

ORIGINAL ARTICLE

Correlation of p210 BCR-ABL transcript variants with clinical, parameters and disease outcome in 45 chronic myeloid leukemia patients

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Summary

Purpose: The aim of this study was to search the BCR/ABL1 fusion gene in 45 chronic myeloid leukemia (CML) Syrian patients using nested reverse transcription polymerase chain reaction (RT-PCR) and compare our results with those of conventional cytogenetics and molecular cytogenetics methods.

Methods: 45 bone marrow or peripheral blood samples from untreated CML patients in chronic phase (CP) were obtained at diagnosis, and analyzed by nested RT-PCR, conventional cytogenetics and molecular cytogenetics methods.

Results: 45 patients examined were positive for some type of BCR/ABL1 fusion gene rearrangement. Out of 45 studied CML patients, 23 (51.1%) expressed b3a2 fusion transcript,

21 (46.7%) b2a2 transcript, and 1 (2.2%) a rare b2a3 transcript. No patient co-expressed both b3a2/b2a2 types.

Conclusions: The distribution BCR-ABL1 transcript types found in Syria were similar to that of Indian Far-Eastern, African or European populations and the M-BCR rearrangement types were not dependent on white blood count (WBC), platelet count, hemoglobin level or gender of the patients. Overall, we could show that patients with b3a2 rearrangements were younger than patients with b2a2 transcripts, thus our young patients may have a worse prognosis.

Key words: BCR breakpoint, chronic myeloid leukemia, M-BCR/ABL1 variants, Philadelphia chromosome, RT-PCR

Introduction

CML is a myeloproliferative disorder, cytogenetically characterized by the presence of Philadelphia (Ph) chromosome, also called derivative chromosome 22 [der(22)], which results from the reciprocal translocation t(9;22)(q34.1;q11.2); it is present in more than 95% of the CML patients [1]. At the molecular level, this rearrangement involves the breakpoint cluster region (BCR) gene in chromosome 22q11.2 and the c-abl proto-oncogene 1 (ABL1) gene in chromosome 9q34, resulting in the hybrid BCR/ABL1 gene [1,2].

Most patients with CML express e13a2 or

e14a2 mRNAs that result from a rearrangement of the major breakpoint cluster region (M-bcr) generating the 210-kDa (p210^{BCR-ABL}) fusion proteins b2a2 or b3a2 respectively [3,4]. However, in 5% of the CML cases, both b3a2 and b2a2 transcripts can be found [1]. The b3a2 variant is produced by the fusion of exon 14 of BCR gene with exon 2 of ABL1 gene while the b2a2 variant is the product of a fusion of BCR exon 13 and ABL1 exon 2 [5]. The incidence of one or other so-called M-BCR/ABL1 (M stands for major) rearrangement in CML patients varies in the literature [6,7].

Several reports have suggested that the type of the chimeric mRNA (b2a2 or b3a2) type is associated with differences in the clinical and hematological characteristics of CML patients, despite the fact that others failed to confirm any significant correlation [8-12]. One of the most interesting findings is the association of b3a2 fusion transcript with higher platelet counts with some evidence in favor [13-15] and some against a good prognosis [16-18].

This study aimed to determine (i) the frequency of expression of p210 BCR-ABL fusion transcript variants in Syrian CML patients by using nested RT-PCR and (ii) hematological characteristics at diagnosis in order to manage the treatment in connection with prognostic implications from this study.

Methods

Sample collection

Forty-five CML patients in CP were included in this study. The diagnosis of CML was based on hematological and morphological criteria of blood and bone marrow, banding cytogenetics, molecular cytogenetics and molecular analyses to confirm the presence of Ph chromosome and/or gene-fusion BCR-ABL. Written informed consent was obtained from all the patients or family members. This study was approved by the Bio-Safety & Bioethics committee of the Institutional Ethics Committee of Syrian Atomic Energy Commission.

RNA extraction and RT-PCR for BCR/ABL1 fusion transcripts

Total RNA was extracted from peripheral blood samples using the InviTrap RNA kit (Invitek, Berlin, Germany) according to the manufacturer's recommendations. cDNA was prepared from 5 µg of total RNA with the Genequality BCR/ABL1 kit especially developed for p210 (AB Analitica, Padova, Italy), and BCR/ABL1 fusion transcript was analyzed according to the manufacturer's instructions (AB Analitica, Padova, Italy).

Statistics

Chi square test was applied for comparing results obtained for M-BCR-ABL variants of the CML patients by gender. Student's t-test was used to compare the distributions of numerical value variables (age, WBC, platelet count, and hemoglobin level) between patients with b3a2 and b2a2 transcripts. P-values <0.05 were considered significant.

Results

All 45 patients examined were tested positive for some type of M-BCR/ABL rearrangement. Twenty-one (46.7 %) of them expressed the b2a2 BCR-ABL transcript, 23 (51.1%) b3a2 transcript, and one patient (2.2%) b2a3 transcript, which is a rare type of M-BCR/ABL. However, no co-expression of any of those variants was found in any of the 45 patients.

The b3a2 was present in 43.5% (10 of 23) of female and 56.5% (13 of 23) of male patients, and b2a2 was expressed in 42.9% (9 of 21) of female and 57.1% (12 of 21) of male patients. No correlation between the obtained M-BCR/ABL variants and the gender of the CML patients was found ($p=1$) (Table 1).

Patients with b3a2 transcript were younger than patients with b2a2 transcript, however, this difference was not significant ($p=0.66$) (Table 1).

The WBC count was higher in the subgroup of patients expressing b3a2 than in those with b2a2 transcript. This difference is not significant, too ($p=0.64$). The platelet count was higher in the subgroup of patients expressing b2a2 than those with b3a2 transcript ($p=0.28$). The hemoglobin levels were not significantly different in both groups (b2a2 and b3a2) ($p>0.05$) (Table 1).

Discussion

In the present study, we explored the incidence and the distribution of M-BCR/ABL1 transcript types in 45 Syrian CML-CP patients. All studied CML patients expressed the p210 M-BCR-ABL1 transcripts. The frequency of b3a2 and

Table 1. Patient hematological data based on M-BCR-ABL variants in 45 newly CML cases in chronic phase

M-BCR/ABL variant	Gender		Mean age (years)	Hematological data		
	Male	Female		WBC ($\times 10^9/l$)	PLT ($\times 10^9/l$)	Hb (g/dl)
b2a2 n = 21 (46.7 %)	12	9	39	143.1	293	11.2
b3a2 n = 23 (51.1 %)	13	10	36	115	257	10.2
p value	1		0.66	0.64	0.44	0.28

WBC: white blood count, PLT: platelet count, Hb: hemoglobin

b2a2 was found to be 51.1% and 46.7%, respectively, which is close to the data derived in a similar study performed in India [19]. No co-expression of these two variants was found in any of the patients.

The incidence of the M-BCR/ABL1 rearrangements in CML patients varies in different reported series (Table 2). In Far-Eastern countries (Japan, Korea, India, and Thailand), the incidence of b3a2 transcripts was found to be higher than that of b2a2 transcripts [19,20-22]. Iranian CML patients showed a frequency of 21% to 62% of b2a2 to b3a2 transcripts [23].

Recently, a Tunisian study [24] showed frequencies of 36.36% and 63.63% for b2a2 and b3a2 transcripts, respectively [23]. Also, in Sudanese study [7] revealed frequencies of 53.5% to 41.9 % for b2a2 vs b3a2, respectively. Surprisingly, those values were relatively close to such from Mexican population [25].

The distribution of M-BCR transcript variants in CML patients has been reported in some European populations [12,26-29] and is in accordance with that in Far-Eastern countries (Table 2). For example, Reiter et al. [27] found the incidence of b2a2 and b3a2 transcripts in CML patients with Ph chromosome was 31.6% and 68.4%, respectively. Also, Verschraegen et al. [15] found a similar frequency of b2a2 and b3a2 transcripts, as 30.2% and 67.9 %.

However, in Western countries, the study of M-BCR transcripts incidence registered different frequencies with higher percentage of b2a2 transcript (Table 2); in 250 Mexican patients with CML, conventional RT-PCR technique revealed that 83.00% were positive for M-BCR (48.00% b2a2 and 35.00% b3a2) [25]. However, this is a similar frequency found in Argentinean population [31] which showed 41.7% for b2a2 and 37.5% for b3a2. Also, a study on an Ecuadorian population found 5% for b3a2 and 95% for b2a2 [6].

These differences could be caused by differences in the sensitivities of the techniques used, but ethnic differences should be strongly taken into consideration [6].

In the present study, the frequencies of 46.7 and 51.1 % for b2a2 and b3a2, respectively, were established, and the b3a2 transcript here was a little higher than b2a2 transcript, whereas in Far-Eastern, African and European countries the b3a2 transcript was quite higher [24].

The co-expression of both b3a2 and b2a2 transcripts could be explained as the result of an alternative splicing mechanism rather than by the presence of two different clones; and, as the dis-

Table 2. Incidence of b2a2 and b3a2 transcripts in Eastern, African, European and Western countries

Countries	b2a2 (%)	b3a2 (%)
Present study	46.7	51.1
India [19]	41.25	56.25
Tunisia [24]	36.36	63.63
Korea [20]	32.34	67.66
Japan [21]	30.20	67.5
USA [15]	30.2	67.9
Thailand [22]	31	61
Austria [27]	31.6	68.4
Serbia [29]	25	73.5
Iran [23]	21	62
Sudan [7]	53.5	41.9
Germany [12]	38	36
Ecuador [6]	94.6	5.4
USA [30]	48	35
Argentina [31]	41.7	37.5

ease progresses, only one of them would prevail [32]. Relevant studies indicate that co-expression of both transcripts b2a2 and b3a2 in patients with CML is 8.3% [31,32]. Some others have reported an incidence of up to 20 % for the expression of both transcripts [33]. However, our study did not find any co-expression of these two variants in the patients, which may be due to ethnic differences.

The reports on a relationship between M-bcr breakpoint location and disease outcome, platelet count, and WBC in p210 CML patients are controversial.

In CML, the fusion gene variant is thought to be related to the clinical course and outcome in each patient. A number of studies have examined whether the position of the breakpoint within the M-bcr region (5' M-BCR vs 3' M-BCR or b2a2 vs b3a2) influences the duration CP or of survival [16,34-39]. According to the literature, CML patients with b2a2 fusion transcripts have a longer CP and a better response to interferon than those with b3a2 fusion [34,37]. Also, prospective studies with large series failed to confirm any significant correlation between M-bcr breakpoint location and disease outcome [16,35-36].

We have tested our patients for possible associations between the M-BCR/ABL1 variants and biological and clinical parameters. The only possible correlation, even though statistically not significant, was that in our study patients with

b3a2 transcript were younger than patients with b2a2 transcript and therefore they may have a worse prognosis. A Tunisian study [24] revealed that patients with b3a2 transcript were older than patients with b2a2 transcript. Also, the same study revealed the majority of male CML patients expressed the b2a2 and b3a2 variants, which was confirmed in our study.

Many studies revealed no correlation between the M-BCR/ABL1 breakpoint location and the platelet count; the same was valid in this study [17,38,40-41]. However, some studies revealed an increase of the platelet count in patients with b3a2 transcript and this was statistically significant [13-14,24].

On the other hand, the association between the M-BCR/ABL1 variant and the WBC in P210 CML patients was supported at least partly [42,43], however in our patients and also in Tunisians this association was not detected [24].

The inverse correlation between WBC with platelet count and the length of BCR sequence in-

cluded in the BCR-ABL1 fusion gene is in accordance with the high frequency of thrombocytosis found in neutrophilic-CML patients [44].

A few studies are currently available regarding the significance of BCR-ABL1 transcript variant; some preliminary reports suggested that knowledge of the transcript type may have clinical application or help to further understand the pathobiology of t(9;22)-positive leukemic cells [24].

In conclusion, our findings indicate that the b3a2 is more frequent than b2a2 transcript in the studied CML-CP Syrian patients and these patients were younger than patients with b2a2 transcript. At present no implications for treatment or prognosis can be deduced from these results.

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