Higher Incidence of Co-Expression of BCR-ABL Fusion Transcripts in an Eastern Indian Population

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Abstract

Background

Chronic myeloid leukaemia (CML) is a hematopoietic stem cell disorder, caused by a balanced reciprocal translocation (t(9;22) (q34;q11))that lead to the formation of BCR (Break point Cluster Region)-ABL (Abelson) fusion transcripts known as Philadelphia (Ph) chromosome. Prevalence of BCR-ABL fusion transcripts in Indian CML population is poorly understood and few studies have been reported from India. The aim of present study was to determine the frequencies as well as prognostic effects of the three fusion transcripts i.e. b2a2, b3a2 and e1a2 in an Indian population.

Methods

RNA was isolated from total 123 sample 27 bone marrow (BM) sample and 96 Peripheral blood (PB) sample of CML patient followed by cDNA synthesis. Real-time quantitative reverse-transcription polymerase chain reaction (qRT-*PCR*) was performed using TaqMan→ assay (ABI, CA, USA) to monitor BCR-ABL transcript.

Results

Ph' chromosome was observed in 103 patients whereas it was not detected in 20 cases. qRT-PCR revealed that the b3a2 fusion transcripts was the most common transcript in CML patients (63.41%) while b2a2 fusion transcript was present in 16.26% cases. Co-expression of b3a2+b2a2 fusion transcript was observed in 0.81% cases whereas co-expression of b3a2+e1a2 fusion transcript was found in 1.63% cases. There was no co-relation observed between b3a2 fusion transcript and platelet count. The fusion transcript b2a2 was observed in relatively younger patients compared to b3a2 fusion transcript. Although this correlation was not statistically significant.

Conclusion

The co-expression of BCR-ABL fusion transcripts was higher (63.41% aggregate of b3a2) in the present population in contrast to other populations reported. This finding was consistent with the frequency data reported from Sudan.

Background

Chronic myeloid leukaemia (CML) is a hematopoietic stem cell disorder characterized by splenomegaly, leucocytosis with myelocyte neutrophil predominance, low neutrophil alkaline phosphatase (NAP) score, hypercellular BM with granulocytic/megakaryotic hyperplasia.[1] CML is a myeloproliferative neoplasm

with an incidence of 1-2 cases per 100,000 adults and approximately 15% of newly diagnosed cases of leukemia in adults. CML is caused due to the formation of Philadelphia chromosome characterized by a balanced reciprocal translocation, t(9;22)(q34;q11) involving BCR (Break point Cluster Region) gene on chromosome 22 and ABL (Abelson) gene on chromosome 9 in the hematopoietic stem cells.[2–4] This balanced reciprocal translocation results in the formation of the BCR-ABL oncogene, which is translated into a protein with constitutive tyrosine kinase activity, possibly the most effectively therapeutically targeted oncoprotein.[31] The exact causal factor of this translocation is not understood well but few studies reported ionizing radiations and exposure of benzene as important risk factors. An interesting study revealed that the higher incidence of CML was observed in population, survived atomic bomb attack.[5]

The ABL gene is translocated within BCR gene results into the formation of chimeric gene (BCR/ABL) on chromosome 22 (Rowley, 1973) which is translated into a protein with constitutive tyrosine kinase activity (Jain P, 2016). The translocation results into the formation of 3 categories of transcripts; the M-BCR (b2a2, b3a2, b2a3, and b3a2), m-BCR (e1a2) and μ-BCR (e19a2).[26,30] All of these fusion transcripts show enhanced tyrosine kinase activity.[6–9] In general b3a2, b2a2 and e1a2 are the most frequent whereas others are rare in the reported populations.[29] In some cases, co-expression of b2a2 and b3a2 occurs due to alternate splicing was also observed.[10] The significance prognostic of the BCR-ABL transcripts has been reported from patients treated with interferon. The first-line treatment of CML patient are approved by the United States Food and Drug Administration for Tyrosine kinase inhibitors (TKIs) like imatinib, nilotinib, dasatinib, and bosutinib for CML in chronic phase (CML-CP).[32] Response has been reported in patients carrying the e14a2 (b3a2) transcript as compared with the e13a2 (b2a2) transcripts after treatment with standard-dose imatinib[33].

The prevalence of the BCR-ABL fusion transcripts in CML cases were not well explored in India. A study reported the incidence of CML in India is 0.8 to 2.2 out of 100,000 male populations and 0.6 to 1.6 out of 100,000 female populations.[11] There are five reports available on the frequency of the BCR-ABL fusion transcripts from India (Mumbai, Kolkata, Delhi and Chandigarh). [1, 12, 13, 14, 15] The main aim of the present study was to determine the frequency of different BCR-ABL fusion transcripts in an Eastern Uttar Pradesh cohort of India and their correlation with the disease prognosis.

Methods

The present study was approved by the ethical committee of the institute of medical sciences, Banaras Hindu University. Detail clinical history and blood sample were collected from each patient after receiving written informed consent for the study and publication.

For the current study, 123 CML cases (80 males and 43 females) were registered from the out-patient and in-patient department of the Sir Sundar Lal Hospital of the Institute of Medical Sciences, Banaras Hindu University. The patient's median age at the time of diagnosis was 40 years. All registered patients were

under treatment and were administered with one or the other tyrosine kinase inhibitor. 0.5 to 4 ml of BM blood was collected from each of the patient. 0.3 ml of it was used for whole blood culture, 200µl was used for RNA isolation, and rest was used for DNA isolation. Cytogenetic analysis was also performed for all the patients. For cytogenetics analysis whole blood culture was performed in RPMI 1640 culture medium containing 10% fetal bovine serum. Metaphases were arrested with colchicine treatment and harvested after incubation of 72 hours at 37°C. Under a microscope (Carl Zeiss Microscopy Gmbh, Göttingen, Germany), chromosome G-banding was performed, and metaphases with 450 G-band resolution were observed, and karyotyping was performed using Ikaros karyotyping system Metasystems software (Carl Zeiss Microscopy Gmbh, Göttingen, Germany).

The RNA was isolated using Tri Reagent BD (Sigma Aldrich→). The integrity of RNA was checked on 1.5% agarose gel. After isolation of RNA, DNase-I treatment was done using DNase-I treatment Kit (Applied Biosystems) and the concentration of RNA was checked using spectrophotometer. The cDNA was synthesised using cDNA synthesis Kit (Applied Biosystems). The integrity of cDNA was also checked by expression of beta actin gene using specific primers.[16] Presence of b2a2, b3a2 and e1a2 fusion transcripts were confirmed in each sample by, qRT-*PCR* using TaqMAN Gene expression Assay (Applied Biosystems).

Results And Discussion

Karyotype revealed the presence of Philadelphia chromosome in 103 CML cases whereas 20 cases have no chromosomal abnormality. qRT-*PCR* identified the presence of various type of BCR-ABL fusion transcript in CML cases (Table-1). The observed frequency of fusion transcripts b3a2 transcript was 63.41% and of b2a2 was 16.26%. The frequency of co-expressing b3a2+e1a2 fusion transcript was in 1.63%, while co-expression of b3a2+b2a2 fusion transcript was detected in 0.81% of the patients. Surprisingly, in 3.25% cases, none of these fusion transcripts were detected possibly due to having the rarest fusion transcripts (Figure 1).

The fusion transcript b3a2 was most prevalent in present population followed by fusion transcript b2a2. This finding was consistent with other reported population although b2a2 fusion transcript was frequently reported in children (figure 2).[1, 12, 13, 14, 15] Interestingly, the present study observed the co-expression of fusion transcript e1a2+b3a2 (1.63%) was higher than other previously reported Indian as well as other population except Sudan.[17] The frequency of fusion transcript b3a2+b2a2 and b3a2+e1a2 was strikingly lower in observed population than other reported population. There are very few studies which reported the co-expression of M-BCR and µ-BCR. One such study carried out in Sudan population reported very high frequency (20.2%) of such co-expressions in CML cases.

The present study also explored to establish a correlate of fusion transcript with platelet count as many previous suggested that patient with b3a2 fusion transcript have higher platelet count.[3, 4, 18, 19, 20, 21, 22, 23, 24]. No such correlation was observed in the present study which might be due to low sample size or this population is different from others.

Age and sex biasedness were also taken in account and observed a higher incidence of CML in males compared to females in this population which is inconsistent with previous studies. [25] The fusion transcript b2a2 was observed frequently in younger patient as contrast to fusion transcript b3a2 but this data was statistically not significant.

Microscopy, karyotyping, and cytogenetic analysis

Total 45 metaphase plates were captured under the microscope and karyotyping was done with 450 G-banding resolution (figure 3) using automated karyotyping work station having Metasystem's software (Ikaros®, Carl Zeiss® Microscopy Gmbh, Göttingen, Germany). Chromosomes were analysed following guidelines provided by the International System for Human Cytogenetic Nomenclature (ISCN 2016).

Frequency of BCR-ABL1 Fusion transcripts Studies in different Country worldwide

Depending upon the location of the breakage, a CML patient may have anyone the various fusion transcripts or may have co-expressions of two or more fusion transcripts. Various studies have been conducted worldwide to study prevalence of fusion transcripts in CML patients. This is focuses mainly on the prevalence of fusion transcripts in various populations worldwide and their prognostic significance. Data of 50 studies which have been conducted worldwide is described in (Table 2) and this table gives the percentage of prevalence of different fusion transcripts in different countries.

In the present analysis on 51 studies from 23 countries we see that there is a high variability in the frequency of different BCR-ABL transcripts in different populations. In general, b3a2 is the most common transcript worldwide followed by b2a2. But in a few reports on the studies conducted in Sudan [34] U.K [35] Germany [36] Mexico [37, 38] Ecuador [39] and U.S.A [40, 41] b2a2 transcript was most prevalent. In one of the studies from Sudan, co-expression of e1a2+b2a2 is the most prevalent [42]. A study from India showed that among children, b2a2 transcript is most prevalent [43] whereas study on children in France showed that b3a2 transcript is the most prevalent one [44]. Rest of the variants b3a2+b2a2, e1a2+b2a2, e1a2, e19a2, e1a2+b3a2 and e1a2 are either not found or in very low frequency except in a Sudanian population, and therefore are not being tested in many populations. These variations in frequency of transcript could be a chance due to low sample size in many studies or could be due to some factor(s) acting on it. One major factor could be some SNP(s) acting as genetic modifiers. Apart from fusion transcripts reported from various studies in table 2, there are certain rare, unusual fusion transcripts such as e6a2, e8a2, e15a2 also found along with common fusion transcripts [43].

Conclusion

The present study reports the frequency of BCR-ABL fusion transcript in CML cases in Eastern Uttar Pradesh population showing higher level of co-expression of the BCR-ABL fusion transcripts (63.41% aggregate of b3a2). This is the first observation of greater co-expression of BCR-ABL fusion transcript in

the present Eastern Indian population. There was no correlation observed between patient with b3a2 transcript and higher platelet count which is contrary to previously reported studies.

Declarations

Acknowledgements

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Conflicts of Interest: None

Abbreviations

CML: Chronic myeloid leukaemia; BCR: Break point cluster region; ABL: Abelson; Ph: Philadelphia; qRT-*PCR*: Quantitative reverse-transcription polymerase chain reaction; NAP: neutrophil alkaline phosphatase; BM: bone marrow

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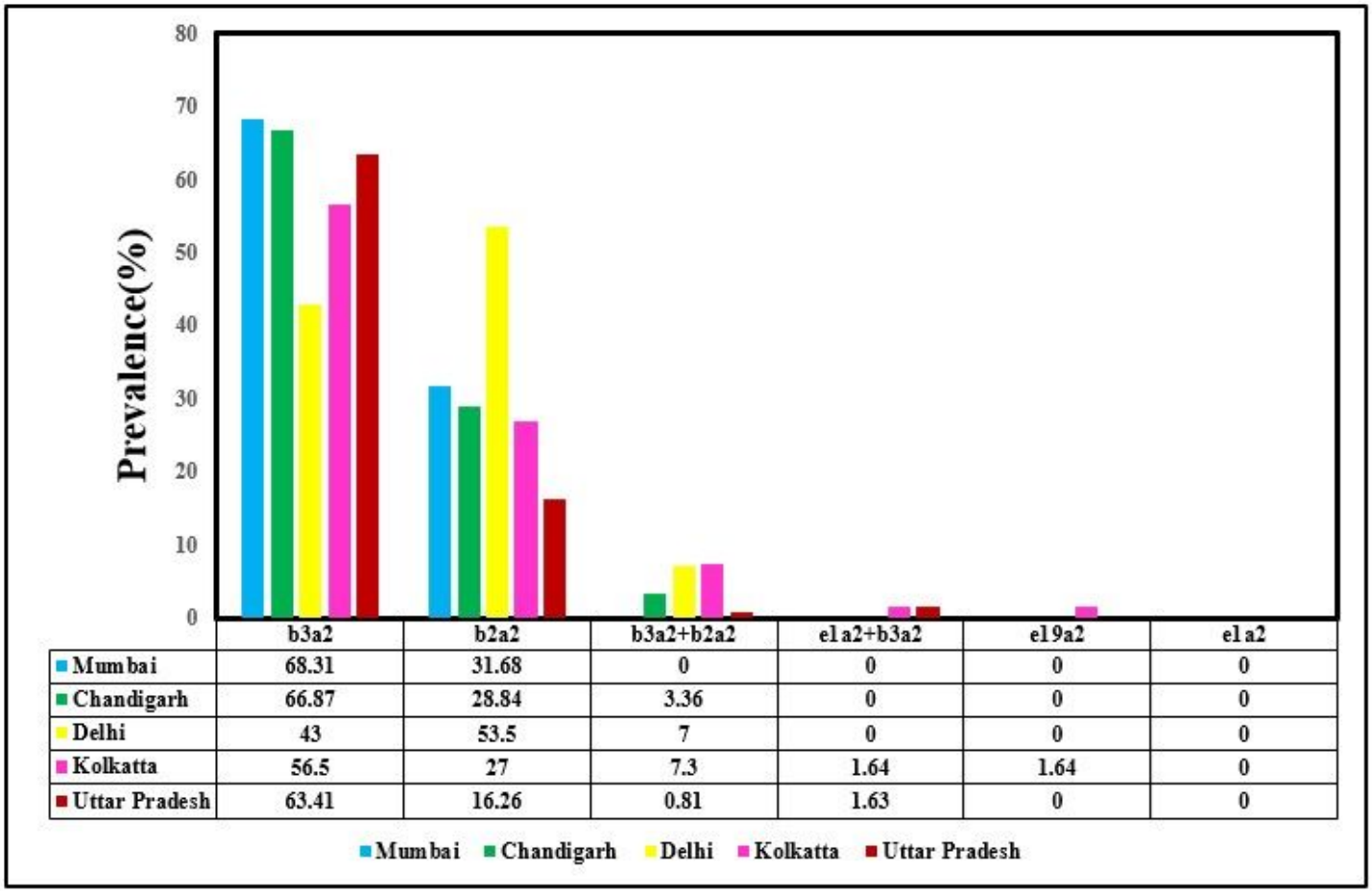
Tables

Tables 1-2 are available in the Supplementary Files section.

Figures

Fig. 1



	b3a2	b2a2	b3a2+b2a2	e1a2+b3a2	e19a2	e1a2
Mumbai	68.31	31.68	0	0	0	0
Chandigarh	66.87	28.84	3.36	0	0	0
Delhi	43	53.5	7	0	0	0
Kolkatta	56.5	27	7.3	1.64	1.64	0
Uttar Pradesh	63.41	16.26	0.81	1.63	0	0

Figure 1

Comparison of the frequency of BCR-ABL fusion transcripts in the present and other studies of India

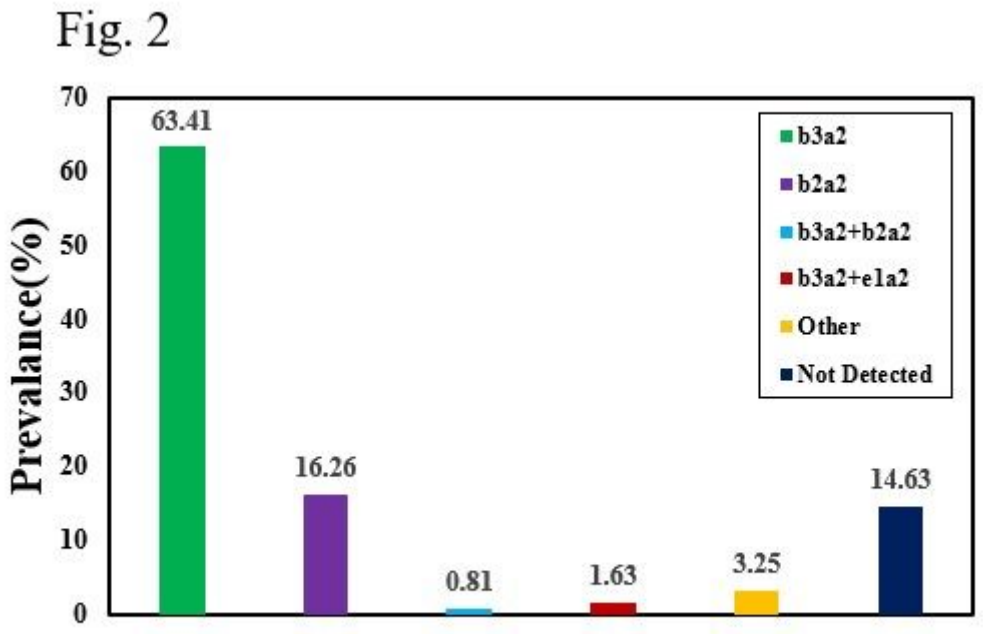


Figure 2

Prevalence of different BCR-ABL fusion transcripts observed in the present study (Uttar Pradesh)

Fig. 3

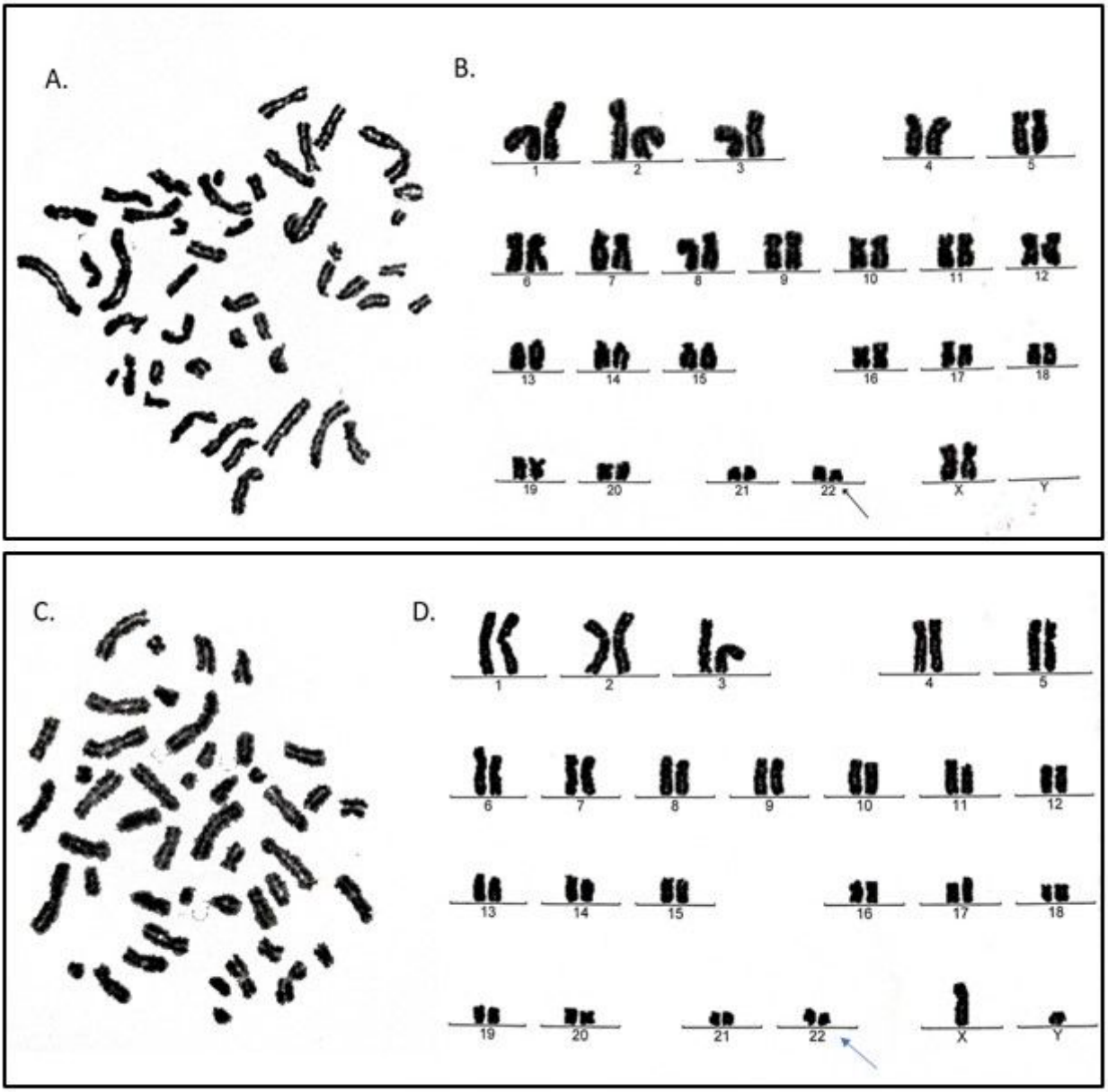


Figure 3

A & C Metaphase shown in human chromosome. B & D Karyogram of a Philadelphia chromosome human Female & Male karyotype

Supplementary Files

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