ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Comparative Expression Analysis of Breakpoint Cluster Region−Abelson Oncogene in Leukemia Patients

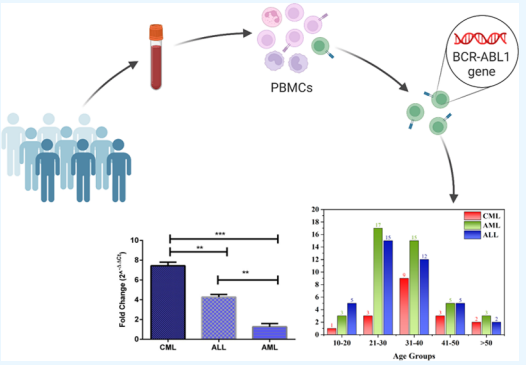
Farah Arshad, Amjad Ali,* Gauhar Rehman, Sobia Ahsan Halim, Muhammad Waqas, Asaad Khalid, Ashraf N. Abdalla, Ajmal Khan,* and Ahmed Al-Harrasi*

Cite This: *ACS Omega* 2023, 8, 5975−5982

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Leukemia is a proliferative disorder of myeloid and lymphoid cells that may lead to death. Different types of leukemia have been reported, and several genetic and environmental factors are involved in their development. The Philadelphia chromosome causes the most common mutation known as breakpoint cluster region−Abelson oncogene (BCR-ABL1), which shows abnormal protein tyrosine kinase (PTK) activity. Basically, this activity is accountable for activating multiple pathways, including the inhibition of cell differentiation, controlled proliferation, and cell death. As a result of the absence of kinase activity, this mutation leads to the uncontrolled proliferation of leukocytes, causing chronic myeloid leukemia (CML), acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), and chronic lymphocytic leukemia (CLL). This study aimed to evaluate the level of BCR-ABL1 expression in patients with these types of leukemias through qPCR.



In brief, PBMCs were isolated from blood samples of patients, RNA was extracted from PBMCs, cDNA was synthesized, and the transcript levels of BCR-ABL1 in patients with each type of leukemia were determined by qPCR. The clinical, demographical, and experimental data were analyzed among CML, AML, and ALL patients. Results: The BCR-ABL1 expression levels are variable in all studied groups and are 90, 30−35, and 1−2.5% in CML, ALL, and AML, respectively. Demographic characteristics such as gender, BMI, age, family history, and clinical parameters along with CBC are also associated with the prevalence and diagnosis of leukemia. In a comparative expression analysis, the expression of BCR-ABL1 is onefold high in AML, but four- and sevenfold high in ALL and CML, respectively, as compared with normal levels. Conclusions: In this study, a significant difference was observed in the expression levels of BCR-ABL1 between CML (p = 0.0043) and ALL (p = 0.0006) and between CML and AML groups, and a high expression of BCR-ABL1 was noted in CML as compared with ALL and AML.

1. INTRODUCTION

Leukemia refers to a group of blood cancers that comprise escalating and malignant diseases of blood-forming organs, recognized by a warped development and proliferation of leukocytes. Generally, leukemia is referred to as a cancer of the bone marrow and blood, in which abnormal white blood cells (blasts) are produced by hematopoietic cells, and they grow and spread rapidly in the bloodstream and crowd out healthy blood cells.[1] These abnormal leukocytes accumulate in the liver, brain, lymph nodes, spleen, and testes and alter the normal function of the body.[2] This complex disease was first identified in the 19th century. The Philadelphia chromosome was the first recurring genetic change revealed to be connected with a particular human cancer, chronic myeloid leukemia (CML). It was originally found as an unusually small chromosome in CML cells. Later, it was determined to be a chromosomal rearrangement including the ABL gene on chromosome 9, a tyrosine kinase, and the BCR gene on chromosome 22. The symptoms of leukemia may include fever, swollen lymph nodes, bruising and bleeding, joint pain, swelling of the abdomen, loss of appetite, tiredness, weight loss, and frequent infections.[3] These signs and symptoms vary with each type of leukemia. Leukemia is divided into four major types of chronic and acute leukemia, which are chronic myeloid leukemia (CML), acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), and chronic lymphocytic leukemia (CLL), besides some less common types. Lymphomas comprise a wide group of tumors that affect the lymphoid systems, referred to as tumors of lymphoid and hematopoietic tissues.[4]

Received: December 10, 2022
Accepted: January 6, 2023
Published: January 29, 2023

The accurate and exact cause of leukemia is still unknown because a combination of genetic and environmental factors is believed to play a role. However, it seems that the chances of acquiring specific forms of leukemia in both adults and children are increased by exposure to excessive levels of ionizing radiation, which may cause cancer by removing electrons from the nucleus of an atom, thereby damaging the DNA in cells. Risk factors include artificial ionizing radiations, petrochemicals, T-lymphocytic viruses and HIV, hair dyes, prior chemotherapy, smoking, and some major genetic disorders.[5] On the genetic level, DNA mutations are major highlighted factors. One major factor that causes changes in DNA is known as chromosomal translocation. It occurs due to a truncated reciprocal chromosomal translocation within chromosomes 9 and 22, resulting in the juxtapositioning of the chromosome 9 ABL-oncogene and the housekeeping gene BCR on chromosome 22, which leads to the formation of the BCR-ABL1 hybrid gene known as the Philadelphia chromosome.[4] It was the first identified chromosomal abnormality related to blood cancer in 1960. On the transcription of this hybrid gene, the BCR-ABL mRNA assembled and translated into a chimeric pathogenic protein BCR-ABL. It is referred to as p210 or p185 because of the molecular weight of this protein.[6] The ABL and BCR genes translated into a membrane-associated protein, known as protein tyrosine kinase (PTK), which is regulated in an auto-inhibitory fashion. Tyrosine kinases are essential regulators of this signal transduction cascade, involved in cell proliferation, differentiation, migration, metabolism, and programmed cell death. Tyrosine kinases catalyze the phosphorylation of specific tyrosine residues in target proteins, utilizing adenosine triphosphate (ATP). BCR-ABL1 synthesizes a protein that is always on and constantly activated and increases unregulated cell division (blood cancer).[7] This is because of a conformational change in the kinase domain due to the substitution of a myristoylated cap region on the truncated site of the BCR protein. However, the BCR region expresses threonine or serine kinases, but the function of tyrosine kinase is related to drug therapy.[8] The level of BCR-ABL1 expression varies in all types of blood cancer due to the clinically important isoforms of its protein. The expression rate of the Philadelphia chromosome is 90% in CML, 25−30% in ALL, and 1−2% in acute myeloid leukemia (AML).[9] Compared with traditional cytogenetic examination, the sensitivity of molecular diagnostic techniques such as fluorescence in situ hybridization (FISH) and quantitative reverse transcription-polymerase chain reaction (QRT-PCR) for identifying BCR-ABL-positive cells is much higher. Among the available methods for monitoring Ph + leukemias, QRT-PCR has emerged as the gold standard because of its greater sensitivity. The diagnosis of leukemia typically involves the laboratory testing of blood samples from patients, which includes determining the presence of the Philadelphia abnormality, documenting the mounting of unexplained leukocytosis by FISH, routine cytogenetics, and molecular testing by real-time (RT)-PCR. RT-PCR allows highly accurate and sensitive detection of BCR-ABL1 transcripts compared with FISH, because FISH studies may yield false-positive results.[10] Quantitative RT-PCR is ideal for monitoring BCR-ABL1 variants using blood and bone marrow samples. False-positive and -negative results can occur due to contamination or poor RNA quality, which can be reduced by proper handling and laboratory experience.[9] It may be difficult to diagnose leukemia based on BCR-ABL in patients who are Philadelphia negative but have a high number of leukocytes and splenomegaly. This is due to the atypical behavior of the BCR-ABL1 gene and is known as ph-negative leukemia. These patients have a chronic cell lineage of eosinophils, neutrophils, and basophils due to myeloid hyperplasia. Molecular and cytogenetic studies show that the BCR-ABL1 arrangement and the Ph chromosome help in suitable diagnosis.[11] RT-PCR detects qualitative and quantitative relapse after or before allogeneic stem cell transplantation. It is now an accepted method for the monitoring, detection, and expression studies of leukemia. The current method uses the control gene to compare gene expression, multiplied by 100, to show the results in percentage folds, but this technique varies between laboratories. In 2005, the International Real-Time Quantitative PCR (RQ-PCR) standardization project was started using a reference baseline to introduce a standardized scale.[12]

Leukemia can be treated by several different medical approaches based on the type of leukemia and the health status, age, gender, and family history of the patient. Treatment may comprise a combination of targeted therapy, chemotherapy, radiation therapy, and bone marrow transplant with palliative and supportive care.[13] Leukemia accounted for roughly 2.5% of all new cancer incidence and 3.1% of cancer-related death. A total of 474,519 new cases of leukemia were recorded in 2020. The global age-standardized rate of incidence was 5.4 per 100,000, and there was an approximately fivefold range globally. In terms of mortality, 311,594 associated deaths were recorded in 2020. There was a lower geographical variation globally in death from leukemia, as the majority of the locations in Asia, Europe, America, Australia, and New Zealand reported a mortality rate of roughly 2.5−4.0 per 100,000.[14] In Pakistan, the main step for the treatment of blood cancer is chemotherapy, which sometimes includes radiation therapy. The complete duration of treatment varies with the type of cancer and from person to person. Bone marrow treatment is mainly used for children who are at a high risk of relapse. The outcomes have been improved in developed countries due to modern treatments. The mortality rate for blood cancer is higher in elders as compared with children and young adults.[15] The 5-year survival rate for leukemia is 66% in CML, 83% in CLL, 27% in AML, and 71% in ALL children and adults.[16] In Pakistan, the most diagnosed type of leukemia is AML, whereas ALL and CML occur at a lower ratio than AML. The prognosis of acute myeloid leukemia is poor. The death rate due to blood cancer is 2.97% in Pakistan, and it occurs predominantly in male patients.[17]

2. MATERIALS AND METHODS

2.1. Study Setup. Blood samples and demographic and clinical information of leukemia patients were collected from the leukemia center of the Jinnah Hospital, Lahore, while molecular testing of leukemia patients was conducted at the Molecular Virology Laboratory, Centre for Applied Molecular Biology, University of Punjab, Lahore. Two different types of proforma were designed, one for the relevant details of patients and the second for patient consent.

2.2. Study Population. One hundred leukemia patients (under treatment with chemotherapy, targeted therapy, and combination therapy), along with seven healthy controls, were enrolled in this study. Out of 100 patients (male and female), 18, 39, and 53% had CML, ALL, and AML, respectively. Among them, 98% of the patients were found to be BCR-ABL-positive using the fluorescence in situ hybridization (FISH)

https://doi.org/10.1021/acsomega.2c07885
ACS Omega 2023, 8, 5975−5982

Table 1. List of Primer Sequences Used in PCR

gene	primer	sequence
BCR-ABL1	forward	5′-CTGAATGTCATCGTCCACTCA-3′
	reverse	3′-CGCGTCTTTGCTTTATTCACA-5′
GAPDH	forward	5′-TGAAGCTCGGAGTCAACGGATTTGGT-3′
	reverse	5′-CACTGTGGGCCATGAGGTCCACCAG-3′

Table 2. Baseline Clinical and Demographic Parameters of Patients[a]

study groups	healthy	AML	CML	ALL
number of patients	07	43	18	39
age	39 ± 10	31.30 ± 13	35.6 ± 10	31.8 ± 12
BMI	22 ± 0.6	21.3 ± 2.8	21.8 ±1.6	19.6 ±1.3
TLC	9.8 ± 2.3	34.09 ± 2.1	67.1 ±1.7	31.07 ±4.6
hemoglobin	15.7 ± 0.7	8.27 ± 0.26	10.26 ± 0.72	10.48 ± 0.33
Platelets	264 ± 90	179.76 ± 15.7	162.61 ± 23.9	120.08 ± 7.1
ΔCt	0.34 ± 1.6	1.049 ± 0.4	7.70 ± 3.1	4.469 ± 1.4

[a]AML = acute myeloid leukemia; CML = chronic myeloid leukemia; ALL = acute myeloid leukemia.

procedure. For the comparative analysis between normal individuals and patients, blood samples from healthy individuals were excluded from further analysis. This study was approved by the Ethics Committee of the University.

2.3. Collection of Blood Samples. Blood samples of patients with different types of leukemia (CML, AML, and ALL) were collected under aseptic conditions. The selected sites of blood collection were the antecubital fossa and the feet. About 3−5 mL of blood was collected in a labeled vacutainer for PBMC isolation.

2.4. Isolation of PBMCs. For the extraction of PBMCs, 10 mL of histopaque-1077 (Sigma-Aldrich, Germany) was added to the blood samples. Centrifugation of blood samples was performed at 3000 rpm for 30 min for the dissociation of nucleoproteins and debris. Three layers were formed, and the whitish buffy layer that was formed between the medium and the histopaque was then aspirated. This interface layer was carefully separated into a new falcon tube. After this, these cells were washed with 10 mL of sterile PBS solution by centrifugation at 2000 rpm for 15 min to obtain the maximum yield and then resuspended in 1 mL to store at 4 °C for further use.[18]

2.5. RNA Extraction. The Wizol reagent was used to extract the total amount of RNA from isolated PBMCs. In brief, 1000 mL of the Wizol reagent was added to 300 mL of cells and incubated at room temperature for 15 min. Then, the cell suspension was centrifuged at 3000 rpm for 5 min, and the supernatant was shifted to a fresh Eppendorf tube. A chloroform solution (500 μL) was added to the Eppendorf tube and vortexed for 15−20 s. Then, blood samples were incubated at room temperature for 2−4 min and centrifuged at 4 °C and 12,000 rpm for 15 min. Then, the upper watery layer was carefully separated into a new RNase-free Eppendorf tube without disturbing the interface. Isopropanol (500 μL) was then added, and these samples were incubated at room temperature for 10 min. On completion of incubation, the above step of centrifugation was repeated. The supernatant was discarded, and the RNA pellet was washed with isopropanol by centrifugation at 7500 rpm for 5 min. The pellet was allowed to dry and then dissolved in 20 μL of DEPC-treated or injection water.

2.6. Complementary DNA (cDNA) Synthesis. For the synthesis of cDNA, RNA from each sample was reverse transcribed using reverse transcriptase enzyme. In brief, 9 μL of diluted RNA sample, 2 μL of dNTPs, and 1 μL of BCR-ABL1 gene reverse primer were added into a PCR tube. Then, tubes were incubated in a thermocycler for 5 min at 65 °C to melt the secondary structures of mRNA. Afterward, PCR tubes were cooled on ice. A defined composition of the reaction mixture was added into PCR tubes and incubated at 42 °C for 1 h and then 85 °C for 5 min in the thermocycler. The same procedure was followed for the cDNA synthesis of the GAPDH gene. The primer sequences of BCR-ABL1 and GAPDH genes are shown in Table 1. After this protocol, cDNA was quantified by a NanoDrop spectrophotometer (2000c; Thermo Scientific).

2.7. Primer Designing. Primer-BLAST[19] was used to design primers for the selected gene (BCR-ABL1). The BCR-ABL1 sequence was retrieved from the National Center for Biotechnology Information (NCBI) database with accession number NM_021574.3. The lengths of the primers were kept at 21 base pairs with a GC content of 40−50% using the NCBI primer Blast tool. The optimum selected primers for the BCR-ABL1gene are reported in Table 1.

2.8. Quantitative PCR (qPCR). The synthesized cDNA of the BCR-ABL1 gene was used to perform qPCR (Roter Gene-QIAGEN). The expressions of the target gene, BCR-ABL1, and the housekeeping gene, GAPDH, were analyzed using SYBER Green (Fermentas Thermo Scientific USA). qPCR was performed in a 10 μL volume of the reaction mix. The relative expression level of mRNA was obtained by the normalization of the mRNA level of the target gene BCR-ABL and reference gene GAPDH. Amplification results and CT values of the target and reference genes were analyzed and compared. For the expression of mRNA, ΔCt was calculated by the following formula:

$$\Delta Ct = Ct_{(\text{target gene})} - Ct_{(\text{reference gene})} \quad (1)$$

Quantities and relative fold values of the BCR-ABL1 gene among CML, AML, and ALL were calculated as shown in eq 2.

$$\text{fold change} = 2 - \Delta\Delta Ct \quad (2)$$

2.9. Statistical Analysis. For statistical analysis, demographic and clinical features of the study population were introduced as the mean and standard mean error using Microsoft excel. GraphPad prism software (version 6.01, San Diego)[20] was used to calculate the comparative fold change in

https://doi.org/10.1021/acsomega.2c07885
ACS Omega 2023, 8, 5975−5982

gene expression. Comparison of the mean of all groups and determination of the relative fold change were performed by ordinary one-way ANOVA. A p-value < 00.5 was considered statistically significant among all groups. Tabular and graphical presentations were generated by Microsoft excel and GraphPad Prism Software.

3. RESULTS

A total of 100 patients were involved in this study, along with seven healthy controls. All patients were treated by chemotherapy and targeted therapy. Among the 100 patients, 18 patients had CML, 39 patients had ALL, and 43 patients suffered from AML; however, we did not find a single patient with chronic lymphoid leukemia (CLL).

3.1. Clinical Parameters and Demographic Features of Leukemia Patients. The main findings, demographic studies, and clinical parameters of leukemia patients are listed in Table 2. Results are listed as the average and mean standard deviation. All patients belonged to Lahore and surrounding areas. The analysis showed that most of the patients were diagnosed with AML. The clinical characteristics were significantly different among the different types of leukemia. The TLC was high in leukemia patients (AML:34.09, CML:67.10, ALL:31.07), with low platelet and hemoglobin counts. The body mass index showed that most of the patients were normal or underweight. Hemoglobin and platelets were decreased in AML, CML, and ALL patients compared with healthy individuals. Similarly, the ΔCt values of the AML, CML, and ALL patients show fluctuations (1.04, 7.70, and 4.46, respectively) when compared with healthy individuals (0.34).

3.2. Gender-Wise Distribution of Leukemia Patients. According to our analysis, the prevalence of blood cancer was predominant in male patients as compared with female patients. Among 18 patients with CML, 11 were male and 7 were female. The ALL group had 39 patients, among whom 29 were male and 10 were female. However, the number of patients with AML was higher, with 9 female and 34 male patients (Figure 1).

3.3. Age-Wise Distribution of Leukemia Patients. In this study, most of the patients belong to the young age group, i.e., less than 40−45 years. However, the least occurrence of leukemia is found in the age group of less than 10 years and in the old age group. A large number of cases were observed in adults and in young male and female patients. Thus, age may be a helpful predictor to determine the prevalence of ALL, CML, and AML in patients (Figure 2). AML was observed more often in the age groups of 21−30 (17) and 31−40 (15) years, while ALL (21−30:15, 31−40:12) is also prevalent among these age groups. Furthermore, the highest number of CML patients was recorded in the age group of 31−40 (9) years.

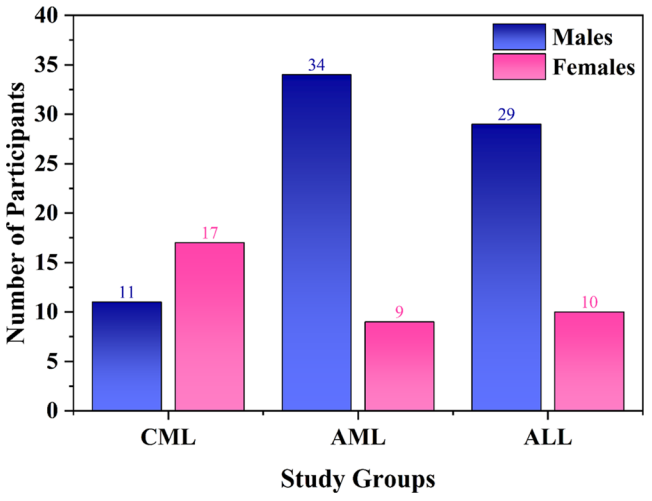


Figure 1. Graphical representation of the gender-wise distribution of CML, AML, and ALL patients.

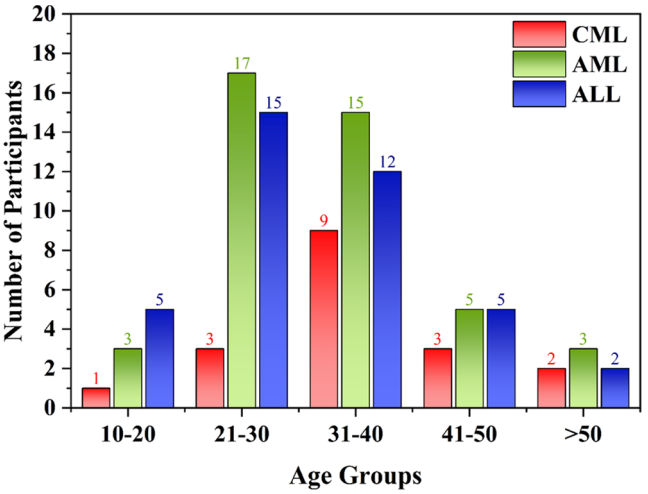


Figure 2. Distribution of CML, AML, and ALL among patients of various age groups.

3.4. Analysis of Body Mass Index (BMI). In this study, BMI analysis showed a variable number of people among different BMI categories. Most of the patients were found with normal BMI. A number of male and female patients were underweight in this study. A few people were categorized as overweight and obese. The CML group has almost equal numbers of normal and underweight patients, whereas the ratio of overweight patients was the same in all leukemia groups (Figure 3). In the overweight and obese groups, the ratio of CML, AML, and ALL was almost the same, while the number of ALL cases was found to be high in the obese group.

3.5. Association of Leukemia with Other Diseases. Leukemia is associated with several other diseases, including diabetes, hypertension, heart problems, skin allergy, and recurring lung infections. In our study, a range of patients were suffering from hypertension in all of the studied leukemia

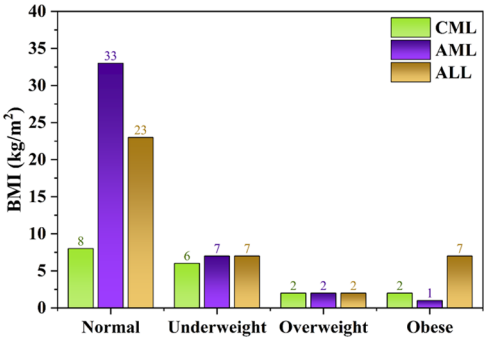


Figure 3. Graph showing the body mass index of the patients in the leukemia study.

https://doi.org/10.1021/acsomega.2c07885
ACS Omega 2023, 8, 5975−5982

groups, where the highest number was recorded in the AML (21) and ALL (16) groups. The AML group had the highest rate of skin allergies (16), whereas diabetes was more common in the ALL group (14). The rate of heart problems was low in all groups (Figure 4).

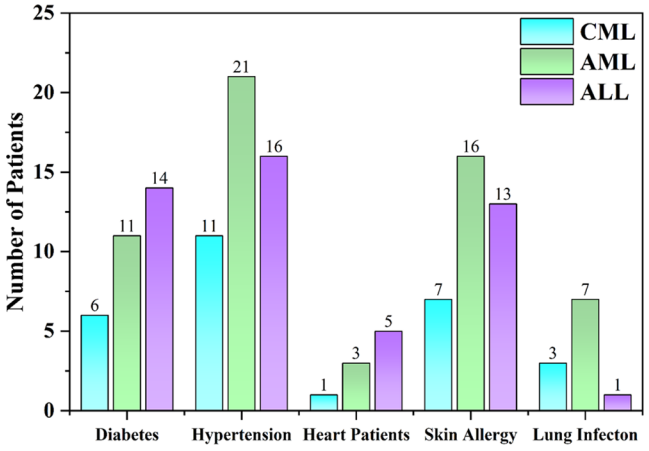


Figure 4. Prevalence of leukemia-related diseases.

3.6. Analysis of Complete Blood Count (CBC). Hematological and serological parameters were obtained from a complete blood count test and used to analyze and differentiate among the leukemia groups.

3.7. Analysis of Total Leukocytes Count in the Different Studied Groups. The normal range of TLC is 4500−12,000 g/L in normal and healthy people. The increase in the level of TLC was variable among the studied groups. CML patients had the highest number of leukocytes with significant differences (p = 0.0057 and p = 0.003) between the CML and ALL and the CML and AML groups, respectively (Figure 5).

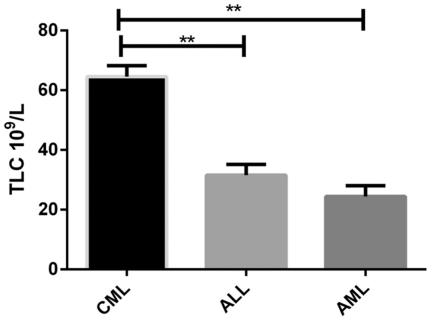


Figure 5. Analysis of the total leukocyte count (109/L) among the different studied groups. Error bars indicate the standard error of mean; significance level was determined by ordinary one-way ANOVA followed by Tukey's multiple comparison tests.

3.8. Analysis of the Hemoglobin Level in the Studied Participants. The normal hemoglobin level is in the range of 14−18 g/dL. Fluctuation of hemoglobin levels was observed among cancer patients. After analysis, we noted a decreased level of hemoglobin in all of the studied groups, CML, ALL, and AML, with no significant difference in the range among these groups. Likewise, a significantly lower hemoglobin level was found in AML patients (Figure 6).

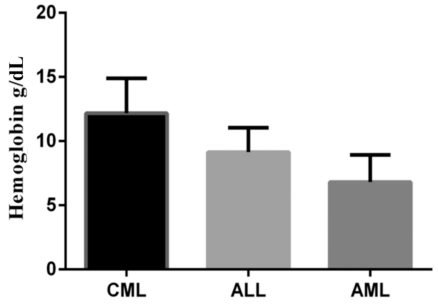


Figure 6. Analysis of hemoglobin levels (g/dL) among different studied groups. Error bars indicate the standard error of mean; the significance level was determined by ordinary one-way ANOVA followed by Tukey's multiple comparison tests.

3.9. Analysis of the Platelet Count in Leukemia Patients. The normal range of the platelet count is 150,000−450,000 μL in healthy individuals. We found a decrease in the platelet count in the CML, AML, and ALL groups. ALL patients had the lowest platelet counts; however, there was a significant difference (p = 0.005 and p = 0.002) between the CML and ALL and the ALL and AML groups, respectively (Figure 7).

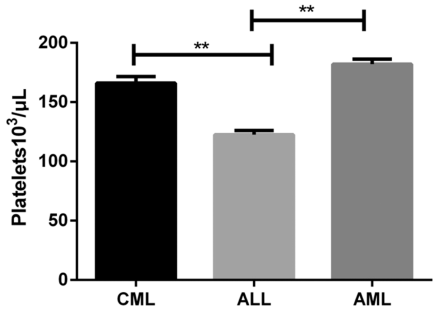


Figure 7. Analysis of the platelet count (10^3/L) among different studied groups. Error bars indicate the standard error of mean; the significance level was determined by ordinary one-way ANOVA followed by Tukey's multiple comparison tests.

3.10. Expression Analysis of the BCR-ABL1 Gene in Leukemia Groups. We evaluated the effects of BCR-ABL1 gene expression, which triggers the uncontrolled division of abnormal white blood cells in leukemia. The fold change reflects whether a gene is upregulated or downregulated in a gene expression analysis. The gene expression was studied in all leukemia groups, including CML, AML, and ALL. BCR-ABL1 expression was analyzed in 18 CML, 39 ALL, and 43 AML patients. The transcript level of BCR-ABL1 in PBMCs was quantified by RT-PCR, which was based on the threshold value (Ct value). The qPCR results were in the form of a sigmoidal curve that estimates the copy number of amplicons and PCR efficacy. ΔCt values were calculated for all groups. The mean values of $2 - \Delta\Delta Ct$ for CML, ALL, and AML were 7.7, 4.46, and 1.04, respectively. The obtained results depict significant differences in the Ct values between CML and ALL (p = 0.0043) and between CML and AML (p = 0.0006), which indicates high expression of BCR-ABL1 in CML as compared with ALL and AML (Figure 8).

3.11. Comparison of FISH and qPCR Results. Hematological screening of all leukemia groups (CML, AML, and ALL) includes the fluorescence in situ hybridization

https://doi.org/10.1021/acsomega.2c07885
ACS Omega 2023, 8, 5975−5982

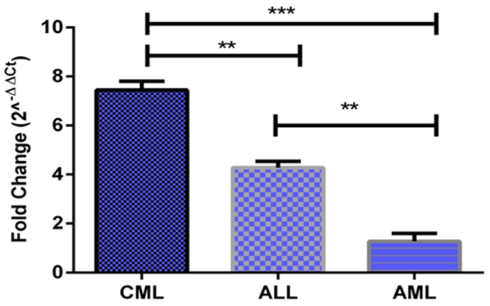


Figure 8. Comparative gene expression of BCR-ABL1 among different studied groups. Error bars indicate the standard error of mean; the significance level of fold change was determined by ordinary one-way ANOVA followed by Tukey's multiple comparison tests.

(FISH) test to confirm mutations in the BCR-ABL1 gene. According to FISH results, there were a large number of Philadelphia-positive (BCR-ABL1 mutation present) patients; however, some of them were Philadelphia-negative (BCR-ABL1 mutation absent). After the real-time PCR analysis, a few patients from the Philadelphia-negative group were indicated as Philadelphia-positive, which showed the specificity and accuracy of qPCR (Table 3).

Table 3. Comparison of FISH and qPCR Results[a]

sample ID	FISH results	qPCR results
10	Ph −ve	Ph +ve
29	Ph −ve	Ph +ve
43	Ph −ve	Ph +ve
68	Ph −ve	Ph +ve

[a]Ph*: Philadelphia.

4. DISCUSSION

Leukemia has become a global health burden and has the highest mortality rate among all cancer types. According to worldwide analysis, ∼1.5−2% of the population is diagnosed with leukemia. Several factors are involved in the occurrence of leukemia. The oncogene BCR-ABL1 produces tyrosine kinase, which causes leukemia cells to grow and proliferate without control. The expression of BCR-ABL1 varies among different types of leukemia: CML, AML, and ALL. The current study included 18, 39, and 43 patients with CML, ALL, and AML, respectively. We did not find any patients with CLL. There are few studies regarding the expression level of BCR-ABL in AML and ALL. In the Pakistani population, AML is the most common type of blood cancer, accounting for ∼50% of all leukemia types. Demographic characteristics or host factors of leukemia patients, such as gender, body mass index (BMI), age, family history, clinical parameters, and CBC, are associated with different groups, CML, AML, and ALL, and their treatments.

In the US, the prevalence of leukemia in men is twice that in women. Similarly, in Europe, leukemia is more common in male children and adults.[16] Furthermore, some studies show no difference in the gender-wise distribution of leukemia.[21] In the current study, the male/female ratio was 70:30, which shows a notable difference in the occurrence of leukemia among female and male patients. According to a recent study by the Leukemia and Lymphoma Society, the death rate due to leukemia is higher in men than in women.[22]

Age is a major factor in leukemia, showing a higher prevalence in adults and young people.[23] According to our results, CML is more common in the 30−40 year age group, whereas AML and CML are more commonly diagnosed in adults and young men and women. In children, ALL is more common than CML and AML. According to the worldwide distribution of leukemia, the occurrence of myeloid leukemia is high.[24] In our study, most leukemia patients had normal weight or were underweight based on the BMI ranges, among whom more men had normal weight and more women were underweight. According to Vos and colleagues, hypertension, diabetes, anemia, lung disorders, constant fever, weight loss, and skin infections are associated with leukemia.[16] Our study identified a strong association of leukemia with hypertension and skin allergies. Diabetes and heart problems were more common in ALL patients, while AML patients were more prone to skin allergies and lung infections, whereas the prevalence of hypertension was high in AML and CML patients. Compared with other studies, our findings revealed that hypertension, skin allergies, diabetes, and lung infections are common in leukemia patients.

The serological and hematological results of the study varied among CML, AML, and ALL patients. According to our results, the total leukocyte count was increased in all leukemia types because leukemia causes abnormal proliferation of white blood cells. CML patients had the highest TLC count compared with ALL and AML, and a significant difference was observed between the CML and ALL ($p = 0.0057$) and the CML and AML groups ($p = 0.003$).

In leukemia, the hemoglobin (Hb) level showed random fluctuation, which may be increased or decreased, whereas the normal hemoglobin levels in women and men were 12 and 15 per deciliter of blood (g/dL), respectively. A decrease in Hb is mostly found in myeloid leukemias such as CML and AML due to anemic conditions.[25] In our study, AML patients had a significantly lower level of Hb, although ALL and CML patients also had a decreased ratio of Hb. According to Arico et al., the platelet count mostly decreases in blood cancer, especially in CML and ALL; however, it may increase in rare cases.[21] Our study found that the platelet count is decreased in CML, ALL, and AML patients, which may lead to thrombocytopenia. In CML patients, the platelet count was the lowest compared with AML and ALL patients.

The Philadelphia chromosome is the hallmark of chronic myeloid leukemia, and the expression of BCR-ABL1 is found to be ∼90% in CML patients.[26] BCR-ABL1 is not restricted to CML: 11−34% of ALL patients and ∼1−2.5% of AML patients harbor the BCR-ABL1 oncogene. BCR-ABL1-positive AML is unique due to some distinct features. We observed the level of BCR-ABL1 expression in PBMCs in CML, AML, and ALL. A previously reported study suggested the highest expression of the BCR-ABL1 gene in CML patients.[27] Interestingly, we found 7.7-, 4.469-, and 1.049-fold higher expressions of the BCR-ABL1 gene in CML, ALL, and AML patients compared with normal individuals. A comparative analysis among all study groups shows the highest fold expression of BCR-ABL1 in CML as compared with ALL and AML. On comparing our results, CML showed a sixfold higher expression of BCR-ABL1 than AML and a threefold higher expression compared with ALL. According to Konoplev et al., the incidence of Ph + AML ranges from 1 to 2.5% of AML

https://doi.org/10.1021/acsomega.2c07885
ACS Omega 2023, 8, 5975−5982

cases, showing lower influence of BCR-ABL1 translocation on leukemia. This study indicates a twofold higher expression of BCR-ABL1 in AML patients, which is the latest research result in Pakistan. Our study revealed a onefold higher expression of BCR-ABL1 in AML.[28]

To analyze the relation of BCR-ABL1 among CML, ALL, and AML, the correlation among $2^{-\Delta\Delta Ct}$ values was determined by Pearson's correlation coefficient. The value of the coefficient of correlation showed a significant difference in the Ct values between CML and ALL ($p = 0.0043$) as well as between CML and AML ($p = 0.0006$), which showed a constant association of BCR-ABL1 with CML. In 2016, the WHO classification of leukemia malignancies included BCR-ABL1-positive AML as a provisional entity that shows the correlation between AML and Philadelphia translocation.[29]

The above quantitative study was performed through qPCR based on the SYBER green principle. Before analysis, these samples were categorized as Philadelphia-positive or Philadelphia-negative by FISH analysis, which revealed a slight difference between the sensitivity of FISH and qPCR to detect the BCR-ABL1 oncogene. In our studied population, 61 patients were Philadelphia-positive (with the BCR-ABL mutation) and 39 patients were Philadelphia-negative (without the BCR-ABL mutation). Among the Ph-negative patients, four samples were found to be BCR-ABL1-positive by qPCR, initially marked as Ph-negative by FISH, which showed the highest sensitivity of qPCR. According to Mrozek et al., FISH has 0.1−5% sensitivity, whereas qPCR has 0.001−0.01% accuracy and sensitivity.[25] Therefore, this study also revealed that qPCR is extremely sensitive, accurate, and precise for the detection of leukemia in patients.

In our study, all patients underwent different treatments, including chemotherapy, targeted therapy, and combined chemotherapy. Targeted therapy with the tyrosine kinase inhibitor (TKI) imatinib was common in CML patients, which showed a positive response of CML patients toward targeted therapy. The main therapies for AML patients were combined chemotherapy and targeted therapy. However, chemotherapy and sometimes radiotherapy was recommended for ALL patients. According to the American Cancer Society, chemotherapy, radiation therapy, targeted therapy, combination therapy, and stem cell transplant are the main therapies for blood cancer treatment. According to the latest survey, chemotherapy and targeted therapy are the most commonly adopted methods for curing and treating leukemia in Pakistan.[17]

5. CONCLUSIONS AND LIMITATIONS

The oncogene BCR-ABL1 plays a fundamental role in the pathogenesis and susceptibility of leukemia. The translocation of the Philadelphia chromosome has variable effects in leukemia. In this study, our results depict a significant difference in the transcription of BCR-ABL1 in CML, AML, and ALL patients who were under different treatments of chemotherapy. Chronic myeloid leukemia (CML) shows the highest expression level of BCR-ABL1 transcripts, followed by acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML). The expression of BCR-ABL1 was the lowest in AML as compared with CML and ALL. However, further studies are needed to explore the molecular mechanism underlying the associated factors. In this study, a limited number of samples (due to availability) or a smaller population was studied. It can be concluded that, along with the altered expression of BCR-ABL1, other metabolic and demographic factors play vital roles in producing a response toward chemotherapy and targeted therapy. In addition to these highlighted parameters, a number of other factors, such as transcripts of BCR-ABL1 and differences in isoforms and their regulation pathways, are worth studying. However, due to limitations of resources and information available, these could not be accomplished.

AUTHOR INFORMATION

Corresponding Authors

Amjad Ali − *Department of Biotechnology and Genetic Engineering, Hazara University, Mansehra 21120 Khyber Pakhtunkhwa, Pakistan; Molecular Virology Laboratory Centre for Applied Molecular Biology (CAMB), University of the Punjab, Lahore 54590, Pakistan;* orcid.org/0000-0001-5857-0653; Email: amjad.genetics@hu.edu.pk

Ajmal Khan − *Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Sultanate of Oman;* orcid.org/0000-0001-7851-6080; Email: ajmalkhan@unizwa.edu.om

Ahmed Al-Harrasi − *Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Sultanate of Oman;* orcid.org/0000-0002-0815-5942; Email: aharrasi@unizwa.edu.om

Authors

Farah Arshad − *Molecular Virology Laboratory Centre for Applied Molecular Biology (CAMB), University of the Punjab, Lahore 54590, Pakistan*

Gauhar Rehman − *District Medical Specialist Category-D Hospital Talash Dir Lower, Lower Dir 23120 Khyber Pakhtunkhwa, Pakistan*

Sobia Ahsan Halim − *Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Sultanate of Oman*

Muhammad Waqas − *Department of Biotechnology and Genetic Engineering, Hazara University, Mansehra 21120 Khyber Pakhtunkhwa, Pakistan; Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Sultanate of Oman*

Asaad Khalid − *Substance Abuse and Toxicology Research Center, Jazan University, Jazan 45142, Saudi Arabia; Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center for Research, Khartoum 11111, Sudan*

Ashraf N. Abdalla − *Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia;* orcid.org/0000-0003-4770-9319

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c07885

Author Contributions

Conceptualization: A.A., A.K., and A.A-H.; methodology: F.A. and G.R.; software: S.A.H.; formal analysis: M.W.; investigation: A.K.; resources: A.A., A.K., and A.A-H.; data curation: A.N.A.; writing—original draft preparation: F.A. and A.A.; writing—review and editing: S.A.H., M.W., and A.K.; visualization: A.K.; supervision: A.A., A.K., and A.A-H.; funding acquisition: A.N.A. and A.A-H.

Funding

This project was supported by a grant from the Oman Research Council (TRC) through the funded project (BFP/RGP/HSS/19/198).

https://doi.org/10.1021/acsomega.2c07885
ACS Omega 2023, 8, 5975−5982

Notes

The authors declare no competing financial interest.

This study was approved by the Institutional Ethics Committee of Hazara University Mansehra, 21120, Pakistan (HU/ORIC/2022/1091). for studies involving humans.

Informed consent was obtained from all subjects involved in the study.

■ ACKNOWLEDGMENTS

The authors thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work through Grant Code: 22UQU4331128DSR66.

■ REFERENCES

(1) Papaemmanuil, E.; Gerstung, M.; Bullinger, L.; Gaidzik, V. I.; Paschka, P.; Roberts, N. D.; Potter, N. E.; Heuser, M.; Thol, F.; Bolli, N.; et al. Genomic classification and prognosis in acute myeloid leukemia. *N. Engl. J. Med.* **2016**, *374*, 2209−2221.

(2) Rüegg, C. Leukocytes, inflammation, and angiogenesis in cancer: fatal attractions. *J. Leukocyte Biol.* **2006**, *80*, 682−684.

(3) Collins, S. J. Retinoic acid receptors, hematopoiesis and leukemogenesis. *Curr. Opin. Hematol.* **2008**, *15*, 346−351.

(4) Mitelman, F.; Johansson, B.; Mertens, F. The impact of translocations and gene fusions on cancer causation. *Nat. Rev. Cancer* **2007**, *7*, 233−245.

(5) Nacheva, E. P.; Grace, C. D.; Brazma, D.; Gancheva, K.; Howard-Reeves, J.; Rai, L.; Gale, R. E.; Linch, D. C.; Hills, R. K.; Russell, N.; et al. Does BCR/ABL 1 positive Acute Myeloid Leukaemia Exist? *Br. J. Haematol.* **2013**, *161*, 541−550.

(6) Verma, D.; Kantarjian, H. M.; Jones, D.; Luthra, R.; Borthakur, G.; Verstovsek, S.; Rios, M. B.; Cortes, J. Chronic myeloid leukemia (CML) with P190BCR-ABL: analysis of characteristics, outcomes, and prognostic significance. *Blood* **2009**, *114*, 2232−2235.

(7) Druker, B. J.; Sawyers, C. L.; Kantarjian, H.; Resta, D. J.; Reese, S. F.; Ford, J. M.; Capdeville, R.; Talpaz, M. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N. Engl. J. Med.* **2001**, *344*, 1038−1042.

(8) Hofmann, W.-K.; Jones, L. C.; Lemp, N. A.; de Vos, S.; Gschaidmeier, H.; Hoelzer, D.; Ottmann, O. G.; Koeffler, H. P. Ph+ acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. *Blood* **2002**, *99*, 1860−1862.

(9) Mullighan, C. G.; Goorha, S.; Radtke, I.; Miller, C. B.; Coustan-Smith, E.; Dalton, J. D.; Girtman, K.; Mathew, S.; Ma, J.; Pounds, S. B.; et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* **2007**, *446*, 758−764.

(10) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods* **2001**, *25*, 402−408.

(11) Urruticoechea, A.; Alemany, R.; Balart, J.; Villanueva, A.; Viñals, F.; Capella, G. Recent advances in cancer therapy: an overview. *Curr. Pharm. Des.* **2010**, *16*, 3−10.

(12) Copland, M.; Jørgensen, H. G.; Holyoake, T. L. Evolving molecular therapy for chronic myeloid leukaemia—are we on target? *Hematology* **2005**, *10*, 349−359.

(13) Nowak, D.; Stewart, D.; Koeffler, H. P. Differentiation therapy of leukemia: 3 decades of development. *Blood* **2009**, *113*, 3655−3665.

(14) Huang, J.; Chan, S. C.; Ngai, C. H.; Lok, V.; Zhang, L.; Lucero-Prisno, D. E., III; Xu, W.; Zheng, Z.-J.; Elcarte, E.; Withers, M.; Wong, M. C. S. Disease Burden, Risk Factors, and Trends of Leukaemia: A Global Analysis. *Front. Oncol.* **2022**, *12*, No. 904292.

(15) Aziz, Z.; Iqbal, J.; Akram, M.; Saeed, S. Treatment of chronic myeloid leukemia in the imatinib era: perspective from a developing country. *Cancer* **2007**, *109*, 1138−1145.

(16) Vos, T.; Abajobir, A. A.; Abate, K. H.; Abbafati, C.; Abbas, K. M.; Abd-Allah, F.; Abdulkader, R. S.; Abdulle, A. M.; Abebo, T. A.; Abera, S. F. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990−2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **2017**, *390*, 1211−1259.

(17) Usmani, S. Z.; Yunus, S. A.; Jamal, Y. Overview of chronic myeloid leukemia patients in Pakistan in the pre-imatanib era. *Asian Pac. J. Cancer Prev.* **2009**, *10*, 1039−1040.

(18) Panda, S. K.; Ravindran, B. Isolation of human PBMCs. *Bio-Protoc.* **2013**, *3*, e323.

(19) Ye, J.; Coulouris, G.; Zaretskaya, I.; Cutcutache, I.; Rozen, S.; Madden, T. L. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinf.* **2012**, *13*, 1−11.

(20) Prism, G.6 GraphPad Software RRID: SCR_002798. 2019. https://www.graphpad.com/scientificsoftware/prism.

(21) Arico, M.; Schrappe, M.; Hunger, S. P.; Carroll, W. L.; Conter, V.; Galimberti, S.; Manabe, A.; Saha, V.; Baruchel, A.; Vettenranta, K. Clinical outcome of children with newly diagnosed Philadelphia chromosome−positive acute lymphoblastic leukemia treated between 1995 and 2005. *J. Clin. Oncol.* **2010**, *28*, 4755−4761.

(22) Klonoff-Cohen, H.; Navarro, A.; Klonoff, E. A. Late effects awareness website for pediatric survivors of acute lymphocytic leukemia. *PLoS One* **2018**, *13*, No. e0193141.

(23) Corbin, A. S.; Buchdunger, E.; Pascal, F.; Druker, B. J. Analysis of the structural basis of specificity of inhibition of the Abl kinase by STI571. *J. Biol. Chem.* **2002**, *277*, 32214−32219.

(24) Haznedaroğlu, İ. C. Current management of chronic myeloid leukemia with tyrosine kinase inhibitors. *Turk. J. Hematol.* **2013**, *30*, 247−255.

(25) Larfors, G.; Hallböök, H.; Simonsson, B. Parental age, family size, and offspring's risk of childhood and adult acute leukemia. *Cancer Epidemiol., Biomarkers Prev.* **2012**, *21*, 1185−1190.

(26) Eghtedar, A.; Verstovsek, S.; Estrov, Z.; Burger, J.; Cortes, J.; Bivins, C.; Faderl, S.; Ferrajoli, A.; Borthakur, G.; George, S.; et al. Phase 2 study of the JAK kinase inhibitor ruxolitinib in patients with refractory leukemias, including postmyeloproliferative neoplasm acute myeloid leukemia. *Blood* **2012**, *119*, 4614−4618.

(27) White, H. E.; Matejtschuk, P.; Rigsby, P.; Gabert, J.; Lin, F.; Wang, Y. L.; Branford, S.; Müller, M. C.; Beaufils, N.; Beillard, E.; et al. Establishment of the first World Health Organization international genetic reference panel for quantitation of BCR-ABL mRNA. *Blood* **2010**, *116*, e111−e117.

(28) Konoplev, S.; Yin, C. C.; Kornblau, S. M.; Kantarjian, H. M.; Konopleva, M.; Andreeff, M.; Lu, G.; Zuo, Z.; Luthra, R.; Medeiros, L. J. Molecular characterization of de novo Philadelphia chromosome-positive acute myeloid leukemia. *Leuk. Lymphoma* **2013**, *54*, 138−144.

(29) Network, C. G. A. R. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N. Engl. J. Med.* **2013**, *368*, 2059−2074.

https://doi.org/10.1021/acsomega.2c07885
ACS Omega 2023, 8, 5975−5982