ORIGINAL ARTICLE

IMMUNOGLOBULIN GENE REARRANGEMENT IN DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Acute lymphoblastic leukaemia (ALL) is mainly a childhood malignancy but affects both children and adults. **Objective:** The study was conducted to evaluate a qualitative PCR based method for detection of clonal immunoglobulin gene rearrangement as a marker of minimal residual disease in patients of acute lymphoblastic leukaemia at the end of induction. **Method:** It was a descriptive study conducted at Armed Forces Institute of Pathology, Rawalpindi from Aug 2009 to Feb 2010. For prospective analysis, genomic DNA was extracted from peripheral blood/bone marrow aspirates and unstained bone marrow smears. A total of 50 patients of acute lymphoblastic leukaemia who showed positive immunoglobulin gene rearrangement by qualitative PCR at the time of diagnosis were included. These patients were then investigated for minimal residual disease at the end of induction. 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. Test for minimal residual disease was conducted by PCR amplification of DNA from remission marrow cells (at day 29 of chemotherapy) with the help of the primer sets used at the time of diagnosis. 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. Of 50 patients, 28 (56%) were positive for Ig gene rearrangement on PCR at the end of induction, 17 (34%) patients were found to be negative for minimal residual disease, 2 (4%) patients died during induction therapy, and 3 (6%) patients did not come for follow-up. **Conclusion:** Molecular approaches have allowed us to detect low level of residual disease which is not detected by cytomorphological methods. Minimal residual disease (MRD) by PCR used in this study would definitely help in monitoring of MRD in all patients with leukaemia.

Keywords: Ig gene rearrangement, ALL, MRD, PCR

J Ayub Med Coll Abbottabad 2013;25(1-2):159–61

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is a clonal lymphoid disorder.[1] It is mainly a childhood malignancy[2] but affects both children and adults with peak incidence between 2–5 years[3]. ALL is the most common cause of death in children from cancer.[4] Although most patients with ALL achieve clinical remission with chemotherapy, residual malignant cells still persist.[4] They represent minimal residual disease (MRD). This MRD is responsible for subsequent relapses. They are detected by highly specialized techniques, which have much higher sensitivity than any cyto-morphological method.[5] Polymerase chain reaction (PCR) is highly sensitive and simple method for the detection of minimal residual disease.[6]

Normally during development, B-cell precursors undergo immunoglobulin heavy chain gene rearrangement process at an early stage.[7] 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).[8] These regions are further subdivided into large number of various 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.[9]

In polyclonal B-cell proliferation antibodies are produced from large number of B-cells and these antibodies are specific for each antigen. So every B-cell shows a unique IgH gene rearrangement at the molecular level. Monoclonal proliferation of B cells however show the same IgH gene rearrangement at molecular level[10], since they are derived from the leukaemic clone.

The documentation of this study will help in establishing the PCR based technique using clonal markers for MRD and will also enable to evaluate the utility of the newer and advanced criteria in patients.

MATERIAL AND METHODS

It was a descriptive study conducted at Armed Forces Institute of Pathology Rawalpindi from Aug 2009 to Feb 2010. The subjects were 50 cases of ALL that showed Ig gene rearrangement at diagnosis. Informed all of the cases came to AFIP Rawalpindi for bone marrow aspiration and were diagnosed by standard morphology and cytochemical methods. All newly

diagnosed patients of ALL who showed a positive Ig gene rearrangement at the time of diagnosis were included in the study. Patients already on treatment were excluded.

All cases were analysed pre- and post-chemotherapy (on day 29) when they were in remission morphologically. The morphological diagnosis of remission was based on presence of <5% blasts in bone marrow, absence of blasts in the peripheral blood and recovery of peripheral blood values towards normal in all cases of ALL.

About 3 ml of venous blood in EDTA or bone marrow aspirate in EDTA was collected. DNA was extracted from blood/bone marrow/unstained bone marrow smears 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 and 2 mM Na_2EDTA (*p*H 8.0). The smear was then strapped off and transferred to a clean 1.5 ml Eppendorf tube. After scrapping 25–30 µl 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.[11]

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 amplified products were loaded and analysed on 6% polyacrylamide gel.

The presence of Ig gene rearrangement in leukaemic blasts was searched in the bone marrow or peripheral blood sample obtained at the time of diagnosis. Tests for minimal residual disease were conducted by PCR amplification of DNA from remission marrow cells with the use of primer sets corresponding to the clonal rearrangement identified at the time of diagnosis. Descriptive statistics were used to calculate the frequencies and percentages of patients with all regarding the status of minimal residual disease at the end of remission induction.

RESULTS

Polyacrylamide gel electrophoresis showed rearranged and un-rearranged immunoglobulin gene at the time of diagnosis and at the end of induction by using a single pair of primers. A discrete band of amplified DNA ranging 90–200 bp showed the positive result. The samples without IgH gene rearrangement showed a diffuse smeared pattern in the 90–200 bp regions. Hence in polyclonal proliferation of B cells, a diffuse smear was seen, since each B cell had a different VDJ, size, length and sequence. On the other hand monoclonal population had the same VDJ size, length, and sequence since they were derived from the leukaemic clone.

MRD status at the end of induction showed that 28 (56%) patients were positive for MRD at the end of induction, 17 (34%) were negative for MRD, 2 (4%) patients died during induction therapy, and 3 (6%) patients were lost to follow up. In view of these results 28 (56%) patients who were morphologically in remission showed positive immunoglobulin gene rearrangement on PCR.

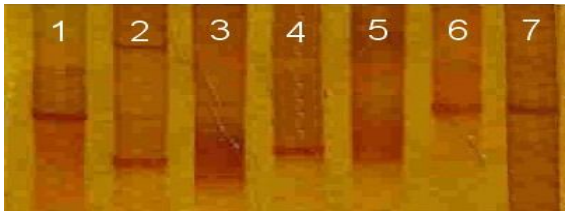

Figure 1: Polyacrylamide gel electrophoresis of amplified Ig gene rearrangement

Lane 1: Known positive control of Ig gene rearrangement,
Lane 2 & 3: Sample of the same patient showing positive result at the time of diagnosis and negative result at the end of induction,
Lane 4 & 5: Sample of the same patient showing positive result at the time of diagnosis and negative result at the end of induction,
Lane 6 & 7: Sample of same patient showing positive result at the time of diagnosis and at the end of induction.

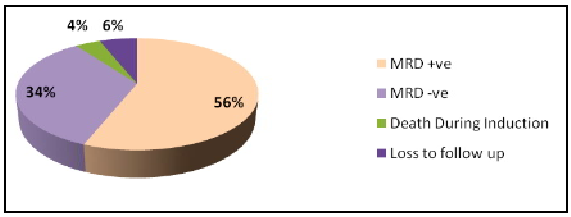

Figure-2: MRD status at the end of induction

MRD +ve patients=28 (56%), MRD –ve patients=17 (34%), Death during induction=2 (4%), Loss to follow up=3 (6%)

DISCUSSION

When ALL is diagnosed in a patient, the total number of leukaemic blasts is about 10^{12-14} Most patients achieve clinical remission four weeks after chemotherapy.[15] Clinical remission means that malignant cells are still present in the body but they are not detected by classical cytomorphological methods. Hence at the time of remission up to 10^{10} malignant cells can be present[4] and they represent minimal residual disease.

The diagnosis of lymphoid neoplasms includes morphology and cyto-chemical staining as well as demonstration of monoclonal proliferation. Study of the presence of immunoglobulin gene rearrangement by PCR can provide useful information on monoclonal status of lymphoid proliferation. The monoclonal proliferation states that all the cells derived from the monoclonal population have the same pattern of IgH gene rearrangement at molecular level.

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.[16] PCR primers are far away from

each other in the un-rearranged 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.[17] The main problem with PCR based assay is a relatively high rate of false negative results. This problem can be avoided by using additional primers for detection of Ig gene rearrangement.[18]

Zhou[19] *et al* studied MRD in 284 children with B-lineage ALL at the end of induction. MRD was detected in 176 children while 108 children were found to be negative for MRD and he also found a higher risk of relapse in MRD positive patients. In a study by Brisco *et al*[20] 88 patients of ALL were studied for minimal residual disease at the end of induction using PCR based assay. Thirty-eight patients were found to be positive for MRD while 50 patients were negative for MRD at the end of induction. In a prospective study few researchers investigated MRD using PCR at the end of induction in 51 patients of ALL. Out of the initial 51 patients, 34 were assessable for MRD at the end of induction. MRD was found in 76% of patients and 24% had no detectable disease.[21]

Using PCR capable of detecting low level of residual disease with the help of single pair of primers to the FR3 region and the JH region, approximately 28 (56%) patients who were morphologically in remission showed positive Ig gene rearrangement on PCR at the end of induction. This suggests that morphology alone is not sufficient for detection of MRD. PCR therefore, can detect the residual leukaemic cells which are not detected by any cyto-morphological method.

There is a vast spectrum of applications of molecular techniques used in haematological malignancies. PCR may significantly improve diagnosis and prognosis of patients and better monitoring of MRD based treatment modalities. The usefulness of this test is that it provides simple and rapid means of detection of minute amount of malignant residual cells at the end of induction which are not detected by classical cyto-morphological methods.

CONCLUSION

A vast majority of the patients while in morphological remission were shown to have clear evidence of MRD at the molecular level. The PCR based detection of clonal Ig gene rearrangement appears to be a quick, simple, sensitive and technically feasible method.

REFERENCES

1. Pui CH. Acute lymphoblastic leukemia. In: Lichtman MA, Kipps TJ, Kaushansky K, Beutlar E, Saligson U, Parchal JT, (Eds). Williams' Haematology. 7th ed. USA: McGraw-Hill; 2006. p. 1321–42.
2. Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL. A systematic literature review of the clinical and epidemiological burden of acute lymphoblastic leukaemia (ALL). Eur J Cancer Care 2005;14:53–62.
3. Pui CH, Robinson LL, Look AT. Acute lymphoblastic leukaemia. Lancet 2008;371:1030–43.
4. Moppett J, Burke GAA, Steward CG, Oakhill A, Goulden NJ. The clinical relevance of detection of minimal residual disease in childhood acute lymphoblastic leukaemia. J Clin Pathol 2003;56:249–53.
5. Foroni L, Gameiro PM, Hoffbrand AV. Minimal residual disease in acute leukemia. In: Hoffbrand AV, Catovsky D, Tuuddenham EGD. (Eds). Postgraduate Hematology. 5th ed. Oxford: Blackwell; 2005. p.561–74.
6. Li A, Zhou J, Zuckerman D, Rue M, Dalton V, Lyons C, *et al*. Sequence analysis of clonal immunoglobulin and T-cell receptor gene rearrangements in children with acute lymphoblastic leukemia at diagnosis and at relapse: Implications for pathogenesis and for clinical utility of PCR-based methods of minimal residual disease detection. Blood 2003;102:4520–6.
7. Drayson MT, Moss PA. Normal lymphocytes and non-neoplastic lymphocyte disorders. In Hoffbrand AV, Catovsky D, Tuuddenham EGD. (Eds). Postgraduate Hematology. 5th ed. Oxford: Blackwell; 2005. p. 330–57.
8. Parslow TG. Immunoglobulins and Immunoglobulin genes. In: Parslow TG, Stites DP, Terr AI, Imboden JB. (Eds). Medical Immunology. 10th ed. USA: Mc Graw-Hill; 2001. p.95–114.
9. Tonegawa S. The molecules of the immune system. Sci Am 1985;253:122–31.
10. Dean M, Norton JD. Immunoglobulin gene fingerprinting: an approach to analysis of B lymphoid clonality in lymphoproliferative disorders. Br J Haematol 1992;77:274–81.
11. Sambrook J, Fritsch EF, Maniatis T. (Eds). Molecular Cloning: A Laboratory Manual. USA: Cold Spring Harbour Laboratory Press; 1989.
12. Teital MA, Pandolfi PP. Molecular Genetics of Acute Lymphoblastic Leukemia. Annu Rev Pathol 2009;4:175–98.
13. Bennet JH. Case of hypertrophy of spleen and liver in which death took place from suppuration of blood. Edin Med Sur J 1845;64:413–23.
14. Mortuza FY, Papaioannou M, Moreira IM, Coyle LA, Gameiro P, Gandini D, *et al*. Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. J Clin Oncol 2002;20:1094–104.
15. Pui CH, Campana D, Evans WE. Childhood acute lymphoblastic leukaemia-current status and future perspectives. Lancet Oncol 2001;2:597–607.
16. Potter MN, Steward CG, Oakhill A. The significance of detection of minimal residual disease in childhood acute lymphoblastic leukaemia. Br J Haematol 1993;83:412–8.
17. Ahmed S. Immunoglobulin gene rearrangement in lymphoid malignancies: A simple method for routine use. Pak J Pathol 2006;17:109–10.
18. Khalil SH, Siegrist, K, Akhtar M. Application of polymerase reaction to detect rearrangement of immunoglobulin heavy chain genes in lymphoproliferative disease. Ann Saudi Med 1997;17:395–8.
19. Zhou J, Goldwasser MA, Li A, Dahlberg SE, Neuberg D, Wang H, *et al*. Quantitative analysis of minimal residual disease predicts relapse in children with B-lineage acute lymphoblastic leukemia in DFCI ALL Consortium Protocol 95-01. Blood 2007;110:1607–11.
20. Brisco MJ, Condon J, Hughes E, Neoh SH, Sykes PJ, Seshandri R *et al*: Outcome prediction in childhood acute lymphoblastic leukemia by molecular quantification of residual disease at the end of induction. Lancet 1994;343:196–200.
21. Jacquy C, Delepant B, Van Daele S, Verman JL, Zenebergh A, Brichard B, *et al*. Prospective study of minimal residual disease in childhood B-lineage acute lymphoblastic leukaemia: MRD level at the end of induction is a strong predictive factor of relapse. Br J Haematol 1997;98:140–6.

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