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Case Report

A Neonatal Case of Infantile Malignant Osteopetrosis Presenting with Thrombocytopenia and Hypotonicity: A Novel Mutation in Chloride Voltage-Gated Channel 7 Gene

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Abstract

Autosomal recessive osteopetrosis is also known as infantile malignant osteopetrosis (IMO). The clinical course is often serious and if left untreated, it is fatal in the 1st year of life. Diagnosis is challenging and often delayed or misdiagnosed. Herein, we present an infant girl who was diagnosed with IMO during evaluations for her hypotonicity and thrombocytopenia. A novel mutation of the chloride voltage-gated channel 7 (CLCN7) gene was also reported. A 10-day-old female patient was referred to our hospital for evaluation of hypotonicity. Her physical examination was normal, other than hypotonicity. Laboratory analysis revealed thrombocytopenia and hypocalcemia. In the progress, while she was followed in outpatient clinic, hepatosplenomegaly was detected at the age of 3 months. IMO was suspected with the findings of hepatosplenomegaly, cytopenia, hypocalcemia, difficulty of obtaining bone marrow, peripheral smear findings, and hearing loss. The X-ray of the bones was consistent with IMO. A novel pathogenic homozygous c.1504>T (p.Arg502Trp) mutation in CLCN7 gene was revealed. IMO is a rare disorder and it is important to differentiate this entity for better clinical outcome. The presence of neurological and hematological findings, organomegaly, hearing loss, and vision disorders must attract attention to IMO.

Keywords: Chloride voltage-gated channel 7 gene, Leukoerythroblastosis, Novel mutation, Osteopetrosis, Thrombocytopenia

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Osteopetrosis is a heterogeneous group of diseases that may affect the skeletal system in varying degrees and have multisystemic consequences. It occurs as a result of mutations in different genes that play a role in osteoclast function and differentiation. Due to complete or partial dysfunction of osteoclasts, bone resorption is impaired and bone mineral density has increased.[1,2] It is divided into three groups according to the clinical severity as mild,

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moderate, and severe.[3] The mild form shows autosomal dominant inheritance; it is also known as the adult form. It usually occurs in adolescence or adulthood. It occurs as a result of a single allelic mutation in the chloride voltage-gated channel 7 (CLCN7) gene.[1] The clinical course varies from asymptomatic cases to cases with severe clinical manifestations. It progresses with severe pain, fracture, and neurological disorders as a result of thickening in the vertebra, pelvis, and skull base.

Moderate form has a dominant or recessive inheritance. Age of onset is variable. Findings are often severe. In the recessive form, as a result of mutation in the carbonic anhydrase enzyme gene, renal tubular acidosis and calcification in the brain are observed.[2,3] Mild hearing loss and hematological findings are observed in some biallelic mutations of CLCN7, sorting nexin 10 protein (SNX10), receptor activator of nuclear kappa B (RANK), and T-cell immune regulator 1 (TCIRG1) genes.

The most severe form of the disease is autosomal recessive osteopetrosis (ARO). It is also known as infantile malignant osteopetrosis (IMO). Symptoms of the disease usually occur in the 1st month of life. The clinical course is often severe and if left untreated, it is fatal in the 1st year of life. About 70% of all IMO cases are TCIRG1 and CLCN7 mutations.[1] Other mutations are detected in SNX10, osteopetrosis-associated transmembrane protein 1, pleckstrin homology domain-containing family M member 1, RANK, and RANK ligand genes. Patients present with different clinical findings: Increased bone density narrows the medullary cavity and cranial nerve foramina, leading to extramedullary hematopoiesis, cytopenia, hepatosplenomegaly, blindness, deafness, and nerve palsies, respectively.[1-3] Diagnosis is challenging and often delayed or misdiagnosed. Thus, it is important that physicians should keep in mind this rare disease.

Herein, we present an infant girl who was diagnosed with IMO during evaluations for her hypotonicity and thrombocytopenia. The diagnostic difficulties and treatment options are discussed. A novel mutation of the CLCN7 gene was also reported.

Case Report

While a 10-day-old female patient was being followed up at an external center due to the transient tachypnea of the newborn, she was referred to us after her hypotonicity was noticed. The patient, who was born from the 3rd pregnancy of a 32-year-old mother at 38 weeks of gestation by cesarean, and weighing 3320 g with Apgar scores of 6 and 8 at the 1st and 5th min, respectively, was regularly followed up for antenatal care. There was a first-degree cross-cousin marriage between the parents, and she had two healthy siblings. Bodyweight was 3180 g (10–50 p), height 50 cm (50–90 p), and head circumference 35 cm (50–90 p). Her physical examination was normal, other than hypotonicity. In the laboratory tests, the hemoglobin (Hb) was 10.7 g/dL, mean corpuscular volume 93 fL, reticulocyte count 6.5%, platelet count $49x10^9$/L, mean platelet volume 10.4 fL, and white blood cell count 14×10^9/L. The peripheral blood smear revealed 55% neutrophil, 40% lymphocytes, polychromasia, poikilocytosis, anisocytosis, and normocytic erythrocytes without atypical cells. On biochemical analysis, hypocalcemia was detected (7.4 mg/dL). Further examinations of the patient who had hypotonicity, thrombocytopenia, and hypocalcemia were planned. Vitamin B12, folic acid, homocysteine, and thyroid hormone levels were normal. Initial examinations were performed concerning metabolic diseases, which were normal. No pathology was found in the cranial magnetic resonance imaging examination (MRI). In the studies for thrombocytopenia, the mother's thrombocyte count was normal. Regarding neonatal alloimmune thrombocytopenia, there was no history of siblings with neonatal thrombocytopenia. It was planned to follow-up thrombocytopenia and performs bone marrow examination if thrombocytopenia continues. Serological studies for viruses were all negative except cytomegalovirus (CMV). Anti-CMV immunoglobulin M was positive and ganciclovir treatment was initiated. Retinitis was not detected on ophthalmologic examination. During the follow-up, she could not pass the hearing test (otoacoustic emission) that was repeated twice. Her Hb level dropped and thrombocytopenia continued. She needed red blood cell and thrombocyte suspensions transfusions. Initially, her cytopenias were considered due to CMV infection and the ganciclovir treatment she was taking. Further evaluation was performed for hypocalcemia (Table 1). Due to increased levels of parathormone (398 ng/L) (normal range; 18.5–88 ng/L), calcitriol and calcium supplement were initiated. The patient, whose general condition was

Table 1. Bone metabolism tests

Calcium (mg/dl)	Ionized calcium	Phosphorus (mg/dl)	ALP (IU/L)	PTH (ng/L)	25(OH)D vit (ng/ml)	Albumin (gr/dl)
7.4	0.8	3.9	405	398	11.4	3.1

ALP: Alkaline phosphatase, PTH: Parathormone, 25(OH)D vit: 25-hydroxyvitamin D.

good, was discharged to be followed up under outpatient clinic conditions.

In progress, hepatosplenomegaly was detected at the age of 3 months. CMV DNA (polymerase chain reaction) test was negative twice and ganciclovir treatment was ceased. She was reevaluated for hepatosplenomegaly. Liver and spleen sizes increased on abdominal USG. Laboratory examination revealed Hb 8.3 g/dL, WBC 18.6 × 10^9/L, absolute monocyte count 6.5 × 10^9/L, and platelet count 71 × 10^9/L, and lactate dehydrogenase 1435 U/L. Peripheral blood smear was consistent with leukoerythroblastosis as there were myeloid and erythroid precursor cells, teardrop-shaped red cells, and polychromasia. Bone marrow aspiration was performed for possible malignant diseases, but it resulted in a dry tap. Because of splenomegaly and monocytosis, juvenile myelomonocytic leukemia was considered in the differential diagnosis. Fetal Hb was in the normal range (8%) for his age and t (9;22) was negative. IMO was suspected with the findings of hepatosplenomegaly, cytopenia, hypocalcemia, the difficulty of obtaining bone marrow, peripheral smear findings, and hearing loss. The X-ray of the bones revealed diffuse sclerosis within all bones (Fig. 1). This finding was consistent with IMO. Bone marrow aspiration was repeated and it was hypocellular and hematopoiesis was reduced. The measurement of visual evoked potentials revealed decreased vision.

For molecular analysis, genomic DNA was extracted from the peripheral blood. A novel homozygous missense variant (c.1504C>T; p.Arg502Trp) was detected in the CLCN7 gene (Fig. 2). The variant has not been previously reported in any public databases. The Mutation Taster, PROVEAN, and SIFT software programs predicted this variant as disease causing. According to guidelines, it is also predicted as a likely pathogenic variant.[4] Segregation analysis was performed using Sanger sequencing. It was shown that her parents and sister were heterozygous for the same variant, but her brother had wild-type sequence (Fig. 2). When she was 4 months of age, she had a tonic–clonic seizure, and

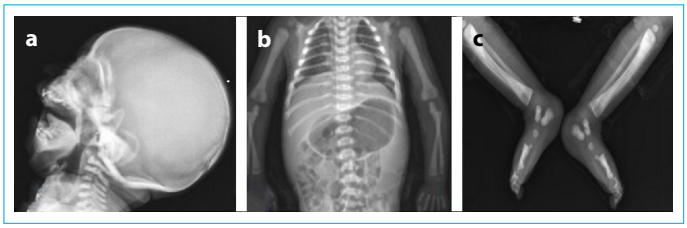

Figure 1. (a) The base of the skull and facial bones are sclerotic. **(b)** Diffuse sclerosis is present within all bones. There is metaphyseal widening and undermodeling. Metaphyseal lucent bands are seen at the ends of long bones. **(c)** Anterior-posterior radiographs of lower limbs demonstrate "bone-within-bone" appearance in the long bones, tarsal bones, and short tubular bones of feet.

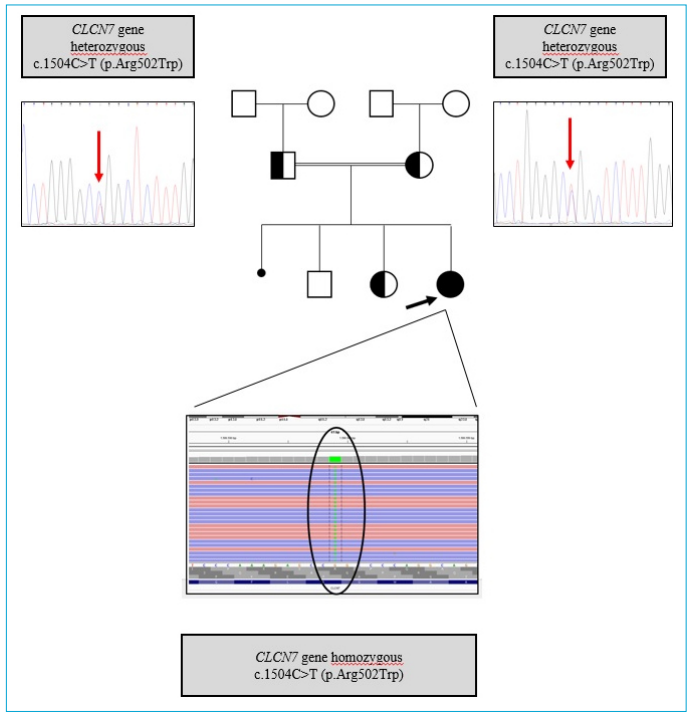

Figure 2. A novel homozygous missense variant (c.1504C>T; p.Arg502Trp) was detected in the chloride voltage-gated channel 7 gene. Segregation analysis demonstrated that her parents and sister were heterozygous for the same variant, but her brother had wild-type sequence.

in cranial MRI, the appearance was suspicious for neurodegenerative diseases. In electroencephalography (EEG), sharp wave discharges were observed in temporo-parieto-occipital regions of both hemispheres. Anticonvulsant treatments were started. Since the patient's findings were compatible with the neuropathic subgroup, hematopoietic stem cell transplantation (HSCT) was not indicated.

The patient was last seen at the outpatient clinic when he was 8.5 months old, and she had growth retardation, hearing, and vision loss. When the records of the patient were examined, it was learned that she died at the age of 10 months in an external center due to respiratory failure.

Discussion

IMO is a rare disease and there are delays in diagnosis and initiation of the treatment.[1] The incidence of IMO is 1:250,000 live births. Higher rates in specific regions were reported because of a high frequency of parental consanguinity.[1,3,5] The most common mutations that cause the disease are located in the TCIRG1 gene and account for more than 50% of ARO cases. The second most frequent form of ARO is CLCN7 gene mutations (17% of the cases).[1-4] The CLCN7 gene mutations may give rise to different clinical features. Biallelic mutations may cause a more severe

form associated with primary neurodegeneration in some patients.[3,5] The reduction of the bone marrow space due to increased bone tissue causes bone marrow insufficiency.[1-3] Therefore, patients present with anemia and thrombocytopenia, compensatory extramedullary hematopoiesis, and hepatosplenomegaly. Due to increased extramedullary hematopoiesis, CD34+ cells can be detected in peripheral blood. Our patient was presented with thrombocytopenia and hypotonicity in the neonatal period. Anemia, hepatosplenomegaly, and leukoerythroblastosis were detected in the clinical course.

Neurological damage occurs by two mechanisms as follows, primary and secondary.[1-3] In the first one, also known as a neuropathic form of the disease, severe seizures and developmental delay because neurodegeneration is observed. In the second form, cranial nerve damage develops secondary to sclerosis of the skull base, leading to hearing and vision loss. About half of those with biallelic CLCN7 mutations show neurodegenerative features.[1,5] Our patient demonstrated both primary and secondary neurological damage findings, as hypotonicity, neurodevelopmental delay, and vision and hearing loss. Cranial MRI and EEG results were consistent with the primary neuropathic form of IMO.

Prompt diagnosis is crucial for the clinical outcome of the patients. In our case, we suspected the diagnosis of IMO at the age of 3 months and genetically diagnosed it at the age of 5 months. Since physicians do not have sufficient knowledge related to this entity, most of the patients are diagnosed over the age of 8–9 months. This delay in diagnosis leads to the emergence of irreversible neurological damage and advanced disease.[6] The definitive diagnosis of IMO is made with targeted molecular genetic analysis. However, radiographic findings are characteristic, and physicians can rely on these results.[5] Diffuse sclerosis of bones, a thickened calvarium, transverse lucent metaphyseal bands, longitudinal diaphyseal striations, and flask-shaped metaphyseal dilatation of the long bones are the most common radiographic changes in IMO. Increased medullary density leads to a "bone-within-bone" appearance.[7] Diffuse sclerosis within all bones, metaphyseal widening and undermodeling, as well as "bone-within-bone" appearance in the long bones were present in the X-ray of our patient. IMO was considered with hypotonicity, hearing loss, hypocalcemia, hematological findings, hepatosplenomegaly and peripheral smear findings; and direct radiography image supported the diagnosis. She was 5 months old when the diagnosis was confirmed by mutation analysis. Our patient had a novel homozygous missense variant (c.1504C>T; p.Arg502Trp) in the CLCN7 gene. In silico prediction tools classify this variant as a pathogenic/likely pathogenic variant, as it is located within a conserved region (GERP score: 4.48, range – 12.3–6.17).

Genetic tests are significant in confirming the diagnosis, detecting the disease subtype, and predicting response to specific treatments. At least 10 genes that have been shown to be pathogenic in humans have been identified. CLCN7 encodes the H(+)/Cl(–) exchange transporter 7, which provides chloride conductance across lysosomes in osteoclasts, ensuring the acidification that is necessary for the cell function. Mutations in CLCN7 have been reported in the three subtypes of osteopetrosis. In recent years, novel CLCN7 gene mutations have been reported in some patients from different geographical regions with mild, moderate, and severe clinical findings.[8-10] To establish a relationship between genotype and phenotype, simple and reliable tests showing the effects of CLCN gene mutations on protein function are needed.

Medical treatments do not change the clinical course of the disease. Calcium replacement and blood transfusions can be performed as supportive treatment. Steroid, Vitamin D, and interferon-gamma have been used in treatment, but positive results have not been obtained.[7] HSCT is the only curative treatment.[1-3,6] Bone manifestations of IMO such as bone sclerosis, bone marrow failure, and extramedullary hematopoiesis can be prevented or reversed by HSCT.[5,7] Since neurodegenerative processes which are intrinsic to neurons are not prevented or improved by HSCT, it is not recommended in the neuropathic form of the disease with neurodegenerative findings.[1,4] Therefore, it is significant to differentiate this rare subset of disease. On the other hand, some CLCN7 gene mutations are clearly associated with neurodegenerative disease. Even HSCT is performed in these patients before the development of neurological findings; neurological deterioration cannot be prevented.[4] Therefore, a careful neurological evaluation, including EEG and cranial MRI, is mandatory and should be repeated before the conditioning for HSCT. Disease-free survival was reported as 73–79% in a full match sibling donor and 38–43% in non-relative transplants.[11] As a consequence, in the long-term efficacy of HSCT, early diagnosis of the IMO and timing of HSCT play a significant role. IMO is lethal within the first decade of life unless HSCT is performed. Unfortunately, there is not a curative treatment for patients with neuropathic forms of the disease. Because our patient's findings were consistent with neuropathic form, although she had a full match sibling donor, HSCT was not indicated.

Conclusion

IMO is a rare disorder and it is significant to differentiate this entity for better clinical outcomes. The presence of

neurological and hematological findings, organomegaly, hearing loss, and vision disorders should attract attention to IMO. Genetic analysis confirms the definitive diagnosis. Early diagnosis and HSCT may prevent the complications of the disease. Since rarity and heterogeneity of osteopetrosis may lead to diagnostic difficulties, we aimed to increase awareness regarding this disorder. Inform consent was obtained from the patient's family.

Disclosures

Informed consent: Written informed consent was obtained from the patient for the publication of the case report and the accompanying images.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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