

Clinico-Laboratory Profile of Vitamin B₁₂ Deficiency in Children

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Abstract :

Introduction: Nutritional vitamin B₁₂ deficiency is common in developing countries and has a wide range of neurological and hematological presentations. **Aim:** To study the clinical features and laboratory findings of vitamin B₁₂ deficiency in children and correlate the findings with the severity of deficiency. **Materials and Methods:** 52 children between the age group 1 month-6 years presenting with anemia and vitamin B₁₂ deficiency were included in this study. Their clinical findings and laboratory parameters were evaluated and correlated with the severity of vitamin B₁₂ deficiency. **Results:** Out of the 52 patients enrolled 23 (44.2%) were boys and 29 (55.8%) were girls. 75% of children were in the age group 6 months to 2 years. All came from the lower socio-economic class. Deficiency was seen in children who had been breastfed by vitamin B₁₂ deficient mothers, on inadequate complimentary feeding and those on vitamin B₁₂ deficient diets. 32 (61.5%) patients had severe vitamin B₁₂ deficiency. Apart from pallor, hyper pigmentation of knuckles (100%), hypo pigmented hair (100%), delayed development milestones (75%), tremors (37.5%), failure to thrive (31.2%) and generalized hypotonia, (25%) were the common features associated with severe vitamin B₁₂ deficiency. 91.7% of patients with severe anemia had severe vitamin B₁₂ deficiency. Thrombocytopenia, leucopenia, pancytopenia, and macrocytosis were more common in severe deficiency. **Conclusion:** Vitamin B₁₂ deficiency was more common in children between the age group of 6 months to 2 years belonging to the lower socioeconomic class on a diet deficient in vitamin B₁₂. Hematological and neurological manifestations were more common in those with severe deficiency. Early diagnosis and treatment is important to prevent hematological complications and neurological impairment in these patients. Preventive measures like dietary management and vitamin B₁₂ supplementation have to emphasize.

Key words: Anemia, Children, Nutritional deficiency, Vitamin B₁₂.

Introduction:

Vitamin B₁₂ is a water soluble vitamin which is naturally found in animal products like fish, meat, poultry, egg, milk and milk products. It is generally not present in plant foods. Milk and their products are the important sources for vegetarians. Human milk is an adequate source for breastfeeding infants, provided the maternal serum vitamin B₁₂ levels are adequate.^(1,2) The most important cause of vitamin B₁₂ deficiency children is dietary deficiency especially in those who are strict vegetarians. Other causes of deficiency include intrinsic factor deficiency (Congenital pernicious anemia), selective vitamin B₁₂ malabsorption (Imerslund-Gräsbeck syndrome), gastric

or distal ileal surgical interventions and increase in vitamin B₁₂ consumption (Diphyllobothrium latum infections).^(3,4,5)

Vitamin B₁₂ is required for red blood cell formation, neurological function and DNA synthesis. It is a cofactor for methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase catalyzes the conversion of homocysteine to methionine. Methionine is required for the formation of S-adenosylmethionine, a universal methyl donor for almost 100 different substrates, including DNA, RNA, hormones, proteins and lipids. L-methylmalonyl-CoA mutase converts L-methylmalonyl-CoA to succinyl-CoA in the degradation of propionate, an essential biochemical reaction in fat and protein metabolism.^(3,5,6) Succinyl-CoA is also required for hemoglobin synthesis.⁽²⁾ Deficiency of vitamin B₁₂ leads to megaloblastic anemia and neurological manifestations such as irritability, developmental retardation, hypotonia and tremors.⁽⁵⁾

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The present study was undertaken to study the various clinical manifestations and laboratory findings in children with vitamin B₁₂ deficiency and to correlate them with the severity of deficiency.

Material and Methods:

A prospective study was conducted in Civil Hospital, Ahmedabad, between May 2015 to April 2016. Approval of the Institutional Ethics Committee was obtained and informed consent from parents of the children enrolled in the study was taken. Patients in the age group of 1 month to 6 years presenting with anemia (haemoglobin <11gm% as per WHO criteria) and serum vitamin B₁₂ levels <300 pg/ml were included in the study.⁽⁷⁾ Patients with vitamin B₁₂ deficiency due to causes other than nutritional deficiency were excluded from the study. 52 patients admitted in the pediatric wards of Civil Hospital, Ahmedabad were enrolled.

A detailed history including birth history, breastfeeding, complimentary feeding and dietary history and development history was taken. The socioeconomic status of the patients was established based on the Modified Kuppaswamy scale. Complete examination including general and systemic examination and anthropometry was done. Development was assessed based on the Denver II Developmental Screening Test. Laboratory investigations included complete hemogram, peripheral blood smear, serum iron and serum vitamin B₁₂ levels of patients. Complete

hemogram and serum B₁₂ levels of the lactating mothers were also done. Anemia was defined as haemoglobin <11gm% , leucopenia as a total white blood cell count of less than 4000/mm³ and thrombocytopenia as a platelet count less than 150 ×10³/mm³.^(1,7) Serum iron levels <22 mcg/dl and serum vitamin B₁₂ levels less than 300pg/ml were considered as iron and vitamin B12 deficiency, respectively.^(1,7) Serum vitamin B₁₂ levels between 200- 300 pg/ml were considered as mild, 100-200pg/ml as moderate and <100 pg/ml as severe vitamin B₁₂ deficiency.⁽⁷⁾ The results were then analysed.

Result:

Out of the 52 patients included in the study, maximum number of children i.e., 20 (38.4%) were in the age group of 6 months-1 year, closely followed by 19(36.5%) in the age group of 1-2 years. 23 (44.2%) of the patients were boys; while 29 (55.8%) were girls. 32 out of 52 patients (61.5%) had severe vitamin B₁₂ deficiency while 20 patients(39.5%) had mild to moderate deficiency. 75% of children with severe deficiency were in the age group 6 months to 2 years (Table 1). Maximum number of the girls (28.1%) with severe vitamin B₁₂ deficiency were in the age group of 6 months-1 year, while maximum number of the boys (25%) with severe B₁₂ deficiency were in the age group of 1-2 years. All the patients were from the lower socioeconomic class.

Table 1 : Age wise distribution of patients with vitamin B₁₂ deficiency

Age (in years)	Patients with vitamin B ₁₂ deficiency (S.vit B ₁₂ <300pg/dl) n=52(%)	Patients with mild to moderate vitamin B ₁₂ deficiency (S.vit B ₁₂ =100-300pg/dl) n=20(%)	Patients with severe vitamin B ₁₂ deficiency (S.vit B ₁₂ <100 pg/dl) n=32(%)
3(9.4%)	2(10%)	5(9.6%)	1-6 months
12(37.5%)	8(40%)	20(38.5%).	6-12 months
12(37.5%)	7(35%)	19(36.5%)	1-2 years
5(15.6%)	3(15%)	8(15.4%)	2-6 years

Table 2: Correlation of clinical features with severity of vitamin B₁₂ deficiency

Clinical features	Patients with mild to moderate vitamin B ₁₂ deficiency n=20 (%)	Patients with severe vitamin B ₁₂ deficiency n=32(%)
Hypopigmented hair	7 (35%)	32 (100%)
Hyperpigmentation of knuckles	11 (55%)	32 (100%)
Delayed milestones	1 (5%)	24 (75%)
Hepatomegaly	4 (20%)	16 (50%)
Coarse tremors	2 (10%)	12 (37.5%)
Failure to thrive	0	10 (31.2%)
Generalized hypotonia	0	8 (25%)
Glossitis/angular cheilosis	7 (21.8%)	1 (5%)

The clinical features most commonly found in patients with vitamin B₁₂ deficiency, apart from pallor were hypopigmentation of hair, hyperpigmented knuckles, development delay, hepatomegaly, coarse tremors, failure to thrive and generalized hypotonia. The findings were more common in those with severe deficiency compared to those with mild to moderate deficiency (Table 2).

years, 4 (50%) were still breastfed. The diet of all the children was deficient in vitamin B₁₂.

Majority of the patients with vitamin B₁₂ deficiency (61.5%) had moderate anemia. 15.4% had mild while 23% had severe anemia. Out of the 5 patients between 1-6 months, 1(20%) had severe anemia who presented with congestive cardiac failure, 2(40%) had moderate

Table 3: Correlation of severity of anemia with severity of vitamin B₁₂ deficiency

Severity of anemia ⁽⁷⁾	Patients with vitamin B ₁₂ deficiency n=52(%)	Patients with mild to moderate vitamin B ₁₂ deficiency n=20(%)	Patients with severe vitamin B ₁₂ deficiency n=32(%)
Mild (Hemoglobin=10-10.9 g %)	5 (25%)	8 (15.4%)	3 (9.4%)
Moderate (Hemoglobin=7-9.9 g %)	14 (70%)	32 (61.5%)	18 (56.3%)
Severe (Hemoglobin<7 g %)	1 (5%)	12 (23.1%)	11 (34.3%)

Out of 52 patients, 42 patients' mothers were lactating; out of which 22 (52.4%) had severe vitamin B₁₂ deficiency and 10 (23.8%) had mild to moderate deficiency. Out of the 22 mothers with severe deficiency, 20 (90.9%) were vegetarians and in those with mild to moderate deficiency, 7 (70%) were vegetarians. Out of 20 patients in the age group of 6 months- 1 year, complimentary feeding was not started in 14 (70%) and 6 (30%) were on inadequate complimentary feeding. Out of 19 children between 1-2 years, 18 patients (94.7%) were breastfed and out of 8 children from 2-6

anemia while 2(20%) had mild anemia. In the age group of 6 months to 1 year, 11 (55%) patients had severe anemia, 8(40%) had moderate anemia and 1(5%) had mild anemia. In children between 1-2 years, 14 (73.6%) had moderate anemia whereas 5 (26.4%) had mild anemia. In children between 2-6 years of age, all had moderate anemia (100%). Also, 91.7% of patients with severe anemia had severe vitamin B₁₂ deficiency (Table 3). Thrombocytopenia, leucopenia, pancytopenia and macrocytosis were more common in those with severe vitamin B₁₂ deficiency (Table 4).

Table 4: Correlation of hematological findings with severity of vitamin B₁₂ deficiency

Hematological findings	Patients with mild to moderate vitamin B ₁₂ deficiency n=20(%)	Patients with severe vitamin B ₁₂ deficiency n=32(%)
Anemia (Hemoglobin<11gm%)	20 (100%)	32 (100%)
Thrombocytopenia (Platelets<150 × 10 ³ /mm ³)	2 (10%)	7 (21.8%)
Leucopenia (White blood cells <4,000/mm ³)	1 (5%)	6 (18.7%)
Pancytopenia	0	5(15.6%)
Macrocytosis (Mean corpuscular volume > 88fl)	9 (45%)	22 (68.7%)
Hypersegmented neutrophils(Nucleus with 6 or more lobes)	0	3 (9.3%)

Associated iron deficiency was present in 36 (69.2%) patients with vitamin B₁₂ deficiency. 30 (57.6%) patients had associated infections or sepsis. 16 (30.7%) patients suffered from severe acute malnutrition while 12 (23%) had moderate and 4(7.7%) had mild malnutrition .12 (23%) patients had rickets.

Discussion:

Vitamin B₁₂ deficiency generally occurs in young children (<2 years of age) from lower socio-economic status, due to inadequate dietary intake of vitamin B₁₂.^(1,2,3) Out of the 52 patients in our study, 44.2% were boys and 55.8% were girls, This is in contrast to the study by Taskesen et al⁽⁸⁾ where 57% were boys and 43% were girls. Jain et al⁽⁹⁾ have also reported increased incidence in males (71%) compared to girls (29%). The median age of presentation in our study was 11.5 months which is similar to the median age of 11 months observed in the study by Jain et al.⁽⁹⁾ Taskesen et al⁽⁸⁾ have reported a median age of presentation of 13 months while in the study by Chandra et al⁽¹⁰⁾, the mean age was 18 months. In our study, as in other previous studies, all patients were from lower socio-economic class.

In our study, apart from pallor the most common manifestations of severe vitamin B₁₂ deficiency were hyperpigmentation of knuckles (100%), hypopigmented hair (100%), development delay (75%), hepatomegaly (50%), coarse tremors (37.5%), failure to thrive (31.2%), generalized hypotonia (25%) and angular chelosis/ glossitis (21.8%). The previous studies^(3,4,5,9,10,11,12) have reported these clinical features in a varying percentage of patients i.e. hypotonia (60-100%), skin pigmentation (10%-100%), hypopigmented hair (33.33%-100%), tremors (9%-100%) and neurodevelopmental retardation (40-86%). In the study by Katar et al⁽¹³⁾, the most frequently reported symptoms of vitamin B₁₂ deficiency were pallor, hypoactivity, attention deficit and stomatitis. Taskesen et al⁽⁸⁾ reported clinical findings like anorexia(92.8%), seizures(28.5%), athetoid head movements (19%) and apathy (14.2%) which were not observed in our study. In the study by Jain et al⁽⁹⁾, the most common findings were apathy and hypotonia (66.7%), motor retardation (59.3%), social retardation (51.9%), tremors (14.8%) and athetoid movements (3.6%). Vitamin B₁₂ deficiency should therefore be considered in the differential diagnosis of children with neurological manifestations such as developmental

delay or regression, hypotonia and tremors. Skin hyperpigmentation, hypopigmented hair and glossitis are useful nonneurologic signs suggesting vitamin B₁₂ deficiency.

Vitamin B₁₂ deficiency has various hematological manifestations, the most important being anemia. In our study, anemia was a pre-requisite for enrollment, and thus found in all patients. We observed that majority of the patients had moderate anemia. 91.7% patients with severe anemia had severe vitamin B₁₂ deficiency. Thrombocytopenia was found in 21.8%, leucopenia in 18.7% and pancytopenia in 15.6% of patients with severe vitamin B₁₂ deficiency, all of which were more common compared to patients with mild to moderate vitamin B₁₂ deficiency. In a study by Incecik et al⁽¹¹⁾, anemia was reported in 100%, thrombocytopenia in 50% and leucopenia in 33.3% of patients with vitamin B₁₂ deficiency while Jain et al⁽⁹⁾ reported 83% patients with anemia and 71% patients with macrocytosis. In the study conducted by Taskesen et al⁽⁸⁾, 83.3% patients had anemia, 28.5% had thrombocytopenia, 16.6% had leucopenia and 26.6% had pancytopenia. No previous studies have compared the incidence of various haematological findings with the severity of vitamin B₁₂ deficiency. The presence of pancytopenia, thrombocytopenia, leucopenia and macrocytosis in the peripheral blood are useful clues suggesting vitamin B₁₂ deficiency.

In our study, out of 42 lactating mothers, 22 (52.4%) had severe vitamin B₁₂ deficiency of which 20 (90.9%) were vegetarians and 10 (23.8%) had mild to moderate deficiency, of which 7 (70%) were vegetarians. Increased incidence of vitamin B₁₂ deficiency in mothers who are vegetarians has been similarly noted in the study by Goraya et al⁽¹²⁾, in which, out of 15 lactating mothers, 9 (60%) had severe vitamin B₁₂ deficiency and 6 (40%) had mild to moderate deficiency and all mothers were vegetarians. Koc et al⁽¹⁴⁾ reported severe vitamin B₁₂ deficiency in 48% of mothers. Jain et al⁽⁹⁾ observed that 83.3% of mothers in their study had low serum vitamin B₁₂ levels of which 35% mothers were vegetarians and the rest were non-vegetarians. However, the dietary intake of vitamin B₁₂ of non-vegetarian mothers was inadequate in all except one. In

our study, there was evidence of delayed initiation of complimentary feeding in 70% of patients aged 6 months to 1 year while the rest of the 30% were on inadequate complimentary feeding. In children aged 1-2 years, 94.7% were breastfed, whereas in children aged 2-6 years, 50% of them were breastfed. In conclusion, the diet of all the patients in our study was deficient in vitamin B₁₂. In the study by Jain et al⁽⁹⁾, 71% of the patients were exclusively breastfed whereas 29% were predominantly breastfed with minimal complimentary feeding. During pregnancy, vitamin B₁₂ is actively transported to the fetus through the placenta and its serum level in the fetus is twice the level in the maternal serum. Neonatal stores of this vitamin are generally sufficient to last upto 6-12 months of age. Infants with poor intake of vitamin B₁₂ tend to develop deficiency once these stores get depleted. Infants with low vitamin B₁₂ levels may develop anemia and irreversible psychomotor retardation and severe neurological problems within the first few years of life.^(1,3,5) Exclusively breast-fed infants whose mothers consume a vegetarian diet with minimal intake of foods of animal origin, including milk, are at risk. These high risk infants should be actively sought during the routine infant visits. Appropriate dietary advice and vitamin B₁₂ supplementation to mothers at risk of vitamin B₁₂ deficiency will help prevent the deficiency in their infants.

In the present study, co-existent iron deficiency anemia was present in 69.2% patients, 57.6% patients had associated infections or sepsis, 61.5% were malnourished and 23% had rickets. In the study by Goraya et al,⁽¹²⁾ 25.9% had associated infections, 74% were malnourished and 7.4% had rickets.

Early diagnosis and treatment is important in cases of vitamin B₁₂ deficiency to prevent neurological and haematological complications. Diet rich in vitamin B₁₂ and vitamin B₁₂ supplements are important in the prevention and treatment of nutritional vitamin B₁₂ deficiency. Strategies to improve vitamin B₁₂ status in children should be developed such as creating awareness regarding intake of proper diet and vitamin B₁₂ supplements by pregnant and lactating mothers, proper method of weaning and timely introduction of

adequate complementary feeding in infants and introducing a modified meal plan in anganwadis and schools to incorporate vitamin B₁₂ rich food to children. In the National Nutritional Anemia Prophylaxis Programme, vitamin B₁₂ supplementation should be given along with iron and folic acid to prevent vitamin B₁₂ deficiency in children.

Conclusion:

In conclusion, severe vitamin B₁₂ deficiency was more common in children belonging to the lower socioeconomic class in the age group of 6 months to 2 years. It was more common in children who had been breastfed by vitamin B₁₂ deficient mothers, on inadequate complimentary feeding and those on vitamin B₁₂ deficient diets. Severe anemia was more commonly associated with severe vitamin B₁₂ deficiency. Severe vitamin B₁₂ deficiency was more commonly associated with hyperpigmentation of knuckles, hypopigmented hair, coarse tremors, delayed development milestones, failure to thrive and generalized hypotonia. It was also more commonly associated with thrombocytopenia, leucopenia, pancytopenia, and macrocytosis. As severe and longstanding vitamin B₁₂ deficiency can lead to severe anemia and irreversible neurological deficits, early diagnosis and treatment is important. Preventive measures like dietary management and vitamin B₁₂ supplementation have to be emphasized.

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