

Low function of natural killer cells in treated classic Menkes disease

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ABSTRACT

Background. Menkes disease (MD) is a rare lethal X-linked, multisystem disorder of copper metabolism resulting from mutations in the ATP7A gene. Features such as Ehlers-Danlos syndrome, trichopoliodystrophy, urologic and skeletal changes have been reported. We present a case of classic MD treated with copper infusions who suffered from persistent natural killer (NK) cell dysfunction.

Case. A 2-year-old, Caucasian male child presented at 8-month-old of age with persistent hypotonia, kinky hair and developmental regression. Diagnosis of MD was based on low serum levels of copper [5 mg/dl (18-37)] and ceruloplasmin [18 ug/dl (75-153)] and gene-targeted deletion/duplication analysis performed by the reference laboratory. Brain MRI showed mild hypoplasia of the cerebellar vermis and vascular tortuosity typical of MD. Copper chloride treatment was immediately initiated. The child became more alert with excellent eye contact and purposeful movements. The child was hospitalized for recurrent respiratory infections, each time caused by enterovirus as confirmed by multiplex polymerase chain reaction (PCR). Extensive immunologic studies were negative, except for a severe NK cell dysfunction on multiple occasions (0.6 NK Iytic Units; N >2.6).

Conclusion. We postulate that NK cell dysfunction in a classic MD can be explained by the deficient incorporation of copper in the endoplasmic reticulum resulting in an abnormal Fenton chemistry within phagosomes.

Key words: Menkes disease, copper, natural killer cell, recurrent infection.

Classic Menkes disease (MD) is a rare lethal X-linked multisystem disorder of copper metabolism caused by mutations in the ATP7A gene and leading to low serum copper and ceruloplasmin levels. The ATP7A gene encodes a transmembrane copper-transporting P-type ATPase (MNK) localized to the trans-Golgi network (TGN). MNK protein is essential for systemic copper absorption and provides copper to secretory cuproenzymes traversing the TGN.¹ Parenteral copper administration is successful in normalizing copper and ceruloplasmin levels. It also improves the muscle

tone and motor activity and most importantly prevents seizures as long as some MNK activity is preserved.² However, the primary clinical manifestations such as peculiar kinky hair (trichopoliodystrophy), hypopigmentation, connective tissue disturbances (Ehler Danlos syndrome) are frequently not improved by parenteral copper supplementation.³⁻⁵

Susceptibility of children with MD to recurrent infection is well documented and thought to be due to hematological consequences of copper deficiency and defective immune functioning due to copper deficiency. We present a male child with classic MD, who manifested with recurrent viral infections due to a proven natural killer (NK) cell dysfunction, which occurred despite continued parenteral copper supplementation. To our knowledge, such a feature has never been reported earlier in patients with MD.

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Case Report

A 3-year-old, Caucasian male child presented at 8-month-old of age with irritability, hypotonia, kinky hair and developmental regression. He was diagnosed with MD based on low serum levels of copper {5 mg/dl (18-37)} and ceruloplasmin {18 ug/dl (75-153)} and Pilli Torti on light microscopy. Gene-targeted deletion/duplication analysis performed by the reference laboratory (Dr. Stephen Kaler Laboratory) revealed a novel deletion of 363 bases involving the 3'end of ATP7A exon 12 (encoding the final 4 amino acids) plus a segment of intron 12 (c.2617_2626+342del)(p.Leu873_Gly876del). Brain MRI showed mild hypoplasia of the cerebellar vermis and vascular tortuosity typical of MD. Parenteral copper chloride treatment was immediately initiated. Following the initiation of copper treatment, the child became less irritable and more alert with excellent eye contact and purposeful movements. Peculiar hair, hypopigmentation, and connective tissue disturbances failed to improve.

The patient also required several in-patient admissions since the initiation of copper treatment. Recurrent viral upper respiratory tract infections were the main reasons for most of these admissions, each time caused by enterovirus as confirmed by multiplex polymerase chain reaction (PCR) testing on the nasopharyngeal secretions. In addition, he was also admitted for multiple episodes of fever of unknown origin. Extensive immunologic

studies including serum immunoglobulin levels were negative.

On two occasions, peripheral blood was obtained to measure plasma copper levels and serum ceruloplasmin levels. Both times, NK quantification and measurements of Natural Killer (NK) activity were performed.⁶ The first time at 13 months, plasma copper level was borderline low (64 mcg/dl; N: 75-153) while serum ceruloplasmin level was normal (20 mg/dl; N: 18-37). The second time at 23 months, both copper (76 mcg/dl; N: 75-153) and ceruloplasmin (19 mg/dl; N: 18-37) were normal. On both occasions, flow cytometry showed that the percentage of circulating CD16+CD56+ NK cells was normal (3%; N: 3-16) and NK cell function was decreased (0.6 NK lytic Units; N>2.6) (Table I). This finding of NK cell dysfunction has not been reported previously in MD. For reporting this case, informed consent was obtained from the mother of the patient.

Discussion

MD is a progressive disorder with varying severity of the clinical course. Severe forms of MD usually result in early childhood death. Either intravenous copper sulfate administration or subcutaneous administration of copper histidine, if initiated early, can successfully modify the disease progression and improve long-term clinical outcomes.² Though copper supplementation, intracellular cytoplasmic, nuclear and mitochondrial

Table I. Serial NK cell activity, plasma copper and ceruloplasmin levels.

Variables	Age 13 months	Age 23 months	Reference range
Plasma copper (mcg/dl)	64	76	75-153
Serum ceruloplasmin (mg/dl)	20	19	18-37
CD16+ CD56+ NK cells (%)	3	3	3-16
50:1 E:T Ratio (% lysis)	7	7	≥ 20
25:1 E:T Ratio (% lysis)	3	3	≥ 10
12.5:1 E:T Ratio (% lysis)	2	2	≥ 5
6.25:1 E:T Ratio (% lysis)	1	1	≥ 1
NK Lytic units	0.6	0.6	> 2.6

NK: natural killer cell, E: T ratio: Effector-to-target ratio.

copper levels are improved, but still, the trans face of the Golgi complex with secretory functions remain deficient in copper. Thus, functions of cuproenzymes in cytoplasm [Superoxide Dismutase 1(SOD), laccase], nucleus (metal-regulatory transcription factor 1) and mitochondria (cytochrome oxidase, SOD1/2) improve, while secretory enzymes (dopamine beta-hydroxylase, tyrosinase, peptidyl-alpha amidating enzyme, diamine oxidase, monoamine oxidase, lysyl oxidase, and SOD3) remain deficient⁷, which could explain the persistent clinical manifestation despite normalization of copper and ceruloplasmin levels through parenteral copper supplementation.

Susceptibility to infections, including pulmonary and urinary tract infections and septicemia, has been reported in MD. Underlying systemic copper deficiency in MD and resultant neutropenia and humoral immunodeficiency are most probably the reason for increased susceptibility to infections in untreated patients.^{7,8} Our index case was hospitalized several times after normalization of copper and ceruloplasmin levels. Most of these admissions included respiratory tract infections. Further evaluations confirmed rhin-enterovirus through PCR as the cause of these respiratory infections. Extensive immunologic studies were negative, except for a severe NK cell dysfunction (0.6 NK lytic Units; N >2.6). This finding of NK cell dysfunction has been unreported previously in human or animal models of MD. Previously, animal studies have shown that ATP7A copper transporter is required for macrophage-mediated killing of infectious organisms by enabling the transport of copper from Golgi complex to cytoplasmic vesicles.⁹ It has also been postulated that phagosomal copper catalyzes the production of hydroxyl radicals from hydrogen peroxide via Fenton-like chemistry. We speculate that NK cell dysfunction in classic MD can be explained by the deficient incorporation of copper in trans face of the Golgi complex leading to a reduced Fenton chemistry within phagosomes.¹⁰

NK cell dysfunction in classic MD has never been reported. We speculate that reduced NK cell function was the underlying mechanism for susceptibility to recurrent infections in the index case. We consider that NK cell dysfunction in classic MD can be explained by the deficient incorporation of copper in trans face of the Golgi complex.¹⁰ Therefore, evaluation of NK cell function should be considered in patients with classic MD.

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