Journal of Inflammation Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Investigation of Serum Pro-Inflammatory Markers and Trace Elements Among Short Stature in Eastern Uttar Pradesh and Bihar Populations

Abhay Kumar Yadav[1], Nitish Kumar Singh[1], Ankur Singh[2], Ashish Ashish[3], Suchitra Singh[1], Sachchida Nand Rai[4], Santosh Kumar Singh[4], Royana Singh[1]

[1]Department of Anatomy, Institute of Medical Science, Banaras Hindu University, Varanasi, UP, 221005, India; [2]Department of Pediatrics, Institute of Medical Science, Banaras Hindu University, Varanasi, UP, 221005, India; [3]Multidisciplinary Research Unit, Institute of Medical Science, Banaras Hindu University, Varanasi, UP, 221005, India; [4]Centre of Experimental Medicine and Surgery, Institute of Medical Science, Banaras Hindu University, Varanasi, UP, 221005, India

Correspondence: Royana Singh, Department of Anatomy, Institute of Medical Science, Banaras Hindu University, Varanasi, UP, 221005, India, Email royanasingh@bhu.ac.in

Purpose: Short stature is prevalent among children worldwide, particularly in developing countries. Various trace elements, including zinc, magnesium, iron, copper, chromium and selenium, are crucial for proper body development. The aim of this study is to explore the relationship between trace elements and TNF-α and IL-6 to elicit and possible pathway responsible for short stature.

Methods: Two hundred and twenty samples were recruited for this study, 100 short statures and 120 controls were randomly selected. Six trace elements were measured using graphite furnace atomic absorption spectrometry. The concentrations of IL-6 and TNF-α in serum were assessed utilizing the Enzyme-Linked-Immunosorbent Assay (ELISA). Superoxide dismutase was also analysed to determine the oxidative stress response.

Results: The study revealed notable distinctions in serum trace element levels of short stature. They exhibited significant lower levels of zinc and magnesium, alongside higher levels of copper. The altered Cu/Zn ratio seemed to have a positive correlation with short stature. Conversely, no significant disparities were observed in iron, chromium, and selenium levels. Furthermore, a significant rise was noted in proinflammatory marker TNF-α and cytokine IL-6. Additionally, superoxide dismutase was low in the short statures In silico study shows a high affinity of Zinc with TNF alpha. It may be suggested that inflammation at any time during childhood, with the rise in TNF alpha tightly binds with zinc and may have led to a decrease in zinc serum levels, altered redox homeostasis and resulted in short stature.

Conclusion: The altered Cu/Zn ratio along with high TNF alpha and IL6 may be used as a marker for short stature in the initial years of growth in children before they reach maturity at the age of 18. Thereafter, introducing zinc supplementation could potentially enhance stature by mitigating TNF-alpha level. Further experimental studies will help to establish the exact role of zinc with TNF alpha in short stature.

Keywords: short stature, zinc, trace element, superoxide dismutase and inflammation

Introduction

Short stature is a condition where the height of the individual is less than the 3rd percentile of the corresponding mean height for a given sex and age.[1] Many instances of short stature can be attributed to normal variations or familial factors, with some cases involving constitutional delay.[2] Short stature is a common disease that occurs in developing countries, the reason behind has been attributed to large number of children suffering from malnutrition. India has a large population of children suffering from diarrhea, typhoid, malaria, and pneumonia.[3] These factors result in alteration of the milieu of the trace elements in children. Trace elements like zinc, iron, and copper, magnesium, selenium and chromium play a vital role in body development.[4] These trace elements are essential for maintaining the structure and function of the anti-oxidant enzyme too. The main anti-oxidant systems associated with trace elements work as cofactors

Received: 13 June 2024
Accepted: 27 August 2024
Published: 5 September 2024

for many anti–oxidant enzymes like SOD, glutathione peroxidase and catalase.[5] Zinc helps the development of growth and DNA synthesis and other metabolic activity like export and transport of metals. Zn is required for many body structure formations in the human body, like bone absorbing 30%, skeleton mussels absorbing 60%, and other body organs absorbing 10%.[6] Copper is an essential trace element that is required for the multiple metal transitions required for the mammalian enzyme. Copper and zinc are essential for the oxidation-reduction reaction, maintain the structure, and work as cofactors for many metalloenzymes.[7–10] Iron found in the human body is basically in two forms: ferrous and ferric, and it helps maintain the function of immune cells and energy metabolism.[11] Essential trace elements such as zinc (Zn), iron (Fe), magnesium (Mg), copper (Cu), and selenium (Se) are involved in immune responses. Any changes in these trace elements can lead to increased inflammation and oxidative reactions. Alterations in oxidative and inflammatory processes affect the Th1 and Th2 cells, which initiate the production of pro-inflammatory factors.[12] A prior investigation demonstrated that reduced serum zinc levels lead to inflammatory conditions, impairing the maturation of B cells and T cells. Consequently, both B cells and T cells proceed to generate pro-inflammatory markers.[13] Figure 1 shows the impact of zinc on levels of anti-oxidant and pro-inflammatory markers.

Trace element deficiency influences proinflammatory markers (TNF-α and IL-6). These trace elements may play a critical role in manifestations of short stature. Literature regarding the effect of trace elements and proinflammatory markers in short stature is limited, and thus the fundamental mechanisms involved are yet to be elucidated. Therefore, the aim of our study was to investigate the correlation between the trace elements (Cu, Zn, Fe, Cr, Mg, and Se) and proinflammatory markers (TNF-α and IL-6) in short stature children among eastern U.P. and Bihar population.

Material and Method

Sample Collection

A short-stature patient sample was collected from the Department of Pediatrics, IMS BHU. Informed consent was obtained from the parents of both the control group and the short-stature group. If the patients were capable, consent was

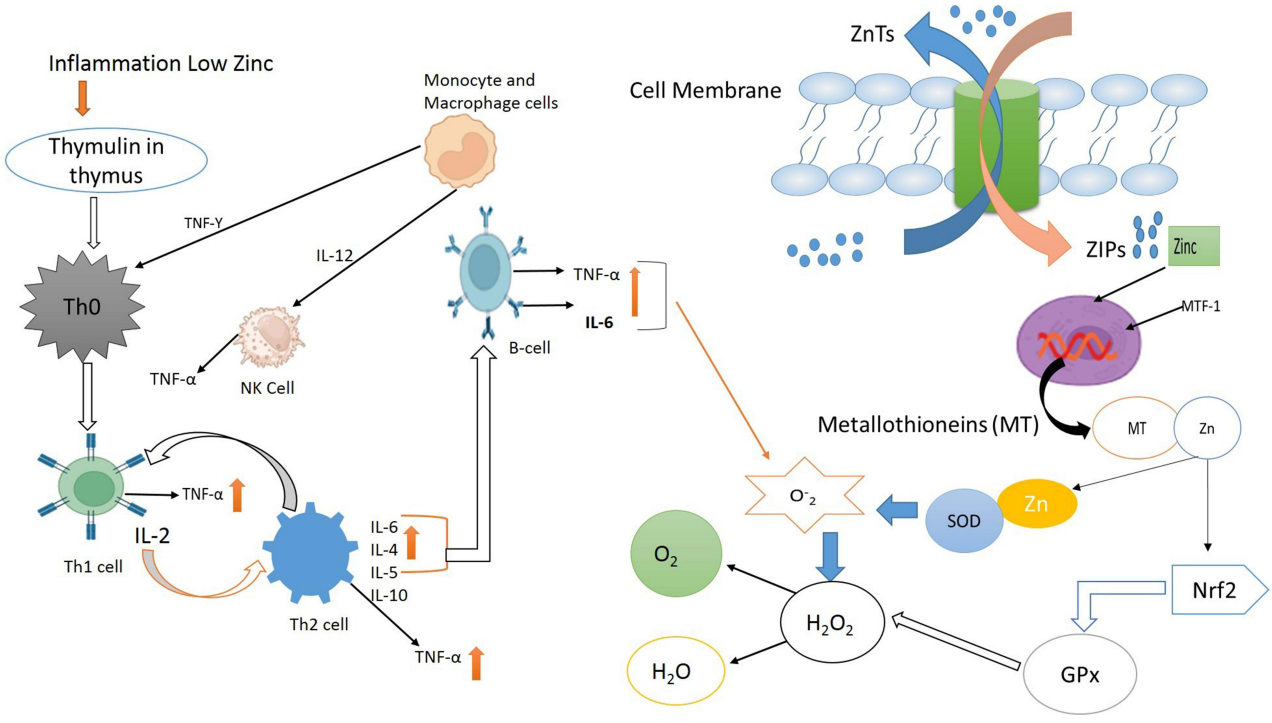

Figure 1 This figure illustrates how Zinc impacts the levels of anti-oxidant and pro-inflammatory markers through its mechanisms of action. Zinc plays two major roles: protecting the naive structure of the macromolecule, which suffers from oxidant agents, and decreasing the concentration of oxidant agents. In cases of zinc deficiency, cell-mediated immunity altered, and Th1, Th2, B cells, and natural killers produce pro-inflammatory TNF-α and IL-6. (Znts, Zinc transporter proteins, ZIP, Zrt/Irt-like proteins, MTF-1, Metal responsive transcription factor 1 and MT, metallothioneins).

also obtained from them. The study was approved by the ethical committee's Institute of Medical Science Banaras Hindu University (IMS BHU) ethical later number (No. Dean/2020/EC/2035). The procedures followed the principles established in the Helsinki Declaration. Additionally, we ensured that all patient data would remain confidential and that the patients' interests would not be compromised.

The anthropometric determinations as well as the blood sampling were performed on the same day. Height in meters (m) and weight in kilograms (kg) was taken using a calibrated wall- mounted stadiometer with a digital weighing machine attached to it (IndoSugicals, India). The BMI calculation was based on the formula: weight in kilograms (kg) divided by height in meters (m) squared. Further Z-score was also calculated using Peditools. (Chou et al 2020)

According to CDC guidelines 2–20 years those children whose heights were less than the 3rd percentile or 2 standard deviations below were considered short stature. Mid-parental height and target height were also calculated for a comprehensive assessment. To determine bone age, an X-ray of the non-dominant (left) hand, with including the wrist joint, was performed, utilizing Greulich and Pyle's atlas of skeletal development.

Inclusion criteria: Children of both genders were included in the study who met the criteria of short stature as per the Peditool 2020, having no syndromic features.

Exclusion criteria: Children who were not included Children with chronic and endocrine abnormalities. Children have skeletal abnormalities, such as achondroplasia and hypochondroplasia. Children have chromosomal abnormalities.

Blood samples were collected through venipuncture in a plane vial, and serum separation was done at 3000 rpm for 5 min. The serum sample was stored at −80°C until analysis was done.

Superoxide dismutase (SOD) Assay

The method for assaying superoxide dismutase (SOD), as developed by Marklund and Marklund,[14] is based on the enzyme's ability to catalyze the dismutation of superoxide radicals. This protective function was assessed by measuring the inhibition of pyrogallol autoxidation under specific conditions. The procedure involved preparing a reaction mixture with pyrogallol and the sample containing SOD, followed by incubation. The absorbance of the mixture was then measured at 420 nm using a spectrophotometer, and the SOD level was calculated based on the observed inhibition of pyrogallol autoxidation.

Proinflammatory Maker Analysis

The concentrations of IL-6 and TNFα in serum were assessed utilizing the enzyme-linked immunosorbent assay (ELISA) method. IL-6 and TNF-α measurements, identified by catalogue Numbers EKL1156 and ELK1190, respectively. The applied protocol was given in detail in the kit.

Atomic Absorption Spectrophotometer

The serum samples of short-stature trace elements Zn, Fe, Mg, Se, Cu, and Cr were evaluated using graphite furnace atomic absorption spectrometry (Perkin Elmer AAS PinAACle 900 FF flame AAS analytical instrument). Samples were diluted (1:10) times. The matrix modifier was added to the required sample. Sample diluent was prepared with 0.4% Triton-X 100 and 1% HNO_3. Zn, Fe, and Cu concentrations were measured at wavelengths of 213.86, 248.33, and 324.75. On the other hand, Se, Mg, and Cr concentrations were measured at wavelengths of 196.03, 285.21, and 357.87, and the absorbance concentration of the measured element was µg/dl.

Calibration Curve

Cu, Se, Cr, and Mg Standard solution calibrations were prepared for the final concentration of stock (1 ppm) in 1% HNO_3. AAS 20 ppb was the final concentration of the serial dilution of standard 1, standard 2, and standard 3 (4, 6 and 8 ppb). Similarly, Zn and Fe standards were prepared at 1 ppm and serial dilutions of standard stock concentrations were 50 ppb, and serial dilutions were standard 1, standard 2, and standard 3 (10, 20, and 30 ppb). The absorbance of the standard and blank plots of the graph against the parallel concentration and the AAS instrument accepting the 95% calibration curve was acceptable.

Binding Site Prediction and Docking of Zn^{2+} Ion with TNF-Alpha

The MIB2 server was used to predict the binding site and evaluate the binding ability of Zn^{2+} ions to TNF-alpha. The MIB2 server predicts binding sites and docks metal ions using both protein sequences and structures. The 3D structure of TNF-alpha (PDB ID-2az5) and Zn^{2+} ion was submitted to the MIB2 service. Based on the highest binding score, the best docking site of Zn^{2+} ions at TNF-alpha was chosen. The retrieved docked structure was then visualized and analyzed with the Discovery Studio Visualizer.

Statistical Analysis

The data set was tested using the Kolmogorov–Smirnov test for normality and homogeneity of variance before going through statistical analysis. Data were normally distributed continuous variables biomarker and Anthropometric was done by independent samples *t*-test. A two-tailed P less than 0.05 was considered statistically significant. For Trace element analysis Mann–Whitney *U*-test was done to compare the non-normally distributed variable mean of both the short-stature group and the control group. Correlations between trace elements and proinflammatory maker (TNF-α, IL-6) were investigated using the Spearman correlation test, where two-tailed P values less than 0.05 were considered significant.

Result

The results show that the two groups have some significant differences in their anthropometric measurements. The average age of participants was similar between the groups, with one group having an average age of 9.1 ± 3.7 years and the other 9.6 ± 4.3 years (P = 0.496). However, there were significant differences in height, with one group having a mean height of 113.7 ± 19.7 cm and the other 131.6 ± 20.3 cm (P = 0.0001). The Height-for-Age Z-score (HAZ) also differed significantly between the groups, with one group having a mean HAZ of −3.2 ± 1.3 compared to −0.4 ± 1.2 in the other (P = 0.0001). Similarly, weight and Weight-for-Age Z-score (WAZ) showed significant differences, with one group weighing 20.3 ± 9.0 kg and the other 28.9 ± 11.9 kg (P = 0.0002), and their WAZ being −3.5 ± 1.7 versus −0.9 ± 1.2 (P = 0.0001). While the Body Mass Index (BMI) was not significantly different between the groups, with values of 15.0 ± 3.1 kg/m² and 15.8 ± 2.0 kg/m² (p = 0.168), the BMI-for-Age Z-score (BMIAZ) did show a significant difference, with one group having a BMIAZ of −1.7 ± 2.1 compared to −0.9 ± 1.1 in the other group (P = 0.021) in Table 1.

Short stature group, exhibited a marked high level of Copper (Cu) with a mean of 93.40 µg/dl compared to 84.11 µg/dl (P = 0.001), while Zinc (Zn) levels were significantly lower (78.56 µg/dl) in contrast with the control group (100.00 µg/dl) with a P value of 0.0001. Iron (Fe) levels indicated no statistically notable difference with mean values of 84.06 µg/dl for short stature and 86.49 µg/dl for controls (P = 0.336). Chromium (Cr) concentrations were similar between the groups, with means of 0.972 µg/L for short stature and 0.928 µg/L for controls (P = 0.111). Magnesium (Mg) levels were

Table 1 Anthropometric Characteristics of Short Stature and Control Group

Parameter	Short Stature Mean ±SD N = 100	Control Group Mean ±SD N = 120	P value
Age (year)	9.1 ± 3.7	9.6 ± 4.3	0.496
Height (cm)	113.7 ± 19.7	131.6 ± 20.3	0.0001
HAZ	−3.2 ± 1.3	−0.4 ± 1.2	0.0001
Weight (kg)	20.3 ± 9.0	28.9 ± 11.9	0.0002
WAZ	−3.5 ± 1.7	− 0.9 ± 1.2	0.0001
BMI (kg/m^2)	15.0 ± 3.1	15.8 ± 2.0	0.168
BMIAZ	−1.7 ± 2.1	−0.9 ± 1.1	0.021

Notes: The data are presented as mean ± Standard deviation (SD). N represent the number of subject in each group, with WAZ indicating Weight-for-age *Z* score, HAZ indicating Height-for-age *Z* score, BMI representing body mass index and BMIAZ representing BMI-for-age *Z* score. A statistically difference from the control group at a significance level of P <0.05.

remarkably low (1.454 mg/dl) in short stature compared to control category (1.616 mg/dl) with a P value of (P = 0.011). In short stature verses control, Selenium (Se) levels had no notable difference, showing mean values of 91.93 µg/L and 92.86 µg/L, respectively (P = 0.231) (Table 2) and Figure 2

The levels of superoxide dismutase (SOD) were observed to be significantly low in the short stature category (17.41 ± 1.36 U/mL) when compared to the control category (73.30 ± 5.97 U/mL), (Complementarily, interleukin-6 (IL-6) levels were markedly elevated in the short stature group (84.84 ± 7.21 pg/mL) compared to the control group (8.29 ± 0.53 pg/mL). Furthermore, Tumour necrosis factor-alpha (TNF-α) levels were substantially higher in the short stature group (10.21 ± 0.896 pg/mL) compared to the control group (0.654 ± 0.181 pg/mL) (Table 3).

Correlation analysis between various trace elements and the cytokines TNF-alpha and IL-6 showed that Copper (Cu) had a weak negative correlation with TNF-alpha (r = −0.209, P = 0.069) and IL-6 (r = −0.072, P = 0.523), though these correlations were not statistically significant. The correlation analysis revealed no statistical significance between serum zinc levels and interleukin-6 (IL-6) levels (r = −0.039, P = 0.730). However, a significant negative correlation was observed between serum zinc levels and tumor necrosis factor-α (TNF-α) levels (r = −0.380, P = 0.001), indicating a potential association between reduced zinc levels and increased TNF-α levels in Figure 3. Iron (Fe) exhibited a very weak positive correlation with both TNF-alpha (r = 0.159, P = 0.156) and IL-6 (r = 0.147, P = 0.192), neither of which were significant. Chromium (Cr) had a very weak negative correlation with TNF-alpha (r = −0.129, P = 0.251) and a weak positive correlation with IL-6 (r = 0.158, P = 0.159), both non-significant. Magnesium (Mg) displayed a weak positive correlation with TNF-alpha (r = 0.161, P = 0.151) and IL-6 (r = 0.062, P = 0.579), neither of which were significant. Selenium (Se) had a weak positive correlation with TNF-alpha (r = 0.145, P = 0.158) and IL-6 (r = 0.107, P = 0.343), and these correlations were also not statistically significant.

The correlation analysis between the Cu/Zn ratio and the inflammatory markers TNF alpha and IL-6 yielded different outcomes. For TNF alpha, there was a positive correlation with the Cu/Zn ratio (r = 0.219), which approached statistical significance (P = 0.050). In contrast, the correlation between the Cu/Zn ratio and IL-6 was nearly negligible (r = 0.006) and not statistically significant (P = 0.954) in Table 4.

The MIB2 server predicted a total of 13 docking sites between Zn^{2+} ions and the TNF-alpha protein. Among these sites, the site with the highest binding score of 4.928 was identified as the most favorable for Zn^{2+} ion binding. This selected binding site encompasses amino acid residues GLU127 and ASP130 within TNF-alpha. Interaction analysis revealed that Zn^{2+} ions form electrostatic interactions specifically with GLU127 and ASP130 residues within the binding

Table 2 Trace Element Comparison Between Short Stature Verses Control

Parameter	Case N = 100	Control N = 120	Normal Range	P value
Cu (µg/dl)	93.40 ± 17.95	84.11 ± 13.96	57–160	0.001
Min – Max	(57.82–126.5)	(55.78–110.9)		
Zn (µg/dl)	78.56 ± 20.08	100 ± 18.06	60–120	0.0001
Min – Max	(32.21–108.1)	(41.07–127.1)		
Fe (µg/dl)	84.06 ± 15.70	86.49 ± 15.55	50–120	0.336
Min – Max	(50.84–116.2)	(52.48–120.8)		
Cr (µg/L)	0.972 ± 0.204	0.928 ± 0.245	0.1–1.2	0.111
Min – Max	(0.29–1.33)	(0.9–1.56)		
Mg (mg/dl)	1.454 ± 0.325	1.616 ± 0.373	1.7–2.1	0.011
Min – Max	(1.01–2.76)	(1.00–2.57)		
Se (µg/L)	91.93 ± 7.58	92.86 ± 8.63	90–140	0.231
Min – Max	(72–106)	(73–107)		
Cu/Zn Ratio	1.28 ± 0.46	0.87 ± 0.27	0.7–1.0	0.001
Min – Max	(0.54–2.91)	(0.48–2.10)		

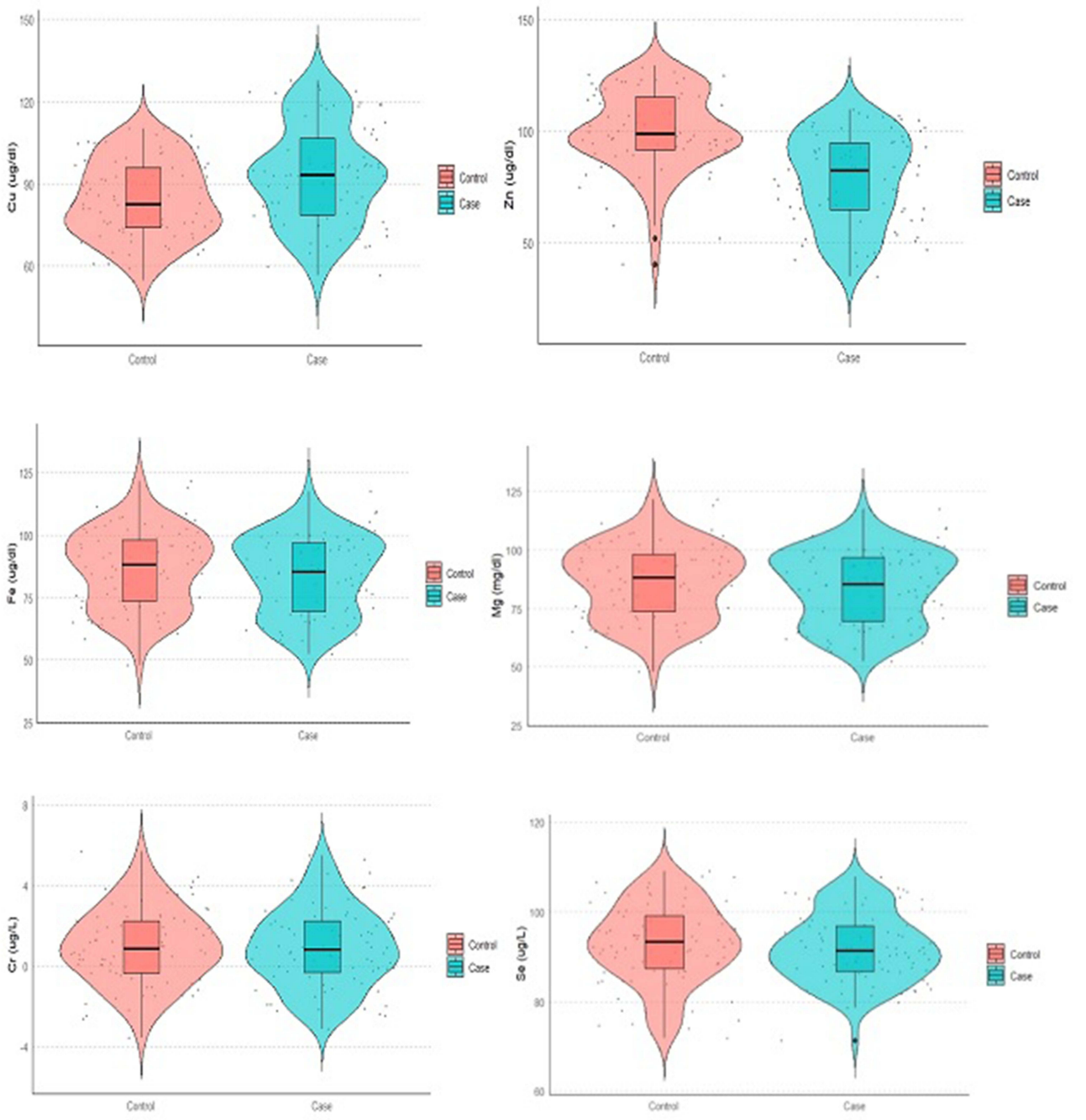

Figure 2 The violin plot shows cases and controls compared in terms of compression. Key statistical variables including the median (shown by a horizontal black line), interquartile range (represented by a box), and the overall data range after outliers have been excluded (represented by thin black lines) are displayed in each violin plot as boxplots. The colorful areas on the plot show an estimate of the kernel density and shed light on the data's distribution.

site on TNF-alpha. These interactions suggest the formation of a complex between Zn^{2+} ions and the TNF-alpha protein (Figure 4).

Discussion

Zinc and Copper are one of the most important trace elements that help in growth, development, and other activities of body parts. A significant lower zinc and higher Copper levels in short stature individuals were observed in our study, similar results also reported in the previous study.[15–19] Gibson et al had also reported significant Zn deficiency in short

Table 3 Compression Between Proinflammatory Cytokine IL-6, TNF-α and Oxidative Stress in Short Stature Verses Control

Parameter	Case N = 100	Control N = 120	P value
SOD (U/mL)	17.41 ± 1.36	73.30 ± 5.97	0.0001
IL-6 (pg/mL)	84.84 ± 7.21	8.29 ± 0.53	0.001
TNF-α (pg/mL)	10.21 ± 0.896	0.654 ± 0.181	0.0001

stature in comparison to the control sample.[20] High serum copper levels in short stature have been reported by other scientist too.[21,22]

The trace elements, however, showed no correlation with BMI. BMI had non-significant difference between the case and control. However, BMIZ (BMI for age Z-score) shows a slight significant difference between case and control, which is similar to a previous study by Hamza RT et al 2012 where no significant difference was observed amongst short stature children and adolescent BMI with Zinc.[23] In another study, Sugawara et al (2022) suggested Zn levels are associated with anthropometric measurements, especially body weight and BMI, but had showed no association with short stature.[22]

Zinc is necessary for several other cellular functions necessary for growth, such as hormone control, protein synthesis, cell division and detoxifying ions of heavy metals from the cells of the liver and kidney. These processes can be hampered by a zinc deficit, which can cause delayed maturation and growth retardation and also affect the decreased immune system.[24–26] For optimal health, a certain amount 2–4 gram of zinc trace element needs to be consumed daily as it is utilized in the liver, brain, muscles, bones, eyes and kidneys.[27]

Zn is bound and transported by albumin (60%) and transferrin (10%) in blood plasma. Since transferrin also carries iron, too much iron might impede the absorption of zinc, and vice versa. Regardless of zinc intake, the plasma's zinc content is mostly constant.[28,29]

No significant association was observed for the trace element Fe (iron) in individuals with short stature and control group. Similar observations were made by previous authors.[30]

Magnesium is another important trace element that is essential for normal fetal development. Deficiency of this trace element was found in short stature girls and boys group which impaired growth development.[31–33]

Similary significant reduction in magnesium levels was observed in our study. No significant differences amongst Selenium (Se) and Chromium (Cr) levels were observed in accordance with previous studies.[34–36]

The Cu/Zn ratio, however, showed a significant difference p value 0.001.[37–39]

The increased Cu/Zn ratio denotes the impairment of the liver and can affect the level of malnutrition markers like albumin and prealbumin notably, a robust and significant association was identified, with erythrocyte sedimentation rate (ESR) showing a primary link to the Cu/Zn ratio and a secondary connection with serum Cu levels. This suggests that the Cu/Zn ratio could potentially serve as an inflammatory marker study reported by Liu Y et al. The study, reported by

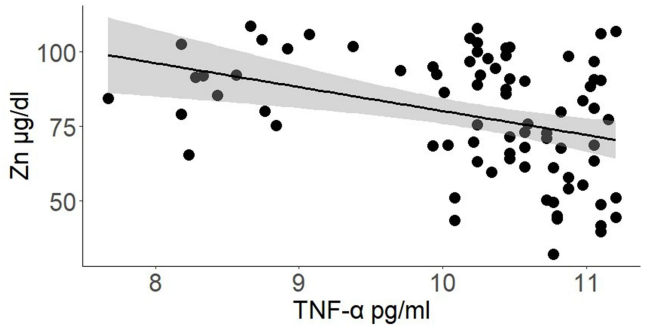

Figure 3 Correlation analysis between serum zinc and TNF-α in short stature (r = - 0.327, p = 0.003).

Table 4 This Table Shows the Correlation Analysis of Serum Proinflammatory Markers TNFα, IL-6, and Trace Elements in Short Stature

Variables	TNF alpha (pg/mL)		IL-6 (pg/mL)	
	Correlation Coefficient (r)	P-value	Correlation Coefficient (r)	P-value
Cu (µg/dl)	−0.209	0.061	−0.072	0.523
Zn (µg/dl)	−0.380	0.001	−0.039	0.730
Fe (µg/dl)	0.159	0.156	0.147	0.192
Cr (µg/L)	−0.129	0.251	0.158	0.159
Mg (mg/dl)	0.161	0.151	0.062	0.579
Se (µg/L)	0.145	0.158	0.107	0.343
Cu/Zn Ratio	0.219	0.050	0.006	0.954

Notes: IL-6, interleukin 6, Zn, Zinc, Cu, Copper, Fe, Iron, Cr, chromium, Mg, Magnesium, Se, Selenium, TNF-α, tumor necrosis factor –α, r, correlation coefficient. The Spearman coefficient is statistically significant if it is less than p<0.05.

Escobedo-Monge MF et al in Cystic fibrosis patients Increase Cu/Zn ratio a may be an indicator of zinc deficiency and high inflammation response.[38,40]

One of the oxidative stress marker showed a highly significant decrease, i.e., SOD in our study Aly GS et al found oxidative stress marker levels SOD, Glutathione Peroxidase, MDA and Catalase statistically significantly decreased in

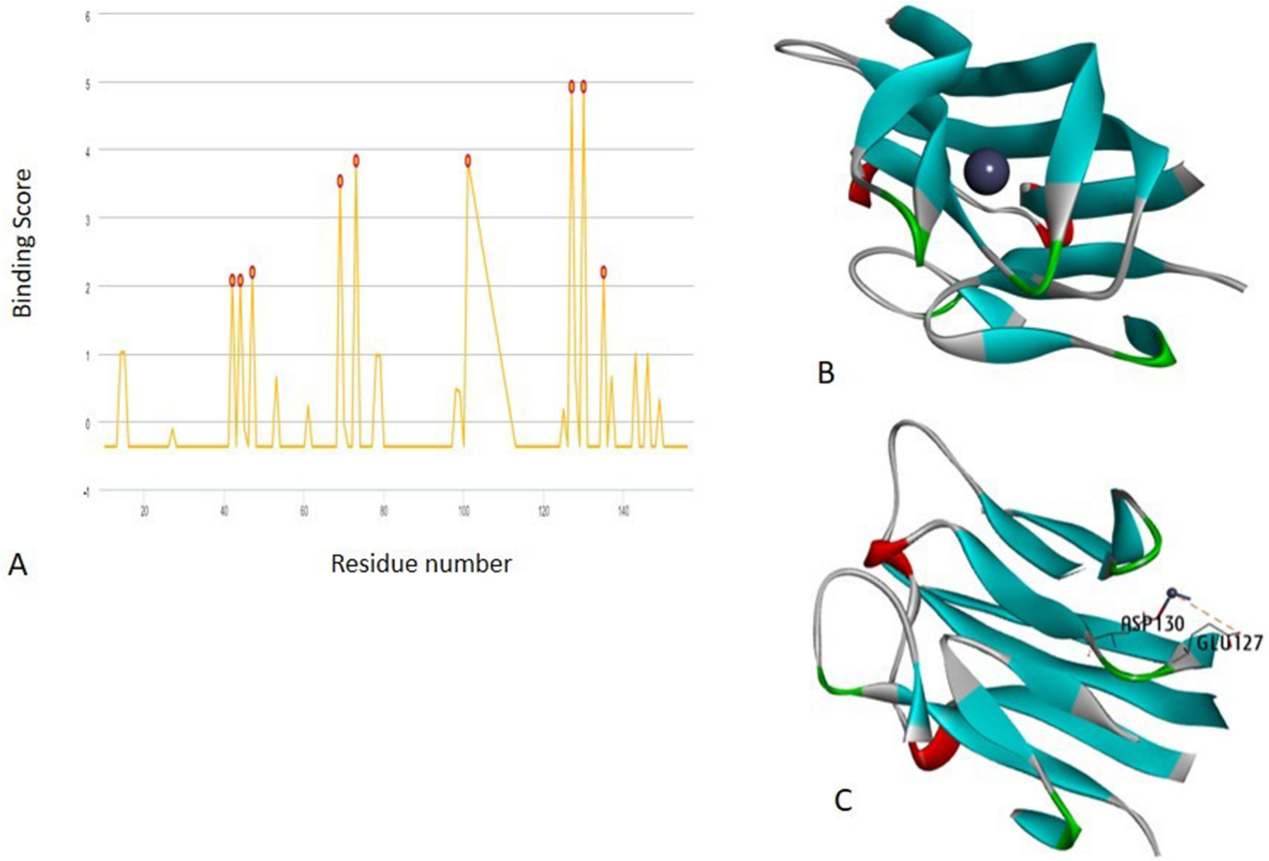

Figure 4 Zn^{2+} ion binding site prediction in TNF-alpha protein. (**A**) Binding score of TNF-alpha amino acid residues. (**B**) Zn^{2+} ion binding pocket in TNF- alpha protein (**C**) Electrostatic interactions of GLU 127 and ASP 130 with Zn^{2+} ion.

short stature. Additionally, the trace element copper (Cu) and zinc (Zn) were found to be significantly lower in short stature.[41]

Costarelli L et al and Garcia O et al stated that low zinc intake was associated with obesity, which was associated with an elevated risk of metabolic disturbance and inflammation.[41,42] Zinc plays a significant role in regulating inflammation through various mechanisms. It exhibits anti-inflammatory properties by effectively preventing the activation of NF-kB, a transcription factor involved in the expression of numerous proinflammatory genes.[43]

Pro- inflammatory markers TNF-α and IL-6 were raised significantly in short stature in our study. Our result aligns with Noori N et al and Ekim M et al found similar results.[44,45]

Growth can be affected by inflammation by disturbing growth hormone and insulin-like growth factor signalling pathways. In patients suffering from chronic liver disease, TNF-α appears as a primary factor contributing to the development of growth hormone resistance.[46–48]

A study reported in an animal model showed that IL-6 does not affect growth but does decrease levels of IGF-1.[49]

Sprietsma JE et al had observed in individuals with asthma and a deficiency in zinc are likely to experience heightened inflammation. Both asthma and zinc deficiency are linked to an inclination towards the proinflammatory Th2 response, leading to an upregulation of the production and release of various proinflammatory cytokines TNF-α and IL-6. Therefore, asthmatics who are also deficient in zinc may exhibit increased inflammatory responses.[44] Negative correlation between Zinc (Zn) and TNF-α levels aligns with the result reported by Feitosa MC et al, where they identified a negative correlation between Zinc and TNF-α ($r = -0.44$, $p = 0.015$[50] (Figure 3).

We suggest that short stature associated with inflammation, which leads to zinc deficiency, which, in turn, triggers the activation of proinflammatory markers-TNF-alpha and IL6 cytokine. These in turn increased the reactive oxygen species triggering oxidative stress. The SOD in turn is utilized to neutralize these reactive oxygen species with the help of Zinc. Zinc also triggers glutathione peroxidase to neutralize the hydrogen peroxide into water and oxygen. The imbalance caused by the disproportionate increase in the reactive oxygen species due to zinc deficiency leads to altered redox homeostasis and in turn low SOD (Figure 1).

In silico analysis highlights the specific region key amino acid residues of TNF-alpha: GLU127 and ASP130 involved in interactions with Zn^{2+} ions. Forming a complex the strong binding draws a strong suggestion that zinc might have been utilized by TNF alpha increase and thus low level in the serum. There on, it turns on a vicious cycle of low zinc, inflammation and further increase in TNF alpha. The above suggestions, however, need more experimental backup to facilitate insight into the exact mechanism of how low zinc or altered Cu/zn ratio lead to short stature.

We further suggest that as a maker of short stature zinc levels alone or Cu/Zinc ratio may be more reliable if TNF alpha and IL6 levels are also accounted for in patients. Thereafter, zinc supplements in the management of these children could alter their stature to normal.

Conclusion

Zinc deficiency is a prevalent issue in developing nations and has been linked to impaired absorption of various nutrients, exacerbating the deficiency itself or it could be a consequence of childhood inflammation due to any infection is yet to be elucidated. However, it is certain that this deficiency contributes to systemic inflammation, which adversely affects proper growth and development. Consequently, initiating daily zinc supplementation in children diagnosed with short stature early on may potentially trigger a growth spurt.

Acknowledgment

This work was done with support from the multidisciplinary research unit, IMS BHU under ICMR-DHR, New Delhi.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Wit JM, Clayton PE, Rogol AD, Savage MO, Saenger PH, Cohen P. Idiopathic short stature: definition, epidemiology, and diagnostic evaluation. *Growth Horm IGF Res*. 2008;18(2):89–110. doi:10.1016/j.ghir.2007.11.004
2. Song KC, Jin SL, Kwon AR, et al. Etiologies and characteristics of children with chief complaint of short stature. *Ann Pediatr Endocrinol Metab*. 2015;20(1):34–39. doi:10.6065/apem.2015.20.1.34
3. Akhtar S. Zinc status in South Asian populations--an update. *J Health Popul Nutr*. 2013;31(2):139–149. doi:10.3329/jhpn.v31i2.16378
4. Delemarre-van de Waal HA. Environmental factors influencing growth and pubertal development. *Environ Health Perspect*. 1993;101(Suppl 2):39–44. doi:10.1289/ehp.93101s239
5. Caglayan A, Katlan DC, Tuncer ZS, Yüce K. Evaluation of trace elements associated with antioxidant enzymes in blood of primary epithelial ovarian cancer patients. *J Trace Elem Med Biol*. 2019;52:254–262. doi:10.1016/j.jtemb.2019.01.010
6. Kambe T, Tsuji T, Hashimoto A, Itsumura N. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. *Physiol Rev*. 2015;95(3):749–784. doi:10.1152/physrev.00035.2014
7. Lin CN, Wilson A, Church BB, Ehman S, Roberts WL, McMillin GA. Pediatric reference intervals for serum copper and zinc. *ClinChimActa*. 2012;413(5–6):612–615. doi:10.1016/j.cca.2011.12.005
8. Jomova K, Makova M, Alomar SY, et al. Essential metals in health and disease. *ChemBiol Interact*. 2022;367:110173. doi:10.1016/j.cbi.2022.110173
9. Wang W, Mo W, Hang Z, et al. Cuproptosis: harnessing transition metal for cancer therapy. *ACS Nano*. 2023;17(20):19581–19599. doi:10.1021/acsnano.3c07775
10. Roy Z, Bansal R, Siddiqui L, Chaudhary N. Understanding the role of free radicals and antioxidant enzymes in human diseases. *Curr Pharm Biotechnol*. 2023;24(10):1265–1276. doi:10.2174/1389201024666221121160822
11. Means RT. Iron Deficiency and iron deficiency anemia: implications and impact in pregnancy, fetal development, and early childhood parameters. *Nutrients*. 2020;12(2):447. doi:10.3390/nu12020447
12. Srivastava S, Tiwari V, Singh S, Karoli R, Bhattacharya P, Gupta N. Low serum levels of zinc, selenium, and vitamin D3 are biomarkers of airway inflammation and poor asthma control: a two-centre study. *Cureus*. 2023;15(6):e41082. doi:10.7759/cureus.41082
13. Zou Y, Huang LC, Zhao D, He MJ, Su D, Zhang RH. Assessment of serum Vit A, D and zinc nutritional status and related dietary and exercise behaviors of children and adolescents in rural and urban area. *Front Nutr*. 2023;9:1088155. doi:10.3389/fnut.2022.1088155
14. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*. 1974;47(3):469–474. doi:10.1111/j.1432-1033.1974.tb03714.x
15. Klatka M, Błażewicz A, Partyka M, Kołłątaj W, Zienkiewicz E, Kocjan R. Concentration of selected metals in whole blood, plasma, and urine in short stature and healthy children. *Biol Trace Elem Res*. 2015;166(2):142–148. doi:10.1007/s12011-015-0262-2
16. Saati AA, Adly HM. Assessing the correlation between blood trace element concentrations, picky eating habits, and intelligence quotient in school-aged children. *Children (Basel)*. 2023;10(7):1249. doi:10.3390/children10071249
17. Skalny AV, Mazaletskaya AL, Ajsuvakova OP, et al. Serum zinc, copper, zinc-to-copper ratio, and other essential elements and minerals in children with attention deficit/hyperactivity disorder (ADHD). *J Trace Elem Med Biol*. 2020;58:126445. doi:10.1016/j.jtemb.2019.126445
18. Hamed AM, Kassem YT, Fayed HK, Solaiman AM. Serum zinc levels in hospitalized children with pneumonia: a hospital-based case–control study. *Egyptian J Bronchology*. 2019;13(5):730–737. doi:10.4103/ejb.ejb_30_19
19. Gibson RS, Manger MS, Krittaphol W, et al. Does zinc deficiency play a role in stunting among primary school children in NE Thailand? *Br J Nutr*. 2007;97(1):167–175. doi:10.1017/S0007114507250445
20. Al-Samarrai AH, Adaay MH, Al-Tikriti KA, Al-Anzy MM. Evaluation of some essential element levels in thalassemia major patients in Mosul district, Iraq. *Saudi Med J*. 2008;29(1):94–97.
21. Claster S, Wood JC, Noetzli L, et al. Nutritional deficiencies in iron overloaded patients with hemoglobinopathies. *Am J Hematol*. 2009;84(6):344–348. doi:10.1002/ajh.21416
22. Sugawara D, Makita E, Matsuura M, Ichihashi K. The association between serum zinc levels and anthropometric measurements and nutritional indicators in children with idiopathic short stature. *Cureus*. 2022;14(5):e24906. doi:10.7759/cureus.24906
23. Hamza RT, Hamed AI, Sallam MT. Vitamin D status in prepubertal children with isolated idiopathic growth hormone deficiency: effect of growth hormone therapy. *J Investig Med*. 2018;66(5):1–8. doi:10.1136/jim-2017-000618
24. Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro RA, Weill R, Boccio JR. The role of zinc in the growth and development of children. *Nutrition*. 2002;18(6):510–519. doi:10.1016/s0899-9007(01)00812-7
25. Tabatadze T, Zhorzholiani L, Kherkheulidze M, Karseladze R, Ivanashvili T. Association between short stature and hair elements. *Georgian Medl News*. 2015;247:25–30.
26. Siklar Z, Tuna C, Dallar Y, Tanyer G. Zinc deficiency: a contributing factor of short stature in growth hormone deficient children. *J Trop Pediatr*. 2003;49(3):187–188. doi:10.1093/tropej/49.3.187
27. Belgemen T, Akar N. The vital functions of zinc and the genes associated zinc metabolism. *Med Faculty J*. 2004;57:161–166.
28. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: an integrative review. *J Res Med Sci*. 2013;18(2):144–157.
29. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. *J Res Med Sci*. 2014;19(2):164–174.
30. Dawood ET, Mohammed HA, Metwalley KA. Zinc and iron levels in children with idiopathic short stature. *J Curr Med Res Pract*. 2021;6(4):368–372. doi:10.4103/JCMRP.JCMRP_164_19
31. Díaz Gómez NM, Doménech Martínez E, Barroso Guerrero F. Elementos traza y factores de crecimiento en el período perinatal [Trace elements and growth factors in the perinatal period]. *An Esp Pediatr*. 1996;44(4):351–356.
32. Zadik Z, Sinai T, Zung A, Reifen R. Effect of nutrition on growth in short stature before and during growth-hormone therapy. *Pediatrics*. 2005;116(1):68–72. doi:10.1542/peds.2004-1129
33. Naji Z. Serum trace elements (zinc, copper and magnesium) in Iraqi patients with thalassemia major receiving desferrioxamine and its relation with growth state. *Iraqi J Med Sci*. 2012;10(4).

34. Azab SF, Saleh SH, Elsaeed WF, Elshafie MA, Sherief LM, Esh AM. Serum trace elements in obese Egyptian children: a case-control study. *Ital J Pediatr*. 2014;40(1):20. doi:10.1186/1824-7288-40-20
35. Strumylaite L, Kregzdyte R, Kucikiene O, et al. Alzheimer's disease association with metals and metalloids concentration in blood and urine. *Int J Environ Res Public Health*. 2022;19(12):7309. doi:10.3390/ijerph19127309
36. Li YQ, Wang Q, Liu R, et al. Associations of exposure to multiple metals with the risk of age-related cataract in Anhui, China: a case-control study. *Environ Sci Pollut Res Int*. 2023;30(2):4680–4693. doi:10.1007/s11356-022-22494-y
37. Bassene HS, Tall FG, Ehmndour MD, et al. Interest of serum Copper/Zinc ratio in oxidative stress assessment on a population of senegalese sickle cell children. *Int J Biol Med Res*. 2020;11(2):7029–7035.
38. Escobedo-Monge MF, Barrado E, Parodi-Román J, Escobedo-Monge MA, Torres-Hinojal MC, Marugán-Miguelsanz JM. Copper and Copper/Zn Ratio in a series of children with chronic diseases: a cross-sectional study. *Nutrients*. 2021;13(10):3578. doi:10.3390/nu13103578
39. Reza BM, Soheila K, Reza MA, Seyed Shaheen H, Ali KA, Akbar VA. Evaluation of copper, zinc and copper-zinc ratio in the serum of pulmonary tuberculosis children. *Pediatric Oncall J*. 2011;4(4).
40. Liu Y, Tang R, Xu Q, et al. High blood Cu/Zn Ratio is associated with nutritional risk in patients undergoing maintenance hemodialysis. *Biol Trace Elem Res*. 2022;200(12):4977–4987. doi:10.1007/s12011-022-03104-x
41. Costarelli L, Muti E, Malavolta M, et al. Distinctive modulation of inflammatory and metabolic parameters in relation to zinc nutritional status in adult overweight/obese subjects. *J Nutr Biochem*. 2010;21(5):432–437. doi:10.1016/j.jnutbio.2009.02.001
42. Aly GS, Shaalan AH, Mattar MK, Ahmed HH, Zaki ME, Abdallah HR. Oxidative stress status in nutritionally stunted children. *Egyptian Pediatr Assoc Gazette*. 2014;62(1):28–33. doi:10.1016/j.epag.2014.02.003
43. García OP, Ronquillo D, Del Carmen Caamaño M, et al. Zinc, iron and vitamins A, C and e are associated with obesity, inflammation, lipid profile and insulin resistance in Mexican school-aged children. *Nutrients*. 2013;5(12):5012–5030. doi:10.3390/nu5125012
44. Jeon KI, Jeong JY, Jue DM. Thiol-reactive metal compounds inhibit NF-kappa B activation by blocking I kappa B kinase. *J Immunol*. 2000;164 (11):5981–5989. doi:10.4049/jimmunol.164.11.5981
45. Noori NM, Moghaddam MN, Teimouri A, Shahramian I, Keyvani B. Evaluation of serum level of tumor necrosis factor-alpha and interleukin-6 in patients with congenital heart disease. *Niger Med J*. 2016;57(4):233–237. doi:10.4103/0300-1652.188353
46. Ekim M, Ikinciogullari A, Ulukol B, et al. Evaluation of nutritional status and factors related to malnutrition in children on CAPD. *Perit Dial Int*. 2003;23(6):557–562. doi:10.1177/089686080302300607
47. Picardi A, Gentilucci UV, Zardi EM, et al. TNF-alpha and growth hormone resistance in patients with chronic liver disease. *J Interferon Cytokine Res*. 2003;23(5):229–235. doi:10.1089/107999003321829944
48. Rinawi F, Assa A, Almagor T, Ziv-Baran T, Shamir R. Prevalence and predictors of growth impairment and short stature in pediatric-onset inflammatory bowel disease. *Digestion*. 2020;101(6):674–682. doi:10.1159/000501924
49. Denson LA, Held MA, Menon RK, Frank SJ, Parlow AF, Arnold DL. Interleukin-6 inhibits hepatic growth hormone signaling via upregulation of Cis and Socs-3. *Am J Physiol Gastrointest Liver Physiol*. 2003;284(4):G646–G654. doi:10.1152/ajpgi.00178.2002
50. Wong CP, Rinaldi NA, Ho E. Zinc deficiency enhanced inflammatory response by increasing immune cell activation and inducing IL6 promoter demethylation. *Mol Nutr Food Res*. 2015;59(5):991–999. doi:10.1002/mnfr.201400761