ORIGINAL ARTICLE

Chromosomal Study For Prognostic Grouping In Chronic Lymphocytic Leukemia

Ayesha Junaid[1], P. Nagesh Rao[2] and Malik Muhammad Adil[3]

ABSTRACT

Objective: To determine the frequency of various cytogenetic aberrations in newly diagnosed chronic lymphocytic leukemia (CLL) patients, and their detection rate by cytogenetic and fluorescent *In situ* hybridization (FISH) technique separately.

Study Design: A case series.

Place and Duration of Study: Clinical and Molecular Cytogenetics Laboratories, University of California, Los Angeles, USA, from November 2007 to July 2008.

Methodology: Analysis was made on 100 diagnosed chronic lymphocytic leukemia patients. Cytogenetics and FISH technique were performed on blood or bone marrow samples.

Results: Nineteen out of 100 cases (19%) showed karyotype abnormalities; whereas 55 showed abnormalities using the CLL - specific FISH probes. The most frequent abnormality detected by standard cytogenetics was trisomy 12. The most common abnormality detected by FISH was a deletion of 13q14 (40 out of 55 cases; 72% of the abnormal).

Conclusion: For prognostic grouping of CLL patients, FISH must always be requested which may even replace standard karyotyping. These chromosomal markers help in choosing the therapeutic options.

Key words: *Fluorescent in situ hybridization (FISH). Cytogenetics. Trisomy 21. Chronic lymphocytic leukemia (CLL).*

INTRODUCTION

Chronic lymphocytic leukemia (CLL), the most common haematological malignancy in adults,[1-3] has substantial genetic heterogeneity resulting in varied survival expectancy.[4,5] In blood, CLL is characterized by the accumulation of monoclonal CD 5+ B-cells with the appearance of small mature lymphocytes.[6-8] CLL is responsible for more than 5000 deaths yearly in the United States.[9] Novel biologic parameters such as ZAP 70 and CD 38 are added to the clinical staging systems to predict an indolent or aggressive outcome.[10]

In order to assign patients into therapeutically relevant prognostic sub-groups molecular karyotyping (FISH) has become increasingly important.[11] The most frequent chromosomal abnormalities in B-cell CLL are deletions on 13q14, 11q22-23, and 17p13, trisomy 12 and re-arrangement of 14q32.[12,13] Each of these cytogenetic aberrations (11q-, 13q-, 17p-) and (+12) has been correlated to have significant prognostic implications for CLL patients.[14] Conventional cytogenetic analysis underestimates the frequency of specific chromosome aberrations in B-CLL because of the low rate of spontaneous mitoses and the poor response to mitogen stimulation. Many studies have shown that the fluorescent *in situ* hybridization (FISH) technique on non-dividing cells (I-FISH) in CLL identifies genomic aberrations at a higher frequency than does classical karyotyping, including stimulated cultures using standard B-cell specific mitogens.

Although during the past two decades there has been considerable progress in the understanding of the pathophysiology of CLL, the most important un-resolved issue, however, is the need for standardization of tests performed by genetic laboratories for these cellular markers. This analysis was carried out to determine the frequency of cytogenetic aberrations in chronic lymphocytic leukemia in newly diagnosed cases, and their detection rate by cytogenetic and FISH techniques separately. This would help in establishing prognostic grouping for financially restricted CLL patients by choosing and prioritizing the molecular techniques to be employed.

METHODOLOGY

A total of 100 consecutive cases of CLL which were reffered to the Clinical and Molecular Cytogenetics Laboratories UCLA, between November 2007 and July 2008, were selected, for which both cytogenetics and FISH studies were requested. All the cases were referred by a haematopathologist to the cytogenetics laboratory with an initial diagnosis of CLL.

Cytogenetic studies were performed following routine protocols on bone marrow / blood samples using 24

[1] *Department of Pathology/Medicine Emergency[3], Shifa Inernational Hospital, Islamabad.*

[2] *Department of Clinical and Molecular Cytogenetics, University of California, Los-Angeles, USA.*

Correspondence: *Dr. Ayesha Junaid, 2nd Floor, Block-2, Kohsar Complex, F-5/2, Islamabad.*
E-mail: ayesha_junaid497@hotmail.com

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hours (hr) cultures with no additives and 72 hours cultures supplemented with Pokeweed mitogen. Harvesting at 24 and 72 hours was accomplished using Colchicine incubation for 20 minutes. Conventional Giemsa Trypsin (GTG) banding was done. Analysis of 20 metaphase cells was performed.

FISH analysis was carried out on un-stimulated peripheral blood mononuclear cells and bone marrow specimens alloqouated from the karyotype specimens. The cells were dropped, air dried and aged in saline sodium citrate (SSC) for 30 minutes. The slides were then dehydrated in graded ethanol (70%, 85% and 100%) for 2 minutes each. Vysis Abbott molecular multi-colour probes were added and sealed on the slides, which were hybridized and denatured in HyBrite and post-hybridization washes and counterstaining with DAPI-II was done as per manufacturer's instructions. For CLL, probe set including the loci: LSI p53 (17p13)/LSI ATM (11q22) and LSI D13S319 (13q14/ LSI 13q34/ CEP12 (chromosome 12) and the dual colour IGH "breakapart" (14q32) were used. The analysis was done on ZEISS fluorescence microscope equipped with appropriate filters. Three hundred nuclei were analyzed for each probe. Probe and analytical validation was established for each probe lot by international reference methods.[15] The data was analyzed using Statistical Program of Social Sciences (SPSS).

RESULTS

Sixty five out of the 100 patients were males and 35 were females making a gender ratios of 1.8:1. The ages ranged from 43-90 years of age with median age of 67 years. Nineteen showed karyotype abnormalities, where as 55 were identified to be abnormal using the CLL specific FISH probes.

The most frequent abnormality detected by standard cytogenetics was trisomy 12 (5 out of 19, 26 %). Only 2 of 19 showed a deletion of 13q (10.5% of the abnormal), while one showed deletion of 11q (1/19, 05% of abnormal). None of the 100 patients was positive for the other recurrent and reported cytogenetic abnor-malities such as deletions of 6q or 17p by the cytogenetic studies. Balanced translocations like t (4; 8) and t (11; 13) were seen in 2 cases. One case showed t (11;14), as an additional abnormality along with a loss of 13q by FISH analysis. Monosomy 12 and 17 and trisomy 14 was observed in one case each (Table I).

Table I: Frequency distribution of the chromosomal abnormalities in chronic lymphatic leukemia.

Chromosomal abnormality	Number of positive cases by cytogenetics analysis	Number of positive cases by FISH analysis
Trisomy 12	5	17
13q14 deletion	2	42
17p deletion	0	5
11q22 deletion	1	6
14q32 del/re-arranged	0	22
Miscellaneous	14	5

The most common abnormality detected by using the commercial FISH probes was the deletion of 13q14 (42/55; 76% of the abnormal), followed by a loss/re-arrangement of IGH locus (22/55; 40% of the abnormal). The next most frequent aberration detected by FISH was trisomy 12 which was positive in 17 of 55 positive cases (31%). Deletion of the long arm of chromosome 11 was found in 6 out of 55 abnormal cases (11%). Short arm of chromosome 17 was deleted in 5 out of 55 cases (09%).

Out of miscellaneous findings in CLL patients for FISH analysis, an additional signal for chromosomes 11,13,14 and 17 was seen in 5 cases.

DISCUSSION

CLL patients with complex karyotype are long established to have a relatively poor prognosis.[16, 17] Also some CLL cell genetic abnormalities, such as deletions in the short arm of chromosome 17 or in the long-arm of chromosome 11, are independent predictors of adverse outcome.[18] Conventional cytogenetic analyses reveal chromosomal aberrations in only 40-50% of patients, because detection of abnormalities is limited by the low mitotic activity of CLL cells *in vitro*. On the other hand FISH analysis on interphase cells identifies chromosomal changes in approximately 80% of patients with CLL and presence of specific chromosomal abnormalities has proven to be a prognostic indicator for disease progression and survival.[19] The frequencies of different genetic aberrations in this review match those that are defined in the literature to be prevalent in CLL. Trisomy 12, the most common abnormality detected by standard karyotyping (26%) is strongly correlated with poor prognosis.[20] Comparatively, detection of this aberration was higher by FISH and was observed in 31% cases. Interstitial deletion of long arm of chromosome 13 was the most common abnormality detected by FISH (76%) analysis, but was seen in only 10% by karyotype studies.

Deletion 13q is associated with a favourable prognosis in CLL. Both deletions of 11q and 17p are associated with rapid disease progression and inferior survival.[18] Patients with these genetic abnormalities are the candidates for clinical trials, experimental therapies and or stem-cell transplantation. Our review showed that frequency of their detection by FISH was almost double the frequency as was identified by karyotype analysis. The frequencies reported by the Housten group who undertook the same comparative analysis between the two techniques in 2005 shows concordance with our analysis and also confirms that detection rate of the genomic aberration is almost twice of the karyotype.

Nine cases showed loss of the Y-chromosome in their karyotypes and 7 of them were negative by the FISH analysis for any genetic abnormality. All of those 9

individuals were above 75 years of age and thus the loss of the Y-chromosome is possibly related to the more common age related sex chromosome loss.

One case showed t (11;14) by karyotyping representing probably leukemic phase of mantle cell lymphoma, the FISH analysis of this case exhibited a 13q deletion. This deletion at 13q14 is observed in up to 52% cases of MCL (T11,14 13q). One less likely possibility, for both of those abnormalities present simultaneously is of 2-5% of CLL cases which are positive for translocation (11;14) and deletion 13 q. However, the prognostic implication of simultaneous presence of both these changes in CLL is not clear.

Regarding the diagnostic sensitivity of both techniques, it was found that 36% cases were negative (chromosomally normal) by cytogenetic analysis but were abnormal by FISH analysis. Of the 100 consecutive CLL cases analyzed, 10 cases did not grow metaphases for chromosome analysis. This is not uncommon despite using well-established protocols as there is inherent resistance of B-lymphocytes to propagate well in the laboratory. [21] The technical failure results in extra cost to the laboratory and to the patient as well. Furthermore, due to sub-microscopic size of most of these aberrations in CLL, even if the malignant cells grow *in vitro*, the results can still turn out to be normal. The minimum size of a deletion that can be visualized by standard karyotyping is in the range of 7-10 MB. Array CGH studies have revealed that 13q deletions are variable in size, but they are all well below the resolution of normal microscopy. This is why the detection rate is higher by FISH studies. Similar results have been recognized for the 11q and 17p deletions. Only one third of the patients of CLL showing abnormalities even if B lymphocytes grow *in vitro*.

FISH evaluates interphase nuclei and a large number of cells in a short period of time as compared to the subjective and time consuming karyotypic analysis. The technique is sensitive for analysis of chromosome aberrations in CLL without the need of *in vitro* growth and provides accurate information regarding the genetic features of CLL.[22,23]

Molecular testing like array CGH (a-CGH) though sensitive but has its limitations like inability to detect balanced translocations and availability in only few set ups. So, a- CGH remains impractical in near future. Also the genomic array technology might be less sensitive than FISH techniques in detecting intraclonal genetic changes that sometimes are found during CLL clonal evolution.[24] Furthermore, as no therapy induced cytogenetic response (CR) is in clinical demand for CLL unlike CML, FISH analysis reveals cryptic chromosomal abnormalities, which remain unknown by conventional cytogenetics. Some reports document an additional detection of clonal aberrations in 35% samples referred for possible CLL by FISH not identified by conventional cytogenetics.[25] There is an increased use of FISH technique recently, to identify specific abnormalities useful in both the diagnosis and management of lymphoid disorders. FISH can detect genomic abnormalities about twice as frequently as chromosomal banding in over 80% of CLL patients.

CONCLUSION

A complete panel testing by FISH should be given preference over standard karyotyping for prognostic grouping in chronic lymphocytic leukemia. In cases where a choice has to be made depending upon cost or time factor, FISH stands far ahead of standard karyotyping in unraveling the genetic lesions.

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