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Research Article

Association of Zinc Deficiency with Anaemia in Under-five Children of Rural Bangladesh: An Observational Study

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Abstract

Introduction: Anemia is a major public health challenge worldwide that occurs with a higher prevalence in under-five children who live in rural areas. Like other micronutrients, zinc deficiency may be a new determinant of anaemia. **Objectives:** This study was designed to find out if there is any association between zinc deficiency and anaemia in preschool children.

Methods: This cross-sectional study was conducted in the rural Bangladesh from July 2018 to December 2019. Total 428 under-five children were enrolled, of them 61.45% (263) were anaemic. Etiological exploration was conducted among anaemia respondent (n=163) through essential blood tests (CBC, Hb electrophoresis, Ferritin, Vitamin B12 assay, and Folate assay) and of them 36% remained as undifferentiated. These subjects and who are willing to respond were enrolled for further Zinc assay as per study flowchart. Then serum zinc was estimated through the colorimetric method by a semi-automated biochemistry analyzer. Zinc status was defined as deficiency (<69 μg/d), normal (≥69 to <108 μg/dL) and high (≥109 μg/dL). Chi-square (χ^2) tests were done to find out the significant factors. Ethical clearance was obtained from IBSc, Rajshahi University. The data were analyzed through SPSS 23 software and at a 95% confidence interval and p values < 0.05 were considered significant.

Results: The overall prevalence of anaemia was 61.45%. Etiologically, iron deficiency anemia (34%) was observed as the leading pattern followed by inherited hemoglobin disorder (32%) and zinc deficiency (24%). Zinc status (n = 67) revealed, nearly 72 % of anaemic children belonged to low zinc category, 22% had normal status and remaining 6% had high zinc level. Among low zinc category (72%), a proportionate 55% anaemic children independently associated with zinc deficiency alone, which was statistically significant ($p < .001$).

Conclusion: The study indicates nearly one fourth of anaemic population had low level zinc and of them nearly threefourth of the anaemia sufferer independently associated with zinc deficiency alone.

Keywords: Zinc; Deficiency; Association; Anaemia

Introduction

Anaemia in childhood is a key public health challenge globally, affecting almost half of all under-five children [1,2] and causing a wide variety of illnesses, including poor physical growth, inadequate behavioral and cognitive development, low school performance, poor immune function, and infection [3]. Poor nutrition or anaemia during the first 1000 days of life may cause irreversible cognitive and physical damages that result in nutritional

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disability and consequently adverse impacts on economic and social growth [4]. Anaemic children are at risk of death when they are complicated by infection and malnutrition [5]. Anaemia in children has a number of triggering elements that can happen alone but more often co-occur. These factors can be nutritional (micronutrient deficiencies) or hereditary, infectious, or chronic inflammations [6]. Anaemia substantially and significantly correlated with age, sex, rural residence, low-birth-weight (LBW), duration of lactation, infant and young child feeding (IYCF) practices, poor dietary iron and micronutrient intake, under-nutrition (e.g., stunting, wasting, and underweight), infectious disease (e.g., malaria, tuberculosis, intestinal parasitic infestation, etc.), poor household, food insecurity, socioeconomic status, maternal education, and maternal anaemia [7,8]. Recent data from South Asia indicates that anaemia affects nearly 79% of Indian children aged 6 to 35 months, with a rural predominance and higher among young children [9]. Bangladesh Demographic Health Survey 2011, using heamoglobin estimation only, reported a 51.9% prevalence of anaemia among young and preschool children, with a higher prevalence of 53.1% among rural children [10]. But the National Micronutrients Status Survey 2011–2022, based on Heamoglobin estimation, ferritin, and other micronutrient levels, revealed anaemia prevalence of 37.0% and 22.8% in rural and urban areas, respectively, in preschoolers [11]. Despite this decreasing trend of anemia, micronutrient deficiencies (iron, folate, vitamin B12, etc) remain a significant contributor to childhood anaemia in Bangladesh [11,12]. Studies in other settings have found that zinc deficiency may be a new determinant of anaemia [13-15]. Zinc involves several coenzyme systems, DNA synthesis, and lots of biochemical mechanisms in the human body, and its deficiency may adversely affect the different body systems, including the hemopoietic system [16-18]. Zinc deficiency in Bangladesh is a major micronutrient disorder with a high (48.6%) prevalence and nearly five times more common than iron deficiency (10.7%) among the under-fives [19]. This may be a prediction of an interrelationship between zinc deficiency and anaemia [16-20]. Therefore, we assume, zinc deficiency in preschool children has an association with the high prevalence of anaemia in rural Bangladesh, in addition to other causes. But till no such studies are conducted. Therefore, we planned for the study.

Methods and Materials

Study design, settings, and population

A cross-sectional exploratory study was carried out in rural areas of northern Bangladesh. Eleven Upazillas (subdistricts) from five districts were specifically chosen as study locations.

Inclusion criteria: The target population was rural children aged 6 to 60 months.

Exclusion criteria: Children who lived in an urban (municipality) region, required hospitalization or had a history of blood transfusion within the past three months from the data collection.

Sample size and sampling strategy

A total of 428 sample sizes were calculated. Firstly, the sample size was calculated using the following formula

(Cochran's principle 1977).

$N = p\ (1 - p)\ z^2/e^{\ 2}$

Where, N = Sample size, z = 1.96 (95% confidence level), p = 33% estimated prevalence of anaemia in the under-five population based on available information, and e = 0.05 (at a 5% acceptable margin of error).

The formula giving the number of sample sizes would be 340. In practice, we might need to enroll more people to adjust for missing data and/or level problems. Finally, the boost-up sample size formula was:

n1 = n / (1-d), n = 22, d = 20% (Sakpal, 2010). Thus, as per the given formula, the final sample size was 428. A convenient sampling method was followed to select the study children.

Data collection: tools and techniques

A pre-designed and pre-tested questionnaire was used for data collection. Face-to-face interviews were conducted with the attending guardian to acquire qualitative data. Following the interview anthropometry and clinical assessments were performed during the same session.

Laboratory procedures, laboratory diagnosis and quality control

Five milliliters (ml) of venous blood were extracted with a sterile syringe; three milliliters (ml) of blood were preserved in an ethylene ediamine tetraacetic acid (EDTA) vial, and the remaining two milliliters were placed in another test tube for serum extraction. On the same day, the EDTA blood was analyzed with an 'Automated Hematology Analyzer' (Nihon Kohden, Tokyo, Japan) for a Complete Blood Count (CBC): heamoglobin estimation, haematocrit, red cell indices (i.e., MCV, MCH, MCHC, RWD), total white blood cells (WBC), differential count of WBC and total platelet count.

Laboratory Diagnosis

Anaemia was further categorized morphologically as normocytic (MCV 80-96 fl), microcytic (MCV 80 fl), and macrocytic (MCV >96 fl) based on mean corpuscular volume (MCV) (Brancaleoni et al., 2016 and Gruchy, 2016). When there was microcytic anaemia, the serum ferritin concentration was measured first. If the serum ferritin level was low (<12 ng/ml), it was diagnosed as iron deficiency anaemia. If normal or raised ferritin level with microcytic

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anaemia was found, then Hb-electrophoresis was carried out, and the diagnosis of an inherited heamoglobin disorders was made according to the diagnostic algorithm based on Hb-electrophoresis. If both ferritin and Hb-electrophoresis were found to be normal, this would be classified as undifferentiated microcytic anaemia. On the other hand, macrocytic anaemia was evaluated through the assaying of both vitamin B_{12} and folic acid for megaloblastic anaemia if any. Consequently, undifferentiated microcytic anaemia and normochromic anaemia patients, who responded for further analysis were enrolled for the estimation of serum zinc level (µg/dL) to reach zinc associated anaemia according to the conceptual flow-chart. In addition, those guardians of anaemic children who were willing to participate in the zinc study also enrolled in the study. The final etiological patterns were determined after serum zinc assay.

Estimation of serum zinc

Serum zinc was estimated by the colorimetric method. In which a semi-automated biochemistry analyzer (Homolizer 3000, Human, Germany) was used for measuring zinc by using a readily available colorimetric reagent kit (Italy). Zinc reacts with 5-Br-PAPS [(2-5-Brom-2-pyridylazo)-5-(N-propyl-N-sulfopropyl-amino)- phenol] and produces a stablecolored chemical compound. The degree of color is directly proportional to the level of zinc ions in the serum. Zinc ions were calculated by measuring the optical density (OD) values of a zinc compound at 560 nm. Serum zinc concentration categories were defined as: low (<69 µg/dL), normal (≥69 to <108 µg/dL), and high (≥109 µg/dL.

Statistical analysis

SPSS version 23.0 for windows software was used to compute and analyze the data. Descriptive data for the study population included frequency, mean, standard deviation, and percentage. Univariate analysis was performed on sociodemographic, nutrition, and health-related factors as well as for serum zinc level, and chi-square (2) tests were used to assess significant factors at 95% confidence intervals. *A p-value of < 0.05 was considered as statistically significant.*

Ethical approval

The Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee (IAMEBBC) of Rajshahi University granted ethical approval (memo no. 83/320/IAMEBBC/IBSC, date: August 27, 2017).

Conceptual framework of the study (Figure 1)

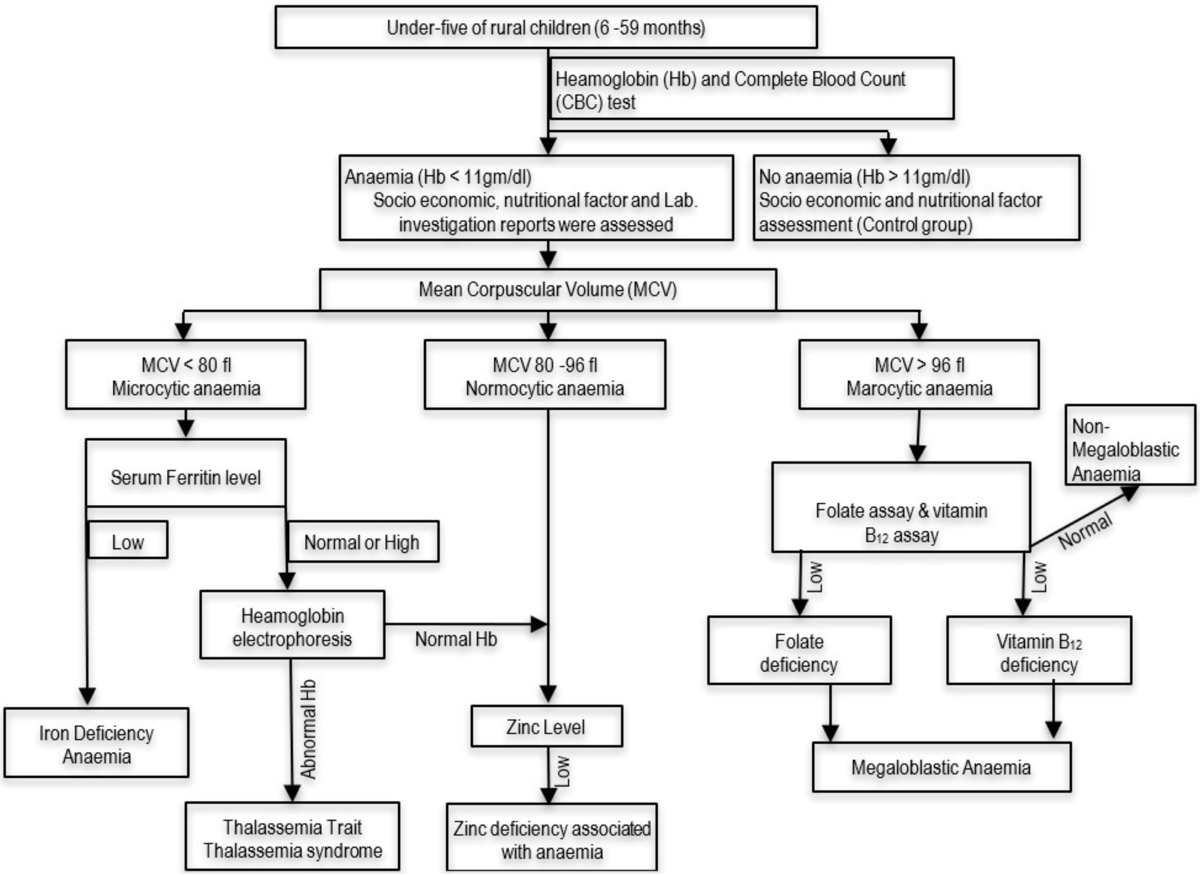

Figure 1: Conceptual flow-chart.

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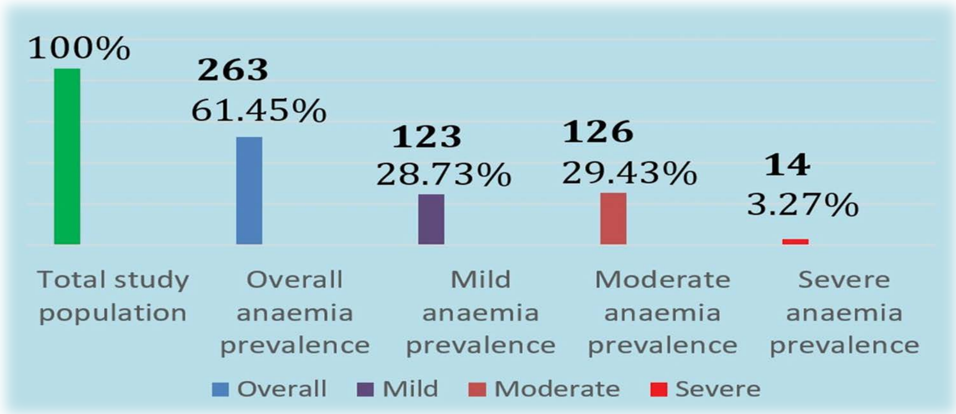

Figure 2: The overall prevalence of anaemia under the study (N = 428).

Results

Figure 2 shows the overall prevalence of anaemia. A total of 428 under-five children aged 6–59 months were enrolled from rural areas of 11 Upazillas under the five districts in the northern part of Bangladesh. Among the study children, the overall prevalence of anaemia was 61.45% (N = 428). Regarding the clinical type of anaemia, proportionate prevalence of mild, moderate and severe anaemia were observed at 28.73%, 29.43%, and 3.27%, respectively.

Table 1: Socio-demographic characteristics of study subjects in relation to anaemia (N = 428).

Variables	Distribution of study population (%) (N = 428)	Anaemia category (n=263)	Prevalence of anaemia (%)	Chi squire & *p* value
Gender				
Male	245 (57.2%)	156	63.67%	χ^2=1.197,df 1 *p* = 0.274
Female	183 (42.8%)	107	58.45%	
Age(in month)				
6-24	224 (52.3%)	159	70.98%	χ^2=21.385, df 2 *p* < 0.001
>24-36	80 (18.7%)	47	58.75%	
>36-60	124 (29%)	57	45.97%	
Educational level of mother				
Primary enrollment or below	193(45.1%)	137	70.98%	χ^2=17.295, df 2 *p* < 0.001
Secondary enrollment	155(36.21%)	90	58.06%	
Higher secondary or above	80(16.3%)	36	45.00%	
Family size				
≤2	398 (93%)	248	62.31%	χ^2=1.785 φ 1
>2	30(7%)	15	50.00%	*p* = 0.182
Occupation of household head				
Service (Public or Private)	168 (39.3%)	100	59.52%	χ^2=8.139 δφ 3 *p* = 0.043
Agriculture	136(31.8%)	77	56.62%	
Small Business	94(22%)	61	64.89%	
Day Labor	30(7%)	25	83.33%	
Monthly (family) income				
5000 BDT or below	42 (9.6%)	30	71.43%	χ^2=37.51, δφ 3 *p* < 0.001
5001-10000 BDT	116(27.1%)	94	81.03%	
10001- 20,000 BDT	118(27.6%)	69	58.47%	
More than 20,000BDT	153(35.7%)	70	45.75%	

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The socio-demographic features of the study children (N = 428) and their associations with anaemia are shown in Table- 1. The study children had a 6:4 male-female ratio. Among male study children (n = 245), nearly 64% were anaemic, while it was nearly 58% among female children (n = 183). The difference in prevalence between the genders was not significant statistically (p = 0.274). The median age of the study subjects was 24 months. Among the age categories, 6–24 months occupied nearly half (52%), followed by 29% who belonged to the age group of >36–60 months and 19% of respondents from the age group of >24–36 months . Among them, the 6–24-month and >24–36-month aged categories had a higher prevalence of anaemia which was 71% and 59%, respectively, while in the age category (>36–60 months), it was 46%. Age was observed as a statistically significant (p = <0.001) factor. On the basis of mothers' education status, 45% had primary enrollment, which was the highest, followed by 36% who had secondary enrollment, and the remaining 16% had higher-level education. Among all primary-educated mothers, the highest prevalence (71%) of anaemia was followed by the lowest prevalence (59%) at the secondary level, while the children of higher-educated mothers had a 45% prevalence of anaemia. The differences in education status were significantly associated with the occurrence of childhood anaemia (p < 0.001). Family size with ≤2 children (93%) and >2 children (7%) was not significantly associated with anaemia (p = 0.23). In regard to the family heads' occupation, service holder (39%), which was the highest, was followed by agriculture (31.8%), small business (22%), and day laborer (7%). The prevalence of anaemia in children from service-holder, agricultural, small business, and day-laborer families was observed at 59.52%, 56.62%, 64.89%, and 83.33%, respectively. Parents occupations were significantly associated (p = 0.043) with anaemia with the highest prevalence among day laborers and the lowest among small business families (Table 2). Regarding four categories of monthly family income, distributions of study children were almost the same in the middle two groups (second and third) family income groups (i.e., 27.1% and 27.6% in the (5001–10,000/-Bangladeshi currency Taka-BDT) and (10,001–20,000 BDT) groups, respectively), 35.7% of family income was more than 20,000 BDT, and the remaining 9.6% of children's family income was 5,000 BDT or below. The highest prevalence of anaemia was observed at 81.03% in the income group (BDT 5001–10,000/-), followed by 71.43% prevalence in the earning category (BDT 5000 or below). While prevalence was 58.47% in the 1000–20.000 BDT and 45.75% in the BDT > 20000 income group. Monthly family income was significantly (p = 0.001) associated with anaemia.

Table 2: Distribution of patterns of anaemia in pre-zinc and post zinc assay state (N=428).

Characteristics	Distribution of anaemic population (%)	Sub-group population	Calculated Prevalence in study population (N = 428)
Patterns of anaemia (Pre zinc assay state)			-
Heamoglobin disorder	50 (30.67%)	n =163	
Iron deficiency anaemia	53 (32.52%)		
Undifferentiated anaemia	59 (36.20%)		
Macrocytic anaemia (non-megaloblastic)	01 (0.61%)		
Patterns of anaemia (Post zinc assay state)		n = 154	
Iron deficiency anaemia	53 (34.41%)		21.20%
Heamoglobin disorder	50 (32.47%)		19.80%
Zinc deficiency associated anaemia	37 (24.03%)		14.70%
Undetermined anaemia	13 (8.44%)		5.60%
Macrocytic anaemia (non-megaloblastic)	01 (0.65%)		0.22%

Based on serum ferritin and Hb-electrophoresis, vitamin B12 and Folic acid assay The etiological patterns before the zinc assay were detected (n = 163) as iron deficiency anaemia at nearly 32.52%, inherited heamoglobin disorder at 30.67%, and macrocytic anaemia as 1%. The rest of the major bulk of 36.20% remained as undifferentiated anaemia. In the post serum zinc assay state, the final estimated prevalence (N = 428) of iron deficiency anaemia, inherited heamoglobin disorders, zinc deficiency-associated anaemia and undetermined anaemia were found to be 21.20%, 19.80%, 14.70%, and 5.60% respectively (Table-2). Among anaemic population (n = 154), the etiological distribution was found to be 34.41% in iron deficiency anaemia, 32.47% in inherited heamoglobin disorders, 24.03% in zinc deficiency-associated anaemia and 8.44% in undetermined anaemia.

It is evident from Table 4 that serum zinc levels have an association with anaemia. Among the study subjects (n = 67), nearly 72% of anaemic children belonged to the group of low zinc levels, while 22% were found in the category of normal zinc levels, and 6% were found in the high zinc level category (Figure 5).

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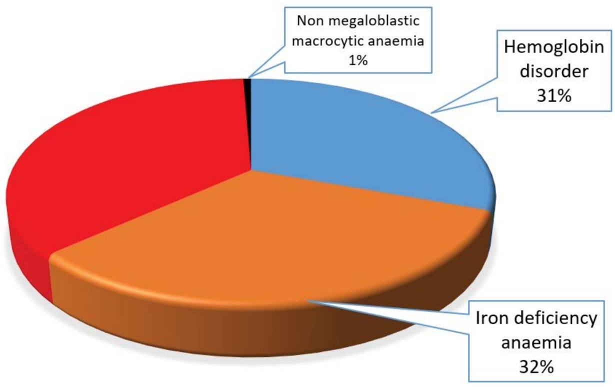

Figure 3 shows the etiologic patterns of anaemia in pre zinc assay state (n = 163).

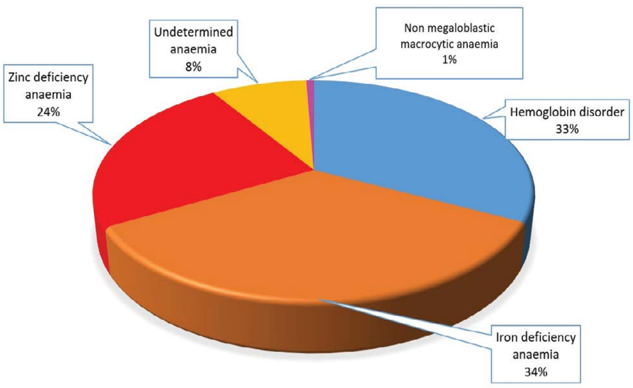

Figure 4 shows the etiologic patterns of anaemia in post zinc assay state (n = 154).

Figure 5 Shows the distribution of serum zinc levels among the study subjects (n = 67), nearly 72% of anaemic children belonged to the group of low zinc levels, while 22% were found in the category of normal zinc levels, and 6% were found in the high zinc level category.

Table 4 shows the association of serum zinc level with anaemia in supgroup (n= 67). Regarding of 72% low zinc level associated anaemia (Figure 3), proportionately 55% anaemia was independently associated with zinc deficiency alone; 10% co-occurred with iron deficiency anaemia and

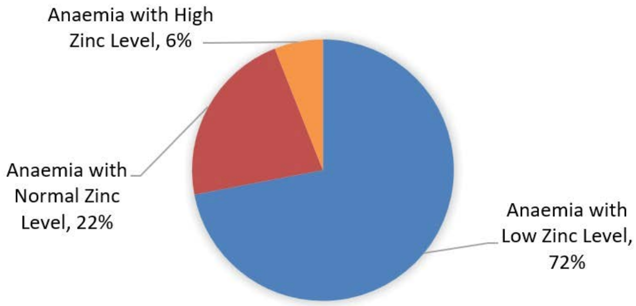

Figure 5: Zinc status and distribution of anaemia (n = 67).

the rest, 6% was occur together with inherited heamoglobin disorders. This indicated that a low serum zinc (<68 mcg/dl) was linked with a high prevalence (72%) of anaemia in children (n = 67), which was statistically significant ($p < 0.001$).

Discussion

This cross-sectional, community-based study was designed to find out if there is any association between zinc deficiency and childhood anaemia. A total of 428 data points were collected from 10 rural upazillas in the five districts of the northern region of Bangladesh. In the current study, the overall prevalence of anaemia in under-five children (N = 428) was 61.45%, with the proportionate prevalence of mild, moderate, and severe anaemia being 28.73%, 29.43%, and 3.27%, respectively. The prevalence was highest at 71% among the 6–24-month-old children. The etiological exploration of anaemic children (n = 154) was conducted through serum ferritin, Hb-electrophoresis, and serum zinc level. The estimated prevalence of anaemia on the basis of etiological factors was identified as 21.20% of iron deficiency anaemia, 19.80% of inherited heamoglobin disorders, 14.70% of zinc deficiency-associated anaemia and 5.60% as undetermined anaemia (Table 2, Figure 1). In our anaemic population, iron deficiency anaemia constitutes 34.41% of the most common etiological pattern anaemia (Table 3), followed by inherited heamoglobin disorders with 32.47%. While zinc deficiency alone occupied 24.03% of the

Table 3: Association of serum zinc level with anaemia (n= 67).

Serum zinc status	Co-occur with iron deficiency anaemia	Co-occur inherited Hb disorder	Anaemia associated zinc deficiency alone	Undetermined anaemia	Sub group anaemic population (n=67)	Chi-square & *p*-value
Low zinc level (<68- mcg/dl)	7(10.45%)	4(5.97%)	37(55.22%)	0	48(71.6%)	χ^2= 36.24, df=9, *p < 0.001*
Normal zinc level (>68-107 mcg/dl)	2(2.98%)	2(2.98%)	0	11(8.96%)	15(22.4%	
High zinc level (>107 mcg/dl)	1(1.49%)	1(1.49%)	0	2(2.98%)	04(6.0%)	

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total distribution (Table 3, Figure 2) of anaemia which was explored as the third common factor etiologically, However, the reason for the high occurrence of iron deficiency anaemia and zinc deficiency-associated anaemia may be due to the fact of micronutrient deficiencies as a result of rural child care practices, rural food habits and food restrictions culture for infants, a high volume of cow milk consumption during infancy and early childhood, and the socioeconomic context of rural societies. Further analysis of serum zinc status and childhood anaemia (n = 67) revealed that nearly 72% of anaemic children belonged to the group of low zinc levels, while 22% were found in the category of normal zinc levels, and 6% were found in the high zinc level category (Figure 5). Among 72% of zinc deficiency-associated anaemia, a proportionate 55% are solely related to zinc deficiency alone, 10% co-occur with iron deficiency anaemia and 6% occur together with inherited heamoglobin disorders (Table 3). Therefore, it can be concluded that low zinc levels (<68 mcg/dl) had a significant association ($p < 0.001$) with anaemia both independently and combinedly. It may be due to the underlying fact that a high frequency of zinc deficiency is prevailing among rural under-five children resulting from low consumption of a zinc-rich diet (animal protein) due to poverty, traditional low dietary diversity, food restriction culture for children, and phytate-rich rural family diets, in which, infant and young child feeding traditionally depended upon the elderly people of the family, who traditionally feed babies with rice porridge (locally sugi) and diluted cow milk. Our findings are compatible with the findings of studies conducted by [21] in China and in a setting in New Zealand by [20], which reported that anemic children had significantly low serum concentrations of zinc. In study settings in India, reported that serum zinc deficiency had a significant association with anaemia, stunting, and wasting [22]. A similar observation was noted in a study in Guatemala, where zinc deficiency was observed as a potential risk factor (OR 3.4) for developing anaemia in children. A study reported that zinc deficiency was an independent risk factor for anaemia [23].

Strengths and Limitations of the Study

This study was cross-sectional in nature and followed a convenient sampling technique because of resource limitations. In addition, some anemic patients were found to be non-responders during the zinc assay. Therefore, the study may not reflect the exact scenario. The study, probably the first of its kind in Bangladesh, provides an overview of childhood anaemia in rural settings by using a wide range of parameters, including CBC, Hb electrophoresis, B_{12} assay, folate assay, ferritin, and a newer parameter, serum zinc assay.

Conclusion

Under the study, zinc deficiency has been identified significantly as a notable risk factor for anaemia in early childhood in our settings. Zinc deficiency alone has an etiological association with anaemia which requires further large-scale experimental studies to examine this association.

Conflict of Interest: None declared

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