

Dietary Protein, Vitamin C, and Iron Intake As Predictors of Hemoglobin Levels in Children With Special Needs”

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Abstract.

Hemoglobin (Hb) is a key indicator of oxygen transport and overall child health, particularly during periods of rapid growth and cognitive development. Anemia remains a significant global public health problem, often driven by nutritional deficiencies, especially iron. Vitamin C enhances iron absorption, while protein supports hemoglobin synthesis, indicating a synergistic role of these nutrients. Children with special needs are at higher risk of nutritional inadequacies due to feeding challenges and restricted diets. However, evidence on how dietary intake influences hemoglobin levels in this population is still limited. Aims: This study aimed to analyze the role of dietary protein, vitamin C, and iron intake as predictors of hemoglobin levels in children with special needs. Methods: A cross-sectional observational study was conducted among 34 students at SLB Kutoarjo between January and May 2024. Dietary intake data were collected using a 3-day food record completed by caregivers and analyzed with NutriSurvey software. Hemoglobin data were obtained from school health records. Statistical analysis included correlation and multiple regression tests to assess the relationship between nutrient intake and hemoglobin levels. Results: The findings revealed that the average intake of protein, iron, vitamin C, and total energy was below recommended levels. Despite this, the mean hemoglobin level (13.38 ± 1.33 g/dL) was within the normal range. Regression analysis showed that the combined intake of protein, iron, vitamin C, and folate explained 25% of the variation in hemoglobin levels ($R^2 = 0.25$), but the adjusted R^2 was low (0.036), indicating weak explanatory power. No individual nutrient showed a statistically significant association with hemoglobin levels ($p > 0.05$). Conclusions: Dietary protein, vitamin C, and iron intake were not significant predictors of hemoglobin levels in children with special needs. These findings highlight that hemoglobin status is influenced by complex interactions beyond dietary intake alone. Further research with larger samples and additional biomarkers is needed to better understand anemia risk in this population.

Keywords: Hemoglobin; Children with special needs and Dietary intake.

I. INTRODUCTION

Hemoglobin (Hb) is a key biomarker of oxygen transport and an essential indicator of children's health, particularly during school age when growth and cognitive development are rapid. Anemia, defined as low hemoglobin concentration, remains a major global public health issue, affecting nearly 40% of children worldwide, especially in low- and middle-income countries [1,2]. In children, anemia is associated with impaired cognitive development, reduced academic performance, weakened immunity, and decreased physical capacity, with potential long-term consequences extending into adulthood [3]. These impacts highlight the importance of identifying modifiable determinants of hemoglobin levels, particularly nutritional factors. Nutritional deficiencies are the leading cause of anemia, with iron deficiency being the most significant contributor. Iron is essential for hemoglobin synthesis as a core component of the heme molecule. However, iron metabolism is influenced not only by intake but also by bioavailability and dietary composition [4]. Vitamin C plays a critical role in enhancing non-heme iron absorption by converting ferric

iron into its more absorbable ferrous form, while protein supports hemoglobin formation by providing amino acids for globin synthesis [5,6].

These nutrients function synergistically, indicating that their combined intake may be more important than individual effects in determining hemoglobin levels. Despite their biological importance, the relationship between nutrient intake and hemoglobin levels is not always consistent. Recent studies suggest that hemoglobin is influenced by multiple factors beyond diet, including inflammation, infection, and overall health status [2]. Inflammatory processes can increase hepcidin levels, which inhibit iron absorption and utilization, thereby weakening the association between dietary intake and hemoglobin concentration [3]. Additionally, limitations in dietary assessment methods may contribute to inconsistent findings, indicating that hemoglobin status reflects a complex interaction of nutritional and non-nutritional factors. Children with special needs are particularly vulnerable to nutritional deficiencies due to feeding difficulties, restricted diets, and potential absorption issues. Conditions such as autism spectrum disorder and cerebral palsy are often associated with selective eating behaviors and limited dietary diversity, increasing the risk of inadequate intake of essential nutrients. However, research examining the relationship between nutrient intake and hemoglobin levels in this population remains limited. Therefore, this study aims to investigate the effect of protein, vitamin C, and iron intake on hemoglobin levels among children with special needs, in order to provide evidence for targeted nutritional interventions.

II. METHODS

This study is a cross-sectional study and Observasional conducted at SLB Kutoarjo. Data taken from January-May 2024. This study was conducted after obtaining Ethical clearance permits at KEPK FKIK UMY number 180/EC-KEPK FKIK UMY/V/2024

1. Respondent recap of food intake

For the recap of children's food intake, it is written by the guardian. The recap data is carried out for 3 days. After receiving instructions on how to write on the food intake recap form Data rekapan makanan di analisis menggunakan NutriSurvei.

2. Hemoglobin data

Data of hemoglobin is taken from record Children's at the Sekolah Luar Biasa Muhammadiyah Kutoarjo.

3. Data Analysis

Data presented as average \pm Stdev. Data analyze using Regression and correlation Test .

III. RESULT AND DISCUSSION

1. Sample Characteristics and Demographic Distribution

2.1 Class Distribution and Gender Composition

The strong gender imbalance is observed, particularly in Class 1 (100% male). Overall, males dominate the sample (24 males vs. 10 females), which introduces sampling bias that may affect generalizability and nutritional interpretation. Gender differences in iron metabolism and anemia risk are well documented, especially post-puberty, although less pronounced in younger children [7]. Samples consists of 34 students distributed across four classes as table 1.

Table 1. Number of student in different class

	class- 1 (N =4)		Class 2 (N =9)		class 3 (N =13)		Class 4 (N= 8)	
	male	Female	male	Female	male	Female	male	Female
	4	0	7	2	7	6	6	2
Age(y.o)	7.5 \pm 0.55		8.7 \pm 0.45		9.7 \pm 0.82		10.7 \pm 0.81	

2.2 Age Distribution

Table 1 showed that mean age increases progressively across classes. This reflects a typical developmental gradient, allowing for age-based interpretation of nutritional needs. However, the dataset does not stratify nutrient intake or Hb by age group, limiting age-specific conclusions.

3. Anthropometric and Physiological Profile

As showed in table 2. Below.

Table 2. Characteristic of students

	Mean
BMI (Kg/M ²)	17.1953±3.72665
Head circumference (Cm)	50.2982±4.14683
Systole (mm.Hg)	96.1053±14.93486
Diastole (mmHg)	67.4737±16.79701
Hemoglobin level (mg%)	13.3842±1.32760

3.1 Body Mass Index (BMI)

This value falls within the normal range for children, though the relatively large standard deviation suggests heterogeneity. Without BMI-for-age z-scores, it is difficult to classify undernutrition or overweight prevalence accurately [8]. Mean BMI was 17.20 ± 3.73 kg/m²

3.2 Head Circumference

This parameter is less commonly used in school-aged children and is more relevant in early childhood development. Its inclusion lacks clear analytical justification unless linked to neurological or developmental outcomes. Mean was 50.30 ± 4.15 cm.

3.3 Blood Pressure

These values fall within normal pediatric ranges, although the high variability suggests possible measurement inconsistency or heterogeneity in physiological status. The average of Systolic was 96.11 ± 14.93 mmHg and Diastolic was 67.47 ± 16.80 mmHg.

3.4 Hemoglobin (Hb) Levels

This indicates generally normal hemoglobin status, as WHO defines anemia in children aged 5–11 years as Hb < 11.5 g/dL [1]. However, mean values can mask subclinical deficiencies or distribution skewness. Mean Hb was 13.38 ± 1.33 g/dL.

4. Nutritional Intake Analysis.

Table 3. The average of daily intake predictor of Hemoglobin

	Average	AKG
Iron (ug)	6.5737±2.33455	8-13
VitC (mg)	31.5158±21.51226	50-75
B12 (ug)	2.0874±0.68729	24.7-47.4
Protein (gr)	48.7916±10.90467	50-70
Calori	1190.9816±196.00854	1400-2000
Folat	76.0742±29.30100	417-547

4.1 Macronutrient Intake

Protein intake is slightly below recommended levels, suggesting marginal insufficiency. Protein plays a key role in hemoglobin synthesis and iron transport [5]. Mean was 48.79 ± 10.90 g/day, meanwhile recommended was 50–70 g/day.

Total Energy showed the mean was 1191 ± 196 kcal/day, but the Recommended was 1400–2000 kcal/day. This indicates a substantial caloric deficit, potentially affecting growth and nutrient absorption.

4.2 Micronutrient Intake

Iron intake is consistently inadequate, which is concerning given its central role in hemoglobin synthesis. However, Hb levels remain normal, suggesting possible compensatory mechanisms or bioavailability factors. Mean of iron was 6.57 µg/day, but the recommended was 8–13 µg/day. Vitamin C intake is insufficient, which may impair iron absorption, particularly non-heme iron [6]. Mean was 31.52 mg/day, but Recommended was 50–75 mg/day. B 12, appears to be a unit inconsistency (likely µg, not mg). If interpreted correctly, intake may actually be adequate. This highlights a critical data validity issue. Mean was 2.09 ug/day, but recommended was 24.7–47.4 ug/day.

Folate intake is severely deficient, which is significant given its role in erythropoiesis. Mean was 76.07 µg/day, meanwhile recommended: was 417–547 µg/day.

5. Regression Analysis: Predictors of Hemoglobin

Table 4. Predictors number of variable

Model	R	R ²	Adjusted R ²	the Estimate of standart . Error
Enter	.500 ^a	.250	.036	1.30349
a. Predictors: (Constant), Iron, Folat, VitC, protein.g				

5.1 Model Summary

Table 4 showed , $R = 0.500$; $R^2 = 0.250$; $\text{Adjusted } R^2 = 0.036$; $\text{Standard Error} = 1.303$. While R^2 suggests that 25% of Hb variance is explained, the $\text{Adjusted } R^2$ drops drastically to 3.6%, indicating overfitting and weak explanatory power. This discrepancy strongly suggests: were small sample size ; too many predictors relative to observations ; low statistical power.

5.2 Predictor Significance

There was no variable is statistically significant ($p > 0.05$).

Discussion**1. Overview of Principal Findings**

The present study investigated the relationship between dietary nutrient intake (iron, folate, vitamin C, and protein) and hemoglobin (Hb) levels among primary school children. Despite clear evidence of suboptimal nutrient intake, particularly for iron, folate, vitamin C, and total caloric consumption, the statistical analysis revealed no significant association between these nutrients and hemoglobin levels ($p > 0.05$ for all predictors). Furthermore, although the regression model yielded an R^2 value of 0.25, the adjusted R^2 dropped sharply to 0.036, indicating weak explanatory power after accounting for model complexity. At first glance, these findings appear paradoxical, given the well-established biological roles of iron and micronutrients in erythropoiesis. However, when interpreted critically within the broader literature and methodological framework, the results align with emerging evidence suggesting that hemoglobin concentration is influenced by a complex interplay of nutritional, physiological, and environmental factors, rather than isolated dietary variables alone.

2. Nutritional Inadequacy and Its Implications**2.1 Evidence of Chronic Dietary Deficiency**

The dataset clearly demonstrates that the children's intake of several essential nutrients falls below recommended dietary allowances (RDA) , Iron intake was below recommended levels; folate intake was severely deficient, vitamin C intake was inadequate and energy intake was substantially below requirement. Such findings are consistent with global trends in low- and middle-income countries, where dietary insufficiency remains a major contributor to micronutrient deficiencies in children [2]. A large national survey in India reported that over 60% of children had at least one micronutrient deficiency, with iron deficiency being the most prevalent [9]. Similarly global meta-analyses indicate that iron deficiency anemia (IDA) remains a moderate to severe public health problem in school-aged children, particularly in developing regions [10].

2.2 Biological Consequences of Nutrient Deficiencies

Iron, folate, and vitamin B12 are essential for red blood cell (RBC) production. Iron is directly involved in hemoglobin synthesis, while folate and vitamin B12 are required for DNA synthesis during erythropoiesis. Deficiencies in these nutrients typically result in reduced hemoglobin synthesis, impaired RBC maturation, increased risk of anemia. Recent literature emphasizes that anemia is rarely caused by a single nutrient deficiency, but rather by multiple interacting micronutrient inadequacies [11]. Therefore, the simultaneous deficiencies observed in this dataset (iron + folate + vitamin C) should theoretically predispose the population to anemia.

3. The Paradox of Normal Hemoglobin Levels

Despite inadequate nutrient intake, the mean hemoglobin level (13.38 g/dL) remains within normal range. This could be physiological adaptation occurred, that children may adapt to low nutrient intake through increased iron absorption efficiency, reduced iron losses, mobilization of iron stores. Also, hemoglobin reflects current functional iron status, not total body iron. Children may still have adequate ferritin reserves despite low intake. Nutritional deficiencies often take time to manifest clinically. The observed data may

represent a preclinical stage of deficiency. This phenomenon is supported by evidence showing that iron deficiency can exist without anemia, particularly in early stages (latent iron deficiency) [12].

4. Lack of Significant Association: Statistical and Methodological Explanations

4.1 Sample Size and Statistical Power

The study includes only 34 participants, which is insufficient for multivariate regression analysis involving four predictors. Statistical guidelines recommend at least 10–15 observations per predictor variable to achieve stable estimates [13]. The extremely low adjusted R^2 (0.036) strongly suggests Overfitting; high variance in coefficient estimates; low statistical power. This limitation alone may explain the absence of significant associations.

4.2 Measurement Error in Dietary Assessment

Dietary intake is typically assessed using recall-based methods (e.g., 24-hour recall or FFQ), which are prone to recall bias; Underreporting or overreporting; Day-to-day variability. Such errors attenuate correlations and reduce the likelihood of detecting significant associations [14].

4.3 Multicollinearity Among Nutrients

Micronutrients do not act independently for vitamin C enhances iron absorption, protein supports iron transport and folate and B12 interact in RBC formation. These interrelationships may lead to multicollinearity, inflating standard errors and obscuring individual predictor effects.

4.4 Hemoglobin as a Limited Outcome Variable

Hemoglobin is widely used but has limitations due to not specific to iron deficiency, it is influenced by hydration, infection, and inflammation, it does not detect early deficiency. Studies show that biomarkers such as ferritin, transferrin saturation, and CRP provide more accurate assessment of iron status [4].

5. Role of Individual Nutrients

5.1 Iron

Iron is the primary determinant of hemoglobin synthesis. However, the lack of statistical significance ($p = 0.156$) suggests that intake alone does not reflect absorption, bioavailability varies (heme vs non-heme iron), dietary inhibitors (phytates, calcium) may reduce absorption. A recent systematic review highlights that dietary iron intake explains only a portion of hemoglobin variability, particularly in populations with infections or inflammation [15].

5.2 Vitamin C

Vitamin C enhances non-heme iron absorption by reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). However, its non-significant effect ($p = 1.000$) may reflect that insufficient variation in intake, threshold effects (minimum level needed), interaction with other dietary factors. Interestingly, recent evidence suggests that vitamin C supplementation may not significantly increase hemoglobin when iron intake is adequate, challenging traditional assumptions [15].

5.3 Folate

Folate deficiency is strongly associated with megaloblastic anemia. However, the lack of association may be due to mild deficiency not yet affecting Hb, compensation by other nutrients, measurement limitations. Large-scale surveys indicate that folate deficiency does not always correlate directly with anemia prevalence, especially when iron deficiency is dominant [8]. Folate and Vitamin B12 involved erythropoiesis [16].

5.4 Protein

Protein supports hemoglobin synthesis indirectly through amino acid availability. However, its non-significant effect suggests that protein intake may be near adequate threshold, other limiting nutrients (iron, folate) play a larger role.

6. Multicausal Nature of Anemia

6.1 Beyond Nutrition

Modern research emphasizes that anemia is multifactorial, involving: Infections (e.g., malaria, helminths); Inflammation ; Genetic disorders; Socioeconomic conditions. A meta-analysis identified multiple risk factors, including birth weight, maternal anemia, and disease burden beyond dietary intake [17].

6.2 Inflammation and Iron Metabolism

Inflammation increases hepcidin levels, which inhibits iron absorption; Traps iron in storage sites. Thus, even adequate dietary intake may not translate into improved hemoglobin levels.

7. *Comparison with Previous Studies*

The findings of this study are consistent with several recent investigations such as Studies show weak or inconsistent associations between dietary intake and Hb; Multi-nutrient deficiencies are more predictive than single nutrients; Biomarkers outperform dietary data in predicting anemia. For example, a study on micronutrient deficiencies found that iron deficiency often coexists with other deficiencies, complicating the relationship with hemoglobin [18]. Diet quality strongly predicts micronutrient status, the highlight that dietary diversity is a stronger predictor than individual nutrients. Also multi-nutrient interactions are critical [19,20].

8. *Public Health Interpretation*

Despite the lack of statistical significance, the findings have important implications, such as there was a hidden risk reflect to the population as shows Chronic nutrient inadequacy and Risk of future anemia. The preventive opportunity at the early intervention is critical before anemia develops some project such as the school feeding programs; Micronutrient supplementation; Dietary diversification. Systematic reviews confirm that food-based and supplementation strategies effectively reduce anemia risk in children [12].

9. *Methodological Strengths and Limitations*

The strengths showed multi-variable analysis; Inclusion of key nutrients; real-world population data. The Limitations were small sample size; cross-sectional design; lack of biochemical biomarkers; potential dietary measurement error; No control for confounders. These limitations significantly restrict causal inference.

10. *Theoretical and Research Implications*

This study reinforces a key concept in nutritional epidemiology that hemoglobin is a downstream indicator influenced by complex biological systems, not merely dietary intake. So future research should adopt for longitudinal designs; Biomarker integration; Systems-based approaches

IV. CONCLUSION

In conclusion, the absence of significant associations between nutrient intake and hemoglobin levels should not be interpreted as evidence of no relationship. Instead, it reflects that Methodological constraints; Biological complexity; Multicausal determinants of anemia. The findings highlight the need for more robust, multidimensional research frameworks to accurately capture the relationship between nutrition and hematological outcomes in children.

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