PROGNOSTIC IMPACT OF DEL (13q14) AS AN EXAMPLE OF CYTOGENETIC ABNORMALITIES IN NEWLY DIAGNOSED EGYPTIAN MULTIPLE MYELOMA PATIENTS

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Abstract:

Background: A deeper understanding of cancer cells and molecular risk stratification is provided by research into the genetics of MM. There are molecular anomalies that put 20% of patients with newly diagnosed multiple myeloma (NDMM) at high risk. All MM patients are said to have cytogenetic abnormalities, sometimes even as the illness progresses. **Aim:** To assess the prevalence and prognostic importance of del(13q14) detected by interphase Fluorescence In Situ Hybridization (FISH) in Egyptian patients with newly discovered multiple myeloma. **Patients and methods:** The study comprised 66 patients of Multiple Myeloma (MM) with a recent diagnosis. They all gave presentations at the medical oncology clinics at Cairo University's National Cancer Institute (NCI). All cases were subjected to special laboratory investigations (FISH) for detection of del(13q) using 13q Locus specific probes (Vysis). **Results:** Del(13q14) was detected in 13 patients (19.7%) and was associated with significantly worse disease-free survival, but did not impact overall survival. **Conclusion:** FISH technique is preferable for detection of cytogenetic abnormalities.

Keywords: Cytogenetic, MM, FISH.

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INTRODUCTION:

An M protein in the blood and/or urine, as well as signs of organ damage connected to the plasma cell neoplasm, are typically linked with plasma cell myeloma (PCM), a bone marrow (BM)-based multifocal neoplastic proliferation of plasma cells. With a median age of 70 years at diagnosis, it accounts for around 1% of all malignancies and 10% of hematological malignancies ***(1)***.

Currently, High-dose chemotherapy is suggested, followed by autologous hematopoietic stem cell transplantation for patients below the age of 65, with approximately 28% of patients surviving five or more years. Despite this relative treatment success, Multiple Myeloma (MM) is still a fatal condition ***(2)***.

Many prognostic factors for MM have been identified. Chromosomal abnormalities, either numerical or structural, are a key factor in MM risk classification. However, as malignant plasma cells often have a low proliferation index, only 35% of patients present abnormal karyotypes by classical metaphase cytogenetics in advanced stages of the illness. As a result, practice recommendations currently include using interphase fluorescence in-situ hybridization (iFISH) as the initial cytogenetic study for MM ***(3)***.

Multiple myeloma is characterized by the heterogeneity of chromosomal translocations, deletions, duplications, and genetic mutations are examples of genetic anomalies, which reflect the heterogeneity of patients. Numerous cytogenetic marker combinations have been examined.

Hyperdiploidy, loss of 17p, gain of 1q21, and translocations involving the immunoglobulin loci are hallmark abnormalities. ***(3,4)***.

One of the earliest described cytogenetic abnormalities in MM is Up to 50% of individuals with MGUS have monosomy 13 or del(13q), which suggests an early event in the etiology of MM. Early studies proposed that it had an adverse prognostic effect on MM. Still, Later research revealed that the prognostic impact was mostly visible when the anomaly was discovered using traditional karyotyping as opposed to FISH ***(5)***.

While each of these abnormalities has its prognostic importance, the simultaneous presence of several chromosomal aberrations is a strong MM survival prognosis factor ***(2)***.

PATIENTS AND METHODS:

The study comprised 66 patients of Multiple Myeloma (MM) with a recent diagnosis. They all presented to the medical oncology clinics at Cairo University's National Cancer Institute (NCI) from

January 2017 to January 2020. According to the World Health Organization, the diagnosis of MM was based on morphology, chemistry, cytogenetic analysis, and radiography criteria ***(6)***.MM cases who received treatment and non-Egyptians were excluded from the study.

The NCI's ethics committee review board granted approval for this study under Helsinki guidelines for protecting human subjects. All patients provided their written, voluntarily informed consent.

All cases were subjected to the detailed history with an emphasis on age, anemia symptoms, fever, bleeding tendencies, bone aches, and enlarged abdominal organs complete clinical examination with emphasis on lymph nodes, spleen, liver, and any bone lesions, laboratory investigations including complete blood picture, serum protein electrophoresis using Sebia electrophoresis, serum calcium, creatinine, urea, albumin, total protein, LDH and β2-microglobulin using Cobas 6000 and Special laboratory investigations (FISH): detection of del(13q) using 13q Locus specific probes (Vysis).

All patients were followed up by clinically assessing the treatment response and immune electrophoresis at the end of the first treatment cycle.

Response to treatment was categorized as follows:

when plasma cells are in complete remission (CR), BM are < 5% and the absence of an M band in serum electrophoresis and partial response (PR): where the plasma cells in BM < 50% of the baseline and the M band is decreasing > 50% of the quantitative IgG.

Clinical end-points:

The Overall survival (OS) was calculated by censoring for patients who were still alive at the last follow-up and measuring from the date of diagnosis to the date of death. The period from the date of CR to the date of relapse or death from any cause was known as disease-free survival (DFS).

Samples:

The samples were collected at presentation, i.e., before receiving any medications. Two ml of venous blood were withdrawn on ethylene diamine tetraacetic acid (EDTA) (1.2 mg/mL) as an anticoagulant for performing CBC and preparing Leishman-stained PB smears. Two ml of venous blood were withdrawn in a serum tube for performing the chemistry profile, including β2-microglobulin, calcium, creatinine, urea, albumin, and total protein. Bone marrow aspiration and biopsy were performed under complete aseptic conditions. Two milliliters of venous blood were taken in a sodium heparin tube for performing the conventional karyotyping and FISH analysis.

STATISTICAL ANALYSIS:

IBM SPSS® Statistics version 26 (IBM® Corp., Armonk, NY, USA) was used for the statistical analysis. The right way to express numerical data was using the mean, standard deviation, or median and range. Frequency and percentage were used to express qualitative data. The link between qualitative variables was investigated using the Pearson's Chi-square test or the Fisher's exact test. Quantitative data were compared between two groups using the Mann-Whitney test (non-parametric t-test) for data that were not normally distributed or the t-test for data that were normally distributed. The Kaplan-Meier method was used for the survival study, and two survival curves were compared with the log-rank test. A p-value of 0.05 or lower was used for multivariate analysis, and it was deemed significant.

RESULTS:

The study group's average age was 56.2±10.3 years, ranging from 42 to 85 years, 51.5% of them were male (Table 1).

Table (1): Demographic characteristics of the studied group (n=66)

	Value
Age (years)	56.2±10.3
Sex	
Male	34 (51.5%)
Female	32 (48.5%)

The most common presentation was anemia (86.4%), followed by pathological fracture (16.7%), hepatomegaly (3%), lymphadenopathy (3%) and splenomegaly (1.5%) (Table 2).

Table (2): Clinical characteristics of the studied group (n=66)

Pathological fracture	11 (16.7%)
Hepatomegaly	2 (3.0%)
Splenomegaly	1 (1.5%)
Lymph nodes	2 (3.0%)
Anemia	57 (86.4%)

Two monoclonal bands were detected in only four patients (6.1%), while a single monoclonal band was found in the remaining patients. IgG was the most common type of heavy chain detected in serum, and only one patient had IgM heavy chain. M-protein was detected in the urine of only ten patients (Table 3).

Table (3): M-protein components in serum and urine

	Number (%)
Monoclonal Bands	
One	62 (93.9%)
Two	4 (6.1%)
Heavy Chains	
IgG	57 (86.4%)
IgA	6 (9.1%)
IgM	1 (1.5%)
Free Light Chain	2 (3.0%)
M-protein in Urine	
Positive	10 (15.2%)
Negative	56 (84.8%)

Beta2-microglobulin levels were greatly variable between 1.00 and 26.47 µg/mL with a median of 3.82 µg/mL. Albumin levels were within the normal range. Lactate dehydrogenase was elevated (> 280 U/L) in 18 patients (27.3%) (Table 4).

Table (4): Baseline laboratory findings of the studied group

	Value
Beta2-microglobulin (µg/mL)	3.82 (1.00-26.47)
Albumin (mg/dL)	3.43±0.82
Lactate dehydrogenase (U/L)	247.2±72.8
Hemoglobin (gm/dL)	10.0±2.2
Total leukocytic count $x10^3/mm^3$	7.09±2.36
Platelet count $x10^3/mm^3$	249±84
Blood Urea (mg/dL)	35.5 (13-142)
Serum Creatinine (mg/dL)	0.9 (0.5-7.8)
Serum Calcium (mg/dL)	9.5±1.4
Total protein (mg/dL)	8.52±1.96

Table 5 shows no relation between Del(13q14) and all demographic, clinical, and laboratory characteristics of the studied patients.

Table (5): Relation between Del(13q14) with characteristics of the patients in the studied group

		Del(13q14)		
		Positive (n=13)	**Negative (n=53)**	**p-value**
Age (years)		55.5±7.8	56.4±10.9	0.800
Sex	Male	8 (23.5%)	26 (76.5%)	0.420
	Female	5 (15.6%)	27 (84.4%)	
Pathological Fracture	Yes	4 (36.4%)	7 (63.6%)	0.206
	No	9 (16.4%)	46 (83.6%)	
Urine M-protein	Positive	1 (10.0%)	9 (90.0%)	0.672
	Negative	12 (21.4%)	44 (78.6%)	
Modal Chromosome Number	< 46	2 (15.4%)	7 (13.2%)	0.171
	46	6 (46.2%)	37 (69.8%)	
	> 46	5 (38.5%)	9 (17.0%)	
Beta2-microglobulin (µg/mL)		3.5 (1.7-12.2)	4 (1-26.47)	0.524
Total leukocytic count x10^3/mm^3		7.54±1.55	6.98±2.52	0.443
Lactate dehydrogenase (U/L)		268.0±38.6	242.1±78.5	0.096
Albumin (mg/dL)		3.29±1.09	3.46±0.76	0.527

Del(13q14) was detected in 13 patients (19.7%) and was associated with significantly worse disease-free survival, but did not impact overall survival (Table 6,7).

DISCUSSION

The study included 66 patients (34 males and 32 females) with a mean age of 56.2±10.3 years, ranging from 42 to 85. The FISH technique revealed 47 cases (71.2%) with genetic aberrations. Therefore, FISH technique yielded a significant importance value for detecting abnormalities.

An essential tool for assessing the genetic abnormalities of MM is conventional cytogenetic examination of the bone marrow (BM). However, obtaining high-quality metaphases for examination isn't always attainable because of the myeloma's in-vitro hypo-proliferative nature. For 11 patients in the current investigation, cytogenetic analysis was not feasible (16.7%) due to the absence of mitosis. Moreover, some abnormalities are cryptic to be detected by classical cytogenetics, such as t(4;14) and t(14;16) ***(7).***

Molecular cytogenetic methodologies such as FISH, Numerous recurrent chromosomal and genetic alterations in MM have been found using comparative genomic hybridization (CGH), single nucleotide polymorphism (SNP) arrays, and next-generation sequencing (NGS), which can be divided into three types: point mutations, copy number abnormalities (CNAs), and chromosomal translocations ***(8).***

In agreement with the current work, previous studies proved the superiority of FISH techniques in detecting aberrations in MM cases. In a group of 45 NDMM patients, karyotyping detected abnormalities in 75.6% of cases. Incorporating A total detection rate of 91.1% was achieved using FISH. The most frequent abnormalities detected by FISH were IgH gene rearrangements, particularly those involving fibroblast growth factor receptor 3 (FGFR3)/IGH and RB1 deletion/monosomy 13 (43.4%) (Lim et al., 2013). A retrospective study 354 out of 381 individuals with MM who underwent conventional cytogenetic analysis for anomalies had positive results, according to the findings (92.9%). Chromosomal aberrations were detected in 31.9% with conventional cytogenetics, and 45.8% of patients screened with FISH (Aydin et al., 2020). Another study of 93 patients with By utilizing karyotyping, MM discovered structural and numerical abnormalities in 22% of cases, whereas FISH analysis identified abnormalities in 50% of cases ***(9).***

FISH was found to be advantageous in hematological malignancies in general. However, it cannot completely replace conventional cytogenetic study. FISH demonstrated a greater diagnostic yield in 201 patients with hematological malignancies, CC discovered cases in 17.9% of the instances, with a positive result in 39.8% of the cases ***(10).***

It is worth noting that FISH analysis of BM aspirates is technically complex because to the possibility that the neoplastic plasma cells (PCs) only make up a small part of the total nucleus. Many methods have been suggested to get beyond this obstacle. Before performing FISH analysis, some labs purify PCs from the BM aspirate using antibody-based sorting methods. Another method that has been suggested is to first map PCs using May-Grünwald Giemsa stain and image analysis software, then analyze PCs using FISH after they have been previously mapped. In the current study, applying any of these techniques to improve the yield of specimen analysis was not feasible. This might limit the power of detecting all chromosomal aberrations in our sample. Still, Most pathology laboratories don't routinely follow guidelines for cell sorting and image analysis ***(7).***

In the current study, Del(13q14) was detected in 13 patients (19.7%) of the studied group. It was linked to noticeably poorer disease-free survival (p=0.022). On the contrary, it did not impact overall survival (p=0.799).

Zojer et al. (11) used iFISH to ascertain the prevalence and clinical significance of 13q14 deletions in newly diagnosed and relapsed MM patients. They detected a deletion in 48 of 104 patients (46.2%) with ND MM and 11 of 15 patients (73.3%) with relapse of MM ***(21).*** A 13q14 deletion compared to those without the deletion, was linked with a considerably poorer rate of response to conventional-dose chemotherapy and a shorter overall survival. The independent prognostic effect of 13q14 deletions for reduced survival was verified by multivariate analysis.

Li et al. (12) found del(13q14) rate of 51.5% with FISH compared to only a 5% detection rate with conventional cytogenetics. They found that a 13q14 deletion rate > 25% was an independent unfavorable factor **(12)**. Another study in China investigated 100 patients with MM, and they showed chromosome 13q deletions in 24% with conventional cytogenetics and 19% with FISH. Univariate analysis showed that the positive del13 was associated with significantly shorter OS ***(13).***

CONCLUSION:

FISH technique is preferable for detection of cytogenetic abnormalities. Del(13q14) was detected in 13 patients (19.7%) and was unrelated to overall survival but was associated with noticeably lower disease-free survival.

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