Atypical BCR-ABL fusion transcript (e6a2) in pediatric acute lymphoblastic leukemia

Dushyant Kumar, Manoj Kumar Panigrahi, Deepti Dewangan, Sarjana Dutt, Khaliqur Rahman, Anurag Mehta

ABSTRACT

Introduction: Among precursor-B-acute lymphoblastic leukemia cases, BCR-ABL translocation occurs in around 20–30% of adults and in $\leq 5\%$ of children. Minor breakpoint transcripts (e1a2) are found in about 70% of positive BCR-ABL cases and major breakpoint transcripts (e13a2, e14a2) in about 30% cases. However, other atypical transcripts are sometimes observed.

Case Report: A rare form of chimeric BCR-ABL fusion transcript (e6a2) was detected in a pediatric patient with precursor-B-acute lymphoblastic leukemia by reverse transcriptase polymerase chain reaction. Sequence analysis of the fusion region of the amplified cDNA fragment showed an in-frame joining of exon 6 of the BCR gene and exon 2 of the ABL gene, giving rise to an e6a2 BCR-ABL transcript. This finding was also confirmed by fluorescent in situ hybridization.

Conclusion: The findings in this case shows that atypical BCR-ABL transcripts are detectable in acute lymphoblastic leukemia patients without M-BCR-rearrangements. Reverse transcriptase polymerase chain reaction using primers that allow for amplification of all known BCR-ABL transcripts is an appropriate method to detect these rare variants.

International Journal of Case Reports and Images (IJCRI)

International Journal of Case Reports and Images (IJCRI) is an international, peer reviewed, monthly, open access, online journal, publishing high-quality, articles in all areas of basic medical sciences and clinical specialties.

Aim of IJCRI is to encourage the publication of new information by providing a platform for reporting of unique, unusual and rare cases which enhance understanding of disease process, its diagnosis, management and clinico-pathologic correlations.

IJCRI publishes Review Articles, Case Series, Case Reports, Case in Images, Clinical Images and Letters to Editor.

Website: www.ijcasereportsandimages.com

CASE REPORT

OPEN ACCESS

Atypical BCR-ABL fusion transcript (e6a2) in pediatric acute lymphoblastic leukemia

Dushyant Kumar, Manoj Kumar Panigrahi, Deepti Dewangan, Sarjana Dutt, Khaliqur Rahman, Anurag Mehta

ABSTRACT

Introduction: Among precursor-B-acute lymphoblastic leukemia cases, BCR-ABL translocation occurs in around 20–30% of adults and in ≤5% of children. Minor breakpoint transcripts (e1a2) are found in about 70% of positive BCR-ABL cases and major breakpoint transcripts (e13a2, e14a2) in about 30% cases. However, other atypical transcripts are sometimes observed. **Case Report:** A rare form of chimeric BCR-ABL fusion transcript (e6a2) was detected in a pediatric patient with precursor-B-acute lymphoblastic leukemia by reverse transcriptase polymerase chain reaction. Sequence analysis of the fusion region of the amplified cDNA fragment showed an in-frame joining of exon 6 of the BCR gene and exon 2 of the ABL gene, giving rise to an e6a2 BCR-ABL transcript. This finding was also confirmed by fluorescent in situ hybridization. **Conclusion:** The findings in this case shows that

atypical BCR-ABL transcripts are detectable in acute lymphoblastic leukemia patients without M-BCR-rearrangements. Reverse transcriptase polymerase chain reaction using primers that allow for amplification of all known BCR-ABL transcripts is an appropriate method to detect these rare variants.

Keywords: BCR-ABL, PCR, FISH, e6a2

How to cite this article

Kumar D, Panigrahi MK, Dewangan D, Dutt S, Rahman K, Mehta A. Atypical BCR-ABL fusion transcript (e6a2) in pediatric acute lymphoblastic leukemia. International Journal of Case Reports and Images 2014;5(1):45–49.

doi:10.5348/ijcri-2014-01-437-CR-10

Dushyant Kumar¹, Manoj Kumar Panigrahi¹, Deepti Dewangan², Sarjana Dutt³, Khaliqur Rahman⁴, Anurag Mehta⁵

Affiliations: ¹M.Tech, Jr. Scientist, Molecular Biology Lab, Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India; ²B. Tech, Jr. Scientist, Molecular Biology Lab, GenX Diagnostic, Delhi, India; ³Phd, Associate Director, Research and Development, Oncquest Laboratory Ltd., Delhi, India; ⁴MD, Hematopathologist, Hematology, Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India; ⁵MD, Director, Lab Services and Blood Bank, Rajiv Gandhi Cancer Institute and Research Centre, Delhi, India.

Corresponding Author: Dushyant Kumar, Jr. Scientist (Molecular Biology Lab), Rajiv Gandhi Cancer Institute and Research Centre Sector-7 Rohini, Delhi, India 110085; Ph: +91 9891099329; Fax: +91 1127051059; E-mail: dushyant. leo@gmail.com

Received: 24 April 2013

Accepted: 20 June 2013

Published: 01 January 2014

INTRODUCTION

BCR-ABL fusion transcripts are found in around 20–30% of adults and in ≤5% of pediatric acute lymphoblastic leukemia (ALL) cases [1–7]. BCR-ABL is of interest in ALL for two reasons. Firstly, those affected are known to have a poor prognosis under conventional therapy [8, 9] and are therefore considered high risk patients and primarily candidate for intensified therapy regime, including allogeneic transplantations. Secondly, BCR-ABL positive patients can have a better prognosis under treatment regime with ABL tyrosine kinase inhibitors such as imatinib. [10] Commonly three typical BCR-ABL mRNA transcripts e1a2, e13a2, e14a2, are found in about 99% of BCR-ABL positive cases but several atypical ones (e13a3, e14a3, e1a3, e19a2, e6a2) have also been observed [11] (Figure 1). However, these variants were observed exclusively in chronic myeloid leukemia (CML) cases, and very little is known about atypical transcript in ALL.

Herein, we report such an atypical BCR-ABL mRNA transcripts e6a2 by reverse transcriptase polymerase

chain reaction (RT-PCR) in pediatric ALL in Rajiv Gandhi cancer institute and research centre (India).

CASE REPORT

A five-year-old boy was presented to our hospital with complaint of lymphadenoid and abdominal pain. On examination he was diagnosed with multiple lymph nodes and splenomegaly. His complete blood count examination showed, hemoglobin 9.2 g/dL, total leukocyte count 3600, peripheral smear showed 12% blasts, with lymphoid morphology, 2% polymorphs and platelet count 8000/mm³. No eosinophils, basophils and monocytes were observed in his blood. Bone marrow examination showed 69% blast of lymphoid morphology which was myeloperoxidase (MPO) negative.

Immunophenotyping: This was carried out using flow cytometer (BD FACSCalibur, BD Biosciences, USA) on bone marrow sample. Using CD45 and side scatter gating 73.41% cells were gated. These cells were dim positive to negative for CD45. These were also positive for CD34 (84.37%), CD10 (76.89%), CD19 (81.65%), cyto CD79a (92.21%), cytoCD22 (97.16%), TDT (47.99%), CD123 (85.01%), and HLA-DR (94.75%). Based on immunophenotype features, it was diagnosed as precursor-B-ALL. The sample was analyzed for common ALL translocations by RT-PCR.

RT-PCR: After red blood lyses RNA was isolated using QIAamp RNA Blood Mini Kit Cat. No. 52304. RNA quantity and quality was checked by ultraviolet spectrophotometer and by running on 1.2% MOPS buffer gel. The cDNA synthesis was carried out by RevertAid H Minus First Strand cDNA synthesis Kit (#K1632) by Thermo Scientific. PCR done for BCR-ABL fusion transcript using primers

BCR 12 FP -5'-TGCTGACCAACTCGTGTGTG-3' (BCR exon 13)

BCR 1 FP- 5'-AACTCGCAACAGTCCTTCGAC-3' (BCR exon1)

ABL 3 -5'-CCATTCCCCATTGTGATTATAGC-3' (ABL exon 3)

FISH: Interphase fluorescence in situ hybridization (FISH) done by using Pathvysion dual color dual fusion translocations probe (o8L10-001) Kit Abbott Molecular. In which spectrum green was for BCR gene and spectrum orange was for ABL gene.

Sequencing: Amplified product was sequenced with the Big Dye Terminator v1.1 cycle sequencing Kit (Applied Biosystems) and run in an ABI PRISM 310 Genetic Analyzer and results were evaluated.

Results: The RT-PCR showed an atypical large amplicon of 1026bp correspond to e6a2 breakpoint using the primers BCR1 FP and ABL 3 RP (Figure 2A), Unlike 384 bp products for e1a2 breakpoint by using the same primers. Patient sample also analyzed for other three common ALL translocations (12;21,1;19,4;11) and were found negative.

Fluorescence in situ hybridization showed by using dual color dual fusion probe we found that there is fusion between BCR and ABL gene (Figure 2B) 55 of the 100 interphase nuclei of the bone marrow smear showed fusion of BCR and ABL gene.

Sequencing result showed identical fusion between exon 6 of BCR gene and exon 2 of ABL gene result in an e6a2 fusion transcript were found (Figure 2C)

Figure 1: BCR and ABL breakpoint regions and resulting fusion transcripts. (A) BCR gene with breakpoint regions. The BCR exons that can be fused in-frame with ABL exons 2 or 3 are e1, e6, e12, e13, e14, e19, and e20. Thus four breakpoint cluster regions (bcr) can be distinguished in the BCR gene: minor (m-) BCR between e1 and e2, major (M-) BCR between e12 and e15, micro (μ-) BCR between e19 and e21, and the region between e6 and e7, here analogously denoted “nano” (v-) BCR. BCR exons that could theoretically be spliced without a reading frame shift, i.e. with a nucleotide number divisible by three, are marked with an asterisk (*). The size of the introns is not to scale, (B) ABL and BCR mRNAs with location of primers, (C) BCR-ABL mRNA fusion transcripts with breakpoints [12].

DISCUSSION

Using RT-PCR, we were able to detect atypical e6a2 BCR-ABL transcript in a pediatric patient with precursor-B-ALL, which also be demonstrated by FISH as well as sequencing and no M-BCR rearrangement was seen by RT-PCR. This is an unusual transcript has only been reported in seven patients with myeloid leukemias,

Figure 2: (A) Agarose gel shows the typical BCR-ABL transcripts, e13a2 (277bp), e14a2 (352bp), e1a2 (384bp) (lane 3,4,5) lane 6 shows the atypical one sample product at 1026 bp. Lane 2 is the internal control and lane 1, 7 are the 50–1000 bp molecular ladder lane 8 is Non template control and 9 and 10 for normal sample internal control and BCR-ABL fusion product. (B) BCR-ABL fusion gene transcript by Pathvysion dual colour dual fusion probe, (C) Sequencing result shows the fusion between exon 6 of BCR gene and exon 2 of ABL gene.

4 with chronic-phase CML, 1 with acute basophilic leukemia [13]. To the best of our knowledge, this patient is the first Indian pediatric BCR-ABL⁺ precursor-B-ALL with a breakpoint outside M-BCR and the first case to express an e6a2 BCR-ABL. It has been hypothesized that CML patients with e6a2 BCR-ABL transcript may have a worse prognosis possibly because they lack the (GET)/dbf-like domain of BCR [14]. However, there is one study done by Burmeister et al. in which they have shown that an adult ALL patient with BCR-ABL⁺ (e6a2) breakpoint showed complete remission after induction of allogeneic transplant and still in complete cytogenetic remission on imatinib [15]. Our patient showed good prednisolone response with an absolute blast count of 32 cells/mm³ on day eight of steroid administration. And complete cytogenetic remission on imatinib until 70 days. Still the patient is on imatinib. Till date there is no data on adult and pediatric precursor-B-ALL with BCR-ABL (e6a2) fusion transcript has been reported in India.

CONCLUSION

Our result suggests that most of the atypical transcripts known from chronic myeloid leukemia can also found

in BCR-ABL⁺ ALL. The e6a2 is the rarest atypical BCR-ABL transcript in pediatric Precursor-B-ALL. Reverse transcriptase polymerase chain reaction based BCR-ABL diagnosis should be design in such a way that it can detect all typical (e13a2, e14a2, e1a2) as well as atypical (e13a3, e14a3, e1a3, e6a2, e19a2) fusion transcripts. Patients with these transcripts also respond to tyrosine kinase inhibitor therapy. A complementary cytogenetic analysis remains mandatory.

Acknowledgements

The author thanks Dr. Kabir Sachdeva for his valuable assistance for guiding me to publish this case report.

Author Contributions

Dushyant Kumar – Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Critical revision of the article, Final approval of the version to be published

Manoj Kumar Panigrahi – Analysis and interpretation of data, Drafting the article, Final approval of the version to be published

Deepti Dewangan – Analysis and interpretation of data, Drafting the article, Final approval of the version to be published

Khalique Rahman – Conception and design, Critical revision of the article, Final approval of the version to be published

Sarjana Dutt – Analysis and interpretation of data, Critical revision of the article, Final approval of the version to be published

Anurag Mehta – Conception and design, Critical revision of the article, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

Copyright

© Dushyant Kumar et al. 2014; This article is distributed under the terms of Creative Commons attribution 3.0 License which permits unrestricted use, distribution and reproduction in any means provided the original authors and original publisher are properly credited. (Please see www.ijcasereportsandimages.com/copyright-policy.php for more information.)

REFERENCES

1. Rieder H, Bonwetsch C, Janssen LA, et al. High rate of chromosome abnormalities detected by fluorescence in situ hybridization using BCR and ABL probes in adult acute lymphoblastic leukemia. *Leukemia* 1998;12(9):1473–81.

2. Copelan EA, McGuire EA. The biology and treatment of acute lymphoblastic leukemia in adults. *Blood* 1995;85(5):1151–68.
3. Secker-Walker LM, Craig JM, Hawkins JM, Hoffbrand AV. Philadelphia positive acute lymphoblastic leukemia in adults: Age distribution, BCR breakpoint and prognostic significance. *Leukemia* 1991;5(3):196–9.
4. Annino L, Ferrari A, Cedrone M, et al. Adult Philadelphia-chromosome-positive acute lymphoblastic leukemia: Experience of treatments during a ten-year period. *Leukemia* 1994;8(4):664–7.
5. Secker-Walker LM, Pentrice HG, Durrant J, Richards S, Hall E, Harrison G. Cytogenetics adds independent prognostic information in adults with acute lymphoblastic leukaemia on MRC trial UKALL XA. MRC Adult Leukaemia Working Party. *Br J Haematol* 1997;96(3):601–10.
6. Pui CH, Crist WM, Look AT. Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia. *Blood* 1990;76(8):1449–63.
7. Pui CH, Evans WE. Acute lymphoblastic leukemia. *N Engl J Med* 1998;339(9):605–15.
8. Schlieben S, Borkhardt A, Reinisch I, et al. Incidence and clinical outcome of children with BCR/ABL-positive acute lymphoblastic leukemia (ALL). A prospective RT-PCR study based on 673 patients enrolled in the German pediatric multicenter therapy trials ALL-BFM-90 and CoALL-05-92. *Leukemia* 1996;10(6):957–63.
9. Gleissner B, Gökbuget N, Bartram CR, et al. Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: A prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. *Blood* 2002;99(5):1536–43.
10. Wassmann B, Gökbuget N, Scheuring UJ, et al. A randomized multicenter open label phase II study to determine the safety and efficacy of induction therapy with imatinib (Glivec, formerly STI571) in comparison with standard induction chemotherapy in elderly (>55 years) patients with Philadelphia chromosome-positive (Ph+/BCR-ABL+) acute lymphoblastic leukemia (ALL) (CSTI571ADE 10). *Ann Hematol* 2003;82(11):716–20.
11. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood* 1996;88(7):2375–84.
12. Thomas Burmeister, Richard Reinhardt. A multiplex PCR for improved detection of typical and atypical BCR-ABL fusion transcripts. *Leukemia Research* 2008;32(4):579–85.
13. Grégoire MJ, Latger-Cannard V, Staal A, et al. Identification of an acute basophilic leukaemia carrying a rare e6a2 BCR-ABL transcript. *Acta Haematol* 2006;116(3):216–8.
14. Colla S, Sammarelli G, Voltolini S, Crugnola M, Sebastio P, Giuliani N. e6a2 BCR-ABL transcript in chronic myeloid leukemia: Is it associated with aggressive disease? *Haematologica* 2004;89(5):611–3.
15. Thomas Burmeister, Stefan Schwartz, Almut Taubald, et al. Atypical BCR-ABL mRNA transcripts in adult acute lymphoblastic leukemia. *Haematologica* 2007;92(12):1699–702.

ABOUT THE AUTHORS

Article citation: Kumar D, Panigrahi MK, Dewangan D, Dutt S, Rahman K, Mehta A. Atypical BCR-ABL fusion transcript (e6a2) in pediatric acute lymphoblastic leukemia. *International Journal of Case Reports and Images* 2014;5(1):45–49.

Dushyant kumar is Scientist in Molecular Biology Department at (Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India). His research interests include Leukemia. He intends to pursue PhD in future.

Manoj kumar Panigrahi is Jr. Scientist in Molecular Biology Department at Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India. His research interests include Leukemia. He intends to pursue PhD in future

Deepti Dewangan is currently Jr. Scientist in Molecular Biology Department at GenX Diagnostic, New Delhi, India. Her research interests include Flow Cytometry. She intends to pursue PhD in future

Sarjana Dutt is Associate Director in R&D Department at Oncquest Laboratory Ltd. Her research interests include (Molecular Biology of Cancer). She intends to pursue (more research work on cancer) in future. She has published 8 research papers in academic journals.

Khaliqur Rahman is Hematopathologist in Hematopathology Lab at Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India. His research interests include Flow Cytometry. He has published 8 research papers in academic journals.

Anurag Mehta is Director Lab Services at Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India. His research interests include Molecular Biology of Cancer. He has published 15 research papers in academic journals.

Access full text article on
other devices

Access PDF of article on
other devices

Edorium Journals: An introduction

Edorium Journals Team

About Edorium Journals

Edorium Journals is a publisher of high-quality, open access, international scholarly journals covering subjects in basic sciences and clinical specialties and subspecialties.

Invitation for article submission

We sincerely invite you to submit your valuable research for publication to Edorium Journals.

But why should you publish with Edorium Journals?

In less than 10 words - we give you what no one does.

Vision of being the best

We have the vision of making our journals the best and the most authoritative journals in their respective specialties. We are working towards this goal every day of every week of every month of every year.

Exceptional services

We care for you, your work and your time. Our efficient, personalized and courteous services are a testimony to this.

Editorial Review

All manuscripts submitted to Edorium Journals undergo pre-processing review, first editorial review, peer review, second editorial review and finally third editorial review.

Peer Review

All manuscripts submitted to Edorium Journals undergo anonymous, double-blind, external peer review.

Early View version

Early View version of your manuscript will be published in the journal within 72 hours of final acceptance.

Manuscript status

From submission to publication of your article you will get regular updates (minimum six times) about status of your manuscripts directly in your email.

Our Commitment

Six weeks

You will get first decision on your manuscript within six weeks (42 days) of submission. If we fail to honor this by even one day, we will publish your manuscript free of charge.

Four weeks

After we receive page proofs, your manuscript will be published in the journal within four weeks (31 days). If we fail to honor this by even one day, we will publish your manuscript free of charge and refund you the full article publication charges you paid for your manuscript.

Mentored Review Articles (MRA)

Our academic program "Mentored Review Article" (MRA) gives you a unique opportunity to publish papers under mentorship of international faculty. These articles are published free of charges.

Most Favored Author program

Join this program and publish any number of articles free of charge for one to five years.

Favored Author program

One email is all it takes to become our favored author. You will not only get fee waivers but also get information and insights about scholarly publishing.

Institutional Membership program

Join our Institutional Memberships program and help scholars from your institute make their research accessible to all and save thousands of dollars in fees make their research accessible to all.

Our presence

We have some of the best designed publication formats. Our websites are very user friendly and enable you to do your work very easily with no hassle.

Something more...

We request you to have a look at our website to know more about us and our services.

We welcome you to interact with us, share with us, join us and of course publish with us.

Edorium Journals: On Web

Browse Journals

CONNECT WITH US