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RESEARCH ARTICLE

FREQUENCY OF *BCR-ABL* TRANSCRIPTS AMONG SUDANESE PATIENTS WITH PERSISTENT MYELOID LEUKOCYTOSIS: A DIAGNOSTIC AIDE FOR CHRONIC MYELOID LEUKEMIA IN LOW-INCOME COUNTRIES

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ABSTRACT

BCR-ABL leukemic fusion gene types in chronic myeloid leukemia (CML) correlate with the disease clinical course and outcome. There is limited reported data regarding the frequency and types of *BCR-ABL* leukemic gene in the Sudan. This study aims to determine the frequency and types of *BCR-ABL* fusion transcripts and investigate their possible diagnostic role in patients with persistent myeloid leukocytosis. Following informed consent, one hundred and fifty-one patients with persistent myeloid leukocytosis for >8 weeks and in the absence of infection were enrolled. Peripheral blood was used for RNA extraction and RT-PCR technique with specific primers was used for *BCR-ABL* amplification. Ninety-seven per cent of patients (147/151; 97.4%) had *BCR-ABL* transcripts. The frequencies of *BCR-ABL* transcripts were 53.7% for b3a2, 27.3% for b2a2 and 19% for other atypical and co-expressed transcripts. Platelets counts were significantly higher in patients with the b3a2 positive transcript compared to those with the b2a2 (p=0.02). All patients with *BCR-ABL* transcripts and persistent myeloid leukocytosis were diagnosed as CML and were treated accordingly with good response. *BCR-ABL* fusion gene is seen in the great majority (97.4%) of Sudanese patients with persistent myeloid leukocytosis and is a good diagnostic aide for CML.

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal disorder of bone marrow pluripotent stem cells (Fungaro *et al.,* 2007). The disease accounts for around 15% of leukemias with an incidence of 1-2 cases/100,000 population (Manero *et al.,* 2003). The commonest age group at presentation is 45-55 years, but all age groups including children can be affected (Faderl *et al.,* 1999a). CML has three clinical stages: a chronic phase that is characterized by an indolent onset without symptoms in 50% of patients. This is followed invariably by an accelerated phase that is characterized by basophilia, increased peripheral blood blasts and promyelocytes and is refractory to treatment. In about 75% of patients, the accelerated-phase disease is followed by a blastic phase, which resembles acute leukemia and causes death within 3 to 6 months (Faderl *et al.,* 1999b). CML was the first neoplasm to be associated with a specific chromosomal rearrangement called the Philadelphia chromosome (Ph') (Lemos *et al.,* 2005). The Ph' chromosome is an abnormal chromosome 22 that results from reciprocal translocations between chromosomes 9 and 22 [t (9; 22) (q34; q11)] (Abruzzese *et al.,* 2003). This translocation results in fusion of the telomeric segment of Abelson (*ABL*) gene from the long arm of chromosome 9 to the centromeric part of the breakpoint cluster region (*BCR*) gene on chromosome 22 forming a new chimeric gene (*BCR-ABL*) on chromosome 22 (Kurzrock *et al.,* 2003). 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 BCR-ABL) 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 (Verma *et al.,*2009).

The types of the fusion gene in CML are thought to be related to the disease clinical course and outcome. Imatinib mesylate is the first line of treatment in developed countries, Hydroxyurea is still in use in many developing countries due to its low cost and good complete hematological remission (CHR). Imatinib mesylate (Gleevec) binds to the ATP-binding pocket of BCR-ABL chimeric protein and stabilizes the inactive form of BCR-ABL protein 5.6 [Braziel *et al.,* 2002; Savage and Antman 2002; Stone , 2004; Sharma *et al.,* 2010; Goh *et al.,* 2006). The incidence of *BCR-ABL* transcripts in CML patients varies in different reported series. There is limited information about the frequency of *BCR-ABL* transcripts in Africa (el-Awady *et al.,* 2001; Osman *et al.,* 2010). In this study we aimed to determine the frequency and type of *BCR-ABL* fusion transcripts and investigate their possible diagnostic role in patients with persistent myeloid leukocytosis in a developing country. It is hoped that this will improve patients management and save time and cost.

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MATERIALS AND METHODS

Following informed consent, one hundred and fifty-one individuals were enrolled. Patients presented with persistent myeloid leukocytosis for >8 weeks without clinical or laboratory evidence of infection (low CRP and low Erythrocytes Sedimentation Rates). Two mls of EDTA-peripheral blood samples were collected for hematological and molecular studies. Hematological and molecular analyses were performed at the Department of Clinical Pathology & Immunology, Institute of Endemic Diseases, University of Khartoum; Alneelain University Medical Research Center and Alzahrawi Medical Laboratories, Khartoum, Sudan. Results of BCR-ABL transcripts were confirmed by random selection of samples and re-checking at different study laboratories.

RNA extraction

Leukocytes were prepared from peripheral blood samples after the application of erythrocytes lysis buffer (150mM NH4Cl, 1mM KHCO3 and 0.1mM EDTA; pH 7.3). Total RNA was extracted from mononuclear cells by Tri reagent (Sigma-Aldrich Corp, Saint Louis. USA) as described in the manufacturer's leaflet. The integrity of RNA was determined by gel electrophoresis and UV trans-illumination prior to reverse transcription.

RT-PCR

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 MgCl2, 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. Primers specific to the (M-bcr) and (m-bcr) were used to amplify the cDNA as follows: primers for M-bcr [First round]:

BCR-b1-A (GAAGTGTTTCAGAAGCTTCTCC);
ABL-a3-B (GAAGTGTTTCAGAAGCTTCTCC). Second round
M-bcr primers: BCR-b2-C (CAGATGCTGACCAACTCGTGT),
ABL-a3-D (TTCCCCATTGTGATTATAGCCTA).

Primers for (m-bcr): [First round]
BCR-e1-A (GACTGCAGCTCCAATGAGAAC);
ABL-a3-B (GTTTGGGCTTCACACCATTCC).
Second round m-bcr primers:
BCR-e1-C (GACTGCAGCTCCAATGAGAAC);
ABL-a3-D(TTCCCCATTGTGATTATAGCCTA).

For the first round of nested PCR, 3µl of cDNA product was amplified in a reaction mixture containing 0.2mM dNTP mix, 1.9µM MgCl2, 0.5u *Taq* polymerase, 1X PCR buffer, 0.5 mM of each primer and H_2O to 25 µl. PCR cycling condition was 94°C for 30s, 64°C for 60s, 72 °C for 60s for 35 cycle and 72°C for 10 minutes final extension. For the second round of nested PCR, 1µl aliquot of the first round of nested PCR product was amplified with specific primers using the same PCR reaction mixture and the PCR cycling condition of the first round of nested PCR. The primers used allow the detection of all known types of BCR-ABL transcripts. The expected bands were as follows: 360 bp...b3a2; 285 bp...b2a2; 186 bp...b3a3; 111 bp...b2a3; 207 bp...el a3; and 381 bp...e1a2 .

Statistical analysis

Statistical analysis was performed using Epi-Info 3.5.1 shareware. Evaluation of patient's data in relation to particular BCR-ABL transcript type was performed using the t-test. Results with p value < 0.05 were considered statistically significant.

RESULTS

The male: female ratio was 1 and the median age of patients was 45 year. All patients were tested for *BCR-ABL* transcripts, 147/151 (97.4%) were positive for at least one of the *BCR-ABL* transcripts whereas the remaining 4/151 patients were negative. The b3a2 or b2a2 -typical CML *BCR-ABL* transcripts- were detected in 81% of *BCR-ABL*-positive patients (b3a2 =79/147 (53.7) (b2a2 =40/147 (27.3). The b2a3 was detected in 7/147 (4.8%) of patients. Co-expression in (M-bcr) transcripts were detected in 11/147 (7.5%) of patients, while the b3a2/b2a2 and the b2a2/b2a3 transcripts were detected in 5.4% (8/147) and in 1.3% (2/147) of patients respectively (Fig. 1). M-bcr/m-bcr transcripts were seen in 8/147 (5.4%) of patients [b3a2/e1a2 3/147 (2%); b3a2/e1a3 2/147 (1.4%); b2a2/e1a2 2/147 (1.4%) and b2a3/e1a2 1/147 (0.6%)]. The hematological profiles of *BCR-ABL* positive patients were as follows: Hb mean 92.0 ±44.0 gram /L; total white cells (TWBC) 149 ±92.0 $X10^9$/L; platelets 369 ±238 $X10^9$/L; Basophils 2.0%; Myeloblasts <5.0%. Platelets counts were significantly higher in patients with the b3a2 transcripts compared to those with the b2a2 transcripts (p=0.02), while the other hematological parameters showed no significant differences. No hematological differences could be detected between patients with the (M-bcr) transcripts and those with co-expression transcripts (Hb p=0.3; platelets p=0.7; TWBCs p=0.8; basophils p=0.5; myeloblasts p=0.4).

DISCUSSION

There is no conclusive evidence that the *BCR-ABL* fusion transcript types have any influence on the clinical outcome. The clinical picture and the presence of these transcripts can greatly improve the diagnosis of patients with persistent myeloid leukocytosis. In this study we determined the frequencies of *BCR-ABL* transcripts among Sudanese patients with persistent myeloid leucocytosis as in an aide to diagnosis. The increase in the b3a2 compared to b2a2 transcript in our patients in the (M-bcr) is in agreement with other studies from other parts of the world (Kim *et al.,* 2001; Goh *et al.,,* 2006; Jiang *et al.,,* 2007; Yaghmaie *et al.,,* 2008). But, our findings are in disagreement with the reports from Sudan [Osman *et al.,,* 2010; Muddathir *et al.,,* 2013). Difference in M-bcr frequencies has been noted before. Difference with the Sudanese studies could probably be due to the fact that Sudan is a vast country with huge numbers of ethnic groups. Atypical *BCR-ABL* transcript were seen in 19% of patients, although higher compared to some studies ((Jiang *et al.,,* 2007), it is in agreement with others (Yaghmaie *et al.,* 2008). Platelets counts were higher in b3a2-positive patients compared to b2a2-positive patients, probably supporting the suggestion that different types of *BCR-ABL* transcripts are associated with different hematological characteristics (Perego *et al.,,* 2000; Qin *et al.,,* 2003; Uchida *et al.,,* 2009). In Sudan and other developing countries, the diagnosis and management of CML, relies mainly on the clinical and hematological characteristics with limited access to cytogenetic and molecular status. This probably adversely affects patients' management and prognosis. Introduction of molecular diagnosis can greatly improve patients' management and save patients' time and money when investigating persistent myeloid leucocytosis.

Conclusion

BCR-ABL fusion gene is seen in the great majority (97.4%) of Sudanese patients with persistent myeloid leukocytosis without infection and is a good diagnostic aide for CML.

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Abbreviations

CML - Chronic myeloid leukemia
BCR-ABL Breakpoint Cluster Region-Abelson

Competing interests: Non.

Authors contributions

MHAA and EAG Khalil conceived the idea of the study, collected and analyzed samples and data and wrote the manuscript. MEAE helped with samples analysis and manuscript drafting. AMM looked after the patients, helped draft the manuscript.

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