Diagnosis, Treatment and Prevention of Nutritional Anemia in Children: Recommendations of the Joint Committee of Pediatric Hematology-Oncology Chapter and Pediatric and Adolescent Nutrition Society of the Indian Academy of Pediatrics

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Justification: Anemia in children is a significant public health problem in our country. Comprehensive National Nutrition Survey 2016-18 provides evidence that more than 50% of childhood anemia is due to an underlying nutritional deficiency. The National Family Health Survey-5 has reported an increase in the prevalence of anemia in the under-five age group from 59% to 67.1% over the last 5 years. Clearly, the existing public health programs to decrease the prevalence of anemia have not shown the desired results. Hence, there is a need to develop nationally acceptable guidelines for the diagnosis, treatment and prevention of nutritional anemia.

Objective: To review the available literature and collate evidence-based observations to formulate guidelines for diagnosis, treatment and prevention of nutritional anemia in children.

Process: These guidelines have been developed by the experts from the Pediatric Hematology-Oncology Chapter and the Pediatric and Adolescent Nutrition (PAN) Society of the Indian Academy of Pediatrics (IAP). Key areas were identified as: epidemiology, nomenclature and definitions, etiology and diagnosis of iron deficiency anemia (IDA), treatment of IDA, etiology and diagnosis of vitamin B12 and/or folic acid deficiency, treatment of vitamin B12 and/or folic acid deficiency anemia and prevention of nutritional anemia. Each of these key areas were reviewed by at least 2 to 3 experts. Four virtual meetings were held in November, 2021 and all the key issues were deliberated upon. Based on review and inputs received during meetings, draft recommendations were prepared. After this, a writing group was constituted which prepared the draft guidelines. The draft was circulated and approved by all the expert group members.

Recommendations: We recommend use of World Health Organization (WHO) cut-off hemoglobin levels to define anemia in children and adolescents. Most cases suspected to have IDA can be started on treatment based on a compatible history, physical examination and hemogram report. Serum ferritin assay is recommended for the confirmation of the diagnosis of IDA. Most cases of IDA can be managed with oral iron therapy using 2-3 mg/kg elemental iron daily. The presence of macro-ovalocytes and hypersegmented neutrophils, along with an elevated mean corpuscular volume (MCV), should raise the suspicion of underlying vitamin B12 (cobalamin) or folic acid deficiency. Estimation of serum vitamin B12 and folate level are advisable in children with macrocytic anemia prior to starting treatment. When serum vitamin B12 and folate levels are unavailable, patients should be treated using both drugs. Vitamin B12 should preferably be started 10-14 days ahead of oral folic acid to avoid precipitating neurological symptoms. Children with macrocytic anemia in whom a quick response to treatment is required, such as those with pancytopenia, severe anemia, developmental delay and infantile tremor syndrome, should be managed using parenteral vitamin B12. Children with vitamin B12 deficiency having mild or moderate anemia may be managed using oral vitamin B12 preparations. After completing therapy for nutritional anemia, all infants and children should be advised to continue prophylactic iron-folic acid (IFA) supplementation as prescribed under Anemia Mukt Bharat guidelines. For prevention of anemia, in addition to age-appropriate IFA prophylaxis, routine screening of infants for anemia at 9 months during immunization visit is recommended.

Key words: *Cobalamin; Deficiency; Folic acid; Hemoglobin; Iron; Vitamin B12*.

Nutritional anemias develop when the hema topoietic nutrients required for hemoglobin synthesis and/or maintenance are insufficient to meet the demands of an individual. The vast majority of nutritional anemias are due to the deficiency of iron, vitamin B12 (cobalamin, Cbl) and/or folic acid [1]. In addition, the role of other nutrients like vitamin D, vitamin A, vitamin C, pyridoxine and proteins in erythropoiesis is being recognized [2].

The statistics from the National Family Health Survey (NFHS-5; 2019-21) have raised a red flag highlighting the rising prevalence of anemia across all ages [3]. The highest spike in anemia was reported among children (6-59 months) with a rise to 67.1% (NFHS-5) from 58.6% (NFHS-4, 2015-16) followed by girls aged 15-19 years [4]. The Comprehensive National Nutrition Survey (CNNS) conducted in 2016-2018, revealed that 41% of preschoolers (1-4 years), 24% school-age children (5-9 years) and 28% of adolescents (10-19 years) in India have anemia [5]. The etiology of anemia was nutritional in 68.9%, 50.9% and 65.1% in children aged 1-4 years, 5-9 years and 10-19 years, respectively [5]. Iron deficiency was common in under-five children, while folate and vitamin B12 deficiency was higher among school going and adolescent age groups. Folate or vitamin B12 deficiency anemia accounted for more than a third of anemia in these three age groups, and 10-18% of children and adolescents with anemia had combined iron and folate or vitamin B12 deficiency [4].

Deficiency of hematopoietic micronutrients not only results in anemia but also leads to impairment of cognitive function in children, which affects learning. Unfortunately, these changes may be irreversible in younger children emphasizing the need for timely prevention, diagnosis and treatment. Additionally, nutritional anemias have a deleterious impact on the physical strength of the individuals as well as decreased productivity and economic loss to the country [6,7].

In the majority of children with nutritional anemia, diagnosis is straightforward and can be established with minimum diagnostic workup. Occasionally, in children with co-existing chronic inflammatory states and chronic illnesses like chronic kidney disease, the diagnosis can be challenging [8]. Several tests have been added to our armamentarium that can assist in the accurate diagnosis, but these must be used judiciously. In addition, the role of newer oral and parenteral preparations of iron and vitamin B12 for treatment needs to be clearly defined. Taking cognizance of the above, a need was felt to develop guidelines for diagnosis, treatment and prevention of nutritional anemias in children.

OBJECTIVE

To review the available literature and amalgamate evidence-based observations to formulate guidelines for diagnosis, treatment and prevention of nutritional anemia in children.

PROCESS

These guidelines are a joint venture of the Pediatric Hematology-Oncology (PHO) Chapter and the Pediatric and Adolescent Nutrition (PAN) Society of the Indian Academy of Pediatrics. In September 2021, experts from both chapters interested in varying aspects of nutritional anemia were invited to join the group. Task groups were constituted to address the key issues, including *i*) Need for guidelines; *ii*) Epidemiology and definitions; *iii*) Nomenclature; *iv*) Etiology and diagnosis of iron deficiency anemia (IDA); *v*) Treatment of IDA; *vi*) IDA not responding to oral therapy; *vii*) Etiology and diagnosis of vitamin B12 and folate deficiency; *viii*) Treatment of folate deficiency and vitamin B12 deficiency anemia; and *ix*) Prevention of nutritional anemia. Two to three experts were assigned each key issue and were required to review the relevant literature, including the recent publications in the related field.

Online meetings were organized from 15 to 18 November 2021, wherein the experts assigned the individual tasks made a presentation. The group discussed each topic and the presenting faculty was then asked to make modifications based on the inputs received during deliberations. The revised versions were discussed amongst the group members for any further modifications. The group prepared the final document with recommendations for each assigned topic. The level of evidence (LoE) of each recommendation was graded as per the Oxford Centre for Evidence-Based Medicine (OCEBM) 2011 Guidelines [9]. The final draft guidelines were circulated to all the committee members for comments, modifications and final approval. The statements below are the consensus recommendations of the expert group.

Scope: While formulating these guidelines, the primary focus is the health benefits resulting from treatment and preventive nutrient supplementation. The age group under focus is from 6 months to 18 years. The recommendations are easy to understand and apply in day-to-day clinical practice. In smaller towns and rural areas, investigations may not be available. These guidelines aim to guide the management of children with suspected nutritional anemia in areas where facilities for laboratory diagnosis are scant. It is expected that these guidelines will enable the medical officers, pediatricians, and post-graduate trainees to manage children and adolescents with nutritional anemia scientifically. These guidelines are intended as an additional shot in the arm for tackling the increasing prevalence of nutritional anemia in children.

RECOMMENDATIONS

1. Definitions and Hemoglobin Cut-offs

The World Health Organization (WHO) suggests the use of hemoglobin cut-off levels below two standard deviations (SD) from the population mean (in a representative healthy population) to define anemia and its severity [10] as shown in **Table I**. However, whether these cut-offs are representative and applicable for children residing in low-income and middle-income countries (LMIC) is being questioned. It is argued that cut-off levels for defining anemia in children in LMIC, including in India, may be lower than the WHO cut-offs [11]. Using Comprehensive National Nutrition Survey (CNNS) 2019 data, Sachdeva, et al. [11] have recently published age and sex-specific hemoglobin percentiles. For these percentiles, the authors included only those children from CNNS for whom the serum levels of ferritin, folate, vitamin B12, and retinol were normal. They also excluded children with elevated C-reactive protein (CRP), variant hemoglobin and a history of smoking. The hemoglobin cut-offs thus derived are 1-2 g/dL lower than the WHO suggested cut-offs at all ages [12]. Although this data used by the authors is representative of the population, it has not yet been adopted by the national public health programs

Nutritional anemia is classified based on the morphological features of red blood cells (RBCs). Cases with iron deficiency have microcytic anemia and are characterized by RBCs with low mean corpuscular volume (MCV). The lower limit of MCV (fL) in children below 2 years is 70 fL (**Table II**). For children between 2-10 years, a lower limit of MCV is 70 plus age in years. In older children and adolescents (≥10 years), an MCV cut-off value <80 fL can be used to define microcytosis, as for adults. Anemia resulting from deficiency of vitamin B12 or FA is characterized by large RBCs (high MCV). The cut-off value for MCV to define the upper limit of MCV in children aged 2-10 years is 84 plus 0.6 X Age (years). Beyond 10 years, the upper limit of MCV to diagnose macrocytic anemia is 90 fL [13]. Not infrequently, children are described to have dimorphic anemia when deficiency of iron coexists with deficiency of vitamin B12 and/or folic acid resulting in two populations of RBCs seen on examination of peripheral smear [14]. RBC histogram on the electronic cell counter will show two peaks [15].

Table I Hemoglobin Thresholds (g/dL) to Define Severity of Anemia as per the World Health Organization

	Non-anemic	*Mild*	*Moderate*	*Severe*
Children 6-59 mo	>11	10-10.9	7-9.9	<7
Children 5-11 y	>11.5	11-11.4	8-10.9	<8
Children 12-14 y	>12	11-11.9	8-10.9	<8
Men aged ≥15 y	>13	11-12.9	8-10.9	<8
Women aged ≥15 y	>12	11-11.9	8-10.9	<8
Pregnant women	>11	10-10.9	7-9.9	<7

Recommendation

The group recommends using the existing hemoglobin cut-offs (age- and gender-specific) provided by the WHO until more reliable population-based age and gender-specific hemoglobin nomograms become available. **(LoE2)**

2. Iron Deficiency Anemia

2.1 Diagnosis of Iron Deficiency Anemia

In most clinical situations, a presumptive diagnosis of IDA can be made based on dietary history, clinical features and the peripheral blood picture suggestive of microcytic hypochromic anemia with anisopoikilocytosis. Unlike thalassemia trait, basophilic stippling is only rarely seen. The classical triad of low MCV, low mean corpuscular hemoglobin (MCH) and low mean corpuscular hemoglobin concentration (MCHC) for age is consistent with

Table II Cut-offs for Laboratory Estimates for the Diagnosis of Nutritional Anemia

Parameter	*Cut-off*
Microcytosis, MCV (fL)	
Upto 2 y	<70
2-10 y	<70 plus age (years)
>10 y	< 80
Macrocytosis, MCV (fL)	
2-10 y	>84 plus 0.6 X Age (years)
>10 y	>90
Serum ferritin (µg/L)	
<5 y	< 12 (IDA)
>5 y	< 15 (IDA)
With infection	< 30 (IDA)
Serum transferrin saturation	<16% (IDA)
Reticulocyte hemoglobin content (CHr) (pg)	<29 (IDA)
% hypochromic cells	>5% (IDA)
Free erythrocyte protoporphyrin level (FEP) (µg/dL)	
<5 y	>70 (IDA)
>5 y	>80 (IDA)
Serum folate (ng/mL)	<4 (Folate deficiency)
RBC folate (ng/mL)	<100 (Folate deficiency)
Vitamin B12 (pg/mL)	< 200 (Vitamin B12 deficiency)
Homocysteine (mol/L)	>15 (Folate deficiency)
Methyl Malonic Acid (nmol/L)	>750 (Vitamin B12 deficiency)

IDA–iron deficiency anemia, MCV-mean corpuscular volume.

the diagnosis of IDA. The diagnosis is often confirmed in a clinical setting by assessing response to empirical iron therapy [16,17]. Investigations to establish the diagnosis of IDA become necessary when an alternative diagnosis cannot be excluded clinically and in children who fail to respond to iron therapy. In such situations, the following investigations are useful:

Serum ferritin: Serum ferritin is a reliable indicator of body iron stores. It is the earliest marker of iron deficiency and has been widely recommended as the initial investigation. A systemic review of guidelines on diagnosis and treatment of iron deficiency observed that serum ferritin assay is recommended for diagnosis of IDA by all 22 guidelines included in this review [18]. However, the recommended cut-off of serum ferritin to diagnose ID is variable across these guidelines. WHO also strongly recommends using serum ferritin to diagnose IDA [19]. Serum ferritin <12 µg/L in children under-five years of age and serum ferritin <15 µg/L in individuals over 5 years of age in the absence of any active inflammation are suggestive of IDA. Similar cut-offs have also been recommended more recently by the British Society of Hematology [20]. In the presence of infection or inflammation, serum ferritin <30 µg/L is suggestive of IDA in children [19].

Serum iron, total iron binding capacity (TIBC) and tranferrin saturation (TS): Iron studies are recommended when serum ferritin results are equivocal. Serum iron levels are low in iron deficiency and IDA. However, there is a day-to-day variability in iron levels as they are influenced by recent intake. Hence, estimation of serum iron alone in the diagnostic workup of IDA is not recommended. It is estimated to calculate TS or TIBC [20]. Ten of the 22 guidelines in the systematic review recommended using TS as an alternative or complimentary to the estimation of serum ferritin [18]. The recommended threshold of TS to diagnose iron deficiency is <16% in young adults, although age-specific cut-offs can be used in children where the cut-offs used are slightly higher than adults [18,20,21].

Newer red cell indices: Since the lifespan of reticulocytes is very short, the measurement of reticulocyte hemoglobin helps determine the availability of iron to form hemoglobin. Additionally, unlike serum ferritin, reticulocyte hemoglobin is not affected significantly by inflammation. Reticulocyte hemoglobin is measured by two methods, viz, reticulocyte hemoglobin content (CHr), and reticulocyte hemoglobin equivalent (Ret-HE). An acceptable correlation has been demonstrated between Ret-HE and CHr in multiple clinical studies. Ret-HE has gained popularity for diagnosing iron deficiency and IDA and evaluating a patient's response to oral iron treatment [20,22,23]. Since CHr and Ret-HE reflect the hemoglobin content in reticulocytes, low values reliably indicate early iron-deficient erythropoiesis prior to the onset of anemia, serving as the earliest indicators of IDA. Although there are no standardized cut-offs of Ret-HE to determine IDA, commonly recommended cut-off in children vary between 25-30 pg [20]. A cut-off of <29 pg has been recommended by the British Society of Haematology for diagnosing IDA in children [20]. These indices can also be used to assess the response to the treatment being one of the earliest parameters to increase in response to treatment. It needs to be borne in mind that Ret-HE and CHr are also low in children with thalassemia trait [11,12].

Another novel parameter is the percentage of hypochromic red cells (Hypo%), which reflects iron status over the preceding 3 months. It may be useful for distinguishing thalassemia trait from ID. More than 5 % hypochromic red cell is the cut-off for defining iron deficiency [20]. Hypo% is also a valuable parameter to diagnose functional iron deficiency, i.e. inadequate incorporation of iron in the erythroid precursors despite having adequate iron stores as per the ferritin or bone marrow iron stores. This is frequently seen in chronic kidney disease [24].

Soluble transferrin receptor (sTfR): Soluble transferrin receptors (sTfR) are derived from actively developing red cells and reflect active erythropoiesis. A cut-off of sTfR >27.3 nmol/L has been proposed to detect IDA. A low ratio of sTfR/serum ferritin can help distinguish IDA from anemia of chronic disease [25]. However, due to poor sensitivity in early and intermediate iron deficiency, this test is routinely not recommended (20, 26).

Free erythrocyte protoporphyrin level (FEP): As iron deficiency limits the final step in heme synthesis, there is an accumulation of FEP in the red cell precursors. FEP is elevated in iron deficiency and rapidly falls as a response to treatment. The cut-off level of FEP is >80 µg/dL for children over 5 years of age and >70 µg/dL for children younger than 5 years of age [16]. Spuriously high levels of FEP are reported in the presence of hyperbilirubinemia [27]. Moreover, FEP is raised in thalassemia trait and hemoglobin E disease, two conditions common in our country [28]. Recent guidelines have not recommended its use for diagnosing iron deficiency [20].

Bone marrow studies: We do not recommend bone marrow aspiration and staining for iron using Perls Prussian blue stain, as the test is invasive and is rarely justifiable as a battery of non-invasive tests are available to assist the diagnosis [17,18].

Recommendations

- In most clinical situations, a presumptive diagnosis of

IDA can be made based on red cell indices and the blood picture suggestive of microcytic hypochromic anemia (**Table II**). The classical triad of low MCV, low mean corpuscular hemoglobin (MCH) and low mean corpuscular hemoglobin concentration (MCHC) for age is consistent with the diagnosis of IDA. Response to iron therapy should be documented for confirmation of diagnosis (**LoE 2**).

- Serum ferritin should be the first test to diagnose ID/ IDA where investigative workup is indicated (**LoE 1**).
- In situations where serum ferritin results are equivocal, transferrin saturation may be used (**LoE 2**).
- CHr and Ret-HE, and percentage of hypochromic cells, wherever available, can be used to diagnose IDA (**LoE 1**).

2.2 Treatment of Iron Deficiency Anemia

The treatment of IDA aims at providing iron therapy in the appropriate dose, formulation and duration to restore hemoglobin to normal range and replete body iron stores. The treatment of IDA should also be comprehensive and should include the identification of any secondary cause of ID and its management (this is beyond the scope of these guidelines). Treatment must also address dietary modifications, if required, and a periodic follow-up for assessment of therapeutic response. High quality evidence for the management of iron deficiency is limited in adults as well as in children.

2.2.1 Route of Iron Therapy

Oral iron therapy is the route of choice almost always. Oral iron may be initiated based on history, hemoglobin, MCV, and peripheral smear where possible, without the need for biochemical investigations. It is safe, effective, economical and leads to rapid improvement in hemoglobin if administered in the correct dose and followed up appropriately. It is convenient for parents and is well tolerated by almost all patients. Parenteral iron is rarely ever required in children.

Oral iron preparations: Oral iron preparations are available mainly in ferrous and ferric forms; the ferrous form is better absorbed [29]. The three most common ferrous iron preparations viz., ferrous sulphate (FS), ferrous fumarate and ferrous gluconate provide 20% (anhydrous 30%), 33% and 12% elemental iron, respectively. Elemental iron is the form of iron in the supplement that is available for absorption by the body. While prescribing oral iron, the amount of elemental iron in the preparation should be noted, and the dose should be calculated accordingly. All these three oral iron formulations have essentially equivalent bioavailability [30, 31]. Iron polymaltose complex (IPC) and ferrous ascorbate are other available iron preparations. The use of iron polymaltose complex (IPC) over ferrous iron preparations does not offer any therapeutic benefit. Patil, et al. [32] have demonstrated higher hemoglobin rise with comparable doses of ferrous ascorbate compared to IPC with a similar side effect profile. Ferrous sulphate has also shown an advantage over IPC in children with IDA in terms of therapeutic efficacy [33]. In another study, IPC was found to be inferior to iron bisglycinate for treating IDA [34]. Ferrous ascorbate has also shown better therapeutic efficacy compared to colloidal iron in children with IDA [35]. Given a similar adverse effect profile with lesser cost, FS may be preferred to IPC and ferrous ascorbate. Entericcoated and delayed-release iron supplements were developed to improve the compliance as their gastrointestinal side effect profile is better. However, they are not absorbed as well as the standard preparations because the release of iron occurs much below the site of absorption and these preparations are expensive [31]. The fractional iron absorbed from enteric-coated preparations is significantly less than from uncoated preparations [36]. **Box 1** summarizes the various iron compounds used to treat IDA in children.

2.2.2 Dose and schedule of oral iron therapy

The recommended therapeutic dose of iron for children is usually 3-6 mg/kg/day [37,38]. However, it has been noted that doses of 2-3 mg/kg/day of elemental iron are also efficacious and can improve patient compliance as they reduce the side effects such as abdominal pain and constipation. Powers, et al. [39] have recently shown a very good hematological response using a 3 mg/kg dose. The British Society of Gastroenterology (BSG) has recommended the beginning dose of oral iron for adults as one tablet of iron sulphate, fumarate, or gluconate, which usually has 66 mg elemental iron or less [31]. This, for an average weight adult, would mean about 1-1.5 mg/ kg.

Whether iron should be administered as a single dose or in divided doses has conflicting evidence. Some authors [40] have demonstrated better absorption with a single morning dose and alternate-day dosing, while others [41] have demonstrated more intense reticulocytosis and better reticulocyte hemoglobin in patients receiving a twice-daily dose. Another study demonstrated equal efficacy of once or thrice daily iron dosing [42]. Overall, emerging evidence supports the treatment of IDA with a single daily dose of iron. The administration as a single dose ensures long-term compliance and administration of the drug in the evening atleast one and a half to two hours after dinner improves gastrointestinal tolerance [31]. Alternate day administration may be advised in cases with intolerance, although it may

Box I Various Oral Iron Formulations

Ferrous vs Ferric

1. All iron salts have to be reduced to ferrous form to enter mucosal cells. Bioavailability of ferric preparations is 3 to 4 times less than that of conventional ferrous preparations. Hence, ferrous salts are preferred.
2. Ferrous salts are among the cheapest preparations available

Ferrous sulphate

1. 20% elemental iron
2. Mainly as tablet forms; syrup form or elixirs (in sorbitol base) are not stable

Ferrous fumarate

1. 33% elemental iron
2. Similar efficacy and gastrointestinal tolerance to ferrous sulphate, more stable, tasteless
3. Less soluble in water / soluble in mild acid- gastric juice

Iron-Amino acid chelates

1. Ferric or ferrous ion with amino acid conjugates
   - Ferrous bisglycinate (20% elemental iron)
   - Ferric trisglycinate
   - Ferrous glycine sulphate (India)
2. No effect on colour or taste of food
3. Relatively high bioavailability in the presence of dietary inhibitors
4. Environmentally stable
5. Chance of toxicity on overdose less

Iron Polymaltose complex (IPC)

1. Non-ionic iron and polymaltose in stable complex
2. Equivalent bioavailability to FS
3. Absorption better when taken with meals
4. No teeth staining
5. Poisoning risk less as intestinal transport gets saturated at higher dose
6. Slower improvement in hemoglobin

result in a slower response. Absorption of the drug is better if given on an empty stomach, but oral iron is better tolerated after a meal [43]. Iron should not be administered with milk, curd, calcium syrup/ tablets and is best given with water if needed. Tea, coffee, and drugs interfering with absorption, such as proton pump inhibitors and antacids should be avoided with iron.

2.2.3 Duration of iron therapy

Recent BSH guidelines highlighted that duration required to replenish the body iron store is unclear. They have persisted with 'traditional' 2-3 months after hemoglobin normalizes [31]. Continued iron therapy in a therapeutic dose for this period allows replenishment of stores so that iron-restricted erythropoiesis is resolved [38].

We recommend that iron therapy be continued for 2-3 months after hemoglobin normalizes. The group recommends that the family/ caregiver be educated about the need for continuing iron supplementation after correcting anemia. Before stopping the therapeutic dose, one should ensure that the family feeding practices have been rectified and the cause for secondary iron deficiency, if any, has been ameliorated. Since iron prophylaxis is recommended till adolescence the prophylactic dose should be adminis-tered as recommended by the national program [1].

2.2.4 Assessing response in iron deficiency anemia

After initiating therapy, the first response expected is a decrease in irritability, lethargy and a sense of well-being. Appetite generally improves within 24 hours [37]. Compared to mild and moderate anemia, the rise in reticulocytes is much higher in severe anemia, as is also seen with the rise in hemoglobin. Peak reticulocyte count is seen on days 5-10 following initiation of iron therapy, and wherever available, reticulocyte response should be assessed in addition to the rise in hemoglobin. Hemoglobin rises on an average by 0.25-0.4 g/dL/day, or hematocrit rises 1%/day during the first 7-10 days. Thereafter, a slower rise of 0.1-0.15 g/dL/day is seen in hemoglobin.

The timing for scheduled follow-up visits also varies depending upon the severity of anemia. Children with severe anemia who do not receive a blood transfusion and are initiated on oral iron therapy need to be called on day 7 and day 14 for clinical evaluation and complete blood count (CBC) or earlier if the child develops any danger signs like edema, fast breathing, lethargy or irritability and any adverse effects such as vomiting or diarrhea. Children with moderate or mild anemia need to be called for a repeat hemoglobin or CBC on day 14 of therapy.

In the case of moderate/severe anemia, once improvement in hemoglobin has been documented, further followup should be done at 2-4 weekly intervals till hemoglobin is >7g/dL, and then monthly. The usual rate of increase in hemoglobin is ≥1 g/dL in 14 days. By 8-12 weeks, hemoglobin rises by 3-5 g/dL and in most cases, anemia is corrected [32,35,44].

Follow-up visits should also be utilized to reinforce the need for food diversity and increased consumption of iron and vitamin C rich food items, including poultry items (like eggs, meat, organ meats like liver), fish, citrus fruits, potatoes and tomatoes. Consumption of tea, phytates (whole grains, cereals, soy, nuts and legumes) and phosphates should be curtailed.

2.2.5 Role of co-administration of cobalamin, folic acid, or vitamin C along with iron

Folic acid is usually a part of all oral iron-containing preparations and hence routinely given to all children with IDA. Oral vitamin B12 may be added where there is a poor response to oral iron alone or in children with combined

vitamin B12 and iron deficiency anemia (dimorphic anemia). Combined deficiency is expected in up to 30% of children with nutritional anemia. The presence of near-normal MCV, dimorphic red cells in the peripheral smear, features of megaloblastic anemia such as hyper-segmented neutrophils, thrombocytopenia, macro-ovalocytes, etc. warrants investigations for associated folate and/or vitamin B12 deficiency. If documented, the addition of folic acid and vitamin B12 in the therapeutic dose is required. Contrary to earlier observations, a randomized clinical trial in adults has shown no benefit of adding vitamin C to iron therapy [45]. Hence, vitamin C is not recommended in pharmaceutical forms. However, the addition of citrus fruits to the diet immediately prior to medication aids in iron absorption.

Recommendations

- We recommend ferrous salts (ferrous sulphate or ferrous fumarate, or ferrous gluconate) for treating IDA in children (**LoE 2**).
- We do not recommend the use of prolonged-release or enteric-coated iron tablets or liposomal iron formulations for the treatment of IDA (**LoE 1**).
- We recommend an oral dose of 2-3 mg/kg/day of elemental iron along with folic acid for treating IDA given for upto 2-3 months after hemoglobin normalizes (**LoE 1**).
- We recommend single daily dosing of oral iron. Divided doses can be given in cases of gastrointestinal adverse effects (**LoE 1**).
- While on treatment, patients with severe anemia should be called for first follow-up on day 7 while patients with moderate and mild anemia should be called for follow-up on day 14 (**LoE 2**).

3. Iron Deficiency Anemia Not Responding to Oral Iron Therapy

Standard texts and review articles have listed causes of failure of an adequate response, but which cases qualify to be labeled as poor responders /non-responders is not well defined [37,38,46]. The absence of hemoglobin rise of less than 1 g/dL after 2 weeks of daily oral iron therapy in adults has been described as predictive of lack of response [31]. Okam, et al. [47], in a pooled analysis of five studies in women, have also defined non-responder as having less than 1 g/dL rise in hemoglobin by day 14 of therapy. Bhatia, et al. [48,49], while evaluating Indian children for iron refractory iron deficiency anemia (IRIDA), have used criteria of less than 1 g/dL rise in hemoglobin after 4 weeks of daily iron therapy to define non-response, as has been used by others as well. While most cases with moderate and severe anemia will have hemoglobin rise of 1 g/dL or more by day 14, it may not be so for cases with mild anemia.

3.1 Evaluation of Non-responders

Ensuring compliance to therapy with adequate dosages should be an integral part of follow-up evaluation and a pre-requisite before labeling non-response to oral iron therapy. A detailed review of dietary history and any intercurrent illnesses that might interfere with iron absorption should be done. The following points should be revisited:

i) Conditions interfering with iron absorption like celiac disease, *Helicobacter pylori* infection, autoimmune gastritis, or anemia of chronic disease should be ruled out by appropriate testing as indicated in a given child [50, 51].

ii) Patients should be evaluated for gastrointestinal blood loss: Three consecutive stool samples should be evaluated for occult blood. Cow milk protein intolerance, Meckel diverticulum and inflammatory bowel disease should be considered in case of a positive stool occult blood [52-54].

iii) Evaluation of other causes of microcytic hypochromic anemia: Celiac disease is a common cause of refractory IDA in children in certain states. All cases of refractory IDA should be screened for celiac disease using tissue transglutaminase antibodies (tTG) along with serum IgA, whenever suspected [55]. Children with betathalassemia trait (BTT) have a blood picture of microcytic hypochromic anemia and can mimic IDA. BTT and IDA can be differentiated by following investigations [56,57]:

- RBC count >5 million/mm^3 suggests BTT, RBC count< 5 million/mm^3 suggests IDA
- Red cell distribution width co-efficient of variation (RDW CV) <14% BTT, RDW CV >14% in IDA
- Mentzer index (MCV divided by RBC count) is <13 in BTT and >13 in IDA
- High performance liquid chromatograhy will confirm the diagnosis of BTT by showing elevated hemoglobin A2 (>3.5 %).

iv) Anemia of chronic disease (ACD) is usually normocytic normochromic. However, in some cases, it may be microcytic hypochromic. In a patient where a chronic infection is suspected, the diagnostic evaluation needs to be directed towards the diagnosis of the underlying disease [58]. Additionally, in children with suspected ACD evaluate for underlying chronic renal disease or connective tissue disease.

v) Iron refractory iron deficiency anemia (IRIDA) is a rare inherited disorder in which absorption of oral iron is markedly impaired. IRIDA is caused by loss of function of the *TMPRSS6/matriptase 2* gene, which causes iron deficiency due to inappropriately high hepcidin levels with markedly reduced iron absorption and increased sequestration of iron in macrophages. Patients present with a mild hypochromic, microcytic anemia with very low serum iron levels and low transferrin saturation. Serum ferritin levels are mostly within the normal range or even slightly elevated following treatment with intravenous iron. The diagnosis of IRIDA is confirmed by demonstrating biallelic mutation in the *TMPRSS6* gene. These patients can be treated with a trial of intravenous iron therapy [49,59].

3.2 Parenteral Iron Therapy

Most cases with IDA can be successfully managed by oral iron supplementation. However, in some situations, parenteral iron therapy is required (see below). As the new intravenous (IV) iron formulations are devoid of major adverse effects, these are the preferred preparations for parenteral iron therapy [47]. The intramuscular route is not recommended as it is painful and can lead to staining of the overlying skin.

3.2.1 Indications for parenteral iron therapy

The conditions where the use of intravenous iron may be required [7,38,47] are noted in **Box II**. In a retrospective review, amongst children aged 3 months to 18 years requiring intravenous iron in a US hospital, most cases (73.8%) were related to kidney diseases. Of the remaining 38 cases, 13 were unresponsive to oral iron due to poor compliance or side effects, 13 due to malabsorptive states, seven due to ongoing blood losses, and two had IRIDA [60].

Box II Conditions Where the Use of Intravenous Iron May be Required [37,38,47]

- Poor adherence or intolerability due to gastro-intestinal side effects of oral iron
- Need for rapid replenishment of hemoglobin and iron stores rather than over the course of several months, e.g., in preoperative situations
- Ongoing blood loss that exceeds the capacity of oral iron to meet needs (heavy uterine bleeding, mucosal telangiectasias).
- Iron malabsorption due to pre-existing anatomic or physiologic condition, e.g., short bowel syndrome
- Coexisting inflammatory state that interferes with iron homeostasis
- Chronic kidney disease
- Genetic forms refractory to oral iron (IRIDA etc.)

3.2.2 Preparations of intravenous iron

The following preparations of iron are available for parenteral use:

- *Iron sucrose*: It is the most common form of IV iron used in children [60]. Adverse events, including anaphylaxis, are rarely reported, so a test dose or routine pre-medications are not indicated. The recommended dose ranges from 1-4 mg/kg elemental iron IV infusion over 1 hour every week with a maximum dosing of 200 mg elemental iron per infusion for adolescents and 100 mg of elemental iron per infusion for children. Most patients require multiple infusions to complete the replacement of their calculated iron deficit.
- *Ferric gluconate*: It is approved for children with chronic kidney disease on dialysis and erythropoietin-stimulating agents aged ≥6y. No test dose or routine premedications are indicated. The maximum dose is 125 mg elemental iron per infusion. Adverse events are rarely reported.
- *Iron dextran*: Low-molecular weight (LMW) iron dextran is commonly given as a single replacement dose (e.g., up to 1000 mg elemental iron in adults).
- *Ferric carboxymaltose* (FCM): It is increasingly used for adults who are intolerant to oral iron therapy and also permits administration of the full replacement dose in a single infusion in the majority of patients. Data in the pediatric population is limited but promising [61]. Hypophosphatemia is a common complication with its use. It is used in a dose of 15mg/kg single rapid (over 15 minutes) intravenous infusion without any test dose.

United States Food and Drugs Administration (FDA) has approved low molecular weight iron dextran, ferric gluconate, iron sucrose and FCM for use in children [62]. The use of iron dextran for intravenous iron therapy is no longer recommended and it has been discontinued. Iron sucrose, FCM and ferric derisomaltose (isomaltoside), are approved for treatment of iron deficiency in India.

3.2.3 Dose calculation for parenteral iron therapy

Hemoglobin iron deficit (mg) = Body weight (kg) X (Ideal hemoglobin – desired hemoglobin) X (2.145) + iron to replenish stores, if desired (mg).

Volume of product required (mL) = Bodyweight X (14 - hemoglobin) x (2.145) ÷ Concentration of elemental iron in the product used (mg/mL) [62-64].

For example, a 6-year-old child weighing 20 kg with hemoglobin of 6 g/dL would need 343.2 mg of elemental iron, equivalent to 1.7 mL of iron sucrose injection

containing 200 mg/mL of elemental iron (20 X 8 X 2.145 ÷ 200). This child will be given 343.2 mg of elemental iron in four divided weekly doses; 0.43 mL iron sucrose dissolved in 100-200 mL of normal saline and infused over 1-3 hours every week X 4 weeks.

An intravenous dose of iron for undernourished children should be calculated with present weight, while for obese patients, ideal/lean body weight should be used for calculation. Lean body weight can be calculated by the formula: Lean body weight = BMI_{50} X Ht^2 [65].

3.2.4 Precautions

Routinely no premedication is indicated. Patients with comorbidity of bronchial asthma, drug allergy and inflammatory arthritis require premedication with 1-2 mg/kg of parenteral methylprednisolone before intravenous iron administration.

3.2.5 Response to parenteral therapy

Subjective improvement in uncomplicated cases occurs within a few days of intravenous therapy. Reticulocytosis peaks at about 10 days. Symptomatic improvement occurs in 1-2 weeks parallel to a rise in hematocrit. However, the complete response may take approximately four to six weeks after the dose is given. The follow-up evaluation should be done between six to eight weeks after administration because intravenous iron interferes with most iron studies [66].

Recommendations

- We recommend that children with moderate to severe iron deficiency anemia who do not show an increase in hemoglobin of 1 g/dL from baseline at the end of two weeks and those with mild anemia not showing Hemoglobin rise by 1 g/dL at 4 weeks of supplementation should be evaluated for non-response to oral iron (**LoE 2**).
- In situations when parenteral therapy is indicated, we recommend intravenous iron therapy. Intramuscular and transdermal administration is not recommended (**LoE 2**).
- We recommend iron sucrose, FCM and ferric derisomaltose (isomaltoside) preparations for parenteral use (**LoE 1**).
- We recommend that follow-up evaluation be done between six to eight weeks after intravenous iron administration (**LoE 2**).

4. Macrocytic Anemia of Nutritional Etiology

Macrocytic anemia can be attributed to both vitamin B12 (cobalamin, Cbl) and folic acid deficiency in children. Vitamin B12 deficiency is more common than folic acid deficiency. Vitamin B12 deficiency is more common in infants born to vitamin B12 deficient mothers and in adolescents [67-69]; the problem is widespread in the Indian population as the majority are vegetarians.

Infants deficient in vitamin B12 may present with developmental delay, tremors, or infantile tremor syndrome. Neurologic symptoms include paresthesias, hypotonia, seizures, sensory deficits, developmental delay or regression, irritability, or cognitive delay [70,71]. Severe deficiency may cause bleeding manifestations due to thrombocytopenia. Mild enlargement of liver and/or spleen may be found. Thus, these patients may mimic cases with acute leukemia and aplastic anemia [72]. Dermatological manifestations like hyperpigmentation of knuckles, icterus and angular stomatitis are commonly seen in children with underlying vitamin B12 deficiency. These may occur even before the development of hematological and neurological complications and hence these findings may aid early diagnosis.

4.1 Diagnosis of Macrocytic Anemia

a. Mean corpuscular volume (MCV) cut-off

Macrocytosis is a common finding in vitamin B12 deficiency. MCV is only a screening test (see **Table II**).

b. Peripheral smear findings

Peripheral smear shows the presence of macrocytes/macro-ovalocytes. The presence of hypersegmented neutrophils (≥5 lobed nuclei) in more than 5% of neutrophils and leucopenia are commonly seen [73,74]. In case of severe deficiency, thrombocytopenia may be observed. A low reticulocyte is usually seen. With severe anemia, circulating nucleated erythroid precursors, Cabot rings, and Howell Jolly bodies can appear. Circulating megaloblasts may also be seen [73].

c. Serum folate assay

Serum folate is estimated by an automated chemiluminescent assay. The normal serum folate level is 6-20 ng/mL. The group suggests a level of 4 ng/mL (10 nMol/L) to define folate deficiency as recommended by WHO [75].

d. Red Blood Cell Folate Assay

Red blood cell (RBC) folate content is unaltered after the reticulocyte stage in the maturation of RBC. Hence, it is less susceptible to transient fluctuations in folate levels. It reflects the folate status over the 120 days lifespan of the RBC. RBC folate level below 100 ng/mL suggests folate deficiency [73, 75]. RBC folate may be falsely low in

pernicious anemia while it may be falsely high in hemolytic anemia, IDA and after blood transfusion.

e. Serum Vitamin B12 (cobalamin) Assay

The endogenous forms of vitamin B12 include Cbl and holotranscobalamin (HoloTC), which represents the active fraction of plasma Cbl. Cbl assay quantitates both the 'inactive' forms (transcobalamin I- and transcobalamin III-bound or holohaptocorrin) and the 'active' form (transcobalamin II-bound or holotranscobalamin) of vitamin B12 in serum. Levels between 200 to 300 pg/mL (148 to 221 pmol/L) are considered borderline. Levels >300 pg/mL (>221 pmol/L) are considered normal and exclude significant vitamin B12 deficiency. Levels <200 pg/mL (< 148 pmol/L) are suggested to be used to define vitamin B12 deficiency [73,75]. In the presence of inflammation and malignancies, serum Cbl may be falsely high [73,76]. Cbl levels may be spuriously low in certain conditions like pregnancy, intake of drugs like phenytoin, technical issues, or coexistent folate deficiency [73,77].

The levels described above for defining vitamin B12 or folate deficiency should be cautiously interpreted. The low levels only substantiate the diagnosis, but normal levels do not exclude the diagnosis in children with a consistent clinical picture [72,73].

f. Holotranscobalamin (Holo TC) Assay

The HoloTC assay has better sensitivity and specificity in identifying B12 deficiency than serum Cbl assays. The expected values for HoloTC in healthy individuals are 35-171 pmol/L. A cut-off of less than 35 pmol/L is suggested for defining deficiency [73,74]. Though it has been used in studies, it is not available widely in India.

g. Assessing Functional Deficiency of Vitamin B12 and/ or Folate

Testing for metabolites in vitamin B12 and folate pathways, including homocysteine (Hcy) and serum methyl malonic acid (MMA), are useful to evaluate vitamin B12 /folate deficiency in the following situations:

- Levels of vitamin B12 and/ or folate are normal or borderline low in cases with a consistent clinical profile.
- Discordant laboratory findings and clinical picture (unexplained macrocytic anemia or unexplained neurologic/neuropsychiatric symptoms with low normal laboratory values of B12 and /or folate)

Vitamin B12 is a cofactor in the conversion of methylmalonyl-CoA to succinyl-CoA and a deficiency of vitamin B12 leads to elevated levels of MMA. Both vitamin B12 and folate are required for the metabolism of homocysteine to methionine, and hence deficiency of either can lead to accumulation and elevated levels of homocysteine. In case both MMA and homocysteine are elevated, vitamin B12 deficiency is likely. If MMA is normal and homocysteine is raised, folate deficiency would be more likely [73,74].

The accuracy of Cbl and/or folate levels alone has limitations. Hence, it is recommended to evaluate for functional folate/ vitamin B12 deficiency by estimating serum MMA or Hcy level to confirm a diagnosis of vitamin B12 and/or folate deficiency where the diagnosis is doubtful [73,74]. The normal range of plasma MMA is 70 to 270 nmol/L and of homocysteine is 5 to 15 µmol/L. Plasma total homocysteine is more sensitive, but plasma MMA is more specific. The British Society of Hematology suggests MMA >750 nmol/L is indicative of vitamin B12 deficiency, and Hcys above 15 mol/L could be indicative of folate deficiency [73]. MMA may be spuriously high in children with renal disease, small bowel overgrowth and hemoconcentration. Hcy may also be elevated in hypothyroidism, renal failure and certain genetic polymorphisms. If Hcy and MMA are found to be normal with borderline Cbl levels, no further tests are needed [73,74].

h. Bone Marrow Examination

Bone marrow aspiration (BMA) to prove megaloblastic anemia is not required when the clinical picture, complete blood count (CBC) and peripheral blood findings are diagnostic [73,74]. In the presence of atypical clinical features or a CBC which does not corroborate the diagnosis, it is preferable to perform a bone marrow aspiration prior to initiating therapy to exclude other causes, like aplastic anemia, acute leukemia, or myelodysplastic syndrome, which could mimic megaloblastic anemia [72]. BMA findings do not help distinguish between vitamin B12 and folate deficiency.

i. Investigations for Suspected Pernicious Anemia

Pernicious anemia has hardly ever been reported in children in India. Anti-intrinsic factor antibody (anti-IF Ab) test is used to evaluate pernicious anemia in adults. It has a high positive predictive value (95%) and a high specificity (98-99%). However, sensitivity is only 40-60%, as seen in studies amongst adults. The sensitivity of gastric parietal cell antibody is 80% for the diagnosis of pernicious anemia. However, it is positive in 10% of normal individuals as well. Hence, a positive antibody is not definitive for pernicious anemia [73]. Even in developed countries where nutritional deficiency is rare, pernicious anemia and the presence of anti-IF Ab is rarely reported in children.

j. Tests for Genetic Disorders or Inborn Errors of Metabolism causing Megaloblastic Anemia

Disorders affecting uptake and metabolism of folate or vitamin B12, as well as thiamine-responsive megaloblastic anemia, orotic aciduria, and 3-phosphoglyceride dehydrogenase deficiency, may also cause megaloblastic anemia. These conditions warrant evaluation for specific mutations and biochemical assays which are beyond the scope of these recommendations.

Recommendations

- A peripheral smear with the presence of macrocytes and hypersegmented neutrophils along with an elevated MCV should arouse suspicion of underlying vitamin B12 or folate deficiency (**LoE 2**)
- Cbl and folate assays should be done simultaneously due to the close relationship in metabolism (**LoE 1**)
- We recommend that serum folate levels should be estimated before the RBC folate level (**LoE 1**).
- RBC folate levels need to be estimated if there is a strong suspicion of underlying folate deficiency despite normal folate levels or as corroborative evidence for folate deficiency, wherever available (**LoE 2**).
- Plasma total Hcy and/or plasma MMA should be considered as supplementary tests to diagnose biochemical vitamin B12 deficiency in a suspected clinical situation with borderline serum Cbl or folate level (**LoE 2**).
- We do not recommend routine testing of anti-gastric parietal cell or anti-intrinsic factor antibody (**LoE 1**).

4.2 Treatment of Vitamin B12 and Folic Acid Deficiency Anemia

4.2.1 Starting replacement therapy

Once the clinical features, dietary history and laboratory findings (anemia with raised MCV) corroborate to suggest nutritional macrocytic anemia, replacement therapy should be initiated with vitamin B12 and folic acid. An assay of serum vitamin B12 and folate is advisable prior to therapy. When it is not possible to estimate serum levels, treatment with both vitamin B12 and folic acid should be provided. Vitamin B12 alone should be started in the initial 10-14 days, followed by the addition of folic acid. Folic acid should not be instituted alone if vitamin B12 deficiency has not been excluded, as the neurological symptoms due to vitamin B12 deficiency may precipitate or worsen [78,79]. Treatment of children with dimorphic anemia should include iron, folic acid and vitamin B12.

Treatment regimens in children have not been well studied, and the preferences of the route of administration and dose of vitamin B12 are varied [73,74,80-82]. Currently available guidelines are restricted to the adult population [73].

4.2.2 Route of vitamin B12 administration

Parenteral [(intramuscular (IM) deep subcutaneous (SC) intravenous (IV) infusion) administration of vitamin B12 rapidly and reliably restores the vitamin B12 stores [83]. Parenteral vitamin B12 is preferably administered by IM or deep SC injection as vitamin B12 is rapidly excreted after IV administration. Lately, sublingual [84,85] and intranasal [86] routes of vitamin B12 administration have also been tried in children with vitamin B12 deficiency. Due to the paucity of robust scientific evidence, we do not recommend intranasal and transdermal routes of vitamin B12 therapy in children. Oral vitamin B12 supplements are convenient and cheaper compared to parenteral vitamin B12 and can ensure better compliance in the long term. Evidence in adults suggests that oral vitamin B12 may be given as an alternative to parenteral vitamin B12 with comparable efficacy and safety [87-90].

Comparative studies of oral vs. parenteral vitamin B12 in children are scant, although there are a few prospective studies and case series assessing the response to oral vitamin B12 [91-97]. A comparative group study in children with symptomatic vitamin B12 deficiency showed comparable efficacy of parenteral and oral cyanocobalamin (CN-Cbl) in terms of hematological response measured by a rise in hemoglobin, MCV and hematocrit [98].

As most cases of vitamin B12 deficiency in India are of dietary origin, injectable vitamin B12 should be used only in cases where a fast response is required or compliance with oral medication is not ensured. Faster and assured response will be particularly required in children with neurological manifestations and pancytopenia. Children with severe thrombocytopenia should be treated with IV vitamin B12 as IM administration may lead to hematoma formation. In patients being started on injectable treatment, after the initial response, maintenance therapy with oral vitamin B12 should be considered [83,92,93, 98].

Varied protocols of vitamin B12 supplementation have been tried in children with neurological manifestations of vitamin B12 deficiency using parenteral [99,100] or oral vitamin B12 [93,101], with different doses and duration of therapy. However, due to the need for quick and assured response in children with neurological manifestations, we recommend initial treatment with parenteral vitamin B12 [73,74].

In patients with malabsorption due to irreversible

causes of vitamin B12 malabsorption, including surgical cases, underlying autoimmune conditions, or genetic defects in vitamin B12 absorption and transport, therapy with parenteral vitamin B12 may be preferred [83]. Alternately, as shown by some studies, life-long daily oral or sublingual vitamin B12 may be given to children with irreversible causes of malabsorption [87]. In two separate randomized controlled trials, which included adults with vitamin B12 deficiency due to chronic atrophic gastritis or perniciones anemia, oral vitamin B12 was as effective as parenteral vitamin B12 [88,89].

4.2.3 Dose of vitamin B12

As the route of therapy is varied, so is the dose and duration of vitamin B12 therapy in children depending upon the age (infant, child, adolescent). Daily oral vitamin B12 in varying doses of 100 µg - 1000 µg [73,92,93,95,98] and parenteral vitamin B12 in doses of 25-1000 µg [73,74,80-82] have been used in children for treating vitamin B12 deficiency anemia. Oral vitamin B12 needs to be given in a fasting state as food interferes with the absorption of vitamin B12 [102]. Based on the various studies, our recommendation is:

Oral: Oral vitamin B12 therapy (500 µg in infants, 1000 µg in older children) to be used to treat macrocytic anemia due to dietary vitamin B12 deficiency. This dose may be given every day for a week, every other day for the next week, 2 times a week, once a week, once every 15 days for a month and then once a month to complete at least 3 months duration of therapy [92].

Alternatively, daily oral vitamin B12 therapy (500 µg in infants, 1000 µg in older children) can be given for 3 months [92].

Parenteral: We recommend starting treatment with 25 µg of vitamin B12 given daily by IM (or deep SC or IV) route for the initial 2-3 days. This is followed by 100 µg (50 µg in infants) of parenteral vitamin B12 given daily for the next 7 days (till 3 weeks in children with neurological features), followed by 100 µg vitamin B12 IM/deep SC/IV on alternate days for next 7 days, and followed by 1000 µg vitamin B12 given IM/deep SC/IV every week over the next 1 month.

The starting dose of vitamin B12 in younger children, especially those who are malnourished or have severe anemia, must be lower (25-50 µg) as there is a risk of life-threatening hypokalemia [103] as well as a risk of neurological deterioration in the form of worsening or appearance of tremors and other involuntary movements [104].

Further maintenance therapy (1000 µg monthly) can be given by oral or parenteral route as necessary [87].

The intravenous route to be used where the patient has thrombocytopenia as giving IM or SC injections may lead to hematoma formation.

4.2.4 Duration of vitamin B12 therapy

Children with hematological manifestations due to vitamin B12 deficiency need treatment for at least 3 months [74,83,92]. Children with neurological manifestations should be treated for at least 6 months [83,92,105]. Children with an irreversible underlying cause of vitamin B12 deficiency (e.g., pernicious anemia, inherited disorders of vitamin B12 metabolism), underlying malabsorption disorders and those with strong cultural practices (e.g., strict veganism) leading to vitamin B12 deficiency require lifelong vitamin B12 therapy [73,74,83].

4.2.5 Choice of pharmacological compound

Cyanocobalamin (CN-Cbl) is the synthetic form of vitamin B12, which requires conversion to metabolically active coenzymes viz., methlycobalamin (Me-Cbl) and 5'-deoxyadenosylcobalamin (Ado-Cbl) (106). Hydroxo-cobalamin (HO-Cbl) is the long-acting form of Vitamin B12, needs less frequent injections and is hence preferred in children with persicious anema or inherited disorders of metabolic Vitamin B12 processing [107,108]. Currently, there is insufficient evidence to suggest the benefits of using Me-Cbl or Ado-Cbl over CN-Cbl or HO-Cbl in terms of bioavailability, biochemical effects, or clinical efficacy. Me-Cbl is the formulation most readily available in the market and can be prescribed for treating anemia due to Vitamin B12 deficiency.

4.2.6 Side effects of vitamin B12 administration

Unlike oral formulations, injections are more allergenic; HO-Cbl is more allergenic than CN-Cbl, but allergic reactions occur with all cobalamin forms and routes [107,108]. Side effects like nausea, itching, chills, fever, hot flushes, nausea, dizziness, or rarely anaphylaxis can occur. A sensitivity history should be obtained from the patient prior to administration of parenteral vitamin B12. In case of allergic reactions, hydrocortisone can be used for premedication or desensitization can be tried [108]. IV vitamin B12 should be administered as an infusion over 45-60 minutes. Some children may even experience transient worsening of neurological symptoms [109,110]. CN-Cbl should be avoided in children with optic nerve atrophy or Leber's disease, as CN-Cbl may cause optic nerve damage [107].

There is a need to monitor serum potassium levels in the initial few days of therapy as there is a risk of transient hypokalemia after starting vitamin B12 therapy, especially in those with severe anemia [73,103,111].

4.2.7 Treatment of Infants with vitamin B12 deficiency

Infants born to vegetarian mothers deficient in vitamin B12 who receive exclusive breastfeeding for a prolonged period are particularly prone to develop vitamin B12 deficiency [104,109-111]. We recommend checking mothers' anemia and vitamin B12 status while treating symptomatic infants with vitamin B12 deficiency. These infants must be closely followed up for neurological development. Additionally, if the mother is found to be vitamin B12 replete, such infants should be assessed to exclude any genetic causes of vitamin B12 metabolism, which may need specific treatment depending upon the underlying condition.

Treatment of infantile tremor syndrome (ITS) involves therapeutic supplementation of vitamin B12 along with other micronutrients. Severe tremors may require treatment with one (or more) of the following drugs - oral propranolol, phenobarbitone, phenytoin, or uncommonly with carbamazepine or steroids [112].

4.2.8 Treatment of folic acid deficiency

Anemia due to dietary folic acid deficiency in children above one year of age and adults should be treated using oral folic acid 1-5 mg daily [74]; in infants, oral doses up to 50 µg/day may suffice. A meta-analysis showed that daily doses of ≥0.8 mg of folic acid are typically required to achieve the maximal reduction in plasma homocysteine concentrations produced by folic acid supplementation [113]. Doses of 0.2 and 0.4 mg are associated with 60% and 90%, respectively, of this maximal effect [113]. However, more recently, a dose of 0.2 mg/d over at least 6 months was shown to have optimal effects [114]. The British Society for Hematology Guidelines [73] recommend 5 mg of FA to be given for 4 months to treat megaloblastic anemia due to dietary folic acid deficiency in adults. In children with underlying malabsorption, higher daily doses of 5 mg are preferred. The duration of folic acid therapy is for 3-4 months in order to replenish the depleted stores. Vitamin B12, even if found to be normal, should be continued alongside in doses of at least 1-2 µg/day to prevent neurological deterioration.

If the anemia or macrocytosis does not resolve with folic acid therapy and/or the child develops new neurologic symptoms, serum vitamin B12 must be tested again. Such children should be treated with parenteral vitamin B12 [115]. Reduced folate form, viz, folinic acid, is used to treat certain specific folate metabolism disorders or toxic effects of chemotherapy [116].

4.2.9 Assessing response to treatment

Clinical improvement with a sense of well-being starts within 24 hours of treatment. Glossitis starts improving by day 2 and resolves by the end of the second week of treatment. The clinical evaluation can be scheduled within the first week for those with severe anemia and in the second week for children with moderate or mild anemia. However, if there is no improvement, worsening of symptoms, or appearance of neurological signs, he/she needs to be assessed earlier.

In addition to symptomatic improvement, we recommend laboratory evaluation with complete blood counts (CBC) and MCV. The timing of the first follow-up visit for laboratory investigations (hemoglobin, CBC) can be day 7 for those with severe anemia, day 14 for moderate anemia and day 28 for mild anemia. Where there are concerns regarding absorption of oral vitamin B12, we can assay serum vitamin B12 levels.

The most useful objective parameter is the rise in reticulocyte count which starts within 48-72h and peaks at the end of the first week after starting treatment; the reticulocytosis is usually proportional to the severity of the anemia. MCV begins to fall by day 14 and usually normalizes by week 6-8. Hypersegmented neutrophils disappear by 10-14 days of treatment. The CBC, including mean corpuscular volume (MCV), should be completely normal by 6-8 weeks. In the case of suboptimal response, it is important to look for coexisting iron deficiency as well as alternate diagnoses.

Neurologic improvement also begins within the first week and is typically complete in 6-12 weeks [74]. An alternate diagnosis should be looked for in case of worsening neurological symptoms despite treatment. Permanent neurological sequelae with vitamin B12 deficiency are common, especially in infants and younger children [104,109,111].

As per existing evidence, we do not recommend estimating serum vitamin B12 and holotranscobalamin II to assess response to treatment as their levels may rise rapidly after administration.

4.2.10 Evaluation of a child with sub-optimal response

In case of inadequate response, the compliance and dose of medication should be rechecked. If initially started on oral therapy, it may be preferable to switch to parenteral vitamin B12 therapy and re-evaluate the response. It is recommended to evaluate for other causes of macrocytic anemia like hypothyroidism, thiamine-responsive macrocytic anemia, chronic hemolytic anemia, hypoplastic anemia, myelodysplastic syndrome, genetic disorders of vitamin B12 and (or) folate metabolism and underlying liver disease. We also recommend evaluating coexisting iron deficiency and testing for other causes of anemia [74].

Recommendations

- In the presence of discordance between the laboratory results and strong clinical features of vitamin B12 deficiency, treatment should not be delayed to avoid neurological impairment (**LoE4**)
- Mild to moderate macrocytic anemia due to dietary vitamin B12 deficiency can be treated with oral vitamin B12 **(LoE3)**.
- The group recommends initial parenteral Vitamin B12 therapy in children with neurological manifestations, underlying malabsorption of vitamin B12**,** severe anemia / pancytopenia or disorders of vitamin B12 metabolism **(LoE3)**.
- In patients being started on parenteral treatment, after the initial response, maintenance therapy with oral Vitamin B12 can be considered **(LoE1).**
- Children without neurological manifestations due to vitamin B12 deficiency need treatment for at least 3 months, and those with neurological signs and symptoms need at least 6 months of therapy (**LoE3**).
- Treatment of folic acid deficiency should be initiated only after excluding co-existing vitamin B12 deficiency (**LoE2)**.
- Folic acid deficiency is treated with oral folic acid 1-5 mg per day for 3-4 months (**LoE1**).

5. Indication for Blood Transfusion in Nutritional Anemia

Blood transfusion (packed red cells/whole blood) is rarely required to treat nutritional anemias in children. Iron/ vitamin B12/folic acid deficiency results in chronic compensated anemia where intravascular volume is maintained despite severely low hemoglobin. Blood transfusion is often dangerous as it increases the risk of transfusion-associated circulatory overload and may delay bone marrow recovery. The only indication of packed red cell transfusion in children with nutritional anemia is the presence of cardiac decompensation in severe anemia, where rapid restoration of oxygen-carrying capacity is vital for survival [117,118]. Severe anemia with no cardiac decompensation may be managed successfully with supplementation therapy, provided compliance is ensured. As recommended by WHO, the indications for blood transfusion for children are with hemoglobin ≤4 g/dL or hemoglobin between 4-6 g/dL associated with cardiac decompensation [119]. In case of an absolute indication, packed red cells may be transfused at a volume of 5-10 mL/ kg over 4 hours under close cardiac monitoring with the administration of furosemide, if required.

6. Dietary Counselling in Nutritional Anemia

All children being treated for nutritional anemia should be provided an appropriate diet in addition to micronutrient supplementation. During the second year of life, a daily milk intake of 400-600 mL is appropriate to meet calcium needs and intakes above 800 mL per day can predispose to iron deficiency [120]. If the infant/ child is being given excess milk, it should be replaced by complementary feeds (see below). The family needs to be counseled to improve the diet of the patient. This will not only help faster recovery from anemia, it will also prevent further recurrence. Nutrition education and counseling to improve dietary diversity and quality is a key intervention for the prevention of anemia in children [121-123]

After 6 months of life, infants should be started on appropriate complementary feeding. Green leafy vegetables such as palak (spinach), cholai (amaranth leaves), cabbage, sarson (mustard leaves), drum stick leaves, turnip, radish, beetroot, lotus stems, sweet potato, tomatoes, etc. are good sources of iron. Oranges, lemon, watermelon, pomegranate, apple, grapes, guava, musk melon, and goose berries are rich in iron. Dry fruits such as dates, raisins, walnuts, almonds as well as ragi seeds also are good sources of iron. Use of jaggery or palm sugar instead of refined sugar should be encouraged. Non-vegetarian food such as red meat is a good source of heme iron which has better bioavailability than non-heme iron. Nutritional counselling should consider regional and local variations in the diet, varied cultural practices, methods of food processing and meal preparation and affordability [1,124].

Intake of foods rich in vitamin B12 and folic acid should be adequate. Food rich in vitamin B12 includes meat, poultry, shellfish, eggs, dairy products, mushrooms and fermented foods like curd and yogurt. Foods like leafy green vegetables (spinach, lettuce), broccoli, sprouts, lemons, bananas, melons, liver, oranges, beetroot, mango, okra, and flaxseed contain useful amounts of folate. To prevent loss of folate, foods should not be overcooked and should not be repeatedly washed in large amounts of water [125].

Recommendations

- We recommend that caregivers of all infants and children being treated for nutritional anemia should be counselled to improve the diet. All infants must be exclusively breastfed till 6 months of age. Age-appropriate, adequate and diverse complementary foods after 6 months are recommended to optimize the micronutrient intake in children. For children older than 12 months of age, intake of milk should be less than 600

mL per day and bottle feeding should be discontinued to limit milk intake (**LoE 1**).

7. Prevention of Nutritional Anemia

For prevention of anemia in children following strategies are required:

- All infants should be exclusively breastfed till 6 months of age as mother's milk is a good source of iron compared to cow's milk [126]. After 6 months of age, infants should be started on complementary feeding.
- Iron supplementation: Under public health programs, iron supplementation across age groups is recommended [1]. Iron supplementation is particularly needed for high-risk age groups, i.e., infants born preterm or low birth weight, pre-schoolers and adolescents, to replenish the iron sores. Preterm infants do not have adequate hepatic iron stores and require larger amounts of iron for catch-up growth. Targeted laboratory screening for iron deficiency anemia (IDA) should be performed in infants and children with risk factors like malnutrition, prematurity, low birth weight, poor diet or symptoms of IDA. The published literature supports the role of early iron supplementation in preterm or low birth weight (LBW) infants in improving hemoglobin level and iron stores, with a lower risk for IDA in infancy [126-128]. American Academy of Pediatrics (AAP) recommends iron supplementation (dose of 2 mg/kg/day of elemental iron, maximum 15 mg) in breastfed preterm or low birth weight infants as early as two weeks of age and continued in infancy until adequate iron intake from complementary food is assured [128]. Oral iron supplementation in a dose of 2-4 mg/kg/day (elemental iron) in LBW infants from 2-4 weeks of life to 12 months of age is recommended by National Neonatology Forum, India [129].
- Older infants (>6 months) and toddlers are at high risk of nutritional anemia due to inadequate and poor-quality complementary diet. Therefore, screening for anemia using hemoglobin estimation should be done at 9-12 months when these infants visit for measles vaccination. This contact point should be utilized to assess the adequacy of complementary food intake and provide appropriate counselling if required. Further, daily iron supplementation in a dose of 1-2 mg/kg/day can be considered in them individually, especially where dietary intake is inadequate. In young children and adolescents, additional screening may be done at any time if there is clinical suspicion or risk factor for anemia secondary to nutritional or a non-nutritional cause. Additionally, siblings of any child diagnosed with IDA must also be assessed for ID and managed accordingly. Daily iron supplementation is also suggested for adolescent children, especially girls (60-100 mg elemental iron), to replenish the iron stores for pubertal growth and development [130].
- Iron prophylaxis for prevention of anemia in children and adolescents at the community level: In the last 5 decades, the national programs for the prevention and control of anemia have evolved from National Nutritional Anemia Prophylaxis Program (NNAPP) in 1971, National Nutritional Anemia Control Programme (NNACP) in 1993, National Iron Plus Initiative (NIPI) program in 2013 to the current Anemia Mukt Bharat (AMB) program in 2018 [12]. The focus of all government programs has been primarily on combating iron and folate deficiency by strengthening the distribution of iron and folic acid (IFA) supplements to the beneficiaries. Under AMB program, screening for anemia is recommended for all children 6-59 months, in-school and out-of-school children (5-9 years), school-going adolescents 10-19 years in government/ government-aided schools and all children with clinical signs and symptoms of anemia (12). Iron folic acid supplementation in an age-appropriate dose is provided to infants and preschool children and school-going children and adolescents biweekly and weekly, respectively. WHO (2016) guidelines recommend the use of daily iron-folic acid supplementation for all beneficiaries in areas where the prevalence of anemia is >40% [130]. Daily therapy results in poor adherence due to the long duration of treatment and increased side effects. Recently, Cochrane systematic review has reported that intermittent iron supplementation is better for improving hemoglobin concentration and decreasing the risk of anemia in children <12 years compared to placebo or no intervention [131].
- WASH strategies and deworming: There is enough evidence to suggest that children exposed to poor sanitation and hygiene conditions and open defecation have lower hemoglobin levels due to an increased risk of intestinal infection and chronic gut inflammation [132, 133]. It has been observed through clinical trials that the implementation of adequate water, sanitation, and hygiene (WASH) strategies have been successful in reducing the prevalence of anemia in children [134,135]. There is evidence to support the use of mass administration of anti-helminths in decreasing the prevalence of anemia [136,137]. To intensify efforts toward soil-transmitted helminths (STH) control in India, the National Deworming Day program is implemented. Biannual mass deworming (albendazole tablet) is carried out for children and adolescents (1-19 years) every year under the AMB program [12]. Under

this strategy, under-five children, out-of-school children and adolescents are provided deworming tablets at Anganwadi centers by AWWs, while school-going children and adolescents are provided the same through school teachers.

- Delayed umbilical cord clamping: To build and regain the iron stores in infants after birth, delayed umbilical cord clamping (by at least 3 minutes, not earlier than 1 min, or until cord pulsations cease) is recommended by WHO and AMB guidelines [12,138], especially for infants born to anemic mothers [139]. Efforts are required to operationalize this practice at all healthcare levels.

Recommendations

- Iron supplementation is recommended for preterm and low birth weight infants to replenish the iron stores and prevent iron deficiency anemia and continued in infancy until adequate iron from complementary food is assured. (**LoE 1**)
- All children, especially those with risk factors for the development of anemia, should be screened for anemia at 9-12 months, 5 years and adolescence.
- In view of operational feasibility and better compliance, intermittent iron-folic acid supplementation (under the Anemia Mukt Bharat program) is recommended for all infants (>6 months), pre-schoolers, school-age children and adolescents for the prevention of nutritional anemia. (**LoE 1**).
- Delayed cord clamping is recommended for deliveries in all health care facilities. (**LoE 1**)

CONCLUSIONS

Considering the increased prevalence of nutritional anemias in children despite the introduction of preventive programs by the Government of India, the expert group felt a need to provide evidence-based guidelines for managing nutritional anemias in children. Most of the available guidelines for managing nutritional anemias are for adult populations. The committee noted the lack of robust scientific evidence for certain recommendations and felt these key areas need to be studied scientifically in Indian settings (**Box III**).

We endorse the hemoglobin cut-offs recommended by the World Health Organization to define anemia in children. Keeping the feasibility and reliability of available laboratory tests, we suggest that IDA be diagnosed based on examination of CBC, including RBC indices and peripheral smear; diagnosis can be confirmed by the therapeutic response to oral iron. Most cases of IDA can be treated with oral iron. Our guidelines also provide answers to seemingly minor but clinically relevant issues such as duration of treatment in IDA, evaluation of response to treatment and management of non-responders. There is sparse evidence for managing vitamin B12 and folic acid deficiency anemia, and we could only retrieve one guideline for the adult population. We have attempted to provide treatment guidelines for macrocytic anemia due to deficiency of vitamin B12 and FA in children as evidence-based as possible. We recommend treatment with vitamin B12 without further delay where there are clinical features of vitamin B12 deficiency even if laboratory tests are equivocal to prevent neurological impairment. Mild to moderate macrocytic anemia due to dietary vitamin B12 deficiency can be treated with oral vitamin B12. However, patients with neurological manifestations, those with pancytopenia, severe anemia, or malabsorption warrant parenteral vitamin B12 therapy. In patients being started on parenteral treatment, after the initial response, maintenance therapy with oral vitamin B12 can be considered. Treatment of FA deficiency should be initiated only after excluding co-existing vitamin B12 deficiency. We recommend screening children for anemia at 9-12 months of age, 5 years and during adolescence, in addition to intermittent iron FA supplementation as advocated under the Anemia Mukt Bharat Program.

Box III Areas for Future Research in Nutritional Anemias

- Single dose versus divided doses of oral iron for treatment of iron deficiency anemia in children
- Therapeutic response to different doses and preparations of oral iron
- Management of non-responders to oral iron
- Use of intravenous iron in children
- Diagnostic role of methyl malonic acid and holotranscobalamin in children with borderline vitamin B12 levels.
- Therapeutic response to oral vitamin B12 therapy in children.
- Optimum therapeutic dose and schedule of vitamin B12 in children

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