

ORIGINAL ARTICLE

ELEMENTS REGULATION DURING CARTILAGE AND BONE DEFORMITY - POTENTIAL CLINICAL INDEX IN EARLY DIAGNOSIS, MONITORING AND PROGNOSIS IN CHILDREN OF KASHIN-BECK DISEASE

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Background: Kashin-Beck Disease (KBD) is a chronic deforming osteoarthritis starting in early childhood and affecting the cartilage metabolism and endochondral ossification. Selenium (Se) deficiency has been postulated as the major environmental etiological factor for KBD by many studies. Other minerals such as the Manganese (Mn) and calcium (Ca) which don't have uniform distribution in environment are also important elements involved in bone and cartilage formation but their regulation in KBD has been rarely reported. The study was done to investigate the role of Mn and Ca in addition to Se in KBD. **Methods:** In this study, the Se, Mn and Ca levels were investigated in children from different groups (KBD group, Healthy group from KBD endemic areas (inner control group), Healthy group (outer control group) from Non KBD areas and KBD group with selenium supplementation). The contents of Mn, S and Ca in serum and hair were analyzed by inductively coupled plasma mass spectrometry. **Results:** The increased Mn levels of serum and hair in KBD children were observed compared with normal groups. The Mn and Ca have similar trends in different groups but Se and Mn displayed reversed trends. **Conclusions:** The Mn and Ca contributed to KBD pathogenesis combined with Se in regulation of growth and development. The relative ratio of Mn to Se can be a potential clinical index in early diagnosis, monitoring and prognosis of KBD in children.

Keywords: Kashin-Beck disease, selenium, manganese, calcium, relative ratio

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INTRODUCTION

Kashin-Beck Disease (KBD) is an endemic osteochondropathy characterized by focal chondronecrosis in the deep zone of epiphyseal growth and articular cartilage and causes abnormal endochondral ossification. It results in growth retardation and secondary chronic deforming osteoarthritis. In the world, KBD is distributed in mountainous and hilly zones of Eastern Siberia of Russia, China and Northern Korea.

The latest report revealed that there are 0.64 million patients with KBD and 104.74 millions of people at risk in China.¹ KBD primarily affects 3-12 years old children. In China, KBD is distributed in the low selenium (Se) zone from the northeast to southwest, the Se contents of cultivated and natural soils in KBD areas are lower than those in non KBD areas.² Se is an essential trace element for humans and animals. It has been proposed that endemic Se deficiency is the major environmental hypothesis for KBD aetiology.³ Epidemiological studies revealed that low Se contents of cereals, soil and drinking water caused lower Se contents of blood, urine and hair in KBD children as compared to healthy.^{4,5} The investigation of mineral content in Tibetan foods showed Se deficiency but Manganese (Mn) exceeded toxicity threshold.⁶ In another report, the Mn content in wheat of KBD area

was higher than that in non KBD area but without significant difference.⁷

In Russia, Ivanov and Voshchenko proposed that Mn and phosphorus were involved in the aetiology of KBD. They found that the Mn content of diet in KBD areas was higher than that in non KBD areas.⁸ Mn intoxication was caused by redundant content in soils, plants and water.^{9,10} While the investigation in Transbaikalia of Russia revealed that the Mn content of water in endemic area was higher than that in domestic drinking water but not exceeded the daily physiological requirement.¹¹ Although the Se deficiency is considered as the major element to be involved in aetiology of KBD, other elements, such as Mn and calcium (Ca), may contribute to the pathogenesis due to its importance for normal skeletal growth and development, and the metabolism in vivo. In this study, Mn and Ca contents combined with Se deficiency in KBD and Non KBD area was investigated.

MATERIAL AND METHODS

In this experimental study four groups were compared for Se, Mn and Ca levels. Three groups were taken from KBD endemic area and the fourth group from non KBD endemic area. Bin County was randomly selected from the KBD endemic counties of the Shaanxi province and 60 male KBD patients aged 6-14 years old, diagnosed by clinical criteria¹²

including radiological changes and physical symptoms were included. They were divided into two groups. One group as KBD group, the other one, i.e., experimental group was given Na₂SeO₃ as Se supplement group with 1 mg per week for 6 months. In the same county, 30 children without KBD were selected as internal-control group (KBD area control group). Another 30 healthy children (Non-KBD area Control group) were included as healthy group from Chang'an County of Shaanxi province which is a non KBD area. The serum and hair samples were collected from all participants. The contents of elements in serum and hair were analysed by inductively coupled plasma mass spectrometry (ICP-MS; Sciex Elan 6000, Perkin-Elmer; hair samples were assayed at Biological Trace Element Research Institute, University of California, USA). The data were analysed by SPSS 19.0. This investigation was approved by the Human Ethics Committee of Xi'an Jiaotong University.

RESULTS

There were four groups in the study. The Se, Mn and Ca levels of the four groups in hair and serum were compared using ANOVA. There were significant differences between KBD group and internal-control group (Non-KBD) for Se in serum, Mn in hair and serum (Figure-1, 2). The Se content of hair in KBD group was similar to KBD area control group, while lower than that in Non KBD area control group. The serum Se was lower and Mn contents of hair and serum were much higher in KBD group compared to both control groups in KBD and Non KBD areas. After giving sodium selenite for 6 months, Se contents in hair and serum of the experimental groups were increased but were still lower than normal; Mn contents in hair and serum were decreased but still higher than normal. Both of them have significant difference between KBD group and Se supplement group in hair and serum. The Se and Mn contents of hair and serum in different groups displayed reversed trends (Figure1, 2).

The elements which are involved in growth and development including Se, Mn, Ca, have the combined trends with significant difference in one way ANOVA analysis (Figure-1, 2). And Mn showed opposite results in children hair and adult chondrocyte. The Mn and Ca level were higher in KBD children than in healthy and KBD area control group, but decreased after Se supplementation. To illustrate the level and relationship between Mn and Se, Mn and Ca, the relative ratio were calculated as Mn to Se, and Mn to Ca/100 (Figure 3) in hair. Relative ratios (RR) of Mn to Se (MS) were higher in KBD group; moderated in Na₂SeO₃ supplementation group and similar to KBD area control group, but still higher than in non KBD area control group. The relative ratios of Mn to Ca(MC) were changed lightly. The relative ratios of Mn to Se in serum (Figure-4) have obtained the similar result.

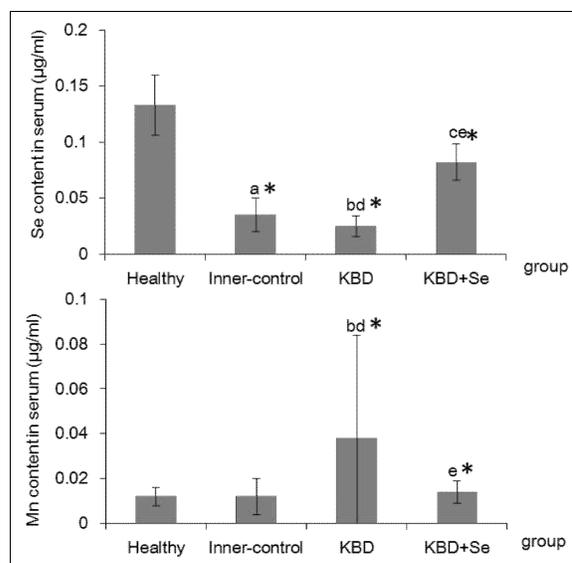


Figure-1: Selenium and Manganese levels (µg/ml) in serum of children.

a: Healthy-Inner control; b: Healthy-KBD; c: Healthy-KBD+Se; d: Inner control-KBD; e: KBD-KBD+Se; **p*<0.01.

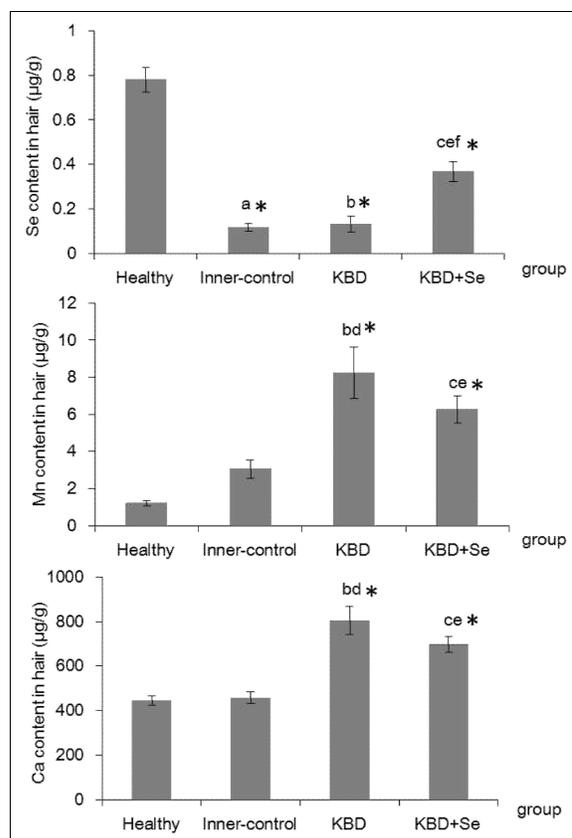


Figure-2: Selenium, Manganese and Calcium levels (µg/ml) in hair of children.

a: Healthy-Inner control; b: Healthy-KBD; c: Healthy-KBD+Se; d: Inner control-KBD; e: Inner control-KBD+Se; f: KBD-KBD+Se; **p*<0.01.

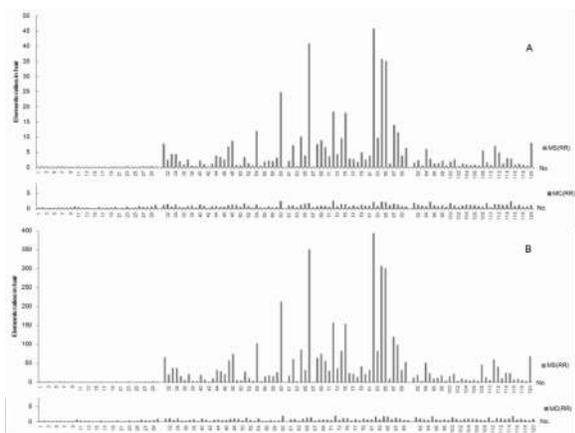


Figure-3: The relative ratio of Mn to Se and Mn to Ca/100 in hair of children.

Healthy group (case 1 to 30), Inner control group (case 31 to 60); KBD group (case 61 to 90); and Na₂SeO₃ supplementation group (case 91 to 120). A: MS (RR) and MC (RR) compare to crowd; B: MS (RR) and MC (RR) compare to healthy.

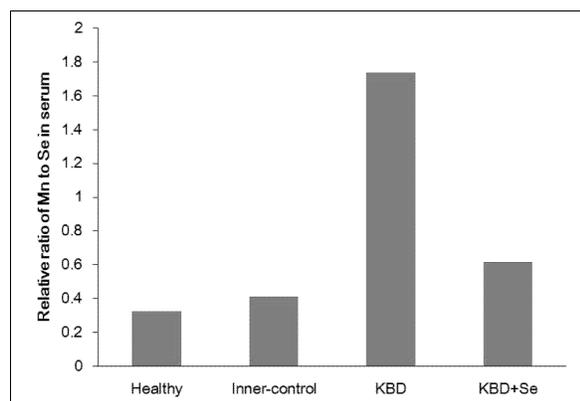


Figure-4: The relative ratio of Mn to Se in serum of children.

DISCUSSION

The differential expressed proteins in KBD adult chondrocyte related to Se, Mn and Ca were studied by 2-DE and MALDI-TOF/TOF MS analysis from our previous study.¹³ Mn is a component of the antioxidant enzyme MnSOD, and as important substance or cofactor in glycolysis. The PGAM1, PGK1 and UGPA were involved in it. The selenium is involved in antioxidant enzyme catalysis and is related to collagen formation. The CNN2 and CAP-G altered in KBD chondrocyte are related to calcium.

In this investigation, Se contents were lower in vivo and cereals in KBD; Mn contents were lower in cereals in KBD area. While, Mn and Ca levels were higher in vivo of KBD children, and the MnSOD expression in KBD adult cartilage was decreased. After applying Se supplementation, Mn and Ca contents were moderated in vivo. The result displayed the regulation complexity in absorption of elements, due to the unordinary contents in previous

reports, and the difference in cereals and in vivo. The Mn concentration of whole blood in children under 18 years old was $14.8 \pm 3.8 \mu\text{g/L}$; and $56.4 \pm 16.4 \mu\text{g/L}$ of neonate blood was observed in Japan.^{14,15} We observed that Mn concentration of KBD children was higher than that of normal but lower than normal neonate. It may result from the requirement of Mn and Ca storage, increased absorption and reduced excretion similar to the regulation in neonate, and displayed specific compensatory response in matrix degradation and bone formation or re-modelling in deformity, since the patients has the enlarged and deformed joint. The Se and Mn were involved in formation and regulation of cartilage matrix, and Se could be helpful in the positive regulation on abnormal matrix mineralization to moderate the joint malformation, because both of Ca and Mn were improved in KBD children after Se supplementation in this study. The selenium deficiency resulted in reduction of bone mineral density in rats has been reported.¹⁶ In selenium deficiency region, Keshan disease coexists with Kashin-Beck disease in some areas, selenium deficiency has been reported as the main risk factor to it. According to previous study¹³, Mn and Ca have been involved in the home stasis of metabolism under Se deficiency. In vitro, the calcium binding protein calponin increased and calcium sensitive protein capping protein decreased in KBD adult chondrocyte, differently, the capping protein increased in osteoarthritis, the calcium dependent protein Annexin A2 was increased compare to normal.¹⁷

Mn is an essential element and a necessary dietary constituent for human health. Bone is the largest storage of Mn in human body by passive accumulation. It is necessary for normal homeostatic processes in skeletal growth and development, glucose utilization, reproduction, cellular protection from free radical species, carbohydrate and lipid metabolism. Mn is the cofactor for some important enzymes, such as cholinesterase, mitochondrial superoxide dismutase, phosphoglucomutase, pyruvate carboxylase and glycosyltransferases.

The glycolysis has been reported in KBD progress in our previous study¹³, Phosphoglyceratemutase 1 (PGAM1) and Phosphoglycerate kinase 1 (PGK1) down regulated in KBD chondrocyte. The first step of glycolysis is D-Glucose conversion to alpha-D-Glucose-6-phosphate by the family of hexokinases, and then alpha-D-Glucose-6-phosphate translocated from cytoplasm into the lumen of the endoplasmic reticulum. Phosphoglucomutase participates in both synthesis and breakdown of glucose; it has the catalytic activity between alpha-D-Glucose-1-phosphate and alpha-D-Glucose-6-phosphate. However, the

phosphoglucomutase expression was not changed, but the PGAM1 and PGK1. Interestingly, another enzyme UTP-glucose-1-phosphate uridylyltransferase (UGPA) also had altered expression in our previous study, it catalyze the UTP and alpha-D-Glucose-1-phosphate to UDP-glucose.

Mn is involved in activation of glycosyltransferases and sulfotransferases which are required for the synthesis of proteoglycan or glycosaminoglycan (GAG), and be used in the formation of cartilage and bone. Manganese deficiency results in abnormal skeletal development have been reported in animal. Its deficiency impairs the biosynthesis of GAGs results abnormal development of cartilage and malformation of bones displayed perosis and deformities in chicken.¹⁸ Its content was higher in KBD children hair and serum than in other three groups in this study, the increased Mn concentration in KBD children may indicate the higher requirement and increased absorption due to the proteoglycan and collagen degradation. Previously studies reported that the combination of glucosamine, chondroitin and manganese could modulate articular cartilage matrix metabolism in vivo and moderate knee osteoarthritis with symptoms and radiographic index.^{19,20} KBD adult cartilage displayed the degenerative changes with increased degradation of proteoglycan and collagen in cartilage,^{21,22} and decreased glycosaminoglycan in urine was found in KBD children.²³ It indicated the specific higher requirement response to bone malformation and repair. Sodium hyaluronate administration promotes the proliferation and inhibits apoptosis of KBD chondrocytes in vitro²⁴ due to the effects on glycosaminoglycan and type II collagen synthesis.²⁵

The prolyl hydroxylase catalyze hydroxyproline of collagen, and the new synthesized collagen in cartilage was soluble one. The decreased hydroxyproline contents in urine were observed in Se deficiency KBD children.²⁶ The metabolism of collagen in KBD was improved revealing increased hydroxyproline content in urine after Se supplementation.²⁷ There was positive correlation between Mn content of serum and metaphyseal attack rate in KBD children,²⁸ the improvement rates of metaphysis and extremities in KBD children with Se supplementation for one year were higher than control.²⁹ Also, the metaphysis repair rate of KBD children was increased after taken Se-rich wheat.³⁰

The hydroxyproline is the specific amino acid in collagen. Both of KBD and OA have the increased degradation of collagen type II.

Interestingly, the altered P4HA1 was found in KBD chondrocyte, but not in OA. P4HA1 is the important catalase in 4-hydroxyproline catalyze, and involved in collagen I formation. Although the collagen I expression increased at the upper zone of KBD adult cartilage, the expression of it was lower in KBD chondrocyte than in normal one, also, the increased expression of collagen type I has been reported in OA.

Collagen type I has been involved in cartilage repair and stiffness, the content of it may related to proliferation and generation activity.³¹ In this process, the amino acid compound with Se may play important role in regulation of collagen formation due to collagen type II contains cysteine-rich domain in the NH₂-terminal propeptide.³² Selenocysteine served as cysteine donor in it, also, Cysteine could be the termination codon as UGA, and delete selenocysteine RNA resulted in abnormal skeletal development in rat;³³ by the other side, Mn is the necessary element between cysteine and cysteine catalysis in keeping oxidation and anti-oxidation homeostasis in cell.

Both Mn and Se are required in protecting the cell against oxidative stress. Mn SOD catalyzes the superoxide radicals to hydrogen peroxide and then be converted into water by GPx. The elevated superoxide production promotes bone resorption and joint degeneration.³⁴ MnSOD was down regulated in KBD cartilage opposite to the increased Mn level in serum and hair. MnSOD has been observed with lower level in foetal cells.^{35,36} It demonstrated that higher MnSOD level could suppress cell proliferation and be associated with differentiation.³⁷ The lower expression of MnSOD may be due to the dysfunction of chondrocyte, under repair process, or in aged cells.

Selenium is the component of glutathione peroxidase (GPx). Selenomethionine is the primary form of Se in cereals to protect cell from apoptosis.³⁸ Se deficiency prevented the GPx synthesis result in peroxides accumulation and subsequent damage of mitochondria membrane.³⁹ The Se concentration and GPx activity of serum were increased in KBD with Se supplementation.⁴⁰

In this investigation, we observed that the Mn and Ca level were moderated in KBD by given Se supplementation.

CONCLUSION

In conclusion, the Mn and Ca status are involved in the regulation and pathogenesis of KBD in presence of Se deficiency. Mn may play an important role in response to the development of KBD by joining the deforming skeleton. The relative ratio of Mn to Se could be used as clinical index in early diagnosis, monitoring and prognosis of KBD children.

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AUTHOR'S CONTRIBUTION

MWJ: Conducted the study, data collection, manuscript writing, GX: Supervised the study, UF: Literature review, data analysis and proof reading.

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