Serum Vitamin D Components and Whole Blood Trace Elements Storage in Chinese Short Stature Children

Bei Xu (✉ xb1990625@126.com)
Mianyang Central Hospital

Yue Feng
Mianyang Central Hospital

Lingling Gan
Mianyang Central Hospital

Yamei Zhang
Mianyang Central Hospital

Wenqiang Jiang
Mianyang Central Hospital

Jiafu Feng
Mianyang Central Hospital

Lin Yu
Mianyang Central Hospital

Research

Keywords: Mass Spectrometry, 25-Hydroxyvitamin D2, 25-Hydroxyvitamin D3, Trace elements, Short stature

DOI: https://doi.org/10.21203/rs.3.rs-117268/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Background:** Literature data on nutritional status in short stature children are unfortunately still scarce. The aim of the study was to compare the serum levels of 25(OH)D2, 25(OH)D3, total 25(OH)D and whole blood trace elements between short stature and healthy controls in Chinese children.

**Methods:** A case-control study including 370 short stature (SS) children and 398 healthy controls (HC) was performed in Mianyang Central Hospital from January 2017 to June 2020. Serum 25(OH)D2 and 25(OH)D3 were accurately measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and were summed to obtain total 25(OH)D. Whole blood concentrations of calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), selenium (Se) and lead (Pb) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) method.

**Results:** 25(OH)D2 and total 25(OH)D levels in the SS group were significantly lower than the HC subjects (both $P<0.05$). Besides, both 25(OH)D2 and 25(OH)D3 were positively correlated with total 25(OH)D in the SS and HC groups. 25(OH)D2 levels had weakly negative association with 25(OH)D3 among healthy subjects, whereas no correlation between 25(OH)D2 and 25(OH)D3 levels was found in short stature group. Otherwise, a significant elevation was observed in Zn ($P<0.001$), Fe ($P<0.001$), and Se ($P=0.027$) in SS patients, while a statistically significant decrease in Cu ($P=0.002$) and Mn ($P<0.001$) was found. Additionally, the significant correlation of serum levels of 25(OH)D2, 25(OH)D3, total 25(OH)D and trace elements were observed between SS and HC group.

**Conclusion:** SS children had more severe deficiency of 25(OH)D2 and total 25(OH)D than healthy subjects. In addition, deficiency of Cu and Mn, but elevation of Zn, Fe and Se were observed. Achieved information about vitamin D and trace element status in SS subjects could further improve the nutritional status of these patient groups.

Introduction

Short stature, which affects about 3% of the population, poses a considerable public health problem globally [1]. Short stature is defined as a height less than or equal to 2 standard deviations (SD) below corresponding mean for a given age, sex and population or height below third percentile [1–2]. Although it is not a disease, individuals with general health but short stature are under physical and psychological stress in the modern society. Especially for children, severe short stature has been found to be vulnerable to diverse developmental, social and educational problems [3].

Growth is not only determined by genetic factors, but also the environments [4]. Although environmental factors’ influence is relatively small compared to that of the genetic factors, it is the more important area to maximize the growth potential due to that environmental factors can be modified through intervention [5]. Short stature is caused by various underlying environmental factors, including nutritional problems and excess or deficiency of trace elements [6].
Vitamin D is one of essential nutrient, which is the most crucial factor because it plays a key role in maintaining and synthesizing body tissues during the growth period [7]. Vitamin D deficiency can reduce skeletal mineralization and bone growth rate [8]. Infants and young children are the most affected individuals because of their rapid growth. The major circulating form of vitamin D is 25-hydroxyvitamin D [25(OH)D], which is the best indicator to monitor for vitamin D status with a circulating half-life of 2–3 weeks [9]. 25(OH)D is estimated as total of 25-hydroxyvitamin D2 [25(OH)D2] and 25-hydroxyvitamin D3 [25(OH)D3] [10–11], but above 2 types of 25(OH)D are obtained from different sources. Generally, 25(OH)D3 is endogenously produced in the skin through the effect of UV-B on 7-dehydrocholesterol, whilst 25(OH)D2 is derived from the diet as ergosterol [12]. More and more evidences have considered 25(OH)D2 is equally as effective as 25(OH)D3 for bone health [13]. Therefore, both clinical and laboratory experts recommend 25(OH)D2 and 25(OH)D3 should be simultaneously detected, thus to ensure that vitamin D status is completely evaluated. Unfortunately, there are few studies to quantify the levels of 25(OH)D2 and 25(OH)D3 in short stature children. Thus, there is an urgent need to determine the relationship of 25(OH)D2 and 25(OH)D3 levels between subjects with and without short stature.

Essential trace elements (TEs, such as zinc or selenium) deficiency and potentially harmful TEs (such as lead or arsenic) excess are both known to have adverse consequences in general population, especially in children [14]. They are essential components of biological structures and have an important effect on and play a key role in a variety of the processes necessary for life throughout mediate vital biochemical reactions [15]. Recently, it is still unclear if the trace elements’ excess or deficiency are correlated with short stature. Considering this, our study aimed to compare the levels of various TEs between short stature children and normal children visiting a growth clinic.

Therefore, considering that 25(OH)D2 and 25(OH)D3 concentrations in short stature children are unclear, meanwhile nutrient status of trace elements is also yet unknown, the purpose of this study was to measure levels of 25(OH)D2, 25(OH)D3, total 25(OH)D and eight trace elements including calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), selenium (Se) and lead (Pb) in short stature children in Western China using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and inductively coupled plasma mass spectrometry (ICP-MS) respectively. Achieved information about vitamin D and trace element status in short stature subjects could assist in the process of developing vitamin D supplements or microelement correction strategy, and it would further improve the nutritional status of these patient groups.

Materials And Methods

Reagents and instrumentation

Vitamin D detection reagent kit was purchased from Jinan Yingsheng Biotechnology Co. Ltd (Jinan, China). Nitric acid (HNO₃, 65%) and TritonX-100 were both obtained from Kelong Reagent Co. Ltd (Chengdu, China). Eight elements standard solutions (Ca, Mg, Cu, Zn, Fe, Mn, Se and Pb) and internal
standard (IS) solution (Germanium and Bismuth) were all provided by PerkinElmer (Shelton, USA). All solutions were prepared with deionized water provided by a water purification system (18.25 MΩ cm, Elix®, Merck Millipore). All chemicals used for analysis were of highest purity.

The LC-MS/MS analysis was performed on a Shimadzu LC-30AD UHPLC system (Shimadzu, Kyoto, Japan) coupled to an Applied Biosystems Sciex API 4500 Qtrap (Applied Biosystems/MDS Sciex, Toronto, CA, USA) equipped with an electrospray ionization (ESI) interface. Data acquisition and analysis were performed using the Analyst software version 1.6.2 (Applied Biosystems, Foster City, CA, USA).

Trace elements of whole blood samples were analyzed by NexION 300Q ICP-MS (PerkinElmer, Shelton, USA). Data acquisition and analysis were performed using the NexION software. Analyses were performed using a standard addition procedure. Germanium (Ge) and and Bismuth (Bi) were used as internal standards for matrix and instrument drift corrections.

**Study participants**

A total of 370 eligible short stature children in ages from 1 to 13 years, including 231 males and 139 females, were recruited between January 2017 and June 2020 in Mianyang Central Hospital, Mianyang, Sichuan Province, China. In order to eliminate confounding variables, patients with histories of endocrine diseases such as growth hormone deficiency or hypothyroidism, malnutrition, chromosomal abnormalities, skeletal dysplasia, psychological and emotional disorders, or family short stature were excluded from the study. 398 healthy subjects were taken as control group, including 240 males and 158 females. The study was approved by the Medical Ethics Committee of Mianyang Central Hospital.

**Blood samples**

Blood samples of study subjects were collected in BD Vacutainer® (Becton-Dickinson, USA) SSTTM II Advance tubes and BD Vacutainer® Trace Element Serum tubes for determination of 25(OH)D2, 25(OH)D3 and TEs, respectively. For 25(OH)D2 and 25(OH)D3 determination, blood samples were centrifuged at 3000 rpm for 15 min and serum samples were collected and stored at -80 °C until thawed for assays. For TEs, the whole blood samples were stored at 4 °C before analysis.

**Determination of 25(OH)D2 and 25(OH)D3**

200 μL of serum samples, 10 μL of internal standard (IS) solution and 1000 μL of extraction solution were mixed, vortexed and then centrifuged to collect the supernatant. The resulting solutions were dried under nitrogen gas, and re-dissovled by 125 μL of reconstitution fluid. After vortex-mixing, all the solutions were transferred to a 96-well sample plate, which was then sealed and transferred to the autosampler. Calibrators and quality controls were prepared by the same procedure.
Chromatographic separation of the samples was performed on a Shimadzu LC-30AD UHPLC system equipped with a Kinetex 2.6 μm C8 100A column. Mobile phase A was water with 0.1% acetic acid, and mobile phase B consisted of methanol with 0.1% acetic acid. 15 μL of the sample solutions was injected into the LC system using a column temperature of 45 °C and a flow rate of 0.6 mL/min. Mass spectrometer detection and quantification were carried out in the positive mode using multiple reaction monitoring (MRM) mode. The optimized parameters for mass detection were as follows: curtain gas was 35 psi; temperature was 550 °C; ion spray voltage was 5500 V; gas 1 and gas 2 (nitrogen) were set both at 60 psi; the dwell time was 100 ms.

Measurement of trace elements

The concentrations of trace elements were measured using the methods validated by our laboratory previously [16]. Briefly, the whole blood samples were directly analyzed in 1/50 (v/v) dilution with the sample diluent solution which contained 0.5% (v/v) HNO3 and 0.5% (v/v) Triton X-100. All diluted samples were centrifuged at 4000 rpm for 10 min, and stored at -4 °C before analysis. TEs' analyses were performed using a standard addition procedure. Addition of IS elements' solution to sample solutions was applied via a three-way pipe.

Statistical analysis

Data were analyzed using the statistical package SPSS 22.0 (SPSS Inc, Chicago, USA). Normally distributed continuous variables were expressed as mean ± standard deviation (SD), and LSD-T test was used for comparing the mean value of data. Non-normally distributed variables were showed as median (interquartile rang, IQR). and comparisons between the two groups were analyzed by Mann-Whitney U tests. For statistical comparisons between multiple groups, one-way analysis of variance (ANOVA) was performed. The association between vitamin D status and trace elements was examined by Spearman correlation coefficients. A P-value of less than 0.05 was considered statistically significant.

Results

Storage of 25(OH)D2, 25(OH)D3 and 25(OH)D in serum

There is no consensus on the optimal level of 25(OH)D in blood, which depends on the person's age, the person's sensitivity to sunlight, the latitude, the season, the time of day and how much skin is directly exposed to sunlight. Most agree that a 25(OH)D concentration <20 ng/mL is indicative of a vitamin D deficiency, whereas a 25(OH)D concentration of 21-29 ng/mL is considered to indicate a vitamin D insufficiency. Concentrations >30 ng/mL are considered to be sufficient [17]. As shown in Fig.1A, the rates of the vitamin D sufficiency, insufficiency and deficiency were 12.1%, 60.1% and 27.8% in SS children, and 23.1%, 48.8% and 28.1% in healthy subjects respectively. Additionally, the levels of 25(OH)D2, 25(OH)D3
and the sum of them in body serum in both SS and healthy cohorts were present in Fig.1B-1D. There were significant differences of 25(OH)D2 and total 25(OH)D levels between the SS and healthy control groups (both \( P<0.05 \)), whilst similar concentrations of 25(OH)D3 (\( P>0.05 \)) were observed.

**Associations of 25(OH)D2, 25(OH)D3 and total 25(OH)D**

As shown in Fig.2, 25(OH)D2 levels in HC and SS cohorts were positively associated with total 25(OH)D. Otherwise, higher 25(OH)D3 levels were strong positively associated with higher total levels of 25(OH)D (both \( P<0.001 \)). There was no correlation between 25(OH)D2 and 25(OH)D3 levels in short stature group, whilst in healthy subjects, 25(OH)D2 levels had weakly negative association with 25(OH)D3.

**Whole blood concentrations of trace elements**

The levels of trace elements in healthy controls and short stature patients showed in Fig.3. Five elements including Cu, Zn, Fe, Se, and Mn had statistically significant differences. Compared with the control subjects, a significant elevation was observed in concentrations of Zn (\( P<0.001 \)), Fe (\( P<0.001 \)), and Se (\( P=0.028 \)) in short stature patients, while a statistically significant decrease was found in Cu (\( P=0.002 \)) and Mn (\( P<0.001 \)) levels.

**Gender's influence on vitamin D components and trace elements levels**

The influence of gender differences on the 25(OH)D2, 25(OH)D3 and total 25(OH)D levels was further examined (Table 1). In the female subjects, short stature participants had significant lower concentrations of 25(OH)D3 and total 25(OH)D than healthy controls. Whereas in the male subjects, short stature children had significant decreased levels of 25(OH)D2 and total 25(OH)D compared with healthy participants.

The relationship between the element concentrations and the genders was also performed (Table 1). ANOVA analysis revealed that among these four subgroups, the concentrations of Ca (\( P=0.183 \)) and Mg (\( P=0.644 \)) were similar, whereas the levels of Cu (\( P=0.007 \)), Zn (\( P<0.001 \)) Fe (\( P<0.001 \)), and Pb (\( P<0.001 \)), Se (\( P=0.009 \)), and Mn (\( P<0.001 \)) were all significantly different (data not shown). In healthy participants, comparisons between the two participant subgroups revealed that male and female subjects had different basic levels of trace elements in Pb (\( P<0.001 \)) and Se (\( P=0.011 \)) (Table 3). Overall, SS and HC groups had different levels of Cu, Zn, Fe, Se, Mn for male (all \( P<0.05 \)), while of Zn, Fe and Mn (all \( P<0.05 \)) for female.
Table 1
Levels of vitamin D components and trace elements in different subgroups

| Index                | Healthy control | Short stature |
|----------------------|-----------------|---------------|
|                      | Female          | Male          | Female          | Male          |
|                      | N=157           | N=239         | N=136           | N=231         |
| 25(OH)D2(ng/mL)      | 1.74(0.72,4.88) | 2.65(1.00,6.67)| 2.36(1.25,4.13) | 1.78(1.11,4.33) |
| 25(OH)D3(ng/mL)      | 21.13±8.58      | 20.86±7.82    | 19.52±5.06      | 20.49±5.41    |
| 25(OH)D(ng/mL)       | 24.85±8.97      | 25.30±8.56    | 22.80±5.49      | 23.73±6.24    |
| 25(OH)D status       |                 |               |                 |               |
| Deficiency           | 49(31.2%)       | 63(26.4%)     | 39(28.7%)       | 63(27.3%)     |
| Insufficiency        | 70(44.6%)       | 122(51.0%)    | 83(61.0%)       | 137(59.3%)    |
| Sufficiency          | 38(24.2%)       | 54(22.6%)     | 14(10.3%)       | 31(13.4%)     |
| Ca (mmol/L)          | 1.84±0.13       | 1.82±0.12     | 1.82±0.11       | 1.81±0.11     |
| Mg (mmol/L)          | 1.70±0.14       | 1.72±0.13     | 1.70±0.16       | 1.72±0.15     |
| Cu (μmol/L)          | 17.28±2.57      | 17.67±2.69    | 16.76±2.41      | 17.09±2.05    |
| Zn (μmol/L)          | 87.62±14.77     | 85.98±15.64   | 92.88±13.62     | 94.70±13.43   |
| Fe (μmol/L)          | 8117.48±634.93  | 8111.96±686.33| 8284.51±662.41  | 8369.73±682.77 |
| Pb (μg/L)            | 15.48±5.67      | 18.25±8.86    | 15.86±6.07      | 18.67±6.79    |
| Se (μg/L)            | 114.53±15.01    | 110.92±14.77  | 113.94±14.09    | 115.07±13.56  |
| Mn (μg/L)            | 20.00±6.58      | 20.33±6.27    | 17.35±6.28      | 16.46±5.39    |

Data were expressed by Mean ± SD, median (IQR), or n (%);

- a P<0.05 compared with healthy control in females;
- b P<0.05 compared with healthy control in males;
- # P<0.05 compared with females in the same group.

Correlations of vitamin D components and trace elements
The correlations of serum levels of vitamin D components and trace elements were demonstrated in Table 2. The results showed that significant correlations varied between short status and healthy controls.

### Table 2

Spearman’s correlation analyses of the serum levels of 25(OH)D2, 25(OH)D3, and 25(OH)D and trace elements

|                | 25(OH)D2 (ng/mL) | 25(OH)D3 (ng/mL) | 25(OH)D (ng/mL) |
|----------------|------------------|------------------|-----------------|
| **Short stature** |                  |                  |                 |
| Ca (mmol/L)    | 0.091, 0.080     | 0.071, 0.175     | 0.107, 0.040    |
| Mg (mmol/L)    | -0.065, 0.215    | -0.108, 0.038    | -0.093, 0.075   |
| Cu (μmol/L)    | 0.024, 0.649     | 0.208, <0.001    | 0.219, <0.001   |
| Zn (μmol/L)    | -0.099, 0.059    | -0.079, 0.132    | -0.116, 0.026   |
| Fe (μmol/L)    | -0.109, 0.036    | -0.045, 0.386    | -0.099, 0.058   |
| Pb (μg/L)      | 0.096, 0.068     | 0.009, 0.869     | 0.083, 0.115    |
| Se (μg/L)      | 0.052, 0.322     | 0.025, 0.638     | 0.026, 0.614    |
| Mn (μg/L)      | 0.016, 0.756     | -0.156, 0.003    | -0.120, 0.022   |
| **Healthy control** |                |                  |                 |
| Ca (mmol/L)    | -0.025, 0.616    | -0.142, 0.005    | 0.127, 0.011    |
| Mg (mmol/L)    | -0.039, 0.436    | -0.063, 0.215    | -0.077, 0.128   |
| Cu (μmol/L)    | 0.059, 0.245     | 0.071, 0.159     | 0.079, 0.117    |
| Zn (μmol/L)    | -0.164, 0.001    | -0.111, 0.027    | -0.192, <0.001  |
| Fe (μmol/L)    | -0.124, 0.014    | -0.058, 0.249    | -0.146, 0.004   |
| Pb (μg/L)      | 0.103, 0.040     | -0.084, 0.096    | -0.001, 0.990   |
| Se (μg/L)      | -0.043, 0.393    | 0.125, 0.013     | 0.090, 0.073    |
| Mn (μg/L)      | 0.044, 0.379     | 0.034, 0.495     | 0.093, 0.065    |

Data were expressed by (r, P)

### Discussion

Interest on height is greatly emphasized in the modern society due to the development of media and widespread perception that long stature is superior. Our study applied LC-MS/MS and ICP-MS methods...
respectively to measure serum 25(OH)D2, 25(OH)D3 and total 25(OH)D levels, as well as whole blood TE concentrations in SS children, giving more information about the nutritional status of these short patient groups.

Some data had once suggested 25(OH)D3 that accounts for approximately 95% of the total circulating 25(OH)D pool \[18\], is a more potent supplement than 25(OH)D2 for increasing total vitamin D levels \[19\]. However, even if 25(OH)D2 is generally present in significantly smaller quantities compared with 25(OH)D3, it is uncertain whether 25(OH)D2 and 25(OH)D3 as well as their metabolites are biologically equivalent at the vitamin D receptor. Furthermore, the way one metabolite affects the levels of the other vitamin D metabolites remains unknown. Therefore, quantification of 25(OH)D2 levels is extremely important to monitor treatment effectiveness. Measurement of 25(OH)D2 and 25(OH)D3 simultaneously is much more necessary than only evaluating the total 25(OH)D status.

Several methods including chemiluminescence, radioimmunoassay (RIA), and high-performance liquid chromatography (HPLC) have been developed for 25(OH)D status measurement \[9\]. But there are still significant drawbacks of them. For example, suboptimal cross-reactivity of the antibody with 25(OH)D2 would cause under recovery of 25(OH)D2 in chemiluminescent immunoassays with unsatisfactory accuracy and precision, while RIA methods are unable to distinguish between the two metabolites of 25(OH)D2 and 25(OH)D3, which could not meet our requirement \[20\]. Although HPLC techniques with UV detection are capable of determining 25(OH)D2 and 25(OH)D3 levels simultaneously, most of these methods require large sample volumes (0.5-2 mL) and time-consuming procedures before quantification \[21\]. LC-MS/MS is able to overcome the defects mentioned above, and has been the “gold standard” measuring method \[9\]. Thus, one strength of our study was the use of sensitive and specific LC-MS/MS vitamin D metabolite assays that separately analyze 25(OH)D2 and 25(OH)D3 concentrations.

In Pakistani children, vitamin D deficiency had been the second leading cause of short stature \[22\]. Vitamin D regulates circulating insulin-like growth factor 1 (IGF-1) and the gene expression of its receptor as well as various other binding proteins \[23\]. In addition, polymorphisms of vitamin D receptor gene which influence biological efficiency of vitamin D, is also associated with adult or babies’ height \[24\]. Consistent with this result, our findings indicated that the prevalence of vitamin D deficiency and insufficiency among children in Western China was high, and it was more severe in children with SS (Fig. 1). Our study further evaluated the levels of different vitamin D metabolites. To our best knowledge, our findings were the first to show that 25(OH)D2 levels in the SS subjects were significantly lower than the healthy controls. The underlying reasons might be the insufficient intake of vitamin D2 supplementation from exogenous sources, the limited substrate-dependent formation of 25(OH)D2 by 25-hydroxylase from vitamin D2, or the excessive conversion of 25(OH)D2 to the biologically active 1,25-dihydroxyvitamin D2 form in the kidney. Unexpectedly, the concentrations of 25(OH)D3 between the two groups had no significant different values. This might be explained by the vitamin D supplementation, many of which are in the form of D3 today. Otherwise, our study demonstrated 25(OH)D2 and 25(OH)D3 were positively correlated with total 25(OH)D in both the SS group and the healthy group (Fig. 2).
Unfortunately, the findings were not compatible with other publications, which reported higher 25(OH)D2 levels had no associations with higher levels of 25(OH)D. The possible reason is vitamin D concentrations and metabolism vary substantially by race/ethnicity\[25\]. Additionally, 25(OH)D2 levels had weakly negative association with 25(OH)D3 among healthy subjects in our study, which was similar to the reports that higher 25(OH)D2 was associated with lower levels of 25(OH)D3 in large healthy cohorts\[21,26–27\]. No correlation between 25(OH)D2 and 25(OH)D3 levels was found in SS group. To understand possible reasons for these associations, it is helpful to remember that cholecalciferol has about a 2-fold higher affinity for vitamin D binding protein compared to ergocalciferol, and 25(OH)D3 has a higher affinity than does 25(OH)D2, likely yielding different amounts of free vitamin D metabolite with different serum half-life periods (D3 > D2) available for hydroxylation\[28–29\]. The short stature disease state might influence the enzymatic preference for substrate and/or positive and negative feedback mechanisms, thus changing the rates of synthesis of 25(OH)D2 vs 25(OH)D3, as well as their associations. Moreover, 25(OH)D2 of male, 25(OH)D3 of female and total 25(OH)D of both male and female in healthy controls were all higher than the relative groups in SS group (Table 1). These findings suggested that we should take gender into consideration when further studies were conducted.

ICP-MS exhibits a good precision, an excellent sensitivity, and multi-isotopic and multi-elemental capabilities\[30\]. It was the most suitable technique to obtain such reliable reference values for TEs. Compared with the control subjects, a significant elevation was observed in Zn (\(P < 0.001\)), Fe (\(P < 0.001\)), and Se (\(P = 0.027\)) in SS patients, while a statistically significant decrease was found in Cu (\(P = 0.002\)) and Mn (\(P < 0.001\)) (Fig. 3). Zn is essential for cell replication and DNA synthesis\[6\], and its deficiency is considered to cause growth retardation\[31–32\]. Several evidences suggested SS group had significantly decreased Zn concentrations in whole blood and plasma\[33\]. In contrast, Yoshida, K. et.al claimed that low Zn level and Zn deficiency were not associated with idiopathic SS in Japanese children\[6\]. Multiple analytical techniques and biological fluid might be one of the causes for the contradictory results\[16\]. Se has a wide range of pleiotropic effects including production of active thyroid hormone by incorporating into selenoproteins\[34\]. Fe acts as essential nutrient utilized in almost every aspect of cell function\[35\], and interacts with other trace elements (Cu and Zn)\[36\]. Adequate dietary supply of Se and Fe is required for a healthy thyroid during development and adolescence\[37\]. However, our result presented higher Se and Fe levels in SS group. Excessive accumulation might be related to multiple factors, such as adequate intake and environmental exposure. Further research is still warranted to elucidate the importance of Se and Fe levels in children with SS. In the present study, Cu and Mn concentration in SS children was significantly lower than that in the control group, which was in accord with previous researches in hair and whole blood\[6,31\]. Cu deficiency induces anemia, decreases absorption of vitamin B1, thus has an effect on various biological progress including growth\[33\]. Lower maternal blood Mn is associated with lower birth weight\[38\]. And animal experiment confirmed that low Mn diet could impair fetal growth and development\[39\]. Physiological systems involved in metabolic homeostasis also exhibit a gender difference. Significant differences of trace element concentrations were found between female and male subjects in both HC and SS patients in our findings (Table 1). The recognition and identification of
gender-specific biological processes will lead to better understanding of trace element alterations in short
stature, and drive novel discovery to develop corresponding element correction strategies based on
gender differences.

Moreover, our results displayed that significant correlations between SS patients and healthy controls
(Table 2). Serum levels of 25(OH)D2 were negatively associated with Fe, while 25(OH)D were negatively
associated with Zn, but positively associated with Cu in both SS and HC groups. However, the significant
correlation of serum levels of 25(OH)D2, 25(OH)D3, 25(OH)D and the other trace elements differed
between SS and HC group. Unfortunately, literature on the association of vitamin D status and trace
elements are still scarce. Therefore, further studies are need to illuminate the correlations of vitamin D
status and trace elements, especially the different patterns in SS children and healthy controls.

Our article was the first to evaluate the vitamin D components and essential trace elements storage in SS
children in West China. Nevertheless, our study also has some limitations. First, our study was limited by
its small sample size and its retrospective nature. Second, our data were collected at a single institution.
Third, we did not collect data on the use of vitamin D and trace element supplementations in our
participants. Similarly, we did not collect information on the person's sensitivity to sunlight, the latitude,
the season, the time of day and how much skin is directly exposed to sunlight, all of which could be
associated with vitamin D status. Next step, we plan to carry out a study with large sample sizes,
prospective design, multiple centers and rigorous inclusion of incident patients to reflect the nutritional
status of vitamin D and trace elements in short stature patients in West of China. Although reliability of
this study results was not so satisfactory, it might serve as an important reference for the design and
conduction of related researches.

**Conclusion**

The presented study revealed essential differences between the content of vitamin D and trace elements
in SS children compared with healthy children. Our results suggested that SS patients had more severe
deficiency of 25(OH)D2 and 25(OH)D than healthy subjects. Otherwise, 25(OH)D2 and 25(OH)D3 were
positively correlated with total 25(OH)D, but no correlation between 25(OH)D2 and 25(OH)D3 levels was
found in SS group. Furthermore, deficiency of Cu and Mn, but elevation of Zn, Fe and Se were also
observed in SS children. Both the alterations in the content of vitamin D, selected metals and their mutual
interactions may play an important role in the pathogenesis of short stature children. Therefore, further
research is needed in this area.

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Medical Ethics Committee of Mianyang Central Hospital (approval no.
P2020040). All participants provided informed consent prior to participation.
Consent for publication
All authors read and approved the final manuscript.

Availability of data and materials
The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing of interest.

Funding
This work was financially supported by the Sichuan Health and Health Committee Support Program (20PJ255).

Authors’ contributions
All authors contributed to the study conception and design and takes responsibility for the integrity of the data and the accuracy of the data analysis. Data collection were performed by BX, LG, YZ, and analysis were performed by YF. Material preparation and the first draft of the manuscript was written by WJ, JF, LY and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
Not applicable.

Authors' information
Affiliations: Department of Clinical Laboratory, Mianyang Central Hospital
Bei Xu, Yue Feng, Lingling Gan, Yamei Zhang, Wenqiang Jiang, Jiafu Feng, Lin Yu

References
1. Klatka M, Błazewicz A, Partyka M, et al. Concentration of selected metals in whole blood, plasma, and urine in short stature and healthy children. Biol Trace Elem Res. 2015;166(2):142–8.

2. Wit JM, Clayton PE, Rogol AD, et al. Idiopathic short stature: Definition, epidemiology, and diagnostic evaluation. Growth Horm IGF Res. 2008;18(2):89–110.

3. Siegel PT, Clopper R, Stabler B. Psychological impact of significantly short stature. Acta Paediatr Scand Suppl. 1991;377:14–8. discussion 19.

4. Hammond GK, Barr SI, Mccargar LJ. Teachers' perceptions and use of an innovative early childhood nutrition education program 1, 2. J Nutr Educ. 1994;26(5):233–7.

5. Mi LE, Jung PM, Seok AH, et al. Differences in dietary intakes between normal and short stature Korean children visiting a growth clinic. Clin Nutr Res. 2012;1(1):23–9.

6. Yoshida K, Urakami T, Kuwabara R, et al. Zinc deficiency in Japanese children with idiopathic short stature. J Pediatr Endocrinol Metab. 2019;32(10):1083–7.

7. Thibault H, Souberbielle JC, Taieb C, et al. Idiopathic prepubertal short stature is associated with low body mass index. Horm Res. 1993;40(4):136–40.

8. Mansoor S, Habib A, Ghani F, et al. Prevalence and significance of vitamin D deficiency and insufficiency among apparently healthy adults. Clin Biochem. 2010;43(18):1431–5.

9. Saenger AK, Laha TJ, Bremner DE, et al. Quantification of serum 25-hydroxyvitamin D(2) and D(3) using HPLC-tandem mass spectrometry and examination of reference intervals for diagnosis of vitamin D deficiency. Am J Clin Pathol. 2006;125(6):914–20.

10. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80:1689S-96S.

11. Holick MF, Biancuzzo RM, Chen TC, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab. 2008;93:677–81.

12. Ahmed LHM, Butler AE, Dargham SR, et al. Association of vitamin D2 and D3 with type 2 diabetes complications. BMC Endocr Disord. 2020;20(1):65.

13. Holick MF, Biancuzzo RM, Chen TT, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab. 2008;93(3):677–81.

14. Tonelli M, Wiebe N, Hemmelgarn B, et al. Alberta Kidney Disease Network. Trace elements in hemodialysis patients: a systematic review and meta-analysis. BMC Med. 2009;7:25.

15. Al-Fartusie FS, Mohssan SN. Essential trace elements and their vital roles in human body. Indian J Adv Chem Sci. 2017;5(3):127–36.

16. Xu B, Zhang YM, Chen Y, et al. Simultaneous multielement analysis by ICP-MS with simple whole blood sample dilution and its application to uremic patients undergoing longterm hemodialysis. 2020, 80(3):247–255.

17. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008;87(4):1080S–1086S.
18. Farrell CJL, Martin S, Mcwhinney B, et al. State-of-the-Art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. Clin Chem. 2012;58(3):531–42.

19. Romagnoli E, Mascia ML, Cipriani C, et al. Short and long-term variations in serum calcitropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. J Clin Endocrinol Metab. 2008;93:3015–20.

20. Carter GD, Carter R, Jones J, et al. How accurate are assays for 25-hydroxyvitamin D? data from the International Vitamin D External Quality Assessment Scheme. Clin Chem. 2004;50:2195–7.

21. Demetriou ET, Travison TG, Holick MF. Treatment with 50,000 IU vitamin D2 every other week and effect on serum 25-hydroxyvitamin D2, 25-hydroxyvitamin D3, and total 25-hydroxyvitamin D in a clinical setting. Endocr Pract. 2012;18:399–402.

22. Jawa A, Riaz SH, Khan Assir MZ, et al. Causes of short stature in Pakistani children found at an Endocrine Center. Pak J Med Sci. 2016;32(6):1321–5.

23. Emmanouilidou E, Galli-Tsinopoulou A, Kyrgios I, et al. Common VDR polymorphisms and idiopathic short stature in children from northern Greece. Hippokratia. 2015;19(1):25–9.

24. Choi SK, Park MS, Song JK, et al. Association of polymorphisms in the vitamin D receptor promoter with idiopathic short stature. J Korean Med Sci. 2013;28(9):1329–33.

25. Budhathoki S, Hidaka A, Yamaji T, et al. Plasma 25-hydroxyvitamin D concentration and subsequent risk of total and site specific cancers in Japanese population: large case-cohort study within Japan Public Health Center-based Prospective Study cohort. BMJ. 2018;360:k671.

26. Swanson Christine M, Nielson Carrie M, Shrestha S, et al. Higher 25(OH)D2 is associated with lower 25(OH)D3 and 1,25(OH)2D3. J Clin Endocrinol Metab. 2014;99(8):2736–44.

27. Hataikarn Nimitphong S, Saetung S, Chanprasertyotin, et al. Changes in circulating 25-hydroxyvitamin D according to vitamin D binding protein genotypes after vitamin D3 or D2 supplementation. Nutr J. 2013;12(1):39.

28. Glendenning P, Chew GT, Inderjeeth CA, et al. Calculated free and bioavailable vitamin D metabolite concentrations in vitamin D-deficient hip fracture patients after supplementation with cholecalciferol and ergocalciferol. Bone. 2013;56:271–5.

29. Romagnoli E, Mascia ML, Cipriani C, et al. Short and long-term variations in serum calcitropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. J Clin Endocrinol Metab. 2008;93:3015–20.

30. Muñiz CS, Fernández-Martin JL, Marchante-Gayón JM, et al. Reference values for trace and ultratrace elements in human serum determined by double-focusing ICP-MS. Biol Trace Elem Res. 2001;82:259–72.

31. Tabataadze T, Zhorzholiani L, Kherkheulidze M, et al. Association between Short Stature and Hair Elements. Georgian Med News. 2015;247:25–30.

32. Siklar Z, Tuna C, Dallar Y, et al. Zinc deficiency: a contributing factor of short stature in growth hormone deficient children. J Trop Pediatr. 2003,49(3):187–188.
33. Klatka M, Blazewicz A, Partyka M, et al. Concentration of selected metals in whole blood, plasma, and urine in short stature and healthy children. Biol Trace Elem Res. 2015;166(2):142–8.
34. Rayman MP. Selenium and human health. Lancet. 2012;379(9822):1256–68.
35. Sangani RG, Ghio AJ. Iron, human growth, and the global epidemic of obesity. Nutrients. 2013;5(10):4231–49.
36. Lönnerdal B. Excess iron intake as a factor in growth, infections, and development of infants and young children. Am J Clin Nutr. 2017;106(Suppl 6):1681S–1687S.
37. Köhrle J. Selenium and the thyroid. Curr Opin Endocrinol Diabetes Obes. 2015;20(5):392–401.
38. Eum J-H, Cheong H-K, Ha E-H, et al. Maternal blood manganese level and birth weight: a MOCEH birth cohort study. Environ Health. 2014;13(1):31.
39. Hansen SL, Spears JW, Lloyd KE, et al. Feeding a low manganese diet to heifers during gestation impairs fetal growth and development. J Dairy Sci. 2006;89(11):4305–11.