HAX-1 deficiency: Characteristics of five cases including an asymptomatic patient

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Summary

Background: **Mutations in the HAX-1 gene cause an autosomal recessive form of severe congenital neutropenia (SCN), which particularly manifests with recurrent skin, lung and deep tissue infections from the first few months of life.**

Objective: **We retrospectively evaluated the clinical and laboratory findings of the patients diagnosed with SCN carrying *HAX1* gene mutations.**

Methods: **A total of five patients with SCN, carrying a *HAX1* gene mutation, were evaluated in terms of clinical and laboratory findings. Mutation analysis of the candidate genes (*HAX1, ELANE* and *CSF3R*) was performed.**

Results: **All of the patients lived in Turkey; four of them were of Kurdish origin and one was Turkish. Of the five patients, three were girls and two were boys, and the mean age of the patients was 8.8 years old (range 4-15 years). The mean age of diagnosis was 25.8 months (range 2 months-5 years). The infections diagnosed included recurrent gingivitis, stomatitis, and skin and soft tissue abscesses. Developmental retardation and epilepsy were present in only one patient, whereas speech retardation was present in two. All of our patients had a *HAX1* mutation, and are still alive and none of them has shown malignant transformation yet.**

Conclusion: **Complete blood count should be performed and absolute neutrophil count should be evaluated in patients with recurrent severe infections. In the event that neutropenia is detected, they should be investigated in terms of SCN and mutation analysis should be performed.** ***(Asian Pac J Allergy Immunol 2016;34:73-6)***

Keywords: *Severe congenital neutropenia, diagnosis, mutation, HAX-1, ELANE*

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Introduction

Severe congenital neutropenia (SCN) is a rare heterogeneous group of diseases that develops due to maturation arrest in the myeloid series in the bone marrow. It particularly manifests with recurrent skin, lung and deep tissue infections from the first few months of life. The diagnosis is usually made based on the presence of a neutrophil count < 500/mm^3 for at least 3 months and demonstration of maturation arrest in the myeloid series. It may transform into acute myeloid leukaemia and myelodysplastic syndrome.[1,2]

Severe congenital neutropenia also shows genetic heterogeneity, and demonstrates autosomal dominant, autosomal recessive, X-linked, and sporadic inheritance.[1] The autosomal dominant type is the most common (60%) and is associated with heterozygous mutations in the neutrophil elastase (*ELANE*) gene.[1,3-5] Autosomal recessive forms have been linked to mutations in the *HAX1* and *G6PC3* genes.[1,6-9] HAX1 is primarily located in the mitochondria and controls the integrity of the inner mitochondrial membrane potential and protects the myeloid cells against apoptosis.[10] Moreover, it has been shown to interact with viral and cellular proteins in many different functions.

The present report aimed to evaluate the clinical and laboratory findings of five SCN patients with a *HAX1* gene mutation.

Methods

A total of five patients with SCN, carrying a *HAX1* gene mutation, were evaluated in terms of

clinical and laboratory findings. Informed consent was received from the parents. Approval for the study was obtained from the committee for ethical research (protocol number 2014/7066). Mutation analysis of the candidate genes (*HAX1, ELANE* and *CSF3R*) was performed at Medizinische Hochschule Hannover.

Genomic DNA was extracted from peripheral blood using standard techniques. Mutational analyses were performed by sequencing the polymerase chain reaction (PCR)-amplified exons of *ELA2, HAX1* and *CSF3R* using a semiautomatic sequencer. Restriction digests of PCR-amplified DNA were performed.[9,11,12]

Results

All of the patients lived in Turkey; four of them were of Kurdish origin and one was Turkish. Three of the five patients were girls and two were boys. Case 3a and 3b are siblings. The mean age of the patients was 8.8 years (range 4-15 years). The symptoms began in the newborn period and early infancy in 4 of the cases (Case 1, 2, 3b, 4). Case 3a was identified upon screening for positive family history. The mean age of diagnosis was 25.8 months (range: 2 months-5 years).

Three patients were from a consanguineous marriage. The infections diagnosed included recurrent gingivitis, stomatitis, and skin and soft tissue abscesses. The patients who have gingivitis and tooth decay were monitored by a dentist. Developmental retardation and epilepsy were present only in Case 4, whereas speech retardation was present in Cases 1 and 4. Absolute neutrophil count in peripheral blood was $<500/mm^3$ and bone marrow aspiration revealed promyelocytic maturation arrest in all cases. While the same type of mutation was identified in Cases 1, 2, 3a and 3b, the mutation was different in Case 4, which explains the central nervous system involvement. Four of the patients received trimethoprim-sulphametaxosol (TMP-SMX) prophylaxis orally. There was no response to G-CSF (up to 30 μcg/kg) in Case 4, despite the absence of mutations in the G-CSF receptor gene (*CSF3R*). All of our cases are still alive and none of them had shown malignant transformation. Bone marrow transplantation (BMT) was planned for case 4. Clinical and demographic characteristics of the patients are demonstrated in Table 1.

Discussion

Severe congenital neutropenia is associated with more than one gene including *ELANE*, *HAX1*, *WAS*, *GFI1*, and *G6PC3* genes. The prevalence of mutations in these genes shows variation among populations and ethnic groups. While the prevalence of mutations in the *ELANE* gene is 44-63% in North America and 35% in France,[13-15] Alizadeh et al. determined the prevalence of *HAX1* mutations to be 41% in Iran. They attributed the higher prevalence of *HAX1* mutation to consanguineous marriages [1] and most of the reported patients were of Kurdish origin.[10] We also detected mutations in the HAX1 gene in the present patient series, whereas other mutations were not identified. There was consanguineous marriage in three of our patients and four of the patients were Kurdish origin living Southeastern Anatolian. Consanguineous marriages account for about 60% of marriages in this region of Turkey, so the risk of an autosomal recessive disease increases. There has been no study about this region of Turkey and this disease.

HAX1 deficiency may clinically manifest with recurrent gingivitis, stomatitis, and soft tissue and skin abscess, pneumonia, otitis, mastoiditis, and tooth decay.[1,10,11,16-18] There were similar manifestations in the present cases as well. However, one of the patients (Case 3a) was asymptomatic, which was remarkable; the patient (Case 3a) had no infection and had not been hospitalised by this time. Her sister (Case 3b) was diagnosed with SCN, so her family was evaluated with whole blood count. We found neutropenia in case 3a. Because the neutropenia was persistent in case 3a, mutation analysis for SCN was performed and a mutation of the *HAX1* gene (Trp44X) was found. This is interesting, as this patient is still infection-free and does not need G-CSF.

Neurological abnormalities were first reported by Carlsson[19] and may be seen in cases with autosomal recessive inheritance (*HAX1* and *G6PC3*). The *HAX1* gene has two different isoforms: isoform A and B. While neurological signs are not present in mutations associated with isoform A (Trp44X, Glu59X, Glu60fs), neurological abnormalities including mental retardation, epilepsy and development retardation are seen in mutations associated with both isoforms (Arg86X, Gln123fs, Val144fs, Gln190X).[1,10,16-19] Among the present patients, only Case 4 had mutations that involved

Table 1. Demographic characteristics, clinical and laboratuary findings of the patients

	Case 1	Case 2	Case 3a	Case 3b	Case 4
Gender	Male	Female	Female	Female	Male
Age	5 age	7 age	11 age	17 age	4 age
Beginning of symptoms	1 mounth	2 mounth	Asymptomatic	5 mounth	20 days
AGE OF DIAGNOSIS	3 age	1 age	5 age	7 mounth	14 mounth
PARENTAL CONSANQUINITY	No	No	Yes	Yes	Yes
INFECTIONS	Stomatitis,gingivitis,perianal cheek and skin abscess, purulent otitis	Gingivitis,stomatitis, recurrent skin abscesses, bronchopneumonia	Asymptomatic	Recurrent skin abscesses, destructive pneumonia	Gingivitis,stomatitis, recurrent skin infections, pneumonia
Developmental retardation	(-)	(-)	(-)	(-)	(+)
EPILEPSY	(-)	(-)	(-)	(-)	(+)
Speech retardation	(+)	(-)	(-)	(-)	(+)
BONE MARROW	Maturational Arrest	Maturational arrest	Maturational arrest	Maturational arrest	Maturational arrest
MUTATION	Trp44X	Trp44X	Trp44X	Trp44X	Val144fs
G-CSF REQUIREMENT	(+)	(+)	(-)	(+)	(+)
RESPOND TO G-CSF	(+)	(+)	(+)	(+)	(-)
Malignancy	(-)	(-)	(-)	(-)	(-)

the two isoforms of *HAX1* (Val144fs) and had mental-motor retardation and epilepsy. Although Case 1 had mutations in isoform A (Trp44X) of *HAX1*, speech was 6 months behind age norms with no epilepsy.

The use of recombinant G-CSF is recommended to keep the absolute neutrophil count >1000/mm^3, and approximately 95% of the cases benefit from G-CSF therapy. It is reported that morbidity and mortality rates showed a dramatic decrease with this treatment.[10] Whereas 42% of the patients were reported to die before the introduction of G-CSF,[20] death due to sepsis decreased annually by 0.9% along with the use of G-CSF.[21] It is used at a daily dose of 1-120 mcg/kg and the majority of patients usually respond to a dose <25mcg/kg. The median dose required in patients with *HAX1* mutation is 6mcg/kg, whereas it is 8.5mcg/kg in those with defects in the *ELANE* gene.[17] Among the present cases, Cases 1, 2, 3a and 3b were responsive to G-CSF and Cases 1, 2 and 3b have been receiving the drug at a dose of 5mcg/kg. Case 3a was not on regular therapy because of the absence of infection. Case 4 was scheduled for bone marrow transplantation because of unresponsiveness to the dose > 8mcg/kg.

Severe congenital neutropenia is a premalignant condition. A substantial proportion of the patients develop leukaemia. Leukaemia can develop in patients with both *ELANE* and *HAX1* mutations, and the risk is enhanced in patients having one or more of the genetic anomalies including monosomy 7, RAS mutation, trisomy 21 or G-CSF receptor mutation independent of the genetic subtype.[22] The most common type of leukaemia is AML and, ALL and CMML may also be seen.[22-24] In 2006, 374

patients that have been registered in SCNIR and received long-term G-CSF therapy were evaluated; cumulative leukaemia incidence was reported to be 21% after 10 years and 35% after 15 years. The 10-year cumulative incidence of leukaemia increased to 40% in patients with lower response to G-CSF (those who needed a dose of > 8 mcg/kg/day).[10,17,21] No malignant transformation has been defined in our cases yet.

Conclusion

Complete blood count should be performed and absolute neutrophil count should be evaluated in patients with recurrent severe infections. In the event of neutropenia being detected, they should be investigated in terms of severe congenital neutropenia and mutation analysis should be performed. This is important for both the definite diagnosis of severe congenital neutropenia, determination of subtype and treatment planning and also for genetic counselling.

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