Original Article

Prevalence of Abelson murine leukemia viral oncogene homolog-breakpoint cluster region fusions and correlation with peripheral blood parameters in chronic myelogenous leukemia patients in Lorestan Province, Iran

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Abstract

Context: Chronic myelogenous leukemia (CML) is a chronic malignancy of myeloid linage associated with a significant increase in granulocytes in bone marrow and peripheral blood. CML diagnosis is based on detection of Philadelphia chromosome and "Abelson murine leukemia viral oncogene homolog" (ABL)-"breakpoint cluster region protein" fusions (ABL-BCR fusions). **Aims:** In this study, patients with CML morphology were studied according to ABL-BCR fusions and the relationship between the fusions and peripheral blood cell changes was examined. **Materials and Methods:** All patients suspected to chronic myeloproliferative disorders in Lorestan Province visiting subspecialist hematology clinics who were confirmed by oncologist were studied over a period of 5 years. After completing basic data questionnaire, blood samples were obtained with informed consent from the patients. Blood cell count and morphology were investigated and RNA was extracted from blood samples. cDNA was synthesized from RNA and ABL-BCR fusions including b3a2 and b2a2 (protein 210 kd or p210), e1a2 (protein 190 kdor p190), and e19a2 (protein 230 kdor p230) were studied by multiplex reverse transcription polymerase chain reaction method. Coexistence of e1a2 and b2a2 (p210/p190) fusions was also studied. The prevalence of mutations and their correlation with the blood parameters were statistically analyzed. **Results:** Of 58 patients positive for ABL-BCR fusion, 18 (30.5%) had b2a2 fusion, 37 (62.71%) had b3a2 fusion and three (3.08%) had e1a2 fusion. Coexistence of e1a2 and b2a2 (p210/p190) was not observed. There was no significant correlation between ABL-BCR fusions and white blood cell count, platelet count, and hemoglobin concentration. **Conclusions:** The ABL-BCR fusions in Lorestan Province were similar to other studies in Iran, and b3a2 fusion had the highest prevalence in the studied patients studied.

Key words: Breakpoint cluster region-Abelson murine leukemia viral oncogene homolog positive, chronic, leukemia, myelogenous, peripheral blood

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Introduction

Malignancy and clonal proliferation of common myeloid progenitors result in chronic myeloproliferative neoplasms involving myeloid lineage, including monocytic, erythroid megakaryocytic series.[1,2] Unlike acute leukemias, these neoplasms do not show disruption in cell maturation and differentiation, and thus, a high count of differentiated cells of myeloid series is observed in bone marrow and peripheral blood of patients.[3,4] Moreover, increase in immature and mature granulocytes (with blast count <10%) as well as increased platelets and monocytes is known as chronic myeloproliferative morphology.[5,6] According to the latest World Health Organization classification, myeloproliferative neoplasms in clued chronic myelogenous leukemia (CML), polycythemia vera, essential thrombocythemia, and chronic idiopathic myelofibrosis. It should be noted that other disorders such as chronic neutrophilic leukemia and hypereosinophilic syndrome have also been included in this classification.[7,8]

CML is the most popular chronic myeloproliferative disease. It comprises approximately 20% of leukemias annually and can be seen in both juvenile and senior patients. However, CML has a higher incidence in middle-aged patients (ranging 40–60 years of age) with a higher incidence in women relative to men (1.7 vs. 1) (9). In CML, genetic changes in myeloid progenitor cells cause inconsistencies in growth and differentiation so that the ability of the cells to divide has been maintained, but they lack full differentiation capacity to normal blood cells. Reciprocal translocation between long arms of chromosomes 9 and 22, which leads to the formation of Philadelphia chromosome, is among the most important genetic changes. In Philadelphia chromosome, 3' fragment of Abelson murine leukemia viral oncogene homolog (ABL) gene from 9q34 chromosome juxtaposes to breakpoint cluster region (BCR) gene of 22q11 chromosome, which gives rise to ABL-BCR hybrid gene.

The transcribed chimeric gene (BCR-ABL mRNA) generates a hybrid protein with potent tyrosine kinase activity.[9,10] This protein contains the N-terminal of BCR as well as C-terminal of ABL gene and can transform the hematopoietic precursor cells *in vitro*. This fusion plays a key role in activation of tyrosine 177 residue and initiation of the leukemogenesis process.[11]

Breakages in ABL gene often occur in intron number 1 and expose the exon number 2 (known as a2). Breakage in BCR gene occurs in three regions: Major BCR or M-BCR: Breakage in introns 13 or 14 and expositions of exons 13 and 14 (b2 and b3), minor BCR or m-BCR: Breakage in intron number 1 and exposition of exon 1 (e1) and μ-BCR: Breakage in intron number 19 and exposition of exon 19 (e19). Several ABL-BCR fusions occur based on BCR gene breakage region, including b2a2 and b3a2 (generating P210), e1a2 (generating P190) or e19a2 (generating P230).[1,9,12] Philadelphia chromosome has been recognized as a prerequisite for diagnosis of CML by WHO.[7,13] Few studies have been conducted to determine the type of ABL-BCR fusions.[14] Yaghmaie *et al*. studied 79 Iranian CML patients in 2007, and reported 62% and 20% incidence of b3a2 and b2a2 fusions, respectively.[15]

In this study, patients showing morphology of CMLwere assessed for ABL-BCR fusions, and the relationship between these fusions and changes in their peripheral blood cells was studied. It is worth noting that the type of fusion can be important for prognosis and response to treatment, so that the patients with b2a2 and b3a2 fusions generating aP210 have a better prognosis than other cases of ABL-BCR fusion.[9,10]

Materials and Methods

All patients with chronic myeloproliferative morphology referred to subspecialist hematology clinics as well as offices of oncology specialists in Lorestan Province gave informed consent to be evaluated for different ABL-BCR fusions within 5 years. This research was accepted by the Ethics Committee of the Research Center at Lorestan University of Medical Sciences. These patients had a white blood cell (WBC) count >25,000/μl with a sharp increase ingranulocytes and their precursors. Blast count was <10% with neutrophils and myelocytes forming the majority of WBC. 5–10 ml peripheral blood of patients was drawn on ethylene diamine tetra acetic acid anticoagulant (Sigma, Germany). Mononuclear cells were separated by Ficoll (Sigma - Aldrich, Germany) within 2 h, and RNA was extracted using QIAzol Lysis Reagent (Qiagen, Germany) as manufactured protocol. RNA level was determined by optical density, the absorption was measured in 260 nm and the quality of extracted RNA was confirmed using electrophoresis and revelation of 18s and 28s bands.

cDNA was synthesized from extracted RNA by cDNA synthesis kit (Fermentas, Germany) according to kit protocol. Multiplex reverse transcription polymerase chain reaction (RT-PCR) was used to assess different ABL-BCR fusions, including b2a2, b3a2, e1a2 b2a3, b3a3, or e19a2. The primers used to detect the relevant fusions[16] and other PCR information are presented in Tables 1-3.

K562 cell line was used as positive control (b3a2) and samples from healthy subjects were used as negative control. Finally, the data were subject to statistical analysis using SPSS

Version 16 (SPSS Inc, Chicago, Ill) and descriptive statistics (mean, standard deviation, and frequencies) as well as multiple analysis of variance (MANOVA).

Results

Fifty-eight patients among those with chronic myeloproliferative morphology were positive for Philadelphia chromosome. Of 58 patients, 18 (30%) had b2a2 fusion and 37 (63.9%) had b3a2 fusion [Figure 1]. In Figure 1, multiplex RT-PCR results, various bands related to b2a2 and b3a2 fusions as well as positive and negative controls have been shown. Three patients (5.1%) had e1a2 fusion but were excluded from the study to avoid errors in statistical analyzes. Simultaneous expression of b3a2 and b2a2 (p210/p190) was not observed as well. Mean age of participants was 48 ± 1.457 years, and there were 24 male and 31 female patients [Table 4], which was an interesting finding.

MANOVA was used for comparison between four variables of hemoglobin (Hb), platelet count, age and WBC count in patients with b2a2 and b3a2 fusions [Table 5]. Box's test

Table 1: Sequence of primers for multiplex polymerase chain reaction

Primer	5' to 3' sequence
CA3-	5´TGTTGACTGGCGTGATGTAGTTGCTTGG 3´
C5e-	5´ATAGGATCCTTTGCAACCGGGTCTGAA 3´
B2B	5´ACAGAATTCCGCTGACCATCAATAAG 3´
BCR-C	5´ACCGCATGTTCCGGGACAAAAG 3´

BCR-C: Breakpoint cluster region-C

Table 2: Size of amplified region using primers listed for breakpoint cluster region-Abelson murine leukemia fusions

Primer	Product	Size of amplified fragment (kb)
B2B/C5e-	BCR	808
B2B/CA3-	b3a2	385
	b2a2	310
BCR-C/CA3-	e1a2	481

The 808 kDa band served as an internal control for protocol and was positive in all patients and healthy controls. Lack of this band indicated failure of the procedure.
BCR-C: Breakpoint cluster region-C

Table 3: Multiplex polymerase chain reaction thermal program

PCR stage	Cycle period	Cycle temperature (°C)
1	10 s	100
2	1 min	96
3	2 min	58
4	1.5 min	72
5	20 s	100
6	20 s	97
7	35 s	56
8	15 s	62
9	10 s	75
10	35 s	73
11	31 times from Stage 5 to 10	
12	10 min	72

PCR: Polymerase chain reaction

Table 4: Prevalence of fusions in studied patients according to gender

	Fusion		Total number
	b2a2	b3a2	
Gender			
Male			
n	10	14	24
Percentage	41.7	58.3	100.0
Female			
n	8	23	31
Percentage	25.8	74.2	100.0
Total number			
n	18	37	55
Percentage	32.7	67.3	100.0

Table 5: Mean±standard deviation age of hematologic parameters and age based on fusion type

	Fusion	Mean±SD	Number
WBC	b2a2	131,875.00±94,676.07	18
	b3a2	126,856.75±70,431.42	37
	Total number	128,499.09±78,323.46	55
PLT	b2a2	364,538.88±35,3450.59	18
	b3a2	392,918.91±23,5832.25	37
	Total number	383,630.90±276,744.44	55
HB	b2a2	11.12±2.42	18
	b3a2	10.84±1.81	37
	Total number	10.93±2.01	55
Age	b2a2	46.38±11.83	18
	b3a2	48.91±15.82	37
	Total number	48.09±14.57	55

Mean±SD in patients with b2a2 and b3a2 fusions; WBC: White blood cell; PLT: Platelets; HB: Hemoglobin; SD: Standard deviation

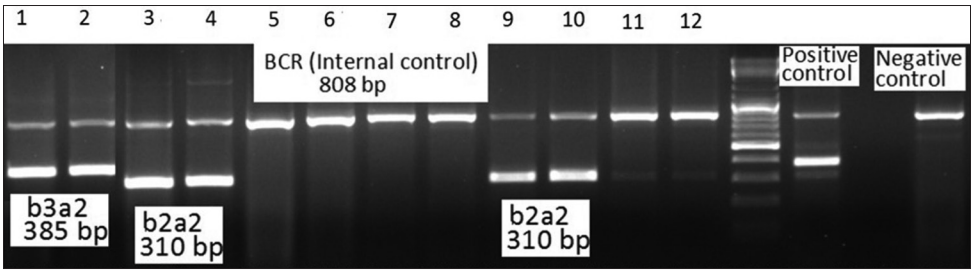

Figure 1: Patients 1 and 2 had b3a2 fusion and patients 3, 4, 9 and 10 had b2a2 fusion (generating 210 kDa protein). Other patients were negative for Philadelphia chromosome

was used to evaluate the equivalence of covariance matrices with $F = 1.222$, degree of freedom 1 = 10 (df), df2 = 49.5449 and significant = 0.266, which confirmed the equivalence hypothesis of multivariate covariance matrices. Considering the results of multivariate analysis and significance level of indices ($P > 0.05$), it can be stated that isoforms were not significantly different in terms of variables such as age as well as hematological parameters of Hb, platelet count, and WBC [Table 6]. The results of univariate analysis showed that mean age and hematological parameters were not different in various fusion types [Table 7].

Discussion

Chronic myeloid leukemia is a chronic malignancy of myeloid lineage associated with increased WBC count with varying degrees of granulocytic cell immaturity at diagnosis.[17] The number of blasts and promyelocytes fluctuates in untreated patients, platelet count is high on diagnosis and mild normochromic normocytic anemia is observed in patients.[6,18] Detection of ABL-BCR variants plays an important role in the diagnosis and treatment of CML patients.[4,19,20] In this study, patients with CML morphology were assessed regarding ABL-BCR fusions and their correlation with changes in peripheral blood cells, and 100 patients with chronic myeloproliferative morphology were examined for different ABL-BCR fusions. Finally, among 58 Philadelphia-positive patients, frequency of b2a2, b3a2, and e1a2 fusions was 5.30, 71.62, and 80.3, respectively, which indicated that b3a2 fusion generating a P210 kDa fusion protein had the highest prevalence among the studied patients. Comparison between four variables of Hb, platelet count, age, and WBC count in patients with b2a2 and b3a2 fusions indicated no significant correlation between these variables and type of fusion. This is while similar studies have indicated a significant correlation between fusion type and a number of peripheral blood parameters. Wei showed that b3a2 fusion and m-BCR-ABL sequence expression increased platelet count on diagnosis but did not affect hematologic parameters in the expression of b2a2 sequence.[21] Another study by Bennour *et al*. in 2013 showed that the level of increase in platelet count was higher in patients with b3a2 fusion relative to patients with b2a2 fusion whereas there was no significant difference in other parameters.[22] In another similar study, Perego *et al*. studied the relationship between b2a2 and b3a2 fusions and parameters such as age, gender, Hb, platelet, and WBC count and showed that platelet count was higher in b3a2 relative to b2a2 sequence.[23] Rosas-Cabral *et al*. reported a significant correlation between b3a2 fusion and increased platelet count in Mexico.[24] However, in this study, similar to other studies conducted in Iran, no significant correlation was reported between fusion type and hematological parameters in CML patients.

Table 6: Multiple analysis of variance results

	P	df	df error	*F* factor	Significance level
Considered mutations					
Wilks' lambda	0.494	8	104	0.397	0.944
Pillai's trace	0.056	8	106	0.373	0.933
Hotelling's trace	0.059	8	102	0.373	0.933
Roy's largest root	0.045	4	53	0.593	0.669

df: Degree of freedom

Table 7: Estimation of marginal means

Dependent variables	Fusion	Mean±SD	95% confidence level	
			Minimum	maximum
WBC	b2a2	131,875.000±18,625.786	94,516.405	169,233.595
	b3a2	126,856.757±12,991.222	100,799.666	152,913.847
PLT	b2a2	364,538.889±65,764.104	232,632.800	496,444.978
	b3a2	392,918.919±45,869.531	300,916.291	484,921.547
HB	b2a2	11.122±0.478	10.163	12.082
	b3a2	10.843±0.334	10.174	11.513
Age	b2a2	46.389±3.456	39.457	53.320
	b3a2	48.919±2.410	44.084	53.753

WBC: White blood cell; PLT: Platelets; HB: Hemoglobin; SD: Standard deviation

Conclusion

Despite the racial features of people in Lorestan Province, the results of this study were comparable with similar studies in the country.

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Conflicts of interest

There are no conflicts of interest.

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