Journal of the Egyptian Society of Haematology & Research, Vol. 3, No. 2, September: 101-111, 2007

Rearrangement of the Mll Gene in Acute Myeloid Leukemia and Myelodysplastic Syndromes

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ABSTRACT

Background: The MLL gene on 11q23 locus is involved in common translocations in human acute leukemias including acute myelogenous leukemia, T-cell ALL, B lineage ALL, myelodysplastic syndrome, lymphoblastic lymphoma and Burkitt's lymphoma.

Aim of Work: This study was intended to examine the frequency of MLL gene rearrangments (translocations & deletions) by conventional cytogenetics and FISH in adult and pediatric newly diagnosed cases with AML and MDS.

Subject and Methods: The study included 56 newly diagnosed AML (40) and MDS (16) patients with age range 3 days- 77 years, as well as 10 matched controls. All cases were subjected to complete blood picture, bone marrow aspirate for morphologic examination, cytochemical stains and immunophenotyping, karyotyping, and fluorescence in situ hybridization (FISH) using MLL gene split signal probe.

Result: MLL gene rearrangement was detected by FISH in 3/40 cases of AML, while conventional cytogenetics could only reveal one of them with 11q23 abnormality. There was a strong correlation between AML M4/M5 and MLL gene rearrangements. In AML-M4, 2/7 cases showed MLL gene rearrangements, while 1/7 cases of AML-M5 carried rearrangements of MLL gene. All MDS cases included in this study did not have MLL abnormalities by FISH. In AML patients, t (8;21) (q22;q22) was found in 20% either as a sole abnormality (10%) or with additional chromosomal abnormalities, + 8 and +19 in two cases each, t (15;17), +21, 7q-, double Philadelphia, +10, t (8;15), t (8;15;21) and i6p, in one case each. In MDS group of patients, chromosome -7/7q- abnormalities was detected in 3/10; complete loss (2 cases), or partial loss (one case), add5q, +19, -22, +2 and del9q in one case each.

Conclusion: FISH detected MLL gene rearrangement in 3 cases of AML while conventional cytogenetics could only reveal one case. FISH being more sensitive and more specific than conventional cytogenetics should be routinely used to screen for MLL gene rearrangements in AML patients.

Key Words: *AML – MDS – MLL – Gene rearrangement – Cytogenetics – FISH.*

INTRODUCTION

The MLL gene, also known as ALL-1, HRX, and Htrx on 11q23 locus is involved in recurrent translocations in human acute leukemias including acute myelogenous leukemia (AML), T-cell ALL, B lineage ALL, myelodysplastic syndrome (MDS), lymphoblastic lymphoma and Burkitt's lymphoma **[1-3]**. The MLL has been labeled a "promiscuous" oncogene since over 60 partner genes or regions have been identified. Included among these is an internal reduplication and self-fusion of MLL in some patients with trisomy 11 **[4]**.

MLL gene translocations are identified in about 5-6% of AML patients with t (6;11) (q27; q23), t (9;11) (p22;q23) and t (11;19) (q23;p13) being the most common. In AML cases showing MLL gene rearrangement, mostly FAB M4 and M5, the blast cells may express lymphoid markers in addition to myeloid markers. They are identified also in up to 10% of ALL patients with t (4;11) (q21;q23) and t (11;19) (q23;p13). ALL cells typically show a very immature phenotype (pro-B) with aberrant expression of mature myeloid or monocytic membrane markers **[5]**. Duplication or amplification of chromosome band 11q23 including the unrearranged MLL gene is a recurrent abnormality in therapy related MDS and AML **[6]**.

In addition 11q23 translocations occur in up to 80% of leukemia in children under one year of age **[7]**. Finally drugs that target topoisomerase II are very effective therapeutic agents, however they are associated with rearrange-

ments of 11q23 as a consequence of treatment **[8]**. Because MLL translocations are much less frequent in de novo leukemias of older patients but frequent in leukemias following chemotherapeutic DNA topoisomerase II poisons, it has been proposed that leukemia in infants may have an etiology resembling treatment related cases **[7]**.

MLL Gene: The MLL gene consists of 36 exons distributed over 100 kb and produces a 12 kb mRNA that encodes a 3968 aa protein with an estimated molecular weight of 430 kD **[1]**. The MLL protein is widely expressed in the developing embryo and is expressed at lower levels in most adult tissues. The predicted amino acid sequence of MLL indicates that it is homologous to the trithorax gene of D. melanogaster. MLL-functions in a large super complex of at least 29 proteins that is involved in the remodeling, acetylation, deacetylation and methylation of nucleosomes and histones.

Several lines of evidence indicate that one important function of MLL is in the maintenance of HOX gene expression during embryonic development. HOX genes are a large family of genes which are important in mammalian developmental cell fate determination **[4]**. Loss of MLL function in flies leads to homeotic transformation and deletion of MLL in mice leads to embryonic lethality and homeotic transformation **[1]**. Biochemical analysis of MLL suggests that it normally functions as a transcription regulator **[1-3]**.

The known fusion partners of MLL are diverse. This would appear to suggest that the fusion partners per se are not functionally important; rather it is MLL disruption that is the critical molecular event. Arguing against this hypothesis, however, is the fact that simple MLL truncation mutations are not frequently observed in AML and some of the common MLL fusion partners themselves share functional motifs of nuclear transcription factors, proteins involved in transcriptional regulation or other nuclear proteins **[7]**.

All of the breakpoints within MLL occur within an 8.3-Kb genomic region **[9]**. By mapping the translocation breakpoints in de novo leukemia, the breaks appeared to be located preferentially in the 5' half of the MLL breakpoint cluster region (BCR), whereas treatment-related leukemias and infant leukemias break more frequently in the 3' half of the BCR. This has led to the suggestion that a similar mechanism of breakage may be involved in MLL translocations in secondary leukemia and in infant leukemia **[8-10]**.

It has been speculated that MLL rearrangements are the initiating event for infant leukemias as they have been shown to occur in utero. **[11]**.

Patients carrying such a MLL translocation respond badly to therapy and have a poor clinical outcome **[4]**. Although, t (9;11) (p22;q23) involving AF9 provides the best outcome compared to other MLL translocations in AML, survival is relatively short **[12]**. Whereas patients with B-lineage ALL and MLL translocations tend to have a poor prognosis. In contrast, although MLL translocations are relatively rarely associated with T-ALL, these patients tend to have a very good prognosis **[13]**.

The Aim of this study was to report the frequency of rearrangments of the MLL gene in newly diagnosed cases of AML and MDS in relation to specific FAB subtypes using both conventional Cytogenetics and FISH.

MATERIALS AND METHODS

This study included newly diagnosed 56 AML and MDS patients (37 adults, 18 children and one infant), aged from 3 days to 77 years admitted to the National Cancer Institute, Cairo University from January 2004 to August 2006. Patients were subjected to complete blood picture, bone marrow aspirate for morphologic examination, cytochemical stains and immunophenotyping on routine basis; karyotyping, and fluorescence in situ hybridization (FISH) using MLL gene probe (LSI MLL Dual color rearrangement probe, Vysis Inc, Downers Grove IL) were also performed. Ten normal controls [7 adults (4 males and 3 females) and 3 children], age ranged from 9 years to 60 years (Median 29 years) were also included in this study. For controls, cytogenetic analysis was performed on peripheral blood lymphocytes which were cultivated for 72 hours in RPMI supplemented with 20% fetal calf serum and antibiotics. The cells were stimulated with 2% phytohemaglutinin (PHA). FISH study using MLL gene probe was performed on metaphase and/or interphase nuclei.

Cytogenetic methods:

Bone marrow or blood samples preserved in sodium heparin were cultured for each subject as previously described **[14]**. Chromosomes were karyotyped using an image analysis system (Vysis Quips XL Genetic work station) according to the international system of hemo-cytogenetic nomenclature **[15]**.

Fluorescence in situ hybridization:

FISH studies were performed according to the manufacture's instructions. MLL Dual Color Probe (Vysis Inc, Downers Grove, IL) designed for detecting the 11q23 rearrangements, was applied in this study. The probe consists of a centromeric portion labeled in spectrum green and a 190 Kb telomeric portion labeled in spectrum orange. The centromeric probe begins between MLL exons 6 and 8 and extends 350 Kb toward the centromere on chromosome 11, and thus covers the centromeric region of the breakpoint cluster region. The telomeric probe begins between exons 4 and 6 and covers a region primarily telomeric of the breakpoint cluster region.

Interphase nuclei lacking the MLL rearrangement are expected to contain two green/orange fusion signals. In the interphase nucleus showing the MLL rearrangement, the telomeric orange signal would move to the partner chromosome and the centromeric green signal would remain on the long arm of chromosome 11. Consequently, separate green and orange signals represent the MLL rearrangement.

RESULTS

Control group:

Ten normal control subjects matched for age and sex were included in the study all of which showed normal karyotype and FISH results for MLL gene. Fig. (1) shows a control with normal MLL gene.

Patients were divided into 2 groups:

Group 1 (AML):

It comprised 40/56 patients (71.4%) including 25 adults, 14 children and one infant. Their age ranged between 3 days and 77 years (Median 24 yrs). Of the 40 patients, 39 were diagnosed as AML based on FAB criteria. When immunophenotyping was performed, 3 patients of the 39 fulfilled the criteria for biphenotypic leukemia diagnosis being Myeloid/T-ALL in one case and Myeloid/B-ALL in two cases. The 40th patient was diagnosed as MDS based on FAB criteria as the percentage of the blast cells in the BM was 16, but when karyotype was performed, t (8;21) was detected so the diagnosis was changed into AML according to the WHO classification **[16-17]**.

The age, sex, FAB subtype, percentage of blast cells in the BM, karyotype and FISH results using MLL gene probe of group 1 patients are shown in Table (1).

In this study, conventional cytogenetics (CC) revealed 2 patients with 11q23 deletion, while FISH analysis for MLL gene proved the abnormality in one patient as a true 11q23 positive /MLL+. This patient (Table (1) case No. 30) was a male child aged 4.5 years (1/15=6.7%) diagnosed as M5a with 75% blast cells in his bone marrow and showed 11q- in the karyotype which was consistent with the FISH result.

FISH showed MLL gene rearrangements in another 2 adult patients (2/25=8%) in whom 11q23 band was normal by CC: One patient (Table (1) case No. 23) was a male aged 20 years diagnosed as M4 with 34% blast cells in the bone marrow and showed t (8;21) in his karyotype. By FISH, MLL gene deletion was detected. The other patient (Table (1) case No. 28) was a female aged 64 years diagnosed as M4 with 70% blast cells in the bone marrow and showed normal karyotype and MLL gene translocation by FISH.

Using FISH technique, collectively, 3/40 (7.5%) AML patients, showed gene rearrangements (Fig. 2).

Group 2 (MDS):

It comprised 16/56 patients (28.6%), including 12 adults and 4 children. Their age ranged between 4 and 68 years (Median 30 years). MDS diagnosis was based on FAB criteria. The age, sex, FAB subtype, percentage of blasts and normoblasts in the BM, karyotype and FISH results using MLL gene probe for these patients are shown in Table (2).

Using FISH technique, none of the MDS patients in this study showed MLL gene rearrangement.

Table (1): Karyotyping and MLL gene rearrangement in 40 de novo AML cases.

Case no.	Age	Sex	FAB	Blasts in BM %	Karyotype	FISH for MLL gene
1	38	Female	M1	90	46, XX	Neg
2	27	Male	M1	93	46, XY	Neg
3	18	Male	M1	73	46, XY	Neg
4	41	Male	M1	67	Not done	Neg
5	24	Female	M1	81	47, XX + 8 [14] 45X-X [6]	Neg
6	14	Female	M1	80	46, XX, add 4q, t(8;21)	Neg
7	14	Female	M1	82	48, XX, 7q-, +19, ++Ph	Neg
8	12	Male	M2	66	46, XY	Neg
9	10	Male	M2	35	47, XY, +21	Neg
10	31	Male	M2	20	46, XY	Neg
11	16	Female	M2	40	46, XX, t(8;21)	Neg
12	23	Male	M2	34	50, XY, +8, +10, +19, +21	Neg
13	12	Male	M2	44	46, XY	Neg
14	15	Male	M2	63	Not done	Neg
15	9	Male	M2	68	46, XY, add 4q t(8;21)	Neg
16	34	Male	M2	20	45, X, t(8.;21), -Y	Neg
17	29	Male	M2	44	Not done	Neg
18	25	Male	M2	71	48, XY, +i(6p), t(8;21) der 12 +mar	Neg
19	24	Female	M2	16	46, XX t(8;21)	Neg
20	47	Male	M3	9	46, XY t(15;17)	Neg
21	20	Female	M3	2	46, XX t(15;17)	Neg
22	24	Female	M3	15	46, XX t(15;17)	Neg
23	20	Male	M4	34	46, XY t(8;21)	MLL gene del
24	28	Male	M4	55	46, XY	Neg
25	37	Male	M4	77	46, XY	Neg
26	22	Male	M4	57	47, XY, +8	Neg
27	51	Female	M4	33	46, XX, der 7	Neg
28	64	Female	M4	70	46, XX	MLL translocation
29	36	Male	M4	60	46, XY	Neg
30	4.5	Male	M5a	75	46, XY, del 11q	MLL deletion
31	32	Female	M5a	85	47, XX, + der8	Neg
32	37	Male	M5a	70	46, XY, t(8;21)	Neg
33	2	Male	M5a	72	47, XY, +8	Neg
34	9	Male	M5a	82	46, XY	
35	77	Female	M5a	55	Not done	Neg
36	36	Female	M5b	35	46, XX	Neg
37	3 d	Male	M7	55	47, XY, +21	Neg
38	2	Male	Biphenotypic	48	46, XY, +4, t(16;17)	Neg
39	35	Male	Biphenotypic	24	46, XY, del 11q	Neg
40	3	Male	Biphenotypic	38	46, XY, add 1q, t(8;15) add 11p, -17, +mar [12] 46, XY [8]	Neg

N.B. MLL gene probe (LSI MLL Dual color rearrangement probe, Vysis Inc, Downers Grove IL).

Table (2): Karyotyping and MLL gene rearrangement in 40 MDS cases.

Case no.	Age	Sex	FAB	Blasts in BM %	Normoblasts in BM %	Karyotype	FISH for MLL gene
1	58	Male	RARS	4	58 >15% RS	46, XY	Neg
2	29	Male	RA	2	22	46, XY	Neg
3	5	Male	RAEB	16	30	46, XY	Neg
4	68	Female	RA	3	16	46, XX	Neg
5	41	Male	RA	4	15	46 XY	Neg
6	32	Male	RA	3	24	46, XY, +19, -22	Neg
7	4	Female	RAEB	18	30	45, XX, -7	Neg
8	29	Male	RA	4	23	46, XY	Neg
9	38	Male	RAEB	15	22	46, XY	Neg
10	28	Female	RA	2	28	47, XX, +2, 13q+	Neg
11	31	Male	RA	3	22	45, XY, -7	Neg
12	18	Male	RAEB	16	26	46, XY, del 7q	
13	33	Female	RA	1	47	46, XX, add 5q, der 5	Neg
14	25	Female	RA	5	28	46, XX	Neg
15	50	Female	RA	1	76	46, XX	Neg
16	10	Male	RA	3	58	46, XY, del 9q	Neg

RARS: Refractory anemia with ringedsiderblast, RA: Refractory anemia, RAEB: Refractory anemia with excess blasts.
N.B. MLL gene probe (LSI MLL Dual color rearrangement probe, Vysis Inc, Downers Grove IL).

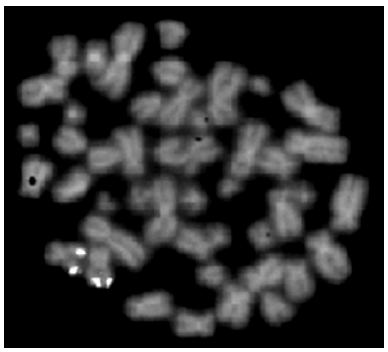

Fig. (1): Negative control for FISH using MLL break apart probe (Vysis).

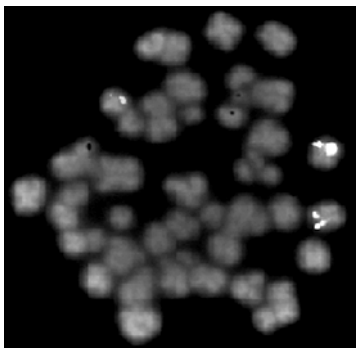

Fig. (2): FISH showing split of signals of the MLL gene probe denoting MLL gene translocation.

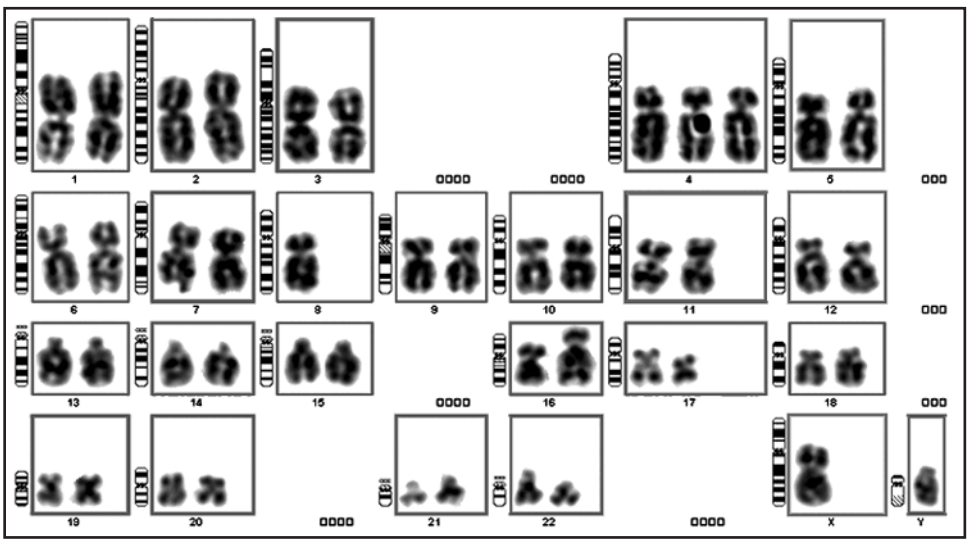

Fig. (3): Karyotype of Case No. 38 showing 47, XY, +4, t (16;17) (q22;11).

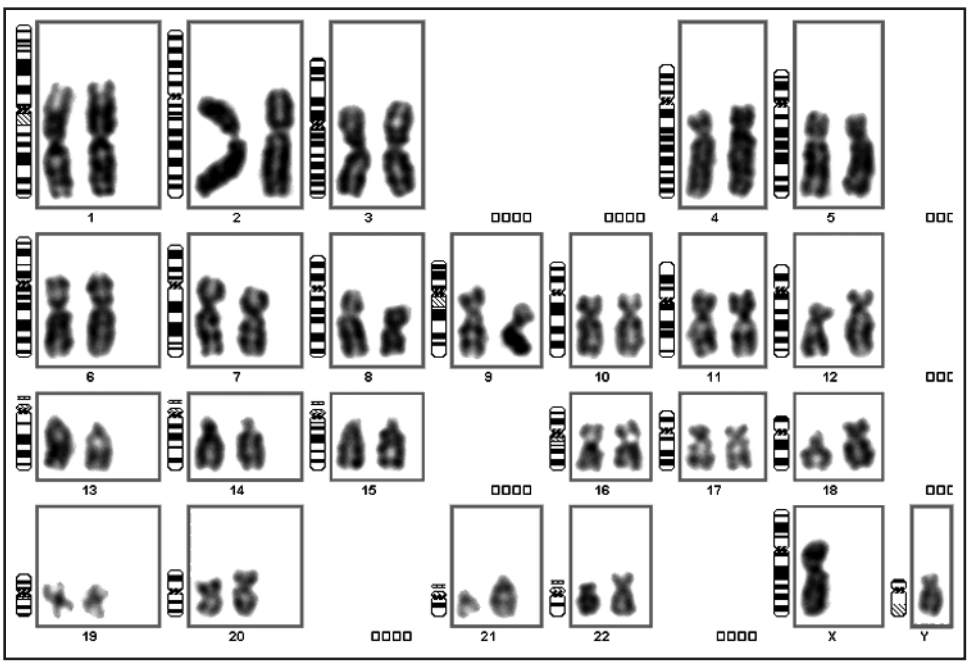

Fig. (4): Karyotype of case No. 11 showing t (8;21) (q22; q22).

DISCUSSION

In the present study, karyotypic analysis revealed normal karyotype in 30% of patients with AML. Normal karyotype was previously represented in 50% and 45% of patients with AML **[18,19]**.

In MDS, karyotypic analysis revealed normal karyotype in 56.3% of patients. Boultwood et al. reported normal karyotype in 50% of patients **[20]** while Chen et al. **[21]** and Pinto et al. **[22]** reported normal karyotype in 63% and 70% of patients with MDS, respectively.

In this study, conventional cytogenetics (CC) failed in 10% of patients. This figure is similar to previously published data **[23]**.

Cytogenetic findings in group 1 (AML patients):

1- Chromosome 11q23 abnormalities:

Structural abnormality of the 11q23 band (11q23 positive) bearing the MLL gene rearrangement (MLL+) is a recurrent chromosome change in leukemia described in AML and ALL with a peak incidence in infant leukemia. It occurs in up to 80% of children under one year of age who have leukemia **[5-24]**.

A proposal by the WHO specifies a separate category for AML with 11q23 positive/MLL+ **[25]**. This notion has been supported recently by biologic studies; microarray analyses have shown that MLL+ acute leukemias (ALs) have a peculiar gene profiling pattern that distinguishes them from all other ALs and that MLL+ leukemic blasts resemble very immature progenitor cells **[26]**.

Extensive cytogenetic and molecular studies have shown that 11q23/MLL is a highly promiscuous locus; more than 50 chromosomal loci have been described as 11q23 chromosome partners, whereas more than 30 MLL partner genes have been characterized **[27]**. To overcome this obstacle in studying MLL gene rearrangements, especially translocation, by FISH technique one can use break apart (split signal) FISH probe which is designed to hybridize upstream and downstream of the common breakpoint cluster region of the MLL gene. In that way MLL gene rearrangements are detected regardless of gene partners. FISH confirms the karyotypic results and helps to detect the precise nature of MLL and 11q23 abnormalities (translocation, deletion, or duplication). It may lead to reinterpretation of the karyotypic result **[28]**.

FISH collectively proved MLL gene rearrangements in 3 of 40 AML patients (7.5%) which is consistent with previously published data **[29,30]**. Another study detected MLL gene rearrangements at 4.7% frequency by FISH technique in adult de novo AML patients in which 11q23 band was cytogenetically normal **[31]**. Mrozek et al. **[32]** reported rearrangements of chromosomal band 11q23 involving the MLL gene at a 3-5% frequency in adult de novo AML.

Using southern blot technique, an Egyptian study published at the National Cancer Institute, revealed MLL gene rearrangements in 17.7% of pediatric AML patients **[33]**. The difference of the MLL gene rearrangement incidence in the present study may be due to different studied age groups as the incidence usually increases in pediatric patients while it is lower in the adults.

Conventional cytogenetics can discriminate between true 11q23 positive/MLL+ and rearrangements clustering within the 11q22-25 regions without MLL involvements **[34]**. Giugliano et al. **[35]** citated that 11q23 might involve genes other than MLL and Cox et al. **[36]** reported 45% 11q23 positive/MLL– in a series of AML patients.

Two studies reported many patients with MLL gene rearrangements in which the karyotype was normal **[37-38]**. Thus, FISH is more sensitive than CC in detecting MLL+ patients **[36-39]**.

In this work a strong association between AML subtypes M4 and M5 and MLL gene rearrangements was estimated. This is consistent with the study of Haferlach et al. **[40]**. Frenny et al. **[41]** reported high frequency of MLL gene rearrangements in M5a (50%) and M4 (20%) while Pan et al. **[39]** showed that MLL rearrangement is highly related to AML-M4/M5.

2- Other cytogenetic abnormalities:

The most frequently observed cytogenetic abnormality in this study was t (8;21) (q22;q22). It was detected in 8 patients (20%). One of these patients was firstly diagnosed as MDS because of the low number of blast cells in the BM. When cytogenetic studies were performed,

and t (8;21) detected, the diagnosis was changed to AML according to the WHO classification **[17]**. The rationale for the WHO classification that incorporated t (8;21) MDS into the category of acute myeloid leukemia with t (8;21) was supported by others **[43]**. The WHO system provides a reliable classification for the majority of AML patients. Further exploitation of cytogenetics and delineation of heterogeneous entities will probably extend its applicability and improve its clinical usefulness **[43]**.

Of the 8 patients with t (8;21) detected in this study, 5 were diagnosed as M2. This study included 12 AML-M2 cases, therefore, t (8;21) was detected in 5/12 (41.8%) of patients with AML-M2. Mitelman and Heim **[52]** showed t (8;21) in 15% of all AML subtypes and in 40% of M2. In this study, one of the patients with M2 having t (8;21) showed loss of Y chromosome; t (8;21) was recognized in one patient with M1, one with M4 and one with M5a. Published data reported t (8;21) predominantly in M2 and also in M4 and recognized that t (8;21) is frequently accompanied by the loss of sex chromosome; Y in males and X in females **[45]**. While others repored t (8;21) in 12.5% of patients with M2, 1.7% of patients with M1 and rarely in other FAB subtypes (M3-M7) **[46]**.

In this study t (8;21) was the sole abnormality in 4 patients (10%). Other studies reported this translocation as a sole abnormality in 20% of patients with AML and reported loss of Y chromosome to be a common additional abnormality **[47]**. In this work t (8;21) was detected as a part of complex karyotype in one patient which was diagnosed as M2. A previous study on pediatric AML patients reported favorable chromosomal abnormalities including t (8;21), t (15;17) and inv16 as a part of complex karyotype in 15/254 cases **[48]**.

In the present study, trisomy 8 was a recurrent chromosomal abnormality in AML. It was detected in 5 patients (12.5%); one patient with M1, one with M2, one with M4 and 2 with M5a. It was the sole abnormality in 3 patients (7.5%) and a part of complex karyotype in one patient. Heim and Mitelman **[45]**, reported +8 as the most frequent abnormality in AML that occurred as a sole abnormality in 5% of patients while, others recognized +8 in 12.7% of patients with AML that occurred as a sole abnormality in 5% **[49,50]**.

In this study, trisomy 21 was detected in 3 patients (7.5%); two patients were diagnosed as M2 and the third was diagnosed as M7 in an infant who had Down's syndrome. Wan et al. **[51]** detected +21 as a sole acquired karyotypic abnormality in AML-M2 and M4 and also reported that approximately 20% of leukemia in Down's syndrome was diagnosed as M7. The incidence of acute megakaryoblastic leukemia (M7) in Down's syndrome is estimated to be 400 times more than that in normal children **[52]**.

In this study the 3 patients with M3 showed a t (15;17) as a sole abnormality (7.5%). A previous study reported that all M3 patients have t (15;17) **[53]**. In agreement with the results of the current study, published data indicated that AML-M3 is associated with reciprocal chromosomal translocations always involving the RARa gene **[54]**, while some authors reported that 70% of patients with M3 had t (15;17) that represented 25% of all karyotypic abnormalities detected in AML **[23]**. The incidence of t (15;17) in different acute promyelocytic leukemia (APL) series varied so much. It may reflect differences in cytogenetic technique. In particular, the use of short term cultures instead of direct preparations seems to substantially increase the yield of chromosomally abnormal mitoses from patients with APL.

In this study two patients showed trisomy 19 as a part of complex karyotype, one patient was diagnosed as M1 and the other as M2. Similar findings have been previously reported **[55]**.

In this study, one patient diagnosed as M1 showed 7q- and double Philadelphia as part of complex karyotype. Complete or partial loss of long arm of chromosome 7 has been recognized in preleukemic MDS or unfavorable AML **[56]**. Monosomy 7 is associated with pediatric AML and MDS **[57]**.

Double Philadelphia was previously reported in a patient with AML belonging to myelomonocytic type of the FAB classification **[58]**. Some authors detected Ph chromosome in 1.2% of patients with AML and reported some patients with double Ph, but still one cannot exclude the possibility that the patient showed ++Ph was an acute blastic crisis (ABC) on top of chronic myeloid leukemia (CML) **[59]**.

In this study one patient diagnosed as M2 showed trisomy 10 as a part of complex karyotype. Others reported +10 as a rare recurring numerical abnormality detected in M0, M1 or M2 and sometimes M6 **[60]**.

In the current study, t (16;17) (q22;q11) was detected in one patient with biphenotypic leukemia M7/T-ALL. Others detected t (16;17) as a sole abnormality in AML-M6 **[61]**.

In this work, one patient diagnosed as biphenotypic leukemia showed complex karyotype in the form of 46, XY, add1q, t (8;15), add11p, -17, +mar. Marker chromosome was detected in another patient diagnosed as M2 as a part of complex karyotype. One patient with AML with variant translocation t (8;21;15) was previously reported **[62]**.

Published data detected monosomy 17 in patients with AML **[44]**. Monosomy 17 and del 17p in patients with AML and MDS as part of complex karyotype that included marker chromosomes was also reported **[63]**.

Some authors detected abnormalities of chromosome 1 in myeloproliferative disorders **[64]**. Others reported unbalanced translocation involving 1q in myeloid disorders **[65]**.

In this work, complex karyotypes were detected in 4 patients (10%), one diagnosed as M1, 2 as M2 and the fourth was diagnosed as biphenotypic leukemia. Others detected complex karyotype in 10% of AML with higher incidence in patients ≥60 years **[66]**. Other studies detected complex karyotype in AML including t (8;21) **[52-67]**. Published data described that 10-15% of patients with AML exhibit complex karyotypes and mentioned that many of these patients cannot be accurately described using conventional chromosome banding analyses due to the low resolution of the technique and the complexity of the rearrangements (e.g. additional material of unknown origin, marker chromosomes and ring chromosomes) that may need further investigations by FISH and CGH **[68]**.

In the current study, isochromosome 6p [i (6p)] and der 12 as parts of complex karyotype were detected in one patient diagnosed as M2. Isochromosome (6p) was previously reported in retinoblastoma tumor cells and in myeloid leukemia **[69-70]**.

Cytogenetic findings in group 2 (MDS):

In the present work 3/16 (18.8%) patients showed abnormalities of chromosome 7. Two patients diagnosed as RAEB and RA showed monosomy 7 as a sole abnormality. 7q- was detected in the third patient who was diagnosed as RAEB. In most of the previously reported series, patients with monosomy 7 were grouped with those with 7q- as both abnormalities lead to the loss of a putative tumor suppressor gene on 7q.

Monosomy 7 and 7q- have been previously reported as common cytogenetic abnormalities in MDS **[62-72]**, while a higher rate of -7 was reported in patients with RA and RAEB **[72]**.

In our series, one patient diagnosed as RA was pseudodiploid as it showed +19 and -22. Some authors detected +19 in patients with MDS, while others reported -22 in a patient with AML preceded by MDS at the time of progression **[73-74]**.

In the current study, trisomy 2 was reported in one patient which was diagnosed as RA in addition to 13q+. Published data reported +2 in MDS as a sole abnormality and suggested that +2 is an early chromosomal abnormality in leukemogenesis as it is found in combination with other chromosomal abnormalities in AML **[75]**. Others reported chromosome 13 abnormality in patients with MDS **[23]**.

In this work, del 9q was reported in one patient diagnosed as RA. Some authors reported del 9q as a sole abnormality to be rare in patients with MDS and AML **[60-76]**.

In our series, abnormalities in chromosome 5 (add 5q) and der 5 was detected in one 33 years old female patient diagnosed as MDS (RA). Some authors recognized 5q- as a common anomaly in patients with MDS with average age from 65 to 70 years **[73]**.

FISH results:

FISH studies revealed no MLL gene rearrangements in all patients with MDS which was consistent with published data. However MLL gene rearrangements, was reported in some patients with therapy related MDS **[77-78]**.

Conclusion:

FISH collectively proved MLL gene rearrangements in 3 of 40 AML patients (7.5%), all

of which were monocytic leukemias and in none of the MDS patients. FISH should be routinely used to screen for MLL gene rearrangements in AML patients because it is more sensitive and more specific than conventional cytogenetics.

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