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Case report

Symptomatic copper deficiency in three Wilson's disease patients treated with zinc sulphate

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ARTICLE INFO

Article history:
Received 6 March 2014
Accepted 6 May 2014
Available online 17 May 2014

Keywords:
Copper deficiency
Wilson's disease
Zinc sulphate

ABSTRACT

Wilson's disease (WD) is caused by excess of copper that leads to accumulation of copper mainly in the liver, brain and needs life-long decoppering therapy. However, overtreatment with anti-copper agents may lead to copper deficiency which may cause neurological and hematological symptoms. Copper is an important cofactor for many enzymes. This report describes three WD patients with diagnosed copper deficiency during zinc sulphate (ZS) treatment. After 5–16 years of therapy all patients developed leucopenia. Spinal cord injury was manifested in two of the patients. One of them also presented myopathy. In conclusion, copper deficiency may occur in different time after treatment onset, therefore regular copper metabolism and hematological monitoring is necessary.

1. Introduction

Wilson's disease (WD) is an inherited copper metabolism disease that leads to accumulation of copper in the liver, brain, cornea, and other organs. The clinical course of WD may be highly variable and includes hepatic, neurological, and psychiatric symptoms [1,2]. WD is an autosomal recessive disorder caused by mutation of the *ATP7B* gene on chromosome 13 [3], which encodes a copper-transporting P-type ATPase [4]. The aim of WD treatment is to remove excess of copper and prevent its re-accumulation [5,6]. There are two different therapeutic approaches in WD. The first group of drugs (d-penicillamine, trientine) are chelating agents which act by promoting the urinary excretion of copper. The second group (zinc salts) interfere with intestinal uptake of copper [7,8].

Clinical symptoms of WD are caused by excess of copper. However, copper is needed because it acts as important cofactor for many important enzymes that have a role in functioning of the nervous system including cytochrome-c oxidase, copper-zinc superoxide dismutase, and dopamine β-hydroxylase [9].

Possible causes of copper deficiency include hereditary conditions such as Menkes Disease and acquired causes: malnutrition, parenteral or enteral feeding without copper supplementation, gastrectomy, proximal bowel resection, over-treatment by zinc salts or copper chelating agent [10].

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http://dx.doi.org/10.1016/j.pjnns.2014.05.002

Copper deficiency may present both hematological (anemia, neutropenia, thrombocytopenia) and neurological (axonal neuropathy, myelopathy, posterior spinal column dysfunction, central nervous system demyelination, myopathy) signs. MRI findings include changes in dorsal columns of spinal cord radiologically similar to those caused by vitamin B12 deficiency and demyelination lesions in brain [10–17]. Electrophysiological studies may indicate axonal neuropathy, central conduction delay in somatosensory pathways, prolonged visual evoked potentials, myopathic changes [17,18].

Copper deficiency in WD may be caused by too excessive treatment with anti-copper agents. We report three patients with WD who were diagnosed with copper deficiency during zinc sulphate (ZS) treatment.

2. Case reports

2.1. *Patient 1*

A 37-year-old woman with WD diagnosed 16 years ago and since then treated with anti-copper agent. She complained of paraesthesias in the fingers and toes for 3 months and she felt weakness of the lower limbs during fast walking over the past 1 month. She was diagnosed in presymtomatic phase of the disease (without hepatic, neurologic signs, no Kayser–Fleischer rings). Diagnosis was confirmed by genetic studies. Her brother was a proband. Since diagnosis she was taking ZS in daily dose 180 mg of elementary zinc. She regularly visited our clinic and earlier she had never had neurological symptoms. Hematological tests were always normal. Copper metabolism did not indicate overtreatment. Additionally, laboratory test results had revealed leucopenia 1 month before symptoms started (Table 1).

On admission to our clinic her neurological examination was normal. Copper metabolism test results showed very low concentration of serum ceruloplasmin and serum copper. Copper urinary excretion was also low. Zinc serum concentration was very high (Table 1). Somatosensory evoked potentials (SEPs) showed impaired conduction in the dorsal column, especially in thoracic spine. Nerve conduction studies were within normal limits. Electromyography (EMG) was suggestive of myopathy. A few low-amplitude, short duration motor units were noted. There was no denervation and recruitment was normal. MRI of cervical spine, showed linear increased T2 signal lesion in the posterior column of the cervical cord from C2 to C7-Th1 (Fig. 1A and C). Brain MRI was normal.

Vitamin B12 level was in normal range. We recognized copper deficiency. Liver tests results were normal, so we decided to withdraw ZS to increase serum copper concentration. After one month white cell blood count was normal. Follow-up copper metabolism test results showed increase serum ceruloplasmin concentration (5 mg/dl) and serum copper concentration (20 μg/dl). She did not report lower limbs weakness and paraesthesias in the fingers and toes were less pronounced. MRI of cervical spine showed marked diminished dorsal columns compared to previous examination (Fig. 1B and D). On the second examination myopathic

Table 1 – Copper metabolism parameters and hematological parameters in three WD patients with copper deficiency.

Patient no	Serum ceruloplasmin (mg/dl) normal range 25–45	Total serum copper (μg/dl) normal range 70–140	Urinary copper excretion (μg/24 h) normal range 0–50	Serum zinc (μg/dl) normal range 50–120	WBC (K/μl) normal range 4.5–10.5	Neutrophils ($\times 10^9$/L), normal range 2.0–7.5	RBC (M/μl) normal range 4.10–5.10	HGB (g/dl) normal range 12–15.5	PLT (K/μl) normal range 140–440
Patient 1									
WD diagnosis (1996)	9.25	35	135	–	5.9	3.8	4.5	13.7	189
Overtreatment (2012)	**0.92**	**<5**	**11**	474	2.9	2.1	4.7	13.5	186
Follow-up after 6 months (2013)	5	20	12.5	–	6.0	4.1	4.5	12.9	197
Patient 2									
WD diagnosis (2008)	7.7	105	394	–	5.2	3.5	4.1	12.2	155
Overtreatment (2012)	**0.5**	**<5**	**6**	192	1.86	0.89	4.0	12.0	256
Follow-up after 12 months (2013)	1.18	5	10.5	–	3.3	2.0	4.2	12.5	274
Patient 3									
WD diagnosis (2007)	14	44	15	–	4.7	3.0	4.9	14.5	244
Overtreatment (2012)	**0.9**	**7**	**12**	247	2.3	0.17	3.2	10.0	190
Follow-up after 12 months (2013)	17	44	10	–	7.3	5.1	5.0	14.7	285

Values marked in bold indicate copper metabolism parameters at the time of overtreatment.

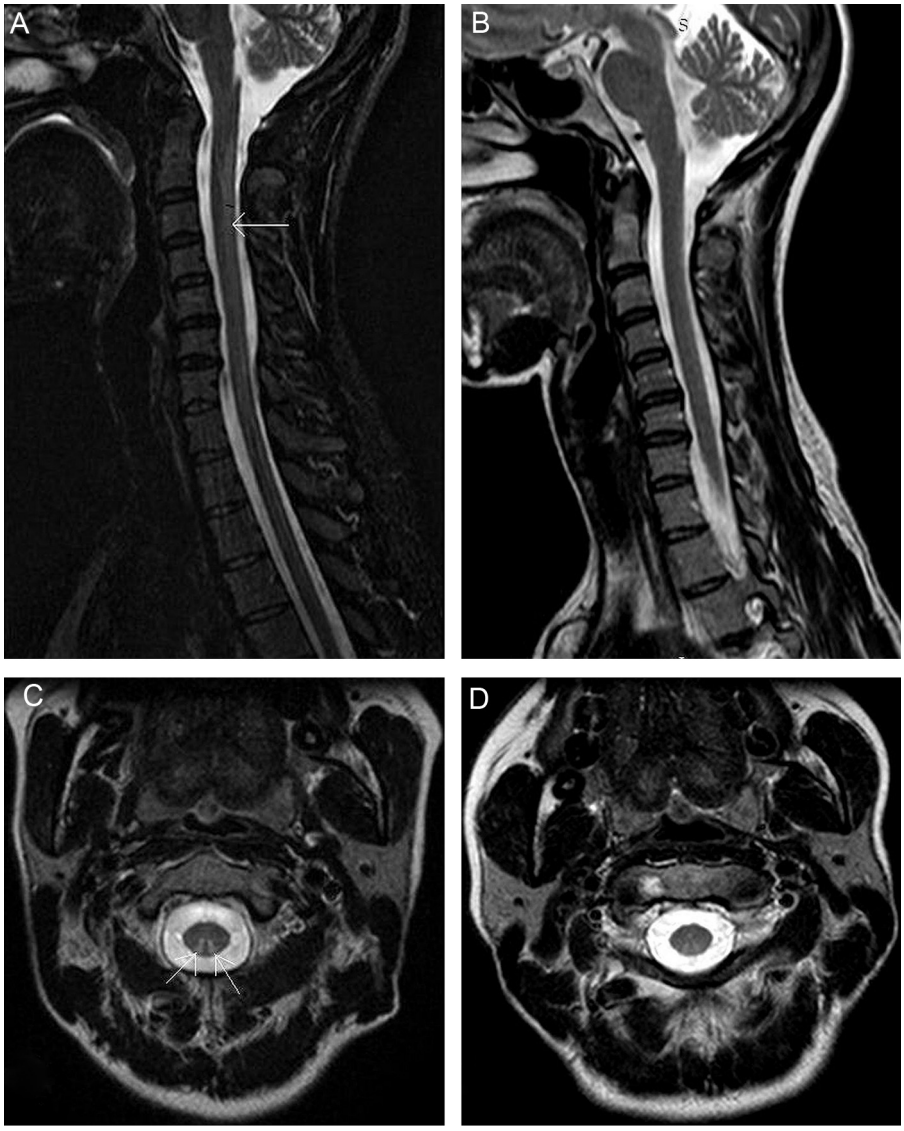

Fig. 1 – (A) Sagittal T2-weighted MRI of the cervical spine: linear increased T2 signal (arrow) in the posterior surface of the cervical cord from C2 to C7-Th1 (March 2013) and follow-up MRI, (B) without evident changes (October 2013), (C) axial T2-weighted image shows the signal hyperintensity (arrows) located symmetrically in the dorsal columns and follow-up MRI with slightly hyperintensity signal in dorsal column (D).

changes on EMG normalized, however SEPs still indicated abnormalities in the dorsal column.

Nine months after interruption in anti-copper treatment after clinical and laboratory improvement, we decided to introduce d-penicillamine instead of ZS.

2.2. *Patient 2*

A 46-year-old woman diagnosed with WD 5 years ago. She started to have recurrent upper respiratory tract infections for the last 6 months. At WD diagnosis she had liver failure and typical for WD biochemical changes (Table 1). Diagnosis was confirmed by genetic studies. She was taking zinc sulphate (180 Zn of elementary zinc daily). Liver function markedly improved. Regular hematological tests were normal. Copper metabolism parameters did not indicate overtreatment.

Due to respiratory infections she had basic laboratory tests done which indicated leucopenia with neutropenia (Table 1). We also found significant, in comparison with previous results, decrease of serum ceruloplasmin concentration (0.5 mg/dl) and serum copper (<5 μg/dl) suggesting copper deficiency during WD overtreatment (Table 1). SEPs were within normal limits. We decided to decrease the dose of zinc sulphate to 135 mg/24 h. Her hematology parameters improved and the patient did not report any infections. Follow-up copper metabolism tests results showed increased level of serum ceruloplasmin 1.18 mg/dl, but still low serum copper concentration (5 μg/dl). We decided to treat this patient

chronically with lower dose of ZS (135 mg elementary zinc) with regular monitoring of copper metabolism parameters.

2.3. *Patient 3*

A 18-year-old woman with presymptomatic WD diagnosed 6 years ago, was admitted to our clinic because of leucopenia with neutropenia and anemia (Table 1). Before admission to our clinic she had diagnostics in hematology center. A bone marrow biopsy was performed. Myelodysplastic syndromes and aplasia were excluded. It was suggested that hematological signs are caused by anti-copper treatment.

She has never presented any WD symptoms. Her mother was diagnosed with neurologic form of WD disease. The WD diagnosis was confirmed by biochemical tests result (Table 1) and radiocopper study was used. Since diagnosis she was treated with ZS in daily dose 180 mg. On admission to our clinic her neurological examination was normal. Copper metabolism test results showed very low serum ceruloplasmin concentration, copper serum concentration and low copper urinary copper excretion (Table 1). Brain MRI did not show typical of WD changes. SEPs showed impaired conduction in the dorsal column. Nerve conduction studies were within normal limits. The patient refused to undergo MRI of cervical spine. Vitamin B12 level, folic acid, liver tests results was in normal range. We decided to withdraw ZS and after two months white cell blood count was normal. Follow-up copper metabolism test results showed higher concentration of serum ceruloplasmin and serum copper. However, urinary copper excretion was still very low and we decided to leave the patient without anti-copper treatment for longer period (Table 1).

3. Discussion

All 3 described patients were treated with Zn for 5–16 years and had clinical and laboratory signs suggestive of copper deficiency.

Copper deficiency in WD has been reported in few patients so far. First reported patient in 1989 with hematologic signs was treated with high zinc salts doses (~275 mg of elementary zinc) [19]. Recently, 6 other WD patients with neurological symptoms of copper deficiency were reported. They presented axonal neuropathy ($n = 3$), myeloneuropathy ($n = 1$), myelopathy ($n = 1$) and CNS demyelination ($n = 1$). One of them also presented kidneys involvement. Three of those reported patients were on zinc monotherapy. Two of them were treated with very high daily doses (~275 mg of elementary zinc). The others were treated with combination of d-penicylamine or trientine and zinc salts [20–25].

Our patients were treated with standard doses of ZS (180 mg of elementary Zn^{2+}) in monotherapy. All of them were regularly monitored with copper metabolism parameters and basic laboratory tests. Two of them had presymptomatic form of WD. All previously reported cases were symptomatic. All had leucopenia which may be seen in course of WD [26]. However, all presented patients had normal hematological tests at the time of diagnosis. Myelopathy together with myopathic changes in electromyography were not to our knowledge reported in WD patient with copper-deficiency before. However, myopathy was reported as possible sign in copper deficiency [17]. These myogenic changes in our patient disappeared after 6 months in treatment disruption.

High zinc concentration may cause copper deficiency because during zinc therapy intestinal absorption of copper decreases. Zinc induces concentration of metallothionein in enterocytes. Copper has higher affinity for metallothionein than zinc and displaces zinc from metallothionein. Then copper is sloughed off into the intestinal tract [13].

In our patients we examined total serum copper concentration, which is decreased in WD. Toxic free copper concentration may be calculated from total serum copper concentration and serum ceruloplasmin concentration [5]. Calculated free copper concentration in our patients is very low (<5 μg/dl) as well as serum copper concentration (<1 mg/dl) .The precise method is direct measurement of free blood copper concentration [27]. More recent, relative exchangeable copper (REC) was demonstrated as accurate tool to determine fraction of free blood copper concentration [28].

According to European and American recommendations on WD treatment monitoring of therapy should be regular. At least twice per year and more frequent at the beginning of therapy. Treatment monitoring should include copper metabolism parameters (serum copper and ceruloplasmin concentration, urinary copper excretion) as well as liver function parameters and complete blood count. By measuring the serum non-ceruloplasmin (free copper) bound copper concentration we may assess treatment efficacy. In inadequately treated patients it is usually above 25 μg/dl (normal range 10–15 μg/dl). In case of overtreatment the values are very low (<5 μg/dl). Additionally, in patient treated with d-penicillamine urinary copper excretion should be between 200 and 500 μg/24 h and for patients on zinc it should be below 75 μg/24 h [5,8,29].

4. Conclusion

Overtreatment with zinc salts may cause deficiency of copper with hematologic and neurologic symptoms. Our cases show that regular monitoring of copper metabolism tests and blood count cells is very important for safety treatment with zinc salts and early recognition of copper deficiency. Patients should be under care of center experienced in monitoring anti-copper treatment for proper analysis of copper metabolism parameters to avoid such treatment complication.

Conflict of interest

None declared.

Acknowledgement and financial support

None declared.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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