Research Article

FLT3 Gene Mutation in Childhood Acute Leukemia: A Preliminary Study

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Introduction. FLT3 is a tyrosine kinase receptor involved in the proliferation and differentiation of hematopoietic stem cells. There are two types of common FLT3 gene mutation, internal tandem duplication and the D835 mutation, which are known to be associated with a poor clinical outcome in acute leukemia patients. Methods. This study evaluates the incidence of FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) in 38 pediatric patients diagnosed with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) in Hospital Universiti Sains Malaysia. DNA extraction was done from archive bone marrow samples to determine FLT3-ITD mutations using polymerase chain reaction. Results. In this pediatric series, the age ranges were 2–14 years. However, no FLT3-ITD mutations were detected in any of the samples. Conclusion. This preliminary study suggested that the incidence of FLT3 gene mutation most probably was very low in pediatrics patients diagnosed with acute leukemia. A further study with larger number of patient samples is necessary to confirm the findings and to further appreciate the prognostic value of FLT3-ITD mutation among pediatrics patients.

1. Introduction

Fms-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase expressed by immature hematopoietic cells. FLT3 ligand is expressed by marrow stromal cells and other cells and synergizes with other growth factors to stimulate proliferation and differentiation of stem cells, progenitor cells, dendritic cells, and natural killer cells. In normal hematopoietic cells, FLT3-ITDs (internal tandem duplications) mutations have not been detected in the cord blood and bone marrow [1–5]. In general, there are 2 types of FLT3 mutations which are internal tandem duplications (FLT3/ITD mutations) in or near the juxtamembrane domain of the receptor and point mutations resulting in single amino acid substitutions occurring within the activation loop of the tyrosine kinase domain (FLT3/TKD mutations). Both mutations are known to be associated with a poor clinical outcome in acute leukemia patients. The incidence of FLT3/ITD mutations varies according to age and clinical risk group, being less common in pediatric acute myeloid leukemia (AML) and in AML arising from an antecedent myelodysplastic syndrome.

FLT3 mutations are genetic changes that have been reported to have prognostic significance in acute myeloid leukemia (AML) [6]. Study by Thiede et al. in 2002 analyzed the prevalence of FLT3-ITD mutations in 979 AML patients, with 20.4% found to be positive. However, the tandem duplication in the FLT3 gene is not a frequent phenomenon in childhood AML patients and so is said to be a poor prognostic
factor [7]. Work by Bang and colleagues had only managed to detect a low frequency of FLT3 ITD and TKD mutation, with 12.8% and 2.7%, respectively, among the 226 Korean pediatrics AML patients [6].

Many methods using genomic DNA can be used to detect the FLT3-ITD mutation [7–9]. The gDNA can be isolated either from bone marrow or peripheral blood of patients by using a simple commercially available DNA purification kit [7, 9]. The FLT3 mutated regions were then amplified by polymerase chain reaction (PCR) using this genomic DNA template. The conventional approach for screening FLT3 involves using PCR followed by gel electrophoresis analysis. However, some very small ITDs go undetected using this technique [10, 11].

2. Materials and Methods

2.1. Patient Samples. This study was carried out on 38 pediatric patients diagnosed with acute leukemia and was followed up from years 2007 to 2011 at Hospital Universiti Sains Malaysia (Hospital USM). Clinical and laboratory data were retrieved from the patient’s records in Unit Record Perubatan, Hospital USM. Archived bone marrow smear at diagnosis of these patients was taken from Hematology Laboratory of Pusat Pengajian Sains Perubatan (PPSP). This study was approved by the Research Ethics Committee (Human), Universiti Sains Malaysia.

2.2. Detection of FLT3-ITD Mutations. DNA was extracted from the archived bone marrow slides using the standard method as in the manufacturer’s protocol GENE ALL. Blood. The concentration and purity were determined by measuring the absorbance at 260–280 nm. FLT3-ITD mutations were detected using conventional polymerase chain reaction (PCR). The PCR master mixtures were prepared using 2 μL (50–100 ng) of DNA samples followed by 18 μL of master mixtures (l.5 mmol/L MgCl2), 0.25 mM dNTPs, and 1.0 μM of ITD oligonucleotide primer (ITD1: 5’GCATTTGGTATGAAAGCCAGC-3’ and ITD2: 5’CTTTCTAGGCATTGGTTAAGACGCACCGC-3’) as in [11, 12] and together with 2.5 units of Taq polymerase. PCR amplification was performed using Mastercycler Eppendorf Gradient S machine. The PCR conditions started with denaturation (94°C, 5 min), followed by 35 cycles of denaturation (92°C, 30 s), annealing (56°C, 30 s), elongation (72°C, 30 s), and final elongation at 72°C for 5 min. For analyses of FLT3-ITD mutations, 20 μL of the PCR product was separated on 1.8% agarose gel. The 329 bp fragment indicates the size of the wild-type FLT3 gene in the absence of ITD, whereas additional upper bands can only be observed in cases with the ITD mutation (Figure 1) [11, 12]. Positive controls were taken from patients already found to have FLT3-ITD mutation from Nurul et al.

2.3. Statistical Analysis. Data analysis was performed using Microsoft Excel. Qualitative data were expressed as frequency and percentage, whereas quantitative data was expressed as mean and range.

3. Results

In this study, 38 patients from both AML and ALL cases were screened for ITD mutations in exons 14 and 15 in FLT3 gene. The patients age is between 2 and 14 years, with mean age of 8 years for AML and 5 years for ALL. Out of these, 23 (61%) patients were male. Twelve (32%) bone marrow samples were obtained from AML, while the remaining was from ALL group. However, no FLT3-ITD mutation was detected in any of these 38 acute leukemia patients.

4. Discussion

The main aim of this study was to establish the prevalence of FLT3-ITD mutations among childhood leukemia patients in this population. However, due to the small number of patient samples, none of our pediatric patients has the FLT3-ITD mutations. Nevertheless, this result may also indicate that the prevalence of FLT3 mutation in childhood acute leukemia is probably very low. Even so, we believe that the data presented in this study could still contribute to a growing knowledge of frequency and clinical significance of FLT3 mutation in childhood AML and ALL. Previous study which involved the largest number of Korean patients with AML showed a relatively low frequency of FLT3 ITD which was consistent with prior reports. Local data on the FLT3 gene mutation among AML Malay adult patients by Nurul et al. have concluded that this type of mutation was found to be uncommon in this group. Furthermore, the presence of ITD was also shown to be associated with an inferior survival outcome [10]. This study also points out that molecular markers should be studied in a number of different groups of patients in order to make definite conclusions of their medical relevance [10].

Furthermore, in the future more sensitive methods such as high resolution melting analysis, sequencing and gene scan analysis can also be applied instead of just using the conventional PCR [7–9].
5. Conclusion

Very low incidence of FLT3 molecular alteration in acute childhood leukemia observed in this study prevents a definite conclusion. Larger prospective studies are necessary to accurately clarify the incidence of this mutation in this population and subsequently confirm the prognostic significance of FLT3 mutations in childhood acute leukemia. Despite this, our study has demonstrated that the FLT3-ITD detection could easily be incorporated into the routine assessment of AML patients as the assay only requires simple PCR amplification technique.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References


