Al-Azhar University Journal
for Virus Research and Studies

Analysis of Clonal Cytogenetic Abnormalities in Persistent Cytopenia: Relation of Degree of Dysplasia and Prognosis

Laila M Aboughazala[1], Dina.A. Fouad[2], Dina.I. Badawy[1], Amany.R. Al_agamy* [3]

[1] Department of Medical Clinical Pathology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt.

[2] Department of Medical Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

[3] Department of Medical Clinical Pathology, Sohag Cancer Institute.

***E-mail: amanysherifallam@gmail.com**

Abstract

We aimed to detect clonal cytogenetic abnormalities in persistent cytopenia by fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) to assess their relations with degree of dysplasia and prognosis. The present study is a cohort prospective study that was conducted on 40 patients with persistent pancytopenia from Ain Shams University Hematology/Oncology clinics. The selection criteria included patients with normal karyotype. Fluorescence in situ hybridization technique was applied using routine probes of MDS (LSI 5q31-q33, LSI 7q33, LSI 17p13, LSI 11q23). Molecular analysis using real time PCR was applied for detection of p53 mutation. The present study revealed 18 patients (45%) with normal cytogenetic analysis by FISH. The most common gene deformity detected is del 17p in four cases (10%), followed by del 5q31 (7.5%), del 7q31 (7.5%), and complex chromosomal abnormalities in the form of del 5q31, del 7q31 and del17p in 2 patients (5%), On the other hand, real time PCR analysis for p53 mutation revealed eight cases (20%) positive for P53 mutation, out of them, five cases (12.5%) showed heterozygous mutation and three cases showed homozygous mutation (7.5%). Notably, Patients with poor prognosis had significantly higher frequencies of TP53 mutations (50% versus 0 %, p =0.01). There was no significant association between prognosis and FISH findings. A considerable proportion of the patients with MDS exhibit chromosomal abnormalities using FISH analysis, even if they have normal karyotype. Likewise, we found that TP53 presented a significant prognostic role in patients with MDS. Thus, integrating karyotyping, FISH and PCR analysis for TP53 mutation will add value to the diagnostic workup of suspected patients as MDS.

Keywords: Myelodysplastic syndrome; Persistent cytopenia; Fluorescence in situ hybridization; Cytogenetics.

1. Introduction

Myelodysplastic syndromes (MDS) are a subset of myeloid malignancies with a wide range of natural histories (1). In the United States, three or four people out of 100,000 are diagnosed with MDS (2). The prevalence of the disease rises with age; the rate is seven to 35 per 100,000 people aged 60 and above. Males are more often affected by MDS than females (3). MDS may be triggered by previous chemotherapy or radiation therapy. MDS was previously thought to be caused by uncontrolled apoptosis in the hematopoietic compartment, which resulted in cell death and cytopenia (4).

Several important studies documenting the prevalence and clinical effects of various genetic lesions in MDS have been published in the last decade (5,6). Bejar et al. was the first study on 439 patients that looked at 18 genes using various techniques (7,8). Following that, two large studies analyzed more genes and identified the mutational environment of MDS in greater detail (5). In a cohort of 738 patients, an European consortium used next-generation sequencing technologies to analyze mutations in 111 genes (5). Since then, several studies have been described the mutational environment of MDS and its possible prognostic and therapeutic consequences (9,10). Despite the variability of some of these findings, mutations in genes like RUNX1, TP53, or EZH2 have been linked to a poor prognosis, while mutations in the splicing factor SF3B1 have been linked to a very good prognosis and long survival (10–13). Since the number of mutations and gene interactions is likely to influence prognosis, further evidence is needed to validate and incorporate genomic testing throughout the clinical environment. These new findings would greatly affect our ability to prognosticate MDS patients and their treatment protocols. Owing to this scarcity in the published literature, we here aimed to perform a most detailed investigation of clonal cytogenetic evolution in a large cohort of 40 patients with persistent cytopenias and healthy controls. Our study aimed to detect clonal cytogenetic abnormalities in persistent cytopenia by fluorescence in situ hybridization (FISH) and assess their relations with a degree of dysplasia and prognosis to characterize clonal evolution patterns in detail.

2. Materials and Methods

We confirm that all study's procedures did not violate any of the principles of the Declaration of Helsinki and other regulatory rules. The study's protocol gained the ethical approval of IRB committee of Al-Azhar and Ain Shams Universities.

2.1 Study design:

The present study is a cohort prospective study that was conducted on 40 patients with persistent cytopenia. They were selected from Ain Shams University Hematology/Oncology clinics through the period from January to December 2019. Patients were deemed eligible if they aged more than 18 years old. Other inclusion criteria include persistent unexplained peripheral blood cytopenia for ≥6 months, normal karyotyping, presence of dysplastic features in peripheral blood and /or bone marrow, bone marrow blasts <20%, and negative for t(8;21), t(15;17), and/ or t(16;16)/inv 16 if acute leukemia with blasts <20% is suspected, We excluded patients with post transfusion sustainable recovery from cytopenia.

2.2 Method:

All patients were subjected to detailed history and clinical examination. Two mL of peripheral blood and bone marrow (BM) aspirate were collected from each patient under complete aseptic conditions. The PB was used for CBC and reticulocyte count One ml on EDTA for PCR, and one ml in a sterile preservative free heparin coated

vacutainer for karyotyping and FISH technique. Few drops were used for making BM films stained with Leishman.

All the patients were subjected to complete blood count (CBC) using Sysmex-XT with identification of grade of cytopenia, reticulocyte count, microscopic examination of Leishman's-stained peripheral blood and bone marrow slides for dysplastic features and assessment of blast cell count, Prussian Blue stain for assessment of iron stores and detection of ring sideroblasts.

For detection of p53 abnormalities, FISH was applied using LSI 17p13 probe, and molecular analysis using real timer polymerase chain reaction (PCR) for TP53 mutation. (Figure 3 and 4).

2.3 Patient's Outcomes:

Follow up of all patients was done using CBC, bone marrow, cytogenetic and molecular analysis. The association between the patient's outcome and chromosomal abnormalities detected by FISH and TP53 mutation was studied.

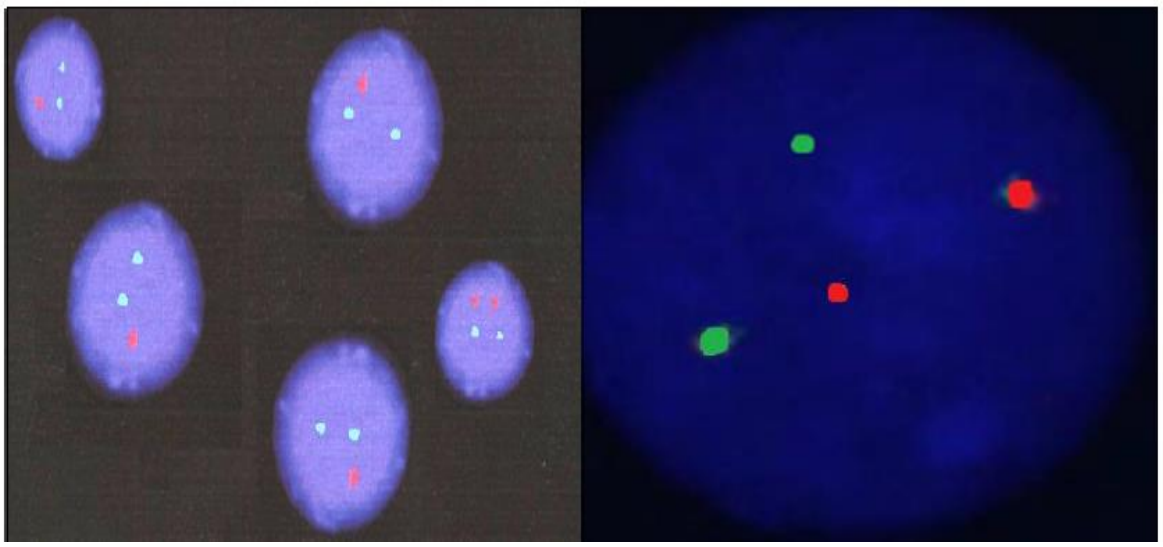
2.4 Statistical analysis:

We employed descriptive quantitative statistics to describe the patients' age, CBC findings, and microscopic examination of peripheral blood and bone marrow slides. While frequencies were used to describe gender, MDS grade, WHO grade of cytopenias, prevalence of FISH abnormalities, presence of TP53 mutation, and prognosis. The hypothesis of significant association between the FISH/TP53 abnormalities with patients' age, CBC findings, and microscopic examination of peripheral blood and bone marrow slides were tested using independent t-test or Mann-Whitney test, based on data distribution. While the hypothesis of significant association between the FISH/TP53 abnormalities gender, MDS grade, WHO grade of cytopenias, and prognosis was assessed with the Chi-square test, with Fisher exact correction when needed. Retrieved data were processed with IBM SPSS statistical software (version 25). P-value <0.05 was regarded as statistically significant.

3. Results

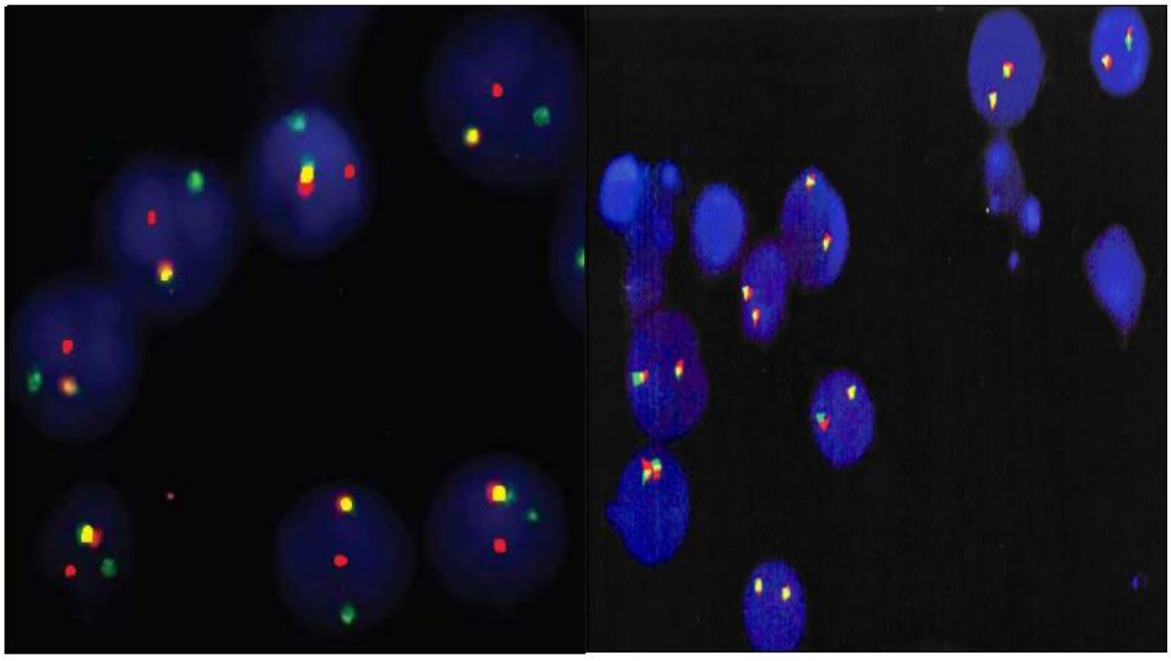
The distribution of all patients was selected from Ain Shams University Hematology/Oncology clinics. The present study was a cohort prospective study that was conducted on 40 patients with persistent cytopenia. The mean age of the included patients was 67 ±7 years old and 80% of the patients were males. The mean hemoglobin level was 7.6± 0.9mg/dL. The mean total leukocyte count (TLC) in our patient was 4± 1.5cell /mm^3. The platelet count showed variations of thrombocytopenia and thrombocytosis with a mean of 127.9± 107.3. Twenty-five patients (62.5%) were graded as grade III. In our patients under study, the mean peripheral blood blast cells were 1 +3. Also under peripheral blood films, we found 20 cases (50%) of our studied patients showed pelger huet cells. Bone marrow slides examined by iron stain (iron stores), 32 of cases showed increase in iron stores (80%). Only five cases showed ring sideroblast. Twenty-nine patients (72.5) classified as MDS with multi-leanage dysplasia (Table 1).

In our patients under study, we found that 22 cases (55%) show normal gene expression by FISH. The most common gene deformity detected is del 17p in four cases (10%), followed by del 5q31 (7.5%), del 7q31 (7.5%), and del5q, del7q, del 17p complex (5%), Figure 3. On the other hand, eight cases (20%) showed P53 mutation, in which five cases (12.5%) showed heterozygous mutation and three cases showed homozygous mutation (7.5%), Figure 4. Concerning the prognosis, four cases (10%) were completely remitted, and twelve patients (30%) gave stable disease with hematological improvement.



(A) **(B)**

Figure (1): A: FISH analysis positive for heterozygous 7q31 deletion identified by the presence of one red signal only representing region 1 band 3 of one copy of chromosome 7, while the other is deleted. The 2 green signals represent the 2 centromeres of both copies of chromosome 7; B: FISH analysis negative for 7q31 deletion identified by the presence of two red signals representing region 1 band 3 of both copies of chromosome 7, The 2 green signals represent the 2 centromeres of both copies of chromosome 7.



(A) **(B)**

Figure (2): A: FISH analysis positive for 11q23 rearrangements identified by the presence of one red, 1 green and 1 yellow; B: FISH analysis negative for 11q23 rearrangements identified by the presence of two yellow signals.

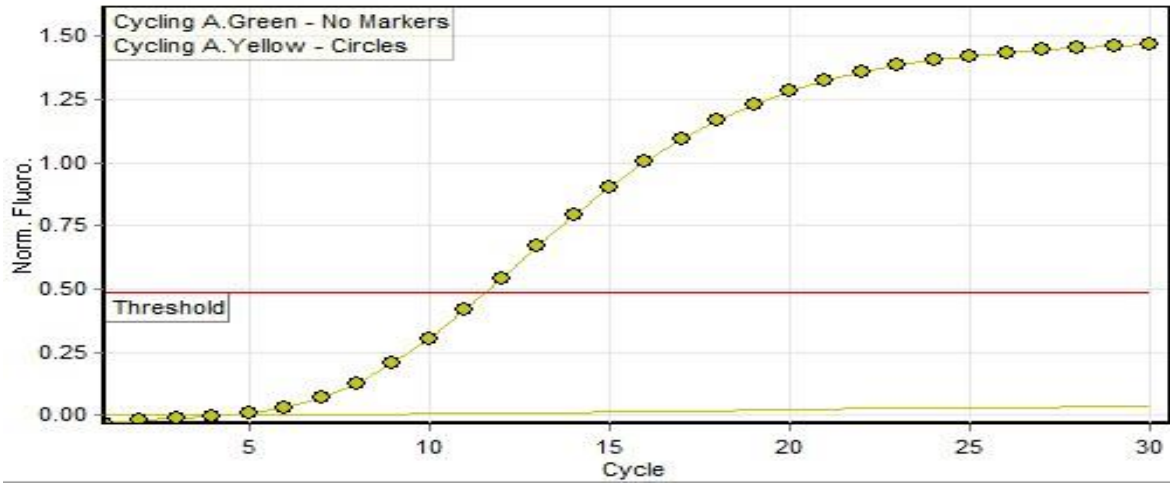


Figure (3): Homozygous mutation of P53 gene detected by real time PCR

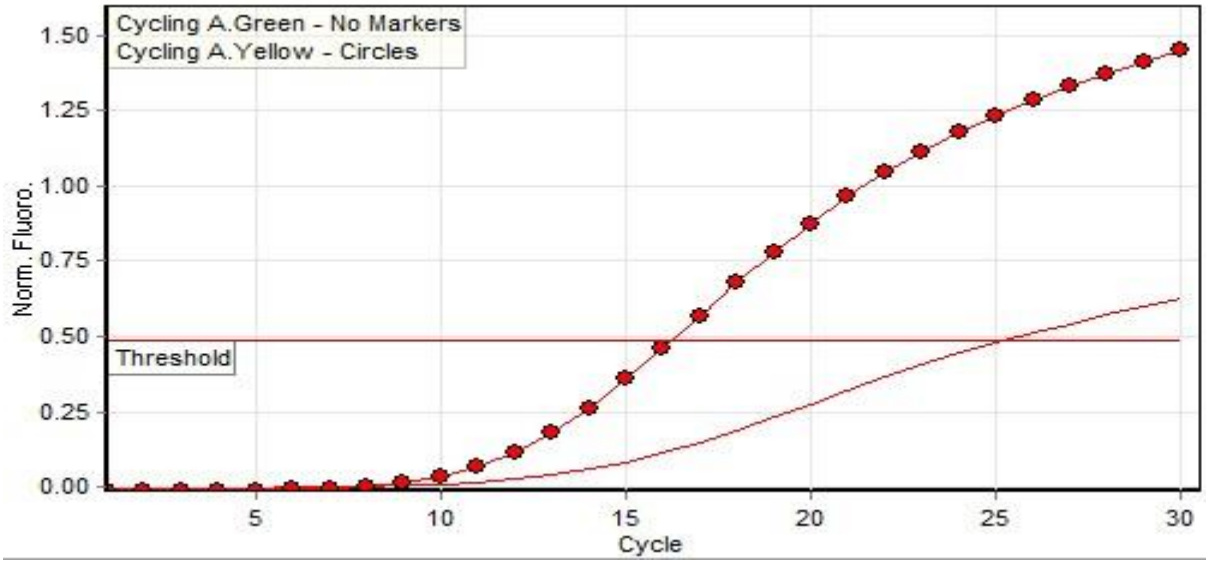


Figure (4): Heterozygous mutation of P53 gene detected by real time PCR

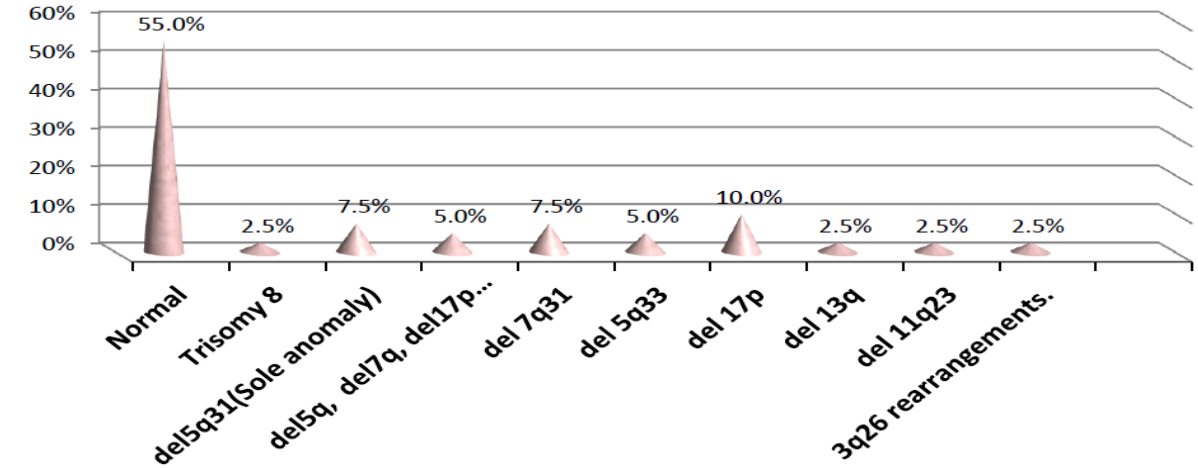


Figure (5): Percentage of gene mutation detected by FISH.

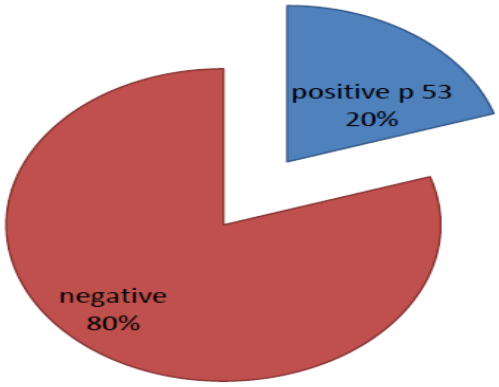


Figure (6): p53 mutation by PCR.

Table (1): Socio-demographic and laboratory parameters of the studied population.

		Cases (40)
Age, mean ±SD		**67 ±7**
Male, No. (%)		**32 (80%)**
HB, mean ±SD		**7.6 ± 0.9**
Total leucocyte count, mean ±SD		**4.0 ± 1.5**
Platelet, mean ±SD		**127.9 ± 107.3**
Grade, No. (%)	**Grade 1**	**10 (25%)**
	Grade 2	**5 (12.5%)**
	Grade 3	**25 (62.5%)**
PB Blasts	**mean ± SD** **Mode (range)**	**1 ± 3** **0 (0-12)**
BM Blasts	**(mean ± SD)** **Mode (range)**	**4 ± 3** **3 (1-15)**
Pelger Huet, No (%)	**Negative**	**20 (50%)**
	Positive	**20 (50%)**
Dysplastic lineages, No. (%)	**1**	**5 (12.5%)**
	2	**12 (30%)**
	3	**23 (57.5%)**
Iron stain (Iron stores), No. (%)	**Negative**	**32 (80%)**
	Positive	**8 (20%)**
Iron Stain Ring sideroblasts	**mean ± SD** **Mode (range)**	**28 ± 11** **19[a] (19-45)**

On the other hand, six cases (15%) did not respond to treatment and other six cases (15%) relapsed after remission. Eight cases (20%) progressed to AML and four cases

(10%) died (bad prognosis). Thus, 60% of cases gave bad prognosis. Patients with poor prognosis had significantly lower TLC (p =0.04), platelets count (p =0.03), pelger huet cells (p =0.05), higher dysplastic lineage (p =0.05), and increased iron stores in bone marrow (p =0.02). Notably, Patients with poor prognosis had significantly higher frequencies of TP53 mutations (50% versus 0 %, p =0.01). There was no significant association between prognosis and FISH findings, Table 2. On the other hand, patients with abnormal FISH analysis had lower peripheral blood blast (p =0.01), BM blast (p =0.02), and increased iron stores in bone marrow (p =0.03), Table 3.

4. Discussion

The role of cytogenetic studies in the assessment of MDS patients has gained momentum over the past few years. The cytogenetic studies can represent a tool in the diagnostic algorithm of MDS and can implicate a prognostic significance. An increased number of studies showed that clonal cytogenetic abnormalities can act as crucial variables in the risk stratification models for MDS (14). Nonetheless, only few studies have assessed the prevalence of clonal cytogenetic abnormalities in MDS and their association with other prognostic indicators. In the present study, we tried to shed the light on the clonal cytogenetic abnormalities in persistent cytopenia by FISH and to assess their relations with degree of dysplasia and prognosis.

The current study was applied on suspected MDS patients with normal karyotyping. Previous studies showed that FISH technique showed higher sensitivity in detecting chromosomal abnormalities than karyotyping, may be referred to expansion of normal clone, cryptic, or submicroscopic chromosomal aberrations. Thus, FISH can play critical role in identifying cytogenetic abnormalities in MDS, even among apparently chromosomally normal patients (15). In the present study, the frequency of FISH abnormalities was 45%. The most commonly encountered abnormalities were del 17p (10%), del5q31(Sole anomaly) (7.5%), del 7q31 (7.5%), and del5q, del7q, del17p (complex) (5%). Such findings highlight that clonal abnormalities defects play a pathogenetic role in the development of MDS, the presence of normal karyotype in patients with persistent cytopenia does not reflect an accurate chromosomal analysis. In line with our findings, Cao and colleagues (16) reported a prevalence of 48% among MDS patients by FISH. The most common of these abnormalities were -5/5q- (15%) and 20q-, +8 (12%). Similarly, Gao and colleagues (17) reported a prevalence of 40% by FISH analysis, mainly in the form of -5/5q- and -7/7q-. In Cai and colleagues (18) and Zakhia and colleagues (19) studies, the prevalence of cytogenetic abnormalities, detected by FISH, was 56.8% and 30%, respectively. Other reports showed a prevalence of 32% (20). Notably, Horiike and colleagues (21) reported a prevalence of 60%. This higher frequency in Horiike and colleagues' study may be attributed to the methodological difference in FISH technique or differences in patients' characteristics.

It is well-established that TP53 plays a detrimental role in the response of the cells to cytotoxic agents. Dysregulated TP53 was found to be closely related to the impaired apoptotic response and unfavorable cellular behavior. TP53 gene defects were also found to increase significantly in patients with hematological malignancies (22). In the present study, we found that the frequency of TP53 mutations was 20%, 12.5% were heterozygous and 7.5% were homozygous mutations. This comes in agreement with Jädersten and colleagues (23), who found a prevalence of 18% in patients with low-risk MDS.

Table (2): Prognosis versus all data collected and laboratory investigations done.

		Good prognosis n= 16 (40%)	poor prognosis n= 24 (60%)	p-*value
Age (mean + SD)		**65.19 + 8.1**	**67.8 ± 5.6**	**0.2**
Gender	**F**	**5 (31.3%)**	**3 (12.5%)**	**0.2**
	M	**11 (68.8%)**	**21 (87.5%)**	
Hb (mean + SD)		**7.6 ± 0.9**	**7.6 ± 1**	**0.8**
Total leucocyte count (mean + SD)		**4.6 ± 1.5**	**3.7 ± 1.4**	**0.04**
Platelet (mean + SD)		**170.4± 151.4**	**99.7 ± 49**	**0.03**
PB Blasts		**0.06 ± 0.25**	**1.54 ± 3.375**	**0.09**
BM Blasts (mean + SD)		**3.06 ±1.0**	**4.1±3.9**	**0.2**
Pelger Huet	**Negative**	**11 (68.8%)**	**9 (37.5%)**	**0.05**
	Positive	**5 (31.3%)**	**15 (62.5%)**	
Dysplastic lineages	**1**	**4 (25.0%)**	**1 (4.2%)**	**0.05**
	2	**6 (37.5%)**	**6 (25%)**	
	3	**6 (37.5%)**	**17 (70.8%)**	
Iron stain (Iron stores)	**Increases store**	**10 (62.5%)**	**22 (91.7%)**	**0.02**
	Normal	**6 (37.5%)**	**2 (8.3%)**	
Iron Stain Ring sidero-blasts (mean + SD)		**28 ± 14**	**27 ± 6**	**0.9**
FISH	**Abnormality**	**6 (37.5%)**	**12 (50%)**	**0.5**
	Normal	**10 (62.5%)**	**12 (50%)**	
PCR	**Positive p53 mutation**	**0 (0%)**	**8(33.3%)**	**0.01**
	Negative p53 mutation	**16 (100%)**	**16 (66.7%)**	

Table (3): All data collected and laboratory finding versus chromosomal patterns.

		Chromosomal abnormality N= 18	Normal N=22	p-value
Age		**66 ± 7**	**67 ± 6**	**0.6**
Gender	**F**	**4(22.2%)**	**4(18.2%)**	**0.7**
	M	**14(77.8%)**	**18(81.8%)**	
Hb (mean + SD)		**7.7 ± 1**	**7.6 ± 0.8**	**0.7**
Total leucocyte count (mean + SD)		**3.7± 1.3**	**4.4 ± 1.6**	**0.1**
Platelet (mean + SD)		**139.3 ± 153.4**	**118.6±45.9**	**0.5**
PB Blasts (mean + SD)		**2.06 ±3.780**	**0.05±0.213**	**0.01**
BM Blasts (mean + SD)		**5± 4**	**3 ± 1**	**0.02**
Pelger Huet	**N**	**7(38.9%)**	**13(59.1%)**	**0.2**
	Positive	**11(61.1%)**	**9(40.9%)**	
Dysplastic lineages	**1**	**2(11.1%)**	**3(13.6%)**	**0.9**
	2	**5(27.8%)**	**7(31.8%)**	
	3	**11(61.1%)**	**12(54.5%)**	
Iron stain (Iron stores)	**IS**	**17(94.4%)**	**15(68.2%)**	**0.03**
	N	**1(5.6%)**	**7(31.8%)**	
Iron Stain Ring sideroblasts		**27 ± 6**	**28 ± 14**	**0.9**
Prognosis	**Good**	**6 (37.5%)**	**12 (50%)**	**0.5**
	Poor	**10 (62.5%)**	**12 (50%)**	
PCR	**Positive p53 mutation**	**6 (33.3%)**	**2 (9.1%)**	**0.05**
	Negative p53mutation	**12 (66.7%)**	**20 (90.9%)**	

Likewise, Belickova and colleagues (24) reported that the TP53 mutations were found in 13% of MDS patients. Other reports showed a prevalence of 17% (25) and 9.4% (26). In a large cohort of 3324 MDS patients, it was found that 378 individuals (11.3%) had TP53 mutations (27). The overall higher incidence in our study is possibly due to the sensitive technique and also sequencing of the whole gene.

Cytogenetic abnormalities in FISH analysis carry significant prognostic implications in wide range of hematological and non-hematological conditions. Previous reports showed that cytogenetic abnormalities in FISH analysis were correlated with relapse, progression, and shorter overall survival (28). However, the present study although there was no significance associated between prognosis and FISH findings. We found that :(del 17p) four cases (10%) showed bad prognosis, (del 5q31) three cases (7.5%) showed good prognosis, (del 7q31) three cases (7.5%) showed bad prognosis, two cases express (del5q, del7q, del 17p complex) (5%) showed poor prognosis, (del 5q33) Two cases (2%) show good prognosis.

Also, Trisomy 8 (good prognostic), del13q (bad prognostic), (del11q23) bad prognostic ,3q26 rearrangements (bad prognostic), that each of them was expressed in only one case (2.5% for each type). This may be referred to several or versed types and subtypes of FISH results in correlation to the number of patients under study. Such findings are similar to the previous reports by Zakhia and

colleagues (19) and Makishima and colleagues (29).

A growing body of evidence showed that TP53 mutations carry a significant prognostic role and its correlated with poor outcomes, mainly due to its dysregulation impacts on cell cycle and apoptosis (22,26). In the present study, we found a statistically significant association between TP53 mutation and poor prognosis. All patients with TP53 mutation had poor prognosis, compared to 50% of the patients without mutations. In concordance with our findings, Haase and colleagues (30) reported that TP53 mutations were independent predictors of poor survival. According to Sebaa and colleagues (25), TP53 mutations were associated with shorter survival. Similar findings were reported by Kulasekararaj and colleagues (26), Duarte and colleagues (31), Kim and colleagues (32), and Kita-Sasai and colleagues (33). In a large cohort study of 3307 patients, it was found that TP53 mutations were independent predictors of poor survival (34). Another cohort over 3324 patients noted similar results (27). A reduced expression of *TP53* after phosphorylation presumably indicated impaired downstream signaling of DNA damage and/or defects in downstream components of the DDR (22).

5. Conclusion:

In conclusion, a considerable proportion of the patients with MDS exhibit chromosomal abnormalities using FISH analysis, even if they have previous normal karyotype analysis. Likewise, we found that TP53 posed a significant prognostic role in patients with MDS. Thus, integrating chromosomal analysis by FISH and TP53 mutation analysis by PCR should add great value in the diagnostic workup of suspected MDS patients. Moreover, patients with TP53 abnormalities should be considered for alternative management strategies, since they are at higher risks of unfavorable prognosis.

Funding: This work didn't receive any fund from any authority and totally funded by the authors.

Conflict of interest: Authors have nothing to disclose.

References

1. Raskovalova T, Jacob M-C,and Park S. Myelodysplastic Syndromes. Vol. 383, The New England journal of medicine. United States; 2020. p. 2590.

2. Rollison DE, Howlader N, Smith MT, Strom SS, Merritt WD, Ries LA, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. Blood. 2008 Jul;112(1):45–52.

3. Goldberg SL, Chen E, Corral M, Guo A, Mody-Patel N, Pecora AL, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol Off J Am Soc Clin Oncol. 2010 Jun;28(17):2847–52.

4. Raza A, Gezer S, Mundle S, Gao XZ, Alvi S, Borok R, et al. Apoptosis in bone marrow biopsy samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. Blood. 1995 Jul;86(1):268–76.

5. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013 Nov;122(22):3616–27; quiz 3699.

6. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241–7.

7. Bejar R, Levine R,and Ebert BL. Unraveling the molecular pathophysiology of myelodysplastic syndromes. J Clin Oncol Off J Am Soc Clin Oncol. 2011 Feb;29(5):504–15.

8. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical Effect of Point Mutations in Myelodysplastic Syndromes. N Engl J Med. 2011 Jun;364(26):2496–506.

9. Thol F, Kade S, Schlarmann C, Löffeld P, Morgan M, Krauter J, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood. 2012 Apr;119(15):3578–84.

10. Malcovati L, Papaemmanuil E, Bowen DT, Boultwood J, Della Porta MG, Pascutto C, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011 Dec;118(24):6239–46.

11. Takahashi K, Patel K, Bueso-Ramos C, Zhang J, Gumbs C, Jabbour E, et al. Clinical implications of TP53 mutations in myelodysplastic syndromes treated with hypomethylating agents. Oncotarget. 2016 Mar;7(12):14172–87.

12. Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jädersten M, Jansson M, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015 Jul;126(2):233–41.

13. Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015 Feb;518(7540):552–5.

14. Tang G, Medeiros LJ, and Wang SA. How I investigate Clonal cytogenetic abnormalities of undetermined significance [Internet]. Vol. 40, International Journal of Laboratory Hematology. Blackwell Publishing Ltd; 2018 [cited 2021 May 15]. p. 385–91. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/ijlh.12826

15. Bernasconi P, Cavigliano PM, Boni M, Calatroni S, Klersy C, Giardini I, et al. Is FISH a relevant prognostic tool in myelodysplastic syndromes with a normal chromosome pattern on conventional cytogenetics? A study on 57 patients. Leukemia [Internet]. 2003 Aug 14 [cited 2021 May 15];17(11):2107–12. Available from: www.nature.com/leu.

16. Cao P, Li Y, Li X, Zhang G,and Chen F. Detecting chromosomal

aberrations in myelodysplastic syndrome with fluorescence in situ hybridization and conventional cytogenetic analysis. J Cent South Univ (Medical Sci [Internet]. 2014 [cited 2021 May 15];39(6):605–11. Available from: https://pubmed.ncbi.nlm.nih.gov/25011965.

17. Gao DG, Li BT, Zhou LN, Chen H,and Zhang B. [Cytogenetic abnormalities of 50 MDS patients by FISH detection and conventional karyotype analysis]. Zhongguo Shi Yan Xue Ye Xue Za Zhi [Internet]. 2013 [cited 2021 May 15];21(5):1190–4. Available from: https://pubmed.ncbi.nlm.nih.gov/24156432.

18. Cai Y, Qin Y wen, Wang C, Yang J, and Yan S ke. Detection of cytogenetic abnormalities involving chromosomes 5,7 and 8 in myelodysplastic syndromes with fluorescence in situ hybridization and its clinical significance. Zhonghua Xue Ye Xue Za Zhi. 2007;28(1):6–10.

19. Zakhia D, Voronel O, Zaiem F, Raval K, Yang J, Schloff D, et al. Comparative assessment of conventional chromosomal analysis and fluorescence in situ hybridization in the evaluation of suspected myelodysplastic syndromes: A single institution experience. Avicenna J Med [Internet]. 2019 [cited 2021 May 15];9(2):55. Available from: /pmc/articles/PMC6530274.

20. Rigolin GM, Bigoni R, Milani R, Cavazzini F, Roberti MG, Bardi A, et al. Clinical importance of interphase cytogenetics detecting occult chromosome lesions in myelodysplastic syndromes with normal karyotype. Leukemia [Internet]. 2001 Nov 29 [cited 2021 May 15];15(12):1841–7. Available from: www.nature.com/leu.

21. Horiike S, Taniwaki M, Misawa S,and Abe T. Chromosome abnormalities and karyotypic evolution in 83 patients with myelodysplastic syndrome and predictive value for prognosis. Cancer. 1988;62(6):1129–38.

22. Cumbo C, Tota G, Anelli L, Zagaria A,and Specchia G, Albano F. TP53 in myelodysplastic syndromes: Recent biological and clinical findings [Internet]. Vol. 21, International Journal of Molecular Sciences. MDPI AG; 2020 [cited 2021 May 16]. Available from: /pmc/articles/PMC7279310.

23. Jädersten M, Saft L, Smith A, Kulasekararaj A, Pomplun S, Göhring G, et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol. 2011;29(15):1971–9.

24. Belickova M, Vesela J, Jonasova A, Pejsova B, Votavova H, Merkerova MD, et al. TP53 mutation variant allele frequency is a potential predictor for clinical outcome of patients with lower-risk myelodysplastic syndromes. Oncotarget. 2016;7(24):36266–79.

25. Sebaa A, Ades L, Baran-Marzack F, Mozziconacci MJ, Penther D, Dobbelstein S, et al. Incidence of 17p deletions and TP53 mutation in myelodysplastic syndrome and acute

myeloid leukemia with 5q deletion. Genes Chromosom Cancer [Internet]. 2012 Dec [cited 2021 May 16];51(12):1086–92. Available from:https://pubmed.ncbi.nlm.nih.gov/22933333.

26. Kulasekararaj AG, Smith AE, Mian SA, Mohamedali AM, Krishnamurthy P, Lea NC, et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol. 2013;160(5):660–72.

27. Bernard E, Nannya Y, Hasserjian RP, Devlin SM, Tuechler H, Medina-Martinez JS, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat Med. 2020;26(10):1549–56.

28. Salido M, Baró C, Oscier D, Stamatopoulos K, Dierlamm J, Matutes E, et al. Cytogenetic aberrations and their prognostic value in a series of 330 splenic marginal zone B-cell lymphomas: A multicenter study of the Splenic B-Cell Lymphoma Group. Blood [Internet]. 2010 Sep 2 [cited 2021 May 25];116(9):1479–88. Available from:http://ashpublications.org/blood/articlepdf/116/9/1479/1490466/zh803510001479.pdf.

29. Makishima H, Rataul M, Gondek LP, Huh J, Cook JR, Theil KS, et al. FISH and SNP-A karyotyping in myelodysplastic syndromes: Improving cytogenetic detection of del(5q), monosomy 7, del(7q), trisomy 8 and del(20q). Leuk Res [Internet]. 2010 Apr [cited 2021 May 25];34(4):447–53. Available from: /pmc/articles/PMC2826525.

30. Haase D, Stevenson KE, Neuberg D, Maciejewski JP, Nazha A, Sekeres MA, et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. Leukemia [Internet]. 2019 Jul 1 [cited 2021 May 16];33(7):1747–58. Available from: https://doi.org/10.1038/s41375-018-0351-2.

31. Duarte FB, Gonçalves RP, Barbosa MC, Rocha Filho FD, De Jesus Dos Santos TE, Dos Santos TN, et al. Tumor suppressor p53 protein expression: Prognostic significance in patients with low-risk myelodysplastic syndrome. Rev Bras Hematol Hemoter [Internet]. 2014 May 1 [cited 2021 May 16];36(3):196–201. Available from: http://dx.doi.org/10.1016/j.bjhh.2014.03.0071516-8484.

32. Kim YJ, Jung SH, Hur EH, Choi EJ, Lee KH, Yim SH, et al. TP53 mutation in allogeneic hematopoietic cell transplantation for de novo myelodysplastic syndrome. Leuk Res. 2018 Nov 1;74:97–104.

33. Kita-Sasai Y, Horiike S, Misawa S, Kaneko H, Kobayashi M, Nakao M, et al. International prognostic scoring system and TP53 mutations are independent prognostic indicators for patients with myelodysplastic syndrome. Br J Haematol [Internet]. 2001 [cited 2021 May 16];115(2):309–12. Available from: https://pubmed.ncbi.nlm.nih.gov/11703325.

34. Stengel A, Kern W, Haferlach T, Meggendorfer M,and Fasan A, Haferlach C. The impact of TP53 mutations and TP53 deletions on survival varies between AML, ALL, MDS and CLL: An analysis of 3307 cases. Leukemia. 2017;31(3):705–11.