Hax-1 deficiency in Turkish Children

Severe congenital neutropenia (SCN) comprises a heterogeneous group of hematopoietic disorders, characterized by granulocytic maturation arrest at the promyelocyte to myelocyte stage, ending with peripheral blood absolute neutrophil count below 0.5 x 10^9/l. SCN can serve as a model for the failure of myelopoiesis where dissection of its pathogenesis has yielded important insights into normal process of myeloid development. Neutrophil elastase, encoded by the ELA2 gene, is produced at the promyelocyte stage of neutrophilic differentiation and stored within the primary granules of mature neutrophils. Mutations were identified in ELA-2 in 60% of autosomal dominant and sporadic SCN. Recently mutations in HAX-1, encoding an anti-apoptotic protein, have been identified as the cause of some autosomal recessive forms of SCN including those present in the original pedigree first reported by Kostmann. HAX1 is a ubiquitously expressed mitochondrial protein with weak homology to bcl-2 that represses apoptosis.

The frequent occurrence of neurological deficits in patients with HAX1-deficiency, the association between HAX1-deficiency and central nervous system (CNS) abnormalities is remarkable. A genotype-phenotype correlation insofar as mutations that affect both HAX1 transcript variants I and II result in neutropenia and neurodevelopmental abnormalities, whereas HAX1 mutations affecting only transcript variant I result in neutropenia without CNS problems.

**Purpose**: The proposed study aims to analyse neutrophil elastase (ELA2) gene and HAX-1 gene mutations in 11 sporadic cases and four familial cases of severe congenital neutropenia (SCN) and their family members. HAX-1 mutation is known to be present in one of the familial cases.

**Methods**: Genomic DNA will be extracted from the patients' and family members' peripheral blood and the coding sequence of the ELA2 gene and HAX1 gene will be amplified by polymerase chain reaction and subjected to direct sequencing. Imaging tests for CNS will be done IQ test and detailed neurological examination, if the patient has neurological problem.
Results: W44X mutation in Exon 2 of HAX1 gene was determined in four of fifteen patients with congenital neutropenia that are in the same family. This mutation was also determined at the same region in five of sporadic cases.

We found heterozygous mutations at W44X, 10 of 49 index family members, and an homozygous mutation one of them. None of these carriers have clinical or laboratory abnormality. ELA 2 mutation could not detected any of the patients. Mild Mental Retardation was determined in 5 of the patients with HAX1 mutation. The results of Cranial MRI were normal in 9 of the patients with HAX1 mutation. Seven of nine patients with HAX-1 mutation had hearing loss, while 2 in 6 patients without any mutations had also hearing loss. Neurological examination of the carriers and patients without HAX 1 mutation were normal.

Conclusion: Contrary of European publications, we suggest that HAX 1 mutations are widespread in our country and it may be related with neurological symptoms.

Keywords: Severe congenital neutropenia (SCN), Kostmann disease, ELA-2 gene, HAX-1 gene, neurological involvement.