International Journal of Contemporary Pediatrics
Ezeanosike OB et al. Int J Contemp Pediatr. 2017 Sep;4(5):1557-1561
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20173763

Original Research Article

Prognostic significance of micro RNA 150 marker in BCR-ABL positive chronic myeloid leukaemia patients on imatinib mesylate

Obumneme B. Ezeanosike[1]*, Onyenmechi J. Afonne[2]

[1]Department of Paediatrics and Pharmacology, Federal Teaching Hospital/Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
[2]Department of Pharmacology, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria

Received: 08 April 2017
Accepted: 02 May 2017

***Correspondence:**
Dr. Obumneme B. Ezeanosike,
E-mail: ezeanosike.obum@gmail.com

ABSTRACT

Background: Micro-RNAs control gene expression by destabilizing targeted transcripts and inhibiting their translation. In chronic myeloid leukaemia (CML), abnormal expressions of miRNAs have been described. The current treatment for newly diagnosed cases of CML is imatinib mesylate which produces rapid haematological responses. It is currently impossible to predict whether a patient will develop resistance to imatinib mesylate. This makes identification of predictors of resistance to imatinib an important goal in management of patients with CML. MicroRNA expression patterns can be used to predict outcome which can be remission or relapse. This study therefore, was set to assess the possible use of microRNA 150 for prognostication.
Methods: Fifty peripheral blood samples previously collected from CML patients who were being treated with imatinib mesylate and stored in the refrigerator at +4°C were analyzed for the expression of microRNAs 150. Total RNA was extracted from guanidium isothiocynate (GITC) lysate of the blood samples using RNeasy mini spin column. The total RNA was converted to complimentary DNA by random hexamer priming using Murine Moloney Leukaemia Virus Reverse Transcriptase. Real time Multiplex PCR was used for detecting Breakpoint Cluster Region-Abelson Murine Leukaemia (BCR-ABL) transcript type.
Results: The patients' samples showed an expression of miRNA-150. Correlation of BCR-ABL ratio with miRNA-150 was done and the Spearman correlation coefficient (Rho) between BCR-ABL1 and miRNA-150 was 0.442 ($p = 0.001$; CI = 0.18-0.65) showing that there was a positive correlation between BCR-ABL1 and miRNA-150. The coefficient of determination was 20% (CI = 3-42%), which implies that about 20% of BCR-ABL1 ratio could be accounted for by the miRNA-150 values.
Conclusions: Therefore, once patients who are on imatinib achieve molecular remission of the CML, the miRNA-150 can be useful in predicting outcome which could be relapse or complete molecular remission but is weak at diagnosis in predicting such outcome.

Keywords: Chronic myeloid leukaemia, Imatinib mesylate, Micro RNA, Prognostic markers

INTRODUCTION

The management of patients with chronic myeloid leukaemia (CML) in advanced phases is challenging. This requires the consideration of different treatment approaches, including targeted therapy with tyrosine kinase inhibitors, cytotoxic chemotherapy, and allogeneic stem cell transplantation.[1] Chronic myeloid leukaemia is

a malignancy typified by robust marrow and extra medullary myeloid cell production.[2] The neoplasm has an incidence of 1-2 cases per 100,000 adults accounting for about 15% of newly diagnosed cases of leukaemia in adults.[3] The pathogenesis of CML is related to the fusion of the Abelson murine leukaemia (ABL) gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22, resulting in expression of a onco-protein, termed BCR-ABL.[4] This resultant constitutively active P210 BCR-ABL tyrosine kinase, led to the detailed unravelling of the molecular pathogenesis of CML.[2] It promotes growth and replication through several downstream pathways such as Rat Sarcoma (RAS) pathway, Rapidly Accelerated Fibrosarcoma (RAF) pathway, c-Jun N-terminal kinases, v-myc avian myelocytomatosis viral oncogene homolog (MYC) and Signal Transducer and Activator of Transcription (STAT) pathways that influence leukaemogenesis by creating a cytokine-independent cell cycle with aberrant apoptotic signals.[5-7] CML therefore, has the propensity to progress from a relatively well tolerated chronic phase to an almost uniformly fatal blast crisis phase in the absence of therapy or sometimes despite it.[2] BCR-ABL is necessary and sufficient to initiate the chronic phase of CML and this provides the rationale for targeted therapy with tyrosine kinase inhibitors and the use of imatinib has dramatically altered the natural history of the disease, improving 10-year overall survival from 20 to 80-90%.[8]

Different mechanisms of resistance for imatinib have been described. This includes the presence of point mutations in the tyrosine kinase domain of *BCR-ABL1*, amplification and over-expression of *BCR-ABL1*, over-expression of efflux transporters (such as *ATP-binding cassette sub-family B member (ABCB1*) also known as multi drug resistance 1 (MDR1) or p-glycoprotein, and *ATP-binding cassette sub-family G member (ABCG2)* and under-expression of uptake transporters such as Solute Carrier Family 224 Member 1(*SLC22A1*).[9,10]

MicroRNAs are non-coding, single-stranded RNAs of 21-25 nucleotides which regulate gene expression by promoting degradation of the messenger RNA (mRNA) or repressing its translation.[11,12] In addition, miRNAs have been implicated in the development of human cancers, either as tumour suppressors or as oncogenes. It is currently impossible to predict whether a patient will develop resistance to imatinib mesylate.

About 20% of patients in early chronic-phase of CML are off therapy after 6 years because of resistance or intolerance, and most patients taking imatinib remain BCR-ABL-positive at the molecular level, indicating primary refractoriness of a leukaemic subpopulation. Patients with advanced disease often do not respond, or they eventually relapse.[13] This makes identification of predictors of resistance to imatinib an important goal in management of patients with CML.[14] In CML, abnormal expression of several miRNAs has been previously described: *miRNA-15a, miRNA-16, miRNA-142, miRNA-155, miRNA-181, miRNA-221, let7a* and the polycistronic *miRNA-17-92* cluster.[15] Micro RNAs can regulate the effectiveness of imatinib in CML, but it is not yet clear how they are able to do this however, a role for microRNA expression as a prognostic factor in various tumours has also been described.[16,17] So, determination of gene expression profiles that will likely provide additional information beyond what is obtainable with current clinical and laboratory approaches, will aid management of the subset of patients at high risk of relapse or resistance, and this may allow therapy to be altered before frank relapse occurs.[18]

METHODS

Briefly, about 5–10mls of the stored peripheral blood were studied. The red cells were lysed and homogenized in 1 ml of guanidinium isothiocyanate (GTC) containing β-mercaptoethanol using the method described by Chomczynski and Sacchi.[19,20] Total RNA was extracted from 350µ of GTC cell lysate using RNeasy Mini kits (Qiagen, Crawley, UK) per the manufacturer's instructions. Total RNA was converted to complementary DNA by random hexamer priming using murine Moloney Leukeamia Virus Reverse Transcriptase. Eppendorf Master Cycler Multiplex PCR machine was used for detecting and quantifying BCR-ABL transcripts and the micro RNA 150 in the samples in accordance with the manufacturer's instructions.

Statistical analysis

Data were analyzed using GraphPad Prism demo (Version 6.00) for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Data were expressed as absolute ABL quantities, BCR-ABL ratios and normalized miRNA values. Summary statistics such as means and standard deviations were calculated, and scatter plots for the correlation analysis were done. Spearman correlation coefficient analysis was determined as appropriate.

RESULTS

Results of ABL quantity was determined for all 50 patient samples and it ranged from 1670 to 669000 units, whereas the BCR-ABL quantity ranged from 78 to 8799 units. The mean BCR-ABL ratio which is a ratio of BCR-ABL to the control gene (ABL) was 20.2 (Table 1). There were 44 diagnostic samples as indicated by the BCR-ABL ratios greater than 1.0 and 6 follow-up samples with BCR-ABL ratios less than 1.0.

The correlation of BCR-ABL ratio with miRNA-150 was done to determine the direction and strength of the relationship between these variables. The scatter plot is as shown in Figure 1.

The Spearman Correlation Coefficient (Rho) value between BCR-ABL1 and miRNA-150 was 0. 442 (p =

0.001; CI = 0.18-0.65). This indicates that there is a positive correlation between BCR-ABL ratio and miRNA-150. The coefficient of determination was 20% (CI: 3-42 %), which implies that about 20% of BCR-ABL1 ratio could be accounted for by miRNA-150 values.

Table 1: Summary statistics of measured variables.

+Variable	Mean	Standard deviation	Minimum	Maximum
ABL quantity	29153.60	94547.48	1670	669000
BCR-ABL1 quantity	1610.46	1526.28	78	8799
BCR/ABL ratio	20.19	15.89	0.03	56.859
miRNA150 quantity	284.82	206.52	104	972
Normalized miRNA 150	4.66	5.71	0.05	31.74

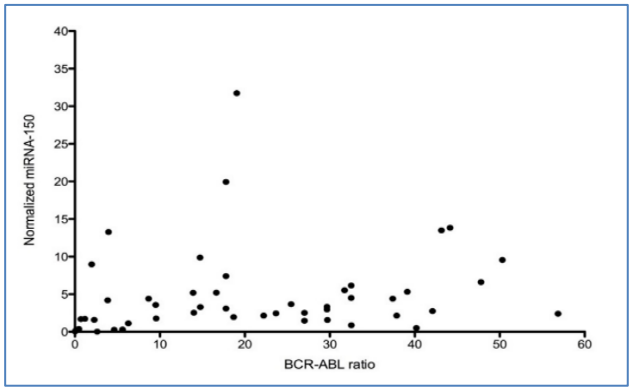

Figure 1: Correlation of BCR-ABL1 ratio with miRNA-150.

The relationship between BCR-ABL1 and miRNA-150 was studied at diagnosis (before remission) and was compared to the molecular remission stage where molecular remission is defined as the 3log reduction in BCR-ABL ratio (Figures 2 and 3).

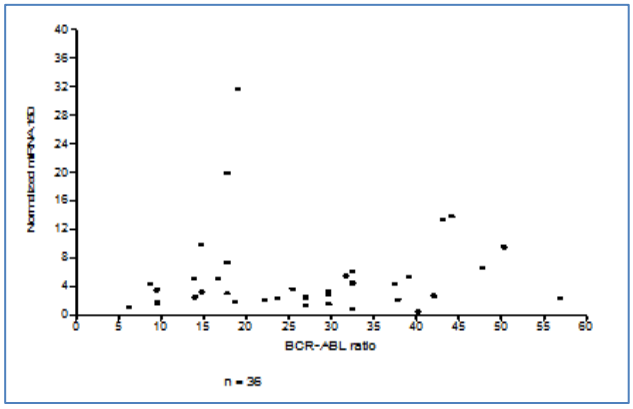

Figure 2: Spearman correlation at diagnosis and before molecular remission.

The Spearman correlation coefficient at diagnosis (before remission) was 0.09 (P = 0.5, CI: -0.25-42); while at remission the Rho was 0.39 (p = 0.16, CI: -0.19 -0.77).

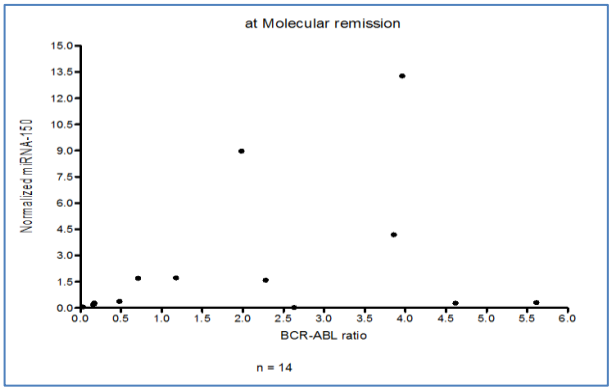

Figure 3: Spearman correlation at molecular remission level.

DISCUSSION

Real time quantitative PCR was used as it is the method of choice.[21] It measures the copy number of BCR-ABL transcripts in each amount of RNA obtained from blood.[22,23] In our samples, BCR-ABL was detected in both diagnostic and follow up samples, ABL being the control gene. The RT-qPCR must detect an endogeneous control transcript to assess the quality and quantity of RNA and to normalize for potential differences between tests.[22] The control gene detected here was ABL. The result is expressed as ratio between BCR-ABL and ABL. The ratios greater than 1.0 suggest the samples were diagnostic samples whereas ratios less than 1.0 suggest they are follow-up samples.[24,25] This study therefore had 44 diagnostic samples and 6 follow up samples.

The presence of BCR-ABL is related to the pathogenesis of CML and is an active tyrosine kinase that regulates growth and replication through pathways that influence leukaemogenesis by creating cytokine-independent cell cycle with aberrant apoptotic signals.[4,26,5-7] The expression of microRNA 150 was detected in our samples. Different sets of miRNAs are expressed in different cell types and tissues and are present in plasma in a remarkably cell free stable form, resistant to harsh

conditions. They have been used as tumour markers for different types of cancers including prostate, breast etc. MicroRNA measured in samples can also be used for diagnostic identification of cancer CML or as circulating biomakers of disease progress so detecting microRNA 150 here is quite significant.

BCR-ABL in this study showed a positive correlation with miRNA-150 at molecular remission level. This implies that once patients who are on imatinib achieve molecular remission of the chronic myeloid leukaemia, the miRNA-150 can be useful in predicting outcome at remission. Such outcomes could be relapse or complete molecular remission. This was also demonstrated by a study which showed that micro RNA 150 levels reduced significantly in CML patients after 2 weeks' therapy with imatinib.[27] Another study showed that decreased levels of *miRNA-150* were detected in patients at diagnosis, in blast crisis and in 67% of haematological relapses and showed significant negative correlation with miRNA-150 target *MYB* and with *BCR-ABL* transcript level.[28] The Rho value at diagnosis and before remission for miRNA-150 is 0.09 (CI: -0.25-42) and the P value is 0.59. That implies that miRNA-150 level at diagnosis is weak in predicting outcome.

CONCLUSION

MicroRNA-150 showed a positive correlation with BCR-ABL1 ratio. Further in vitro studies would be required to elucidate the mechanism of the interaction between miRNA 150 and BCR-ABL. The strong correlation of miRNA 150 and BCR-ABL1 ratio may not be causally related since correlation is not causation, however, it can give an insight into the biology of BCR-ABL1 kinetics in CML

Contributions of this study to current knowledge

This study has contributed the following to our knowledge on CML in Nigeria

- MicroRNA 150 are among the microRNAs expressed in leukaemic patients in Nigeria
- MicroRNA 150 can be used to monitor CML patients on treatment once they achieve remission
- This may help to predict outcome.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the College of Medicine, University of Nigeria Research and Ethics Committee

REFERENCES

1. Ostendorf BN. BCR-ABL Mutation-Guided Therapy for CML Blast Crisis: A Case Report. Biomark Insights. 2015;10(3):25-8.
2. Jamieson CH. Chronic myeloid leukemia stem cells. ASH Education Program Book. 2008;2008(1):436-42.
3. Jemal A. Cancer statistics. CA Cancer J Clin. 2010;60(5):277-300.
4. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243(5405):290-3.
5. Mandanas RA. Role of p21 RAS in p210 bcr-abl transformation of murine myeloid cells. Blood. 1993;82(6):1838-47.
6. Raitano AB. The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. Proc Natl Acad Sci USA. 1995;92(25):11746-50.
7. Shuai K. Constitutive activation of STAT5 by the BCR-ABL oncogene in chronic myelogenous leukemia. Oncogene. 1996;13(2):247-54.
8. Saglio G, Baccarani M. First-line therapy for chronic myeloid leukemia: new horizons and an update. Clin Lymphoma Myeloma Leuk. 2010;10(3):169-76.
9. Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. Lancet Oncol. 2007;8(11):1018-29.
10. Apperley JF. Part II: management of resistance to imatinib in chronic myeloid leukaemia. Lancet Oncol. 2007;8(12):1116-28.
11. Ramkissoon SH. Hematopoietic-specific microRNA expression in human cells. Leuk Res. 2006;30(5):643-7.
12. Agirre X. Down-regulation of hsa-miR-10a in chronic myeloid leukemia CD34+ cells increases USF2-mediated cell growth. Mol Cancer Res. 2008;6(12):1830-40.
13. La Rosee P and Hochhaus A, Resistance to imatinib in chronic myelogenous leukemia: mechanisms and clinical implications. Curr Hematol Malig Rep. 2008;3(2):72-9.
14. San Jose-Eneriz E. MicroRNA expression profiling in Imatinib-resistant Chronic Myeloid Leukemia patients without clinically significant ABL1-mutations. Mol Cancer. 2009;8:69.
15. Lu J. MicroRNA expression profiles classify human cancers. Nature. 2005;435(7043):834-8.
16. Yu Y. Targeting microRNA-30a-mediated autophagy enhances imatinib activity against human chronic myeloid leukemia cells. Leukemia. 2012;26(8):1752-60.
17. Marcucci G, Maharry K, Radmacher MD, Mrózek K, Vukosavljevic T, Paschka P, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study. J Clinic Oncol. 2008;26(31):5078-87.

18. Nashed AL, Rao KW and Gulley ML. Clinical applications of BCR-ABL molecular testing in acute leukemia. J Mol Diagn. 2003;5(2):63-72.
19. Chomczynski P and Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat Protoc. 2006;1(2):581-5.
20. Chomczynski P and Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162(1):156-9.
21. Benes V and Castoldi M. Expression profiling of microRNA using real-time quantitative PCR, how to use it and what is available. Methods. 2010;50(4):244-9.
22. Baccarani M. Chronic myeloid leukemia: an update of concepts and management recommendations of European Leukemia Net J Clin Oncol. 2009;27(35):6041-51.
23. Jennings LJ. Detection and Quantification of BCR-ABL1 Fusion Transcripts by Droplet Digital PCR. J Molec Diagnost. 2014;16(2):174-9.
24. Balasubramanian P. International reporting scale of BCR-ABL1 fusion transcript in chronic myeloid leukemia: first report from India. Acta Haematol. 2012;127(3):135-42.
25. Asuragen Inc. BCR-ABL Testing, BCR-ABL Test, BCR-ABL International Scale. 2016. Available from: http://www.whereareyouontheis.com/#Q1.
26. Zhang Y and Rowley JD. Chronic myeloid leukemia: current perspectives. Clin Lab Med. 2011;31(4):687-98.
27. Flamant S. Micro-RNA response to imatinib mesylate in patients with chronic myeloid leukemia. Haematologica. 2010;95(8):1325-33.
28. Machova Polakova K. Expression patterns of microRNAs associated with CML phases and their disease related targets. Mol Cancer. 2011;10:41.

Cite this article as: Ezeanosike OB, Afonne OJ. Prognostic significance of micro RNA 150 marker in BCR-ABL positive chronic myeloid leukaemia patients on imatinib mesylate. Int J Contemp Pediatr 2017;4:1557-61.