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Original Article

Bone Marrow Karyotype, Flow Cytometry, and FISH Analysis: Essential Tests to Improve the Initial Diagnosis of Patients with Myeloid Malignancies

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A B S T R A C T

Background and Aims: Diagnosing hematologic malignancies requires implementing several tests. This study aims to evaluate the chromosomal changes in patients with myeloid disorders and compare the results of flow cytometry and cytogenetics with the initial diagnosis performed by the oncologist.

Materials and Methods: 115 patients with myeloid disorders, 57.2% males and 42.8% females with a mean age of 50.3 years, previously diagnosed by an oncologist based on the clinical features, complete blood count, and peripheral blood smear interpretations, were considered. Moreover, flow cytometry and cytogenetic analysis were implemented on the bone marrow samples.

Results: Cytogenetic results showed that 30% of patients with myeloid disorders had abnormal karyotypes. 77% of patients with myelodysplastic syndromes, 65% of acute myeloid leukemia, and 30.7% of chronic myeloid leukaemia indices showed normal karyotypes, and the others resulted in common and uncommon abnormalities, including the translocation (13;17), 92, XXYY, and del (4q). Considering the flow cytometry and karyotype results, the improved diagnoses were made for 41 patients who had not been diagnosed initially.

Conclusion: This study showed that, in some cases, an initial diagnosis is inconsistent with the flow cytometry and karyotype analysis results. Also, the flow cytometry results may differ from the karyotype depending on the case. Therefore, combining the results obtained by the cytogenetic investigation, flow cytometry, fluorescence in situ hybridization, and molecular testing is preferable to provide a comprehensive report for the appropriate disease diagnosis and prognosis.

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Introduction

Hematologic malignancy refers to cancers affecting the bone marrow, lymph nodes, and blood cells [1]. In terms of the cytochemistry, morphology, immunophenotype, clinical features, and underlying genetic defects, the World Health Organization (WHO) categorizes myeloid neoplasms into several subtypes, including: 1) myelodysplastic syndromes (MDS), 2) myeloproliferative neoplasms (MPN), 3) acute myeloid leukemia (AML), 4) myelodysplastic/myeloproliferative neoplasm s (MDS/MPN), and 5) myeloid neoplasms associated with eosinophilia and abnormalities of growth factor receptors derived from platelets or fibroblasts [2].

No one can cast a shadow of doubt on the fact that the authentic diagnosis establishes the foundation and preliminary therapy of diseases. A treatment will make sense when the disease diagnosis is made accurately and the relationship between them is crystal clear. Diagnosis of hematologic disorders requires the implementation of several technologies, such as cytogenetics, cellular histology, and the study of cellular immunological markers [3, 4]. Therefore, flow cytometry, bone marrow karyotype, and Fluorescence in situ hybridization (FISH) are recommended diagnostic techniques for hematologic malignancies [5].

One of the immunophenotypic characterization methods of hematological disorders is flow cytometry, which can be used before and after therapy for diagnosis, staging, classification, and monitoring of immunophenotypic features for minimal residual disease [6]. Flow cytometry provides an ambient to quickly and accurately examine the immune system cells, cancer cells, chromosomes, and the number of antigens on the cell surface and cytoplasm [7].

Genetic findings are among the most influential factors that play an essential role in predicting the biological characteristics of malignancies and disease diagnoses [5]. This made academics recommend that the cytogenetic analysis be performed at the initial stage of patient evaluation to specify the clonal proliferation condition. This analysis can be even more vital when a controversial therapy diagnosis happens between a reactive process and a neoplastic, including a selection of appropriate treatment protocol and the choice and time of hematopoietic stem cell transplantation. Moreover, it is vital to identify cytogenetic abnormalities to evaluate the response to the treatment protocol [8]. Due to chromosomal abnormalities' diagnostic and prognostic importance, clinicians carry out the cytogenetic analysis of hematologic diseases for disease classification and prognosis. Conventional cytogenetic (G-banding) is a primary technique that detects chromosomal abnormalities of myeloid disorders, including numerical, structural, or both [9-11].

This method identifies various genetic defects; however, the possibility of occurrence depends on the condition. For instance, the diagnosis of genetic defects may not be accurately made when the results are normal due to cryptic alterations or in the absence of metaphases and

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poor chromosomal morphology. As a result, it is necessary to utilize the techniques to identify genetic anomalies undetected by conventional cytogenetics. However, FISH analysis is a popular method to detect abnormal clones. Either molecular cytogenetics or FISH, which can be performed on poorly or well-spread metaphases and interphase nuclei, can be helpful for disease diagnosis in such situations [12].

The present study aims to evaluate the frequency of various chromosomal changes in patients suffering from myeloid disorders and compare the results of flow cytometry and cytogenetic analysis with the initial diagnosis performed by expert physicians.

Materials and Methods

The statistical society of the present descriptive cross-sectional study consists of 115 patients diagnosed with myeloid disorders, referred to Imam Khomeini Hospital in 2019. Hematologist oncologists initially diagnosed the type of myeloid disorders based on the clinical features, complete blood count, and peripheral blood smear interpretations. Moreover, another laboratory performed flow cytometric immunopheno-typing on bone marrow samples under stable conditions. Immunophenotypic profile for myeloid leukemia included cluster of differentiation (CD)11c, CD13, CD33, CD14, CD16, CD64, CD34, CD71, and CD117.

Conventional cytogenetic

Cytogenetic analysis was implemented on the bone marrow samples using G-banding. Briefly, the procedure included the following process:

1. Cell culture in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with fetal bovine serum 20%;
2. Harvest of metaphase chromosome by adding colcemid to arrest mitotic cells and hypotonic solution to improve the yield and quality of metaphase spreads;
3. Fixation of chromosomes spread on the slide;
4. Banding and staining by using trypsin and Giemsa.

For each case, between 20 and 25 metaphase spreads were analyzed by two expert cytogeneticists, according to the International System for Human Cytogenetic Nomenclature (ISCN) [13].

FISH

In the FISH analysis, some probes are structured, including PML-RARA, BCR-ABL, del TP53, and Trisomy12 (PML/RARa, BCR/ABL and del TP53) probe but Trisomy 12 is a centromeric probe), was applied regarding the physicians' prescription. PML RARa and Trisomy 12 probes were used to diagnose AML, t (9;22) or BCR/ABL for chronic myeloid leukaemia (CML) and del of TP53 for CLL. All FISH analyses were done following the manufacturer's protocol. Briefly, fixed cells were prepared and dropped onto slides together with the appliance of 5 µl of the specific probe to a hybridization area. These slides were held for denaturation in a hybridization chamber at 85 °C for 8 minutes, and subsequently, hybridization was carried out at 37 °C overnight. Then, post-hybridization washes were implemented at 0.4×SSC (at 72°C for 1 minute) followed by 2× SSC for 30 seconds at 25 °C. The slides

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were dried and counterstained with 7µl of 4',6-diamidino-2-phenylindole. The slides were put in a dark ambient for color development and analyzed using an appropriate fluorescence microscope (Leica, Model DM2500, and FISH software (GenASIS). Finally, at least 100 cells were scored for signals for each patient. Informed written consent was obtained from all patients. All the procedures, methods, and experiments complied with the Declaration of Helsinki and were approved by the Ethics Committee of the Tehran University of Medical Sciences (IR.TUMS.IKHC.REC. 1400.315).

Results

The mean age of the patients was 50.3 years (Range: 2.5-88 years). There were 68 men and 51 women. The clinical characteristics of the patients are presented in Table 1.

AML

According to the physician's initial diagnosis, 40 cases with a mean age of 45.2 years were diagnosed as AML. The flow cytometry results of 23 patients with AML were consistent with the initial diagnosis (Table 2). Among them, two patients were observed with normal karyotypes. The findings showed a shift to myeloid series in the flow cytometry technique among 4 cases initially diagnosed with AML. Although 3 of 4 cases showed a normal karyotype, one patient had uncommon t(13;17) chromosomal abnormalities with 24% positive for PML-RARA based on the results obtained from the FISH analysis (Fig. 1). Although the flow cytometry results reported AML in 6 patients, no initial diagnosis was made by physicians. Among these six patients, a severe abnormality was observed in only one case; the rest had a normal karyotype. An uncommon abnormality 92, XXYY, was found in one patient initially diagnosed with leukocytosis by a physician and reported AML by the flow cytometry technique (Table 3). Moreover, the chromosomal translocation t (9;22) was initially observed in FISH and karyotype analysis in a patient diagnosed with AML (Fig. 2).

MDS

Thirty-one cases were recorded with MDS based on the initial physicians' diagnosis or flow cytometry analysis, including 17 females at a mean age of 58.5 years and 14 males at a mean age of 59. Most chromosomal abnormalities were observed among men; only one woman showed chromosomal changes (47, XX, +8). 77.4% of patients of this group showed normal karyotypes, and no chromosomal abnormalities were found. The deletion of the Y chromosome was observed in 3 patients (mean age of 60 years) with no initial diagnosis showing MDS in the flow cytometry. Common chromosomal abnormalities in MDS have also been observed in our patients, including the deletion of chromosome 5, del (17p), trisomy 8, loss of chromosome Y, and complex abnormality (Table 4).

Pancytopenia and anemia (without initial diagnosis)

The 23 patients with the initial diagnosis of pancytopenia/ anemia with an average age of 58.4 years were recorded, including eight men (mean age of 56 years) and 15 women (average age of 54 years). The abnormal

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karyotype 46, XX,+1, der(1;7) and 46, XX,t (15; 17) were found in 2 patients diagnosed with a shift to myeloid series in the flow cytometry. There was no evidence of abnormal karyotype in 86% of patients with an initial diagnosis of pancytopenia/ anemia, but a shift to myeloid series was reported in the flow cytometry. The FISH analysis seems to help physicians make a more accurate diagnosis.

Among five patients with anemia, the translocation t (8; 21) was observed in one woman, resulting in myeloid hyperplasia with a shift to myeloid series in flow cytometry results. There was no abnormal chromosomal analysis for four patients, while flow cytometry results showed evidence of myeloid disorders (Table 5).

Table 1. Clinical characteristics of the patients with myeloid disorders (N=115)

Parameters		Frequency	Percentage
Sex	Female	51	42.8
	Male	68	57.2
Age	< 12 years	2	1.6
	12-60 years	80	66.6
	≥ 60 years	37	31.8
Hemoglobin (gr/dl)	≤12	84	70.6
	12-16	14	11.8
	≥ 16	1	0.8
White blood cell (x1000/mm3)	< 4	46	36.5
	4-10	23	19.3
	≥ 10	50	44.2
Red blood cell (million/mm3)	< 4.2	94	79
	4.2-5.8	23	19.3
	≥ 5.8	2	1.7
Platelets (x1000/mm3)	< 150	77	64.7
	150-450	38	32
	≥ 450	4	3.3

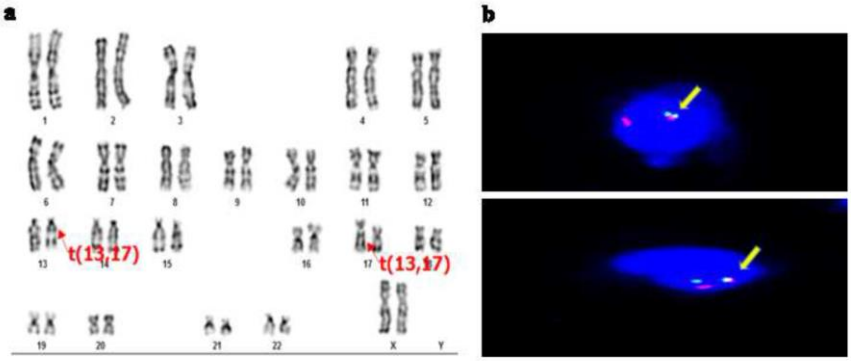

Fig. 1. Bone marrow aspiration findings (a): Cytogenetic analysis showing 46, XX, t(13;17)(q14;q21)[11] /46XX[12] (b): Identification of t(15;17)(15q24;17q21) rearrangement in the interphase nucleus using FISH probe.

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Table 2. The results of the initial diagnosis by the physician, flow cytometry, cytogenetic, and FISH analysis for AML

Initial Diagnosis	Flow Cytometry	Karyotype	Most reported in literature	FISH
AML (N=1)	AML	47,XY,+12[30]/46,XY[5]	Trisomy 12 CLL [14]	Trisomy 12 16% positive t(15;17) 11% positive
AML (N=3)	AML	47,XY,+8	Trisomy 8 AML, MDS, CML [15, 16] Trisomy 21 ALL, CLL & AML [17]	ND
AML (N=1)	AML	49,XY,+6,+21,+22[2],48,XY,+6,+21[2],46,XY[16]	Trisomy 22 Rare abnormality in AML [18] Trisomy 6 AML & MDS [19]	ND
AML (N=1)	AML	46,XY,t(8;21)[3]/46,XY[27]	t(8;21) AML [20] t(8;21) AML [20]	ND
AML (N=1)	AML	45,X,t(8;21),-X	Monosomy X 30-40% of cases with t(8;21) [21]	ND
AML (N=1)	AML	Normal	Normal Karyotype ~50% of AML [22]	t(15;17) 7% Positive
AML (N=1)	AML	Normal	Normal Karyotype ~50% of AML [22]	t(15;17) 25% positive
AML (N=1)	AML	ND	-	t(15;17) 70% positive
AML (N=13)	AML	Normal	Normal Karyotype ~50% of AML [22]	ND
AML (N=3)	Shift to Myeloid Series	Normal	Normal Karyotype ~50% of AML [22]	ND
AML (N=1)	Shift to Myeloid Serie	46,XY,t(13;17)	Rare abnormality [23]	24% Positive
Leukocytosis (N=1)	AML	92,XXYY	92,XXYY Rare abnormality [24, 25]	ND
AML (N=1)	MPD	46,XX,t(9;22)	about 2-3% of cases with AML [26]	71% Positive
AML (N=1)	Not AML	46,XX,inv(3)	AML & MDS [26]	4% Positive
AML (N=1)	Not AML	47,XY,+6[15]/46,XY[3]	Trisomy 6 AML & MDS [19]	t(15;17 10% positive
AML (N=3)	Not AML	Normal	Normal karyotype ~50% of AML [22]	ND
Without diagnosis (N=5)	AML	Normal	Normal karyotype ~50% of AML [22] del(11) 0.7% in *de novo* & secondary MDS and AML [27]	ND
Without Diagnosis (N=1)	AML	45,XY,del(11)(q23),-16,-20,+22[35]/ 46,XY[7]	Monosomy 16 AML [28] Monosomy 20 both myeloid & lymphoid malignancies [29, 30] Trisomy 22 Rare abnormality in AML [18]	ND

FISH= Fluorescence in situ hybridization; AML= Acute myeloid leukemia; ND= Not determined

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Table 3. Comparison of the FISH, karyotype, and flow cytometry results performed on six patients

Tests	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
FISH PML/RAR A (%) positive	11%	7%	25%	70%	24%	10%
Final result of FISH (PML/RARA)	Negative	Negative	Positive	Positive	Positive	Negative
Other Perobes	Trisomy12 16% positive	-	-	-	-	-
Karyotype	47,XY,+12[30]/ 46,XY[5]	Normal	Normal	-	46,XY,t(13;17)	47,XY,+6,add (7) (p22)[15]/46,XY [3]
Flow Cytometry	AML	AML	AML	AML	Shift to myeloid series	Not AML

FISH= Fluorescence in situ hybridization; AML= Acute myeloid leukemia

Table 4. The results of the initial diagnosis by the physician, flow cytometry, and cytogenetic analysis for MDS

Initial Diagnosis	Flow Cytometry	Karyotype	Most reported in literature
MDS (N=1)	Shift to Myeloid Series	45,XY,-5[2]/46,XY[8]	Monosomy 5 Rare abnormality 31]
MDS (N=5)	Shift to Myeloid Series	Normal	Normal Karyotype 40-50% of MDS cases [32]
MDS (N=1)	MDS	46,XY,del(17p)[4]/46,XY[9]	del(17p) AML and MDS [33,34]
MDS (N=1)	MDS	47,XX,+8	Trisomy 8 5-7% of MDS [16]
MDS (N=1)	MDS	Sever Abnormality	-
MDS (N=11)	MDS	Normal	Normal Karyotype 40-50% of MDS cases[32]
MDS (N=2)	Non diagnostic	Normal	Normal Karyotype 40-50% of MDS cases[32]
Chronic Anemia (N=1)	MDS or Megaloblastic Anemia	Normal	Normal Karyotype 40-50% of MDS cases[32]
Without Diagnosis (N=5)	MDS	Normal	Normal Karyotype 40-50% of MDS cases[32]
Without Diagnosis (N=3)	MDS	45,X,-Y[9]/46,XY[10]	-Y MPD, MDS and AML [35]

MDS= Myelodysplastic syndromes

Table 5. The results of the initial diagnosis by the physician, flow cytometry, and cytogenetic analysis for pancytopenia and anemia

Initial Diagnosis	Flow Cytometry	Karyotype	Most reported in the literature
Pancytopenia (N=16)	Shift to myeloid series	Normal	Normal karyotype AML, MDS and CML[22, 32, 36]
Pancytopenia (N=1)	Shift to myeloid series	46,XX,der(1;7)[19]/ 46,XX[5]	der(1;7) 1.5-6% of MDS, 0.2-2% of AML and rarely in MPN [37]
Pancytopenia (N=1	Shift to myeloid series	46,XX, t(15;17)[14]/46,XX[9]	t(15;17) APL [38, 39]
Anemia (N=1)	Shift to myeloid series	46,XX,t(8;21)/46,XX	t(8;21) AML [20]
Anemia (N=4)	Shift to myeloid series	Normal	Normal karyotype AML, MDS and CML [22, 32, 36]

AML= Acute myeloid leukemia; CML= Chronic myeloid leukaemia; MDS= Myelodysplastic syndromes; APL= Acute promyelocytic leukaemia; MPN= Myeloproliferative neoplasms

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Table 6. The results of the initial diagnosis by the physician, flow cytometry, and cytogenetic analysis for CML

Initial Diagnosis	Flow Cytometry	Karyotype	Most reported in the literature
CML (N=3)	CML	Normal	Normal karyotype <2% of CML [40]
CML (N=5)	CML	46,XY,t (9;22)	t(9;22) CML [41]
CML (N=2)	CML	45,X,t (9;22), -Y	t(9;22), -Y CML [42]
CML (N=1)	CML	46,XY,t (8;21)[11]/46,XY[9]	t(8;21) CML [43]
Thrombocytopenia (N=1)	CML	46,XX,del (4q)[8]/46,XX[12]	del(4q) Rare abnormality [44]
Thrombocytopenia (N=1)	CML	Normal	Normal Karyotype <2% of CML [40]

CML= Chronic myeloid leukaemia

Table 7. Distribution of chromosomal abnormalities in blood disorders

Parameters	Patients (%)	AML (%)	MDS (%)	CML (%)	Pancytopenia/ Anemia (%)	Diagnosed with Lymphoid (%)
Karyotype	115 (100%)	40(35)	31(27)	13(11)	23(20)	8(6.7)
Normal	81 (70)	26(65)	24(77)	4(30.7)	20(87)	6(75)
t(9;22)	8 (7)	1(2.5)		7(53)		
Loss of Y	7 (6)		3(10)	2(15.4)		2(25)
Monosomy X	1 (0.8)	1(2.3)				
t(8;21)	4 (3.4)	2(5)		1(7.7)	1(4.3)	
t(15;17)	1 (0.8)				1(4.3)	
Trisomy 8	4 (3.4)	3 (7.5)	1 (3.2)			
Monosomy 5	1 (0.8)		1 (3.2)			
der (1;7)	1 (0.8)				1 (4.3)	
del (4q)	1 (0.8)			1 (7.7)		
del (17p)	1 (0.8)		1 (3.2)			
Trisomy 12	1 (0.8)	1 (2.3)				
Trisomy 21	1 (0.8)	1 (2.3)				
Trisomy 22	2 (1.7)	2 (5)				
t (13;17)	1 (0.8)	1 (2.3)				
Complex karyotype	4 (3.4)	3 (6.8)	1 (3.2)			

AML= Acute myeloid leukemia; CML= Chronic myeloid leukaemia; MDS= Myelodysplastic syndromes

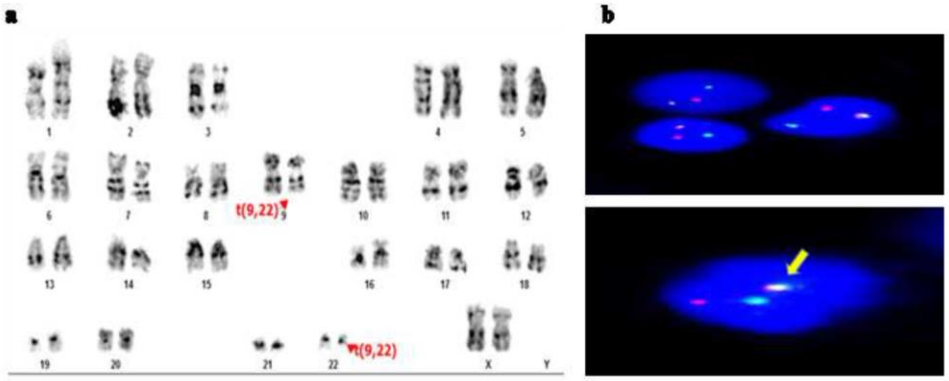

Fig. 2. Bone marrow aspiration findings (a): Cytogenetic analysis showing BCR/ABL t (9;22)(q34;q11) (b): Identification of BCR/ABL rearrangement in the interphase nucleus using FISH analysis.

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Initial diagnosis flow cytometry karyotype most reported in the literature normal karyotype

Normal/Shift to Myeloid / Pancytopenia/ (N=16) AML, MDS and CML/ der(1;7)/ 46,XX,der (1;7)/ 46,XX/ Shift to Myeloid /Series /Pancytopenia (N=1) /1.5-6% of MDS, 0.2-2% of AML and rarely in MPN /46, XX, t(15;17) /t(15;17)[14]/46, XX[9] /Pancytopenia /(N=1) APL

CML

A total of 13 patients at a mean age of 49.8 years, which included ten men (average age 50 years) and three women (average age 49.8 years) were initially diagnosed with CML and myeloid disorders in flow cytometry results. The translocation t (9; 22) was observed in 7 patients of this group. Moreover, karyotype analysis for two patients showed the loss of the Y chromosome. Although flow cytometry analysis showed CML in two patients, they were diagnosed with thrombocytopenia. One of these patients had a normal karyotype, while the other had an abnormal one (46, XX, del(4q)[8]/46, xx[12]). This abnormality del (4q) has not yet been reported as common in hematological malignancies. Moreover, one patient with the CML results in both initial diagnosis and flow cytometry analysis showed abnormal karyotype 46,xy,t(8; 21) [11]/46,xy[9] in cytogenetic analysis (Table 6).

The inconsistent initial diagnosis

Among the 115 cases referred to Imam Khomeini Hospital, eight male patients with a mean age of 39.5 years and initially diagnosed with lymphoma, analyzed by the karyotype and flow cytometry. The mosaic karyotype45, X,-Y/46, and XY were detected in 2 cases, whereas 6 cases were cytogenetically normal. However, the flow cytometry results were inconsistent with the physician's initial diagnosis, in which the results were reported as myeloid hyperplasia or shift to myeloid series. Furthermore, for a patient with an initial AML diagnosis, the flow cytometry and karyotype showed pre-B-ALL and normal cytogenetic analysis, respectively. Table 7 presents the chromosomal abnormalities observed in this research.

Discussion

The diagnosis of myeloid disorders has been improved from a purely morphological diagnosis to a precise evaluation based on immunology and cytogenetics. A large number of chromosomal abnormalities have been observed in hematological disorders associated with specific morphologic, immunophenotypic, and clinical features [45]. Therefore, cytogenetic analysis is a principal method used to evaluate these abnormalities. Depending on the case, the cytogenetic analysis may not be adequate in diagnosing chromosomal abnormalities due to the weakness of conventional cytogenetics in detecting cryptic changes. Accordingly, the FISH analysis is recommended to fill these debilities. Although the Targeted FISH is a rapid high-sensitivity method, it cannot be used for genome-wide and conventional cytogenetic investigation [45, 46]. Furthermore, the results can be accessible in 24 hours by implementing the high-sensitivity FISH method, which is effective for

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managing and treating the patient. On the other hand, this FISH technique can be carried out on interphase cells and poorly spread metaphases. As a result, it can solve the problems of conventional cytogenetics related to this issue [46]. Hence, performing FISH analysis in conjunction with conventional cytogenetics is vital to assess molecular rearrangements, especially when karyotype results have been reported normally. Unfortunately, prescribing FISH analysis is not highly prevalent in observing normal karyotype results. Consequently, only eight patients with FISH analysis prescriptions were recorded in this research. Moreover, the cytogenetic examination helps physicians prognosticate the therapy effects on malignancy and prescribe more efficient treatments. However, in this present study, some uncommon chromosomal abnormalities were observed that put the physician at the bottleneck of decisions. Thus, flow cytometry, FISH, and molecular testing results may help physicians with appropriate diagnosis, and it is essential to apply a combination of cost-effective and accurate methods for any clinical condition.

Normal karyotype may be reported for patients with AML indications detected in 65% of the patients in this study. As a result, it seems that a precise diagnosis is accessible by implementing a molecular examination. Some genetic mutations associated with AML disease included FLT3-ITD, NPM1, and CEBPA gene mutations [47-49]. Therefore, the molecular analysis of these genetic mutations in patients with normal karyotype and AML indication is recommended. PML/RARA rearrangement was detected in two patients who had no evidence of this translocation in conventional karyotype by using the FISH method. These results highlight the importance of performing FISH analysis in all cases with no evidence of cytogenetic alterations regarding its high sensitivity. The translocation t(13;17) was observed in a patient with the AML initial diagnosis and a shift to myeloid series in flow cytometry analysis. In comparison, the FISH analysis showed 24% positive for PML/RARA. However, the significance of t(13;17) has not yet been determined for hematologic malignancies. In 2006, this chromosomal translocation was identified by Turhan et al. in an AML-M4 patient and reported as a novel chromosomal abnormality. This chromosomal structural change was also associated with poor prognosis [23]. Therefore, this rearrangement can be considered a new prognostic marker for AML.

In the present study, an uncommon abnormality 92, XXYY, was found in a patient with leukocytosis, in initial diagnosis, and AML, in the flow cytometry technic. Similar results were demonstrated by Leopoldo Zelante et al. identifying a patient with AML-M1 together with 92 XXYY chromosomes [24]. However, Heim et al. first reported this chromosomal abnormality in 2 patients with ALL and L2 morphology [25]. No structural chromosome changes have been observed in both examined patients and the previous two studies. Therefore, despite the lack of structural changes, the mechanism by which tetraploid leads to leukemia is still unknown. There may be a hypothesis that this chromosomal abnormality could lead to a specific subtype of leukemia [25].

It has been reported that trisomy 12 was seen in about 16% of CLL patients [50]. Unlike the

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previous studies, trisomy 12 was observed in one patient diagnosed with AML, and no other structural changes were observed in the karyotype analysis. Also, the results of FISH analysis for this patient showed trisomy 12=16% and PML/ RARA=11% positive. This novel chromosomal abnormality in AML is found in the present study. Regarding MDS, studies reported that about 40% of patients had a normal karyotype with no changes in the chromosomal analysis [32]. In the present study, approximately 77% of patients with MDS indices showed normal karyotypes, suggesting that there may be hidden genomic changes not determined by conventional karyotype analysis. In a study by Thiel et al., 39% of the samples showed hidden aberrations with CGH array analysis [32]. Therefore, identifying new markers and hidden aberrations is essential to predict disease progression and definitive diagnosis. This information interests scientists, researchers, clinicians, and laboratory directors involved in the quality assurance of cancer cytogenetic services. This study showed that, in some cases, a physician's initial diagnosis is inconsistent with the results of the flow cytometry and karyotype analysis, and the results of flow cytometry differ from the results of karyotype depending on the case. One of the main problems in this type of problem is finding and choosing the right treatment. The AML was differentially diagnosed in one case, while flow cytometry and cytogenetics were reported t(9; 22). The type of treatment may change due to the lack of knowledge about this translocation.

Conclusion

This study showed that, in some cases, an initial diagnosis is inconsistent with the flow cytometry and karyotype analysis results. Also, the flow cytometry results may differ from the karyotype depending on the case. Therefore, the combination of the result obtained by the cytogenetic investigation, flow cytometry, FISH, and molecular testing is preferable to provide a comprehensive report for the appropriate disease diagnosis and prognosis.

Conflict of Interest

The authors declare no competing interests.

Acknowledgment

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