Detection of BCR-ABL protein in chronic myeloid leukemia patients using Immunocytochemistry

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Abstract

Background: Chronic Myeloid leukemia (CML) is a myeloproliferetive disorder associated with chromosomal abnormality, Philadelphia chromosome (Ph), in more than 95% of CML patients. The resulting BCR-ABL fused gene is markers for this type of leukemia. In CML, the product of the fused BCR-ABL gene is typically an oncoprotein termed $P210^{BCR-ABL,}$ a constitutively active tyrosine kinase, activates numerous signal transduction pathways, leading to uncontrolled cell proliferation and reduces apoptosis.

Objective: Primary diagnosis of CML patients by screening the presence of BCR-ABL protein in patients' venous blood lymphocytes using immunocytochemistry technique (ICC).

Method: A total of 42 CML patients, 10 Acute Lymphoid Leukemia(ALL) patients, 2 Acute Myeloid Leukemia (AML) patients, 1 Chronic MyeloMonocytic Leukemia (CMML) patient and 8 healthy individuals were screened. Lymphocyte was separated from heparinized venous blood sample from each subject, smeared and fixed on positive charged slides. Monoclonal antibody specific for BCR-ABL protein was used as primary antibody.

Results: The results showed that all the 42 cases of CML were positive for BCR-ABL protein and all the other cases were negative.

Conclusion: The results indicate that the Immunocytochemistry assay has clinical application as a primary qualitative diagnosis tool of *BCR-ABL* protein.

Key words: BCR/ABL protein - CML-Immunocytochemistry.

IRAQI J MED SCI, 2010; VOL.8 (1):39- 43

Introduction

Chronic Myeloid leukemia (CML) is characterized most frequently by its association with an abnormal chromosome 22, known as the Philadelphia chromosome (Ph) [1, 2]. It is estimated that at least 95% of CML cases possess the Ph [3]. This abnormal chromosome fuses a central portion of the BCR gene to the second exon of the ABL gene (*BCR-ABL*) [4, 5]. The fusion of BCR and ABL on the Ph chromosome occurs in a head-to-tail manner, with the 3` end of ABL joined to the 5` end of BCR [6].

Regardless, the fusion transcript almost always includes exon 2 of ABL (a2). In contrast, in CML, the break on chromosome 22 is restricted in most patients to an area of 5.8-kb termed the major-bcr (M-bcr). Most breaks occur immediately downstream of exon 2 or 3 of the M-bcr region [6] and result in b2a2 or b3a2 fusion transcripts encoded for protein of 210KiloDalton (KD) ($p210^{BCR-ABL}$) [7]. In acute leukemia, however, the breakage can also occur outside M-bcr in about two third of the cases and usually within the 3' end of intron 1 of the BCR gene termed the minor bcr (m-bcr) , resulting in an e1a2 fusion transcript encode for a protein of 190KD ($p190^{BCR-ABL}$).Also, one third of acute leukemia cases show p210 positive[8]. Other unique breakpoint sites have been found. These include a micro

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Received: 17th March 2009, Accepted: 7th October 2009.

3' site termed µ-bcr (BCR exon 19 to BCR exon 20), which encode for a protein of 230KD (p230 $^{BCR-ABL}$). Patients with the e19a2 fusion between BCR exon 19(e19) and ABL exon 2(a2) were classified as having neutrophilic CML [9].

Tyrosine kinase activity is essential to cellular signaling and growth. Constitutively elevated kinase activity has been associated with oncogenic changes in several systems. Both the $p210^{BCR-ABL}$ and the $p190^{BCR-ABL}$ proteins have constitutively activated tyrosine kinase enzymatic activity, with higher levels of activity in the p190 [10].

Interestingly, most of the autophosphorylated tyrosines occur within the BCR segment of BCR-ABL [11]. The tyrosine kinase activity is attributable to the kinase domain found within the ABL segment of the fusion proteins [12]. Indeed, it appears that the degree of transforming activity of BCR-ABL correlates with the degree of tyrosine kinase activity and this activity has been implicated in the growth factor independence that BCR-ABL confers on cells [13].The initial effect of this kinase in primitive hematopoietic stem cells was investigated. It was improve that BCR-ABL protein can regulate protein levels (such as tumor suppressor proteins) by governing secretion through down-regulation of specific tumor suppressor genes [14].

Patients, Materials and methods

Patients:

A total of Sixty three venous blood (VB) samples were included in the study, they were 42 CML, 10 ALL, 2 AML and 1 CMML in addition to 8 samples from healthy individuals were screened as negative control .These samples were screened for the expression of BCR-ABL protein using ICC technique.

Materials and method Methods:

Lymphocytes isolation

Lymphocytes were isolated according to Boyum [15].

Immunocytochemistry procedure

Immunoperoxidase Secondary Detection system (Dako Cytomation, USA, K0673) were used for staining according to the manufacturer instruction.

ICC procedure was done according to Huang *et al* [16].Mouse monoclonal antibody (Mouse anti-Human c-Ab1, BCR-ABL, USBiological, USA) was used as a primary antibody according to the manufacturer instruction. A total of 100cells were counted to determine the percentage of reactivity of BCR-ABL monoclonal antibody. In this study, cells considered as positive when the nucleus was being stained with dark brown color. The percentage of positive cell calculated as following:

$$Percentage\ of\ positive\ cells = \frac{No.\ of\ positive\ cells}{total\ no.\ of\ counted\ cells} \times 100\%$$

The results were scored for the percentage of positive nuclei according to Alessandra *et al.* [17] and as following:

1-Negative: less than 5% positive nuclei.
2-Weak positive: 5%-25% positive nuclei.
3-Moderate positive: 26%-50% positive nuclei.
4-High positive: >50% positive nuclei

Results

The results of immunostained of smears were evaluated on the bases of positive nuclear staining. All CML patients included in this assay were BCR-ABL positive and were consistent with the hematological diagnosis.

CML patients were classified for two groups according to scoring of positive BCR-ABL cells, as in table (1).

Staining pattern of BCR-ABL positive cells was studied using ICC revealed a typical nuclear localization of this protein, figure (1).

No one of ALL, AML, CMML or healthy individuals showed positivity for BCR-ABL cells. That result was consistent with their hematological diagnosis. Also, non- specific binding of monoclonal antibody used in this assay to cell components or the presence of background signal due to endogenous cell biotin were proved through the replacing of the addition of primary antibody to one smear in each slid by phosphate buffer slain. The reproducibility of ICC assay was evaluated by repeating this assay for CML samples that collected at different time points from starting imatinib treatment. The same results were seen.

Table 1: Correlation between score of BCR-ABL positive lymphocytes of CML patients and disease phase and hematological response.

Scoring group	Patients No. (%)	CML -phase		HR	
		CP No. (%)	AP No. (%)	CHR No. (%)	PHR No. (%)
Moderate BCR-ABL positive	12(28.57)	12(100)	---------	12(100)	----------
High BCR-ABL positive	30(71.42)	23(76.66)	7(23.33)	10(33.33)	20(66.66)

-CP=chronic phase, AP=accelerated phase, HR=Hematological response, CHR=complete hematological response, PHR=partial hematological response.

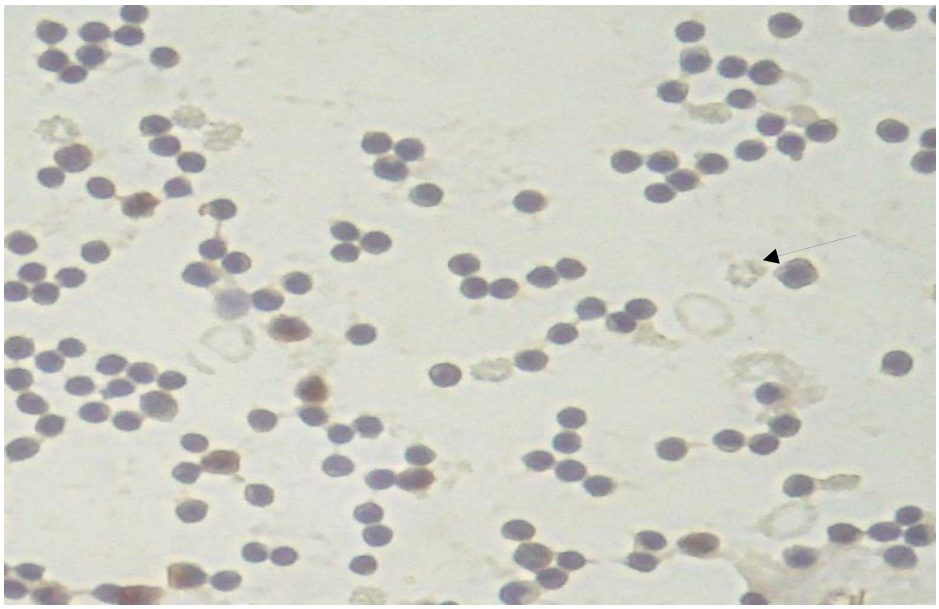

Figure1: Immunocytochemistry staining of BCR-ABL oncoprotein in CML patient's VB lymphocytes using BCR-ABL monoclonal antibody. Antibody binding was visualized by incubating with diaminobenzidine solution (DAB), counterstained with Mayer hematoxylin and evaluated with light microscope. Arrow showed positive BCR-ABL cell. The nuclear stained with dark brown (DAB). High power magnifications of 400X.

Discussion

The genetic alterations in human leukemia and lymphoma results from a creation of hybrid genes which encode chimeric proteins. Those proteins were studied by ICC using antibodies specific for the protein products of gene involved in such alteration [18, 19].

The ICC demonstration of BCR-ABL protein offers several potential advantages over biochemistry techniques such as western blotting or immunoprecipition in which, these techniques are particularly applicable to the study of hybrid genes, since they allow chimeric proteins such as BCR-ABL to be distinguished on the basis of their unique molecular size. However, compared with ICC, biochemical methods are more demanding technically and provide only limited information on subcellular localization of the protein.

Moreover, ICC is rapid and does not require the substantial investment in laboratory equipment [20, 21].

The expression of BCR-ABL was indicated in all CML patients included in this study. That is consistent with what was reported by Volpe *et al.* [22].

Comparing the qualitative results (whether moderate or high) of the immune staining technique showed a full agreement with the results of hematological response. As seen from table (1), all patients (100 %) with moderate BCR-ABL positive cells percentage achieved complete hematological response (CHR), while 33.33% of patients with high BCR-ABL positive cells percentage achieved CHR.

The lack of false positive in patients with other disease or healthy individuals indicate that the BCR-ABL ICC assay has its value as a relative and easy mean for assessing whether or not patients with leucocytosis and /or myeloproliferative syndrome are BCR-ABL positive. The limitations of ICC technique are: (1) the results are semi-uantitative because the amount of visible reaction product is related in a complex manner to the amount of specific antibody binding to the receptor and it is not necessarily a simple linear relationship. (2)Using this assay, the detection of both forms of BCR-ABL protein (p210 or p190) were not possible.

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