case report

Novel t(7;10)(p22;p24) along with NPM1 mutation in patient with relapsed acute myeloid leukemia

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Chromosomal abnormalities/genetic mutations associated with hematological malignancies alter the structure and function of genes controlling cell proliferation and differentiation through multiple and complex pathways, resulting different clinical outcomes. This is a case study of a lady presented with acute myeloid leukemia (AML M1) at our center who relapsed 10 years after the induction therapy. Cytogenetic and molecular analyses were performed in this case at the time of relapse to find out the chromosomal abnormalities and genetic abnormalities like FMS-like tyrosine kinase (FLT3) and nucleophosmin (NPM1) mutation. The cytogenetic analysis of bone marrow established a novel translocation t(7;10) (p22;q24) in 100% of the cells analyzed. Phytohaemagglutinin (PHA)-stimulated blood culture also revealed the same abnormality. Apart from this, the molecular analysis showed NPM1 exon 12 (hotspot) mutation in this patient. This was the first report of novel chromosomal translocation in this subset of AML in which a new translocation along with NPM1 mutation was discussed.

Acute myelogenous leukemia (AML) is a heterogeneous disease with diverse genetic abnormalities and variable responsiveness to therapy, with the prognosis highly conditioned in the presence of specific cytogenetic and molecular abnormalities, resulting in proliferative advantage over tumor cells or impaired myeloid differentiation of tumor cells.[1,2] Nonrandom chromosomal aberration in malignancies provides important insights into the molecular pathogenesis of human cancer.[3] In this study, we reported a case of relapsed AML-M1 with a novel t(7;10) (p22;q24) along with NPM1 mutation that was not reported elsewhere.

CASE

A 27-year-old lady presented in March 1999 at our center with fever, weakness, and loss of pallor. Her hemoglobin was 5 gm%, platelet 34×10^9/L, and total WBC count 5.2×10^9/L. Bone marrow was the diagnostic of AML-M1 subtype. She received chemotherapy with cytosine arabinoside and doxorubicine followed by a high dose of cytosine arabinoside till July 1999. She achieved remission and was at follow-up till June 2005. When she relapsed in the bone marrow with 93% blast in June 2009, she received chemotherapy with FLAG (fludarabine and Ara–C and GCSF) and continued to be in remission till Jan 2011. When she relapsed for the second time, she opted for further palliative treatment. She is alive and is in remission now.

Cytogenetic analysis

The short-term unstimulated culture of bone marrow cells was carried out in the RPMI 1640 medium (Himedia, India) supplemented with 20% fetal bovine serum (Pan Biotech, Germany). Harvesting and GTG banding procedure was performed as per the standard protocol,[4] followed by karyotyping according to International System for Human Cytogenetic Nomenclature 2009[5] using cytogenetic software (Cytovision, USA). PHA-stimulated culture was also carried out in the blood sample of the patient.

Mutation analysis

The molecular analysis was performed to find out mu-

tations like FLT3-internal tandem duplication (ITD) (exon 14 and 15) mutation, tyrosine kinase domain (TKD) mutation (exon 20), and NPM1 (exon 12) mutation by polymerase chain reaction (PCR), agarose gel electrophoresis (AGE), restriction fragment length polymorphism (RFLP), and single strand confirmation polymorphism (SSCP), respectively.

For FLT3-ITD analysis, PCR products (329 bp) were subjected to 3% AGE and analyzed for band shift. In the case of TKD, the PCR product (114 bp) was subjected to restriction digestion by EcoRV and the number of bands was analyzed. SSCP (29:1, 8% gel) was performed for NPM1 exon 12 mutation analysis and band shifts were noted.

RESULTS

The chromosome analysis of the bone marrow cells revealed the karyotype 46, XX, t(7;10)(p22;q24) (**Figure 1**: karyotype of GTG-banded metaphase showing 46, XX, t(7;10) (p22;q24). The arrows indicate clonal structural abnormalities.) as a sole abnormality in all the 32 metaphases analyzed. Microscopic evaluation disclosed the translocation of the long arm of chromosome 10 (10q24) to the short arm of chromosome 7 (7p22), thus the translocation t(7;10) (p22;q24) was defined. As this abnormality was seen in 100% of the cells, the long-term PHA-stimulated lymphocyte microculture was also performed, and the same abnormality was observed in all the cells analyzed (100%). When the molecular analysis was performed at the time of relapse, FLT3-ITD and TKD mutations were not detected but NPM1 mutation was found to be positive, which was evident by the band shift in SSCP (**Figure 2**: SSCP gel picture showing NPM1 [exon 12] mutation. Arrow shows the shifted band).

Figure 1. Karotype of GTG-banded metaphase showing 46, XX, t(7;10)(p22;q24).

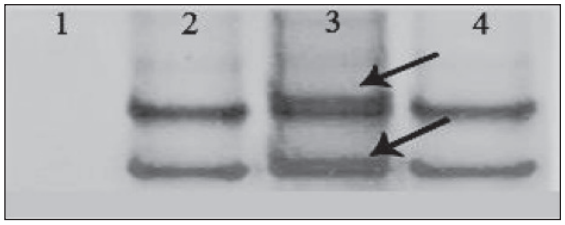

Figure 2. SSCP gel picture showing NPM1 (exon 12) mutation. Lane 1: Negative control (distilled water). Lanes 2 and 4: Control sample showing normal SSCP pattern for NPM1. Lane 3: Patient sample (case) showing NPM1 mutation as band shift.

DISCUSSION

AML is a group of aggressive neoplastic disorders thought to originate from the clonal expansion of a multipotent hematopoietic stem cell. Cytogenetic studies of AML for the past 3 decades indicated that each and every case is important, as there is an increase in the number of balanced and unbalanced rearrangements, particularly in chromosomal translocations associated with distinct cases and characteristic clinical features.[6] Moreover patients at relapse may acquire additional cytogenetic abnormalities, and it is suggested that cytogenetics at relapse tends to be related to the outcome more strongly than cytogenetics at diagnosis. In this article, we have discussed about a case of a new chromosomal translocation t(7;10)(p22;q24).

To our knowledge, this is the first reported case of isolated t(7;10)(p22;q24) in AML.[7] This karyotypic abnormality was detected in 100% of the bone marrow cells at the time of first relapse. Cytogenetic information at the time of diagnosis was not available. The PHA-stimulated culture also revealed the same abnormality in all the cells, showing the possibility of this abnormality to be present at the time of diagnosis also. Translocation involving chromosomes 7 and 10 were frequently reported in acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia in which different regions of chromosome 7 were involved with respect to ours.[8-10] In all these reported cases, 10q24 is a common entity. The translocation involving 10q24 region was not yet reported in AML.

Chromosomal alterations affecting band 10q24 were recurrently associated with hematological malignancies[11] with a gene-rich domain and host to a number of cancer, developmental, and neurological genes. Recurring translocations, deletions, and mutations involving this chromosome band were observed in different human cancers and other disease conditions.[12] The

leukemia-specific genes in this region include HOX11 and NFKB. Gene targeting and overexpression studies demonstrated the importance of clustered homeobox (HOX) gene in hematopoiesis, and substantial evidence exist to suggest that the aberrant expression of HOX gene contributes to the pathogenesis of leukemia,[13] which is the most frequently deregulated gene in T-ALL[14] by t(7;10)(q35;q24) and t(10;14)(q24;q11) and showed a trend for better outcome for patients.[15]

Chromosomal translocation involving 10q24 region was also reported in B cell lymphoma where rearrangement of NFKB2, which is involved in the initiation or acceleration of tumorigenesis, was reported as a result of translocation t(10;14)(q24;q32)[16] and was associated with poor prognosis.

The translocation involving 7p22 region (t[7;21][p22;q22]) were already reported in AML, where USP42 gene is involved leading to deregulation of ubiquitin-associated pathways that may be pathogenetically important in AML.[17] The t(7;10) involving 7p22 region was reported in 3 cases, t(7;10)(p22;q22) in AML (FAB-type M4),[18] t(7;10)(p22;q25) in adenocarcinoma,[19] and t(7;10)(p22;q23) in mycosis fungoides and the Sézary syndrome.[20]

Even though the involvement of 10q24 and 7p22 regions was observed in various malignancies, no reports were seen in the AML case where 10q24 region was translocated to the short arm of chromosome 7p22 and also was not reported in any of the hematological malignancies till date. The presence of frequent chromosomal rearrangements at 10q24 itself shows the importance for the progression of cancer. The present study supported the possible role of both del (10)(q24) and t(7;10)(p22;q24) in the pathogenesis and progression of cancer.

The fluorescence in situ hybridization (FISH) analysis was not carried out in this case because the involvement of the actual putative genes in the translocation was not clear, letting the prognostic significance of this isolated abnormality remain elusive. Thus, the molecular mechanism by which this translocation can cause leukemia and whether this can form an independent prognostic factor is not known. This patient was also analyzed at the molecular level to analyze the most common molecular abnormalities associated with the prognosis of AML such as FLT3-ITD (exon 14 and 15), FLT3-TKD (exon 20), and NPM1 (exon 12) mutations. Studies revealed that FLT3 mutation carries an unfavorable prognosis, and NPM1 mutation confers a favorable prognosis.[21] It was demonstrated that AML patients with intermediate cytogenetic risk and absence of FLt3-ITD mutation, showed a significantly improved overall survival in the presence of NPM1 mutation than those without NPM1 mutation.[22]

The molecular detection of these gene mutations in this patient showed a negative result for FLT3 mutation (both ITD and TKD) and positive for NPM1 mutation. The patient relapsed twice but attained remission and is still alive and is having a better survival (>10 years). In summary, we reported the first case of AML with a novel cytogenetic abnormality t(7;10)(p22;q24) with NPM1 mutation that may contribute to a better outcome. Thus, further molecular characterization of this translocation will unravel the molecular mechanism for the disease progression and will provide further insight into the significance of various gene alterations in leukemogenesis.

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