

ALL type	N	Mean	Std. Deviation	P. Value
L1	6	50.05	6.0089	0.669
Pre-B ALL	10	48.22	3.9527	

Table 20. Platelet counts in different type of ALL.

ALL type	N	Mean	Std. Deviation	P. Value
L1	6	71.17	126.762	0.000
Pre-B ALL	10	20.8	13.547	

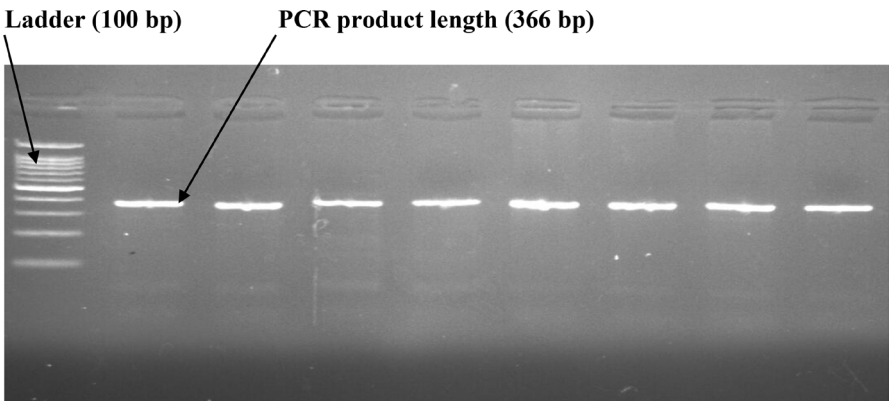

Figure 5. Electro photogram of amplified DNA *BCL2* promoter region (3ul) from 20 ALL children and 20 Health children samples in 2% agarose gel, stained with using Taq PCR Master Mix and visualized with UV light.

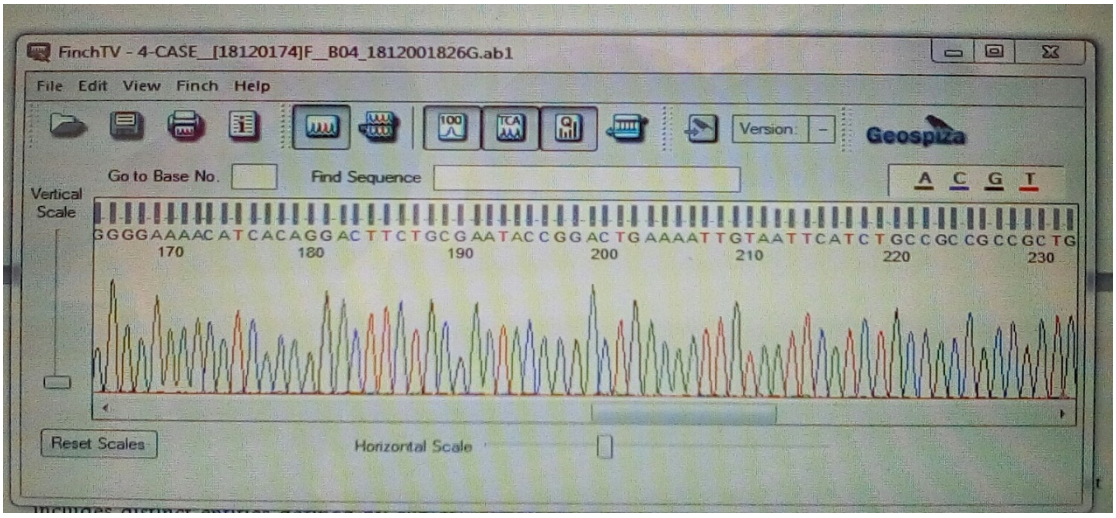

Figure 6. The sequencing result by Finch TV.

Figure 7. Alignments by Bioedit tool.

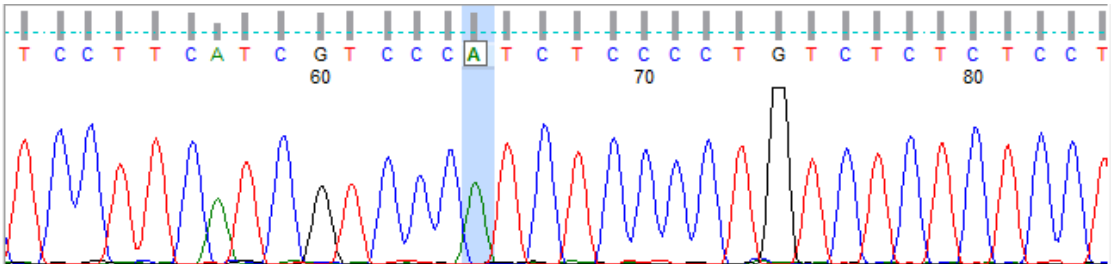

Figure 8. Chromatogram result for one case.

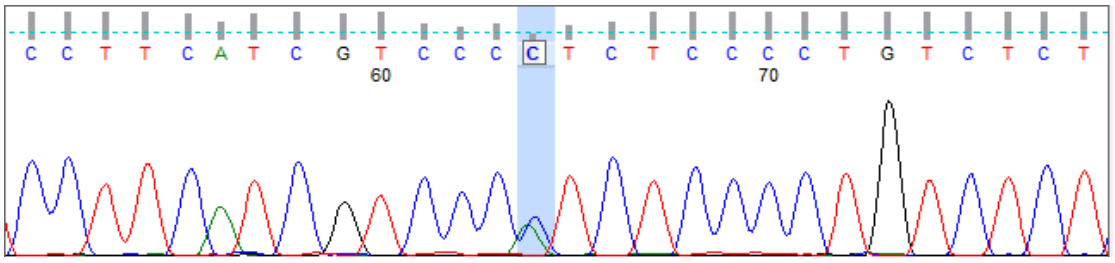

Figure 9. Chromatogram result for one control.

4. Discussion

Pediatric acute lymphoblastic leukemia (ALL) is a heterogeneous disease with subtypes that differ markedly in their cellular and molecular characteristics as well as their response to therapy and subsequent risk of relapse [12]. The dere-

gulation of pro- and anti-apoptotic mechanisms is well known to be closely related with the onset and rate of progression of malignant processes. The *BCL*2 proteins interact to maintain cell survival. [13], showed that greater expression of *BCL*2 mRNA was associated with a worse disease prognosis and a greater likelihood of relapse. Demographic data analysis showed that the age groups of the study population, aged between 0 - 5 years, 6 (30%), aged between 6 - 10 years were 7 (35%) and aged more than 10 years were 7 (35%). Also types of ALL among cases and control it was found that T-ALL 1(5%), L1 were 6 (30%), L2 were 2 (10%) and L3 was 1 (5%). Similar studies with same demographic data were reported by *Schultz et al* [14]. In this study pre-B ALL was found to be 10 (50%) of the total population, in agreement with the reported study by [15]. Regarding CBC parameters between cases and controls, the TWBC was found to be highly significant (p-value 0.000) in cases of ALL. Also it was found that platelets count highly significant (P-value 0.006) lower than the control [16]. Because of ALL patients were firstly diagnosed on the basis of the bone marrow examination in which several factors, including a significant increase in white blood cells and platelet low, which indicates the presence of abnormality in the bone marrow, at the same manner when confirmed the diagnosis of ALL by the flow cytometer techniques concerning CBC parameters between the types of ALL, pre-B ALL and L1, the TWBC was found to be highly significant (p-value 0.001) in pre-B ALL. Also it was found that platelets count highly significant (P-value 0.000) in L1 lower than pre-B ALL [16] while, another parameters of RBCs, Hemoglobin level, RDW-SD showed no significant difference between L1 and pre B-ALL that reflect another major difference among cases group was not only a factors, because we limited sample size analyses to B-precursor and T ALL. In our analyses, TWBCs, platelets count remained important variables in trials conducted by both groups. In contrast, other variables not included previously predictive of outcome have been replaced by genetic analyses of the leukemic blast.

Only six samples were sending for sequencing analysis due to high cost. In this study, sequencing showed that harmful mutation of a homozygous AA allele in one case of pre B-ALL and a heterozygous mutation AC allele in one control, in agreement with previous studies AA allele frequency was related with elevated *BCL*2 expression, the C allele interacts more powerfully with transcription factors and so by activating the P2 promoter region the activation of P1 promoter and so *BCL*2 expression reduces. In this case, an allele causes opposite of that process (Arico and Pui, 2011). On the other hand, Moazami-Goudarzi *et al*., [17] found a relationship between *BCL*2 expression and leukemogenesis in comparison to a healthy control group and hypothesized that the AA allele occurs more frequently than other alleles [17]. Study of rs227195 polymorphism is not done in Sudanese populace in past. Our results were somehow similar to previous studies done on different populations. But at some points the difference in the result may be due to gene pool and different expression. These findings showed that the *BCL*2 promoter region polymorphism is more reliable gene promoter

polymorphism in ALL.

5. Conclusions

BCL2 is considered to be a significant molecular diagnostic marker for the acute lymphoblastic leukemia (ALL).

The single nucleotide polymorphism (SNP) which was detected as AA allele in the promoter region of *BCL2* gene occurs more frequently than other alleles that alter the protein function and expression of this gene in childhood acute lymphoblastic leukemia.

Conflicts of Interest

The authors declare no conflicts of interest.

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