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Serum metallomics study on patients with osteoarthritis based on ICP-MS technique

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The aim of the present study was to analyze the distribution of 21 metallic elements in patients with osteoarthritis from different ethnic nationalities and to examine their mechanisms of action in the development of osteoarthritis. Guoluo Prefecture of Qinghai Province, Altay City of Xinjiang Uygur Autonomous Region, and Hulunbeier City of Inner Mongolia Autonomous Region were selected for random surveys of five ethnic groups, including Han, Tibetan, Mongolian, Kazakh, and Russian. Inductively coupled plasma mass spectrometry was used to test 21 metallic elements in the serum, including lithium, magnesium, aluminum, calcium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, strontium, molybdenum, cadmium, barium, thallium, and lead. Enzyme-linked immunoassay was used to detect human selenoprotein 1, human selenoprotein P, calcitonin, parathyroid hormone, and alkaline phosphatase in the serum. A two independent samples *t* test and χ^2 test were adopted for comparative statistical analysis; the Mann-Whitney U test and partial least squares (PLS-DA) were used for discriminant analysis of metal content. Of the 21 metals, six(Ti, V, Ni, As, Mo, and Tl) were not detected in the serum of osteoarthritis patients and 15 elements were detected, of which the contents of 11 elements differed significantly ($P < 0.05$) among the nationalities. Multidimensional statistics and one-dimensional statistical analysis showed that there was a significant difference ($P < 0.05$) in the serum contents of lithium, selenium, and strontium between osteoarthritis patients from different regions, different ethnic groups, and those of the control group; body hormones corresponding to the three elements were highly correlated with protein detection. Metallic element distribution and content in the serum differed among osteoarthritis patients of different nationalities. Variations in the contents of lithium, selenium, and strontium were correlated with protein metabolism, which may be related to the occurrence and development of osteoarthritis..

Keywords: Osteoarthritis,inductively coupled plasma mass spectrometry,metallic element, metallomics

INTRODUCTION

Osteoarthritis (OA) is a chronic degenerative bone disease characterized by biochemical and metabolic abnormallities of the articular cartilage, degeneration, injury, and cartilaginous hyperplasia that occurs mainly in the elderly. In the United States, OA ranks second in incidence among the population over 50 [1]; among the population over 65 with imaging abnormalities, the incidence of osteoarthritis is approximately 70%[2]. In China, where the population over 60 exceeds 330 million, there are approximately90 million patients with OA, of which 55% are older than60 years and 80% are older than 70 years[3]. In recent years, these figures have grown substantially, which may be related to bad habits, incorrect diet, general weight gain, drug abuse, excessive exercise, and other factors. Several theories have been proposed to explain the etiology of OA, including the intraosseous hypertension theory, cytokines and growth factor theory, cartilage degradation theory, and immune response theory. Research into the etiology of OA has been performed using genomics, proteomics, metallomics, and related disciplines.

Genomics, which uses genome-wide association studies as an effective tool to identify disease-causing genes, is widely used to examine the genetic characteristics of OA. Proteomics analyzes complex gene interactions, gene expression related to cell internal activity and the environment, as well as the dynamic process of protein processing after translation. Metallomics[4],which studies metal-related molecular mechanisms in the organism and the metal ions and metal complexes within cells and tissues, uses inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) to analyze the metallome and for morphological analysis[5]. Metallomics analyzes the metallic element content and distribution in biological fluids, cells, and organs, identifies metal proteins and metal enzymes, and studies the associations between

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metallic elements and biological molecules. It describes the metallic element enrichment process, metabolism, and biological functions in OA patients, and reveals possible relations between alterations in metallic element metabolism and OA pathogenesis.

In the present study, metal metabolomics technology was used to test 21 metallic elements [lithium (Li),magnesium (Mg),aluminum (Al), calcium (Ca), titanium (Ti), vanadium (the V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni),copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo),cadmium(Cd), barium (Ba), thallium (Tl), and lead (Pb)]and related metalloproteins in the serum of osteoarthritis patients from five Chinese ethnic nationalities(Han, Tibetan, Mongolian, Kazakh and Russian) to explore the distribution of metallic elements in OA patients from different ethnic nationalities and its impact on osteoarthritis occurrence and development. Metallic elements with potential relevance to OA were identified and the underlying mechanisms were explored to provide a scientific basis for early prevention, early diagnosis, and early treatment of OA.

MATERIALS AND METHODS

Plasma

A questionnaire was designed based on the control study survey method of epidemiological populations. Guoluo Prefecture of Qinghai Province, Altay City of Xinjiang Uygur Autonomous Region, and Hulunbeier City of Inner Mongolia Autonomous Region were selected as survey areas to investigate patients among a population of over 40 OA patients. Patients were diagnosed according to the 1995 OA diagnostic criteria of the American College of Rheumatology, as well as orthopedist examination, X-ray images, and film-reading in patients from the following five nationalities: Chinese, Tibetan, Mongolian, Kazakh, and Russian. A total of563 OA patients were screened and confirmed (132 Han, 136 Tibetans, 111 Mongolians, 125 Kazakh, and 59 Russian).A total of555 patients from the case group and healthy volunteers(149 Han, 112 Tibetans, 93 Mongolians, 129 Kazakh, 72 Russian) were used as the control group. All subjects signed an informed consent and voluntarily participated and exited, as well as agreeing to a questionnaire and disease-related laboratory inspection.

Reagents and instruments

The instruments used were an Agilent-7700x inductively coupled plasma mass spectrometer (Agilent-7700x ICP-MS, Agilent Technologies Inc.), IA-89 inductively coupled plasma mass spectrometer autosampler (Agilent Technologies Co., Ltd.), U410 type -80°C ultra-low temperature freezer (NBS company, US), A10-type Milli-Q ultrapure water machine (Merck Millipore Santa Clara, USA), and 10mL PTFE digestion tubes(homemade). A human selenoprotein 1 (SEP1) enzyme-linked immunosorbent assay (ELISA) kit and human selenoprotein P (SEP-P) ELISA kit (Boster Bioengineering) were also used.

Nitric acid (excellent pure), hydrogen peroxide (excellent pure), tuning solution(Agilent Technologies Co., Ltd.), multi element internal standard mixing solution(Agilent Technologies Co., Ltd.), and mixed standard solution with 21 elements (Li, Mg, Al , Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Tl, and Pb; U.S. Inorganic Ventures Corporation) were used. For the preparation of all standard solutions and samples, Milli-Q purified deionized water (> 18MΩ.cm) was used.

With the three mass number elements Li^{45}, Sr^{88}, and Tl^{209}, P / A factor tuning of ICP-MS was performed to eliminate the effects of fluctuations in operating conditions. ICP-MS conditions were optimized as follows: the plasma work coil RF power (W)was set at 1550, the carrier gas flow rate (L/min) was 1.03,sampling depth (mm) was7.9,sample lifting speed (rps) was0.1,and the voltages for extraction taper hole 1, extraction taper hole 2, bias, lens, and octupole bias were 4.7 V,(−)200 V, (−)100 V,7.4 V, and 8V, respectively.

Standard curve generation

The multi-element mixed standard solution (Ca: 1000μg/mL, Mg: 500μg/mL, Li, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Tl, and Pb: 10μg/mL) (5mL)was placed in a 50mlvolumetric flask meter and the volume was adjusted to 50mL with ultrapure water, obtaining an intermediate stock solution. Aliquots of the intermediate stock solution of 0.5, 1, 2, 4, and 5mL were diluted to 50mL with ultrapure water, thus obtaining a standard series with concentrations of Li, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As , Se, Sr, Mo, Cd, Ba, Tl, and Pb of 10, 20, 40, 80, and 100μg/L. With Bi^{209}, Lu^{175}, Tb^{159}, Rh^{103}, Ge^{72}, Sc^{45}, Li^{6} as internal standards, measurements were performed under optimal conditions using the above instrument.

Sample preparation and determination

Sterile vacuum negative pressure anticoagulation blood vessels were used to collect morning fasting venous blood from all study subjects. After centrifugation at 3000×*g* for 10 min, the upper

yellow translucent liquid was extracted to obtain the plasma sample, which was placed in EP0.5mLfrozen sample tubes and stored at−80°C for metallomics analysis. The frozen plasma sample was removed from the −80°C refrigerator and thawed at room temperature. Plasma samples were shaken and blended for 30s with a vortex shaker. Then, 0.2mL of plasma sample was added to10mL PTFE digestion tube with 0.3mLHNO_3 and 0.3mLH_2O_2 digestion liquid. The sample digestion tube was tightly sealed, placed in an oven at 130°C for 2h until the solution was clear and transparent. Then, the solution was removed, cooled, and transferred to 5-mLquantitative flasks. After adjusting the volume to the scale with Milli-Q purified deionized water, the solution was transferred to the ICP-MS autosampler, which then automatically determined the 21 elements of Li, Mg, Al, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Tl, and Pb.

For ELISA, plasma was vortexed at 3000×g for 10 min to remove particulates and metalloproteinase selenoprotein 1 (SEP-1), selenoprotein P (SEP-P), calcitonin (CT), parathyroid hormone (PTH), and alkaline phosphatase (ALP) were detected following kit instructions. The standard solution was diluted, and each empty sample volume was 50μL; blank and sample wells were respectively set, wherein sample and enzyme labeled reagent were not added to the blank control well. For sample testing, a volume of 40μL was first added and then10μL of the sample to be tested was added. After incubation at 37°C for 30min, the reaction well was washed and 50μL enzyme labeled reagent was added and incubated at 37°C for 30min. After washing, reagent A and B, each 50μL, were successively added, followed by 15min coloration away from light at 37°C. A total of 50μL stop solution was added immediately to terminate the reaction. UV-visible spectrophotometer was selected for empty air zero adjustment, with OD value measured at 450nm. A standard curve was generated and the content of different metalloenzymes in serum was calculated.

Statistical analysis

Data were expressed as the mean ± standard deviation. Three statistical software programs were used for data analysis, namely Epidata3.1, SPSS17.0 and SIMCA-P12.0. For comparisons between two groups, $p<0.05$ was considered statistically significant. The Mann-Whitney U test and partial least squares (PLS-DA) discriminant analysis were used to analyze metal content.

One-way ANOVA was used to analyze differences between mean values between groups, namely multiple comparisons of mean value. Patients with OA were classified according to ethnic nationality, and the least significant difference (LSD) method was adopted to analyze metallic element distribution in the serum of patients from various ethnic groups.

A two independent samples *t* test and χ^2 test were used for comparative statistical analysis.

RESULTS

The internal standards method was used to eliminate interference. The mass number of the internal standard elements(Bi^{209}, Lu^{175}, Tb^{159}, Rh^{103}, Ge^{72}, Sc^{45}, and Li^{6}) was between 7 and 209, including all mass numbers of elements to be tested (Figure 1).Fluctuations of the internal standard curve throughout the experiment were within the allowable range, except a greater fluctuation due to one argon replacement in the experiment.

ICP-MS was performed with wide linear range, generally in the linear dynamic range of nine orders of magnitude. Taking the content of each trace element in the serum into account, 0–10μg/mL was chosen for Ca and Mg in the standard curve range, whereas 0–100ng/mL was used for other elements. The correlation coefficients and quantitative detection limits of the 21 elements within the scope of the standard curve working range are shown in Table 1.The majority of correlation coefficients of the 21 trace elements were >0.999; the correlation coefficient of zinc was0.9961. The quantitative detection limit was 0.0026–5.04μg /L.

Day and inter-day reproducibility experiments were performed for the same mixed serum samples as shown in Figure 2 to examine the accuracy of the determination method for the elements to be tested. The results revealed that inter-day relative standard deviation (RSD%) of all elements to be tested was higher than the one day RSD%, indicating that the analysis of samples should be completed in a short time to ensure accurate testing. On the other hand, the RSD% of five elements (Al, Ca, Fe, Cu, and Zn) was generally higher than that of other elements, indicating that environmental factors may interfere with the analysis

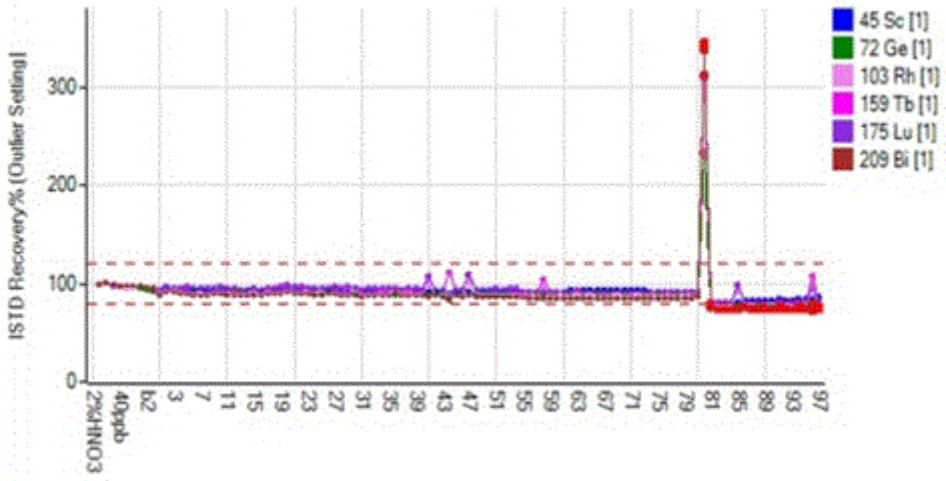


Fig.1. Results of internal standard of ICP-MS.

Table 1. Detection limit and correlation coefficient of 21 types of elements in plasma samples

	R	Dl (μg/L)		R	Dl(μg/L)
Li	0.9998	0.9	Cu	0.9999	1.3
Mg	0.9998	6	Zn	0.9961	0.8
Al	0.9994	8	As	0.9995	6
Ca	0.9998	50	Se	0.9998	1.3
Ti	0.9998	1.5	Sr	0.9995	0.3
V	0.9998	0.05	Mo	0.9997	0.1
Cr	0.9999	0.2	Cd	0.9999	0.07
Mn	0.9998	0.1	Ba	0.9993	0.7
Fe	0.9992	6	Tl	0.9991	0.03
Co	0.9999	0.08	Pb	0.9991	0.4
Ni	0.9994	2			

(1) R, correlation coefficient;(2)Dl, detection limit.

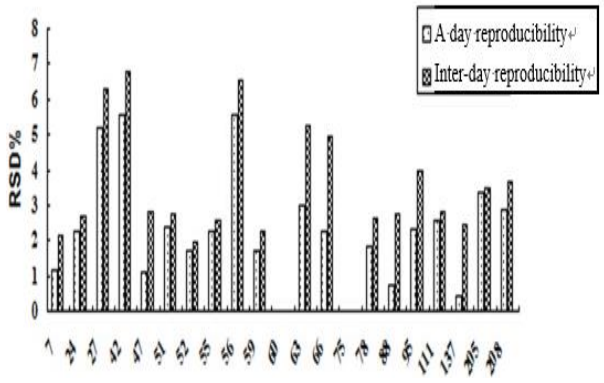


Fig. 2. Results of one day or inter-day reproducibility in 21 elements.

The content of the 21 elements (Li, Mg, Al, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Tl, and Pb) was measured in the serum of patients and healthy controls and the results were shown in Table 2.Six elements (Ti, V, Ni, As, Mo, and Tl) were below the quantitative detection limit of the method; the other 15 elements were detected. There were significant differences (P<0.05) in five elements (Li, Cu, Se, Sr, and Ba) between the OA case group and healthy control group. Se, Sr, and Bacontents were lower, whereas Li and Cu were higher in the serum of OA patients than in controls.

Figure 3shows the serum sample ICP-MS mass spectrum of an OA patient and a healthy control. In

Figure 4, the abscissa shows the m/z detecting metallic element and the ordinate shows the count value per second (CPS). Because there were marked differences in the contents of the 21metallic elements tested in the serum, it was difficult to determine the CPS value of all metallic elements. Therefore, the CPS in Figure 4 is shown in proportion to1 × 10^4(the CPS of some elements is beyond the ordinate range). General differences between the OA group and HC group were identified in the 21 elements tested; however, this did not represent statistically significant differences between the two groups. Therefore, multi-dimensional statistical analysis was needed.

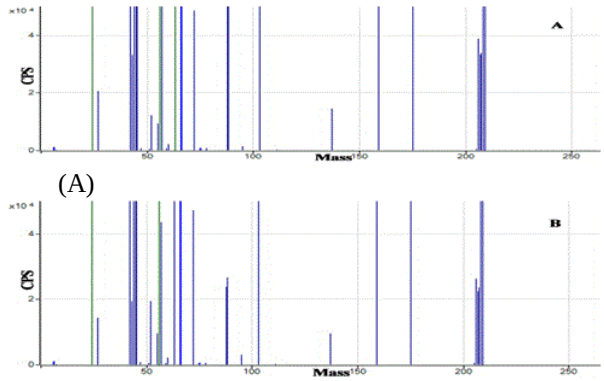


Fig.3. Serum ICP-MS spectrum of OA patients and healthy controls. (A) OA patient sample map; (B) healthy control sample map.

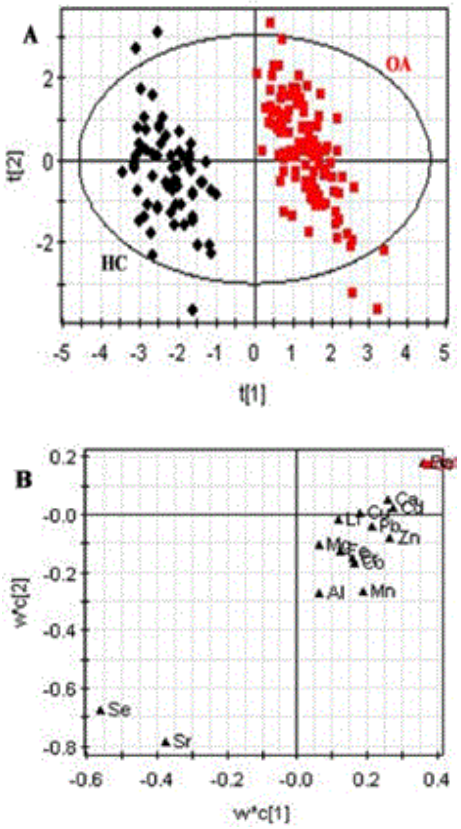
One-way ANOVA was used to analyze the distribution of trace elements in the serum of patients from the five ethnic nationalities (Table 3).Eleven elements (Mg, Ca, Cr, Mn, Fe, Co, Cu, Se, Sr, Cd, and Ba) were significantly affected by nationality. The LSD method for multiple comparisons showed that for magnesium, there were significant differences (P<0.001) between Han and Tibetan, Mongolian and Tibetan, Kazakh and Tibetan, and Russian and Tibetan; for calcium, there was a significant difference (P<0.001) between Han and Mongolian; for chromium, manganese, iron, and cobalt, there were significant differences (P<0.05) between Kazak and Han, Tibetan, Mongolian and Russian; for copper, there were significant differences (P<0.001) between Kazakh and Han, Tibetan, Mongolian and Russian, and significant differences (P<0.001) between Russian and Han, Tibetan and Kazak; for selenium, there were significant differences (P<0.001) between Han and Tibetan, Mongolian, and Kazak, significant differences (P<0.001) between Tibetan and other ethnic groups, significant differences (P<0.001) between Mongolian and other ethnic groups, significant differences (P<0.001) between Kazakh and other ethnic groups, and significant differences (P<0.001) between Russian and Tibetan, Mongolian, and Kazak; for strontium, there were significant differences (P<0.001) between Han and other ethnic groups, significant differences between Tibetan and other ethnic groups, significant differences (P<0.001) between Mongolian and other ethnic groups, significant differences (P<0.001) between Kazak and Han, Tibetan, Mongolian, and significant differences (P<0.001) between Russian and Han, Tibetan, and Mongolian; for cadmium, there were significant differences (P<0.001) between Mongolian and other ethnic groups; for barium, there were significant differences (P<0.05) between Mongolian and Han, Kazak, and Russian, and significant differences (P<0.05) between Russian and Han, Tibetan, and Mongolian.

Figures 4,5,6,7, and 8 show the results of the statistical analysis of multidimensional data. The PLS-DA shot chart(Figures4A and5A) shows that the established PLS-DA model can completely separate the case group from the control group, indicating that the established PLS-DA model has good reliability and predictability. The chart(Figures 6A,7A and 8A) shows that the boundary of the OA group and HC group is not obvious, although differences between the two groups were detected. The PLS-DA shot chart shows differences in content distribution *in vivo* of metallic elements in the OA group and HC group of different ethnic groups.

The load diagram can intuitively reflect the contribution of each element to the model. The corresponding positions of the 21 metallic elements to be tested are shown on the diagram. If the position of an element is on the same side as the position shown by OA, it means that there is a relatively high concentration of the element in the OA group, which can be considered as a risk factor for OA.

By contrast, if the element is on the opposite side of OA, there is a relatively high concentration of the element in the HC group, which can be considered as a protective factor against OA occurrence and development. The PLS-DA shot chart shows that (Figure 4B) for the Han population, the concentrations of lithium and barium are high in the serum of the OA group, which can be considered as a risk factor for OA. The concentrations of selenium and strontium are high in the serum of the HC group, which can be considered as a protective factor against OA. Figure 5B shows that for the Tibetan population, lithium and magnesium are high in the serum of the OA group and considered a risk factor, whereas selenium and strontium are high in the HC group and considered a protective factor against OA.

Figure 6B shows that for the Mongolian population, lithium is high in the OA group and considered as a risk factor, whereas selenium and strontium are high in the HC group and considered as a protective factor against OA.

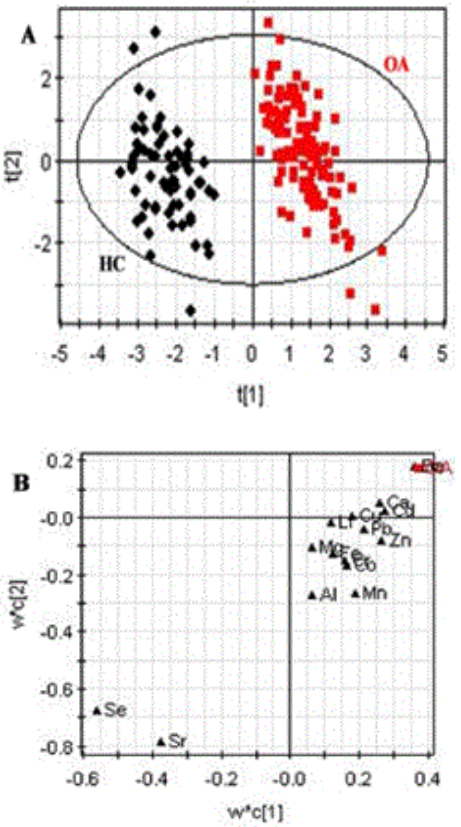


(A)

(B)

Fig.4. Partial least squares discriminant analysis model diagram of serum metallic element spectra discrimination of the Han OA case group and control group.(A) Shot chart; (B) load diagram.

Figure7B shows that for the Kazakh population, lithium and calcium are high in the OA group and considered as a risk factor, whereas selenium and strontium are high in the HC group and considered as a protective factor against OA. Figure 8Bshows that for the Russian population, lithium is high in the OA group and considered as a risk factor for OA, whereas selenium and strontium are high the HC group and considered as a protective factor against OA. The above results reveal that among the three elements lithium, selenium, and strontium, Li was always on the same side with OA, while Se and Sr were always on the opposite of OA



(A)

(B)

Fig.4. Partial least squares discriminant analysis model diagram of serum metallic element spectra discrimination of the Han OA case group and control group.(A) Shot chart; (B) load diagram.

By contrast, if the element is on the opposite side of OA, there is a relatively high concentration of the element in the HC group, which can be considered as a protective factor against OA occurrence and development. The PLS-DA shot chart shows that (Figure 4B) for the Han population, the concentrations of lithium and barium are high in the serum of the OA group, which can be considered as a risk factor for OA. The concentrations of selenium and strontium are high in the serum of the HC group, which can be considered as a protective factor against OA.

VIP was used as a selection indicator of the multi-dimensional model for different elements. According to the experimental value, VIP>1.0 was used as the selection criterion of the model contribution variable. The PLS-DA model short chart and VIP value analysis provided preliminary data on the contribution of each element to the model and their relative concentration distribution among groups.

Table 2. Results of content determination of the 21 types of trace elements inOA and HC ($\bar{x}\pm s$)

	OA	HC	*p*-value*		OA	HC	*p*-value*
Li	145.5±77.8*	96.2±50.8	<0.05	Cu	1282±372*	1240±408	<0.05
Mg	23400±4625	22800±4525	n.s.	Zn	770±372	768±378	n.s.
Al	690±77	377±40	n.s.	As	—	—	—
Ca	104950±23750	104775±24350	n.s.	Se	73.2±28.5*	78.5±29.2	<0.001
Ti	—	—	—	Sr	64.0±33.5*	67.0±33.0	<0.05
V	—	—	—	Mo	—	—	—
Cr	202±104	328±154	n.s.	Cd	1.25±0.08	1.10±0.20	n.s.
Mn	32.0±26.0	39.5±32.5	n.s.	Ba	35.2±21.8*	38.5±31.2	<0.05
Fe	3530±940	4877±1430	n.s.	Tl	—	—	—
Co	2.40±1.02	3.40±1.48	n.s.	Pb	28.5±11.0	27.5±12.5	n.s.
Ni	—	—	—				

(1)*,μg/L;(2) -, not detected; (3)n.s., not significant.

Table 3. Results of content determination of the elements in different nationalities($\bar{x}$).

	Han	Tibetan	Mongolian	Kazak	Russian	*p*-value*
Li	0.16	0.003	0.07	0.14	0.25	n.s.
Mg*	24.8	20.4	24.0	24.5	24.6	< 0.001
Al	1.64	0.19	0.13	0.41	0.32	n.s.
Ca*	100.49	89.70	94.15	105.23	106.58	＜0.001
Cr*	0.04	0.005	0.01	0.76	0.20	< 0.001
Mn*	0.01	0.005	0.01	0.07	0.02	< 0.001
Fe*	1.79	1.90	1.46	2.71	2.84	< 0.001
Co*	0.0003	0.0003	0.0001	0.006	0.002	< 0.001
Cu*	1.21	1.15	1.23	1.46	1.34	< 0.001
Zn	0.64	0.67	0.59	0.64	0.61	n.s.
Se*	0.08	0.04	0.07	0.09	0.08	< 0.001
Sr*	0.07	0.03	0.06	0.08	0.08	< 0.001
Cd*	0.001	0.0004	0.001	0.001	0.0003	< 0.05
Ba*	0.03	0.03	0.03	0.04	0.04	< 0.05
Pb	0.02	0.01	0.02	0.01	0.02	n.s.

(1)*,mg/L;(2)n.s., not significant.

Table 4. The potential differences of elemental analysis with VIP > 1.0 and $P < 0.05$in the cases and the control group of different ethnic groups

		VIP	*P*			VIP	*P*
Han	Li*	1.47	0.020	Kazakh	Li*	1.01	0.034
	Se*	2.19	0.000		Se*	1.19	0.002
	Sr*	1.02	0.035		Sr*	1.78	0.025
	Ba	1.24	0.044		Ca	1.65	0.019
Tibetan	Li*	1.08	0.017	Russian	Li	1.06	0.044
	Se*	1.59	0.000		Se*	1.19	0.001
	Sr*	1.14	0.000		Sr	1.78	0.027
	Mg	1.81	0.046				
	Zn	1.29	0.038				
Mongolian	Li*	1.39	0.041				
	Se*	1.69	0.000				
	Sr*	1.32	0.007				

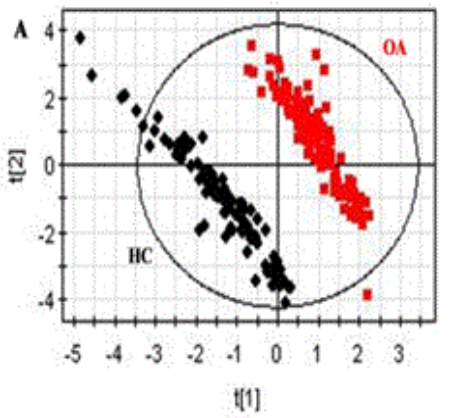
DISCUSSION

Metallic element balance is indispensable to maintain human health. The development and progression of many diseases are associated with abnormal metallic elements. The metallic element content in the body is closely related to factors such as the geographical environment, lifestyle, and dietary structure. In recent years, studies on the correlation between trace elements and OA mostly focused on copper, selenium, zinc and iron[6-8].

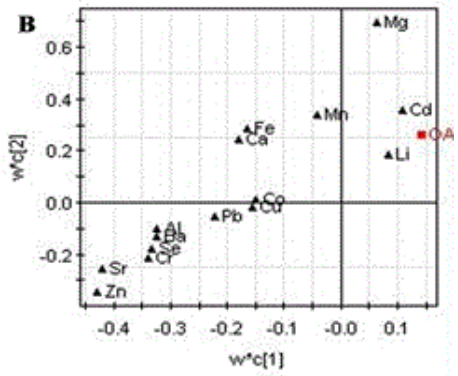
The content and mechanism of trace elements in the plasma of patients with OA are less studied. In this paper, an epidemiological survey was performed on OA patients of five ethnic nationalities, namely Han, Tibetan, Mongolian, Kazakh and Russian, to analyze 21 metallic elements in the serum of OA patients. The results showed differences in the content of 11 metallic elements (Mg, Ca, Cr, Mn, Fe, Co, Cu, Se, Sr, Cd, and Ba) in the serum of different ethnic groups, with significant differences between the OA group and HC groups ($p < 0.05$) in three elements, namely Li, Se, and Sr in various ethnic groups.

Lithium can promote osteoblast differentiation *in vitro*, while it can promote bone regeneration *in vivo*[9].Oral administration of lithium for bipolar disorder has been used in the clinic for more than 50 years[10]. Epidemiological studies have shown that for patients taking lithium, fracture risk is significantly reduced[11]. In the present study, the lithium content in OA patients' serum was higher than that in the healthy control group, which is consistent with Krachler's results [12]. Despite some research on lithium toxicity[13] and physiological and biochemical effects, such as the roles of lithium in reproduction and growth[14], endocrine function [15-16]and enzyme activity[17], the significance of increased lithium in the serum of OA patients has not been reported previously. However, increased lithium may not be the direct cause of OA, since according to test results; lithium concentration in the

serum of OA patients is still far below toxic levels. Lithium chloride can increase osteoporotic bone mass in senile and ovariectomized animals, and also result in increased bone mineral density in normal animals. The literature shows that lithium is involved in the clinical manufacture of bone models, indicating that this element exerts certain effects on bone growth and development. The present results showed that lithium content in the serum of the case group did not reach toxic levels. We suspect that increased lithium affects bone growth and development, leading to the occurrence of osteoarthritis.



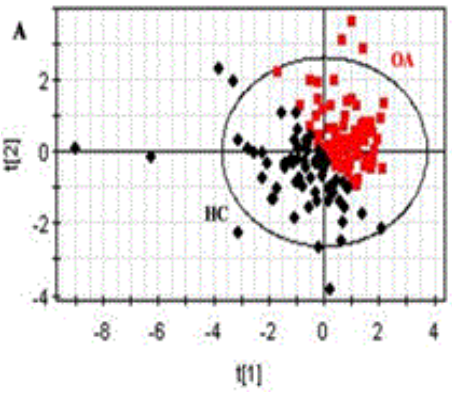
(A)



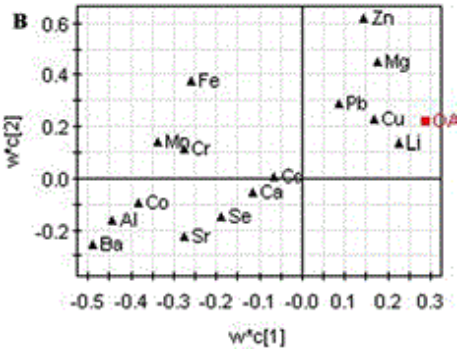
(B)

Fig.5. Partial least squares discriminant analysis model diagram of serum metallic element spectra discrimination of the Tibetan OA case group and control group.(A) Shot chart; (B) load diagram.

Selenium is an essential trace nutrient for the body. Its physiological functions as antioxidant, and in eliminating free radicals and enhancing immunity play an important role in health maintenance. Therefore, selenium has a high value in health care in terms of physical activity and disease prevention and treatment[18].

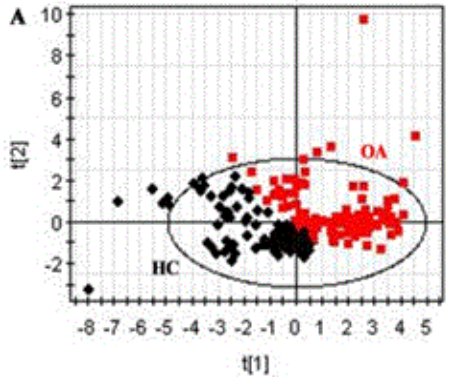


(A)

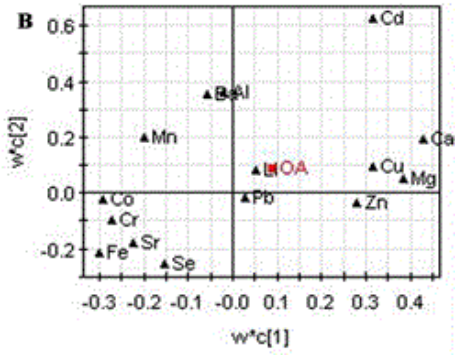


(B)

Fig.6. Partial least squares discriminant analysis model diagram of serum metallic element spectra discrimination of the Mongolian OA case group and control group.(A) Shot chart; (B) load diagram.



(A)



(B)

Fig.7. Partial least squares discriminant analysis model diagram of serum metallic element spectra discrimination of the Kazakh OA case group and control group.(A) Shot chart; (B)load diagram.

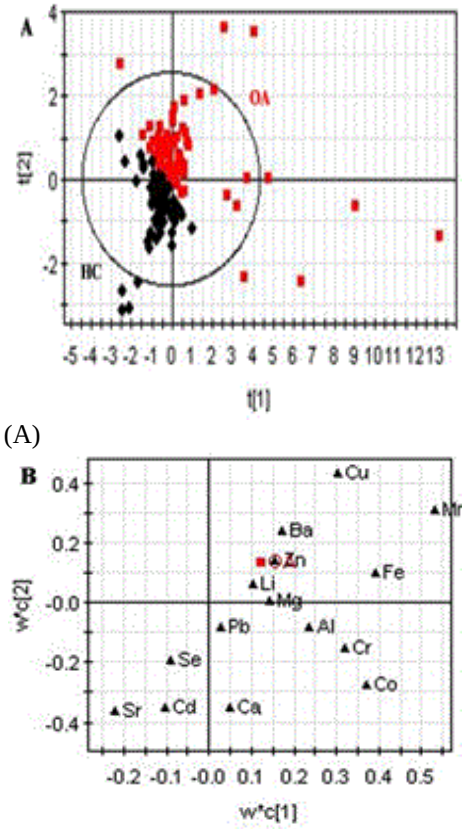


Fig.8. Partial least squares discriminant analysis model diagram of serum metallic element spectra discrimination of the Russian OA case group and control group.(A) Shot chart; (B)load diagram.

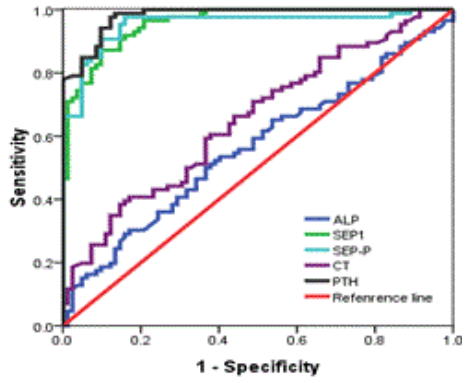


Fig.9. Receiver operating characteristic curve analysis of metalloproteinase of the case and control groups.

A large number of domestic and foreign clinical trials demonstrated that selenium deficiency in the human body can result in organ dysfunction, leading to many serious diseases. More than 40 countries around the world are in selenium deficiency areas, China's hundreds of millions of people are at selenium deficiency or low selenium regions, and these areas feature very high incidence of cancer, liver disease, and cardiovascular disease[19]. In this study, selenium content in the serum of OA patients was significantly lower than that in the healthy control group. The results show that the selenium content in the serum of OA patients was lower than the normal level; therefore, selenium cannot play a protective role, resulting in the development and progression of the disease. Human selenoprotein 1 and human selenoprotein P were further detected in an attempt to explore effect of selenium content reduction on OA occurrence and development.

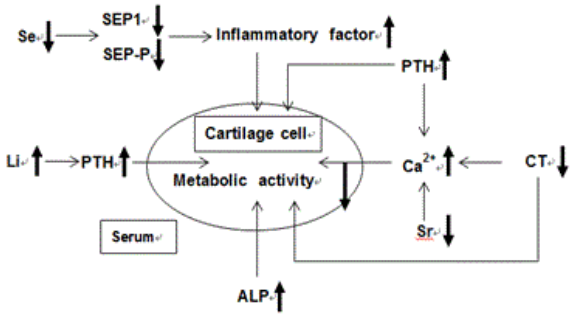


Fig.10. Relation between metal metabolic disorder and OA occurrence and development.

Strontium and calcium belong to the same family as essential trace elements for the human body and important components of bone, which can promote calcium absorption and osteoid formation, regulate metabolism of bone calcium, increase trabecular bone, and improve bone microstructure [20]. Strontium content in serum is 28–44ng/ml[20]. However, excessive strontium can replace calcium in bone tissue and interfere with calcium absorption and metabolism, leading to bone disease. In this study, the average strontium content in the serum of OA patients was 25.6ng/ml, which was significantly lower than that of normal people. The calcium content in serum was significantly higher than that of the control group, indicating that strontium content reduction affected calcium absorption, which might be one factor leading to OA occurrence. Therefore, strontium can be considered as one of the heavy metals potentially threatening human health.

The main reason why metallic element metabolic disorder *in vivo* can cause certain diseases is that metallic elements combine with metalloproteins, enzymes or other biological molecules containing metallic elements, resulting in biological effects. Therefore, in this paper, the biological effects of the three metallic elements lithium, strontium, and selenium on OA were further investigated.SEP1, SEP-P, CT, PTH and ALP contents in OA and HC serum were detected to understand how metallic elements affect OA (Figure 10).Increase in lithium can cause hyperparathyroidism, resulting in excessive secretion of PTH, while elevated PTH content affects the metabolic activity of cartilage cells, resulting in OA occurrence and development.

PTH detection and content determination indicated that PTH has a good ability to identify and diagnose OA and can be used as a sensitive and reliable serum biomarker of OA. Based on the analysis results, we speculate that selenium may cause OA development and progression by affecting selenoprotein metabolism. Selenoprotein is the main carrier for selenium, which refers to protein after peptide synthesis with selenium in the form of selenocysteine (Sec). Because of the active properties of Sec, it plays an important role in redox reactions[21]. SEP-P is the storage protein of blood selenium, which mainly exists in the serum[22]. A study found relevance between SEP-P and KBD, and the main clinical manifestation is bone and joint deformation[23-24]. Among KBD patients, SEP-P's mRNA expression was lower in patients than in controls[25-26]. The results of the present study showed that the selenoprotein content in the serum of osteoarthritis patients was significantly lower than that of the healthy control group, and selenium content in the serum was low. This indicated that the reduction in selenium content affected selenoprotein synthesis, which might be one factor leading to OA. In addition, selenoprotein content reduction in serum might be caused by its degradation, resulting in increased inflammatory factor content in damaged cartilage cells, leading to OA occurrence. Selenoprotein 1 and selenoprotein P detection and content determination indicated that the two metals have a good ability to identify and diagnose OA, which can be used as a sensitive and reliable serum biomarker of OA. Strontium promotes osteoblast growth and inhibits osteoclast activity[27], exerting positive effects on calcium absorption in bone tissue. In this study, strontium content was reduced, while there was no significant difference in calcium content, although it showed an increasing trend. Strontium reduction leads to decreased calcium absorption by cartilage cells and increased calcium content in serum. Both increased PTH and elevated calcitonin (CT) will affect the concentration of calcium ions in serum[28-29]. Increased blood calcium promotes osteogenic activity and new bone formation, causing OA. Metabolic disorders of PTH and CT will also affect the metabolism of cartilage cells, resulting in diseases. We found that the ALP content in the OA group was higher than that in the control group.ALP is mainly generated by cartilage cell secretion[30]. Its elevated level reflects metabolic changes. The elevated level of ALP may lead to metabolic imbalances in cartilage cells, leading to disease.

Metabolic disorders of the three metallic elements lithium, selenium, and strontium directly affect the bioactivities of parathyroid hormone, human selenoprotein 1, and human selenoprotein P, which is inextricably linked to cartilage and subchondral bone protection and restoration in the early stage of OA. Hence, the three metallic elements lithium, selenium, and strontium may be important causes of OA.

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REFERENCES

1. C.Y. Wenham, P.G. Conaghan, *AgeAgeing*, **42**, 272 (2013).
2. W. Wei, W. Kun-zheng, D. Xiao-qian, P. Chuan-yi, W. Chun-sheng, S. Zhi-bin, M. Shu-qiang, *Journal of Medical Colleges of PLA*, **22**(3), 179 (2007).
3. C.G. Helmick, D.T. Felson, R.C. Lawrence, S. Gabriel, R. Hirsch,C.H.Kwoh, M.H. Liang, H.M. Kremers, M.D. May-es, P.A. Merkel, S.R. Pillemer, J.D. Reveille, J.H. Stone, *Arthritis Rheum*, **1**, 26 (2008).
4. H. Haraguchi, *J Anal At Spectrom*, **19**, 5 (2004).
5. A.R. Upton, C.A. Holding, A.A. Dharmapatni, D.R. Haynes, *Rheumatol Int.*, **32**, 535 (2012).
6. M. Yazar, S. Sarban, A. Kocyigit, D. Isikan, *Biol Trace Elem Res*, **106,** 123 (2005).
7. M. Krachler, W. Domej, *Bio Trace Elem Res*, **79**, 139 (2001).
8. M.W. Krachler, K. Domej, J. Irgolic, *Biol Trace Elem Res*, **75**, 253 (2000).
9. Y. Chen, H.C. Whetstone, A.C. Lin, P. Nadesan, Q. Wei, R. Poon, B.A. Alman, *PLoS Medicine*, **4**(7), e249 (2007).
10. C. Livingstone, H. Rampes,*J Psychoph-armacol*,**20**, 317 (2006).
11. P. Vestergaard, L. Rejnmark, I. Mosekilde, *Calcif Tissue Int*, **77**, 1 (2005).
12. M. Krachler, W. Domej. *Biol Trace Elem Res*, **79**,139 (2001).
13. L.S. Richman, A.L. Dzierba, K.A. Connolly, P.M. Bryan, S. Chandra, *JPharm Pract*, **28**, 1 (2015).
14. M. Bauer, M. Adli, T. Bschor, M. Pilhatsch, A. Pfennig, J. Sasse, R. Schmid, U. Lewitzka *Neuropsychobiology*, **62**, 36 (2010).
15. T.C. Oliveira, I.A.C. Neto, M.H. Aguiar-Oliveira, A.F. de Pereira, *Arq Bras Endocrinol Metabol*, **58**, 619 (2014).
16. L. Pesce, P. Kopp, *Int J Pediatr Endocrinol*, **2014**, 8 (2014).
17. P. Clément-Lacroix, M. Ai, F. Morvan, S. Roman-Roman, B. Vaysslère, C. Belleville, K. Estrera, M.L. Warman, R. Baron, G. Rawadi, *National Academy of Sciences*, **102**,17406 (2005).
18. K.G. Patel, P.C. Yadav, C.B. Pandya, H.N. Saiyed, *J Environ Bio I*, **25**(4), 413 (2004).

19. H.J. Zhuo, A.H. Smith, C. Steinmaaus, *Cancer Epidemiol Biomarkers Prev*, **13**, 771 (2004).
20. Y.Wu, S.M. Adeeb, M.J. Duke, D. Munoz-Paniague, M.R. Doschak. *Journal of pharmacy and pharmaceutical sciences*, **16**(1), 52 (2013).
21. X. Ma, X. Zhang, Y. Jia, S. Zu, S. Han, D. Xiao, H. Sun, Y. Wang,*Int Orthop*, **37**, 1399 (2013).
22. B. Hollenbach, N.G. Morgenthaler, J. Struck, C. Alonso, A. Bergmann, J. Kohrle, L. Schomburg, *J Trace Elem Med Biol*, **22**, 24 (2008).
23. L.C. Davies, E.J. Blain, S.J. Gilbert, B.Caterson, V.C. *Duance, Tissue Eng Part A*, **14**(7), 1251 (2008).
24. L.Y. Sun, F.G. Meng, Q. Li, Z.J. Zhao, C.Z. He, S.P. Wang, R.L. Sa, W.W. Man, L.H. Wang, *Osteoarthritis Cartilage*, **22**(12), 2033 (2014).
25. W.Y. Sun, X. Wang, X.Z. Zou , R.X. Song , X.H. Du, J. Hu, *Br J Nutr*, **104**, 1283 (2010).
26. E.L. Kuyinu, G. Narayanan, L.S. Nair, C.T. Laurencin, *J Orthop Surg Res*, **11**, 19 (2016).
27. C.R. Scanzello, S.R. Goldring, *Bone*, **51**, 249 (2012).
28. Y. Zhang, K. Kumagai, T. Saito, *J Orthop Surg Res*, **9**, 68 (2014).
29. F. Eckstein, W. Wirth, M.I. Hudelmaier, S. Maschek, W. Hitzl, B.T. Wyman, M. Nevitt, M.-P. Hellio, *ArthritisResTher*, **11**, R90 (2009).
30. M. Bellido, L. Lugo, J.A. Roman-Blas, S. Castañeda, J.R. Caeiro, S. Dapia, E. Calvo, R. Largo, G. Herrero-Beaumont, *Arthriti ResTher;***12**, R152 (*2010).*