International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Identification of Chromosomal Translocations in Childhood Leukaemia Using Multiplex RT-PCR: A Single Institution Study in Multi-Ethnic Malaysia

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Received: 13/09/2016 *Revised: 03/10/2016* *Accepted: 13/10/2016*

ABSTRACT

Background: Leukaemia accounts for 48% of all childhood cancers in Malaysia. Recent advances in genomics have contributed significantly towards a better understanding of the genetic landscape of leukaemia. Identification of recurrent chromosomal translocations which can be detected in a substantial number of these patients are important for classification of the disease, prognostication, treatment monitoring and also to guide targeted therapy.
Objective: This study aims to report the incidence of leukaemia-specific translocations in Malaysian children who were admitted in tertiary care hospital from 2008 to 2011.
Materials and Methods: A total of 229 bone marrow or peripheral blood samples were collected from children who were newly diagnosed with leukaemia. Their demographic data, bone marrow morphology and also immunophenotyping results were recorded. Multiplex reverse-transcriptase polymerase chain reaction (RT-PCR) was performed using the *Hema Vision 28N* protocols for detection of 28 common translocations.
Results: Among the 229 children, 162 patients were diagnosed with Acute Lymphoblastic Leukaemia (ALL), 52 Acute Myeloid Leukaemia (AML) and 15 Chronic Myeloid Leukaemia (CML). We found that 26.2% of these patients have chromosomal translocations. Overall, 10 fusion gene transcripts and 15 different splice variants were detected. The most common genetic abnormalities found were *BCR-ABL1 8.3%, ETV6-RUNXI* 7.0% and *TCF3-PBX1 2.6%.* Conclusion: Multiplex RT-PCR is an effective and rapid screening tool for detection of recurrent chromosomal translocations in childhood leukaemia. A comprehensive sub grouping of leukaemia by molecular technique is very useful not only for diagnostic purpose, but also for risk assessment, prognostication and personalised treatment.

Key words: Chromosomal translocations, Multiplex PCR, Childhood leukaemia.

INTRODUCTION

Leukaemia is most common malignant disease affecting children and it is also the seventh most common cancer in the general population of multi-ethnic Malaysia which consists of Malay (54.5%), Chinese (24.9%), Indian (7.5%) and others (13.1%). [1] Children under the age of 15 years represented 32.4% of the total Malaysia population and leukaemia accounted for 48% (153/319) and 44.5% (98/220) of childhood cancer cases in male and female,

respectively. [1] The incidence rate of leukaemia is estimated to be 35 per million of children under the age of 15 years in Malaysia. [2] Over the past decade, rapid advances in genomic technologies have contributed significantly towards a better understanding of the molecular basis as well as the genetic landscape of leukaemia. The detection of genetic abnormalities in leukaemia patients not only provides a basis for classification but also served as a guide for targeted therapies and prognosis assessment. [3] The conventional G-band karyotyping is a morphology-based, cytogenetic analysis that is routinely performed to identify numerical and structural chromosomal aberrations in leukaemic cell but submicroscopic genetic alterations which do not affect the chromosome structure remained undetectable. [4] The increasing number of abnormalities which are detectable by molecular techniques has led to the development of multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) which allowed simultaneous screening of multiple fusion transcripts in a single reaction and facilitates the rapid detection of targeted gene abnormalities in a large number of patients. [5] HemaVision is a multiplex RT-PCR-based assay which detects leukaemia associated fusion gene transcripts and its utility as a screening tool in leukaemia patients has been reported in several countries including Austria, [5] Switzerland [3] and Korea. [6]

MATERIALS AND METHODS

In this study, we present the first report on the incidence of leukaemia-specific translocation amongst multi-ethnic Malaysian children who were admitted in a tertiary care hospital as revealed by a multiplex RT-PCR assay (HemaVision 28N, DNA Diagnostic, Denmark) which identifies 28 different chromosomal translocations and more than 145 breakpoints (Table 1).

Bone marrow aspirate or peripheral blood sample was obtained from 229 children aged 15 years and below who were newly diagnosed with leukaemia by morphological, cytochemical and immunophenotypic assessments in the Paediatric Institute, Kuala Lumpur Hospital from 2008-2011. Total RNA was extracted from bone marrow aspirates or peripheral blood samples using QIAamp RNA Mini Blood Kit (Qiagen, Germany). The multiplex RT-PCR assay was performed according to the manufacturer's instructions using purified RNA samples as the template and a positive RT-PCR reaction was further confirmed with a nested PCR assay using translocation-specific primer sets. Results generated with the RT-PCR assay were then interpreted according to the table provided by the manufacturer which specify the translocation and splice variant that were present.

Table 1: List of 28 chromosomal translocations screened using multiplex RT-PCR

Fusion transcript	Chromosomal translocation
SIL-TAL1	del1(p32)
MLL-EPS15	t(1;11) (p32;q23)
MLL-MLLT11	t(1;11) (q21;q23)
TCF3-PBX1	t(1;19) (q23;p13)
NPM1-MLF1	t(3;5) (q25;q34)
RUNX1-MECOM	t(3;21) (q26;q22)
MLL-AFF1	t(4;11) (q21;q23)
ETV6-PDGFRB	t(5;12) (q33;p13)
NPM1-RARA	t(5;17) (q35;q21)
DEK-NUP214	t(6;9) (p23;q34)
MLL-MLLT4	t(6;11) (q27;q23)
RUNX1-RUNX1T1	t(8;21) (q22;q22)
SET-NUP214	t(9;9) (q34;q34)
MLL-MLLT3	t(9;11) (p22;q23)
ETV6-ABL1	t(9;12) (q34;p13)
BCR-ABL1	t(9,22) (q34;q11)
MLL-MLLT10	t(10;11) (p12;q23)
MLL-MLLT6	t(11;17) (q23;q21)
ZBTB16-RARA	t(11;17) (q23;q21)
MLL-ELL	t(11;19) (q23;p13.1)
MLL-MLLT1	t(11;19) (q23;p13.3)
ETV6-RUNX1	t(12;21) (p13;q22)
ETV6-MN1	t(12;22) (p13;q11)
PML-RARA	t(15;17) (q24;q21)
CBFB-MYH11	inv(16) (p13;q22)
FUS-ERG	t(16;21) (p11;q22)
TCF3-HLF	t(17;19) (q22;p13)
MLL-FOXO4	t(X;11) (q13;q23)

RESULTS

During the study period, bone marrow aspirates or peripheral blood samples from 229 children consisting of 172 Malay, 39 Chinese, 13 Indian, 3 Singh and 2 other indigenous races were received by our Haematology Unit, Institute for Medical

Research, Kuala Lumpur. The patients comprised of 128 males and 101 females between the age of 3 months to 15.8 years and the median age was 5.4 years. The number of childhood leukaemia cases that were screened with RT-PCR assay was 162 acute lymphoblastic leukaemia (ALL), 52 acute myeloid leukaemia (AML) and 15 chronic myeloid leukaemia (CML) cases, whereby 26.2% of the patients (60/229) were found to have chromosomal translocation. The positivity rate obtained in this study with RT-PCR assay was similar to other cohort studies involving mostly adult patients which were between 20 - 30% [3,7] whereas a much higher positivity rate was observed among Korean children with leukaemia whereby 46.3% of the patients had genetic abnormalities. [6] Overall, 10 fusion gene transcripts and 15 different splice variants were detected amongst RT-PCR positive cases and the 3 most common genetic abnormalities detected through RT-PCR were *BCR-ABL1* (19/60), *ETV6-RUNX1* (16/60) and *TCF3-PBX1* (6/60) (Figure 1).

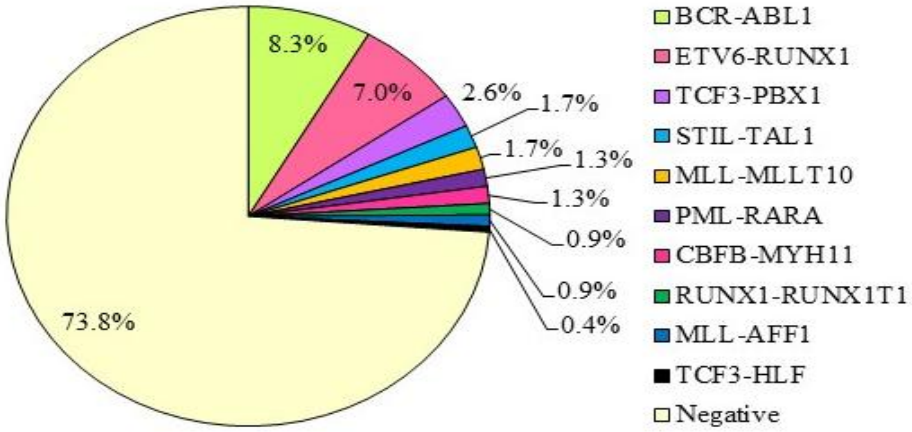

Figure 1: Pie chart showing chromosomal translocations detected by multiplex RT-PCR

With regards to each leukaemia type, RT-PCR-positive result was obtained for 20.4% of ALL patients (33/162), 23.1% of AML patients (12/52) and 100% of CML patients (15/15) (Table 2).

Table 2: Incidence of fusion transcripts detected by multiplex RT-PCR

Fusion transcripts	Chromosomal translocation	Breakpoints	*n* (%)
Acute lymphoblastic leukemia (*n* = 162)			
ETV6-RUNX1	t(12;21) (p13;q22)	ETV6 ex5 - RUNX1 ex3	16 (9.9)
TCF3-PBX1	t(1;19) (q23;p13)	TCF3 ex 16 - PBX ex3	6 (3.7)
STIL-TAL1	del1(p32)	STIL ex1- TAL ex1 d1+d2	4 (2.5)
BCR-ABL1	t(9,22) (q34;q11)	BCR ex1 - ABL1 ex2 (e1a2)	2 (1.2)
		BCR ex14 - ABL1 ex2 (b3a2)	2 (1.2)
MLL-AFF1	t(4;11) (q21;q23)	MLL ex10 - AFF1 ex6(1414)	2 (1.2)
MLL-MLLT10	t(10;11) (p12;q23)	MLL ex7 - MLLT10 ex17	1 (0.6)
Acute myeloid leukemia (*n* = 52)			
CBFB-MYH11	inv(16) (p13;q22)	CBFB ex5 - MYH11 ex34 (A)	3 (5.8)
MLL-MLLT10	t(10;11) (p12;q23)	MLL ex8 - MLLT10 ex4	1 (1.9)
		MLL ex9 - MLLT10 ex9	1 (1.9)
		MLL ex9 -MLLT10 ex10	1 (1.9)
PML-RARA	t(15;17) (q24;q21)	PML ex3 - RARA ex3 (S, bcr3)	3 (5.8)
RUNX1-RUNX1T1	t(8;21) (q22;q22)	RUNX1 ex6 - RUNX1T1 ex2	2 (3.8)
TCF3-HLF	t(17;19) (q22;p13)	TCF3ex15 - HLF ex4	1 (1.9)
Chronic myeloid leukemia (*n* = 15)			
BCR-ABL1	t(9,22) (q34;q11)	BCR ex13 - ABL1 ex2 (b2a2)	6 (40)
		BCR ex14 - ABL1 ex2 (b3a2)	9 (60)

ALL was the most common type of childhood leukaemia as the malignant disease accounted for 70.7% (162/229) of all the cases that were under investigation. The ratio of males to females was 1.7 and the median age was 5.4 years (3 months-

15.8 years). *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%, 16/162) which comprised of 13 Malay, 2 Chinese and 1 Indian. The other genetic abnormalities that were detected in the ALL subgroup were as follows: *TCF3-PBX1* (*n*=6), *STIL-TAL1* (*n*=4), *BCR-ABL1* (*n*=4), *MLL-AFF1* (*n*=2) and *MLL-MLLT10* (*n*=1). There were 2 patients with major *BCR-ABL1* subtype namely b3a2 fusion transcript and another 2 patients had minor *BCR-ABL1* subtype namely e1a2 fusion transcript. The incidence rate of *TCF3-PBX1* (3.7%), *BCR-ABL1* (2.5%) and *STIL-TAL1* (2.5%) was slightly lower than another study undertaken by Yeoh *et al.* [8]

AML, which mainly affect adults, was the second most frequently diagnosed cancer among Malaysian children. The incidence of AML was observed to be higher among females (61.5%) compared to males (38.5%) and the median age was 5.1 years (8 months-15 years). Positivity rate of the RT-PCR for paediatric AML patients was previously reported to be 54.1% [6] and 39% [5] but a much lower positivity rate of 21.2% was obtained in the present study. The fusion genes detected in the AML subset were *CBFB-MYH11* (*n*=3), *MLL-MLLT10* (*n*=3), *PML-RARA* (*n*=3) and *RUNX1-RUNX1T1* (previously known as *AML1-ETO*) (*n*=2). Of note, the co-existence of two fusion genes was observed in one AML patient namely *TCF3-HLF* and *RUNX1/RUNX1T1*. The occurrence of *CBFB-MYH11* (5.7%) and *MLL-MLLT10* (5.7%) were in concordance with the frequency reported by Kumar although the frequency of *PML-RARA* (5.7%) and *RUNX1-RUNX1T1* (3.8%) in the present study was lower. [9] The low incidence rate of *RUNX1-RUNX1T1* was also in contrast to past studies which found that 19.7% (12/61) of the cases exhibited t (8; 21). [10]

The positivity rate of CML (100%) was the highest among the three types of leukaemia investigated and all 15 RT-PCR-positive cases of CML possessed the Philadelphia chromosome as indicated by the presence of *BCR-ABL1* fusion gene. The two *BCR-ABL1* major subtypes detected were b3a2 (*n*=9) and b2a2 (*n*=6) fusion transcripts. Females (66.7%) had a higher incidence rate of CML compared to males (33.3%) and the median age was 6 years (3.5-15.7 years). The results obtained with RT-PCR assay were comparable to a study conducted by Hassan *et al.* whereby 35 out of 36 adult Malay patients diagnosed as CML were positive for *BCR-ABL* fusion gene and 68.6% had a breakpoint at b3a2 junction and 31.4% at b2a2 junction. [11]

DISCUSSION

The findings reported in this study served as a preliminary analysis on the incidence of genetic abnormalities that were detected among paediatric leukaemia patients in Malaysia over a 4-year period with a multiplex RT-PCR. The molecular-based assay was shown to be an effective and rapid screening tool for detecting important genetic abnormalities especially cryptic translocations when compared to cytogenetic analysis which is known to be time-consuming and highly-dependent on the expertise of the laboratory. [3] The RT-PCR assay may also serve as a cost-effective alternative to FISH as it capable of screening 28 translocations in leukaemia. [6] Full genetic assessment in accordance to WHO criteria may be performed using RT-PCR assay with the only exceptions being numerical and structural abnormalities. [6] Nevertheless, there are also other drawbacks associated with the RT-PCR assay such as the limited number of MLL gene rearrangements that were detectable with the assay kit and novel translocations that have yet to be characterized would remained undetected. [5] Diagnostic significance of the RT-PCR assay would need to be further established with larger sample size of Malaysian children in order to elucidate possible population-based differences as well as to obtain an accurate representation of the translocation incidences among multi-

ethnic paediatric leukaemia patients. In conclusion, the multiplex RT-PCR complements existing cytogenetic studies and contributes towards a better care management for each patient by improving diagnostic accuracy, genetic risk assignment and personalised treatment.

ACKNOWLEDGEMENT

The authors would like to thank the Director General of Health, Ministry of Health Malaysia for approval to publish this scientific paper. We would also like to thank the Deputy Director General of Health (Research and Technical Support), and the Director of the Institute for Medical Research for their kind support. This work was supported by IMR operating budget.

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How to cite this article: 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.

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