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Original Article

Assessment of Selenium and Zinc Levels in Malnourished Pediatric Cerebral Palsy Patients.

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Abstract

Background: Cerebral palsy (CP), a neuromotor disorder caused by prenatal to neonatal brain injury, often leads to malnutrition. Children with CP commonly experience deficiencies in key trace elements like selenium and zinc, essential for immune function and neurodevelopment. This study aims to assess selenium status by selenium binding protein (BP) and zinc levels, in malnourished pediatric CP patients and correlate their levels with nutritional status and infection.

Methods: This case-control study included 70 participants (35 malnourished CP children and 35 healthy controls, all under 18 years) from the pediatric department at Zagazig University Hospital. Zinc levels were assessed using a colorimetric method, and selenium-binding protein levels were measured using ELISA.

Results: Anthropometric measurements were all significantly smaller in the cases group than in the control group ($P<0.0001$ for all). The patient group hemoglobin levels were significantly lowered ($P=0.0033$), while white blood cell (WBC) counts were significantly higher ($P<0.0001$), as were C-reactive protein (CRP) levels ($P<0.0001$). Gram-negative bacilli were more commonly detected in the patients group (22.86% vs. 0%, $P=0.005$). Additionally, albumin, zinc, and selenium binding protein (BP) levels were significantly lower in the patients group. Cutoff values for zinc (<67.44µg/dL) and selenium BP (<191.08ng/dL) showed acceptable sensitivity and accuracy as predictors of CP in pediatric malnourished patients. Serum zinc and selenium BP levels exhibited significant positive correlations with growth parameters and negative correlations with CRP.

Conclusion: Malnourished pediatric CP patients show significant deficiencies in zinc and selenium BP, which contribute to growth impairments and increased infection risk.

Keywords: Selenium, Zinc, Malnutrition, Pediatric, Cerebral Palsy

Introduction

Brain injury during prenatal to neonatal period causes CP, a neuromotor condition that affects movement, muscle tone, and posture. Primary brain injury is non-progressive, but subsequent problems can severely impair functional ability in children with CP **[1].**

Malnutrition, characterized by low weight relative to height and signs of wasting or nutritional edema, affects around 20 million children globally. It weakens the immune system, increases infection risk, and contributes to 54% of global child mortality according to world health organization (WHO). A high proportion of children with CP are also growth-retarded and underweight **[2].**

CP children are malnourished, which increases morbidity and death. Countries have variable rates of malnutrition, and the relationship between CP and nutrition is complex. The level of motor impairment, oral-motor dysfunction, eating skills, and gastrointestinal problems affect nutritional status **[3].**

CP children have decreased nutritional and trace element consumption, according to studies. These children had low iron, calcium, ferritin, zinc, and selenium levels. The Oxford study found severe sodium, iron, copper, magnesium, and zinc shortages in CP children **[4].**

As micronutrients necessary for cell metabolism and reproduction, trace elements are essential for immune system function. Iron, zinc, magnesium, and manganese deficiency might impede leukocyte proliferation, especially during acute infections **[5].**

Zinc is essential for neuronal growth, particularly in glutamatergic neurons, where it supports protein structures like N-methyl-D-aspartate (NMDA) receptors. It is essential for the process of neurogenesis, synaptogenesis, and neuronal migration, with deficiencies linked to impaired neurotransmission, behavioral issues, growth retardation, and increased infection risk**.** Selenium (Se), converted into seleno-proteins, aids immune function, detoxifies heavy metals, and protects against oxidative brain damage. Its deficiency is associated with cognitive and motor impairments **[2].**

Selenium helps brain growth and cognition. It promotes synaptic plasticity, zinc regulation, autophagy, and ferroptosis prevention, which aids neurodevelopment **[6].**

This study aimed to assess selenium status by selenium BP and zinc levels in malnourished pediatric CP patients and correlate their levels with nutritional status and infection.

Methods

This case-control research involved 70 children consisting of 35 malnourished CP patients and 35 healthy controls, at the pediatric department of Zagazig University Hospital from December 2023 to July 2024. Informed consent was obtained from all parents following approval from the institutional review board (N 11385-19-12-2023).

In accordance with the World Medical Association's Declaration of Helsinki, the study followed all applicable ethical guidelines for conducting research on human subjects. Cases in our study were children under the age of 18 who had a diagnosis of cerebral palsy (CP) and malnutrition. Diagnosis was confirmed through history-taking, clinical evaluation, anthropometric measurements, and relevant laboratory tests. Anthropometric data, including weight and height, were collected and evaluated using Z-scores, and the Nelson growth charts.

Exclusion criteria included children over 18 years, those with chronic medical conditions other than CP unrelated to CP, malignancies, and recent vitamin or mineral supplementation. Comprehensive A comprehensive blood count (CBC), renal function tests, and liver function tests were performed in the laboratory to examine the nutritional state and general health of the patients.

Blood cultures were performed under strictly aseptic conditions by promptly inoculating three to five milliliters of sterile blood into pediatric blood culture media bottles **[7].**

Sputum samples were obtained spontaneously, and direct smears were made for all specimens to assess the presence of pus cells and epithelial cells. Only superior specimens were grown on chocolate, MacConkey, and blood agars and incubated for 24 to 48 hours at 37°C.

Following the identification of positive cultures, **bacterial identification was** carried out by colony morphology, Gram stained films, biochemical reactions and was confirmed with VITEK® 2 COMPACT Microbial Detection System (bioMérieux ,USA) according to the manufacturer instructions.

Biochemical estimation of serum zinc and selenium levels:

Serum zinc and selenium BP levels were measured from 3 mL of venous blood following sterile collection, centrifugation at 3000 rpm for 15 minutes, and storage at -20°C **[8].**

Zinc levels were assessed using a colorimetric method by using kit from MG (www.biostc.net). Selenium binding protein levels were measured

using the ELISA technique using kit from BT LAB, (Bioassay Technology Laboratory, China.) with results expressed in µg/dL for zinc and ng/dL for selenium BP. We followed manufacturer instructions for element detection.

Statistical analysis:

A version of SPSS 20.0 was used for the data analysis. For variables that followed a normal distribution, quantitative data was presented as mean ± standard deviation. For variables that did not follow a normal distribution, median and range were used. Frequencies and percentages were used to display the categorical data. For continuous variables, we used the Student t-test and the Mann-Whitney U test to look for distribution-based differences; for categorical data, we used the Chi-square test and Fisher's exact test. We used the Shapiro-Wilk test to see if things were normal. To maximize the balance between specificity and sensitivity, we constructed receiver operating characteristic (ROC) curves to assess diagnostic accuracy and determine the optimal cutoff values for selenium BP plasma concentrations and zinc. In order to measure this, the AUC was calculated. Statistical significance was determined by a p-value that was less than 0.05.

Results

The case group had a median age of 46.49 months (range: 19.62 - 67.93 months) and the control group had a median age of 47.09 months (range: 19.07 - 67.98 months, P = 0.9636) and there's no significant difference regarding age.. Significant differences were observed in anthropometric measurements. The median weight was significantly lower in the case group (2.01 kg, range: 1 - 20 kg) compared to the control group (8.06 kg, range: 3.98 - 30 kg, P = 0.0001). The case group had significantly shorter median height (50 cm, range: 39.9 - 120 cm) compared to the control group (89.99 cm, range: 60 - 140 cm, P < 0.0001). Arm circumference was also significantly smaller in the case group (7.9 cm, range: 5.95 - 14.99 cm) compared to the control group (10.9 cm, range: 9.9 - 18.07 cm, P < 0.0001). Head circumference was smaller in the case group (30 cm, range: 24.9 - 44.96 cm) compared to the control group (40.01 cm, range: 37.94 - 46.07 cm, P < 0.0001). **(Table 1).**

Table (1): Comparison between cases and controls regarding basal characteristics and anthropometrics

	Cases (N = 35)	**Control (N = 35)**	**P. Value**
Age (Months)	46.49 (19.62 - 67.93)	47.09 (19.07 - 67.98)	0.9636
Sex			
Male	14 (40%)	12 (34.29%)	0.6268
Female	21 (60%)	23 (65.71%)	
Positive Family history	8 (22.86%)	9 (25.71%)	0.7842
Nutritional edema			
Present	0 (0%)	0 (0%)	-
Not present	35 (100%)	35 (100%)	
Anthropometric measurements			
Weight (Kg)	2.01 (1 - 20)	8.06 (3.98 - 30)	0.0001*
Height (cm)	50 (39.9 - 120)	89.99 (60 - 140)	<0.0001*
Arm Circumference (cm)	7.9 (5.95 - 14.99)	10.9 (9.9 - 18.07)	<0.0001*
Head Circumference (cm)	30 (24.9 - 44.96)	40.01 (37.94 - 46.07)	<0.0001*

Hemoglobin (Hb) levels were significantly reduced in the case group (8.47 ± 0.9 g/dL) compared to the control group (9.45 ± 1.62 g/dL, P = 0.0033). The case group white blood cell count (WBC) was 13.03 ± 3.98 × 10^3/µL, significantly above that of the control group (6.93 ± 1.62 × 10^3/µL, P < 0.0001). The case group had a significantly reduced neutrophil percentage (48%, range: 28–59) compared to the control group (53%, range: 39–60, P = 0.0026). The case group AST levels (34 U/L, range: 11 - 333) were significantly elevated compared to the control group levels (24 U/L, range: 12 - 55, P = 0.0024) in the liver function tests (LFT). The serum albumin level in the case group (3.3 g/dL, range: 2.3 - 5) was markedly lower than that of the control group (3.9 g/dL, range: 3.5 - 5.3, P < 0.0001). The creatinine levels in the case group (0.44 mg/dL, range: 0.1 - 0.7) were substantially lower than those in the control group (0.59 mg/dL, range: 0.2 - 1.1, P = 0.0324).serum selenium binding protein values of the case group (223.23 ng/dL, range: 62.41 - 661.4) were substantially inferior to those of the control group (269.69 ng/dL, range: 123.05 - 1553.74, P = 0.0203). The blood zinc levels in the case group (73.4 µg/dL, range: 29.3 - 147.31) were substantially lower than those in the control group (89.9 µg /dL, range: 55.6 - 183.01, P = 0.0485) **(Table 2).**

Table (2): Comparison between cases and controls regarding lab data

	Cases (N = 35)	**Control (N = 35)**	**P. Value**
CBC			
Hb (g/dL)	8.47 ± 0.9	9.45 ± 1.62	0.0033*
WBC (×10^3/µL)	13.03 ± 3.98	6.93 ± 1.62	<0.0001*
PLTs (×10^3/µL)	315 (143 - 618)	274 (147 - 446)	0.2772
Lymph (×10^3/µL)	5 (1 - 67)	5.2 (1 - 8.6)	0.7642
Neutrophil percentage (%)	48 (28 - 59)	53 (39 - 60)	0.0026*
Neutrophil count (×10^3/µL)	1.2 (0.1 - 6.8)	1 (0 - 5.5)	0.4987
LFT			
AST (U/L)	34 (11 - 333)	24 (12 - 55)	0.0024*
ALT (U/L)	39 (8 - 359)	46 (29 - 83)	0.0658
Total serum bilirubin (mg/dL)	0.88 (0.25 - 1.66)	0.68 (0.33 - 1.14)	0.3749
Direct serum bilirubin (mg/dL)	0.22 (0.03 - 0.3)	0.18 (0.09 - 0.29)	0.8599
Albumin (g/dL)	3.3 (2.3 - 5)	3.9 (3.5 - 5.3)	<0.0001*
KFT			
BUN(mg/dL)	11 (3.5 - 18)	11.7 (4 - 18)	0.3618
Urea (mg/dL)	13 (0.3 - 38)	14 (6 - 21)	0.526
Creatinine (mg/dL)	0.44 (0.1 - 0.7)	0.59 (0.2 - 1.1)	0.0324*
Serum Zinc (µg/dL)	73.4 (29.3 - 147.31)	89.9 (55.6 - 183.01)	0.0485*
Serum Selenium BP (ng/dL)	223.23 (62.41 - 661.4)	269.69 (123.05 - 1553.74)	0.0203*

CBC: Complete Blood Count, Hb: Hemoglobin, WBC: White Blood Cell count, PLTs: Platelets, Lymph: Lymphocytes, LFT: Liver Function Test, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, KFT: Kidney Function Test, BUN: Blood Urea Nitrogen, BP: Binding Protein.

When comparing the case group to the control group, the C-reactive protein (CRP) levels in the cases were considerably higher (27.48 mg/L, range: 25 - 30.81 mg/L) with a p-value of less than 0.0001. In terms of microbiological cultures, the case group had a significantly higher prevalence of G-negative bacilli in blood (22.86%) than the control group (0%, p = 0.005). Furthermore, Pseudomonas Aeruginosa (20%) and Streptococcus (20%) were found in sputum at substantially greater frequency in the case group compared to the control group (both P = 0.0112) **(Table 3).**

Table (3): Comparison between cases and controls regarding microbiological evaluation

	Cases (N = 35)	Control (N = 35)	P. Value
Immunology			
CRP (mg/L)	27.48 (25 - 30.81)	3.1 (0.3 - 6.2)	<0.0001*
Cultures			
Blood			
G-ve Bacilli	8 (22.86%)	0	0.005
Sputum			
Pseudomonas Aeruginosa	7 (20%)	0	0.0112
Strept	7 (20%)	٠	0.0112

CRP: C-reactive protein, G -ve: gram negative.

Serum zinc levels showed strong positive correlation with height (r = 0.351, p = 0.0384), arm circumference (r = 0.463, p = 0.0051), and head circumference (r = 0.475, p = 0.0040) among CP cases. A negative correlation of -0.375 and a p-value of 0.0266 was seen with CRP, however. Blood levels of selenium BP showed a strong negative relationship with C-reactive protein (r = -0.78, p <0.0001) **(Table 4)**.

Table (4): Correlation Analysis between Serum Selenium BP and Serum Zinc with anthropometric measurements and CRP Among CP cases

	Serum Selenium BP		**Serum Zinc**	
	r	**p-value**	**r**	**p-value**
Anthropometric measurements				
Weight	0.077	0.6619	0.306	0.0740
Height	0.081	0.6433	0.351	**0.0384***
Arm Circumference	0.080	0.6479	0.463	**0.0051***
Head Circumference	0.077	0.6603	0.475	**0.0040***
CRP	-0.780	**<0.0001***	-0.375	**0.0266***

In our pediatric subjects serum zinc with a cutoff value of <67.44 µg/dL could be used to detect cerebral palsy associated with malnutrition with an AUC of 0.362, with sensitivity and specificity of 57.14% and 14.29%, respectively. The positive predictive value (PPV) and negative predictive value (NPV) were 40% and 25%, respectively, with an overall accuracy of 35.71% (p = 0.0478). For serum selenium BP with cutoff value <191.08 ng/dL, it may be utilized to identify cerebral palsy linked to malnutrition, exhibiting an AUC of 0.3384, with sensitivity and specificity of 68.57% and 20%, respectively. The PPV was 46.15%, the NPV was 38.89%, and the overall accuracy was 44.29% (p = 0.02) **(Table 5).**

Table (5): ROC curve analysis between Serum Zinc and Selenium BP with CP associated with malnutrition in our pediatric patients:

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P. Value
Serum Zinc (µg/dL)	<67.44	0.362	57.14	14.29	40	25	35.71	**0.0478***
Serum Selenium BP (ng/dL)	<191.08	0.3384	68.57	20	46.15	38.89	44.29	**0.02***

AUC: Area Under the Curve, PPV: Positive Predictive Value, NPV: Negative Predictive Value.

Discussion

According to the American Society for Parenteral and Enteral Nutrition (ASPEN), a child is considered to be suffering from pediatric malnutrition if their dietary intake falls short of their nutritional needs, leading to a slow but steady loss of calories, protein, or micronutrients. Numerous health outcomes, including growth and development, might be negatively impacted by this imbalance **[9].**

Zinc, the second most prevalent trace element in the body following iron, is essential for various cellular functions, including protein synthesis, nucleic acid metabolism, gene transcription, cell proliferation, differentiation, and mitosis. It is integral to the function of over 300 enzymes and 1000 transcription factors **[10].** Selenium, incorporated into various proteins as amino acids, protects cells and tissues from oxidative damage and supports immune system function. It also mitigates heavy metal toxicity, contributes to thyroid hormone synthesis, and is essential for nucleotide synthesis, growth, and reproduction **[11].**

In our study age and sex distribution were not significantly different (P = 0.9636; P = 0.6268). Early malnutrition is often caused by motor deficits that limit oral intake. Severely impacted CP patients may have significantly more feeding issues, requiring earlier medical intervention and study participation **[12, 13].**

Our findings matched **Schoendorfer et al. [14],** who evaluated micronutrient balance in CP children, filling a gap in the literature on undernutrition in this population. **Schoendorfer et al.** conducted a research with 21 children aged 4 to 12 years with severe cerebral palsy (CP), comprising 12 who were fed orally (O) and 9 who received enteral nutrition by percutaneous endoscopic gastrostomy (PEG). The study also incorporated a control group of 16 age-matched typically developing children (C). The main aim was to evaluate the concentrations of protein, energy, minerals, and trace elements between children with severe cerebral palsy receiving either oral or enteral nutrition and a typically developing control group.

In our study, the case group had reduced anthropometric measurements, including weight (P=0.0001), height (P<0.0001), arm circumference (P<0.0001), and head circumference (P<0.0001). Due to motor deficits, eating and nutrient absorption are problematic in children with CP, limiting caloric intake and growth **[13].** In CP, brain damage may impair growth regulation, stunting physical development. The lack of significant family history suggests that physical and metabolic obstacles rather than genetic variables are responsible for developmental deficiencies **[15-17].**

Our study matched **Carman et al. [3]** findings on CP children dietary and micronutrient levels. All anthropometric values were lower in the CP patients compared to normal values detected in the controls. Based on CDC growth charts, 92.6% of CP children were malnourished, with 25% having severe (3rd degree) malnutrition. Waterlow Classification (Wasting, Weight-for-Height, WFH) showed chronic malnutrition in 71.3% of CP patients, while stunting classification (Height-for-Age, HFA) showed it in 93.7%, a significant difference between cases and controls.

Sharawat et al. [18] examined the prevalence, severity, etiology, and effects of malnutrition on the

quality of life in children with cerebral palsy (CP). The study comprised 569 children diagnosed with cerebral palsy, with a mean age of 5.4 ± 2.8 years, of whom 74% were male. The findings indicated that 71% of the subjects were underweight, 44% were wasted, and 72% were stunted. Severe malnutrition was identified in 22% of underweight children, 11% of wasting children, and 21% of stunted children.

We found that cases had significantly lower hemoglobin (P=0.0033) and neutrophil percentage (P=0.0026) but higher WBC counts (P<0.0001) compared to controls. The lower hemoglobin levels observed in cases are likely due to iron and micronutrient deficiencies, exacerbated by inflammation impairing erythropoiesis, consistent with anemia of chronic disease **[19-21].** The elevated white blood cell counts may reflect chronic low-grade inflammation or recurrent infections, commonly seen in CP. The reduced neutrophil percentages might be attributed to immune regulation disturbances caused by malnutrition and deficiencies in essential micronutrients like zinc and selenium, critical for neutrophil function. These findings underscore the systemic impact of malnutrition and inflammation in CP patients **[22, 23].**

In alignment with our study findings, **Khandelwal et al. [24]** examined the hematological and biochemical indicators in children diagnosed with cerebral palsy (CP). Their analysis indicated an average hemoglobin content of 11.48 g/dL, a platelet count of 301.24×10□/L, and a white blood cell count of 11.13×10□/L. A greater prevalence of anemia was seen in male children under nine years of age. Of the 282 CP patients in the research, 14 (4.96%) exhibited a platelet count < 150×10□/L.

In our study, cases had significantly higher AST levels (P=0.0024) and lower albumin levels (P=0.0446). CP feeding problems may cause liver stress and nutritional deficits, causing elevated AST and low albumin. AST increase may be caused by motor impairment-related muscle breakdown **[24],** while low albumin may be caused by protein deficiency **[25].**

Our study findings indicate that serum creatinine levels were markedly lower in cases than in controls (P = 0.0324). Nonetheless, no notable change was detected in blood urea nitrogen (BUN) levels across the groups. Lower serum creatinine levels in pediatric cases may be due to reduced muscle mass, as creatinine is a byproduct of muscle metabolism. Additionally, malnutrition may impair kidney function and alter metabolic processes, further contributing to decreased creatinine production yet the underlying mechanism isn't fully understood.

In agreement with our study, **Gök Dağıdır et al. [26]** reported even lower mean serum creatinine levels in the CP group (0.3022 ± 0.09242 mg/dL) than in the control group (0.4028 ± 0.19959 mg/dL, P = 0.030).

Among our study patients, serum zinc levels were significantly lower in cases (P=0.0485), as were serum selenium BP levels (P=0.0203). Depleted trace elements in CP may be attributed to reduced nutritional absorption and elevated oxidative stress **[27, 28].**

Malnourished CP children had decreased zinc and selenium BP levels before nutritional rehabilitation, with 90% and 100% deficient, respectively, according to **Bebars et al. [2]. Tinkov et al. [29]** found significantly decreased serum zinc and copper levels in CP children, with age affecting trace element levels.

Our cases had significantly higher CRP levels (P=0.0001) and increased frequencies of gram-negative bacilli (P=0.012), Pseudomonas aeruginosa (P=0.041), and Streptococcus species (P=0.0241) in cultures compared to controls.

In line with our study, **Hanna et al. [30]** found elevated ESR, CRP, and TNF-α levels in children with cerebral palsy (CP), supporting the role of inflammation in CP.

In our CP patients, serum zinc positively correlated with height, arm circumference, and head circumference, but negatively with CRP. Serum

selenium BP negatively correlated with CRP significantly.

Kulathinal et al. [31] posited that anthropometric measurements may indicate nutritional status associated with particular biochemical markers, highlighting waist circumference, mid-upper arm circumference (MUAC), weight, and handgrip strength as significant nutritional indicators. **Mbunga et al. [32]** reported a high prevalence of serum zinc (64.6%) and selenium (84.1%) deficiencies in children, with inflammation influencing trace element concentrations. Serum selenium levels were positively associated with linear growth.

In our study, for serum zinc, a cutoff of <67.44 µg/dL resulted in an AUC of 0.362, sensitivity of 57.14%, specificity of 14.29%, PPV of 40%, NPV of 25%, and overall accuracy of 35.71% (p = 0.0478). For serum selenium BP levels below 191.08 ng/dL were associated with an AUC of 0.3384, with sensitivity of 68.57%, specificity of 20%, PPV of 46.15%, NPV of 38.89%, and overall accuracy of 44.29% (p = 0.02). Despite statistical significance, the low AUC values indicate limited discriminative ability for identifying malnutrition-associated CP. The low AUCs for both markers may be attributed to the complex interplay of chronic inflammation and other factors influencing micronutrient levels, which limit their specificity and sensitivity in identifying malnutrition **[14, 29, 33].**

The study limited sample size, single-hospital location, and cross-sectional design limit generalizability and causation. Diet, socioeconomic status, and nutritional inadequacies were not properly assessed. Malnutrition, trace elements, and health outcomes in pediatric CP patients need to be studied using bigger, more diversified samples and longitudinal follow-up.

Conclusion

The study found that malnourished pediatric cerebral palsy (CP) patients have significantly lower serum levels of selenium BP and zinc, along with growth deficits in weight, height, and head circumference. These deficiencies might be linked to weakened immune function and higher infection risk, as indicated by CRP levels and bacterial presence. The study emphasizes the importance of targeted nutritional interventions to address these trace element deficiencies, potentially improving health and reducing secondary complications in CP patients.

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