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Original Research Article

P210 and P190 BCR-ABL fusion transcripts variants frequencies among Philadelphia chromosome-positive chronic myeloid leukemia in Sudan

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Abstract

Breakpoint cluster region-abelson (BCR-ABL) leukemic fusion gene types in chronic myeloid leukemia (CML) correlate with the disease clinical course and outcome. There are variations in the reports of previous studies about the frequencies and distribution of BCR-ABL transcripts in chronic myelogenous leukaemia among Sudanese patients. This research aims to determine the frequencies of BCR-ABL fusion transcript variants in Sudan. One hundred (informed consent) Philadelphia positive chronic myeloid leukaemia patients, in chronic phase, were enrolled in this study. EDTA anticoagulated peripheral blood samples were collected from each participant, RNA was extracted from mononuclear cells by (TRIzol) reagent. BCR-ABL transcripts were detected by qRT-PCR technique with specific primers forP190 and P210 BCR-ABL transcript variants. The typical p210 BCR-ABL transcripts (b3a2 or b2a2) were detected in all patients (100%) the b3a2 transcript was detected in 96/100 (96%) and the b2a2 transcript was detected in 4/100 (4%).co-expression of p210/p190 (b2a3/e1a2) was detected in 6/100 (6%). p190 variant was not detected independently.

Keywords: Chronic myeloid leukaemia, Philadelphia chromosome, BCR-ABL transcript, Sudan.

1. Introduction

Chronic myelogenous leukaemia (CML), is amyeloproliferative neoplasm. CML is a multi-stage disease that progresses from chronic phase (50% of patients are asymptomatic while others have symptoms, e.g., weight loss). Untreated cases shift to an accelerated phase which is characterized by clinical symptoms such as Organomegaly, and laboratory diagnostic features including Leukocytosis and Basophilia. In about75% of patients, accelerated phase shift to ablastic phase which is similar to acute leukaemia and leading to death within 3-6 months[1]. CML was the first neoplasm to be associated with a specific chromosomal rearrangement called the Philadelphia chromosome (Ph'). The Ph' chromosome is an abnormal chromosome 22 that results from reciprocal translocations between chromosomes 9 and 22 [t (9; 22) (q34; q11)] [2]. This translocation of Abelson gene (ABL) located on chromosome 9 and breakpoint cluster region (BCR) located on chromosome 22 lead to the creation of BCR-ABL fusion gene [3]. This BCR-ABL fusion gene activates proto-oncogene tyrosine kinase, which is responsible for dysregulation of cell differentiation, proliferation and apoptosis[4,5]. The BCR-ABL genomic breakpoint location is highly variable[6]. Break points in the ABL gene are relatively consistent, typically in the intron before exon 2. According to the breakpoints in the BCR gene, three BCR-ABL genes can be formed. The first typical BCR-ABL gene that is seen in more than 99% of Ph'-positive CML patients is derived from a disruption of the major break-point cluster region (M-bcr) either between b2 and b3 or b3 and b4 which results in (b2a2) or (b3a2) transcripts. This result in translation of a 210 kDa fusion protein designated as (p210 BCR-ABL). The second breakpoint in BCR gene has been identified in Ph'-positive ALL and in sporadic cases of CML and is located in intron 1 within the minor break-point cluster region (m-bcr). Consequently, only BCR exon 1 (e1) is joined to ABL exon 2 (e1a2) transcript, the translation results in (p190 BCR-ABL) protein. The third break-point

is located in the micro break-point cluster region (µ-bcr) between exon 19 and exon 20 and results in an (e19a2) BCR-ABL transcript and a (p230 BCRABL) protein. Another rare transcript (b2a3, b3a3) that occurs within the (M-bcr) region can also be seen in CML. Co-expression of the transcripts in the (M-bcr) or in the (m-bcr) with one of (M-bcr) have also been reported[7-9].

The types of the fusion gene in CML are thought to be related to the disease clinical course and outcome. Researchers have been, however, unsuccessful in locating any significant correlation[7, 10]. There are variations in the reports of previous studies about the frequencies and distribution of BCR-ABL transcripts in chronic myelogenous leukaemia among Sudanese patients [11-13]. The aim of this study was to determine the frequencies of BCR-ABL fusion transcript variants in Sudan.

2. Materials and methods

One hundred Ph positive CML patients were enrolled in this study. Following informed consent, 3 ml of peripheral blood samples were collected in EDTA from each patient. Haematological and molecular analyses were performed at RICK and Alzahrawi Medical laboratories, Khartoum, Sudan.

2.1 RNA extraction

Leukocytes were prepared from peripheral blood samples after the addition of red blood cells lysis buffer (0.1mM EDTA and 1mM $KHCO_3$, 150mM NH4Cl) pH7.3. Total RNA was extracted from mononuclear cells by TRIzol reagent. Extracted RNA integrity was determined by gel electrophoresis (agarose).

2.2 cDNA synthesis

For cDNA synthesis, 5µl of total RNA were first incubated with 9.5 ml of RNAase free distilled water at 70°C for 10 minutes, cooled on ice and reversely transcribed in a reaction mixture containing (Reverse Transcriptase (RT) buffer: 20 mM Tris HCl, 50 mM KCl, pH 8.3; 5mM $MgCl_2$, 10 mM DTT, 5mM random hexamers, 20 units RNAase, 10 units RT enzyme, 1mM dNTP and H2O to a total volume of 20 µl) at 42°C for 60 minutes. RT enzyme was denatured by incubating the reaction at 99°C for 5 minutes.

2.3 qRT-PCR

Primers and probe sequences were obtained from (Eurofins genomics) company as follows: (BCR2) b2 sense: TGCAGA TGC TGA CCA ACT CG; (BCR3) b3 sense: CGT CCA CTC AGC CAC AT; and a2 (ABL) antisense: TCCAAC GAG CGG CTT CAC. TaqMan probe for e14a2 and e13a2 was: CAG TAG CAT CTG ACT TTG AGC CTC AGG GTC T, which is derived from ABL exon 2 and lies within the fusion region of the b2a2 and b3a2. The ABL Primers and probe were: ABL antisense, GGC CAC AAA ATC ATA CAG TGC A; ABL sense, GTC TGA GTG AAG CCG CTC GT; and TaqMan probe, TGG ACC CAG TGA AAA TGA CCC CAACC. Sequences were contained in ABL exon 2.

Reaction mixtures of 25 µlcontained MgCl2 5 mM, TaqMan buffer A with the ROX dye as the passive reference 12.5 µl, 400µM dUTP, 200µMdATP, dCTP, dGTP, AmpliTaq Gold DNA polymerase 1.25 U, AmpErase Uracil N-glycosylase (UNG) 0.5 U, forward and reverse primers 300 nM, specific TaqMan probe 200 nM and cDNA 6µl (1:3) dilution.Mixture was incubated at 50°C for 2 minutes and 95°C for 10 minutes, with 50 amplification cycles of15 seconds at 95°C and 60 seconds at 65°C. Agarose gel was used to determine the P210 and P190 transcript variants by differences in fragment size of PCR product.

2.4 Ethical consideration

The participants were informed by simple language about the aim of the research, the disease and the benefit from the study. Patients consent were taken if they agreed to participate. The blood samples were collected under aseptic technique by a professional technician to protect the participants from infectious hazards.

3. Results

One hundred CML-Ph positive Sudanese patients' samples were examined by qRT-PCR for BCR-ABL transcript variants. (59/100; 59%) were male with mean age 46.56 years, and (41/100; 41%) were female with mean age 46.51 years.

The typical p210 BCR-ABL transcripts (b3a2 or b2a2) were detected in all patients (100%) the b3a2 transcript was detected in 96/100 (96%) and the b2a2 transcript was detected in4/100 (4%). co-expression of p210/p190 (b2a3/e1a2) was detected in 6/100 (6%). p190 was not detected independently.

4. Discussion

In this study, we explored the frequencies and of major and minor BCR-ABL transcript variants among Ph-positive Chronic Myelogenous Leukaemiain Sudanese patients. P210 transcript variantswere detected in all patients.Which is in agreement with data derived from similar studiesin KoreaSyriaand Sudan [11,14,15].

The distribution of p210 transcript variants (b3a2 & b2a2) show an increase in b3a2 compared to b2a2. Our result is in agreement with previous study done in Sudan[12]. Our study also in agreement with otherstudies inTunisia[16] and Japan[17]. Our result is in disagreement with a study in Ecuador which described an increase in b2a2 compared to b3a2 transcripts [16]. The differences between studies maybe due to the differences in the technique sensitivity[15], or ethnicity [12]. Some studies have also shown that b3a2 had a rapid complete cytogenetic response than b2a2 which may be due to higher BCR-ABL tyrosine kinase inhibitors [18]. Co-expression of p210/p190

transcripts was detected in 6% of the patients. This is in agreement with a local study that found 5.4%[12]. Recent reports have described an association between p190 and rapid disease prognosis[19]. In this study, high frequency of p190 were detected in comparison to internationally reported cases[20], for this reason, more research is needed in this field.

5. Conclusion

P210 transcript variants were detected in all CML-Ph positive patients, while co-expression (b3a2/e1a2) was detected in6%. This study reported differences in the frequencies and distribution of BCR-ABL fusion transcript variants compared to previously studied populations in the same field among Sudanese patients. These variations could be due to multi-ethnicity in the Sudanese patients included in the study.

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