Detection of Wilm's Tumor one gene mutation in Sudanese patient with Acute Myeloid Leukemia

Amr. A. Ahmed[1], Malik. H. Mustafa[2], Munsoor. M. Munsoor[3], Enaam. A. Abdelgader[4], Abdel Rahim .M. Muddathir[5]*, Hanan. B. Eltahir[6]

[1]Alnomais Medical & Pharmacies group, Abha, Saudi Arabia, 61431
[2]Department of Medical lab Sciences, College of applied Medical Science, Taif University, Saudi Arabia
[3]Department of Hematology, Faculty of Medical laboratory Science, Sudan University of Science and Technology, Sudan
[4]Department of Hematology, Faculty of Medicine, Elneelain University
[5]Department of Hematology, Faculty of Medical laboratory Sciences, Alzaiem Alazhari University
[6]Department of Biochemistry, Faculty of Medicine, University of El Imam El Mahdi, Sudan
***Corresponding** Author: Department of Haematology and Blood transfusion
Faculty of Medical Laboratory Sciences, Alzaiem Alazhari University
P. O. Box 845 Cod 11111, Khartoum, Sudan
Tel: 00249912351688, E-mail: abdelrahimm@gmail.com

ABSTRACT

***Introduction*:** The diagnosis, prognosis, and treatment of acute myeloid leukemia (AML) have been transformed to be based on genetic, genomic, and molecular characteristics of the disease. Wilms' tumour (WT1) is an important regulatory molecule involved in cell growth and development. WT1 is highly expressed in the bone marrow or peripheral blood of a variety of leukemia.

Materials & Methods: This was a cross sectional – hospital based study, conducted in Khartoum centre for Radiology & Isotopes (RICK), Fifty one Sudanese patients with AML were enrolled in this study, for all candidates DNA extraction was done , followed by PCR amplification of exon 7 for WT1gene , then RFLP was done for mutation analysis.

Results: The study revealed that 43.14% of cases (22/51 patients) were heterozygous A/G and only 3.92% (2/51) were homozygous G/G for the mutant allele.

***Conclusion**:* We can detect the known WT1 gene mutation with a technique that requires only small sample of DNA extracted from the blood. RFLP analysis enables both homozygotes and heterozygotes for WT1 alleles to be unambiguously identified in blood samples. The technique may be useful for screening patients at high risk for AML.

Keywords: Acute Myeloid Leukemia (AML), Wilms' tumour (WT1) gene, Ristriction fragment Length polymorphism PCR-RFLP

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INTRODUCTION

Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. The diagnosis, prognosis, and treatment of acute myeloid leukemia (AML) has been transformed over the past 15 years from a disease defined, classed, and staged based on histological characteristics alone to a disease classified largely based on genetic, genomic and molecular characteristics.[1,2,5,6]

WT1 was initially discovered as a tumor suppressor in Wilms' tumor (WT), a pediatric kidney malignancy. The WT1 gene is located at chromosome11p13 and encodes for 10 exons that generates a 3 kb mRNA WT1 is an important regulatory molecule involved in cell growth and development. It is expressed in the urogenital system, the central nervous system and in tissues involved in hematopoiesis, including the bone marrow and lymph nodes.[1, 2]

We undertake this study to detect to frequency of WT1 gene mutation in Sudanese patients with AML and to assess the relationships of WT1 gene mutation to the clinical outcome [1, 2].

MATERIALS & METHODS

This was cross sectional, hospital based study. All samples were obtained at the National Radiation and Isotopes Center in Khartoum. The diagnosis of AML was based on the morphological diagnosis a well as the flow cytometery.

After written informed consent, 51 known AML patients were participated in the study, 5.0 ml of blood samples were collected in EDTA evacuated tubes. Genomic DNA was extracted from frozen blood using salt-precipitation method. For exon 7 amplification of WT1 gene

(214 bp) (Figure 1), 100 ng of genomic DNA was mixed with 10 pmol of each primer in a total volume of 20µl containing 2.5mM of each dNTP and 1u of thermostable DNA polymerase. 35 cycles required for amplification (denaturatin in 94°C for 30 seconds, annealing in 55 °C for 1minute and extension in 72 °C for 45 seconds) in a PCR system (Figure 1).[1,4,7]

WT1 SNP Identification by RFLP

After amplification, 10µl of the PCR product was digested for 1hour with AflIII enzyme. Aliquots were electrophoresed on 2% agarose gel. After digestion with AflIII, PCR product was run on every gel stained with ethidium bromide and photographed (Figure 2). [7], the product was run in the gel and interoperated for Homozygous normal type enzyme was cut into two bands 165 bp and 49 bp,while for the Homozygous mutant type was uncut gave band 214 bp ,but for Heterozygous mutant type the enzyme was cut into three bands 214 bp, 165 bp and 49 bp.(Figure 2).

RESULTS

The RFLP analysis revealed that 27 (52.9%) were normal (A/A) of WT1 mutation, 22 (43.1%) were heterozygous (A/G) of WT1 gene mutation and only 2 (3.9%) of patients were homozygous (G/G) of WT1 gene mutation.

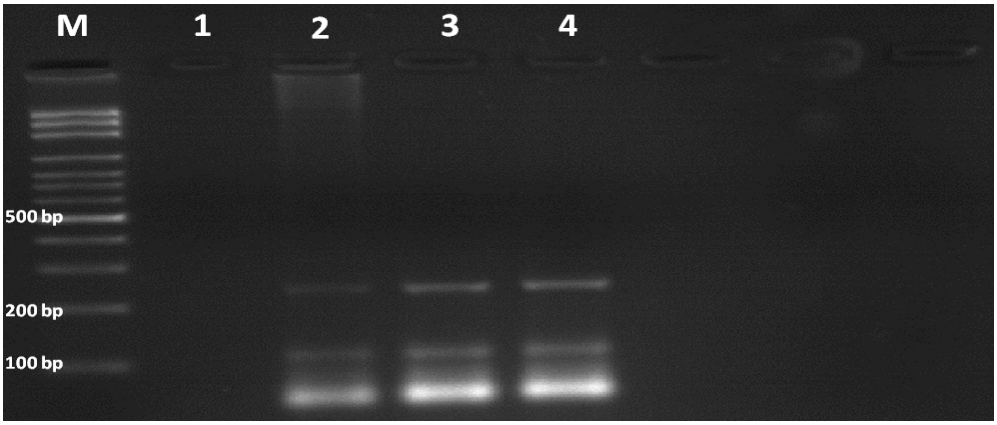

Figure (1): PCR amplification of Exon (7) of WT1 gene.

M: DNA marker Lane1: Negative PCR, Lane 2 – 4 shown positive PCR amplification of exon 7 of WT1 gene give band 214pb.

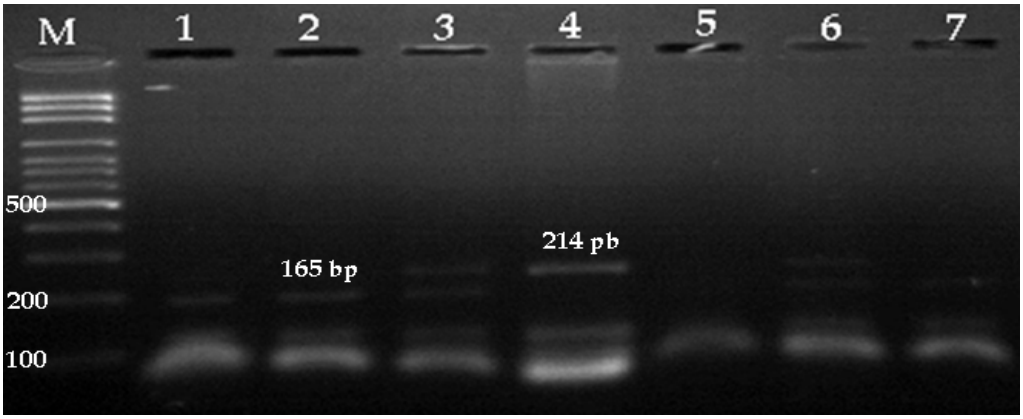

Figure (2): Shown RFLP for samples 1 – 7.

M: DNA marker Lane 1, 2 & 7 shown normal allele (cut band 165pb), lane 3& 6 shown heterozygote mutant allele (cut into 3 band 214,165 & 49 pb), lane 4 shown Homozygote mutant allele (un cut band 214 pb).

DISSCUSSION

In this study we have found the mutation of WT1gene in 24 of 51 (47.1%), AML cases, This mutation frequency is not equivalent to the previous studies of WT1 gene mutation in AML, Subsequent studies revealed that WT1 is mutant in approximately 10 - 15% of primary leukemia, mainly in AML. L. King-Underwood et al, found mutation of WT1 gene in 3 of 20 (15%) cases of AMl And also this mutation frequency is not equivalent to that found in sporadic William's Tumors, suggests that WT1 may be not equally important in both tumor types in Sudanese.[1,2,4]

This study had assessed for the first time the frequency of the mutant WT1gene (47.1%) in Sudanese Patients with AML, It was observed that the frequency of the mutant WT1 gene was significantly higher in Sudanese AML patients, comparing to the other population from previous studies that has been done.[1,4]

CONCLUSION

In the Sudanese population, the Wilms' tumour (WT1) gene A→G mutation appears to be associated with increased risk of developing Acute Myeloid Leukemia. The disease was

found to be associated with the heterozygous genotype. We can detect the known WT1 gene mutation with a technique that requires only small sample of DNA extracted from the blood. RFLP analysis enables both homozygotes and heterozygotes genotyping.

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