

Clinicohaematological Characteristics, Cytogenetic Profile, and Risk Stratification in Myelodysplastic Syndrome: A Study from Pakistan

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ABSTRACT

Objective: To determine the clinicohaematological characteristics, cytogenetic abnormalities, and risk profiles of treatment-naive Pakistani myelodysplastic syndrome (MDS) patients.

Study Design: Descriptive study.

Place and Duration of the Study: Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from June 2019 to 2023.

Methodology: MDS was diagnosed following the World Health Organization (WHO) criteria, with detailed documentation of clinicohaematological parameters and cytogenetic findings. Risk assessment was done using the Revised International Prognostic Scoring System (IPSS-R). Descriptive statistics summarised the patient characteristics, while Chi-square and parametric or non-parametric tests facilitated comparisons. Survival analysis utilised Cox proportional hazard models and Kaplan-Meier survival curves.

Results: A total of 47 MDS patients were assessed, with a median age of 66 years (IQR = 20) and a male predominance (68%). Anaemia (haemoglobin <10 g/dL) was the most frequent presentation, observed in 95.7% of patients. MDS with multilineage dysplasia was the most common subtype, diagnosed in 59.6% of cases. Cytogenetic analyses revealed a normal karyotype in 55.3% of patients, while 44.7% revealed clonal abnormalities, including trisomy 8, monosomy 7, and complex karyotypes. Risk stratification identified 40.4% of patients as low-risk at presentation.

Conclusion: Cytogenetic analysis showed that a normal karyotype was the most prevalent finding, with low-risk disease predominating in risk stratification. These findings provide valuable insights into the clinicohaematological and cytogenetic profiles of MDS patients in the Pakistani population.

Key Words: Myelodysplastic syndrome, Cytogenetics, Revised international prognostic scoring system, World Health Organization, Risk stratification.

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INTRODUCTION

The myelodysplastic syndromes (MDS) comprise haemopoietic clonal disorders of stem cells that include clinically and biologically different groups of malignancies.¹ Although these conditions predominantly affect the elderly, they can also occur in adolescents and children.² The prevalence of familial MDS cases is rare.³

MDS is characterised by ineffective haematopoiesis leading to chronic cytopenia, dysplastic morphology of the blood and bone marrow cells, increase in the blast cells, and the possibility of development of acute myeloid leukaemia (AML).^{3,4} The prevalence of MDS tends to increase with age and is found to be marginally more common in males.⁵

The patients having MDS most commonly present with clinical signs of pallor, weakness, repeated infections, and bleeding.⁶ The diagnosis of MDS primarily depends upon identifying the morphological evidence of dysplasia through microscopic evaluation of blood smear and bone marrow.⁷ Karyotyping, flow cytometry, or molecular genetic testing serve as an important tool in confirming and refining the diagnosis.^{3,6} Primary MDS, which accounts for approximately 80% of all cases, arises spontaneously without a known cause.^{6,7} In contrast, secondary MDS is linked to more complex chromosomal abnormalities and often requires more intricate treatments. Cytogenetic abnormalities are observed in over 50% of MDS patients.⁸

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The 2016 classification guidelines of WHO categorise six categories of MDS with single lineage dysplasia (MDS-SLD), MDS with multilineage dysplasia (MDS-MLD), MDS with ring sideroblasts (MDS-RS), MDS with isolated del (5q), MDS with excess blasts 1 (MDS-EB1), MDS with excess blasts 2 (MDS-EB2), and MDS unclassifiable (MDS-U).⁹ In addition, another type of MDS named as “refractory cytopenia of childhood” has also emerged. MDS-EB is also sub-classified as MDS with excess blasts-1 (MDS-EB-1) and MDS with excess blasts-2 (MDS-EB-2).^{9,10} The 2016 classification of MDS is established according to the results of blood and bone marrow tests.

The present study aimed to characterise the spectrum of demographic, clinical, laboratory haematology, cytogenetic, and survival features in Pakistani MDS patients presenting at a single centre.

METHODOLOGY

The study protocol was reviewed and approved by the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, through its Ethical Review Committee in accordance with the Declaration of Helsinki’s (2013) updated ethical guidelines. Written informed consent was acquired from each of the MDS patients before enrolment into the study and sample collection.

This study spanned a duration of four years, starting from June 2019 till 2023, including enrolment, analysis, and follow-up time. During the study period, a total of 47 Pakistani patients of all ages and both genders with newly diagnosed MDS, who presented at the Department of Haematology, Institute of Pathology, were recruited into this study. Inclusion criteria involved recently diagnosed patients of MDS on supportive therapy with no history of chemotherapy. The exclusion criteria included patients having megaloblastic anaemia and a history of chemotherapy / radiotherapy. The diagnosis of MDS patients and different risk groups was based on clinical and laboratory evaluations.^{9,11} All the patients were of Pakistani origin.

Keeping the signs and symptoms in account, a detailed history was taken, and a complete physical examination was conducted and documented. Complete blood count, peripheral blood film, and bone marrow examination were performed, and the patients were diagnosed with MDS based on the WHO criteria.⁷

The cytogenetic analysis was carried out with the traditional G banding method and karyotypes were determined after analysis of at least 20 metaphase chromosomes using the CytoVision semi-automated image analysis and capture system.

The patient characteristics were summarised using descriptive statistics. Qualitative variables (e.g. gender) were expressed in the form of frequencies (percentages). The normality of quantitative variables was assessed using Shapiro-Wilk and Kolmogorov-Smirnov tests, where quantitative variables with normal distribution were given in the form of mean and standard deviation, while those with skewed distribution were described as median and interquartile range (IQR). The statistical comparison of quantitative data between different

patient groups involved the use of parametric (Student’s t-test) or non-parametric (Mann-Whitney U test) for quantitative variables. All p values were two-sided and considered statistically significant when <0.05 . The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 24 for Windows and GraphPad Prism 8.0.

By means of routine clinical visits, all recruited MDS patients were followed up from the time of diagnosis till the conclusion of the study and overall survival (OS) in each patient was documented. The prognostic significance of demographic (age and gender), clinical (WHO risk category), laboratory (haemoglobin levels, TLC, and platelet count), and cytogenetic variables in determining OS were explored using univariate and multivariate Cox proportional hazard models. The survival curves were plotted using Kaplan-Meier estimates and log-rank statistics were used to evaluate differences between potential prognostic factors (including age groups, gender, haemoglobin level, TLC and platelet counts, and WHO risk categories) and OS curves.

RESULTS

In this study, 47 MDS cases from Pakistan were examined categorically for clinicopathological and cytogenetic changes. The baseline characteristics of MDS patients in this study are presented below. The median age of MDS patients was 66 years (range 24-87 years) with 30 (63.8%) patients older than 60 years. Gender distribution showed a male predominance with 32 (68.1%) males and a male-to-female ratio of 2.1:1. The median age for female patients was 70 years (range 53-80 years), while for male patients it was 64.5 years (range 24-87 years). The most common presenting clinical feature was pallor (95%), followed by symptoms of fatigue (92%), recurrent infections/fever (24.5%), and bruising/bleeding (11%). Most of the patients (65.9%) were transfusion-dependent at the time of presentation. The laboratory and cytogenetic characteristics of MDS patients are summarised in Table I.

Regarding MDS WHO type and risk category, high-risk WHO types (MDS EB1 + EB2) and category were observed in 16 (34%) of the patients. Based on the WHO category for diagnosis, the majority of the patients were found to be in the MLD subgroup ($n = 28$, 59.6%), followed by MDS-EB2 ($n = 11$, 23.4%) and MDS-EB1 ($n = 5$, 10.6%). Considering the risk category, very good risk category was observed for 4.3% of patients, followed by good (61.7%), intermediate (19.1%), and poor (6.4%). The WHO group risk stratification for MDS showed low risk in 40.4% of patients, followed by intermediate risk in 25.5%, high risk in 21.2% of patients, and very high risk in another 8.5% of patients. Association of laboratory parameters of the WHO MDS risk groups was also analysed, which indicated significantly lower haemoglobin levels [Hb (g/dL) = 7.30 vs. 8.01, $p = 0.045$] and platelet counts [platelets ($\times 10^9/L$) = 45 vs. 78, $p = 0.013$] in the high-risk WHO group as compared to the low-risk group.

Cytogenetic analysis showed normal karyotypes in 27 (57.4%) patients, while 20 (42.6%) showed karyotype abnormalities, including trisomy 8 (14.9%), monosomy 7 (6.4%), and complex karyotype (6.4%). However, karyotypes associated with a dismal survival outcome (poor + very poor) were evident in only 14.9% of MDS patients (Table I, Figure 1).

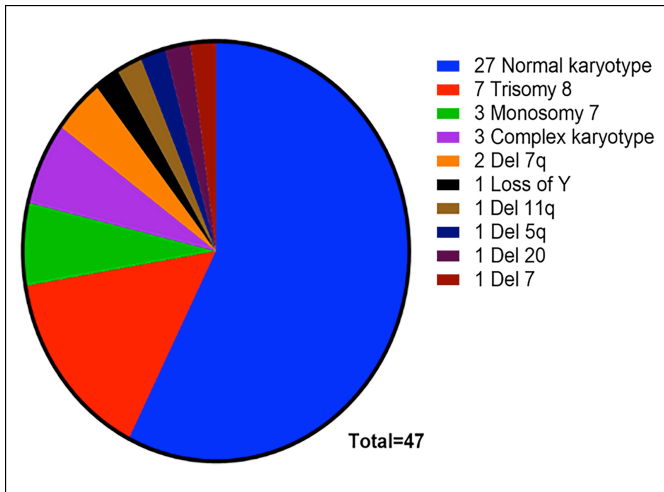


Figure 1: Cytogenetic characteristics of MDS patients. Normal karyotype is the most prevalent, followed by trisomy 8, monosomy 7, and complex karyotype.

Table I: Laboratory and cytogenetic parameters of the MDS patients.

Parameters	Frequency (n, %) / Median (IQR) / Mean ± SD
Haemoglobin (g/dL)	7.77 ± 1.16
Mean ± SD	
<10 g/dL	02 (4.3%)
<10 g/dL	45 (95.7%)
Total leucocyte count (x 10 ⁹ /L)	
Median (IQR)	6.5 (8.6)
>4 x 10 ⁹ /L	33 (70.2%)
<4 x 10 ⁹ /L	14 (29.8%)
Platelets (x 10 ⁹ /L)	
Median (IQR)	77 (77)
≥50 x 10 ⁹ /L	33 (70.2%)
<50 x 10 ⁹ /L	14 (29.8%)
Blasts (%)	
Median (IQR)	5 (6.5)
PB <1%, BM <5%	31 (65.9%)
PB 2-4%, BM 5-9%	05 (10.6%)
PB5-19%, BM 10-19%	11 (23.4%)
WHO category, n (%)	
MDS-MLD	28 (59.6%)
MDS-RS-MLD	03 (6.4%)
MDS-EB1	05 (10.6%)
MDS-EB2	11 (23.4%)
Cytogenetics risk category, n (%)	
Very good	02 (4.3%)
Good	29 (61.7%)
Intermediate	08 (19.1%)
Poor	05 (8.5%)
Very poor	03 (6.4%)
Risk category, n (%)	
Very low	02 (4.2%)
Low	19 (40.4%)
Intermediate	12 (25.5%)
High	10 (21.2%)
Very high	04 (8.5%)

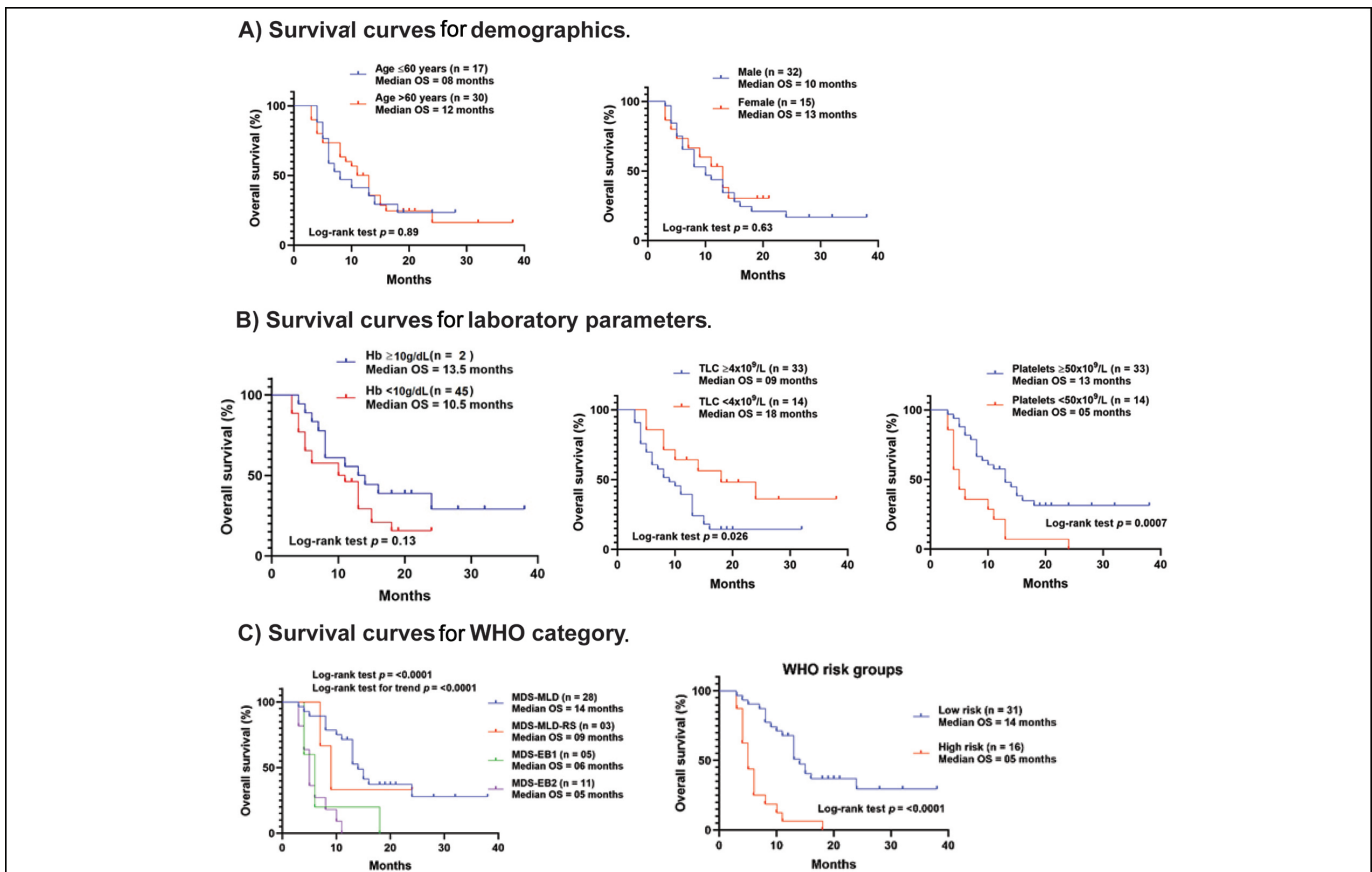


Figure 2: Overall survival curves with reference to (A) Demographics, (B) Laboratory parameters, and (C) WHO risk categories in MDS patients.

Table II: Prognostic factors for overall survival in Pakistani MDS patients.

Variables	Univariate		Multivariate	
	HR (95% CI)	p-value*	HR (95% CI)	p-value*
Age	0.99 (0.97-1.01)	0.47	-	-
Gender (female)	0.84 (0.40-1.76)	0.65	-	-
Haemoglobin (<10 g/dL)	2.96 (0.40-21.76)	0.29	-	-
Platelets (<50 x10 ⁹ /L)	2.98 (1.44-6.17)	0.003	2.98 (1.40-6.30)	0.004
WHO risk category (high-risk)	4.87 (2.38-9.96)	<0.001	4.97 (2.27-10.85)	<0.001
Cytogenetic risk category (high + very high)	2.36 (0.92-6.05)	0.07	1.21 (0.46-3.23)	0.70

*p-values from univariate or multivariate Cox-regression analyses.

The median survival of Pakistani MDS patients was recorded as 11 months (range: 3-38 months). Cox proportional hazard analyses were performed, and Kaplan-Meier survival curves were constructed to evaluate the prognostic impact of relevant demographic, clinical, laboratory, and cytogenetic parameters on the two-year overall survival of patients in follow-up. The results of the Cox proportional hazard analysis are given in Table II, where the overall analysis reflected thrombocytopenia and the WHO high-risk category as significant determinants of poor clinical outcomes in MDS patients, which also persisted in multivariate analysis. In contrast, the cytogenetic risk category of poor and very poor showed only a marginally significant association which did not persist in the multivariate analysis.

With respect to the survival curves of demographics, age >60 years showed higher median OS rates as compared to the alternative groups; however, these differences were not statistically significant. With respect to the survival curves of relevant clinical and laboratory parameters, patients with thrombocytopenia displayed a dismal median survival of only five months as compared to MDS patients without it. Similarly, survival curves of different WHO MDS categories resulted in significant differences where a trend of decreasing OS was evident for high-risk WHO MDS types (p = <0.001) having OS of only 5 months for MDS-EB2 category (Figure 2).

DISCUSSION

Myelodysplastic syndromes primarily affect haematopoiesis and lead to morphological dysplasia and cytopenia. For demographics, the median age of the patients was 66 (26-87) years in the present study. Rashid *et al.*¹² reported 60 years, Sultan and Irfan¹³ reported 64 years, Narayanan¹⁴ reported 67 years, Voso *et al.*¹⁵ reported 71 years, and Greenberg *et al.*¹⁶ also reported 71 years of age. These local and international studies showed similar results as shown in this study. The present study showed a male-to-female ratio of 2.1:1 which is the same as reported by Greenberg *et al.*¹⁶

The most common presenting clinical finding was pallor in patients (mean haemoglobin 7.82g/dL), followed by fatigue seen in the present study having the same findings as those reported by Chaubey *et al.*¹⁷ in the Indian population while a local study conducted by Sultan and Irfan have reported

mean haemoglobin of 7.7 gm/dL which is comparable with the present study.¹³ A study conducted by Voso *et al.* reported 9.9g/dL which is higher than the local studies.¹⁵ This might be because tertiary care medical facilities are not readily available to the patients in Pakistan because it is an underdeveloped country.

Among the presently reported MDS patients, 65.9% were transfusion-dependent at the time of presentation. A similar frequency of 66% was reported by Mahmood *et al.*⁸ 58% by Voso *et al.*¹⁵ while Greenberg *et al.*¹⁶ reported 32% of the patients to be transfusion-dependent patients. This might be because these patients presented earlier than the patients in the present study.

The mean platelet count of the present study population was 77x10⁹/L. The studies conducted locally showed 55.6 x10⁹/L by Mahmood *et al.*⁸ and 90x10⁹/L by Anwar *et al.*¹¹ A study conducted in China by Wu *et al.*¹⁸ showed 60x10⁹/L, another study conducted in Taiwan by Hou *et al.*¹⁹ showed 78x10⁹/L. These all results showed similar findings to the present study.

On cytogenetic analysis, a normal karyotype was seen in 25 patients (57.4%), while 20 (42.7 %) patients showed clonal karyotypic abnormalities at diagnosis. Chromosomal abnormalities were detected in 34.6% of cases by Narayanan,¹⁴ 39% by Voso *et al.*,¹⁵ 47.5% by Chaubey *et al.*,¹⁷ 53.4% by Mahmood *et al.*,⁸ and 42.3% by Rashid *et al.*¹² A complex karyotype, which carries poor overall survival, was seen in 8.5% of the present patients while Mahmood *et al.* reported 10.7%.⁸

In this study, the most common cytogenetic abnormality was trisomy 8 observed in 14.9% of patients, followed by monosomy 7 in 6.4% of the patients. According to the cytogenetic profile of national and international data, Rashid *et al.*¹² reported trisomy 8 to be the most common cytogenetic abnormality with a frequency of 9.9%.¹² They also reported a lower frequency of del 5q in 2.8% of the patients which is consistent with the present study findings. Mahmood *et al.*⁸ reported trisomy 8 in 12.9% followed by monosomy 7 in 5.6% of the patients.⁸ These findings are inconsistent with the present study. Voso *et al.* in the Italian population, reported the most common karyotypic abnormality to be del 5q in 10.5%, while much lower frequencies of 5% for trisomy 8 and 2% for monosomy 7 have been reported.¹⁵ This

difference may be due to the difference in the cytogenetic methodology adopted.

This study also showed 29 (61%) of the patients having MDS-MLD category, while 4 (8%) cases of MDS-EB1, 11 (23%) of MDS-EB2, and 03 (6%) of MDS-RS-MLD. The present findings on the types of MDS are similar to those reported in previous studies, including those conducted by Rashid *et al.*¹² in Pakistan, Elnahass and Youssif²⁰ in Egypt and Li *et al.*²¹ in the Chinese population.

In this study, risk stratification using the R-IPSS revealed that the majority of patients (40.4%) were classified as low risk, followed by 25.5% in the intermediate-risk category. These results are compatible with the findings of Mahmood *et al.* who reported 41% in low-risk category followed by 27.1% in the intermediate category.⁸ These findings are also consistent with those reported by Greenberg *et al.*¹⁶ who observed that 38% of their patients were in the low-risk group, 20% in the intermediate-risk group, and 19% in the very-low-risk category. Conversely, a study by Voso *et al.*¹⁵ in Italy found that 38% of patients fell into the very-low-risk group, 33% into the low-risk group, and 18% into the intermediate-risk group.

The present study showed an overall survival (OS) in MDS-MLD and MDS-RS-MLD better than that observed in patients with MDS-EB1 and EB2. The same findings were observed in a study conducted by Mahmood *et al.* with a median survival of approximately 5-12 months in MDS with multilineage dysplasia and 3-6 months in MDS with excess blasts.⁸

Despite efforts to provide a comprehensive picture of clinicopathological and cytogenetic characteristics of MDS patients, this study has certain limitations. First, it utilised a moderately-sized sample set from a single centre in Pakistan, which restricts the general applicability of its findings to other diverse local clinical settings. Second, only two years of follow-up data were available for most patients. A larger sample size and a longer follow-up data would enable a deeper understanding of clinicopathological and cytogenetic variations prevalent in the MDS patients from this part of the world and their relevance to improving the clinical management of local patients diagnosed with MDS.

CONCLUSION

The cytogenetic analyses showed higher normal karyotype frequency in MDS, while trisomy 8 was the most frequently encountered cytogenetic anomaly. Low-risk disease predominated upon presentation. The overall survival was better in MDS-MLD and MDS-RS-MLD compared to that in MDS-EB1 and EB2. These results are mostly consistent with those of other local and international studies, although some differences may arise due to the variations in the methodologies used to detect the chromosomal abnormality.

ETHICAL APPROVAL:

Ethical approval was obtained prior to data collection from the Institutional Review Board of the Armed Forces Institute of Pathology / NUMS(AFIP/NUMS) with Approval number R:017-016-14244/2019/AFIP/NUMS.

COMPETING INTEREST:

The authors declared no conflict of interest.

PATIENTS' CONSENT:

Informed written consent was obtained from the patients.

AUTHORS' CONTRIBUTION:

AW, SAK: Conceptualisation.

AW, RM, HSM: Formal analyses.

RM, AW, AK: Data curation.

AW, HSS: Writing of the original draft and methodology.

HSS: Software.

AW: Validation.

AW, AK: Investigation.

AK, HSM: Writing, reviewing, and editing.

All authors approved the final version of the manuscript to be published.

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