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Frequency of Paroxysmal Nocturnal Hemoglobinuria Clone in Turkish Myelodysplastic Syndrome Group

Türk Myelodisplastik Sendrom Hasta Grubunda Paroksismal Noktürnal Hemoglobinüri Klonu Sıklığı

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Abstract

Aim: Retrospective, cross-sectional, observational study to examine the frequency and features of paroxysmal nocturnal hemoglobinuria (PNH) clones in patients with myelodysplastic syndrome (MDS). **Methods:** Data were analyzed from the medical files of 41 MDS patients diagnosed and followed up in the hematology department of a referral center between 2006 and 2017. Descriptive data, cytogenetic and hematologic characteristics, prognostic features and PNH clone sizes were assessed. PNH clone sizes were evaluated using the fluorescently labeled inactive toxin aerolysin (FLAER) method **Results:** The study population comprised 22 (53.7%) female and 19 (46.3%) male patients with confirmed MDS; the overall mean±SD age was 68.20±9.84 years (range, 45–85). PNH clones were detected in 8 (19.5%) patients. The numbers of patients with PNH clone sizes >10%, >1%, >0.1% and >0.01% were 1, 1, 1 and 8, respectively ($p<0.001$ for all subgroups). **Conclusion:** These data indicate that PNH clones exist in approximately one-fifth of MDS patients. Further studies on a more extensive cohort are required to better understand the pathophysiological and clinical relationships between MDS and PNH.

Keywords: Paroxysmal Nocturnal Hemoglobinuria, Myelodysplastic Syndrome, FLAER

Öz

Amaç: Türk Myelodisplastik Sendrom (MDS) hasta grubunda retrospektif, cross-sectional, gözlemsel çalışma ile Paroksismal Nokturnal hemoglobinüri (PNH) klon sıklığı ve özellikleri araştırılmıştır. **Yöntemler:** 2006-2017 yılları arasında hematoloji bölümümüzde takip edilen 41 MDS hastasının verileri analiz edildi. Sitogenetik, hematolojik ve prognostik ve karakteristik özellikler, PNH klon varlığı değerlendirildi. PNH klonu taraması için fluorescently labeled inactive toxin aerolysin (FLAER) methodu kullanıldı. **Bulgular:** Çalışma grubu teit edilmiş MDS tanılı 22 (%53.7) kadın ve 19 (%46.3) erkek hasta oluşmuştur, ortalama yaş 68.20±9.84 (range, 45-85) idi 8 hastada (% 19.5) PNH klonu saptandı. PNH klon genişliği >10%, >1%, >0.1%, >0.01% olarak sırasıyla; 1, 1, 1 and 8 hastada ($p<0.001$ tüm alt gruplarda) saptandı.

Sonuç: MDS hasta grubumuzun yaklaşık 1/5'inde PNH klonu saptanmıştır. MDS ve PNH arasındaki klinik ve patofizyolojik ilişkinin daha iyi anlaşılması için geniş vaka serilerinde yapılacak çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Paroksismal Nokturnal Hemoglobinüri, Myelodisplastik Sendrom, FLAER

Introduction

Myelodysplastic syndrome (MDS) is a clonal disease of hematopoietic stem cells characterized by cytopenias due to ineffective hematopoesis. It occurs more commonly in advanced age and can transform into acute myeloid leukemia. Even though an etiologic factor cannot be identified in the vast majority of patients, exposure to benzene, chemotherapeutics, topoisomerase inhibitors

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and radiation are among predisposing factors for MDS (1). Refractory anemia with erythroid dysplasia, ring sideroblasts or cytopenias with multiple dysplasia as well as blastic changes in bone marrow can be observed in MDS, and common clinical symptoms include increased bleeding, frequency of infections, fatigue and weakness. However, some cases can stay dormant without any symptoms . A diagnosis of MDS is confirmed by peripheral smear, bone marrow aspiration and biopsy. Cytogenetic analysis and FISH can also assist in estimating prognosis and determining disease subgroups.

Paroxysmal Nocturnal Hemoglobinuria (PNH) is a chronic, progressive, life-threatening, rare, multi-systemic disease, developing as a result of somatic mutation of hematopoietic stem cell, and characterized by clonal, complement-mediated intravascular hemolysis (2).

PNH is caused by a somatic mutation of the phosphatidylinositol glycan A gene (PIGA), which results in a partial or complete absence of synthesis of glucosylphosphatidyl inositol cell membrane anchors and a subsequent deficiency (or total lack) of CD55 and CD59 cell-surface proteins of progeny cell populations (3). Clinically, PNH manifests with chronic intravascular hemolysis, bone marrow insufficiency and thrombosis, with patients generally grouped according to hemolytic or hypoplastic subtypes. The hemolytic type presents with chronic intravascular hemolysis, while pancytopenia is the most common symptom in the hypoplastic type.

Classical PNH may exist individually or may be accompanied by aplastic anemia or MDS. The gold standard technique for the diagnosis of PNH is based on the determination of proteins such as CD55 and CD59 by flow cytometry using two surface antibodies in at least two cell groups, including granulocytes.

There are no widely accepted evidence-based indications for treatment of PNH. In classic PNH, authors recommend eculizumab for patients with disabling fatigue, thromboses, transfusion dependence, frequent pain paroxysms, renal insufficiency, or other end-organ complications from disease. Watchful waiting is appropriate for asymptomatic patients or those with mild symptoms. In patients with AA/PNH, therapy should be directed toward the underlying bone marrow failure with careful monitoring of the PNH clone using flow cytometry. Patients who meet criteria for severe AA should be managed with either allogeneic BMT or immunosuppressive therapy depending on the age of the patient and the availability of a suitable HLA-matched sibling donor.(4)

Investigations for PNH are recommended in MDS patients with refractory anemia, and an increased incidence of PNH (10–17%) has been reported among patients with MDS. (5-12) We report data from a study that evaluated the frequency of PNH in our MDS patients with refractory anemia.

Methods

Study Design

This retrospective, cross-sectional, observational study was based on clinical chart data from patients treated in the hematology department of a tertiary care center in Istanbul, Turkey between 2006 and 2017. All patients with a confirmed diagnosis of MDS based on relevant diagnostic tests and classified according to WHO and French-American-British (FAB) criteria were included.

Study assessments and methods were approved by the local Institutional Review Board (No. 75/dated 2nd April 2014) and were conducted in accordance with the current version of the Helsinki Declaration. Written informed consent was obtained from all patients for inclusion and publication of anonymized data.

Sampling and PNH Clone Analysis

Blood samples were collected in EDTA tubes by peripheral venipuncture and were kept at room temperature. PNH clone analyses were conducted within 24 hours of sampling. Clones were analyzed in granulocytes according to the fluorescently labeled inactive toxin aerolysin (FLAER) method using commercially available kits (Becton Dickinson, New Jersey, USA, Cedarlane Labs, Burlington/Ontario, Canada).

Outcome Measures

Demographic data, including age, gender, co-existing systemic diseases were collated to characterize the patient cohort. Flow-cytometry-derived PNH clone sizes were evaluated to assess the extent of PNH. Other outcomes measures included the Eastern Cooperative Oncology Group (ECOG) performance status scale (13), the International Prognostic Scoring System (IPSS) score, patient survival periods, and the magnitude of cytopenia (12).

Data Analysis

Statistical analysis was performed using Statistical Package for Social Sciences Program version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive data are expressed in terms of frequency and percentages, mean ± standard deviation and/or median ± interquartile range.

Results

Patients and Disease Charcteristics

The study population comprised 22 (54%) female and 19 (46%) male patients with a confirmed diagnosis of MDS. The mean ± SD age was 68.2±9.8 (range, 45–85) years. The most frequent concurrent systemic diseases

accompanying MDS were hypertension, diabetes mellitus and ischemic heart disease, observed in 22% of patients each.

The distributions of MDS patients categorised with respect to FAB and WHO classifications, and the presence of PNH clones, are shown in Tables 1 and 2, respectively.

Table 1. PNH clones according to FAB-classified MDS type

FAB MDS type	PNH clone		Total
	No	Yes	
MDS-U	1	0	1
RA	19	5	24
RAEB	4	1	5
RAEB-2	1	0	1
RAEB-t	1	0	1
RARS	7	2	9
Total	33	8	41

MDS-U0 myelodisplastic syndrome unclassified, PNH: paroxysmal nocturnal hemoglobinuria, RA: recractory anemia, RAEB: recractory anemia excess blast, RAEB-1: recractory anemia excess blast-1, RAEB-2: recractory anemia excess blast-2, RAEB-t: refractory anemia with excess blasts in transformation, RARS: recractory anemia with ring sideroblasts, WHO: World Health Organization

Table 2. PNH clones according to WHO-classified MDS type

WHO MDS type	PNH clone		Total
	No	Yes	
MDS-U	1	0	1
RA	12	1	13
RAEB-1	5	0	5
RAEB-2	1	1	2
RA-isolated del5q	0	2	2
RARS	3	1	4
RCMD	11	2	13
RCUD	0	1	1
Total	33	8	41

MDS-U: Myelodisplastic syndrome unclassified, PNH: paroxysmal nocturnal hemoglobinuria, RA: recractory anemia, RAEB-1: recractory anemia excess blast-1, RAEB-2: recractory anemia excess blast-2, RARS: recractory anemia with ring sideroblasts, RCMD: refractory cytopenia with multilineage dysplasia, RCUD: refractory cytopenia with unilineage dysplasia, WHO: World Health Organization

Table 3. Cytogenetic profile of MDS patients

	Frequency and percent (%)
11q23 (del)	1 (2.4)
46 XX	16 (39)
46 XY	16 (39)
47 XX+8	1 (2.4)
add (1) p32-36 [18]	1 (2.4)
del (11) (q13q23), der5, der12, der18	1 (2.4)
del (20) (q11,2)	1 (2.4)
del (5) (q31q35) +8, -18	1 (2.4)
del 5 (q12q33[16]	1 (2.4)
del5q	1 (2.4)
Insufficient metaphase	1 (2.4)

Based on the FAB system, MDS was recorded as most frequently calssified as Refractory anaemia (RA), with five patients confirmed as having PNH clones. Using the WHO system, equal numbers of patients overall were calssified with RA or refractory cytopenia with multilineage dysplasia (RCMD); but together these classifications contributed only three patients to the overall total; PNH-positive patients were therefore spread over a wider range of categories under the WHO system.

Cytogenic Characteristics

Cytogenetic findings in this cohort are summarized in Table 3. The percentage of myeloblasts in bone marrow biopsy were <5% in 36 patients (88%), 5–10% in three cases (7%), and 10–20% in two patients (5%). PNH clones were detected in eight (20%) of patients overall.

Outcome Measures

Results from evaluations of all patients according to the IPSS are summarized in Figure 1. Twenty-four cases were identified in the Int-1 risk group (59%), three patients were diagnosed as Int-3 (7%) and 13 MDS patients were in the low-risk group (32%). Performance status on the ECOG scale revealed scores of 1 in 30 patients (73%), 1.5 in one patient (2%), 2 in six patients (15%), and 3 in one

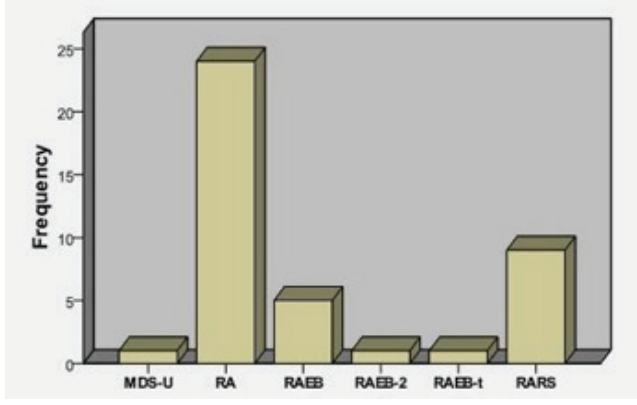

Figure 1. FAB classification of patients

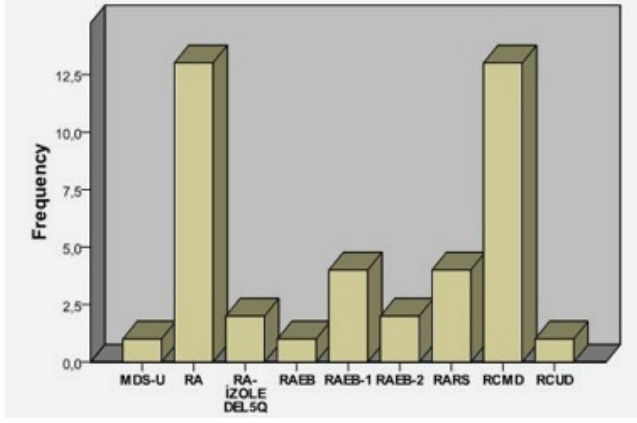

Figure 2. WHO classification of patients

Table 4. Distribution of PNH clone sizes		
Clone size	**Number (%) of patients**	**p-value**
>0.1	1 (2.4)	<0.001
>0.01	1 (2.4)	<0.001
>0.001	1 (2.4)	<0.001
>0.0001	8 (19.5)	<0.001

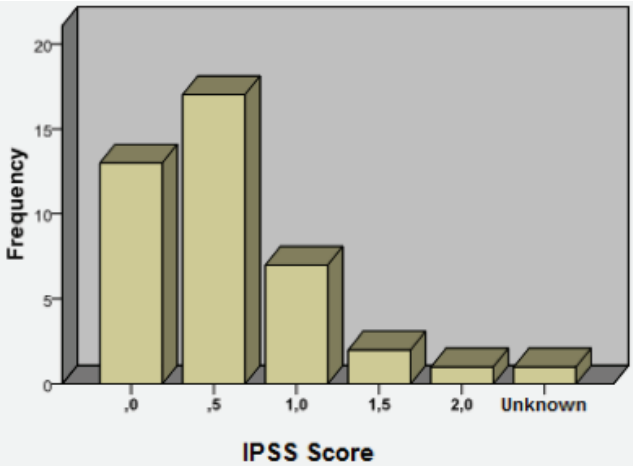

Figure 3. Distribution of International Prognostic Scoring System (IPSS) scores in MDS patients

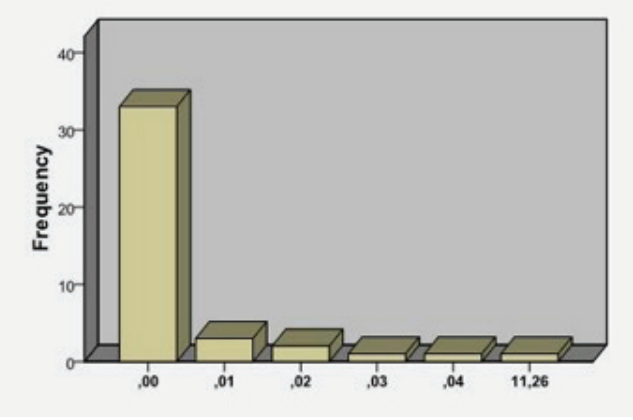

Figure 4. Frequency of PNH clone size

patient (2%). Median survival times were 3.5 years in 24 patients (59%), 5.7 years in 12 patients (29%) and 1.2 years in three cases (7%).

As illustrated in Table 4, it can be postulated that PNH clone size in MDS patients is most frequently very low (<1%).

Discussion

Based on these data, we estimate that PNH, which has previously been linked with MDS and other bone marrow failure syndromes (2, 5), occurs in approximately one-fifth of patients with MDS in Turkey.

Due to small PNH clone sizes, patients with MDS may not display obvious signs of hemolysis. However, PNH clone sizes can now be conveniently and accurately determined using modern flow cytometry methods (5,8,14). The pathogenesis of PNH leaves red cells, platelets and neutrophils vulnerable to attack by the complement system, with subsequent hemolysis and platelet activation resulting in severe end-organ damage and a high risk of thrombosis (15). In particular, thrombosis, a major cause of mortality in PNH, is thought to originate from both activation of the complement system and ADP (16).

The link between the immune system and PNH has not yet been fully elucidated. In addition to deficiencies of CD55 and CD59, immune selection for PNH stem cell proliferation may play a crucial role in PNH pathogenesis (14). Interestingly, PNH cells have been found to be equivalent to normal cells in terms of their capability to elicit an immune response in tissue cultures (15).

Despite a general agreement that PNH clonal expansion occurs in some MDS patients, some controversial issues exist, including: 1) the type of MDS associated with PNH; 2) techniques adopted for routine laboratory measurement of PNH clones; and 3) the pathophysiological interpretation of PNH clones in MDS. Previously reported data indicate that PNH, as opposed to leukemia, is more likely to exist in MDS patients presenting with bone marrow failure (17, 18). However, the prognostic significance of PNH in MDS patients requires further investigation (5,6). Dysregulation of apoptosis may be involved in pathogenesis of PNH, and the resistance of leukocytes to apoptosis in bone marrow may contribute to selection of a PNH clone. Expansion of the selected (PNH) clone may subsequently lead to clinical manifestations (14).

In a previous prospective multicenter study of PNH in patients with bone marrow failure, PNH clones were detected in 17% of MDS patients based on findings from high sensitivity flow cytometry (19). The detection of even small clone sizes can now be accomplished using the FLAER method, as adopted in the current study. Patients with MDS categorized to the refactory anaemia (RA) group, according to FAB classification, have been associated with a higher risk of PNH (20). Even though the number of patients in our cohort was too limited to postulate such a relationship, patients with clone sizes >0.1%, >1% and >10% were categorized into the RA subgroup. We therefore suggest that the prognostic significance and correlation of RA associated with PNH should be investigated in further studies.

According to the National Comprehensive Cancer Network (NCCN) guidelines, the detection of PNH clones >10% in size is sufficient for the confirmation of a diagnosis of PNH, and a positive result for PNH clones in an MDS patient constitutes an indication for

immunosuppressive treatment (21). While we feel that findings from the current study can be extrapolated to the wider MDS patient population, the interpretation of these data is subject by limitations owing to the cohort size and study methodology. Our observations were based on a relatively small number of patients, irrespective of the rarity of PNH in MDS, which makes it difficult to assess pathophysiological links between these two conditions. Moreover, the influence of ethnic or socio-economic factors on our findings cannot be fully assessed. Despite these restrictions we believe that these data provide an overview of MDS and PNH in Turkey, and serve as a reminder for increased awareness of the possible occurrence of PNH in patients with MDS.

Conclusion

In conclusion, this study indicates that PNH clones exist in approximately one-fifth of MDS patients. The pathophysiological association between MDS and PNH needs to be investigated in further clinical studies based on larger, possibly international patient populations.

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