Prospective evaluation for mutational frequency of Wilm's tumor-1 (WT1) gene in de novo cases of acute myeloid leukaemia

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Introduction

- Wilm's tumor 1 gene (WT1) was initially identified as tumour suppressor gene, located at chromosome 11p13, which encodes transcriptional regulatory protein for genes involved in cellular growth and metabolism and its mutation was reported in both syndrome-associated and sporadic cases of renal malignancy.
- Many studies have indicated that WT1 gene is over expressed in acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS), and other leukemic diseases.
- This gene is highly expressed in more than 80% of AML patients in bone marrow (BM) and PB in comparison with normal control.
- WT1 mutations have been found in about 10%- 15% of cases of AML. WT1 mutations cluster is mainly in exon 7 & 9 and less in exons 1, 2, 3, & 8.
- Therefore, *WT1* gene mutation and related significant in AML need to be studied to emphasize its potential role as a marker of disease progression.

Material & Methods

- Objective:** Prospective evaluation of mutation of *WT1* gene in cases of acute myeloid leukaemia.
- Design:** Prospective clinical research
- Setting:** The study was conducted at academic tertiary referral cancer research center.
- All patient of AML which were diagnosed on basis of morphology, cyto-chemistry and immunophenotyping, were collected for this study, a total of 100 consecutive cases of AML and 10 healthy control.
- In all case and control, genomic DNA were extracted from blood/bone marrow sample using Qiagen® DNA extraction kit.
- DNA were amplified with standard PCR master mix and primer pair designed to flank intronic regions for all cases of AML and controls.
- Primer pair used for both exon 7 and exon 9 was standardized manually to get the optimal annealing temperature.
- PCR products were then analysed by Sanger Sequencing using capillary electrophoresis on an ABI Genetic analyser.
- A total 100 case and 10 control, amplified product of both exons 7 and 9 were taken for direct Sanger sequencing.
- The mutation analysis of coding region as well as intron-exon boundaries of the *WT1* exon 7 and exon 9 was carried out in all cases and controls.

Results

- We analysed 100 cases of AML and 10 healthy control for WT1 gene mutation in both exon 7 and 9.
- In total, WT1 gene mutation were detected in 17/100 patients, (exon 7: n=10, exon 9: n=7); in which 8 silent mutation (8%), 2 missense mutation (2%) and 7 intronic variant (7%), leading to frequency of 17%.
- A total 10 patient out of 100 had WT1 mutation affecting exon 7 (10%) whereas 7 out of 100 in exon 9 (7%). In healthy normal control, no variants were detected. In overall cases of AML, 7 intronic sequence variation were detected in both exon 7 and 9. Therefore, total mutational frequency of WT1 gene was 17% (17out of 100) in both exons.

Fig 1. Electrophoretogram of PCR products on a 1% agarose gel
Lane 1-7: PCR product of different samples of exon 9 of WT1 gene
Lane 8: Molecular weight ladder, 100 base pair ladder

Fig. 1. Exon 9 of WT1 gene, (Wild type)

Fig. 2. Exon 9 of WT1 gene, Point mutation (Mutant type),

(Nucleotides of colour coding in chromatogram, showing in Green peak= A, Red peak= T, Black peak= G, and Blue peak= C)

Conclusion

- This study showed significant frequency of WT1 gene mutation in studied sample size.
- Missense mutation, which was detected as new mutation in two case of exon 9, may interrupt DNA binding capacity through amino acid residues or zinc finger motif structures.
- WT1 gene mutation and its RNA expression pattern with disease outcome needs to be studied in more number of cases to establish WT1 as prognostic bio-marker for risk stratification.

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