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ORIGINAL RESEARCH ARTICLE

Clinico-Etiopathogenesis of Vitamin B12, Folic Acid and Iron Deficiency in Severe Acute Malnutrition Children: A Tertiary Care Hospital Experience from Central India

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Abstract

In severe acute malnutrition, micronutrient deficiency as well as protein energy malnutrition is a major obstacle to growth & development. Iron deficiency dominates the spectrum of nutritional anemia. After taking informed consent, 211 SAM children and 211 age-and sex-matched healthy children with normal nutritional status were enrolled for the study. MUAC was used to diagnose SAM. A 5-part automated hematoanalyzer was used to measure the complete blood count and red cell indices, and the peripheral smear method to determine the red cell morphology. We measured serum ferritin, Vitamin B12, and folic acid using the ELISA method. Compared to controls, children with SAM had significantly lower red cell indices, platelet counts, and white cell counts. The most common clinical symptoms seen in SAM children were diarrhea, pneumonia, acute gastroenteritis, and acute respiratory infection. Children with SAM are more likely to suffer from iron deficiency and B12 deficiency. Severe vitamin B12 deficiency was more frequently associated with severe anemia. The severe anemia in SAM children constantly changes the body's defense mechanism, affecting the haematopoiesis. In this study, haematological indices are recommended for predicting severity of anemia, and hematopoietic changes are described, in order to improve anticipatory care and outcome in children with SAM.

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Introduction

It has been demonstrated that micronutrient deficiencies have an impact on society's health and well-being, and are potential targets for supplementation [1]. Because affected populations are not able to achieve their full mental and physical potential, have low work capacity, and are susceptible to infections, it could adversely affect economic and overall development [2]. Although iron deficiency is the most common cause for nutritional anemia. Micronutrient deficiencies, especially vitamin B12 deficiency, are significant contributors to anemia. Furthermore, micronutrient deficiency and protein energy malnutrition are major barriers to growth and development in children with SAM. Iron deficiency is the most common nutritional deficiency causing anemia in children. The condition generally arises when dietary iron intake and iron absorption are insufficient to meet physiological requirements. Being a developing nation, India's diet and infection are contributing factors to causing the disease [3]. The vitamin B12 is essential for the normal functioning of the nervous system and for the production of blood. It is involved in the metabolism of every human cell, especially in the synthesis and regulation of DNA. Vitamin B12 is almost exclusively derived from animal products [1]. Regarding anemia in malnutrition or SAM, much emphasis is placed on supplementing iron and folic acid rather than vitamin B12. In addition, supplementing only folic acid to children with deficiency of both vitamin B12 and Folic acid can worsen their neurological status. In a study by Vaid et al., 58% of SAM patients are vitamin B12 deficient. Vitamin B12 deficiency is most common in young children [4]. The most common cause of macrocytic anemia is vitamin B12 or folate deficiency [5]. Anemia may play a causal role in

the hospitalization of severely malnourished children, given its high prevalence [6].

Materials and Methods

Research was conducted at the multidisciplinary research unit associated with Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital in district Rewa, Madhya Pradesh, India. The parents of each child were informed and written consent was obtained after the study objective was explained. A case control study was performed at Shyam Shah Medical College, Rewa (M.P). The study protocol was approved by the Institutional Human Ethical Committee. The purpose and methods of the research and the voluntary nature of participation in the study were explained verbally and in writing to all children and their parents. There were 211 severely malnourished children, aged 6 months to 60 months, admitted to Gandhi Memorial hospital from May 2018 to February 2020. We selected 211 matched children between the ages of 6 and 60 months with normal nutritional status and no haematological or infectious conditions attending routine vaccination/consultations as controls. Children within these age groups who met any one of the following criteria in accordance with WHO guidelines regarding growth parameters were included in the study. We used the WHO-MUAC reference range in the different age groups for girls and boys.i.e. Weight for height less than − 3SD,Visible severe wasting,Edema of both feet (excluding other causes of edema),Mid arm circumference less than 11.5 cm (in infant more than 6 months of age). Children who had received oral or parenteral vitamin B12 in the preceding six months, or folic acid in the preceding three months, were excluded. Study participants with hemolytic anemia, liver disease, gastrointestinal disorders (inflammatory bowel disorder, celiac disease, and malabsorption), and any other medical conditions were excluded. In order to obtain detailed demographic and socioeconomic information, a pretested questionnaire was applied. Ages of children were gathered from birth records provided by the mothers. Weighing machines and Stadiometers were used to measure body weight and height. Each child's body mass index (BMI) was calculated based on the ratio of weight (kg) to height (m2). With the WHO Growth Standards, BMI data was converted to z-scores, that is, BMI-for-age z-scores (BAZ). Approximately 2 ml of venous blood was collected according to the participants' agreement. A complete blood count was performed using one ml of venous blood. A peripheral blood smear was prepared for the purpose of morphologically differentiating RBCs. Anemia was assessed by measuring hemoglobin (g/dL) (Mild, Moderate and Severe Children aged 6–60 months < 10–10.9 7.0–9.9 < 7.0). Biochemical and hematological investigations were performed in the Multi-Disciplinary Research Unit. The CBC was performed using an automated 5-part hematoanalyzer (SYSMEX XS-800i, Kobe Japan) using the Transasia diagnostic kit. In the analyzer, the blood was mixed well and placed on a rack. A binocular microscope (Olympus CX21ILED) was used to determine macrocytosis and microcytosis in peripheral smears. In addition to hemoglobin (Hb), red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cells (WBC), granulocytes, thrombocytes (platelets), lymphocytes, and monocytes were counted in the instrument. For estimating serum ferritin, B12 & Folic Acid using an ELISA machine (MICROLISA PLUS, Microlab Instrument, India) & ImmunoTag kits from GBiosciences St. Louis, USA. WHO reference values were used for interpreting hemograms in different age groups of girls and boys. As per the WHO guidelines, reference ranges are used for hematological and biochemical indicators. All statistical analyses were conducted with GraphPad Prism software (version 4.00) using data entered in Microsoft Excel 2007. The descriptive characteristics (mean and standard deviation) and percentage were calculated separately for each parameter. The t-test was used to compare proportions and mean between Case and Control. p -value < 0.05 was considered statistically significant.

Result and Discussion

The study examined 422 children, 211 cases of SAM (120 males and 91 females with ages of 21.45 ± 7.93 and 20.21 ± 6.47 months respectively) and 211 controls (120 males and 91 females with ages of 20.1 ± 5.93 and 19.83 ± 5.42 months respectively) of healthy children. The z-score of all SAM cases with oedema was below − 3 SD. The mean duration of exclusive breastfeeding was 8.2 ± 2.3 months. In SAM cases, 86.25% of the parents and 74.4% of the controls parents belong to low socioeconomic status and difference was statistically significant (P = 0.002). Over 86.77% of the study cases' mothers had a primary school education, while 80.09% of the control populations' mothers had a primary school education and difference was not statistically significant (P = 0.06). In SAM population, 61.61% of children were immunized completely, while 38.38% were partially immunized, while in the control population, 67.77% were completely immunized, and 33.22% were partially immunized. Immunization difference was not statistically significant (P = 0.18). Over 185 (86.7%) children with SAM received colostrums. The morphology of

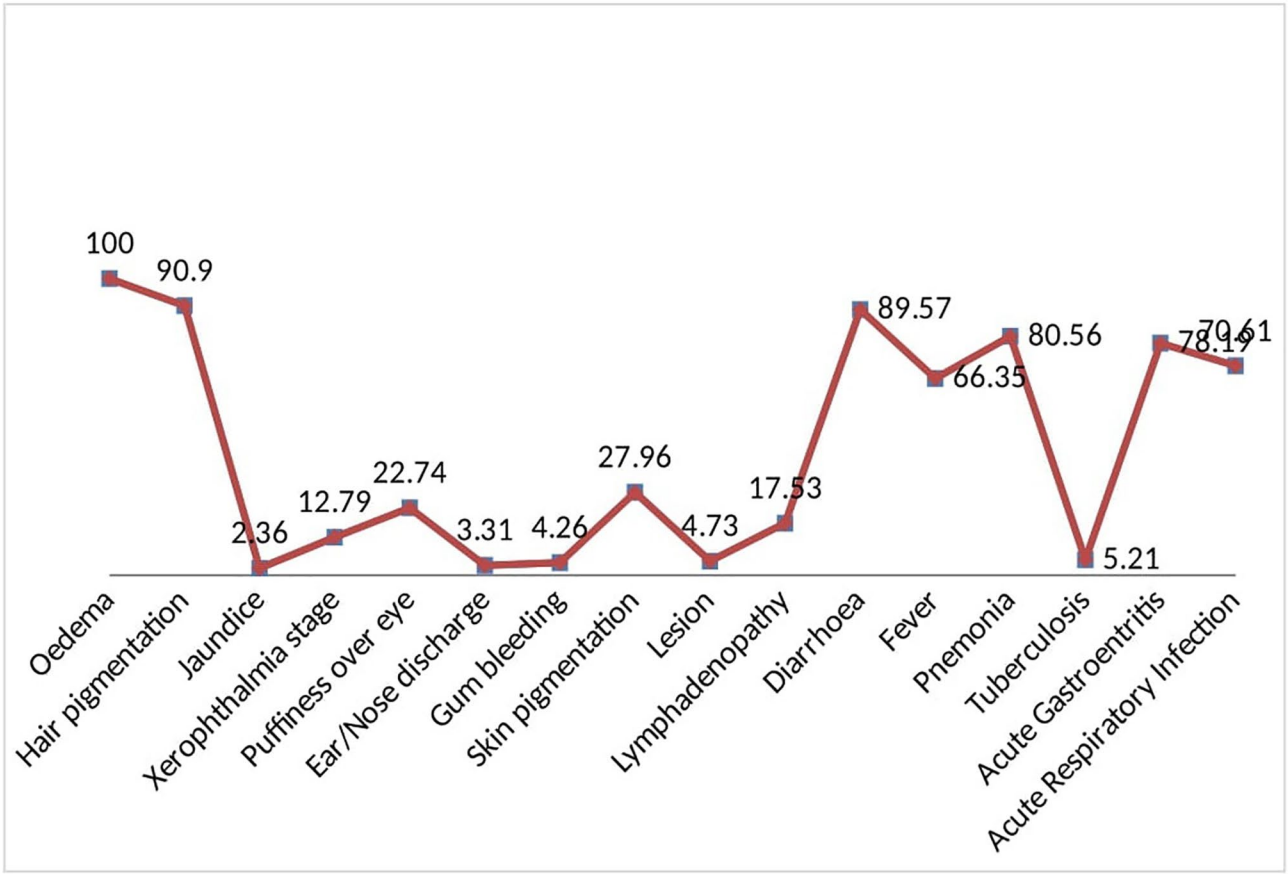

Fig. 1 Clinical Frequency(%) in SAM Children

the cells in various forms of anaemia was studied and it was determined that microcytic hypochromic (45.97%) predominates in SAM children while macrocytic hypochromic is 30.33%. The result was consistent with finding of Arya et al. [7]. There were 56.87% and 27.96% of patients with severe and moderate anemia, respectively. Similar results have also been reported by Thakur et al. [8]. However, this finding did not accord with that of Kumar et al. [9]. Diarrhea was the most common complaint among the children (89.57%), followed by Pnemonia (80.56%), acute gastroenteritis (78.19%), acute respiratory infection (70.61), and fever (66.35%). Affected people come from far flung regions of Madhya Pradesh's Vindhya region and reach a tertiary care hospital after infection rates have raised. As a result, acute gastroenteritis and acute respiratory syndrome percentage rates were high as per the compression of Kumar et al. findings [9]. Children with SAM were predominantly affected by hair pigmentation (90.9%), skin pigmentation (27.96%), eye puffiness (22.74%), lymphadenopathy (17.53%), and xeropthalmia (12.79%). Previous studies have also reported similar results [10–13]. However this finding was not consistent with Munthali et al. findings [14]. Among 211 SAM children, 92.41% had at least one co-morbidity, while 7.58% had no co-morbidity. Figure 1 shows the detailed clinical features of severe acute malnutrition. The mean hemoglobin values for cases and controls were 8.0 ± 2.15 gm/dl and 11.5 ± 2.3 gm/dl, respectively, and the mean hematocrit values were 26.72 ± 3.82% and 31.14 ± 5. 23% respectively and the differences were statistically significant (p = 0.0001). The findings of this study were similar to those reported by Saka et al. [10] and El-Nawawy et al. [15]. The mean values

Table 1 Age and sex wise distribution of case versus control

Subject	Age (Months) (Mean ± SD)	
	Male	**Female**
Case (N = 211)	120 (21.45 ± 7.93)	91 (20.21 ± 6.47)
Control (N = 211)	120(20.1 ± 5.93)	91(19.83 ± 5.42)

Table 2 Anthropometric data of case versus control

Particulars	(Mean ± SD)	
	Case (N = 211)	**Control (N = 211)**
Weight (Kg)	7.62 ± 1.34	11.63 ± 2.3
Height (CM)	67.21 ± 3.26	78.41 ± 4.2
BMI (Kg/M^2)	15.6 ± 1. 37	18.93 ± 2.1

Table 3 Grading of Anemia in Case

Particulars	No. of cases	Frequency(%)
Mild	32	15.16%
Moderate	59	27.96%
Severe	120	56.87%

Table 4 Anemia based on Red cell size

Particulars	No. of cases	Frequency(%)
Microcytic hypochromic	97	45.97%
Microcytic normochromic	15	7.10%
Normocytic hypochromic	6	2.84%
Normocytic normochromic	17	8.05%
Macrocytic normochromic	12	5.68%
Macrocytic hypochromic	64	30.33%

Table 5 Hematological profile of case versus control

Hemogram	Case (N = 211)	Control(N = 211)	*p* value
	(Mean ± SD)		
WBC (ths/µl)	11.10 ± 6.23	8.43 ± 3.5	0.0001
RBC (millions/ µl)	3.64 ± 1.14	4.2 ± 1. 2	0.0001
HB (g/dl)	8.0 ± 2.15	11.5 ± 2. 3	0.0001
HCT(%)	26.72 ± 3.82	31.14 ± 5. 23	0.0001
MCV(fl.)	78.48 ± 29.07	73.39 ± 10. 93	0.0177
MCH (pg)	25.38 ± 7.10	27.02 ± 5. 88	0.0101
MCHC (g/dl)	27.84 ± 6.01	26.18 ± 5. 04	0.0022
PLT(ths/µl)	2.41 ± 1.62	2.58 ± 1. 54	0.2699

of RBC in cases and controls were 3.64 ± 1.14 millions/ µl and 4.2 ± 1.2 millions/ µl respectively and the difference was statistically significant (p = 0.0001).The mean values of mean corpuscular volume (MCV) were 78.48 ± 29.07 fl. and 73.39 ± 10.93 fl. in the cases and controls respectively and the differences were statistically significant (p = 0.0177). For the MCH and MCHC, the mean values were 25.38 ± 7.10 pg and 27.84 ± 6.01 g/dl for cases, and 27.02 ± 5.88 pg and 26.18 ± 5.04 g/dl for controls, both statistically significant (p = 0.0101 and p = 0.0022). Among the controls, the WBC mean value was 8.43 ± 3.125 ths/µl and 11.10 ± 6.23 ths/µl in the case. These values were significant (p = 0.0048). Arya et al. have also reported similar findings [7]. In cases and controls, platelet counts were 2.41 ± 1.62 ths/µl and 2.58 ± 1.54 ths/µl, respectively, and the differences were not statistically significant (p = 0.1699). Detailed hematological parameters are given in Table 5. Cases and controls had mean B12 levels of 132.54 ± 28.75 pmol/L and 134.61 ± 70.75 pmol/L, respectively, and the difference was statistically significant (p = 0.0001). I n case and control, Folic acid levels were 14.67 ± 2.58 nmol/L and 15.11 ± 2.21 nmol/L. There was no statistically significant difference between the two groups (p = 0.0606). The current study confirms that severe acute malnutrition is accompanied by anemia. Anemia associated with severe malnutrition is the result of multiple factors, including inadequate food intake, infection, and dietary

Table 6 Nutritional profile of case versus control

Particulars	Case (N = 211)	Control (N = 211)	*p* value
	Mean ± SD		
B12 (pmol/L)	132.54 ± 28.75	134.61 ± 70.75	0.0001
Folic Acid (nmol/L)	14.67 ± 2.58	15.11 ± 2. 21	0.0606
Serum Ferritin (ng/mL)	28.71 ± 3.81	110.25 ± 9.43	0.0001

imbalance. As a result of a decrease in lean body mass and an adaptation to reduced metabolic oxygen requirements, red blood cells may change. The low values of MCV, MCH and MCHC demonstrate iron deficiency while high level of MCV and MCH indicate vitamin B12 deficiency. Macrocytic anemia is associated with iron deficiency while vitamin B12 deficiency is associated with macrocytosis. Leucocytosis in these children may be caused by infection. Despite the wide array of diseases associated with SAM, the two most common co- morbidities, i.e. diarrhoea and pneumonia, should be managed aggressively and appropriately at the time of hospitalization in order to reduce the high mortality rate associated with SAM. Hypopigmentation of the hair, hyperpigmentation of knuckles, failure to thrive, and generalized hypotonia are symptoms of vitamin B12 deficiency. Predisposing factors are delayed weaning and inappropriate complementary feedings. Vitamin B12 deficiency can cause severe anemia and irreversible neurological deficits if not diagnosed and treated early. Prevention measures such as dietary management and vitamin B12 supplementation must be emphasized. The results of our study support the need for a comprehensive public health strategy to control anemia among Indian children as well as to provide iron supplements. We found similar results to those of Yaikhomba et al. [16]. In case and control groups, serum ferritin was 22.93 ± 2.21 nmol/L and 110.25 ± 9.43 ng/mL, respectively, and there was a statistically significant difference (p 0.0001). A similar finding was reported by Vaid et al. [4]. The nutritional status of the cases versus the controls is shown in Table 6.

Conclusion

Compared to controls, hemoglobin levels and hematocrit levels were lower in children with SAM. Children with SAM also had lower RBC counts, MCV, MCH, and MCHC. When MCV, MCH, and MCHC levels are below the reference level, it indicates iron deficiency; when they are above the reference level, it indicates vitamin B12 deficiency. Low RBC count can be caused by deficiency of iron, vitamin B12, folate, or hemolysis. Lower level of HCT was also indicative of the same. There was a significant difference in leucocytosis among children with SAM compared to controls in the present study. The prevalence of WBC

deficiency was indicating immunosuppression/viral infection. The WBC level of the children was above the cutoff level that indicated inflammation or infection. Anemia was found to be a common co-morbid condition in nearly all patients with severe acute malnutrition. The majority of patients had moderate to severe anemia. Microcytic anemia is more common in SAM children than macrocytic anemia. Iron deficiency and B12 deficiency are more common in SAM children. A severe deficiency of vitamin B12 was more commonly associated with severe anemia. Children with SAM were more likely to have diarrhea, pneumonia, acute gastroenteritis, and acute respiratory infections. A further study is needed to determine prognostic markers of post-discharge malnutrition among children with complicated severe acute malnutrition. Plasma proteomic profiles may be used to predict post-discharge outcomes in children who have been medically treated for complicated SAM and nutritionally stabilized. The malnutrition of mothers contributes to the deficiencies of vitamin B12 in infants, so it is necessary to measure maternal nutritional status, including B12, Folic Acid, and iron levels for a better correlation of nutritional deficiency.

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Declarations

Conflict of interest None.

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