

## Original Research Article

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# A prospective, cross sectional open label clinicopathological study of vitamin B-12 deficiency in adolescent children

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## ABSTRACT

**Background:** Megaloblastic anemia is becoming most common forms of anemia in adolescents during recent years. In India limited studies are available on megaloblastic anemia. The main aim of the study was to determine the incidence, prevalence, and various morphological patterns and complications of vitamin B12 deficiency anemia in adolescents.

**Methods:** Hospital based cross sectional observational study of 100 adolescents anaemic patients (excluding anemia of bone marrow failure, haemolytic anemia associated with jaundice and organomegaly, aplastic anemia, leukaemia and anemia associated with chronic inflammatory disease) admitted in paediatric ward from 2016 to 2017 was done. In every case detailed history, examination along with blood investigation done including complete blood count, blood indices and peripheral blood smear for morphology. In every case serum B12 level was estimated.

**Results:** Out of 100 cases 38% were deficient with female dominance. Significant association ( $p < 0.05$ ) was found between B12 deficiency and vegetarian diet, macrocytic peripheral film, pancytopenia, knuckle pigmentation and neurological symptoms.

**Conclusions:** Deficiency of B12 deficiency is common among the adolescents, which is common in vegetarian's diet patterns. Supplementation through implemented nutritional programmes and education regarding diet patterns may overcome this deficiency.

**Keywords:** Anemia, Adolescents, B12

## INTRODUCTION

Vitamin B12 deficiency has been previously thought to be rare in adolescent children, however, recent studies suggest that condition is more common than previously recognized. Vitamin B12 deficiency often presents with nonspecific manifestations like developmental delay, weakness and failure to thrive.

Treatment may resolve these manifestations, but permanent neurological damage may remain. Familiarity with risk factors, manifestations, and diagnostic studies of vitamin B12 deficiency by paediatric health care provider is crucial to enable early recognition and

treatment. The association of B12 deficiency and anemia and gastrointestinal and neurologic abnormalities referable to the brain, spinal cord, and peripheral nerves has been recognized in several clinical and postmortem case reports and series by Combe, Addison, and Fenwick since the early 19th century.<sup>1</sup> Gardner and Osler coined the term pernicious anemia (PA) to describe a patient with progressive arm numbness and difficulty with buttoning and using tools.<sup>2</sup> Liechtenstein reported the association of PA and spinal cord disease but attributed both to tabes dorsalis.<sup>3</sup> Lichtheim and Minnich recognized the histologic differences in the spinal cord between PA and tabes dorsalis.<sup>4</sup> Russell et al coined the term subacute combined degeneration of the spinal cord.

Minot and Murphy fed PA patients a half-pound of calf liver daily, for which they received the Nobel Prize.<sup>5-6</sup> Castle distinguished the role of gastric (intrinsic) and dietary (extrinsic) factors in PA.<sup>7</sup> Cyanocobalamin was isolated from the liver.

The existence of vitamin B12 deficiency neuropathy was recognized. Lassen et al noted megaloblastic anemia secondary to prolonged nitrous oxide (N<sub>2</sub>O) exposure; the neurologic features were described by Sahenk et al and Layzer et al.<sup>8</sup>

Various clinical manifestations of vitamin B12 deficiency reported in children are

- General
  - a. Weakness
  - b. Fatigue
  - c. Anorexia
  - d. Failure to thrive
  - e. Irritability
- Neurologic/psychiatric
  - a. Developmental delay/regression
  - b. Paraesthesia
  - c. Impaired vibratory and proprioceptive sense
  - d. Hypotonia
  - e. Seizures
  - f. Ataxia
  - g. Dementia
  - h. Paralysis
  - i. Abnormal movements
  - j. Memory loss
  - k. Personality change
  - l. Poor school performance
  - m. Depression
- Hematologic
  - a. Macrocytosis
  - b. Anemia
  - c. Hypersegmentation of neutrophils
  - d. Leukopenia
  - e. Thrombocytopenia
  - f. Pancytopenia
- GIT
  - a. Bleeding per rectal
  - b. Glossitis
  - c. Diarrhoea / vomiting
  - d. Icterus
- Other features
  - a. Skin hyperpigmentation
  - b. Systolic flow murmur

The objectives of the present study were to observe the clinico-pathological pattern of vitamin B12 deficiency in anemia of adolescent children, to detect morphological pattern of vitamin B12 deficiency in adolescent children, to detect severity and degree of vitamin B12 deficiency in anemia of adolescent children and to detect Non-hematological manifestation (neurological/cognitive and gastrointestinal) of vitamin B12 deficiency in adolescent children.

## METHODS

100 Cases admitted in pediatric ward of JLN Medical college Ajmer were enrolled in the study by excluding Anemia of bone marrow failure, Haemolytic anemia associated with jaundice and organomegaly, Aplastic anemia and leukaemia, and Anemia associated with chronic inflammatory disease. In every case detailed history including Clinical, developmental, dietary and detailed clinical examination including General, neurological, haematological and abdominal were done. Written informed consent was taken from caregiver of each patient, following which blood investigations were done including complete blood count, blood indices and peripheral blood smear for morphology. In every case serum B12 level was estimated by automated chemiluminescent immunoassay.

### Estimation of haemoglobin levels

Haemoglobin levels were estimated using Bekmann and Coulter LH 500 automated hematology analyzer. Anemia was said to be present when the haemoglobin (Hb) level in the blood was below the lower extreme of the normal range for the age and sex of the individuals. Adolescents (age 10-18years) attending the outpatient department or admitted in the hospital and having haemoglobin values <12 gm/dl in adolescents of 14 years of age; <13 gm/dl in males 15 years and above and <12 gm/dl in non-pregnant females of 15 years and above as per WHO, adolescents having haemoglobin percentage below 12 gm/dL were considered as anemic. Complete blood count (CBC) remains a practical starting point in the laboratory evaluation and classification of B12 deficiency anemia's and consists of estimation of haemoglobin, RBC indices like, MCV (mean corpuscular volume), MCHC, MCH (mean corpuscular haemoglobin) and peripheral smear, platelets count, total leucocyte count. In every case serum B12 level was estimated by automated chemiluminescent immunoassay (Table 1).

The scholastic performance was obtained from the progress report for the period of past six months based on the grading system.

**Table 1: Normal values in adolescent.**

Normal values	Adolescent
Hb	13.0-16.0 gm/dl
MCV	79-98 fl
MCHC	32-36 %
MCH	25-35 pg
RDW	11.5-14.0 %
Total RBC	4.50-5.30 (x10 <sup>6</sup> /µL)
Platelets	150-450 (x10 <sup>3</sup> /µL)
TLC	4.5-13.0 (x10 <sup>3</sup> /µL)
Serum Vitamin B12 level	Normal values are 200-900 (pg/mL).

Assessment of cognitive function was done based on GHQ (General Health Questionnaire) score and HDRS (Hamilton Depression Rating Scale-17) by the paediatric psychologist and were graded according to the points obtained.

#### Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet. The categorical data was expressed as rates, ratios and proportions and continuous data was expressed as mean  $\pm$  standard deviation (SD). Categorical data was analysed using chi-square and Fisher's exact test. Continuous data was compared by independent sample t test. A p- value of  $<0.05$  was considered as statistically significant.

## RESULTS

A total of 100 adolescent children aged between 10 to 18 years were included with 62% normal serum B12 level( $>200\text{pg/dl}$ ) and 38% were deficient( $<200\text{pg/dl}$ ). In this study 42% were male and 58% were female with nonsignificant but higher incidence (68%) of B12 deficiency in females ( $P=0.9833$ ). In this study 22% of the children were aged 10 to 12 years, 47% were aged 13 to 15 years and 31% were aged 16 to 18 years. Most affected deficient group was 13 to 15 years (60%) ( $p=0.0625$ ). In present study there was significant association between vegetarian and incidence of B12 deficiency ( $p=0.0001$ ). It showed higher (86.84%) chance of B12 deficiency in vegetarian compared to mixed diet (13.16%) (Table 2).

**Table 2: Association of B12 deficiency with diet.**

Diet	B12 deficiency (%)	B12 Normal (%)	Total	$\chi^2$ (chi square)	P value
Vegetarian	33 (86.84)	30 (48.38)	63		
Mix	5 (13.15)	32 (51.61)	37	14.94	0.0001
Total	38	62	100		

In above analysis there was significant association between vegetarian and incidence of B12 deficiency( $p=0.0001$ ). It showed higher(86.84%) chance of B12 deficiency in vegetarian compared to mixed diet.

In present study children were anaemic as mild, moderate and severe anemia was present in 46%, 24% and 30% of children respectively with positive association was found between vitamin B12 deficiency and mild grade anemia ( $p=0.0458$ ).

In present study 48% of the children had normal peripheral smear findings while megaloblastic(macrocytic) anemia was noted in 30% and dimorphic anemia in 22% of the children. The frequency of vitamin B12 deficiency was significantly high in children who had megaloblastic anemia (57%) ( $p=0.00001$ ). There was significant association between B12 deficiency and MCV 90fl (73%) ( $p=0.00001$ ) and MCH between 25-35pg (71%) ( $P=0.00001$ ). In present study significant relation found in B12 deficiency and pancytopenia as total leucocyte count $<5000/\mu\text{l}$  (52%) ( $P=0.00011$ ) and thrombocytopenia  $<1.5$  lac (60%) ( $p=0.00002$ ). In this study with regard to scholastic performance, 53% of the children had secured grade

III, 32% % secured grade II and 15% children had secured grade I. The prevalence of vitamin B12 deficiency was high in children with grades III (55.26%), II (34.67%), I (8.55%) suggesting a nonsignificant but increasing frequency of vitamin B12 levels with decrease in scholastic function ( $p=0.6155$ ). Further, the cognitive function was assessed using GHQ Score and no significant relation was found between B12 deficiency and low GHQ score as cognitive dysfunction ( $p=0.349$ ). Assessment of depression based on HDRS-17 showed significant association between B12 deficiency and score  $>7$ (68.42%) as sign of mild depression ( $p=0.0169$ ) (Table 3).

In present study significant association was found between neurological symptoms (tremor/ataxia/sensory changes) (79%) and B12 deficiency( $p=0.0129$ ) (Table-4). In present study 74% children were found with knuckle pigmentation there was positive relation between B12 deficiency and knuckle pigmentation (92%) ( $p=0.0012$ ) as leading sign (Table 5).

**Table 3: Association between B12 deficiency and depression HDRS (Hamilton Depression Rating Scale-17).**

HDRS-17	B12 deficiency(%)	B12 Normal (%)	Total	$\chi^2$ (chi square)	P value
$\geq 7$	26(68.42)	52(83.87)	81		
$<7$	12(31.57)	7(11.29)	19	5.7	0.0169
Total	38	62	100		

In this study there was significant association ( $p=0.0169$ ) between B12 deficiency and depression (68.42%)  $\text{HDRS} \geq 7$ .

**Table 4: Association between B12 deficiency and neurological manifestations (Tremor/ataxia/sensory changes).**

Neurological sign	B12 deficiency(%)	B12 Normal (%)	Total	$\chi^2$ (chi square)	P value
Present	8 (21.05)	3 (4.83)	11		
Absent	30 (78.94)	59 (95.16)	89	6.17	0.012
Total	38	62	100		

In this study there was significant association (p=0.129) between B12 deficiency and neurological manifestations (21.05%) compare to normal level group (4.83%).

**Table 5: Association between B12 deficiency and knuckle pigmentation.**

Knuckle pigmentation	B12 deficiency (%)	B12 Normal (%)	Total	$\chi^2$ (chi square)	P value
Present	35 (92.10)	39 (62.90)	74		
Absent	3 (7.89)	23 (37.09)	26	10.44	0.00012
Total	38	62	100		

There was significant association between knuckle pigmentation (92.10%) and B12 deficiency (p=0.0012).

## DISCUSSION

In India 70% under five children, 55% women and 24% men were anemic as per NFHS3 (2005-2006).<sup>9</sup> Limited data is available on nutritional anemia in adolescents. As per previous studies from India iron deficiency was most common cause for anemia. But from the past two to three decades prevalence of folate and cobalamin deficiency is increasing and was shown in several studies. Both folate and B12 micronutrients deficiency causes megaloblastic anemia. The present study mainly deals with megaloblastic anemia due to vitamin B12 deficiency and the varied clinical presentations among the adolescent age group.

Children in the age group of 10 to 19 years are specified as school-age and adolescents which is a period of rapid growth and development and is a time during which lifelong behaviors are formed. The primary reasons for deficiencies of vitamin B12 in childhood are that they are not sufficiently present in the diet. In addition, the prevention of absorption of vitamins in foods by various reasons may also lead to deficiencies of these substances. The present study was undertaken to evaluate the haematological, biochemical parameters, neurological and gastro intestinal symptoms to aid in understanding the vitamin B12 deficiency anemia in adolescent children in nutritional anemia. The main aim of the study was to determine the incidence, prevalence, and various morphological patterns and complications of vitamin B12 deficiency anemia in adolescent's children.

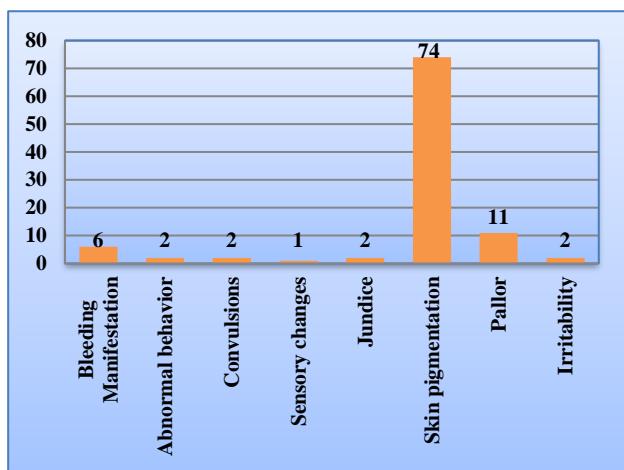
100 cases were enrolled in the one-year study by excluding Anemia of bone marrow failure, Haemolytic anemia associated with jaundice and organomegaly, Aplastic anemia and leukaemia, and Anemia associated with chronic inflammatory disease. In every case detailed history including Clinical, developmental, dietary and detailed clinical examination including General, neurological, haematological and abdominal was done. Written informed consent was taken from caregiver of

each patient, following which blood investigations were done including complete blood count, blood indices and peripheral blood smear for morphology. In every case serum B12 level were estimated. The scholastic performance was obtained from the progress report for the period of past six months based on the grading system.

Assessment of cognitive function was done based on GHQ (General Health Questionnaire) score and HDRS (Hamilton Depression Rating Scale-17) by the paediatric psychologist and were graded according to the points obtained. In present study the vitamin B12 levels were low (<200 pg/mL) in 38% of the study population as found in study done by Chandra et al.<sup>10</sup> In present study there was positive relation between B12 deficiency and knuckle pigmentation (92%) (p=0.0012) as leading sign, which is in accordance with Chandra J et al.<sup>10</sup> In present study 47% were age group 13 to 15 years with high incidence (60%) of B12 deficiency but not significant(p=0.0626). This might be ascertained to increased nutritional requirement in the pre-pubertal and pubertal ages which might put these children at risk for deficiency, which was consistent to study done by Enver Atay et al.<sup>11</sup> In present study there was significant association between vegetarian and incidence of B12 deficiency (p=0.0001). It showed higher (86.84%) chance of B12 deficiency in vegetarian compared to mixed diet (13.16%). This was compared to Khanduri et al in which 87% were vegetarians.<sup>12</sup>

In this study no significant association was found between sex ratio and B12 level, but there was higher (68.42%) incidence of B12 deficiency in female compare to male (31.57%). The present study was in accordance with Khanduri et al and Haq S et al with a female preponderance.<sup>12-13</sup> In present study 76% cases were from lower grade socioeconomic status (III, IV, V) with high incidence (72%) of B12 deficiency but no statistically significant association with vitamin B12 deficiency (p=0.65) as study done by Rafik M et al and Villamor E

et al.<sup>14-15</sup> In present study out of 100 cases 46%, were mild, 24% moderate and 30% children were of severe anemia, with positive association was found between vitamin B12 deficiency and higher incidence (52.63%) of mild anemia ( $p=0.0456$ ). which is in accordance with a similar hospital-based study of Etiology and Varied Clinical Features of Megaloblastic Anemia in Adolescents at Niloufer Hospital by Ravikumar et al.<sup>16</sup> Pallor (87.5%) and fatigue (72.5%) were the most common clinical presentations. This was in accordance to Salma et al (Lahore), Ravi kumar et al (Figure 1).<sup>16</sup> In present study 48% of the children had normal peripheral smear findings while megaloblastic(macrocytic) anemia was noted in 30% and dimorphic anemia in 22% of the children.



**Figure 1: Sign/symptom of vitamin B12 deficiency.**

The frequency of vitamin B12 deficiency was significantly high in children who had megaloblastic anemia (57%) ( $p=0.00001$ ). There was significant association between B12 deficiency and MCV 90fl(73%)( $p=0.00001$ ) and MCH between 25-35pg(71%)( $P=0.00001$ ), in accordance to study of Patra et al and Choudhary SM et al.<sup>17-18</sup>

In present study significant relation found in B12 deficiency and pancytopenia as total leucocyte count $<5000/\mu\text{l}$ (52%)( $P=0.00011$ ) and thrombocytopenia  $<1.5 \text{ lac}$  (60%)( $p=0.00002$ ). This was consistent with R.Sarode et al.<sup>19</sup> Micronutrients are essential to neurocognitive development; Deficiencies of nutrients such as vitamin B12 are more commonly associated with impairment of memory, learning ability and concentration difficulties, yet their role in educational outcomes is unclear. In this study with regard to scholastic performance, 53% of the children had secured grade III,32% secured grade II and 15% children had secured grade I.

The prevalence of vitamin B12 deficiency was high in children with grades III (55.26%), II (34.67%), I (8.55%) suggesting a nonsignificant but increasing frequency of vitamin B12 levels with decrease in

scholastic function ( $p=0.6155$ ). Further, the cognitive function was assessed using GHQ Score and no significant relation was found between B12 deficiency and low GHQ score as cognitive dysfunction( $p=0.349$ ) as in study done by MM Black et al and MW Louwman et al in 2000-03.<sup>20-21</sup> Assessment of depression based on HDRS-17 showed significant association between B12 deficiency and score  $>7$ (68.42%) as sign of mild depression ( $p=0.0169$ ) as in study by J Lindenbaum et al 1988.<sup>22</sup>

The neurological findings such as tremors and parasthesias were low and found only in 4 patients (4%). This was consistent with and DG Savage et al and G. Scalabrino.<sup>23-24</sup> In present study significant association was found between neurological symptoms (tremor/ataxia/sensory changes) (79%) and B12 deficiency( $p=0.0129$ ).

## CONCLUSION

Thus, we recommend B12 deficiency should be suspected in atypical extra hematological presentation of adolescent anemia. The addition of vitamin B12 along with iron and folate tablets in the ongoing school health programmes which can have considerable benefits to prevent vitamin B12 deficiency thereby improving the scholastic/cognitive performance and hematological/extra hematological manifestations. We conclude that Megaloblastic anemia is common in an adolescent which is caused by deficiency of either folate or B12 deficiency. It has a significant correlation with diet pattern as it is more common in vegetarian people and predominantly is seen in low socioeconomic status. Pallor associated with hyper pigmented knuckles and hyper pigmented distal phalanges are most commonly associated with megaloblastic anemia. All cell lines of erythropoiesis are affected, resulting in pancytopenia. Along with iron and folic acid, B12 supplementation is needed through nutritional programmes. Periodic screening of adolescents may detect anemia at early stage and need for other supplements. Education about proper dietary habits is very essential.

## Recommendations

Large scale studies on the prevalence and etiology of Cobalamin deficiency in children and adolescents is needed as in recent years Cobalamin deficiency is increasing globally.

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