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Original Article

Seven Cases of Severe Neutropenia: A Single-center Experience

Ağır Nötropenili Yedi Olgu: Tek Merkez Deneyimi

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ABSTRACT

Objective: Severe congenital neutropenia is a rarely encountered heterogeneous group of disorders characterized by myeloid maturation arrest in the bone marrow. The present study aimed to discuss clinical and laboratory findings, genetic mutations, therapeutic approaches and outcomes in these rarely seen seven cases followed up with the diagnosis of Kostmann syndrome in a single center so as to make a contribution to the literature.

Method: In this retrospective study, data of the seven cases followed up with the diagnosis of Kostmann syndrome were retrieved from the patient files. The diagnosis was established based on an absolute neutrophil count of <500/mm³ persisting for more than 3 months and presence of *HAX-1* gene mutations detected by positive molecular genetic analysis.

Results: All patients were born to consanguineous parents. Six of the seven cases had sibling history. All cases had homozygous HAX-1 mutation. Case 1 had motor-mental retardation and case 5 had urogenital system anomaly. Mortality or malignancy was not encountered in any of the cases despite the absence of prophylactic granulocyte-colony stimulating factor (G-CSF) therapy.

Conclusion: The diagnosis and differential diagnosis of congenital neutropenia must be considered in the patients presenting with neutropenia and recurrent infections. Monitoring of the cases with severe neutropenia like Kostmann syndrome carries extreme importance. Families should be educated in terms of early signs of infection and importance of regular patient monitoring for prophylactic G-CSF-free management of the disease.

Keywords: Congenital neutropenia, *HAX-1*, G-CSF therapy, mortality

ÖZ

Amaç: Ağır konjenital nötropeni, kemik iliğinde miyeloid olgunlaşmanın durması ile karakterize, nadir görülen, heterojen bir grup hastalıktır. Bu çalışmada tek merkezden takip edilen Kostmann sendromu için yedi olguda klinik ve laboratuvar bulguları, genetik mutasyonlar, tedavi yaklaşımları ve sonuçlarının tartışılması, nadir görülen bir hastalık olması ve nadir görülen bir hastalık olan yedi olgunun takip edilmesinin olağandışı olması nedeniyle literatüre katkı sağlanması amaçlandı ediliyor.

Yöntem: Bu retrospektif çalışmada Kostmann sendromu nedeniyle takip edilen yedi olgunun verileri hasta dosyalarından elde edildi. Tanı, 3 aydan uzun süredir mutlak nötrofil sayısının <500/mm³ olması ve *HAX-1* geninin moleküler genetik analizinin pozitif olmasıyla konuldu.

Bulgular: Olguların tümü akraba evliliği olan ebeveynlerden doğmuştu. Yedi olgunun altısında kardeş öyküsü vardı. Olguların tamamında homozigot HAX-1 mutasyonu vardı. Olgu 1'de motor zeka geriliği, olgu 5'te ise ürogenital sistem anomalisi vardı. Profilaktik granulocyte-colony stimulating factor (G-CSF) tedavisi uygulanmamasına rağmen hiçbir olguda mortalite veya maligniteye rastlanmadı.

Sonuç: Nötropeni ve tekrarlayan enfeksiyonlarla başvuran hastalarda konjenital nötropeni tanısı ve ayırıcı tanısı mutlaka göz önünde bulundurulmalıdır. Kostmann sendromu gibi ciddi nötropeni olgularının izlenmesi önemlidir. Aileler, enfeksiyonun erken belirtileri ve hastalığın profilaktik GCSF'siz tedavisi için düzenli hasta takibinin önemi açısından eğitilmelidir.

Anahtar kelimeler: Konjenital nötropeni, *HAX-1*, G-CSF tedavisi, mortalite

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INTRODUCTION

Severe congenital neutropenia (SCN) belongs to a rarely encountered heterogeneous group of disorders characterized by myeloid maturation arrest in the bone marrow affecting both neutrophil homeostasis and function[1]. An absolute neutrophil count (ANC) below 500/mm³ in peripheral blood circulation is considered severe neutropenia. The diagnosis of SCN is established in the presence of severe neutropenia lasting for more than 3 months together with myeloid maturation arrest in the bone marrow. Its prevalence is estimated to be nearly 1-2 to 10/1,000,000 with both genders being affected equally[2,3].

Congenital neutropenia was first defined in 1956 by Rolf Kostmann as "infantile genetic agranulocytosis". In 1975, the same author identified 10 more cases and thereafter the terms of SCN and Kostmann syndrome have been associated and showed up as a group of diseases. Dale et al.[4] firstly demonstrated mutations in the *ELA-2* gene in cyclic neutropenia cases in 1999 and then in SCN cases in 2000; thereby, these two diseases have been classified in the same group. So far, more than 20 gene mutations associated with neutropenia (including *G6PC3*, *GFI1*, *SBDS*, *JAGN1*, *SRP54*, and *DNAJC21* gene mutations) have been identified. Nevertheless, genetic defects remain unidentified in nearly 25% of patients[1].

The most frequent pathogenetic defects are autosomal dominant mutations in the *ELA2/ELANE* gene, which encodes the neutrophil elastase, and autosomal recessive mutations in the *HAX-1* (HS1-associated protein X-1) gene, the product of which contributes to the activation of the granulocyte-colony stimulating factor (G-CSF) signaling pathway[5]. *ELA2/ELANE* gene mutations also playsa role in myeloid differentiation, thus mutation of this gene enhances the risk of developing acute myeloid leukemia (AML)[6,7].

SCN presents particularly with recurrent skin, lung, and soft tissue infections caused mostly by *S. aureus, E. coli,* and *P. aureginosa* which can be seen from the first few months of life. Fifty percent of patients die of infections before the age of one year. In a study, rates of survival over 5 years was reported to be 30%[8]. The disease has been usually fatal with a mean survival of 13 years before the availability of colony-stimulating factors, but the mean survival has been remarkably prolonged along with the use of G-CSF therapy. Nevertheless, some patients (nearly 20%) can develop AML and myelodysplastic syndrome (MDS) after treatment with G-CSF[9,10]. It has been reported that G-CSF therapy can trigger the transformation of the underlying myeloid stem-cell defect into malignancy, or may increase the risk of malignancy by prolonging mean survival time[11]. Today, allogeneic bone marrow transplantation is the only curative therapy, which provides a favorable prognosis when a human leukocyte antigen (HLA)-matched donor can be found[3].

Establishment of a definite diagnosis of congenital neutropenia requires a detailed medical history, physical examination, family history, and realization of laboratory screening tests. These clinical and laboratory data can identify a viral infection, bone marrow malignancy, an iatrogenic cause, an immune deficiency, a metabolic disorder, an autoimmune disorder, or a congenital etiology leading to conduction of a more specific further investigation.

MATERIALS and METHODS

In this retrospective study, the data of seven cases followed between January 2014 and May 2021 with the diagnosis of Kostmann syndrome were retrieved from the patient files. The study was approved by the Mersin University Clinical Research Ethics Committee (approval number: 2022/715, date: 01.11.2022). The diagnosis was established based on an ANC of <500/mm³ persisting for more than 3 months and myeloid series maturation arrest in the bone marrow. In addition, molecular genetic analyses were requested from all patients for *HAX-1*, *ELANE*, *G6PC3*, and *CSF3R* gene mutations so as to make a diagnosis of congenital neutropenia. Information concerning demographic and clinical characteristics of the patients (age, gender, consanguinity, family history, presenting symptoms, age at symptom onset, and at diagnosis, follow-up period, clinical and laboratory findings, and treatments used) were recorded.

Before making the final diagnosis, drug-induced neutropenia, infection-related neutropenia, immune deficiency, metabolic disorder, and malignancies were ruled out for all patients. All patients received prophylactic antibiotic therapy (trimethoprim-sulfamethoxazole at a daily dose of 5 mg/kg). None of the patients received prophylactic G-CSF therapy; nevertheless, G-CSF was commenced at a daily dose of 5-10 µcg/kg in the presence of fever or infection.

Statistical Analysis

ANC was obtained as part of complete blood count, which was measured using Sysmex XN-1000™ Hematology Analyzer (Sysmex America, Inc., Illinois, USA). Molecular genetic analysis for *HAX-1* and *ELANE*

gene mutations was performed by polymerase chain reaction - based DNA line analysis.

RESULTS

Five male, and two patients were included in the study. The median age (91 months; range: 10-150 months) and the median age at diagnosis (40 months; range: 2-81 months) were as indicated. All patients were born to consanguineous parents. Six of the seven cases had sibling history (there were three pairs of siblings excluding case 7). In three of these six patients, Kostmann syndrome was suspected based on the fact that their siblings had Kostmann syndrome, and then the diagnosis was confirmed with genetic testing. In all of these three patients, the parents had refused to undergo prenatal diagnostic tests. Presenting symptoms included recurrent skin abscesses, gingivostomatitis, recurrent upper and/or lower respiratory tract infections, lymphadenitis, and recurrent urinary tract infections (Table 1). Motor-mental retardation was detected in case 1, and urogenital system anomalies including ambiguous genitalia, cryptorchidism, hypospadias and bifid scrotum were detected in case 5. Molecular genetic analysis revealed homozygous *HAX-1* mutation in all of the seven cases. Additionally, case 1 had homozygous KATNIP mutation (Table 1).

Table 1. Baseline demographic characteristics of the cases

	Cases						
	#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**
Age, months	39	135	10	78	91	150	121
Sex	Male	Male	Female	Male	Male	Male	Female
Parental consanguinity	+	+	+	+	+	+	+
Sibling history	+	+	+	+	+	+	-
Presenting symptom	None. Screened because his sibling had Kostmann syndrome	Fever	None. Screened because her sibling had Kostmann syndrome	URTI	Fever	Fever	Pneumonia
Age at diagnosis, months	3	2	2	52	53	81	40
Follow-up, months	36	133	8	26	38	69	81
Presence of an infection at diagnosis	+	+	-	+	+	-	+
G-CSF prophylaxis	-	-	-	-	-	-	-
Congenital anomalies	Motor mental retardation	-	-	-	Ambiguous genitalia Cryptorchidism Hypospadias Bifid scrotum	-	-
Molecular defects	**HAX-1** c.130_131insA Homozygous **KATNIP** c.4120C>T(p.Gln1374Ter) Homozygous	**HAX-1** , c.130_131insA Homozygous;	**HAX-1** c.130_131insA Homozygous	**HAX-1** c.130_131insA Homozygous	**HAX-1** c.130_131insA Homozygous	**HAX-1** c.130_131insA Homozygous	**HAX1** IVS1+1 G>A(G.267 G>A) Homozygous
Current survival status	Alive	Alive	Alive	Alive	Alive	Alive	Alive, lost to follow-up

G-CSF: Granulocyte-colony stimulating factor

Overall, the patients were hospitalized for median 11 (6-35) times over the median 38 (range 18-33)-month follow-up period. The most common cause of hospitalization was pneumonia (39.3%), followed by upper respiratory tract infection (19.6%) and prolonged diarrhea (15.4%). None of the patients developed sepsis or required intensive care (Table 2).

Mortality or malignancy was not encountered in the cases although none of them had received prophylactic G-CSF therapy.

DISCUSSION

SCN is a rare condition with underlying genetic mutations showing variabilities among ethnicities. For example, *HAX-1* mutation accounts for nearly 11% of SCN cases in Europe, whereas this mutation has not been detected in the USA so far, where ELANE mutation is frequent. On the other hand, G6PC3 mutation was detected in 25% of the cases in Israel[12]. In a study from Turkey, *HAX-1* mutation was detected in 36% of the patients[13]. All cases (100%) in the present study had homozygous *HAX-1* mutation.

HAX-1 protein, which is the main pathogenic factor in 15-20% of the cases with SCN, plays an important role in the maintenance of mitochondrial integrity. *HAX-1* deficiency results in impaired mitochondrial membrane potential leading to 2-3 times higher than normal spontaneous programmed cell death rates of neutrophils[14]. *HAX-1* protein is synthesized not only in the hematopoietic cells but also in the fibroblasts and neuronal cells, therefore neurological involvement at varying levels is likely to occur in people who carry this mutation[14,15]. This mutation should be considered when learning difficulty, developmental retardation, epilepsy, and neutropenia are seen in combination. Similar to the case presented by Patiroglu et al.[16], only one of our cases (case 1) had motor-mental retardation together with homozygous *HAX-1* mutation. As a striking fact that motor-mental retardation was not detected either in the sibling or in the remaining five cases despite the presence of homozygous *HAX-1* mutation. Motor-mental retardation in case 1 can be attributed to the presence of homozygous *HAX-1* mutation accompanied by homozygous KATNIP mutation, which in the literature has been associated with Joubert syndrome. So, it would be reasonable to start the mutation analysis starting with *HAX-1* gene and to continue with analyzing ELANE and *G6PC3* genes if *HAX-1* gene mutation was not detected. Since the percentage of consanguineous marriages is very high in Turkey, a sibling with negative result for genetic screening for *HAX-1* gene mutation should be screened also for the other rare genetic mutations to arrive at a more precise conclusion.

In the literature, urogenital anomalies and SCN have been usually associated with G6PC3 mutation[17]. Indeed, one of our cases (case 5) had urogenital system anomalies including ambiguous genitalia, cryptorchidism, hypospadias and bifid scrotum although he had homozygous *HAX-1* mutation alone. In this case, further genetic examinations may identify additional genetic mutations.

Results of demographic surveys indicate that consanguinity is responsible for 22% of SCN cases in general. Given the higher rate of consanguineous marriages in Turkey, we can hypothesize that the prevalence of SCN is high in our country[18]. In fact, in our series, all of the SCN cases were born to consanguineous parents and six of these seven cases had sibling history of SCN. As an important corollary, three of these six cases were suspected of having Kostmann syndrome based on the sibling history of the disease which was confirmed by diagnostic investigations.

Table 2. Number of hospitalizations due to neutropenic infections over follow-up period

Case	URTI	UTI	Pneumonia	Oral aphtha	Anal abscess	AGE	Total
#1	5	2	9	2	1	8	27
#2	5	3	12	8	-	7	35
#3	2	-	4	-	-	-	6
#4	3	-	6	-	-	2	11
#5	2	4	2	-	-	1	9
#6	3	-	8	-	-	-	11
#7	3	3	5	4	3	-	18
Total (n)	23	12	46	14	4	18	**117**

URTI: Upper respiratory tract infection, UTI: Urinary tract infection, AGE: Acute gastroenteritis

Intrinsic stem cell defect is the main cause of SCN. Indeed, some studies have reported that mononuclear cells of the patients with Kostmann syndrome normally synthesize and secrete G-CSF, indicating that G-CSF deficiency is not the underlying defect in this syndrome, while scarce number of studies have argued the opposite[7,8]. For example, Dong et al.[19] revealed the presence of a G-CSF receptor defect. In addition, a relationship was identified between the disease and *HLA B12* gene.

Today, G-CSF therapy accounts for the substantial proportion of SCN treatment modalities leading to an increase in survival rates up to 80% as well as an improvement in the patient's quality of life. However, sepsis-related mortality can be seen in approximately 10% of the patients on G-CSF therapy maintained for more than 10 years[20].

In a multicenter phase III study of 123 SCN patients, the patients were divided into G-CSF -treated and placebo groups In that study, G-CSF therapy was associated with a nearly 50% decrease in the incidence and duration of infectious complications. Again, protocols recommend prophylactic G-CSF therapy so as to maintain ANCs between 1000-1500/mm^3[3]. Contrary to the recommendations, prophylactic G-CSF therapy was not commenced in any of the patients in the present case series. G-CSF therapy was given only in the presence of infection at a daily dose of 5-10 IU/kg to increase the ANC above 1500/mm^3. Nevertheless, mortality was not observed in any of the patients throughout the follow-up period, and all of them are still alive.

Despite the fact that sepsis-related mortality rates decrease with regular G-SCF and appropriate antibiotherapy if infectious complications develop in the SCN patients, the risk of developing MDS or AML during a 10-year follow-up period is reported to be 20%. MDS/AML was reported in 16% of 374 patients registered in the Severe Chronic Neutropenia International Registry which was suggested to be a complication of underlying pathogenic mechanisms that became manifest during the prolonged patient survival rather than the direct effect of G-CSF therapy(11). However, the safety of long-term G-CSF therapy remains to be an important concern. In the present study, none of the patients developed AML or MDS during the median 38 (8-133)-month follow-up period.

CONCLUSION

The diagnosis and differential diagnosis of SCN must be taken into account and ruled out in the patients presenting with recurrent unexplained infection and concurrent neutropenia particularly in countries such as Turkey where consanguineous marriages are common. It should be kept in mind that neutropenia-related complications may have quite serious outcomes. Therefore, monitoring severe cases of neutropenia like Kostmann syndrome carries extreme importance. We believe that infection in SCN patients can be managed without resorting to prophylactic G-CSF therapy to minimize G-CSF-related side effects such as potential malignancies. It is critical to inform families about early predictive signs of infection and regular patient monitoring for prophylactic GCSF-free management of the disease.

Ethics

Ethics Committee Approval: The study was approved by the Mersin University Clinical Research Ethics Committee (approval number: 2022/715, date: 01.11.2022).

Informed Consent: Retrospective study.

Author Contributions

Surgical and Medical Practices: B.D.G., S.Ü., Concept: B.D.G., S.Ü., Design: B.D.G., S.Ü., Data Collection or Processing: B.D.G., H.K., Analysis or Interpretation: B.D.G., H.K., Literature Search: B.D.G., H.K., Writing: B.D.G.

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