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Diagnostic and Etiological Role of Zinc Insufficiency in Children with Iron Deficiency Anemia from Sukkur, Sindh, Pakistan

Rafique Abbas Shar[1]*, Shaista Khan[2], Gul Afshan Soomro[3], Sanaullah Abbasi[4]

[1,2,4] Department of Biochemistry, Shah Abdul Latif University, Khairpur, Sindh, Pakistan
[3] Institute of Chemistry, Shah Abdul Latif University, Khairpur, Sindh, Pakistan
Corresponding Author's E-mail*: rafiqueabbasshar@gmail.com

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Abstract
Iron deficiency anemia (IDA) still represents one of the significant public health problems among children living in developing countries. Coexistent zinc deficiency may occur together with iron deficiency and possibly interfere with erythropoiesis, immunity, growth, and recovery of hematological profile. To measure serum zinc status in children with IDA and to explore its correlation with ferritin and hematological parameters. The present cross-sectional comparative study involved 150 children with IDA and 150 healthy controls aged 1–15 years selected from Civil Hospital/Ghulam Muhammad Mahar Medical College Teaching Hospital, Sukkur, Pakistan. Hematological parameters and ferritin were determined on an automated analyzer, whereas serum zinc was analyzed using 5-Br-PAPS method with ADVIA 1800 Chemistry analyzer. Mean serum zinc level was significantly lower in children with IDA than in control children (79.20 ± 0.73 vs. 112.17 ± 0.48 microgram per deciliter; $p<0.001$). Zinc deficiency was noted in each of the studied age-sex groups. Serum zinc had positive correlations with hemoglobin ($r = 0.51$), hematocrit ($r = 0.42$), mean corpuscular volume ($r = 0.40$), and ferritin ($r = 0.41$). Multivariate linear regression revealed independent contributions of zinc and ferritin levels as predictors of hemoglobin, accounting for 78% of its variance. There was a clear evidence of severe zinc deficiency among our study subjects suffering from pediatric IDA, which supports the notion of multifactorial nature of IDA. Zinc status evaluation during routine hematological assessment of children with anemia deserves attention as a potential source of valuable information.

Keywords: Zinc deficiency; Iron deficiency anemia; Children; Ferritin; Hemoglobin; Micronutrients

Introduction

Anemia constitutes a very common nutritional problem among people around the world, occurring in numerous cases of children and leading to adverse consequences in terms of growth and cognitive development, increased morbidity, and mortality (McLean et al., 2009; Stevens et al., 2013; Kassebaum et al., 2014; World Health Organization, 2008). The global prevalence of anemia is especially high in children in the low- and middle-income countries as a result of nutrition deficiency, recurrent illnesses, lack of dietary diversity, and poor socioeconomic situation (Black et al., 2013; Soofi et al., 2021; Habib et al., 2023). The problem of childhood anemia remains topical for Pakistan, particularly in tertiary healthcare settings of children admitted from poor communities. Iron deficiency anemia constitutes the most common type of childhood anemia, being caused by insufficient dietary iron, inadequate iron absorption, and deficiency of iron reserves that become critical when there is active hematopoiesis in children with growth spurt (Killip et al., 2007; DeLoughery, 2017; Cappellini et al., 2020). IDA is not merely a laboratory condition characterized by low hemoglobin level and absence of iron stores; additionally, it is accompanied by various clinical manifestations, including immune suppression, delayed cognitive function, decreased intelligence, poor school

achievements, fatigue, and restricted growth (Grantham-McGregor & Ani, 2001; Beard, 2001; Lozoff et al., 2006; Georgieff, 2011).

The importance of this complication is increased by the fact that early nutrition disorders have long-lasting impact on child health even after normalization of hemoglobin level. While IDA treatment is usually focused on iron, recent studies point out to the multi-factorial nature of anemia in children with malnutrition due to involvement of other nutrients essential for erythropoiesis, nucleic acid synthesis, immunological regulation, cell proliferation, and antioxidative defense, which affect hemoglobin concentration and hematologic indices. One of the nutrients with particular relevance is zinc, which acts as a cofactor in many enzymes and transcriptional regulators (Vallee & Falchuk, 1993; Shankar & Prasad, 1998; McCall et al., 2000). Zinc deficiency can impair erythropoietic and immune responses and slow down hematological recovery in IDA (Prasad, 2003; Hambidge & Krebs, 2007; Kim et al., 2023). Coincident presence of iron and zinc deficiencies can be explained by identical risk factors influencing their dietary intake, absorption, and metabolic processes. Iron and zinc deficiencies have common dietary sources and share pathways of absorption, which include metal ion transporters and channels such as divalent metal transporters (Gunshin et al., 1997; Sandström, 2001; Kondaiah et al., 2019). In the context of poor diet quality, recurrent infections, and malnutrition in children who rarely consume animal products, concomitant iron and zinc deficiency appears plausible (Allen, 2008; Bhatnagar & Taneja, 2001). Nevertheless, despite obvious interrelationship of iron and zinc, zinc status determination in routine diagnostics of IDA is not widely practiced. While routine diagnosis of anemia in children includes CBC, erythrocyte indices, and ferritin, zinc level is less commonly assessed. This omission may have a serious impact since simultaneous deficiency of iron and zinc leads to further deterioration of hematological and immunological status and poor response to single-nutrient therapy. Moreover, local literature from Sukkur and neighboring regions provides insufficient information regarding relationships between serum zinc and ferritin and hemoglobin level in children with IDA. Therefore, the present study aimed to evaluate serum zinc status in children with IDA and healthy children from Sukkur, Sindh, Pakistan and to investigate the diagnostic and pathogenic role of zinc deficiency relative to ferritin and hematological parameters in this population group. It can contribute to better understanding of the complex nature of IDA and improve the accuracy of clinical assessment of patients with anemia.

Methods

Study design and setting

The present comparative cross-sectional study took place in the Department of Pediatrics Ghulam Muhammad Mahar Medical College Teaching Hospital, Sukkur, Pakistan. The study period was set up to fall within January 2025-December 2025, as required by IRB approval. The aim of the study was to compare serum zinc status and other hematological variables in IDA patients and healthy controls.

Ethical approval and consent

Prior to starting the study, ethical approval was provided by the IRB committee or relevant hospital authority (IRB No. DPS/SMBBMUL/862/2025; date: 10 January 2025). Informed written consent was obtained from the participants' legal representatives before admission to the study. Confidentiality of patient information was maintained during all stages of the research process.

Study population

The study recruited a total of 300 children aged 1-15 years. Half of the patients had IDA, whereas another half represented a healthy control group without anemia.

Inclusion and exclusion criteria

Cases of IDA met the inclusion criterion of age 1-15 years. Cases that would not meet the inclusion criteria include children with chronic diseases, hemoglobinopathies, renal or liver

disorders, chronic inflammation, previous blood transfusion, and zinc therapy. The inclusion criteria of controls consisted in age 1-15 years and absence of anemia.

Clinical and anthropometric assessment

Demographic, anthropometric, and clinical data were collected from the patients. The clinical examination paid special attention to signs and symptoms of anemia (e.g. pallor, lethargy, dyspnea, tachycardia, cardiac murmur, chest pain). Data about diet and nutritional status were collected using a standardized questionnaire. Nutritional status was assessed according to WHO recommendations.

Blood collection and processing

Blood samples were obtained through venipuncture in accordance with aseptic technique. Peripheral blood for hematological tests was collected in anticoagulant tubes. Blood sera were prepared for analysis of ferritin and zinc concentration and frozen at -20°C in polypropylene containers free of trace elements.

Laboratory investigations

Complete blood counts and erythrocyte indices were analyzed using automated hematology analyzers. Serum ferritin was estimated by standardized immunoassay. Serum zinc was measured by the 5-Br-PAPS colorimetric method using the ADVIA 1800 clinical chemistry analyzer (Siemens, USA) at a wavelength of 574 nm. The manuscript reported analytical validation with linearity R^2 values above 0.997, recovery of 97-98%, and relative standard deviation values below 2.5%, indicating acceptable precision and recovery for trace-element analysis.

Statistical analysis

Data were analyzed using SPSS. Continuous variables were summarized as mean +/- SEM, while categorical variables were summarized as frequencies and percentages. Student's t-test was used to compare mean values between IDA and control groups. Analysis of variance was used where group-wise comparisons were required. Pearson correlation analysis was performed to assess relationships between serum zinc and hematological or iron-status variables. Multiple linear regression was used to assess independent predictors of hemoglobin concentration. A two-tailed p-value of less than 0.001 was considered statistically significant where reported in the original manuscript.

Results

Clinical characteristics

Clinical manifestations were frequent among children with IDA. Generalized weakness was the most common symptom, followed by pallor, shortness of breath, and tachycardia. These findings indicate that children presented with both general constitutional symptoms and cardiopulmonary manifestations of anemia.

Nutritional and anthropometric status

Anthropometric assessment showed that mean weight-for-age and height-for-age Z-scores were below WHO reference expectations in both genders. A higher proportion of underweight and stunting was observed among female children in the submitted manuscript, suggesting that IDA in this population existed in a broader background of undernutrition.

Hematological indices and ferritin

The pattern of iron deficiency was observed in the form of decreased hemoglobin, hematocrit, and mean corpuscular volume. An increase in severity of anemia was associated with a progressive fall in hemoglobin, hematocrit, and MCV levels. Low levels of ferritin in serum samples of severe anemia patients indicated iron depletion in the body, which is one of the key characteristics of IDA.

Serum zinc status

The serum zinc levels in children suffering from IDA were significantly lower than those in healthy subjects. Mean serum zinc level was found to be 79.20 +/- 0.73 microgram/dL in children with IDA and 112.17 +/- 0.48 microgram/dL in controls ($p < 0.001$). Deficiency of zinc in serum samples was found to exist regardless of the gender and age of participants. Therefore, zinc deficiency was not limited to any particular group.

Correlation and regression analysis

Correlations between serum zinc level and various hematological parameters were assessed using Pearson correlation test. Serum zinc levels showed a positive correlation with hemoglobin ($r = 0.51$), hematocrit ($r = 0.42$), MCV ($r = 0.40$), and ferritin ($r = 0.41$). Results of multiple linear regression showed that zinc and ferritin were independent predictors of hemoglobin concentration, accounting for 78% of hemoglobin variability.

Table 1. Major clinical manifestations among children with IDA

Clinical manifestation	Frequency (%)	Approximate number (n = 150)
Generalized weakness	95%	143
Pallor	90%	135
Shortness of breath	88%	132
Tachycardia	80%	120

Note. Frequencies were calculated from the percentages reported in the manuscript.

Table 2. Laboratory approach used for zinc and anemia assessment

Parameter	Method / instrument	Key point
Complete blood count	Automated hematology analyzer	Hemoglobin and erythrocyte indices
Serum ferritin	Standardized immunoassay	Assessment of iron stores
Serum zinc	5-Br-PAPS colorimetric method on ADVIA 1800	Wavelength 574 nm
Assay validation	Internal validation	$R^2 > 0.997$; recovery 97-98%; RSD < 2.5%

Note. IDA = iron deficiency anemia; RSD = relative standard deviation.

Table 3. Serum zinc concentration in IDA cases and healthy controls

Group	Number	Serum zinc (microgram/dL), mean +/- SEM	p-value
IDA cases	150	79.20 +/- 0.73	<0.001
Healthy controls	150	112.17 +/- 0.48	Reference

Note. SEM = standard error of the mean. Values are from the submitted manuscript.

Table 4. Correlation of serum zinc with hematological and iron-status variables

Variable correlated with serum zinc	Pearson r	Direction of association
Hemoglobin	0.51	Positive
Hematocrit	0.42	Positive
Mean corpuscular volume	0.40	Positive
Serum ferritin	0.41	Positive

Note. All correlations were reported as positive in the uploaded manuscript.

Table 5. Multiple linear regression summary for predictors of hemoglobin

Model variable	Reported finding	Interpretation
Serum zinc	Independent predictor	Zinc status contributed to hemoglobin variability
Serum ferritin	Independent predictor	Iron-store status contributed to hemoglobin variability
Overall model	$R^2 = 0.78$	Model explained 78% of hemoglobin variability

Discussion

Key findings

The current study highlights the existence of low serum zinc levels in children with IDA compared to healthy participants. The difference in serum zinc levels between the two groups was both clinically and statistically significant. These findings allow interpreting the presence of IDA in the pediatric population in Sukkur, Sindh, as a sign of general micronutrient insufficiency. Positive correlation between serum zinc and hemoglobin, hematocrit, MCV, and ferritin levels and the independent predictive power of zinc for hemoglobin provide evidence regarding the association between serum zinc level and anemia severity in children.

Biological rationale.

This study has great biological plausibility because zinc takes part in DNA synthesis, cell division, enzyme reactions, and immune responses, all of which play a role in erythropoiesis. Rapid cell division required in the process of red-blood cells formation needs adequate trace element supply. Deficiency of zinc may hinder cellular proliferation, slow down heme-dependent enzymatic reactions, and exacerbate anemia (Vallee & Falchuk, 1993; McCall et al., 2000; Kim et al., 2023). Zinc may occur with iron deficiency in children due to similar dietary patterns. Consumption of animal-based products is insufficient, whereas intake of cereal crops is excessive. Poor nutrition, socioeconomic problems, and repeated infections may further complicate trace-element homeostasis. Therefore, low levels of both zinc and ferritin found in serum samples of children with IDA can be explained by a variety of micronutrient and environmental factors.

Implications for practice

These findings have significant implications for healthcare practices. Currently, anemia in children is diagnosed and managed by assessing red-cell indices and treating patients with iron preparations alone. However, in case zinc insufficiency coexists, recovery from anemia will be hampered by zinc-related problems. For instance, poor immunity, poor growth, weak appetite, and lack of erythropoietic support will contribute to incomplete recovery. In addition, it is possible to develop a diagnostic algorithm in which serum zinc would be tested alongside CBC, red-cell indices, and serum ferritin. This approach seems especially appropriate for tertiary hospitals treating patients with multiple problems. Such a strategy can help diagnose children whose serum zinc level needs to be estimated to rule out combined anemia.

Significance for public health

Public health importance of the findings should not be underestimated because iron deficiency is currently viewed as the only reason of pediatric anemia. Nutritional programs need to pay special attention to promoting diversity of diet and encouraging parents to breastfeed and introduce complementary foods. Also, de-worming and infection prevention, as well as education about nutrition, should be encouraged in the region. Zinc-rich foods should be made affordable for families.

Comparison with prior studies

Prior studies also found the relationship between iron and zinc deficiencies, their impact on erythropoiesis, and the roles played by the former in anemia development and the latter in its treatment (Allen, 2008; Hambidge & Krebs, 2007; Kim et al., 2023). In fact, the present study adds information related to the zinc content in serum of IDA patients in Sukkur, and emphasizes the role of serum zinc status in diagnosing anemia.

Weaknesses and strengths

It is necessary to note some weaknesses of the study. It used a cross-sectional design, and therefore cannot assess causation. There was no assessment of trace-element intake through diet and measurement of inflammatory markers, which might have helped interpret the results differently. The study used serum samples collected in one tertiary hospital, and therefore its findings may not be generalized to all children in the country. Strengths of the study include the use of both IDA cases and healthy controls, assessment of multiple variables, implementation of analytical techniques, and exploration of correlations and regressions between different variables.

Conclusion

Children with IDA in Sukkur exhibited signs of zinc insufficiency in terms of serum zinc level, as compared to healthy subjects. Serum zinc was positively associated with hemoglobin, hematocrit, MCV, and ferritin. In addition, zinc was an independent predictor of hemoglobin, as was ferritin. Overall, this study shows that pediatric IDA is a condition of zinc and other micronutrient deficiencies. Testing of serum zinc in selected IDA patients may be helpful for more precise diagnosis.

Declarations

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