ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2021; 7(12): 251-253
www.allresearchjournal.com
Received: 27-10-2021
Accepted: 29-11-2021

Harshita Pandey
Department of Biotechnology, Mewar University, NH-79 Gangrar, Chittorgarh, Rajasthan, India

Dr. Pallavi Mittal
Department of Biotechnology, Mewar University, NH-79 Gangrar, Chittorgarh, Rajasthan, India

Corresponding Author:
Harshita Pandey
Department of Biotechnology, Mewar University, NH-79 Gangrar, Chittorgarh, Rajasthan, India

Identification of chromosomal translocations in children with acute lymphocytic leukemia using multiplex RT-PCR

Harshita Pandey and Dr. Pallavi Mittal

DOI: https://doi.org/10.22271/allresearch.2021.v7.i12d.9255

Abstract
Acute lymphocytic leukemia (ALL) is a heterogeneous disease that requires a risk-stratified approach for proper treatment. Specific chromosomal translocations within leukemic blasts are important prognostic factors that allow identification of relevant subgroups. In this study, we developed a multiplex RT-PCR assay for detection of the 4 most frequent translocations in ALL (BCR-ABL, TEL-AML1, MLL-AF4, and E2A-PBX1). A total of 100 children were diagnosed as ALL patients by morphology, clinical examinations, and flow cytometry assays. Then, they were assessed for specific chromosomal translocations by multiplex RT-PCR assay. Results: The results showed that TEL-AML1 fusion gene was the most frequently encountered genetic anomaly in ALL pediatric patients. Translocation t (4;11) i.e. MLL-AF4 was not detected in any of the cases. Highest frequency of translocations was found in the age group of 1 to 9 years and not any chromosomal translocations were detected below the age of 2 years. Thus, multiplex RT-PCR can be used to detect recurrent chromosomal translocations in childhood leukaemia in an effective manner.

Keywords: Acute lymphocytic leukemia (ALL), Chromosomal translocations, Multiplex RT-PCR

Introduction
Acute lymphocytic leukemia (ALL) is a malignant disorder of the bone marrow which is characterized by the genetic alteration of lymphoid progenitor cells. The disease is the most common malignancy among children, affecting 3–4 out of every 100,000 children each year. The disease occurs more often in children, but is not limited to them (Pui *et al*., 2004) [9]. While the main cause of the disease has not been determined yet, it has been shown that various factors play a role in the development of the disease, including environmental factors, viral infections, genetic changes, and certain syndromes such as Down, Klinefelter, and Bloom. Chromosomal translocations and their related molecular variations have, however, been shown to contribute significantly to pathogenesis and therapeutic response in patients (Paul *et al*., 2016 and Deris *et al*., 2018) [8, 2]. Leukemia and other malignant diseases are often characterized by chromosomal translocations. Several translocations can be used as predictive markers of prognosis or as treatment stratification markers in clinical studies (Metzler *et al*., 2003). Identification of chromosomal translocation and related molecular changes determines the pathogenicity of leukemia cells, as well as optimizes treatment approaches to increase patient survival (Mi *et al*., 2012) [6]. At present, reverse transcriptase-PCR assay (RT-PCR) has been demonstrated to be highly sensitive, specific, and rapid for the detection of genetic alterations, including recurrent chromosomal translocations in leukemia (Elia *et al*., 2003; Yang *et al*., 2010) [3, 11]. Reverse transcriptase polymerase chain reaction (RT-PCR) is an especially useful method in patients who have no analyzable metaphase cells or a few cells of poor quality after standard cytogenetic analysis. This multiplex RT-PCR system, which can handle up to 10 patients with a response time of 2 working days, is thus an important tool that complements cytogenetic analysis in the initial screening of acute leukemia patients and should provide a rapid and precise characterization of leukemia cells, even if there is limited patient material (Pallisgaard *et al*., 1998) [7]. They are essential for identifying cytogenetically cryptic abnormalities. An example is translocation t (12;21) (p13;q22), which involves juxtaposition of similarly banded regions and thus cannot be detected reliably by G-banding (Mrozek *et al*., 2009).

In the current study, a multiplex RT-PCR assay was developed for detection of the 4 most frequent translocations (BCR-ABL, TEL-AML1, MLL-AF4, and E2A-PBX1) in children with acute lymphocytic leukemia (ALL).

Materials and Method

Patient samples

Peripheral blood or bone marrow sample was obtained at diagnosis from 100 ALL pediatric patients in the age group of 0 to 17 years during January 2014 to December 2014 to the Oncquest Laboratories, India. The diagnostic criteria were based on morphologic and clinical examinations, as well as flow cytometry tests.

RNA extraction, RNA quantification, and cDNA synthesis

Fresh peripheral blood was collected from patients in an EDTA tube and mixed well to avoid clot formation. Then RNA was extracted from the sample. Quantity of RNA was checked by spectrophotometer and quality was checked by agarose gel electrophoresis. RNA was stored at –70 °C deep freezer for further use i.e., cDNA preparation.) One µg of total RNA was reverse transcribed to cDNA with 12 µL nuclease free water, 4µL 5X reaction buffer, 1 µL random hexamers, 1µL dNTPs and 1µL reverse transcriptase for 5 mins at 65 °C and the cDNA then used for PCR studies.

Multiplex PCR

Single-round multiplex PCR reaction specific for amplification of four common chromosomal translocations in ALL (TEL-AML1, MLL-AF4, E2A-PBX1, and BCR-ABL) genes was performed to amplify cDNA generated from RNA isolated from ALL patients. Optimal multiplex PCR conditions were as follows: 95 °C for 15 minutes, 35 cycles of 94 °C for 45 seconds, 61 °C for 60 seconds, 72 °C for 90 seconds, and final extension at 72 °C for 5 minutes. Amplified products were stored at 4 °C until further steps. 10 µl of PCR products were detected by automated electrophoresis, QIAxcel Advanced system (QIAGEN, Germany).

Results and Discussion

Molecular genetic testing plays an important role for the diagnosis, risk stratification, planning of the effective therapeutic strategies, and disease monitoring in hematological malignancies. In the present study, the ALL patients comprised 59 (59 %) males and 41 (41 %) females between the age of 1 months to 17 years. Out of 100 cases, 73 cases were normal and only 27 cases were found to have chromosomal translocations by multiplex RT-PCR. Highest frequency i.e., 21 cases (77.77 %) of translocations were found in the age group of 1 to 9 years and not any chromosomal translocations were detected below the age of 2 years. TEL-AML1 fusion gene was the most frequently encountered genetic anomaly in ALL pediatric patients. The incidence rate of TEL-AML1 was (62.96 %, 17/27). The other genetic abnormalities that were detected were BCR-ABL (29.62 %) and E2A-PBX1 (7.40 %). Translocation t (4;11) i.e. MLL-AF4 was not detected in any of the cases. Our results were similar to the study done by Kamaluddin *et al*. (2016) [4] in which ETV6-RUNX1 (previously known as TEL-AML1) fusion gene was the most frequently encountered genetic anomaly in ALL patients. The incidence rate of ETV6-RUNX1 was 9.9 %.

Alqasi *et al*. (2019) [1] found the highest percentage of cytogenetic and molecular genetic abnormalities was related to t (9;22) BCR-ABL in both preB and T-ALL subtypes in children. Limsuwanachot *et al*. (2016) [5] showed that within the positive ALL patients the most prevalent cryptic translocation observed was mBCR-ABL (p190) at 8.41 %. In addition, other genetic rearrangements detected by the multiplex PCR were 4.21 % TEL-AML1 and 2.34 % E2A-PBX1, whereas MLL-AF4 exhibited negative results in all tested samples.

Table 1: Age wise distribution of chromosomal translocations

Age group	Number of cases	Normal	t(9;22)	t(12;21)	t(1;19)	t(4;11)
Below 2 years	3	3	0	0	0	0
2 to 9	72	51	2	17	2	0
10 to 13	16	11	5	0	0	0
14-17	9	8	1	0	0	0

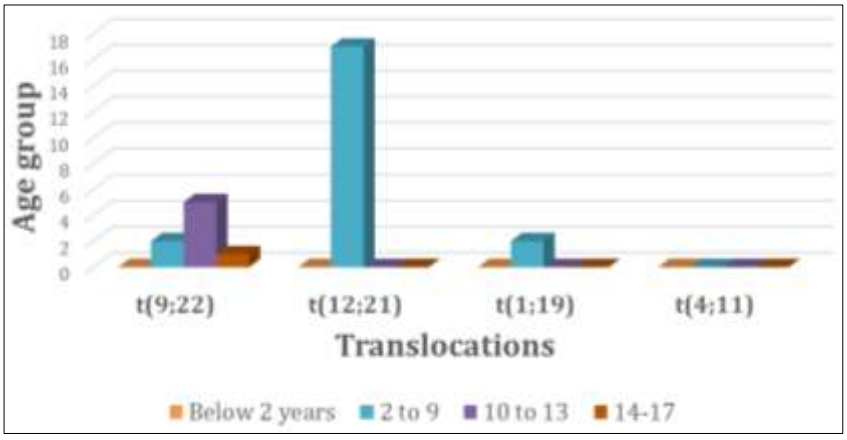

Fig 1: Age wise distribution of chromosomal translocations

Conclusion

Our results conclude that the developed multiplex PCR for routine use is a specific, highly sensitive, and fast method for identifying recurrent chromosomal translocations in ALL patients. By performing this assay, clinicians will be able to assess genetic status of patients before making

clinical decisions. In addition to existing cytogenetic studies, multiplex RT-PCR may aid in the better care management of individual patients through improved diagnostic accuracy, genetic assessment, and tailored treatment.

References

1. Alqasi A, Tavakolifar Y, Rezaeeyan H, Saki N, Bagherpour S, Nasab MA. Cytogenetic and molecular assessment of childhood acute lymphoblastic leukemia patients from 2014 to 2017 in Ahvaz. Clin Cancer Investig J 2019;8:28-32.
2. Deris Zayeri Z, Tahmasebi Birgani M, Mohammadi Asl J, Kashipazha D, Hajjari M. A novel in frame deletion in MSH6 gene in glioma: Conversation on MSH6 mutations in brain tumors. J Cell Physiol., 2018, 1-11.
3. Elia L, Mancini M, Moleti L, *et al*. A multiplex reverse transcriptase-polymerase chain reaction strategy for the diagnostic molecular screening of chimeric genes: a clinical evaluation on 170 patients with acute lymphoblastic leukemia. Haematologica 2003;88:275-9.
4. Kamaluddin NR, Yusoff YM, Esa E *et al*. Identification of chromosomal translocations in childhood leukaemia using multiplex RT-PCR: a single institution study in multi-ethnic Malaysia. Int J Health Sci Res., 2016;6(11):52-56.
5. Limsuwanachot N, Siriboonpiputtana T, Karntisawiwat K, Chareonsirisuthigul T, Chuncharunee S, Rerkamnuaychoke B. Multiplex RT-PCR Assay for Detection of Common Fusion Transcripts in Acute Lymphoblastic Leukemia and Chronic Myeloid Leukemia Cases. Asian Pac J Cancer Prev., 2016;17(2):677-84. Doi: 10.7314/apjcp.2016.17.2.677. PMID: 26925663.
6. Mi JQ, Wang X, Yao Y, Lu HJ, Jiang XX, Zhou JF, *et al*. Newly diagnosed acute lymphoblastic leukemia in China (II): Prognosis related to genetic abnormalities in a series of 1091 cases. Leukemia 2012;26:1507-16.
7. Pallisgaard N, Hokland P, Riishøj DC, Pedersen B, Jørgensen P. Multiplex reverse transcription-polymerase chain reaction for simultaneous screening of 29 translocations and chromosomal aberrations in acute leukemia. Blood., 1998;92(2):574-88. PMID: 9657758.
8. Paul S, Kantarjian H, Jabbour EJ. Adult acute lymphoblastic leukemia. Mayo Clin Proc., 2016;91:1645-66.
9. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med. 2004;350(15):1535-48. Doi: 10.1056/NEJMra023001. PMID: 15071128.
10. Romana SP, Le Coniat M, Berger R. t (12;21): a new recurrent translocation in acute lymphoblastic leukemia. Genes Chromosomes Cancer 1994;9(3):186-191. [PubMed: 7515661.
11. Yang YL, Lin SR, Chen JS, *et al*. Multiplex reverse transcription-polymerase chain reaction as diagnostic molecular screening of 4 common fusion chimeric genes in Taiwanese children with acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2010;32:323-30.