ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by Pharmascope Publications Journal Home Page: www.pharmascope.org/ijrps

Detection of BCR-ABL gene in Chronic Myeloid Leukemia patients treated with Tyrosine Kinase Inhibitors Drugs by Gene Expert System

Maysaa Ali Abdul Khaleq[1], Hussein Ali Saheb[2], Ahmed M Sultan[2], Mohsen A. N. Alrodhan*[2]

[1]Bilad Al-Rafidain University College, Diyala, Baquba, Iraq
[2]College of Pharmacy, University of Al Qadisiyah, Diwaniyah, Iraq

Article History:

Received on: 06.12.2018
Revised on: 20.02.2019
Accepted on: 24.02.2019

Keywords:

CML,
BCR-ABL,
Imatinib,
Nilotinib,
q RT-PCR

ABSTRACT

Chronic myeloid leukaemia (CML) is one of the human fatalities caused by genetic mutation and chromosomal translocation, a *BCR-ABL* fusion gene and as a result, Philadelphia chromosome is formed. The irregular tyrosine kinase activity of the encoded protein by this gene causes the establishment of the disease. This study was conducted at the period from September 2016 to February 2017, 100 Iraqi CML patients were divided into two groups, first group of 50 patients were received Imatinib 400-800 mg/day, second group of another 50 patients were received 800 mg/day Nilotinib, WBC were microscopically counted using improved Neubauer ruled hemocytometer counting chamber. The results of WBC count in different disease duration and stages of treatment in the group of patients treated with Imatinib showed that highest WBC count was observed in newly diagnosed patients while there was a significant reduction in the WBC count after one month, after one year and final dose of treatment were 11.8, 6.4 and 8.1 respectively, on the other hand the results of WBC count in the group of patients treated with Nilotinib showed that the highest WBC count was observed in newly diagnosed patients 87.54 ± 8.71 whereas the WBC count after one month, one year and at the final dose of treatment were 7.3, 6.8, 7.9 respectively with significant reduction in the WBC count. The results of the CML patient's distribution according to BCR-ABL gene analysis in the patient's group treated with Imatinib showed that high concentration in newly diagnosed patients 4.753 as compared with significant reduction with other stages of treatment after one month and after one year 0.94 and 0.09 respectively.

* Corresponding Author

Name: Mohsen A. N. Alrodhan
Phone: 009647801569488
Email: moh.alrodhan@gmail .com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i2.274

Production and Hosted by

Pharmascope.org

INTRODUCTION

Chronic myeloid leukaemia (CML) is a worldwide myeloproliferative disease, result from constitutively expressed of the tyrosine kinase activity of BCR-ABL oncoprotein (Hehlmann R. 2017). The diagnostic hallmark of this somatic mutation is Philadelphia chromosome, which is abnormal short chromosome 22 result from a reciprocal chromosome translocation t (934; 911) the translocation fuses the ABL gene on chromosome 22 thereby producing the chimeric BCR-ABL mRNA. BCR-ABL protein is activated from the ABL tyrosine kinase. It is recently believed to be the cause of this disease (Zhen C and Wang YL 2013, Melo JV et al; 1993). The incidence of CML range between 10 and 15 cases / 100000 annually without geographical differences, the median age in Europe ranges between 60-65 and lower in younger population countries (Buyukasik Y and Hanedaroglu Ilhan O 2010) in Iraq leukaemia ranked fourth after breast, bronchus, and lung cancer and represented 7.28%

of the common cancers with incidence of more than 3/100000 of the population (Al saraj MA 2011). The diagnosis can be suspected on the basis of blood picture, and differential count of there is excessive leucocytosis and typical shift to the left of granulopoiesis and the confirmative diagnosis made by detection and identification of Philadelphia, 299 chromosomes, BCR-ABL mRNA or both in bone marrow cell or in peripheral blood (Beillard E., and Pallisgaard N., *et al.,* 2003). Fluorescence *in situ* hybridization (FISH) and reverse transcription real-time polymerase chain reaction RT-RT qPCR can be used on peripheral blood or bone marrow aspirate, flow cytometry is identifying cases with unrecognized progression to lymphoid blast crisis by their phenotypic features while karyotyping may identify some cytogenetic abnormalities (Jabbour E. and Kantrjian H 2018). Malignancies of the blood characterized by genetic translocation which can be used as tumour markers while monitoring the response to therapy (Rahem MR., Alaawad AS., and Kamoona TH 2018). The use of real-time PCR qPCR is becoming an important research tool for the detection of the molecular event and guide therapeutic decision based on how the patient responded at the molecular level. Many studies have been carried out to quantify the translocation fusion transcripts of BCR-ABL in CML to determine the response to the treatment (Ou J, Vergillo J. and Bagg A 2008) CML is the first malignancies of human that targeted chemotherapy may give amazing results (Soverini S., et al 2018). Three tyrosine kinase inhibitors (TK1) has modulated the outcome in CML; Imatinib mesylate is a potent and well tolerated of BCR-ABL tyrosine kinase ATP competitive, the efficacy of Imatinib may compromise by the development of resistance and second and third generation TK1 rationally developed as Nilotinib and Dasatinib as allosteric inhibitors (Mahon FX 2011). This study aimed to evaluate the molecular monitoring the Iraqi patients with CML on Imatinib and Nilotinib as a TK1 treatment according to ELN guidelines

MATERIALS AND METHODS

Data of 100 Iraqi patients with CML is registered in the national centre of haematology Baghdad/ Iraq at the period from Sep 2016 to Feb 2017. The reported data includes patient name, age, address, date of examination and diagnosis, and report of molecular finding. Some additional data were completed by direct questionnaire. Newly diagnosed, and poor adherence to treatment patient was excluded, permission was obtained from the national centre of haematology, and verbal consent to laboratory tests was obtained from the adults or child parent. Venous blood was collected from all patients in venepuncture to evaluate the BCR-ABL oncogene level according to the ELN guideline for molecular assessment.Four ml was obtained in EDTA tube for complete blood cell count and measurement of BCR-ABL oncogenic level by RT-RT qPCR (Richter J., Soderlund S., Lubking A. *et al.,* 2014, Legos L. 2016). Packed cell volume (PCV), RBC indices, WBC total and differential count, platelet count was measured using full automatic haematology auto analyser (swelab-sweden) BCR-ABL mRNA transcript in blood using peripheral blood collected in EDTA tube according to (Goldbergs S. 2016). The quantitative real-time PCR amplification of specific sequences of the ABL and BCR-ABL was accomplished by reverse transcription (RT) of a complementary DNA (cDNA) followed by qRT-PCR. WBC was obtained by red blood cell lysis of centrifuged buy coated preparation. Then WBC was washed in PBS and processed for RNA extraction then the RNA was reversed transcripted into cDNA with reverse transcriptase enzyme using oligo forward and reverse primers. Control, negative and reference gene were included, RT-PCR was amplified, and cDNA was performed according to (Richter J., Soderlund S., Lubking A. *et al.,* 2014, Legos L. 2016). The level of oncogene BCR-ABL mRNA transcript in WBC was evaluated by qRT-PCR (Cepheid gene expert diagnostic system, USA) that was standardized according to (Hoffman US., Baccarani M. *et al.,* 2016, Sakurai M. 2016) by comparing gene level to specific control gene calibrated to WHO international genetic panel for quantification of BCR-ABL mRNA transcript, by using Xpert BCR-ABL monitor kit and the result was measured as percentage. CML patients were divided into two groups, 50 patients received Imatinib mesylate 800 mg per day and the second group 50 patients received 800 mg Nilotinib per day

RESULTS

The results of studied samples treated with Imatinib showed that out of 50 patients CML 28 (56%) were females and 22 (44%) were males in ratio F: M 1.3:1, while the age ranged from 13-72 years (mean age 45.82).

While the results of studied samples treated with Nilotinib showed that out of 50 CML patients 23(45%) were males, and 27(55%) were females in ratio F: M 1.3:1, mean age were 36.68±13.51.

WBC count

The results of WBC count in different disease duration and stages of treatment in the group of patients treated with Imatinib showed that highest WBC count was observed in newly diagnosed patients mean ± SD *10 ^{9}/L 98.82± 2.89 while there was a significant reduction in the WBC count after

Table 1: Distribution of CML according to age and gender

	Characteristic		Results
Imatinib group	Age		45.82± 16.178
	Gender	Female	28 (56%)
		Male	22(44%)
		female	22 (44%)
Nilotinib group	Age		36.68± 13.51
	Gender	Female	27 (55%)
		Male	23 (45%)

one month, after one year and final dose of treatment were 11.8, 6.4 and 8.1 respectively, on the other hand the results of WBC count in the group of patients treated with Nilotinib showed that the highest WBC count was observed in newly diagnosed patients 87.54± 8.71 whereas the WBC count after one month, one year and at the final dose of treatment were 7.3, 6.8, 7.9 respectively with significant reduction in the WBC count, fig(1).

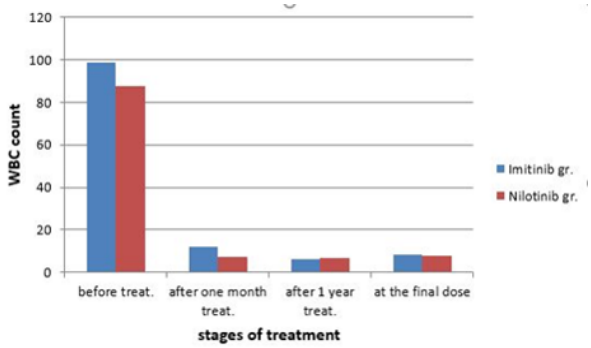

Figure 1: Figure 1: Distribution of CML cases according to WBC count during different disease duration and stages of treatment

BCR-ABL gene analysis

The results of the CML patient's distribution according to BCR-ABL gene analysis in patients group treated with Imatinib showed that high concentration in newly diagnosed patients 4.753 as compared with significant reduction with other stages of treatment after one month and after one year 0.94 and 0.09 respectively.

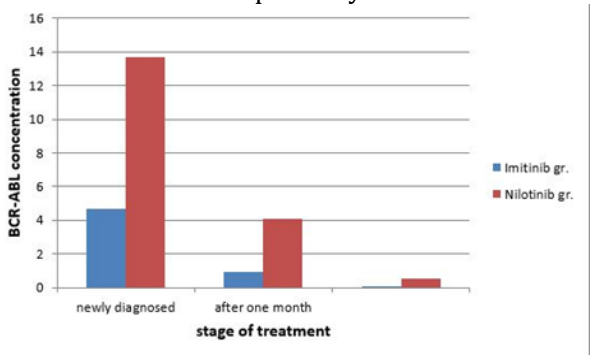

Figure 2: The results of CML patients according to BCR-ABL gene analysis

The highest concentration of BCR- ABL of patients group treated with Nilotinib was in newly diagnosed (13.784) patients, and there was a significant reduction in the gene concentration after one month and after one year 4.191 and 0.530 respectively, fig (2).

DISCUSSION

There is a rapid and continuous update in the treatment and response monitoring of drug used in CML. The aims of measurement of molecular response conducted by qRT-PCR for genetic monitoring of CML patients treated by tyrosine kinase inhibitors can evaluate the response according to ELN guideline on which Iraqi guideline for molecular monitoring was depended on the results of 12 months of treatment by qPCR (An X., Tiwari AK., *et al.,* 2010, Hochhaus A., Saussele S., *et al.,* 2017), the demographic characteristic of studied patients showed that CML is mainly a disease of adults and was similar to most of the studies with CML, study in the united kingdom stated that more than 50% of patient were diagnosed at 65 years and above (Ginzinger DG., 2002). CML is not common in childhood and not reported more than 3 % of paediatric leukaemia (Thompson PA., Kantarjian H *et al.,* 2017, Soverini S., Rosti G *et al.,* 2011, and Patel AA, Patel KM, *et al.,* 2013). In the present study, CML showed to increase the occurrence in young patients; this may be due to genetic and molecular bases of the disease in our population or the nature of the disease development in a younger age group in our society. Our findings agreed with other studies in another area of the world (Höglund M, Sandin F, et al,. 2015) that the CML occurred more frequent in female than male, and that may relate to the pathophysiology of the disease. The PCR method is applicable to study any RNA found in the cell whether it is of normal or abnormal origin. It used in the diagnosis of many cancers. The diagnosis of CML by amplification and identification of leukaemia specific mRNA sequences has an advantage over other molecular methods (Marin D, *et al.,* 2005). In Iraq, the regular follow, up of CML patient on tyrosine kinase inhibitors in the current time change the method of treatment and be compatible to international guideline due to the provision of drugs and availability of facilities to diagnose and detect the BCR-ABL gene level by qPCR.

After one year of treatment with two types of tyrosine kinase' inhibitors (Imatinib and Nilotinib) 90% of CML Iraqi patients of the studied sample showed a major molecular response (MMR), BCR-ABL level reduction and this reflect the proper treatment and follow up of the patients according to ELN guideline. Application of two drugs in our study may overcome the drug resistance. Our findings were in agreement with most studies that more than 50% of patients on TKI treatment response after 12 months (Bauer S, Romvari E. 2013) while at 6 months of treatment more than 70% of CML patients showed optimal response but at this stage of treatment the result may not reflect the real nature of the response, and we cannot depend upon a single reading of the oncogene level, so most of our measurement depends on 12 months (after one year of treatment) for residual disease checkpoint. The treatment of intolerant CML or treatment of Imatinib resistance patient may include other policy such as dose increment of the drug (Imatinib) or the use of the second generation of the TKI such as Nilotinib or another compound. Increasing the dose of the Imatinib may overcome some case of primary Imatinib resistance, but the response is usually short-acting (Apperley JF 2015). The second generation of TKI Nilotinib offers improved potency and greater improvement in Imatinib resistance patients. Many authors stated in clinical trials the efficacy and safety of Nilotinib treatment of a patient with resistance or intolerance to Imatinib.

CONCLUSION

Most of our patient were male and adult. The major molecular response performed after one year of treatment with tyrosine kinase inhibitors according to ELN guideline reflects adequate program and regular follow up of CML Iraqi patients by q RP-CR for detection of BCR-ABL level.

Acknowledgement

We thank the PCR unit in the college of pharmacy – AL Mustansiriyah University, and we also thank the staff of the national centre of haematology Bagdad Iraq.

REFERENCES

Al Saraj MA, Al sabraj SJ., "Iraqi cancer registry Bagdad ministry of health publication house"; 42-66,2011.

An X., Tiwari AK., Sun Y., *et al.,* "BCR-ABL TKI in the treatment of Philadelphia chromosome-positive CML: A review" Leukaemia Research." 34,1255-1268, 2010.

Apperley JF. Chronic myeloid leukaemia. Lancet 2015; 385:1447-59

Bauer S, Romvari E. Interpreting molecular monitoring results and international standardization in chronic myeloid leukemia. J Adv Pract Oncol 2012; 3:151-60

Beillard E., Pallisgaard N., van der Velden VH., *et al.,* "Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program." leukaemia. ;17(12) pp:2474-2486,2003.

Buyukasik Y and Hanedaroglu Ilhan O. "Chronic Myeloid Leukaemia: Practical Issues in Diagnosis, Treatment and Follow-up" 20(2):1-12,2010.

Ginzinger DG., "Gene quantification using real-time qPCR: An emerging technology hits the mainstream" Experimental haematology 30: pp 503-512, 2002.

Goldberg S. "TKI switching patterns during the first 12 months in simplicity, an observational study of CML patients in routine clinical practice." ASH. Abstract: 937.2016.

Hehlmann R. "Research in the heart of haematology: chronic myeloid leukaemia 2017"., Haematol. ;102(3): pp 418-421,2017.

Hochhaus A., Saussele S., Roti G., *et al.,* "CML: ESMO clinical practice guideline for diagnosis, treatment and follow-up."Annals of Oncology, 28, 2017.

Hoffman US., Baccarani M., Hasford T., *et al.,* "Treatment and outcome of CML patients from the Autos population-based registry leukaemia."2016.

Höglund M, Sandin F, Simonsson B. Epidemiology of chronic myeloid leukaemia: An update. Ann Hematoln; 94 Suppl 2: S241-7,2015.

Jabbour E. and Kantrjian H. "Chronic myeloid leukaemia: 2018 update on diagnosis, therapy and monitoring." Am.J. Haematol. 93:442-459.2018.

Legos L., "Second TKI discontinuation in CML patients that failed the first discontinuation and subsequently regained deep molecular response after TKI re-challenges" ASH-abstract: 788.2016.

Mahon FX., "Cessation of tyrosine kinase inhibitors treatment in chronic myeloid leukaemia patients with deep molecular response" Results of the Eur-ski trial. ASH-abstract 787,2016.

Marin D, Kaeda J, Szydlo R, Saunders S, Fleming A, Howard J, *et al.,* Monitoring patients in complete cytogenetic remission after treatment of CML in chronic phase with imatinib: Patterns of residual leukaemia and prognostic factors for cytogenetic relapse. Leukaemia 2005; 19:507-12

Melo JV., Gordon DE., Cross NC., Goldman JM., "The ABL-BCR fusion gene is expressed in chronic myeloid leukaemia." Blood, 8 (1) pp 158-165,1993.

Ou J, Vergillo J. and Bagg A. "Molecular diagnosis and monitoring in the clinical management of patients with chronic myelogenous leukaemia treated with tyrosine kinase inhibitors." Am J Haematol. 83: pp296-302.2008.

Patel AA, Patel KM, Jain AK. Chronic myeloid leukaemia in childhood. GCSMC Med Sci;2: 5-8.2013.

Rahem MR., Alaawad AS., and Kamoona TH. "Evaluation of molecular monitoring and response milestone of patients with chronic myeloid leukaemia to tyrosine kinase inhibitors in middle Euphrates of Iraq." Iraqi journal of haematology. 5 (2): pp143-147.2018.

Richter J., Soderlund S., Lubking A. *et al.,* "Musculoskeletal pain in patients with CML after discontinuation of imatinib a(TKI) withdrawal syndrome" J. Clin. Oncol. :32(25) pp 2821.2014.

Sakurai M., "Long-term treatment with imatinib is associated with decreased estimated glomerular filtration rate and haemoglobin level in patients with CML" c. ASH. Abstract: 1888. 2016.

Soverini S., Maneini M., Bavarol, Cavo M., and Martinelli G., "Chronic myeloid leukaemia: the paradigm of targeting oncogenic tyrosine kinase signalling and counteracting resistance for successful cancer therapy." Molecular cancer. 17: 49.pp1-15, 2018.

Soverini S., Rosti G., Iacobuccl I., *et al.,* "Choosing the best second line TKIs in Imatinib-resistant CML patients harbouring BCR-ABL kinase domain mutations: How reliable is the IC50?" The Oncologist 10.1634, 20

Thompson PA., Kantarjian H., and Cortes JE., "Diagnosis and treatment of CML in 2015." Mayo Clin. Proc.90 (10) pp 1440-1454, 2017.

Zhen C, Wang YL. "Molecular monitoring of chronic myeloid leukaemia: international standardisation of BCR-ABL1 quantitation." J. Mol. Diagnostics. ;15(5): pp556-564.2013.