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FISH Analysis of 9p21 Deletion in Egyptian Childhood Acute Lymphoblastic Leukemia Patients: Relation to Prognosis and Disease Outcome

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Abstract
Background: *Identification of specific abnormal genes involved in the process of leukemogenesis often suggests possible prognostic markers that may be applied into risk stratification and treatment protocol in leukemia. Inactivation of the 9p21 region has been reported in acute lymphoblastic leukemia (ALL) but its prognostic importance in childhood ALL has been debated for a long time. The aim of this work was to detect deletion of 9p21 in pediatric ALL patients to evaluate its impact on patients response to therapy and to correlate it to standard prognostic factors.*
Patients and Methods: *Fluorescence in situ hybridization (FISH) technique was used to detect deletion of 9p21 in 45 newly diagnosed pediatric ALL patients, with follow- up for 12 months to assess their response to chemotherapy and for detection of relapse.*
Results: *9p21 deletion was detected in twelve (26.7%) out of the forty five pediatric ALL patients, and it was significantly associated with poor prognostic criteria; age<1or >10 years, splenomegaly, hepatomegaly, high risk score and bad patient outcome.*
Conclusion: *The incidence of 9p21 deletion is higher in high risk group of pediatric ALL and conveys an inferior outcome.*
Keywords: *del (9p21), childhood ALL, prognosis, outcome.*

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease resulting from the accumulation of genetic alterations of B or T lymphoid precursor cells [1]. It is considered the most common cancer among persons less than 15 years and accounting for more than 30% of all childhood malignancies [2]. A number of clinical and laboratory features evident at diagnosis, have prognostic value for predicting the outcome of patients treated for ALL. The identification of these prognostic factors has provided a mean of stratifying patients into different risk groups and "tailoring" treatment accordingly [3].

In developed countries, 8 of 10 children diagnosed with acute lymphoblastic leukemia will survive 5 years or longer due to treatment assignments based on presenting age, white blood cell count, extramedullary disease, blast cytogenetics, and initial treatment response. These risk stratification categories for ALL are still evolving, however, treatment failures occur in 10-15% of lower risk patients [4].

Pediatric ALL is one of the success stories of cancer research and treatment. Not only this fatal disease is now curable in the majority of patients, who have access to appropriate therapy and support [5], but much of its cellular and molecular biology has been uncovered [6]. Molecular characterization of the genetic changes has yielded a wealth of information on the mechanism of leukemogenesis. These findings have also allowed the development of sensitive techniques such as fluorescence in situ hybridization (FISH) for identification of underlying molecular defects, which can be applied to evaluate disease prognosis, monitor response to treatment and predict minimal residual disease [7].

Genetic alterations of tumour suppressor genes (TSG) such as p53, retinoblastoma (RB) gene, p15 and p16 contribute to leukemic transformation of hemopoietic stem cells or their committed progenitors by changing cellular functions [8,9]. The 9p21 region contains three genes that were found to be involved in the development of different tumours: cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase inhibitor 2B (CDKN2B) and S-methyl-5'-thioadenosine phosphorylase (MTAP). CDKN2A is a tumour suppressor gene that encodes two proteins, and acts through the Rb and mouse double minute 2 (MDM2) pathways. CDKN2B (p15) is a tumour suppressor gene, the action of which through the Rb pathway is complementary to CDKN2A (p16) [10,11]. MTAP is located approximately 100 kb telomeric to CDKN2A and encodes methylthioadenosine phosphorylase, an enzyme involved in purine and methionine metabolism. Loss of MTAP has been suggested to make cancer cells more sensitive to drugs that interfere with folate metabolism. Deletions of 9p21 vary in size and may cover large genomic regions, spanning between 0.1 and >30 Mb. Therefore, the MTAP gene, which is located approximately 100 kb telomeric to CDKN2A, is often co-deleted in ALL [12].

Both $p16^{INK4a}$ and $p15^{INK4b}$ tumor suppressor genes counteract the activity of cyclins, cyclin dependent kinase (cyclin D – CDK 4/6 complexes) and other regulatory proteins of the cell cycle such as p53 and pRb. Dysregulation of cell cycle control is crucial in the development and progression of ALL. Thus, deletions of p16 or p15 located at 9p21 may play a role in leukemogenesis, have a prognostic significance and provide new targets for therapeutic interventions [13].

Investigation of the 9p21 deletion frequency and its prognostic significance in ALL has been hampered by the absence of simple and readily available diagnostic methods. Most of the earlier analyses were based on Southern blotting, which is reliable but laborious and requires large amounts of material, which is not always available from ALL patients [14]. Therefore, some of these studies may have been biased by the preferential selection of samples from patients with high tumour burden and high white blood cell (WBC) count, usually referred to a high risk group leading to under-representation of standard risk samples with low leucocyte counts. Today, the use of high-

resolution genomic arrays, as well as quantitative polymerase chain reaction (PCR) analysis gives new opportunities to identify smaller deletions, but these methods require saved DNA. Interphase fluorescent in situ hybridization (FISH) provides easy and quick detection of translocations and deletions on bone marrow (BM) smears from unselected ALL patients. Other advantages of this method are the availability of smears for FISH analysis from diagnostic bone marrow (BM) samples and small demands on their storage. Interphase FISH is robust and easy to perform, and thus an attractive diagnostic tool [12].

The present work aims to detect deletion of 9p21 in pediatric ALL patients, by FISH technique; in order to evaluate the impact of such deletion on patients response to therapy and to correlate it to standard prognostic factors.

Subjects and Methods

The present study included 45 newly diagnosed pediatric ALL patients (31 males and 14 females) attending the Hematology/Oncology Clinic in the Pediatric Hospital, Ain Shams University. An informed consent was obtained from the legal guardian of each patient before enrollment. This study was approved by the ethical committee of Ain Shams University.

All patients were subjected to full clinical history, thorough clinical examination, work up for ALL included the following: complete blood count (CBC) using Coulter LH 750 (Beckman Coulter, Inc., Fullerton, CA, USA) with examination of Leishman- stained peripheral blood (PB) and BM smears for blast count and morphology, as well as flow cytometric immunophenotyping using Coulter Epics XL 3-color flow cytometer (Coulter Electronics, Hialeah, FL, USA). Detection of 9p21 deletion by FISH technique using LSI p16 (9p21)/CEP9 dual color probe for ALL patients at diagnosis. Follow- up was done for 6-12 months following the diagnosis and the induction therapy to assess response to chemotherapy by clinical evaluation, CBC and BM examination, lactate dehydrogenase (LDH) was performed on Synchron CX-9 (Beckman Coulter, California, USA).

The diagnosis of acute leukemia was based on the morphologic criteria and confirmed by cytochemical stains, immunophenotyping, and cytogenetic analysis.

Sampling

Blood and BM aspiration samples were collected under complete aseptic conditions on ethylene diamine tetra-acetic acid, potassium salt (K_2-EDTA) (1.2 mg/mL) for CBC and immunophenotyping. For cytogenetic examination 2mL of BM aspirate was collected in tubes coated with lithium heparin.

Detection of 9p21 deletion

Cytogenetic analysis by FISH technique using fluorophore labeled locus specific identifier (LSI) probe: LSI p16 (9p21)/CEP9 Dual Color Probe (Vysis, Abbot, molecular diagnostics, USA). The LSI p16 Spectrum Orange probe spans approximately 190 kb and contains a number of genetic loci including D9S1749, D9S1747, p16(INK4A), p14(ARF), D9S1748, p15(INK4B), and D9S1752. The CEP 9 Spectrum Green probe hybridizes to alpha satellite sequences specific to chromosome 9. Slides were prepared from material fixed in methanol-acetic acid. All probes were set up separately on different slides for each patient. Hybridization and detection of hybridization signals were performed according to the manufacturer's protocols. For each probe, at least 100 interphase cells were evaluated using the Chromoscan CytoVision 7.3.1 (Leica Biosystem, Richmond Inc., USA) in order to detect the target abnormalities. Images of FISH were captured through the program Mac Probe 4.4 of Power Gene System (Applied Imaging Corporation, USA). In a normal sample, the expected pattern for a nucleus hybridized with the LSI p16/CEP 9 probe is the two orange, two green signal patterns. If a deletion at the 190 kb region covered by the LSI p16 probe occurs on one chromosome 9 homolog and

both centromeres from chromosome9 are retained, the one orange, two green signal pattern is expected.

Definitions

Complete remission (CR) was defined by clinical and morphological criteria; the presence of 5% or less blasts in a normocellular or hypercellular BM, with granulocytes more than $1.0x10^9$/L and platelets more than $100x10^9$/L [15]. The definition of CNS infiltration was the concomitant presence of leukemic blast cells and at least five leukocytes per microliter in a cytocentrifuged specimen of the cerebrospinal fluid [15]. Relapse after complete remission was defined as the reappearance of leukemic blasts in PB or more than 5% blasts in BM not attributable to any other cause (e.g. BM regeneration after consolidation therapy) [16].

The studied ALL patients were classified into two risk groups (high and standard risk) based on the National Cancer Institute (NCI) criteria [17] that had international acceptance and reproducibility: age, initial white blood cell (WBC) count, and the presence of extramedullary disease at diagnosis. Standard risk group included 31(68.9%) patients [<10 years old and white blood cells (WBCs) <50 $x10^9$/L], while high risk group included 14 (31.1%) patients [< 1 or ≥10 years old, WBC ≥50 x 10^9/L or presence of extramedullary disease].

Statistical Analysis

Analysis of data was done using Statistical Program for Social Science version 20 (SPSS Inc., Chicago, IL, USA). Qualitative data were described in the form of number and percentage. Quantitative variables were described in the form of mean and standard deviation (SD) or median and inter-quartile range (IQR). In order to compare quantitative parametric variables between two groups, Student *t*-test was applied. Comparison between nonparametric variables was carried out using Mann-Whitney U test. Comparison between groups regarding qualitative data was performed by using Chi square (X^2) test. p value <0.05 was considered significant.

Results

Clinical and laboratory data of all studied patients are listed in Table 1

Results of interphase FISH for 9p21 deletion

Metaphase and interphase FISH analysis using fluorophore labeled locus specific identifier (LSI) probe for detection of 9p21 deletion was successfully performed on 45 BM samples. It revealed positive results in 12/45 (26.7%) patients and negative in 33/45 (73.3%). The characteristics of ALL patients positive and negative for 9p21 deletion are summarized in Table 1.

Comparison between 9p21 deletion positive patients and 9p21 deletion negative patients revealed high incidence of 9p21 deletion among patients with poor prognostic criteria including; age <1 year and >10 years (p=0.028), high tumour burden manifested by hepatomegaly and splenom-egaly (p=0.016, p=0.04; respectively). Despite of higher incidence of CNS infiltration (16.7%) in patients with positive 9p21 deletion, no significant difference was found (p=0.475). Moreover, there was no significant difference regarding sex or lymphadenopathy (p=0.593, p=0.187; respectively). Regarding laboratory data, the 9p21 deletion positive group showed a significantly higher WBC (p=0.028) with 41.7% of patients showing WBC ≥50 $X10^9$/L, and percentage of peripheral blood blasts (p<0.001). No significant difference was detected as regards hemoglobin concentration (p=0.901), platelets count (p=0.280), percentage of BM blasts at diagnosis (p=0.150), and after treatment at day 14 and 28 (p=0.269) or LDH level (p=0.787). Immunophenoptypically, no significant difference was observed as regard distribution of precursor B-ALL and T-ALL patients (p=0.736).

Eight patients with positive 9p21 deletion were associated with high risk and four patients had standard risk with a significant statistical difference from patients without 9p21 deletion. (p= 0.002).

Outcome of studied ALL patients in relation to 9p21del

As shown in Table 1, thirty seven (82.2%) patients achieved complete remission with a

favorable outcome till last follow up report, four (8.9%) patients showed incomplete remission, 4 (8.9%) patients revealed an inferior outcome (relapse). On the other hand, no cases revealed testicular relapse.

Comparison between the two groups of patients with and without 9p21 deletion revealed lower incidence of complete remission in patients with 9p21 deletion (58.3% versus 90%), whereas, the incidence of incomplete remission (16.7% versus 6%) and relapse (25% versus 3%) were significantly higher in patients with positive 9p21 deletion compared with negative 9p21deletion (p= 0.03).

Table 1. Descriptive data and comparative analysis of all studied ALL patients

Parameter	All studied patients (n=45)	del (9p21) positive (n=12)	del (9p21) negative (n=33)	positive vs negative del (9p21)
Male, n (%)	31 (68.9)	9 (75)	22 (66.7)	0.593
Age (years), mean ± SD < 1 or ≥10 1-9	 14 (31.1) 9 (20)	 5 (41.7) 7 (58.3)	 4 (12.1) 29 (87.9)	 0.028
Lymphadenopathy, n (%)	26 (57.8)	5 (41.7)	21 (63.6)	0.187
Splenomegaly, n (%)	19 (42.2)	8 (66.7)	11 (33.3)	0.045
Hepatomegaly, n (%)	17 (37.8)	8 (66.7)	9 (27.3)	0.016
CNS infiltration, n (%)	5 (11.1)	2 (16.7)	3 (9.1)	0.475
WBC ($x10^9$/L), mean ± SD <50 $X10^9$/L ≥50 $X10^9$/L	 36 (80) 9 (20)	 7 (58.3) 5 (41.7)	 29 (87.9) 4 (12.1)	 0.028
Hb (gm/dL), mean ± SD <7 gm/dL ≥7 gm/dL	 7 (15.6) 38 (84.4)	 2 (16.7) 10 (83.3)	 5 (15.2) 28 (84.8)	 0.901
Platelets ($x10^9$/L), mean ± SD <30 ≥30	 3 (6.7) 42 (93.3)	 0 (0) 12 (100)	 3 (9.1) 30 (90.9)	 0.28
PB blasts ≥60% <60%	 20 (44.4) 25 (55.6)	 10 (83.3) 2 (16.7)	 10 (30.3) 23 (69.7)	 0.002
BM blasts (≥5%) Day 14, n (%) Day 28, n (%)	 4 (8.9) 4 (8.9)	 2 (16.7) 2 (16.7)	 2 (6.1) 2 (6.1)	 0.269
Immunophenotyping, n (%) Precursor-B ALL T- ALL	 36 (80) 9 (20)	 10 (83.3) 2 (16.7)	 26 (78.8) 7 (21.2)	 0.763
LDH (IU/L) <800 ≥800	 24 (53.3) 21 (46.7)	 6 (50) 6 (50)	 18 (54.5) 15 (45.5)	 0.787
Risk group Standarad High	 31(68.9) 14 (31.1)	 4 (33.3) 8 (66.7)	 27 (81.8) 6 (18.2)	 0.02
Outcome CR IR Relapse	 37 (82.2) 4 (8.9) 4 (8.9)	 7(58.3) 2 (16.7) 3 (25)	 30 (90) 2 (6) 1(3)	 0.03
9p21 deletion by FISH	12 (26.7)	12 (100)	0 (0)	

del: deletion, CNS: cerebral nervous system, WBC: white blood cells count, Hb: hemoglobin, PB: peripheral blood, BM: bone marrow, IPT: Immunophenotyping, LDH: lactate dehydrgenase, CR: Complete remission, IR: incomplete remission, FISH: fluorescence in situ hybridization.

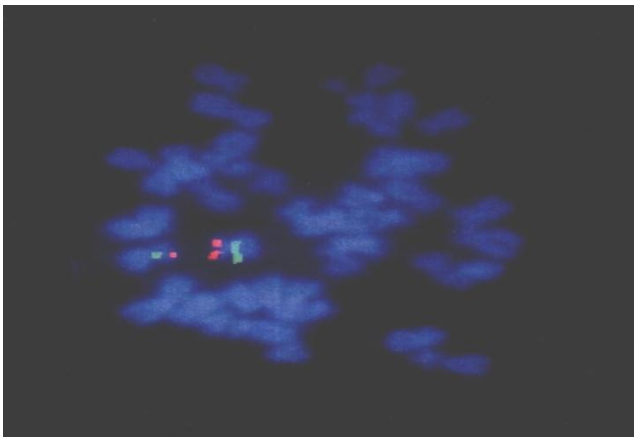

Figure 1. Metaphase FISH analysis negative for 9p21 deletion denoted by the presence of 2 green signals of the centromeres of both copies of chromosomes 9 and 2 red signals of region 2 band 1 of both copies of chromosome 9.

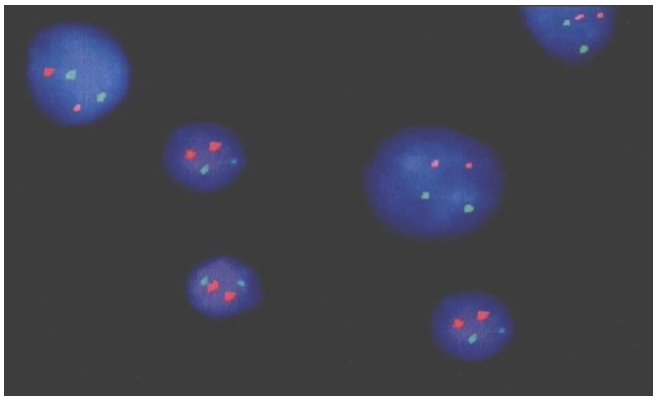

Figure 2. Interphase FISH analysis negative for 9p21 deletion denoted by the presence of 2 green signals of the centromeres of both copies of chromosomes 9 and 2 red signals of region 2 band 1 of both copies of chromosome 9.

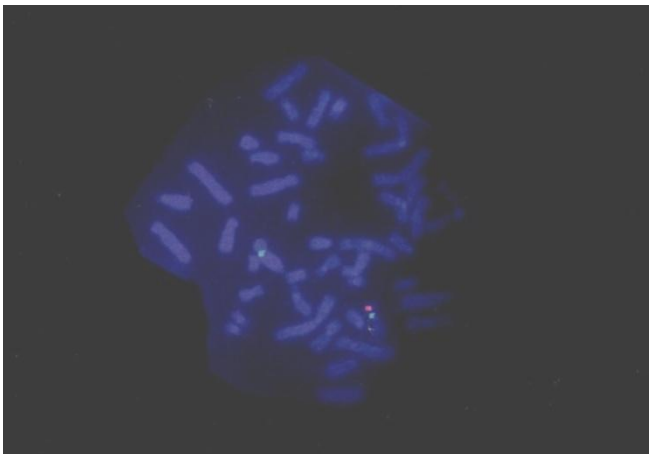

Figure 3. Metaphase FISH analysis positive for heterozygous deletion of region 2 band 1 of chromosome 9 denoted by the presence of two green signals of the centromeres of both copies of chromosomes 9 and one red signals of region 2 band 1 of chromosome 9.

Discussion

It is well established that the identification of cytogenetic abnormalities is very useful for the prediction of outcome in childhood ALL [18]. Although some of these abnormalities are accepted as favorable or poor prognostic factors, the prognostic effects of others have not been well determined [19]. The prognostic importance of 9p21deletion in childhood ALL has been debated for a long time, however, the association between the deletion of 9p21 and poor prognosis was suggested [20].

Out of the studied 45 newly diagnosed childhood ALL patients, twelve (26.7%) were 9p21 deletion positive by FISH technique. This comes in line with four large studies by Woo et al. [18], Mullighan et al. [21], Perez-Vera et al. [22] and Sulong et al. [10], they utilized FISH technique with a commonly used commercial probe to study 9p21 deletion in patients with ALL and the deletion frequency ranged from 20–27%. On the other hand this frequency is higher than that recorded by Kuchinskaya et al. [12] who identified this deletion in (15.7%) of patients, but is lower than a study done by Schiffman et al. [4] who reported this deletion in 29/45 (64.4%) patients and Karkucak et al. [19] who detected 9p21 deletion in 8/22 (36%)of patients. The discrepancy in frequency rate may be attributed to the difference in the studied populations. In addition, the increasing use of high-resolution genomic arrays, as well as quantitative PCR analysis, indicate that the real prevalence of 9p21 loss is higher and reaches 40–50% in children with ALL and points out that deletions of this locus may be both small and large [12].

As regards age of 9p21 deletion positive patients; it ranged from 2 years to15years, with higher incidence (41.7%) among high risk patients (<1 or >10 years) compared with 9p21 deletion negative ALL patients (12.1%). Similarly Karkucak et al, [19] reported age range of 3-14years with 50% <10 years and 50% > 10 years. On the other hand, Woo et al. [18] reported that 28.6% of 9p21 deletion positive patients have age <1+>10 years and 71.4% of them have age (1-10) years.

Kuchinskaya et al. [12] reported that 9p21 deletion was detected in all age groups with a steady rise in the frequency with age.

The prognostic importance of 9p21 deletion in childhood ALL has been debated for long time [12]; Kim et al. [26] reported that the prognostic value of 9p21 deletion is unknown. Schiffman et al. [4] mentioned that the clinical significance of 9p21 deletion in childhood leukemia remains both conflicting and controversial.

In the present study, we recorded higher incidence of CNS infiltration (16.7 %) in 9p21 deletion positive patients compared with ALL patients without 9p21 deletion (9.1%). Kuchinskaya et al. [12] recorded CNS infiltration only in 2.5% (13/514) of studied ALL patients with 9p21 deletion. Moreover, the presence of 9p21 deletion was found to be related to tumor burden as it was significantly associated with hepatomegaly and splenomegaly.

Considering laboratory data, 5/12 (41.7%) of 9p21 deletion positive patients presented with WBCs ≥50 $x10^9$/L, Woo et al. [18] and Kuchinskaya et al. [12] reported initial WBCs count >50 $x10^9$/L in (50%) and (23.6%) in their studied patients respectively.

As regards prognostic risk stratification, the present study showed that 66.7% in high risk category had 9p21 deletion. This come in line with Woo et al. [18] and Karkucak et al. [19] who reported that 64.3% and 66% of their patients respectively in high risk group had 9p21 deletion. Karkucak et al. [19] concluded that 9p21 deletion was the most common abnormality among high- risk patients.

Follow up of the studied patients to detect their response to treatment revealed a significant lower incidence of complete remission and higher incidence of relapse in ALL patients with 9p21 deletion in comparison to patients without this cytogenetic abnormality.

In 2008, Yang et al. [26] reported significantly shorter remission times in children with relapsed ALL and 9p21 deletion at diagnosis. Woo et al. [18] reported that 21.4% of 9p21 deletion positive patients developed relapse. Karkucak et al. [19] reported that the prognostic significance of 9p21 deletion is limited to homozygous deletion, which can be explained by loss of heterozygosity. Unlike homozygous deletion, hemizygous deletion might not be sufficient to turn off the function of involved genes (CDKN2A and CDKN2B). On the other hand, Kim et al [27] suggested that homozygous deletion is a poor prognostic factor in adult but not in childhood ALL.

Kuchinskaya et al. [12] reported that there was no significant difference in outcome between cases with or without 9p21 deletion or between cases with hemi or homozygous deletion of 9p21. However, they recommended that interphase FISH deletion analysis of 9p21 could be used as a first step to detect unfavorable subtle cytogenetic aberrations such as dicentric (9;20) rearrangement. The 9p21 deletion leads to removal of proteins essential to controlling tumors suppression. In addition the rearrangement of tumor suppressor genes might contribute to leukemogenesis in cooperation with other genetic changes possibly by amplifying the malignant potential [4,18].

In conclusion, the results of the present study revealed higher incidence of 9p21deletions among high risk ALL patients aged less than 1 year or more than 10 years old, patients with hyperleuco-cytosis (WBCs ≥ $50x10^9$/L), and with high tumour burden (CNS and/or extramedullary infiltration), as well as lower ability to achieve complete remission suggesting the association of this cytogenetic anomaly with poor prognostic risk factors. Moreover, follow-up of ALL patients with 9p21 deletion showed higher incidence of relapse and poor disease outcome. Further longitudinal studies including larger number of patients with extended follow- up may provide additional information.

Conflict of Interest Statement: Nothing to declare.

Reference

1. Meshinchi S, Arceci RJ. Prognostic factors and risk-based therapy in pediatric acute

myeloid leukemia. Oncologist. 2007;12: 341–355.
2. Ries LAG, Smith MA, Gurney JG, Linet M, Tamra T, Young JL, Bunin GR, editors. Cancer incidence and survival among children and adolescents: United States SEER Program 1975-1995. Bethesda, MD: National Institute of Health, 1999.
3. Carroll WL, Bhojwani D, Min DJ, Moskowitz N, Raetz E. Childhood acute lymphoblastic leukemia in the age of genomics. pediatr Blood Cancer. 2006;46: 570-578.
4. Schiffman JD, Wang Y, McPherson LA, Welch K, Zhang N, Davis R, Lacayo NJ, Dahl GV, Faham M, Ford JM, Ji HP. Molecular inversion probes reveal patterns of 9p21 deletion and copy number aberrations in childhood leukemia. Cancer Genetics and Cytogenetics. 2009;193:9-18.
5. Greaves M. A Natural History for Pediatric Acute Leukemia. Blood. 1993;82 (4):1043-1051
6. Pui CH, Relling MV, Pharm D, Downing JR. Acute lymphoblastic leukemia. N Eng1 J Med. 2004;350:1535-48.
7. Lee L, Livak K, Mullah B. Seven colour, homogenous detection of six PCR products. Biotechniques. 1999;27:342.
8. Krug U, Ganser A, Koeffler HP. Tumor suppressor genes in normal and malignant hematopoiesis. Oncogene. 2002;21:3475-3495.
9. Bertin R, Acquaviva C, Mirebeau D, Guidal-Giroux C, Vilmer E, Cave H. CDKN2A, CDKN2B, and MTAP gene dosage permits precise characterization of mono- and biallelic 9p21 deletions in childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2003;37:44–57.
10. Sulong S, Moorman AV, Irving JAE, Strefford JC, Konn ZJ, Case MC, Minto L, Barber KE, Parker H, Wright SL, Stewart ARM, Bailey S, Bown NP, Hall AG, Harrison CJ. A comprehensive analysis of the CDKN2A gene in childhood acute lymphoblastic leukemia reveals genomic deletion, copy number neutral loss of heterozygosity, and association with specific cytogenetic subgroups BLOOD. 2009;113 (1):100-107.
11. Chim CS, Wong AS, Kwong YL. Epigenetic inactivation of INK4/CDK/RB cell cycle pathway in acute leukemias. Ann of Hematol. 2003;82(12):738–742.
12. Kuchinskaya E, Heyman M, Nordgren A, Soderha S, Forestier E, Wehner P, Vettenranta K, Jonsson O, Wesenberg F, Sahle´n S, Nordenskjo M, Blennow E. Interphase fluorescent in situ hybridization deletion analysis of the 9p21 region and prognosis in childhood acute lymphoblastic leukaemia (ALL): results from a prospective analysis of 519 Nordic patients treated according to the NOPHO-ALL 2000 protocol. British Journal of Haematology. 2011;152:615–622.
13. Lin YC, Diccianni MB, Kim Y, Lin H, Lee CH, Lin RJ, et al. Human p16c, a novel transcriptional variant of $p16^{INK4a}$, coexpresses with $p16^{INK4a}$ in cancer cells and inhibits cell-cycle progression. Oncogene. 2007;26:7017–7027.
14. Calero Moreno TM, Gustafsson G, Garwicz S, Grander D, Jonmundsson GK, Frost BM, Makipernaa A, Rasool O, Savolainen ER, Schmiegelow K, Soderhall S, Vettenranta K, Wesenberg F, Einhorn S, Heyman M. Deletion of the Ink4-locus (the p16ink4a, p14ARF and p15ink4b genes) predicts relapse in children with ALL treated according to the Nordic protocols NOPHO-86 and NOPHO-92. Leukemia. 2002;16:2037–2045.
15. Dohner H, Estey EH, AmadoriS, Appelbaum FR, Buhner T, Burnett A, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz

MA, Sierra J, Tallman MS, Lo Wenberg B, Bloomfield CD. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European Leukemia Net. Blood. 2010;115:453-474.

16. Einsiedel HG, Von Stackelberg A, Hartmann R, Fengler R, Schrappe M, Janka-Schaub G, et al. Long-term outcome in children with relapsed ALL by risk-stratified salvage therapy: results of trial acute lymphoblastic leukemiarelapse study of the Berlin-Frankfurt-Mu¨ nster Group 87. J Clin Oncol 2005;23:7942–7950.
17. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol. 1996;14:18–24.
18. Woo HY, Kim, DW, Park, H, Seong, KW, Koo HH, Kim SH. Molecular cytogenetic analysis of gene rearrangements in childhood acute lymphoblastic leukemia. Journal of Korean Medical Science. 2005;20:36–41.
19. Karkucak M, Gorukmez O, Yakut T, Baytan B, Gorukmez O, Gunes A. Molecular Cytogenetic Findings in Cases with Childhood Acute Lymphoblastic Leukemia. International Journal of Hematology and Oncology. 2012;22 (2):67-70.
20. Mirebeau D, Acquaviva C, Suciu S, Bertin R, Dastugue N, Robert A, et al. The prognostic significance of CDKN2A, CDKN2B and MTAP inactivation in B-lineage acute lymphoblastic leukemia of childhood. Results of the EORTC studies 58881 and 58951. Haematologica. 2006;91:881–885.
21. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007;446:758–764.
22. Perez-Vera P, Salas C, Montero-Ruiz O, Frias S, Dehesa G, Jarquin B, et al. Analysis of gene rearrangements using a fluorescence in situ hybridization method in Mexican patients with acute lymphoblastic leukemia: experience at a single institution. Cancer Genetics and Cytogenetics. 2008;184:94–98.
23. Ramakers-van Woerden NL, Pieters R, Slater RM, Loonen AH, Beverloo HB, van Drunen E, et al. In vitro drug resistance and prognostic impact of $p16^{INK4a}$/ $P15^{INK4b}$ deletions in childhood T-cell acute lymphoblastic leukaemia. British Journal of Haematology. 2001;112:680–690.
24. Kees UR, Burton PR, Lu C and Baker DL. Homozygous deletion of the p16/MTS1 gene in pediatric acute lymphoblastic leukemia is associated with unfavorable clinical outcome. Blood. 1997;89:4161–4166.
25. Carter TL, Watt PM, Kumar R, Burton PR, Reaman GH, Sather HN, et al. Hemizygous p16(INK4A) deletion in pediatric acute lymphoblastic leukemia predicts independent risk of relapse. Blood. 2001;97:572–574.
26. Yang JJ, Bhojwani D, Yang W, Cai X, Stocco G, Crews K, et al. Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. Blood. 2008;112:4178–4183.
27. Kim HJ, Woo HY, Koo HH, Tak EY, Kim SH. ABL oncogene amplification with p16(INK4a) gene deletion in precursor T-cell acute lymphoblastic leukemia/ lymphoma: report of the first case. Am J Hematol. 2004;76(4):360–336.