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Evaluation of the Sensitivity of Conventional Cytogenetic and Fluorescence in Situ Hybridization Methods for the Detection of Cytogenetic Abnormalities in Multiple Myeloma Patients: A Retrospective Study

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Abstract

Background: The identification of genetic abnormalities in multiple myeloma (MM) patients is of particular importance in designing their treatment. Therefore, it is necessary to use diagnostic methods with high sensitivity to detect abnormalities. Conventional cytogenetic and fluorescent in situ hybridization (FISH) methods are commonly used to identify genetic abnormalities. So far, studies have been conducted to investigate the sensitivity of each of these methods alone; nonetheless, the present research aimed to assess and compare the sensitivity of two methods in identifying genetic abnormalities.

Objectives: in this study, the sensitivity of Conventional cytogenetic and FISH methods for identifying genetic abnormalities in MM patients has been investigated and compared.

Methods: This retrospective study included 246 patients who were referred to Kariminejhad Center for the diagnosis of genetic abnormalities from 2009-2019. All patients were diagnosed based on diagnostic tests, as well as the approval of the relevant physician. The diagnosis of cytogenetic abnormality was made based on the two methods of conventional cytogenetic and FISH.

Results: As evidenced by the obtained results, out of 246 patients examined by conventional cytogenetics, only 17.8% had abnormal karyotypes. While out of 67 patients examined by FISH, 64.1% had abnormal results. The results also pointed out that out of 50 patients with normal karyotypes, 31 cases had abnormal FISH results. Moreover, 25% of patients had hyperdiploidy (pseudodiploid or structural abnormality in 23 pairs of chromosomes), which was diagnosed by conventional cytogenetics. Furthermore, 40.90% of subjects had diploid abnormalities (pseudodiploid or structural abnormalities). In addition, FISH detected del 13q in 27.9% and t(11;14) IGH-CCND1 in 18.6% of patients, the most frequently observed compared to other abnormalities.

Conclusion: Considering that the variety of mutations and translocations is high in different parts of the world and new mutations are detected every day, the use of both methods together can help identify genetic disorders.

Keywords: Abnormal cytogenetic, Conventional cytogenetic, Fluorescence in situ hybridization, Multiple myeloma

1. Background

Multiple myeloma (MM) is a hematological malignancy characterized by clonal plasma cell proliferation in Bone Marrow (BM). It is the most common hematological malignancy after non-Hodgkin lymphoma, accounting for about 1% of malignancies (1, 2). The MM is mainly prevalent in adults, and the average age of diagnosis is about 65 years (3). Clinical symptoms associated with MM vary from patient to patient, depending on the pathogenesis of the disease. Nonetheless, the most common symptoms include renal failure, infection, anemia, and hypercalcemia. Molecular and biological markers can affect the clinical symptoms of patients. Therefore, the identification of each of them can help in designing the treatment strategy of patients (4-8).

Recent studies have demonstrated that cytogenetic abnormalities are the main factors in the incidence and progression of MM (9). The evaluation of cytogenetic abnormalities can be considered in order to treat patients and evaluate their response to treatment (10). The response or resistance to treatment can be evaluated using the prognostic feature of cytogenetic abnormality. Moreover, the identification of diagnostic markers in cancers can help in the recovery and management of patients (11-13). Conventional cytogenetics and Fluorescence in situ hybridization (FISH) are two methods by which cytogenetic abnormalities can be diagnosed. Due to the low proliferation of malignant plasma cells, many cytogenetic abnormalities are not detected by conventional cytogenetics. Therefore, the use of FISH can lead to a better diagnosis of cytogenetic abnormality (14, 15). So far, very few studies have evaluated the accuracy and sensitivity of conventional cytogenetics and FISH. In light of the aforementioned issues, the present study aimed to determine the frequency of chromosomal anomalies in patients with the diagnosis of MM by analyzing both conventional cytogenetics and FISH results.

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2. Objectives

in this study, the sensitivity of Conventional cytogenetic and FISH methods for identifying genetic abnormalities in MM patients has been investigated and compared.

3. Methods

This study was performed after obtaining the ethics code from the Ethics Department of Iran University of Medical Sciences and the informed consent of patients. This retrospective study included 246 patients who were referred to the Kariminejhad Center for the diagnosis of genetic abnormalities from 2009-2019. All patients were diagnosed based on the laboratory tests (serum electrophoresis, and clinical findings (bone pain, bone lesion, and bone marrow (BM)), as well as the approval of the relevant physician. The diagnosis of MM was made based on the International Myeloma Working Group criteria. Cytogenetic abnormalities were determined based on two methods: conventional cytogenetic and FISH. The inclusion criteria entailed no history of cancer, no bone and kidney disorders, and complete clinical information. On the other hand, the exclusion criteria included kidney failure, bone disorders, and incomplete clinical information.

3.1. conventional cytogenetic

Chromosomal and/or FISH study for Multiple Myeloma was performed on 2-5 mls of fresh BM sample received in heparin tubes. For chromosomal study, the BM sample was incubated in a Falcon tube to Marrowpan culture medium for 24 and 48 h. After incubation, the culture medium was mixed with colcemid solution (GIBCO) at a concentration of 0.1 ug/ml. After centrifugation, it was incubated with 0.075 M KCl hypotonic solution for 15 min. Following that, the sample was mixed with Carnoy solution and discarded after the centrifugation of the supernatant solution. Thereafter, the slides were prepared from the suspension with 2-3 ml of Carnoy solution. ZEISS microscope was used to find the metaphases in the slides (16).

3.2. FISH method

For the FISH study, a BM smear was prepared using a buffy coat by a pathologist and was stained with the wright dye to confirm the adequacy of the plasma cells. In cases with plasma cell count less than 10%, mononuclear cells were isolated using MACS Miltenyl Biotec CD138 Micro Beads human kits. After preparing the plasma cells, fixation and slide preparation of all samples were performed (17).

3.3. Statistical Analysis

To describe the data, we used mean (SD), median, and midrange quartiles in quantitative variables; however, frequency and percentage were used for qualitative variables. We performed all analyses using SPSS software (version 23). A p-value less than 0.05 was considered statistically significant.

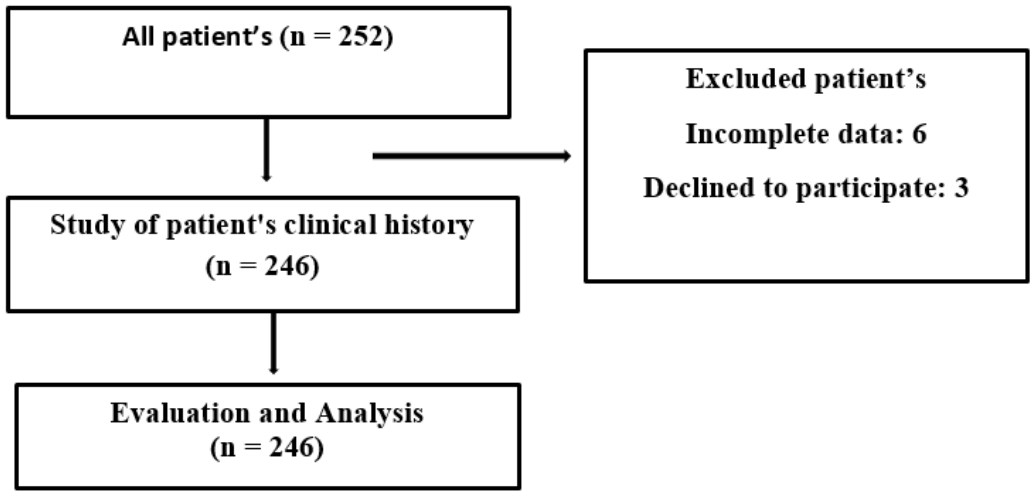

Figure1. Flowchart of patients' selection

4. Results

4.1. Demographical information

In the present study, 252 patients with MM were studied. Six patients were excluded from the study. Out of 246 patients, 147(59.7%) cases were male, and 99 (40.2%) subjects were female. The age range of patients was 25-90 years upon diagnosis, and the mean age was 58.74±13.3. According to Figure 1, all 246 patients who participated in this study underwent conventional cytogenetics. The results illustrated that 44 patients had abnormal karyotypes. In addition to the conventional cytogenetics, out of 246 patients, 67 cases were evaluated by FISH. The

results pointed out that 43 subjects had abnormal FISH. It was also observed that out of 67 patients, 50 cases had normal karyotypes, while out of 50 patients, 31 subjects had abnormal FISH results (Figure 1).

4.2. Frequency of cytogenetic abnormalities based on the number of chromosomes

Cytogenetic abnormalities were examined based on the number of chromosomes. The results suggested that 25% of the studied cases had hyperdiploidy (pseudodiploid or structural abnormality in 23 pairs of chromosomes), and 75% had a non-hyperdiploidy abnormality. Among non-hyperdiploidy anomalies, 40.9% of patients were Diploid, while 25% were Hypodiploidy (Table 1).

4.3. Evaluation of disorders identified by conventional cytogenetic and FISH methods

Cytogenetic abnormalities were examined using two methods: conventional cytogenetic and FISH. The results are displayed in Table 2. The prevalence of all conventional cytogenetic abnormalities was one in 44 samples (2%). The most abnormalities detected by FISH were del 13q (27.9%) and t (11; 14) IGH-CCND1 (18.6%). Abnormalities that had the lowest frequency detected by FISH included t (6; 11), t (8; 14) MYC, and t (6; 14) IGH-CCND3.

4.4. Frequency of abnormal cytogenetic group disorders with normal FISH

Out of 44 patients with abnormalities in conventional cytogenetics, five cases had normal FISH results. Table 3 illustrates the list of abnormalities detected by conventional cytogenetics. Balanced copies of chromosomes 7, 6, and 2 are considered normal in FISH results. Inversion 9 (inv9) was regarded as a normal variation in the karyotype.

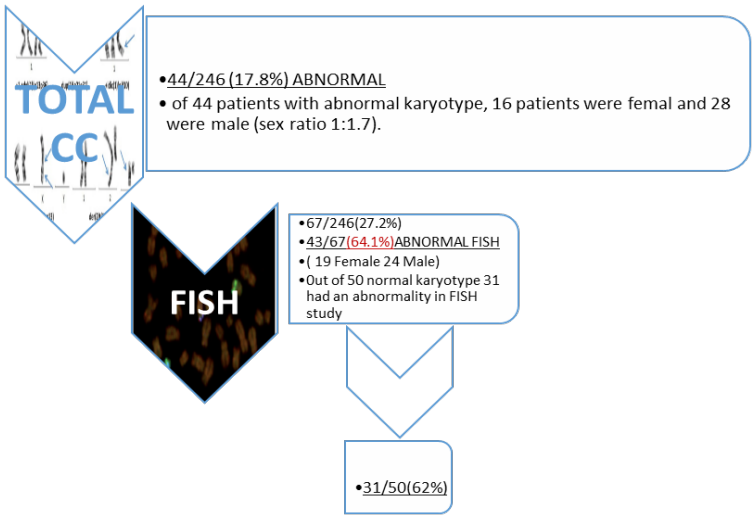

Figure2. scheme of patients' evaluation

Table1. Cytogenetic abnormalities based on the number of chromosomes

Numerical Cytogenetic abnormalities	N (%)
Hyperdiploidy	11.44 (25%)
NONHYPERDIPLOID	75%
Hypodiploidy(=<45)	11.44 (25%)
Near-triploidy(58-80)	3.44 (6.8%)
Tetraploidy	1.44 (2.2%)
Diploid (with structural or numerical abnormalities)	18.44 (40.9%)

Table2. List of abnormal cytogenetic detected by FISH and conventional cytogenetic.

Conventional cytogenetic		FISH	
Translocations	**N(%)**	**Translocations**	**N(%)**
t(6;14)//t(6;11)	1.44 (2%)	del 13q	12.43 (27.9%)
t(3;8)(q10;q10)	1.44 (2%)	del 17p (TP53)	4.43 (9.3%)
t(12;14)(p11.2;p13)	1.44 (2%)	t(11;14)IGH-CCND1	8.43 (18.6%)
t(2;6)(q35;q21),t(6;11)(q22;q23)	1.44 (2%)	t(4;14)IGH-FGFR3	2.43 (4.6%)
t(X;15)(p22;q15),t(8;15)(q10;p10;p15),t(3;14)(p14;q32)	1.44 (2%)	t(6;14)IGH-CCND3	1.43 (2.3%)
t(3;6)(p26;q22)//t(17;19)	1.44 (2%)	t(8;14)MYC	1.43 (2.3%)
t(3;11)(p10;p10),t(17;19)(q22;p13)	1.44 (2%)	t(6;11)	1.43 (2.3%)
t(8;13)(q24;q12)	1.44 (2%)	9q34(ABL) amp	2.43 (4.6%)
t(7;13)(q22;q34)	1.44 (2%)	del 1p	2.43 (4.6%)
t(9;12)(q34;p12),t(20;21)(p10;p10)	1.44 (2%)	Rearrangement IGH 14q32	5.43 (11.6%)
----------	----------	1q gain	4.43 (9.3%)
----------	----------	(15q11.2) (SNRPN)	1.43 (2.3%)

4.5. Frequency of abnormal FISH with normal karyotype

Table 4 presents the abnormal FISH results of patients who had normal Karyotype. In relation to trisomy 7, 9, and 11, 12 patients, and regarding del 13(DLEU1) and del 17p, 10 patients had abnormal FISH. Concerning other cases, only less than 10% of patients had abnormal FISH results.

4.6. Evaluation of sensitivity of FISH and conventional cytogenetic

There was a significant relationship between the obtained results of both mentioned methods. The high sensitivity of the FISH method in identifying cytogenetic disorders, especially hidden types that were not identified in the karyotype, is obvious (Table 5).

Table 3. Frequency of abnormal cytogenetic with normal FISH

Conventional cytogenetic abnormalities	Fish
46,XY,t(1;16)(p21;q24),der(1)t(1;4)(q32;q12),add(2)(q31),-4,-5,del(6)(q13;q23),del(7)(q32),add(10)(q23),add(11)(q21),t(12;14)(p11.2;p13),-13,add(17)(p12)	Balance copies of 7
46,XY,del(6)(q21q23)[3]/46,XY[17]Clonal deletion chromosome 6q	Balance copies of 6
46,XX,per inv(9)(p11q12)	Normal
46, XY, add(4)(q28), add(7)(q31), add(12)(q13)[2]/46, XY[38]Compatible with clonal rearrangement of chromosomes 4, 7 and 12	Balanced copies and no rearrangement of chromosome 2 subtelomeric regions
Loss 7	No loss of 7

Table4. Frequency of abnormal FISH with normal karyotype.

Conventional Cytogenetic NORMAL FISH ABNORMAL GROUP(31/50)	N(%)
1q gain	4.43 (9.3%)
MYC t (8;14)	1.43 (2.3%)
del 13(DLEU1)& del 17p	10.43 (23.2%)
14q32 Rearrengmentand/or deletion	2/43 (4.6%)
Trisomy of 7/9/11 chromosomes	12.43 (27.9%)
t(4;14)	1.43 (2.3%)
Loss 15q(SNRP1)	1.43 (2.3%)

Table 5. Distribution of methods and results test

Items			methods		Total	P-value
			KARYOTYPE	FISH		
RESULT	NORMAL	Count	202	24	226	<0.001
		%	80%	34.8%	72.9%	
	ABNORMAL	Count	44	43	87	
		%	19.3%	64.2%	27.1%	

5. Discussion

The investigation of cytogenetic abnormalities is one of the issues discussed today for different types of cancer, including MM. On the other hand, its identification methods can be of paramount importance (18). The identification of abnormalities can help in designing treatment and better management of patients (14). The results of the present study pointed out that 25% of patients had hyperdiploidy (57-47), which was diagnosed by conventional cytogenetics. Moreover, 40.90% of cases had a diploid abnormality. In the study by Avet-Loiseau et al., hyperdiploidy accounted for about 39% of whole abnormalities (19). In the study by Safavi et al., the prevalence of hyperdiploidy was 53.8% (20). In addition, in the study by Foong, the prevalence of hyperdiploidy was 43.75% (21). This discrepancy in results can be attributed to the number of examined patients, different environmental conditions, as well as the variety of diagnostic kits and materials. It can also be stated that the prevalence of mutations and genetic abnormalities in patients and in different parts of the world is different.

Regarding the abnormalities detected by FISH, the most frequently observed abnormalities were 13q (27.9%) and t(11;14)IGH-CCND1 (18.6%). In the study by Foong et al., the prevalence rates of Biallelic del(13) and Monoallelic del(13) abnormalities were 70.1% and 29.9%, respectively (21). In line with the present study, Avet-Loiseau reported del 13q as the most prevalent abnormality in MM patients (19). Conventional cytogenetic and FISH methods are usually used to investigate chromosomal and genetic abnormalities; most centers also use conventional cytogenetics. This method is more economical in terms of time and cost compared to FISH (22). Nonetheless, recent studies have indicated that the sensitivity of FISH is higher compared to the conventional cytogenetic, and it can identify more abnormalities. On the other hand, it has been shown that the use of these two methods can show different information regarding genetic abnormalities (23).

The results demonstrated that only 17.8% of patients examined by conventional cytogenetics had abnormal results, while this value was obtained at 64.1% in the FISH method. These results suggested that the sensitivity of FISH for identifying genetic abnormalities is higher compared to conventional cytogenetics. Crabtree et al. pinpointed that conventional cytogenetic and FISH methods provided

separate information concerning genetic abnormalities in MM patients; however, in cases where the karyotype was normal, the FISH results were abnormal (24). Kim et al. also implied that the use of conventional cytogenetic and FISH methods in combination with each other could be more effective in the identification of genetic abnormalities in MM patients (23). In the same context, another study illustrated that the FISH method can identify translocations, deletions, and other genetic abnormalities compared to conventional cytogenetics (25). On the other hand, Oh et al. suggested that the use of conventional cytogenetics as a primary tool for identifying genetic abnormalities can be effective (26).

6. Conclusion

As evidenced by the obtained results, the sensitivity of FISH is higher compared to conventional cytogenetics. The FISH method identifies genetic abnormalities that cannot be detected by conventional cytogenetics.

6.1. limitation and future perspective

It is suggested that future studies be conducted on a more extensive statistical population. It is also better to evaluate genetic abnormalities based on the gender and age of patients in future studies.

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Footnotes

Conflicts of Interest: The authors declare that they have no conflict of interest.
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Ethical Statements: This article does not contain any studies with human participants or animals performed by any of the authors. All the procedures performed in the studies involving human participants were in accordance with the ethical standards of the Local Ethics Committee of Iran University of Medical Sciences (IR.IUMS.FMD.REC.1399.607) and the 1964 Helsinki Declaration.

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