Journal of Pioneering Medical Sciences
Received September 23, 2023 Accept January 08, 2023 Publish February 29, 2024.
Volume 13, Issue 1, Pages 94-98

DOI https://doi.org/10.61091/jpms202413116

Immunological and Molecular Investigation of BCR-ABL1 in Samples of Iraqi Chronic Myeloid Leukemia Patients

Farooq I. Mohammad[1,*] and Alaa R. Nadim[2]
[1]College of Science, Kirkuk University, Iraq.
[2]Department of Biology, Kirkuk University, Iraq.

Corresponding author: Farooq I. Mohammad (e-mail: farooqalwais@yahoo.com).

Abstract **Background:** Chronic myelogenous leukemia (CML) is a blood malignancy that affects hematopoietic stem cells. Also, it is commonly identified by the Philadelphia chromosome. This chromosome produces a protein with strong tyrosine kinase activity and functions as a tumorigenic factor. **Objective:** This study aimed to investigate the Immunological and Molecular of BCR-ABL1 gene expression and ABL1 protein in patients admitted to the Oncology and Hematology Center of the Kirkuk Governorate, Iraq. **Materials and Methods:** A study was conducted in Kirkuk Governorate, Iraq, from the beginning of November 2022 to May 2023 on Chronic Myeloid Leukemia patients in the Oncology and Hematology Center, which was hospital-based and cross-sectional. The study comprised fifty-three patients with Chronic Myeloid Leukemia. They were diagnosed using a complete blood count (CBC), a molecular test BCR-ABL1, and an immunological test ABL1 protein. The research did not include patients with missing data and those without consent. Fifty-three peripheral blood samples from 53 CML patients at chronic stages of CML were taken, and using automated Gene Xpert BCR-ABL1 Ultra assay, BCR-ABL1 transcript quantification was conducted on them. **Results:** The research included fifty-three patients in total (27 males, 28 females), with a mean age of 47.3±12.8 years. The average of BCR_ABL1 by RT_ qPCR 3.22±0.09 and the mean of ABL1 by Enzyme-linked Immune sorbent assay (ELISA) reigned is 981±891. **Conclusion:** Values of the quantitative real-time reverse transcription polymerase chain reaction (RT_ qPCR) by Gene Xpert Ultra level range between 38.62 and 0.00 with a mean of 3.22±0.09. The RT_ qPCR level showed that 22% of the patients were in good condition, 11% of them had a strong response to treatment, and other 11% showed a complete cytogenetic response (CCyR), which is also a good outcome; however, 78% of the patients either not taking their medication, or there is true resistance. These two methods used different techniques; the former is a nuclear-derived method that detects the presence of specific genetic materials of any pathogen; in the current research, BCR-ABL1 is the target. The latter is a test that detects and measures antibodies, antigens, proteins, and glycoproteins in biological samples, so ABL1 is the target.

Key Words Leukemia, CML, p190, p210, Treatment, B cell, BCR-ABL1

1. Introduction

CML is a hematopoietic cell myeloproliferative illness in which the BCR-ABL1 translocation is necessary for malignant change. It is responsible for 15 percent of the diagnosed leukemia cases in adults in the United States (1.95 cases per 100 thousand persons yearly). It affects 1 in 100,000 persons in Europe yearly, with 64 years being the average age upon diagnosis. CML is often identified in the treatable chronic phase (CP), but it can advance to an accelerated phase (AP) or lethal blast phase (BP). Hyperactivation of signaling pathways is the key molecular change in CML, which leads to excessive cell proliferation and reduced apoptosis [1], [2].

The ABL proto-oncogene 1 (ABL1) and the fusion gene of the breakpoint cluster region (BCR) encode a constitutively active tyrosine kinase known as BCR-ABL1. The reciprocal translocation of chromosomes 9 and 22 (q34, q11) Forms the Philadelphia chromosome (Ph). Nowell & Hungerford detected a recurrent tiny chromosome in neoplastic cells of CML patients. Relying on the location of the breakpoint, three isoforms differ in length; the longer variation of 210 kDa molecular weight (p210), the shorter variation of 185-190 kDa molecular weight (p190), and the most extended variation (p230) is uncommon and have not been explored in this study. Figure 1 displays a schematic of the differences in structure between p210 and p190. There is little

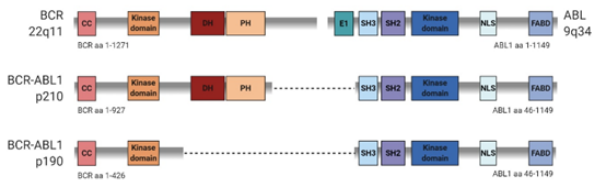

Figure 1: Differences in structure of p190and p210 isoforms of the BCR-ABL1 fusion gene, based on [5] and [6]. Abbreviations; FABD, F-actin binding domain DH, Dbl-homology; E1, ABL1 exon 1; CC, coiled-coil; PH, Pleckstrin-homology; SH2/3, SRC homology 2/3 NLS, nuclear localization signal

understanding of particular mechanisms of Ph chromosome creation and the factors that influence the development of particular variations. Nonetheless, single-strand annealing, RAG activity, and non-homologous end joining have been identified as advocators of the mentioned events [3], [4].

The Ph chromosome is seen mainly in B-cell acute lymphoblastic leukemia and CML. The disorders differ in clinical and molecular characteristics. CML originates from hematopoietic stem cells, and the expression of BCR-ABL1 in leukemic stem cells initiates and promotes leukemogenesis. The first targeted medications introduced in the late 1990s were Tyrosine kinase inhibitors (TKIs), commonly used to control CML in its chronic, moderate phase. Adding TKIs to CML therapy regimens significantly improved disease control and patient outcomes. In rare circumstances, myeloid or lymphoid-origin CML is identified in an advanced, accelerated, or blast phase. During the therapy with TKIs, a shift into the blast crisis may also occur due to rising resistance [7], [8]. Ph+ B-ALL. It is a form of acute lymphoblastic leukemia (ALL) that becomes more common as individuals get older. It develops from B cell-committed progenitors. Besides the BCR-ABL1 translocation, multiple additional genetic abnormalities are present upon diagnosis. It is frequently identified in a more advanced stage than TKIs, and CML has less effect because of the primary or secondary resistance, which results in short all-around survival of patients of Ph+ B-ALL [9].

Prevalence of distinct BCR-ABL1 isoforms varies with disease-p210 and is detected in around 95% of cases of CML, whereas p190 is 70% present in Ph+ B- patients [10]. Although all isoforms retain a complete ABL1-derived kinase domain (KD), current studies show considerable variations in their signaling, subcellular localization, and interactomes. Furthermore, research over the past decade has revealed a wealth of signaling pathways and linage-specific mutations that impose particular vulnerabilities and may serve as potential targets for therapy [11], [12].

CML is diagnosed using cytogenetics, histology, and the identification of the BCR-ABL1 transcript via fluorescence in situ hybridization (FISH) or reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR is used to measure molecular response (MR) to therapy; using the International Scale, it can be defined as the ratio of BCR-ABL1 to ABL1 transcripts. A less than 1% MR suggests full cytogenetic remission, while an MR $\leq 0.1\%$ indicates MMR or MR3. Molecularly undetectable leukemia is characterized as BCR-ABL1 transcript levels < 0.0032% or MR4.5 [13].

2. Materials And Methods

A. Study Design

A cross-sectional study was performed at Kirkuk Governorate, Iraq from the beginning of November 2022 to May 2023 on Chronic Myeloid Leukemia patients in Oncology and Hematology Center. The total of 53 patients and 15 control, who were admitted The research included people who had chronic myeloid leukemia based on a full blood count and the genetic test BCR-ABL1. Patients with missing data and those who did not consent were not included in the research.

B. Molecular Test

The Xpert BCR-ABL Ultra test from the Cepheid Gene Xpert® Dx System the quantitative real-time reverse transcription polymerase chain reaction method was utilized. (RT_ qPCR). The procedure was followed Instruments Company. The GeneXpert system interprets the findings, which are shown in the results display window, automatically using built-in calculation algorithms and the observed fluorescence signals [14], [15].

C. Assay Procedure

Peripheral blood (PB) samples were collected in standardized tubes (EDTA). The kit (CA 94089, used by Xpert BCR-ABL Ultra Cepheid USA), 100 μl of proteinase K (PK) was poured into the conical tube, 4 ml of blood was added to the tube containing (PK), the sample was mixed for 3 seconds by Vortex and stored at room temperature, Lysis reagents (LY) with a volume of 2.5 ml was poured into the same tube and mixed for 10 seconds and stored at room temperature for 5 minutes and then mixed for 10 seconds with Vortex. From the previously prepared sample, transfer 1 ml to another conical tube containing 1.5 ml of the (LY). Mixing of the samples was done for 10 seconds and stored at room temperature for 10 minutes, then 2 ml of ethanol was added and mixed for 10 seconds to remove (PK) and (LY). A washing reagent was added next to the port in the test cartridge. The cartridge was loaded into the Gene Xpert System. The total assay time was approximately 2 hours.

D. Statistical Analysis

Informed consent was obtained from the patients who had concealed entirely the data. Gender and age were recognized among the demographic data.

In order to compare the effect of variables on the treatment activity, the student's t-test was used, a statistical test used to compare the means of 2 groups. It is utilized in hypothesis testing to assess if the treatment or method affects the population of interest or if the two groups differ. Therefore,

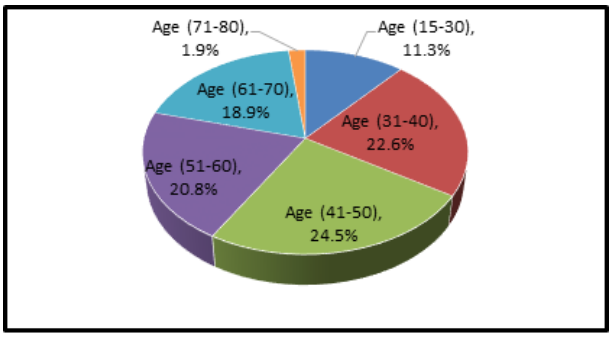

Figure 2: Distribution of the patients group based on the age

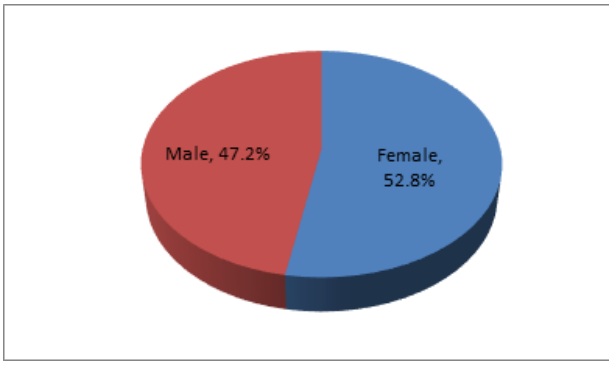

Figure 3: Distribution of the patients group based on gender

the population was split into two groups of participants: 1) A control group that is in normal conditions and 2) A patient group that is taking a treatment course.

In order to investigate whether there are differences between the two groups, a between-subjects design was considered; moreover, it is assumed that the data are continuous and there are no gaps. Also, the two groups are independent and approximately normally distributed. As a summary, a two-tailed t-test was applied, i.e., Null Hypothesis and Alternative Hypothesis (H1: $\mu_1 \neq \mu_2$) should be checked. Moreover, the equality of the variances can also be tested. Two assumptions are checked in order to get meaning comparison, and a signify result should be obtained; as a first step, the t-statistic and p-value were calculated, assuming that the variances are equal. The Significance level (α), which is the null hypothesis likelihood of rejecting if it is true, was taken as 0.05, the degree of freedom (df) is 66, and for specified df = 66, and the Significance level α = 0.05 the t-critical (from t-distribution table) is 1.9966.

3. Results and Discussion

Age of the population is ranging between 20-80 years with a slight female predominance. The age groups illustrated in Figure 2, it is clearly can be shown that the most frequent age was elder than 50 years' old which was 42% compared to 58% who are younger than 50 years old. The patients average age was 47.3±12.8 years, which is higher than the highest mean found in the literature by 5.4 years. Where, the means found in literature are ranging between 39 years to 41.8 years [16]–[21].

The distribution of the population based on gender are illustrated in Figure 3, the percentage of male are 47% compared to female which was 53%. This percentage can help to give an accurate idea on the development of the recovery percentage based on gender.

In vitro BCR-ABL1 transcript quantification was done on 53 peripheral blood samples from CML patients in the chronic phase of the disease utilizing the automated Gene Xpert BCR-ABL Ultra assay (Cepheid, USA). Gene Xpert BCR-ABL Ultra is a highly sensitive test with a detection limit of MR4.5 and below. Due to its sensitivity and availability of international standard methods for determining BCR-ABL1 transcript levels, RT-qPCR is a regularly used monitoring tool in CML [22].

The translocations of the nine chromosomes and 22 resulted in creating a protein coded as BCR-ABL, commonly referred to as the Philadelphia chromosome. Identification of this abnormal protein led to the development use of tyrosine kinase inhibitors (TKIs), which resulted in a wide improvement in nearly all patients with CML [23].

The BCR-ABL genetic test is used to diagnose or rule out CML because the BCR-ABL is found in almost all patients with CML and a few cases in patients with Acute Myelogenous Leukemia (AML). Moreover, this type of test may be used to ensure that the treatment is effective and that, in some cases, the patient resists certain treatments. Despite this remarkable therapeutic progress, many methods were suggested to diagnose, but with altered accuracy and time-consuming.

In the current research, two common, inexpensive, quick, and accurate techniques have been used. The first method is a reverse transcription-polymerase chain reaction (RT-PCR) method in real-time; the statistical analysis of the tested samples is introduced in Table 1. Values of the PCR level range between 38.62 and 0.00 with a mean of 3.22±0.09. The PCR level showed that 22% of the patients were in good condition, 11% of them with a strong response to treatment, and the other 11% showed a complete cytogenetic response (CCyR), which is also a good outcome. However, 78% of the patients either not taking their medication, or there is true resistance.

The other method is the Enzyme-Linked Immunosorbent Assay, shortened by (ELISA). The mean of the results is 981 with a big standard deviation of ±891. These two methods used different techniques; the former is a nuclear-derived method for finding the presence of specific genetic materials of any pathogen; in the current research, BCR-ABL1 is the target. The latter test detects and measures antibodies, antigens, proteins, and glycoproteins in biological samples, so ABL1 is the target.

In order to compare the accuracy of these test methods, the t-test method was used, assuming two-sample unequal variances. The result is introduced in Table 2. It clearly

Variable	BCR_ABL1 by RT PCR	ABL1 by ELISA
Max.	38.62	4673.70
Min.	0.00	113.10
Mean.	3.22	980.95
St.D	0.09	891.70
St. Error mean	8.53	646.81

Table 1: Statistics analysis of BCR_ABL1 by RT PCR and ABL1 by ELISA

Parameter	BCR_ABL1 by RT PCR
t-statistic	-11.00
t-critical	2.00
df	52
P-value (2-tail)	0

Table 2: T-Test: for BCR-ABL genetic test (Two-Sample Unequal Variances Assumed)

can be seen that the absolute t-statistic is greater than the critical t. Moreover, the p-value is zero, i.e., less than the significant level (= 0.05). This means that null hypotheses can be rejected or the means are unequal. There is a fundamental difference between these two methods.

4. Conclusion

Values of the perform (RT_ qPCR) level is ranging between 38.62 and 0.00 with mean of 3.22±0.09. The PCR level showed that 22% of the patients are in good conditions, 11% of them with strong response to treatment, and other 11% shows a complete cytogenetic response (CCyR), which is also a good outcome, however 78% of the patients either not taking their medication, or there is true resistance. the mean of ABL1 by Enzyme linked Immune sorbent assay (ELISA) reigned is 981±891. And then there is a real difference between these two methods.

5. Acknowledgment

We would like to acknowledge the University of Kirkuk/ College of Science / Department of Biology for the support and the Oncology and Hematology Center / Kirkuk for help and scientific advices, Dr. Ahmed Ibrahim Shuker and Dr. Dalia Maher Aljomard/ Dr. Dalia Laboratory/ Kirkuk for help, support and providing the samples. we would like to acknowledge Dr. Engineer Farid Al Arnaout for his assistance whit the statistical results.

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

References

[1] Soverini, S., Mancini, M., Bavaro, L., Cavo, M., & Martinelli, G. (2018). Chronic myeloid leukemia: the paradigm of targeting oncogenic tyrosine kinase signaling and counteracting resistance for successful cancer therapy. Molecular Cancer, 17(1), 1-15.

[2] Foulon, S., Cony-Makhoul, P., Guerci-Bresler, A., Delord, M., Solary, E., Monnereau, A., ... & Tubert-Bitter, P. (2019). Using healthcare claims data to analyze the prevalence of BCR-ABL-positive chronic myeloid leukemia in France: A nationwide population-based study. Cancer Medicine, 8(6), 3296-3304.

[3] Chereda, B., & Melo, J. V. (2016). The biology and pathogenesis of chronic myeloid leukemia. Chronic Myeloid Leukemia, 17-39.

[4] Score, J., Calasanz, M. J., Ottman, O., Pane, F., Yeh, R. F., Sobrinho-Simões, M. A., ... & Grand, F. H. (2010). Analysis of genomic breakpoints in p190 and p210 BCR–ABL indicate distinct mechanisms of formation. Leukemia, 24(10), 1742-1750.

[5] Reckel, S., Gehin, C., Tardivon, D., Georgeon, S., Kükenshöner, T., Löhr, F., ... & Hantschel, O. (2017). Structural and functional dissection of the DH and PH domains of oncogenic Bcr-Abl tyrosine kinase. Nature Communications, 8(1), 2101.

[6] Bernt, K. M., & Hunger, S. P. (2014). Current concepts in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia. Frontiers in Oncology, 4, 54.

[7] Soverini, S., Bassan, R., & Lion, T. (2019). Treatment and monitoring of Philadelphia chromosome-positive leukemia patients: recent advances and remaining challenges. Journal of Hematology & Oncology, 12(1), 1-14.

[8] Hochhaus, A., Baccarani, M., Silver, R. T., Schiffer, C., Apperley, J. F., Cervantes, F., ... & Hehlmann, R. (2020). European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia, 34(4), 966-984.

[9] Abou Dalle, I., Kantarjian, H. M., Short, N. J., Konopleva, M., Jain, N., Garcia-Manero, G., ... & Ravandi, F. (2019). Philadelphia chromosome-positive acute lymphoblastic leukemia at first relapse in the era of tyrosine kinase inhibitors. American Journal of Hematology, 94(12), 1388-1395.

[10] Cutler, J. A., Tahir, R., Sreenivasamurthy, S. K., Mitchell, C., Renuse, S., Nirujogi, R. S., ... & Pandey, A. (2017). Differential signaling through p190 and p210 BCR-ABL fusion proteins revealed by interactome and phosphoproteome analysis. Leukemia, 31(7), 1513-1524.

[11] Müschen, M. (2019). Metabolic gatekeepers to safeguard against autoimmunity and oncogenic B cell transformation. Nature Reviews Immunology, 19(5), 337-348.

[12] Chan, L. N., & Müschen, M. (2017). B-cell identity as a metabolic barrier against malignant transformation. Experimental Hematology, 53, 1-6.

[13] Hochhaus, A., Baccarani, M., Silver, R. T., Schiffer, C., Apperley, J. F., Cervantes, F., ... & Hehlmann, R. (2020). European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia, 34(4), 966-984.

[14] Hochhaus, A., Saussele, S., Rosti, G., Mahon, F. X., Janssen, J. J., Hjorth-Hansen, H., ... & Buske, C. (2017). Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology, 28, iv41-iv51.

[15] Baccarani, M., Deininger, M. W., Rosti, G., Hochhaus, A., Soverini, S., Apperley, J. F., ... & Hehlmann, R. (2013). European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood, The Journal of the American Society of Hematology, 122(6), 872-884.

[16] Amna, M. B., Siddiqui, A. A., Wajid, Z., Moin, A., & Khan, A. (2018). Presentations in patients of chronic myeloid leukemia; an observational study focusing on the association of haematological parameter on gender. Cancer Sci Res Open Access, 5(1), 1-5.

[17] Jbireal, J. M., Azab, A. E., Alzahani, S., & Elshareef, M. (2019). Haematological and cytogenetic changes in CML patients treated with imatinib mesylate in Western Libya. Hematol Transfus Int J, 7(3), 50-7.

[18] Al-abady, I. (2021). Clinico-Hematological Profile in Patients with Chronic Myeloid Leukemia. Annals of the College of Medicine, Mosul, 43(1), 1-9.

[19] Jamal, S. I., Ranjan, A., Choudhary, V. N., Kumar, K., Agrawal, R., & Kumar, S. (2022). Clinico-Hematological Profile and Bone Marrow Correlation of Chronic Myeloid Leukemia: A Multiparameter Study in a Tertiary Care Hospital. European Journal of Molecular & Clinical Medicine, 9(3), 4687-4695.

[20] Lee, J. P., Birnstein, E., Masiello, D., Yang, D., & Yang, A. S. (2009). Gender and ethnic differences in chronic myelogenous leukemia prognosis and treatment response: a single-institution retrospective study. Journal of Hematology & Oncology, 2(1), 1-8.

[21] Ngono, A. P. B., Tipane, P. A., Njonnou, S. R. S., Timnou, A. T., Sango, A. J. F., Ndom, P., ... & Mbanya, D. (2020). Hematobiological Profile of Patients with Chronic Myeloid Leukemia at the Diagnosis in Yaoundé: A Cross-Sectional Study. Open Journal of Blood Diseases, 10(04), 110-123.

[22] Ferrand, C., Deschamps, M., Levitas, A., Lockwood, C., Payton, J. E., Uy, G. L., ... & Day, G. J. (2017). Xpert® BCR-ABL ultra, a high sensitivity assay, demonstrates a limit of detection Reaching MR4. 5 and below, Blood blood 130 (Suppl_1) : 1610.

[23] Aitken, M. J., Benton, C. B., Issa, G. C., Sasaki, K., Yilmaz, M., & Short, N. J. (2021). Two cases of possible familial chronic myeloid leukemia in a family with extensive history of cancer. Acta Haematologica, 144(5), 585-590.