Detection of Immunoglobulin Gene Rearrangement in Acute Lymphoblastic Leukemia by Polymerase Chain Reaction

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**Background:** The objective of the study was to evaluate a qualitative PCR based method for detection of clonal immunoglobulin gene rearrangement in patients of acute lymphoblastic leukemia. It was a descriptive Cross sectional and it was conducted at Armed Forces Institute of Pathology Rawalpindi between Aug 2009- Jan2010. **Method:** 61 patients with acute lymphoblastic leukemia were studied. Genomic DNA was extracted from the peripheral blood, bone marrow aspirate/unstained bone marrow smears. PCR amplification of the IgH gene was done by a VH primer homologous with a highly conserved sequence near the 3’ end of the FR3 region and a consensus sequence JH primer. The amplified DNA was seen by electrophoresis on 6% polyacrylamide gel. **Results:** A sharp clonal band ranging from 90-200 bp indicated a positive reaction. At the time of diagnosis 50 out of the 61 patients (82%) showed clonal immunoglobulin gene rearrangement by qualitative PCR using a single pair of primers. Eleven (18%) patients did not show the Ig gene rearrangement. **Conclusion:** Clonal Ig gene rearrangements can be demonstrated in a vast majority of patients of Acute Lymphoblastic Leukemia by simple PCR using a single pair of primers. **Key words:** Ig gene rearrangement; ALL; PCR

**Introduction**
The diagnosis of Acute Lymphoblastic Leukemia (ALL) is usually made by cytomorphological criteria often supplemented by immunophenotyping. However in certain cases diagnosis may be difficult even with these methods. Study of presence of immunoglobulin gene rearrangement can provide further useful information on clonal status, lineage assignment and to a minor degree extent of the disease. Hence beside cytomorphological methods the diagnosis of lymphoid neoplasms needs the demonstration of monoclonal proliferation of lymphoid cells. The principle of monoclonality states that all cells in a clone have the same immunoglobulin (Ig) gene rearrangement. Methods of establishing monoclonality by demonstrating the Ig gene rearrangement have long been established. Replacement of Southern blot analysis by polymerase chain reaction has made detection of Ig gene rearrangement simple and quick.1,2 This study aims to detect clonal Ig gene rearrangement in leukemic cells using PCR technique in patients of ALL. There are very little data available locally which could help in formulating the Ig gene rearrangement as a marker of clonality in patients of acute lymphoblastic leukaemia. Therefore this study will help in establishing the PCR based technique using Ig gene rearrangement as a clonal marker in a local setting and will also enable to evaluate the utility of the newer and advanced criteria in Pakistani patients.

**Materials and Methods**
It was a descriptive cross sectional study conducted on a group of sixty one patients selected by non-probability purposive sampling. All newly diagnosed untreated patients of acute lymphoblastic leukemia were included in the study. Patients who were already on treatment were excluded. Demographic data including name, age, sex, telephone no. was recorded. Hematological parameters including TLC, Hb, and Platelet count were also recorded. Blood counts were performed on Sysmax KX 21. Percentage of blasts in peripheral blood and/or bone marrow was charted. All of the cases came to AFIP Rawalpindi for bone marrow aspiration and were diagnosed by standard morphology & cytochemical methods. The presence of
Ig gene rearrangement in leukemic blasts was searched in the bone marrow or peripheral blood sample/unstained bone marrow smears obtained at the time of diagnosis. Genomic DNA was extracted from whole blood/bone marrow aspiration samples by using a commercial reagent kit (Gentra, USA). DNA was also extracted from unstained bone marrow smears. The smears were covered with 500 µl of 2 % lauryl sulphate (SDS) solution containing 50 mM Tris & 2mM Na$_2$EDTA (pH 8.0). The smear was then scrapped off and transferred to a clean 1.5milliliter Eppendorf tube. After scrapping 25-30ul proteinase-K (Sigma, USA) was added to the mixture for protein digestion and it was incubated at 37°C overnight. DNA extraction was done by phenol chloroform method.

**PCR Conditions**

The PCR was done by using a VH primer homologous with a highly conserved sequence near the 3' end of FR3 region in conjunction with a consensus J$_H$ primer. The sequences of the primers were: V$_H$ 5'-ACA CGG CCG TGT ATT ACT GT and J$_H$ 5'-GTG ACC AGG GTA CCT TGG CCC CAG. The primers were synthesized by Integrated DNA technologies, USA. The PCR was carried out in a 25 µl reaction mixture containing 5pM of each primer, 0.5 units of Taq polymerase (Fermentas, Lithuania), 30 mM of each dNTP (Fermentas Life sciences Lithuania), 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 100 mg/ml gelatin (Sigma, UK), and 0.3 – 0.5 µg of genomic DNA.

Thermal cycling was done in 9700 (Perkin Elmer, USA) using 30 cycles of: Denaturation 94°C for 30 sec, Annealing 63°C for 30 sec, and extension 72°C for 1 min. The final extension was done for 3 minutes. The amplified products were loaded on 6% non-denaturing polyacrylamide gels measuring 1 mm x 10 cm x 10 cm on Mini-protean electrophoresis apparatus (Bio-Rad, USA). Electrophoresis was carried out at 150V for 40 minutes. The gels were stained by silver nitrate.

**Results**

With PCR analysis a sharp discrete band of amplified DNA ranging in size from 90-200 bp showed the positive result. The samples without IgH gene rearrangement showed a diffuse smeared pattern in the 90-200bp region (Figure 1, 1-4). Ig gene rearrangement were seen in 61/50(82%) patients by using a single pair of primers.

![Figure 1: Polyacrylamide gel electrophoresis of amplified Ig gene rearrangement](image)

Lane 1-4: Rearranged Ig gene  
Lane 5 & 6: Unrearranged Ig gene

**Discussion**

Normally during development, B-cell precursors possess immunoglobulin heavy chain gene rearrangement process at an early stage. B-cell produces immunoglobulin molecules, with the rearrangements of various segments of the immunoglobulin gene at molecular level. This Ig gene comprises various regions (i.e., VH, DH and JH). These regions are further subdivided into large number of other segments. At the expense of antigenic challenge this germ line Ig gene chooses a few segments from each of the regions and rearranges itself. Hence a gene is formed which is much smaller in size than the original germ line gene. This recombination event provides a molecular basis for the production of an innumerable antibodies of vast diversity. V-D-J combination is specific for each lymphocyte. Due to this, the VDJ sequence at the site of junction is fingerprint-like. So each lymphocyte clone can acquire a specific pattern. Since leukaemias are regarded as clonal cell proliferations, these rearrangements are expected to be identical at molecular level in all the cells derived from a particular leukaemia.

Polymerase Chain Reaction can be used to amplify the IgH gene. Un-rearranged and the rearranged IgH gene can be differentiated by using specific primers. PCR primers are far away from each other in the unrearranged IgH gene. When the gene becomes much smaller in size due to rearrangement the PCR primers come closer to each other and amplification takes place.

The main problem with PCR based assay is a relative-
ly high rate of false negative results. This can be avoided by using additional pair of primers for detection of Ig gene rearrangement. Another limitation of PCR based assay is that original sequence of Ig gene may be altered and additional rearrangement may become dominant (clonal evolution) during the course of the disease. This problem can also be overcome by using different sets of primers against different regions of Ig gene. The PCR techniques using specific primers against conserved and consensus regions, of Ig gene have been developed for the detection of clonal Ig gene rearrangement by several groups of investigators. Khalil et al documented 84% monoclonality in patients of ALL by simple polymerase chain reaction using single pair of primers. Ahmed conducted a PCR based assay on patients of ALL using single pair of primers, and found monoclonality in 91.3% Steward et al found monoclonal IgH gene rearrangements in 90% of B-lineage ALL patients at presentation. Trai
nor et al found 83% of monoclonal IgH gene rearrangements in cases of B-lymphoproliferative disease by PCR technique. Liang et al conducted a study for detection of Ig gene rearrangement using PCR in 14 out of 17 patients of acute lymphoblastic leukemia. Arnutti et al investigated clonal IgH gene rearrangements from 31 children with ALL by PCR amplification using primers directed to FR1, FR2 and FR3 and found monoclonality in 93.5% of cases. Yao et al studied IgH gene rearrangement in 40 patients of ALL and found it to be positive in 86% of cases.

Conclusion
In summary we find PCR a useful technique for the detection of Ig gene rearrangement as a clonal marker in peripheral/bone marrow specimens of patients with acute lymphoblastic leukemia. The PCR based detection of clonal Ig gene rearrangement appears to be a quick, simple, sensitive and technically feasible method for use in a Pakistani setting.

References