RESEARCH ARTICLE

Association between serum iron deficiency and motor development in the children of the Santal tribe of West Bengal, India

Sutanu Dutta Chowdhury*

ABSTRACT

Background: Low serum iron levels may be associated with the poor motor proficiency of chronically undernourished Santal children of West Bengal. This study aimed to investigate the relationship between iron status and motor function in these children. *Methods:* A total of 170 randomly selected children aged 5 to 12 years (52.8% boys and 47.1% girls) of the Purulia district of West Bengal were assessed for their iron status, including haemoglobin concentration, serum iron, transferrin, ferritin, transferrin saturation, and total iron binding capacity. Their motor development was assessed using the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2). *Results:* The results showed that the concentration of hemoglobin, serum iron, transferrin saturation, and serum ferritin levels in children who scored above -1 z-score on the BOT-2 were significantly higher ($p < 0.05$) than those in children who scored below -1 z-score. Notably, a considerable number of both boys and girls (66.6%) in the well-below-average category on the BOT-2 exhibited Stage III iron depletion. Furthermore, serum ferritin levels were significantly associated ($p < 0.001$) with total BOT-2 scores for all children. *Conclusion:* These findings indicate that iron depletion is linked to the severity of motor impairment, suggesting that serum ferritin may serve as a sensitive biomarker for predicting motor proficiency.

Keywords: Motor development, Iron status, Iron depletion, Serum ferritin, Transferrin saturation, Santal.

Indian Journal of Physiology and Allied Sciences (2026); DOI: 10.55184/ijpas.v78i02.543 **ISSN:** 0367-8350 (Print)

INTRODUCTION

The Santals are found to be the largest tribal group in the eastern part of India, making up approximately more than 52% of the total tribal population in West Bengal[1]. They primarily inhabit forested and remote areas, facing challenges related to poverty and nutritional deficiencies. Over 80% of Santals are unskilled workers[1]. Earlier studies indicated that a significant percentage of the Santal children suffer from undernutrition[2]. Research measuring the development of motor function in Santal children showed that they have significantly poor motor skills as evaluated using the BOT-2 (Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition). This poor motor competence is linked to inadequate nutrition and lower socioeconomic status[3]. In Purulia district, the nutritional status of Santal children was measured by examining their dietary intake and certain biochemical parameters[4]. The results revealed that a considerable percentage (29%) of Santal children were experiencing from iron deficiency and that their nutritional intake fell below the recommendations set by the Indian Council of Medical Research[4].

Iron deficiency has been a significant nutritional issue in India and the primary reason for iron-deficiency anaemia in infants and young children. It is well-established that iron is essential for the structural as well as functional growth of the brain. Haemoglobin and cytochromes, both of which are iron-containing heme proteins, play crucial roles in tissue oxygen delivery and energy metabolism[5]. Iron and iron-containing enzymes in the brain are vital for glial and neuronal energy metabolism, neurotransmission and myelin synthesis[6,7]. In India, iron deficiency among children is a major nutritional challenge, often attributed to parasitic infections and inadequate nutrient intake[8,9].

The connection between iron status and motor development in Santal children has not been previously explored. Therefore, the present study examined the link between motor proficiency and iron status and the relationship between various phases of iron depletion and motor proficiency in Santal children residing in the Purulia district of West Bengal, India.

METHODS

Study participants

A total of 170 Santal Children, comprising 52.9% boys and 47.1% girls aged 5 to 12 years, were randomly selected from six schools in the Bagmundi and Balarampur areas of

Department of Physiology, Basirhat College, Basirhat, North 24 Parganas – 743412

***Corresponding author:** Sutanu Dutta Chowdhury, Department of Physiology, Basirhat College, Basirhat, North 24 Parganas – 743412, Email: e-mail: sutanu_78@yahoo.com

How to cite this article: Chowdhury SD. Association between serum iron deficiency and motor development in the children of the Santal tribe of West Bengal, India. *Indian J Physiol Allied Sci* 2026;78(2):9-13.

Conflict of interest: None

Submitted: 15/05/2026 **Accepted:** 25/06/2026 **Published:** 30/06/2026

the Purulia district of West Bengal. Following established inclusion and exclusion criteria, an average of 6 to 8 students were randomly chosen from each of the four classes in each school. Data was collected from the children present on the specific day following the necessary approval from the school authorities and their parents. Children who had systemic diseases, infections, or had undergone major surgical operations that could affect their motor performance or biochemical parameters were excluded from the study. The protocol and procedures were approved by human ethical guidelines set forth by the Human Ethical Committee of our institution (Ref. No. WBSU/IHEC/07/2024/11 dated 28.07.2024).

Biochemical estimations

A 5 mL venous blood sample was collected from each child after a 12-hour overnight fast. The samples were placed in vacutainers without anticoagulant for serum separation. They were kept at room temperature for 30 minutes and then incubated at 37˚C for an additional 30 minutes. The serum was separated by centrifugation and a 1-mL aliquot was stored in Eppendorf tubes at -80˚ C for biochemical assessments.

The haemoglobin concentration was assessed using the cyanmethaemoglobin method[10]. Total iron binding capacity (TIBC) and serum iron levels were measured using IRT011 and IRT010 kits (Crest Biosystems, Berlin, Germany) utilizing the ferrozine method[11] in a BS-300 fully automated analyser (Mindray, New Jersey, USA). Serum ferritin was determined using the DiaMetra DK0039 kit (DiaMetra, Perugia, Italy) through an immunoenzymatic determination method[12] in a fully automated Alpha Prime analyser (SFRI Laboratories, Berganton, France). The transferrin level was calculated indirectly by multiplying TIBC by 0.7[13]. Transferrin saturation (TS%) was calculated by dividing serum iron by TIBC and then multiplying by 100[14].

Stages of iron depletion`

The stages of iron depletion were assessed using the method outlined by Lee and Nieman[14]. Stage I iron depletion, also referred to as iron-deficient stores, is characterized by a serum ferritin level of less than 12 µg/L. In stage II, or iron deficiency erythropoiesis, a transferrin saturation level falls below 15%, accompanied by a serum ferritin level of less than 12 µg/L. Stage III, or iron-deficient anaemia, is characterized by a hemoglobin level below 12 g/dL, transferrin saturation level below 15% and serum ferritin level below 12 µg/L.

Motor Competence

The Bruininks-Osretsky Test of Motor Proficiency, second edition (BOT-2) was utilized to assess children's motor abilities. The BOT-2 is a standardized assessment that evaluates motor skill achievement and is commonly used to measure motor abilities in children[15]. The short form of the test has been validated against the full version and includes 14 items taken from eight subtests. The eight subtests evaluate various aspects of gross and fine motor development, including running speed and agility, balance, bilateral competence, strength, upper limb competence, response speed, visuomotor control, and upper limb speed and dexterity. A total standard score, adjusted for the child's age and sex, is used to interpret motor competence. BOT-2 scores are classified into five categories based on z-score values: well above average (z-score > 2), above average (z-score between +1.99 and +1), average (z-score between +0.99 and -1), below average (z-score between -1 and -1.99), and well below average (z-score <-2).

Statistical analysis

Descriptive statistics for various biochemical parameters and total BOT-2 scores were reported as means along with the standard error of the mean. A one-way analysis of variance was employed to examine differences in biochemical parameters across different BOT-2 z-score categories as well as differences in total BOT-2 standard scores among various stages of iron depletion. *Post-hoc* comparisons were carried out using the Bonferroni test, with a significance level (alpha) set at 0.05. Additionally, a Student's t-test was conducted to identify differences in biochemical parameters between two groups: those with total BOT-2 scores < -1 z-score and those with scores > -1 z-score. Chi-square tests were utilized to evaluate the association between different BOT-2 z-score categories and stages of iron depletion. Furthermore, multiple forward regression analysis was performed to investigate the relationship between total BOT-2 scores and biochemical parameters across five incremental models, treating the total BOT-2 standard score as the outcome variable. All statistical analyses were conducted using SPSS 23.0 for Windows (SPSS Inc, Chicago, USA).

RESULTS

The mean concentration of haemoglobin, serum iron, serum ferritin, TIBC, serum transferrin and transferrin saturation were compared between both sexes as shown in Figures 1 and 2. Children with total BOT-2 z-scores below the -1 demonstrated significantly lower value for haemoglobin concentration (p <0.05 for both sexes), serum iron (p <0.001 for boys and p <0.01 for girls), serum ferritin (p <0.001 for boys and p <0.01 for girls) and transferrin saturation (p <0.001 for boys and p <0.01 for girls). Additionally, serum transferrin levels were significantly higher in girls with total BOT-2 z-scores below -1 (p <0.05), and TIBC was also significantly higher in this group (p <0.05).

The haemoglobin concentration, serum ferritin and TS% levels in boys classified as well-below average were significantly lower than those in the below average (p <0.05) and average (p <0.05) categories of the BOT-2 z-score (Figure 3). Similarly, girls in well-below average demonstrated significantly lower haemoglobin concentration (p <0.05), serum ferritin levels (p <0.001) and transferrin saturation levels (p<0.05) compared to the below average category of the BOT-2 z-score (Figure 3). Figure 4 illustrated that TIBC and serum transferrin levels were significantly higher in boys identified as well-below average

category (p <0.05 for TIBC and p <0.01 for serum transferrin when comparing average categories, and p <0.05 for below average categories) compared to those in than average and below average BOT-2 z-score categories. Similarly, for girls in the well-below average group, TIBC and serum transferrin levels were also significantly higher (p <0.05) than those in the below average BOT-2 z-score category. Additionally, serum iron levels for Santal children of the well-below average category were significantly lower (p <0.01) than those in the average category. For both boys and girls, serum iron levels in the well-below average group were significantly lower than in the below average (p <0.01 for boys and p <0.05 for girls) BOT-2 z-score category.

According to the BOT-2 z-score categories based on total BOT-2 scores, 55, 50, and 66.66% of boys (as shown in Table 1) with stage I, II, and III iron depletion were classified as well below average, respectively. Similarly, 75, 75, and 66.66% of girls (as indicated in Table 2) with the same stages of iron depletion fell into this category. The total BOT-2 scores for children with stage II and III iron depletion were significantly lower than those of children with normal iron levels (p < 0.01 for boys, p < 0.001 for girls, and p < 0.001 for the total cohort). Additionally, the scores for stage I iron depletion were also significantly lower than those of children with normal iron levels (p < 0.05 for boys, p < 0.01 for girls, and p < 0.001 for the entire group). This data is illustrated in Figure 5.

Stepwise regression analysis indicated a significant association (p <0.001) between serum ferritin levels and total BOT-2 scores for all children (Table 3).

DISCUSSION

The present study suggests that poor iron status among the surveyed children is linked to their motor functions. There was a significant difference in serum ferritin levels and TS% among children categorized as below average and well-below average in motor development. The serum ferritin has been found to be a strong predictor of motor proficiency.

Numerous authors have reported conflicting results regarding the impact of serum iron levels on motor development. Few studies have directly investigated the connection between physical and mental development and iron deficiency in infants and young children[7,16]. Some studies indicate that the structural and functional development of the brain may be delayed or altered by iron deficiency[16,17], potentially leading to poor neuromaturation and delayed neurodevelopment[18,19]. Indeed, iron-deficiency anemia has been linked to poor motor development in both infancy and childhood[18-20]. Longitudinal studies have consistently established that the children who were anemic during their first two years of life due to low serum iron experienced ongoing deficits in the development of motor functions throughout childhood and adolescence[21]. However, some researchers have failed to establish a clear association between deficiency of iron and motor development[22,23]. Surkan *zzzzzzzzzz*. (2013) showed that treatment of iron-deficient infants didn't recover their motor development[24]. Additionally, other studies have produced mixed findings regarding the association between iron supplementation and motor development[25,26].

The findings of the present study indicate the association of poor iron status of the surveyed children with their motor functions. The connection is evident in the differences in iron status parameters between children who have BOT-2 scores above -1 z-score and those below it. A significant percentage of children in stages II and III of iron depletion demonstrated below-average BOT-2 z-scores. Those in well-below average category increased as the BOT-2 z-scores decreased. Recent research highlights various effects of iron deficiency (ID) on the central nervous system (CNS), which may help clarify our findings. Iron plays a crucial role in myelin production, and a lack of iron can hinder this process[27,28]. Low iron levels likely disrupted or delayed the myelination process in the corticospinal tract, which in turn affected the normal development and refinement of motor skills. Myelination process of the pyramidal tract is only incomplete at birth and continues for many years[29]. This myelination process during early childhood increases conduction velocity along the tract, ultimately enhancing motor performance and aiding in the development of motor skills to adult-like levels[30].

One potential clarification for the long-standing effects of iron deficiency (ID) on motor function is related to diminished dopamine function, particularly in the striatum. Severe dietary iron restriction can lead to reduced brain iron concentration, which changes the dopamine transporter as well as the density of both D1 and D2 dopamine receptors in the striatum. These changes can persist into adulthood, even after iron levels are restored[28,31]. Long-term potentiation (LTP) and long-term depression (LTD) in the striatum, facilitated by the supportive action of D1 and D2 receptors, are believed to play a significant role in regulating motor learning[32]. Thus, the enduring alterations in the densities of D1 and D2 receptors could disrupt the process of motor development in children suffering from iron deficiency. In addition, dopaminergic neurotransmission is crucial for the motor cortico-basal ganglia–thalamocortical loop, which encompasses two pathways in the basal ganglia: the direct and indirect pathways. Normal motor behavior relies on a balance between the outputs of these two pathways, which together facilitate desired movements while inhibiting potentially competing ones[21]. Therefore, the persistent changes in D1 and D2 receptors due to iron deficiency in children may disrupt this subtle balance, ultimately affecting their motor function.

The significant difference in serum ferritin levels and TS% of below-average and well-below average categories of children suggests that poor motor development is related to insufficient iron for erythropoiesis (the production of red blood cells) and body iron storage. Iron is a crucial constituent in several enzymes involved in vital oxidation-reduction reactions, as well as in the synthesis and

breakdown of neurotransmitters[31]. Iron deficiency anemia may contribute to poor motor development, as indicated by lower hemoglobin concentrations in the well-below average category. Additionally, poor motor development has been linked to higher levels of iron transport proteins like serum transferrin and TIBC. When iron levels are low, transferrin's binding affinity for iron increases, as observed in this study through elevated serum transferrin concentrations. TIBC is a measure of transferrin that does not change rapidly with fluctuations in the concentration of serum iron, reflecting the iron level until iron storage is fully depleted.

When examining total BOT-2 scores and sex differences, it was found that children having lower stages of iron depletion (specifically stages II and III) had lower total BOT-2 scores compared to children with regular iron levels. This implies a connection between lower iron levels and diminished motor performance. The regression analyses revealed that serum ferritin is a strong predictor of motor proficiency, as it exhibited a significant association with total BOT-2 scores. Previous studies have indicated that other parameters of iron status, such as serum transferrin, serum iron, TIBC, and TS, are also related to motor function development. However, their relationship with total BOT-2 scores appeared to be much weaker, as their inclusion in regression models did not demonstrate a significant impact on motor function.

A significant portion of surveyed children exhibited stage III iron depletion and poorer BOT-2 scores. Earlier research indicated that these individuals often faced challenging socioeconomic conditions[2,33], which could be a contributing factor to their poor dietary choices and insufficient iron intake. Furthermore, support for iron-deficient children may be lacking, and these circumstances could negatively impact their motor proficiency.

One limitation of the present study is the small number of subjects, which restricts the generalization of the findings to a larger population. Similarly, the impact of potential confounders like socioeconomic status has not been investigated in this study. Additionally, we could not determine the exact age or duration of the onset of motor disability (MD) in our participants. Furthermore, as an observational study, we cannot definitively establish that MD caused the observed effects, despite controlling for a comprehensive range of background factors. Future studies must focus on establishing causation, as well as investigating the period of MD onset and age. These studies should use a diverse strategy, such as randomized controlled trials aimed at averting MD in children. The use of various tests for assessing motor development among the children surveyed also posed challenges for analysis and interpretation. By exploring these avenues, we can gain an improved understanding of the implications of iron status on motor skills and potentially develop targeted interventions to support the developmental needs of children, particularly in vulnerable populations like the Santal tribe. Lastly, the study can be conducted with the full version of BOT-2 instead of the BOT-2 short form to avoid the limitations of the short form. An intervention program may be initiated by focusing on early detection and treatment of iron deficiency in surveyed children, along with nutritional interventions and educational initiatives. This includes promoting iron-rich diets, food fortification, and addressing maternal iron deficiency to prevent low birth weight and ensure adequate iron endowment for infants.

CONCLUSION

In this cohort, higher levels of iron depletion were linked to increased severity of motor impairment. Serum ferritin, transferrin, and hemoglobin were used to recognize iron shortage, with serum ferritin being a strong predictor of motor function.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.