IJBPAS, November, 2015, 4(11): 6312-6326

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DE NOVO CHROMOSOMAL ABNORMALITIES IN ACUTE LEUKEMIA PATIENTS IN NORTHEAST OF IRAN

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ABSTRACT

Backgroun: In this study we investigated cytogenetic analysis and molecular study in patients with acute leukemia to determine the frequency of denovo structural and numerical chromosomal changes in these patients.

Method : During two year study, 60 caseswith provisional diagnosis of acute leukemia were submitted for cytogenetic and molecular diagnostic tests of common recurrent translocations of t(15;17),t(8;21), inv16, t(6;9) t(12;21),t(4;11),t(1;19),t(9;22)(p190)and p210 using real time quantitative PCR (RT-qPCR).

Result: Conventional cytogenetic analysis were also performed in 56(93%) of 60 cases.A total of 56 cases with adequate samples were 19 (34%) cases with a normal karyotype and 37 (66%) had

cytogenetic abnormalities. It was found that14 out of 56 cases with clonal cytogenetic change abnormalities had not any recurrent translocations with molecular assay.

Conclusion: Finally, the frequency of De Novo cytogenetic abnormalities showed high incidence in acute myeloid and lymphoblastic leukemia,despite cytogenetic remains a valuable prognostic stratification of acute leukemia patients, it is has to be integrated by molecular and immunophenotypic investigations.

Keywords: Cytogenetic analysis, Acute leukemia, RT-qPCR

INTRODUCTION

Cytogenetic analysis is an essential diagnostic tool in analysis of acute leukemia to improve our understanding of the disease subtypes. Chromosomal and genetic abnormalities (structural or numerical) are detected in malignant bone marrow cells in more than 75% of patients suffering from hematological malignancies. Nevertheless, chromosomal aberrations have re-designated the classification of acute leukemia and are important to predict the prognosis. It has been shown that chromosomal aberrations are important to design of risk adapted therapeutic pathway for new cases. Over the last few decades, it has been mentioned that molecular tests are a useful merging of cytogenetic analysis that can detect of molecular alteration in sample with normal or failed karyotype (1, 2, 3). The acute leukemias diagnosed by the methods such as morphologic, cytochemical, immunophenotypic, cytogenetic and molecular testing.

Acute leukemia port a variety of genetic aberrations, which can be determined by conventional cytogenetic analysis. These are three classes of abnormalities: 1- balanced chromosomal translocations,2-numeric abnormalities, such as deletions and additions; and 3- submicroscopic genetic abnormalities (4). Cytogenetic results are necessary in the recognition and prognosis of hematologic malignancies. Acute lymphoblastic leukemia is a heterogenous disease that includes B-lymphoblastic leukemia (B-ALL) and T-lymphoblastic leukemia (T-ALL).ALL with many subtypes are manifested by recurrent copy number alteration and structural chromosomal rearrangement. We therefore have classified ALL as either B-ALL or T-ALL (5).Chromosomal abnormalities, were diagnosed in more than 75% malignant bone marrow cells, with an increasing incidence with use of complementary detection methods like molecular cytogenetic (1). One

of the most important aspects of this study was to perform the molecular and cytogenetic analysis to identify genes involved in the pathogenesis of leukemia. It is well understood that, there are some cytogenetic subtypes for AML (Acute Myeloid leukemia) and ALL (Acute Lymphoblastic Leukemia) with different prognosis (1,4). Therefore, in this study we aimed to analyze60 bone marrows in leukemia patients through molecular and cytogenetic methodologies in our molecular pathology laboratory over a 2-year period.

MATERIALS AND METHODS

Patients:

This study was performed on bone marrow samples from 60 continuous patients referred from Sheikh, Omid and Ghaem hospitals between 2013 and 2015 years. Patients were then classified into two groups based on Acute Leukemia (Acute Myeloid leukemia and Acute Lymphoblastic Leukemia).Bone marrow aspirate (>1mL) was accumulated in 5mL sterile sampling tube containing heparin for cytogenetic study and EDTA for Molecular test.

Chromosomal Analysis

Bone marrow aspiration sample were cultured for 24-48hours in RPMI 1640 that supplemented by 20% fetal calf serum, L-glutamin and antibiotics. After 24 hours' incubation at 37 °C, Colcemid was added for 30 minutes. The cells were exposed to a hypotonic solution (0.075 mol/L KCl) for 30 minutes at 37 °C, and then fixed three times in methanol: acetic acid (3:1, v/v) for 30 minutes each. Slides were prepared and then air dried. Conventional cytogenetic analysis was performed using a trypsin-gimsa banding technique to analyze cells in metaphase according to the International system for Human cytogenetic Nomenclature 2012(ISCN 2012)(6,7,8).

Molecular methods:

To identify the most frequent fusion gene in ALL and AML we used reverse transcriptase Real time quantitative PCR reaction RT-qPCR method.

RNA was extracted from bone marrow or peripheral blood by using TRIzol reagent (Invitrogen, Cergy, France), after extraction and isolation the RNA concentration was determined by measurement the density at 260 nm followed by the storage ofRNA at -80.cDNA was then synthesizedfor the RT-qPCR was optimized and according to the BIOMED-1 protocol started(9) All the RT-qPCR reactions were performed on Step one Applied Biosystem (Applied Biosystems, Foster City, USA). As a starting point the protocol describing previously.(Standardization and quality)(10).

For AML, the following translocations were analyzed:t(8;21) with AML1-ETO, t(9;22) with BCR-ABL p190 and BCR-ABL p210, t(15;17) with PML-RARA, inv (16) with CBFB-MYH11and t(6;9) with DEK-CAN.In addition, for diagnosis of ALL translocations were performed such as t(1;19) with E2A-PBX1, t(4;11) with MLL-AF4, t(12;21) with TEL-AML1 and t(9;22) with BCR-ABL p190.

RESULTS

A total of 60 patients, four patients had in adequet samples for complete cytogenetic analysis and therefore analyzed for only molecular investigations. Out of 60 patients, 39 were male and 21 were female with a male to female ratio of1.8%.The difference age range was between 8 months to 70 years. The mean haemoglobin concentration was 11.2 g/dl (range 3.4-25), mean white cell count was 68.4×10^9/L (range 3.3-200), mean platelet count was 84×10^9/L (range 6-207) and Red blood cell was 2.7×10^9/L (range 1.3-4.65).A total of 56 cases with adequet samples were 19 (34%) cases with a normal karyotype and 37 (66%) had cytogenetic abnormalities. In the present study we have collected 60 samples of acute leukemia with the final diagnosis of AML and ALL. In 35 cases of AML and 25 cases of ALL, molecular and cytogenetic investigation was performed and illustrated in table 1 and 2.In the present study abnormal karyotype was seen in 68.5% of patients with denovo AML and 80% in ALL also normal karyotype had seen in 38% of AML patients and 32% in ALL.

Molecular study was performed on all of 60 patients and the results of molecular and cytogenetic studies based on the 2008 WHO classification system are shown in Table 1 and 2.Moreover, complex karyotype classified according to the presence of the chromosomal abnormalities shown in Table 3.

The frequency of recurrent abnormalities are t(8;21) (8.5%),t(15;17)(5.7%) and inv16(11.5%) detected with molecular and cytogenetic assay. The diagnosis of AML and ALL were based on the morphologic and cytogenetic studies of peripheral blood smears and bone marrow aspiration.2008 WHO criteria were use for classification of the acute leukemia. The prevalence of each subtypes have shown in **Figure 1**.

In this study 92% of ALL cases had B-cell phenotype, while only 8% of ALL patients were diagnosed of T-ALL phenotype. The incidence of t(1;19), t(12;21) and t(4;11) were 8%16% 4%respectively.Theaberrationsin our study were translocation, inversion, deletion and

numerical changes and the incidence of each in this study are shown in **Figure 2**.

Table 3 illustrated different levels of chromosomal complexities at 5 different levels according to the Grimwade et al study (29).In the current study 19% of cases were diagnosed with 5 chromosomal abnormalities while only 7% of patient had 3 or more abnormalities.

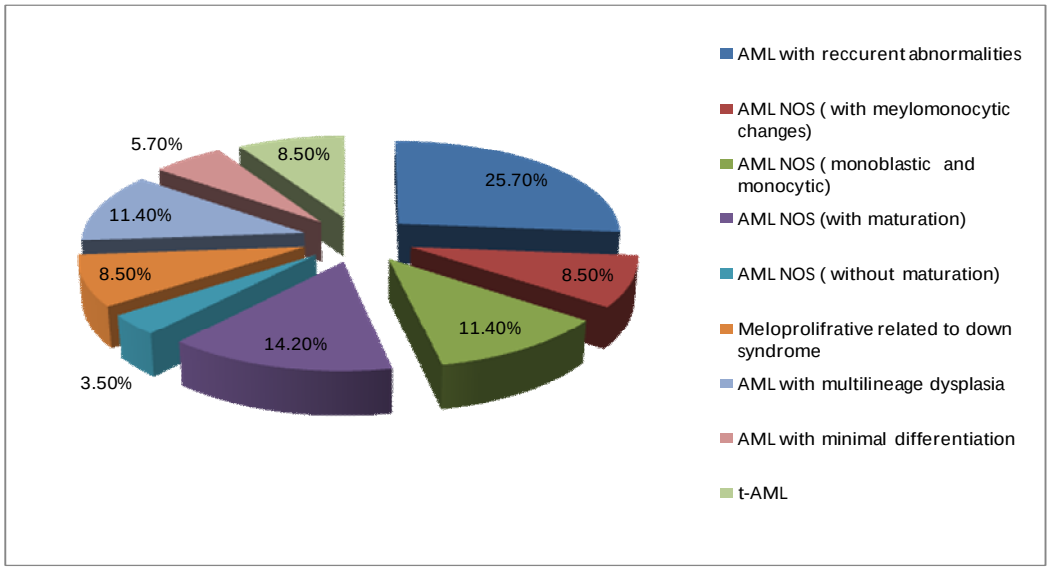

Figure 1: This figure shows the incidence of AML subtypes according to WHO classification.

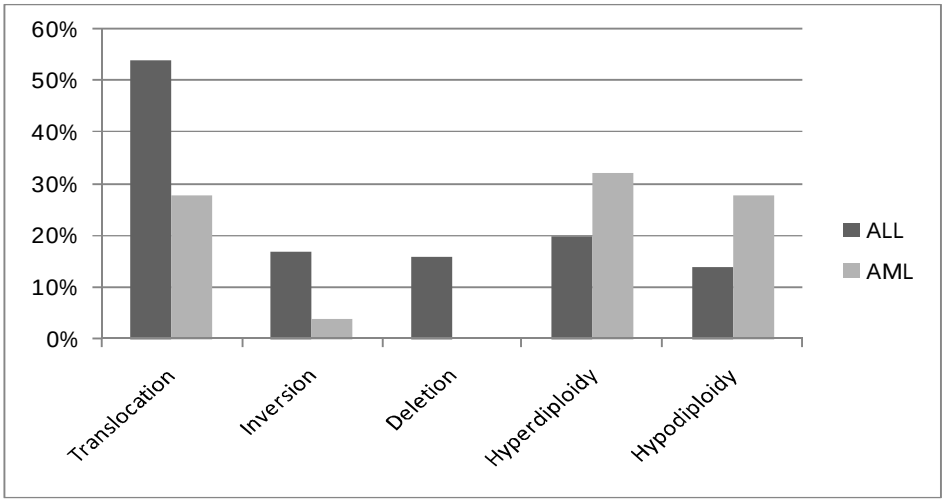

Figure 2: The prevalence of chromosomal abnormalities.

Table 1: Acute Myeloid Leukemia according to 2008 WHO classification system
n=35

No	WHO Classification	Molecular analysis	Karyotype of bone marrow cells
1	AML-with recurrent abnormalities	Inv16 positive	46,XY, del(11)(p14),inv(16)(p12q22)
2	AML-with recurrent abnormalities	t(15;17) pos	46,xy,t(15;17)(q24;q21)
3	AML-with recurrent abnormalities	t(8;21) pos	46,XX,t(1;5)(p36.1;p13),del(3)(p12p21),t(8;21)(q22;q22)
4	AML-with recurrent abnormalities	Inv16 pos	32~45,XY[cp25]/46,XY[10], Inv16 (p12q22)
5	AML-with recurrent abnormalities	Inv16 pos	Mos 52,XY,+8,+10,inv16(p12q22),+20,+21,+22/(17)/hyperdiploidy,XY(3)
6	AML-with recurrent abnormalities	Inv16 pos	46,XY, inv16(p12q22
7	AML-with recurrent abnormalities	t(8;21) pos	46,XY, t(8;21)(q22;q22)
8	AML-with recurrent abnormalities	t(15;17) pos	46,xy,t(15;17)(q24;q21)
9	AML-with recurrent abnormalities	t(8;21) pos	46,XY, t(8;21)(q22;q22)
10	NOS with meyomonocytic change	negative	Mos,44 XX,-3,-13[2]/41,XX,-12,-13,-13,-21,-22[1]/42,XX,-1,-4,-12,-17[1]/43,XY,-16,-17,-19[1]/44,XX,-19,-22[1]/45,XX,-10[1]/45XX,-11[1]/45,XX, -21[1]/46,XX[15]
11	AML with multilineage dysplasia	negative	46,XY
12	NOS -acute leukemia without maturation	negative	46,XY
13	AML with multilineage dysplasia	negative	46,XY
14	NOS with meyomonocytic change	negative	46,XY,t(3;6)(q26.3;q27)
15	AML with myelodysplasia-related change	negative	46,XX
16	NOS with monoblastic change	negative	46,XY,inv(1)(p13q21)
17	NOS-Acute leukemia with maturation	negative	46,XY
18	NOS with monoblastic change	negative	46,XX,del(12)(p11)
19	NOS with monoblastic change	negative	46~72,XXY,+1,+2,+3,+4,+5,+6,+7,+11,+12,+12,+13,+13,+14,+16,+19,+20,+21,+22,+22,+22,inc]cp20]
20	Meloprolifrative related to down syndrome	negative	46~49,XX,der(3)t(3;6)(q26;q15),der(5)t(4;5)(q23;p15),+8,dup(18)(q21q23),+21,+21,+22[cp20]
21	Meloprolifrative related to down syndrome	Negative	46~49,XX,der(3)t(3;6)(q26,q15),der(4),der(5)t(4;5)(q23;p15),+8,dup(18)(q21q23),+21,+21,+22[cp20]
22	NOS acute leukemia without maturation	t(9;22)210 pos b3a2	46,XY,t(9;22)(q34;q11)[17]/46,XY[9]
23	NOS-Acute leukemia with maturation	negative	45,XX,dic(4;17)(p15;p11)[18]/55,XX,+6,+8,+8,+10,+13,+13,+18,+19,+22[4]/ 46,XX[17]
24	Meloprolifrativerelated to down syndrome	negative	48,XX,der(17)t(1;17)(q35;q25.3),+8,+21

25	NOS-Acute leukemia with maturation		46,XY
26	NOS with minimal differentiation	negative	42~46,XY,der(1)t(1;3)(p36;p21)-4,-5,-6,-9,-11,-14,-17,-19,-20,-21,-X[cp17]/42~46,XY,-4,-6,-14,-17[cp10]
27	NOS-Acute leukemia with maturation	negative	42~46,XY, -4,-5,-6,-9,-11,-14,-17,-19,-20,-21,-X[cp17]/42~46,XY,-4,-6,-14,-17[cp10]
28	NOS with meylomonocytic change	negative	46,XY,inc
29	NOS with minimal differentiation	negative	46,XX
30	t-AML	negative	46,XX
31	NOS with MDS related change	negative	47,XY,+8
32	t-AML	negative	46,XX
33	t-AML	negative	46,XY
34	NOS with monoblastic change	negative	47,XY,der(8)t(8;17)(p11;q11),+8,der(17),t(8;17)(q21;p13)[14]/46,XY,del(2)(p23),der(8)t(8;8)(p23;q12),der(21)t(9;21)(q22;q22)[7]/46,XY,der(8)t(8;8)(p23;p12),t(12;18)(q10;q10)[6]
35	NOS -acute leukemia without maturation	negative	46,XY,in(1)(p36;q32)

Table 2: Acute Lymphoblastic Leukemia according to 2008 WHO classification system
n=25

No	WHO Classification	Molecular analysis	Karyotype of bone marrow cells
1	B-ALL	negative	46,XX
2	B-ALL	negative	43-45,XX[8]/46,XX[10]
3	B-ALL	negative	Mos 44-45,XY[4]/46,XY[5]
4	B-ALL	negative	Mos 54-57,XX[14]/46,XX[14]
5	T-ALL	negative	46,XY
6	B-ALL	negative	33~45,XX[11]/46,XX[6]/56~58,XX[3],inc
7	B-ALL	t(12;21) pos	44~45,XY,-15,-20[cp3]/46,XY[4],t(12;21)
8	B-ALL	negative	47,XX,+5 Trisomy5
9	B-ALL	t(1;19) pos	44~45,XX,t(1;19)(q23;p13)[,-2,-5,-6,-13,-18,[cp7]/46,XX[9]
10	B-ALL	negative	46,XX[inc]
11	B-ALL	t(1;19) pos	46,XX,t(1;19)(q23;p13)[8]/92,XXXX[2]/46,XX[19]
12	B-ALL	negative	Unsuccessful Culture
13	B-ALL	t(12;21) pos	46,XY,t(12;21)
14	B-ALL	negative	56-66,hyperdiploidy,
15	B-ALL	negative	48,XX,del(3)(q12),+8+21
16	B-ALL	negative	Unsuccessful Culture
17	B-ALL	negative	Unsuccessful Culture
18	B-ALL	t(12;21) pos	50-66 ,hyperdiploidy,t(12;21)
19	B-ALL	negative	47-60,hyperdiploidy
20	T-ALL	negative	38-44,hypodiploidy
21	B-ALL	negative	46,XX
22	B-ALL	t(4;11)pos	44-45, hypodiploidy
23	B-ALL	t(12;21) pos	55-68, hyperdiploidy
24	B-ALL	negative	46-55, hyperdiploidy
25	B-ALL	negative	Unsuccessful Culture

Table 3

Level of karyotype complexity	
1 abnormality	12%
2abnormality	8%
3 abnormality	7%
4abnormality	3%
5 or more	19%

DISCUSSION

Classification of the acute leukemia was traditionally based on the morphology developed by the French-American-British (FAB) system (11,12). AML and ALL are now classified using the 2008 WHO system which mainly based on the morphology, genetics, clinical features and immunophenotype (13,5). In this study we classified Acute leukemia patients to subtypes according to the WHO (2008) standards (5).

We have analyzed at least 20 cells in metaphase of bone marrow samples. Abnormal karyotype was seen in 68.5% of patients with denovo AML and 80% in ALL, most study reported an abnormal karyotype between 54-78% of cases (14,15). In another study the genetic abnormality was seen in 32.7% of patients with denovo AML (16). It was shown that, in the present study 34% of the patients have normal karyotype in acute leukemia which defined as the absence of clonal chromosomal abnormalities. Approximately, 40 to 50% of denovo AML and up to 10% of t-AML cases had normal karyotype according to the standard cytogenetic analysis (17). In the currentstudy31% of patients with denovoor secondary AML had a normal karyotype(18) that classified as intermediate risk based on the previously published studies (19,20,21,22,23,24,25).Patients with normal karyotype showing different response to treatment and due to this fact ,molecular investigations are crucially important in diagnosis of AML. In the present study, cases with normal karyotype did not show any molecular abnormality, however; there were few patients with known translocations, diagnosed by molecular testing. The incidence of recurrent genetic abnormalities are as follows : t(8;21)(q22;q22),inv16(p13.1;q22) and t(15;17)(q22;q12) in AML patients reported. It is shown that 5% of AML patients with t(8;21) and 10% in (M2) category of the FAB classification system,inv16 is found in 5-8% and t(15;17) is between5-8% of AML patients(13).

One of the most frequent numeric aberrations in AML is trisomy of chromosome 8(+8) that found in single aberration in 6% of newly diagnosed AML and 10% in case as combined alteration. Inour study 20% of AML patients have trisomy 8(+8). Other studies have suggested that the incidence of +8 will increase with age and present in 11% of AML patients aged 81-90 years(14,26,27), while in our study patients diagnosed with +8 are aged between 1-9 years.Tetrasomy of chromosome 8 is one of the infrequently

aberration that observed in hematologic disorders and only 20 cases have been described. This abnormality is more often in M5 and M4 FAB subtypes in AML and strongly associated to poor prognosis(28). In our study,wehad experiencedonly one case with AML-M2 FAB subtype that had tetrasomy of chromosome 8. Other trisomy has been reported in both denovoand secondary AML or MDS is trisomy of chromosome 11(+11) and it is the third common chromosomal abnormality in denovoAML.The international cooperative groups consider AML with trisomy 11 as an intermediate cytogenetic risk(19).In our patients only one case with AML-M5 had trisomy of chromosome 11 in combined with another aberration.Trisomy of chromosome 13(+13) is one of rare but recurring alteration founded in 2.5% of newly diagnosed AML,some evidence show correlation between trisomy 13 with RUNX1 mutation and FLT3 expression in AML(19,29) . In our cases about 7.4% of newly AML patient had trisomy 13 without RUNX1 mutation or FLT3 expression.

In addition, in the present study it was shown that complex karyotype which defined by the number of unrelated abnormalities are present from 1to 5 or greater (30), one aberration for each numerical change (including –X), simple structural change and balanced translocation count one. For each complex structural change; for example, a derivative chromosome showing both an unbalanced translocation and a deletion would be count one aberration. Count one aberration for tetraploidy(31). Approximately 80-85% of ALL patients have indeed B-cell phenotype and 15-25% of ALL in pediatric and adult had T-ALL phenotype(32).Chromosomal translocation including B-cell precursor leukemia are t(12;21)(p13;q22),t(9;22)(q11;q34),t(4;11)(q21;q23) and t(1;19)(q23;p13).The translocation t(12;21) is found in 20% to 25% of the B-ALL patients(33).In our study we have detected 16 % in B-ALL phenotype, another translocation in childhood B-cell ALL is t(1;19)and present in 3-5%of cases(34,35) and in the present study we have seen 8% of such abnormality. The translocation of t(4;11) were present in 10% of newly diagnosed B-cell ALL and in 10-20% of older infants(32).Only one of our ALL patients had t(4;11) and was 9 months infant.

Between all chromosomal aberrations identified in childhood ALL, hyperdiploidy (2n>50) has been most favorable prognosis compare to other cytogenetic changes. It was shown that, patients with lower chromosome

numbers are having poorer prognoses. One study has reported that 51.2% of cases with hypodiploidy and showed adverse outcome, while 32% had hyperdiploidy (36). In the current study, in ALL patients20% of cases were diagnosed of hypodiploidy (2n<46) and 25% with hyperdiploidy.

In acute leukemia, cytogenetic analysis at diagnosis is one of the integral parts of the diagnosis in terms of assessment of prognosis and in predicting of the disease outcome. Molecular analysis is very important for monitoring of residual disease and for evaluation of therapy responses. Considering the challenges and low sensitivity of cytogenetic tests, molecular characterization of acute leukemia patients are rapidly growing in recent years. In conclusion, despite cytogenetic remains a valuable prognostic stratification of acute leukemia patients, it is has to be integrated by molecular and immune phenotypic investigations.

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