Vitamin B\textsubscript{12} status among anaemic adolescents

Amita Surana\textsuperscript{*}, Sandeep Tilwani, Shefali Patel, Hiren Prajapati, Rajeev Prasad

Department of Pediatrics, Surat Municipal Institute of Medical Education and Research, Surat, India

Received: 01 February 2017
Accepted: 28 March 2017

*Correspondence:
Dr. Amita Surana,
E-mail: amita.surana@yahoo.co.in

ABSTRACT

Background: Nutritional anaemia are common health problems. Most studies are done regarding iron deficiency anaemias. There are limited data regarding vitamin B\textsubscript{12} deficiency anaemia especially in adolescents. Aims and objectives of the study were to find the prevalence of vitamin B\textsubscript{12} deficiency among anaemic adolescents and to study various socio-demographic factors and hematological parameters associated with vitamin B\textsubscript{12} deficiency.

Methods: Cross sectional hospital based observational study of 211 adolescents (10 -18 year) with anaemia. Socio demographic characteristics like age, sex, education of mother & patient, socio-economic class, dietary history were noted for each patient. CBC including RBC indices and serum estimation of vitamin B\textsubscript{12} level were done for each patient.

Results: Anaemia was seen in 46.6\% of adolescents. Vitamin B\textsubscript{12} deficiency was seen in 49.76\%. Vitamin B\textsubscript{12} deficiency was significantly associated with male gender (p=0.032) and vegetarians (p=0.047). Moderate to severe degree of anaemia (p=0.016), macrocytosis (p= 0.000) and thrombocytopenia (p=0.007) was more observed in vitamin B\textsubscript{12} deficient patients as compared to vitamin B\textsubscript{12} non deficient groups. Statistically significant fall in mean haemoglobin level and increase in mean MCV values were seen with decreasing serum vitamin B\textsubscript{12} level.

Conclusions: Among anemic male and vegetarian adolescents vitamin B\textsubscript{12} deficiency is a significant health problem. Hematological findings in peripheral blood are more associated with severe degree of vitamin B\textsubscript{12} deficiency. Vitamin B\textsubscript{12} supplementation along with IFA should be addressed through national programmes.

Keywords: Adolescent, Anaemia, Hematological changes, Sociodemographic factor, Vitamin B\textsubscript{12} deficiency

INTRODUCTION

Nutritional anaemia is one of the most important health problem throughout the world especially in developing countries due to its high prevalence in these regions.\textsuperscript{1-2} Preschool children, pregnant women & adolescents constitute vulnerable group of anaemia.\textsuperscript{3} Adolescence is a period of transition between childhood & adulthood, is second to infancy as the period of most rapid growth. During this period with inadequate & improper dietary habits, one is vulnerable to all kinds of nutritional morbidities.\textsuperscript{4} The prevalence of anaemia among adolescents is 27\% in developing countries and 6\% in developed countries. In India, the prevalence in adolescent population ranges from 32-55\%.\textsuperscript{4}

Among nutritional anaemias, iron deficiency is the most common cause and it is a major public health concern for researchers and policy makers.\textsuperscript{5} In addition to iron, adequate amount of other nutrients like folic acid and vitamin B\textsubscript{12} is required for haematopoiesis, deficiency of which leads to megaloblastic anaemia.\textsuperscript{5,6} Vitamin B\textsubscript{12} deficiency is a reversible cause of bone marrow failure and demyelination of nervous tissue for which early recognition and treatment is critical.\textsuperscript{7} Low levels of vitamin B\textsubscript{12} are less commonly associated with infertility.
and thrombosis. It can be a cause of recurrent abortion or preterm delivery in pregnant mothers. Infants born to deficient mothers are at high risk for developing B₁₂ deficiency and irreversible neurological damage.

Most of the studies related to anaemia were focused on iron deficiency anaemia and study population targeted was mainly adolescent females, pregnant mothers or young children. There is paucity of data regarding B₁₂ deficiency among adolescent population. This study was done to find the prevalence of vitamin B₁₂ deficiency among anaemic adolescents and to study various socio-demographic factors and hematological parameters associated with it.

METHODS

A hospital based cross sectional observational study done at SMIMER hospital, Surat, India over a period of 1 ½ year, after approval by institutional ethical committee. The study population comprised of adolescent patients (10-18 year). Informed written consent was obtained from legal representatives of patients.

The criterion of anaemia was accepted as the haemoglobin (Hb) value below 12 g/dl. All the adolescent patients with Hb <12 g/dl were included in study. Patients having acute critical illness, chronic systemic disease, diagnosed cases of hemolytic anaemia, blood transfusion history in past, reticulocyte count >2% or patients unwilling for inclusion in study, were excluded from the study. A detailed history including socio demographic determinants like patient’s age, sex, literacy level; maternal literacy level; socioeconomic status (SES) based on modified Prasad’s classification and dietary history were recorded. Those consuming animal food like meat, fish at-least once per week were considered as non-vegetarians. Age group was divided as early adolescent (10-<13 year), mid adolescent (13 - <16 year) and late adolescent (16-18 year). Anthropometric measurements (weight, height) were noted. Body Mass Index (BMI) was categorised as under-nutrition (BMI <18.5 kg/m²), normal (BMI 18.5-24.9 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI >30 kg/m²).

Hematological parameters like hemoglobin (Hb), total WBC count and platelet count, RBC indices MCV, MCH and MCHC were measured in venous blood by automated cell counter.

Reticulocyte count was done for all patients. Serum vitamin B₁₂ level estimation was done in venous blood using chemiluminescent enzyme immunoassay technique.

Hb level between 10-11.9 mg% was taken as mild anaemia, between 7-9.9 mg% moderate and <7 gm% as severe anaemia. Platelet count <1.5 lac/cmm was taken as thrombocytopenia. Based on MCV value patients were categorised as having microcytic (<80fL), normocytic (between 80 fl-100 fl) and macrocytic anaemia (>100 fl).

Vitamin B₁₂ level below 160 pg/ml were accepted as deficiency level as per laboratory cut off value.

Patients were grouped in two: group A- vitamin B₁₂ <160 pg/ml, group B-vitamin B₁₂ >160 pg/ml. For further analysis group A patients were divided in 3 subgroups; <60 pg/ml, 60-100 pg/ml, 100-160 pg/ml.

For statistical analysis of qualitative data Chi square test and quantitative data independent ‘t’ test and Annova test were applied.

RESULTS

A total of 757 adolescent patients were admitted during study period, out of which 350 had Hb <12 gm%. Hence, prevalence of anaemia in our study was 46.6%. Out of 350 anemic patients, 13 patients took DAMA, 19 patients were critically ill and in 107 patients vitamin B₁₂ estimation were not done due to cost restraint. Finally 211 patients were subjected to statistical analysis. Out of 211 patients, 105 patient had vitamin B₁₂ level <160 pg/ml. Thus prevalence of vitamin B₁₂ deficiency in our study was 49.76%.

In Table 1, mean age of group A was 14.12 years and group B was 14.28 years. Maximum numbers of patients in both groups were found in 13-16 years (mid adolescent). Maximum patients belonged to lower middle class (41.90% vs. 48.11%). Maximum mothers were illiterate (47.61% vs. 41.50%). Most patients were educated up-to primary level (52.38% vs. 55.66%). Under-nutrition was seen in 69.25% patients of group A and 65.71% of group B. Statistical significance between vitamin B₁₂ level and age of patient (p=0.1023), socio-economic class (p=0.25), education of mother (p=0.66), education of patient (p=0.89) and BMI (p=0.9169) was not found.

Group A had 60 (57.14%) males as compared to 45 (42.45%) in group B. Male: Female ratio noted was 1.3: 1. Boys were more affected than girls with vitamin B₁₂ deficiency (p=0.032).

In group A, 80 (76.19%) patients were vegetarian and 26 (24.76%) were non-vegetarian. Statistical significance has been noted with regard to dietary habits between both groups (p=0.047), suggesting that vegetarians were more affected by vitamin B₁₂ deficiency.

In Table 2, Hematological parameters showed none of the patients with low total WBC count. In group A patients, 58(55%) had moderate anaemia, 34 (32%) had mild anaemia and 13 (12.38%) had severe anaemia as compared to 39.62%, 51.86% and 8.4% having moderate, mild and severe anaemia respectively in group B. Statistical significance was noted with regard to severity of anaemia in both groups, suggesting that vitamin B₁₂ deficiency was more seen with moderate to severe anaemia (p=0.016).
Table 1: Characteristic of patients with anaemia (N=211).

| Socio demographic variables | Group A (n=105) | Group B (n=106) | p value |
|-----------------------------|-----------------|-----------------|---------|
| Age groups (in years)       |                 |                 |         |
| 10-13                       | 22 (20.95%)     | 36 (33.96%)     | 0.1023  |
| 13-16                       | 54 (51.42%)     | 44 (41.50%)     |         |
| 16-18                       | 29 (27.61%)     | 26 (27.35%)     |         |
| Mean age                    | 14.12           | 14.28           |         |
| Sex                         |                 |                 |         |
| Male                        | 60 (57.14%)     | 45 (42.45%)     | 0.03286 |
| Female                      | 45 (42.85%)     | 61 (57.54%)     |         |
| Socio economic class        |                 |                 |         |
| Upper                       | 1 (0.95%)       | 1 (0.94%)       |         |
| Upper middle                | 37 (35.23%)     | 42 (39.62%)     | 0.2527  |
| Lower middle                | 44 (41.90%)     | 51 (48.11%)     |         |
| Upper lower                 | 23 (21.90%)     | 12 (11.32%)     |         |
| BMI                         |                 |                 |         |
| <18.5                       | 73 (69.52%)     | 44 (41.50%)     |         |
| 18.5 – 25                   | 36 (34.28%)     | 40 (37.73%)     | 0.9169  |
| >25                         | -               | -               |         |
| Education of mother         |                 |                 |         |
| Illiterate                  | 50 (47.61%)     | 44 (41.50%)     | 0.668   |
| Primary                     | 35 (33.33%)     | 40 (37.73%)     |         |
| Secondary                   | 20 (19.04%)     | 22 (20.75%)     |         |
| Education of patient        |                 |                 |         |
| Illiterate                  | 2               | 2               | 0.8903  |
| Primary                     | 55 (52.38%)     | 59 (55.66%)     |         |
| Secondary                   | 48 (45.71%)     | 45 (42.45%)     |         |
| Diet                        |                 |                 |         |
| Vegetarian                  | 80 (76.195)     | 66 (62.26%)     | 0.047   |
| Mixed                       | 26 (24.76%)     | 39 (36.79%)     |         |

In group A, 44 (41.95%) had thrombocytopenia as compared to 26 (24.52%) in group B, which was statistically significant (p=0.007).

Based on MCV value >100fl, macrocytosis was seen in 17 patients in group 1. None of the patients in group 2 had macrocytosis. In group A, 48 (45.71%) had normocytic anaemia and 40 (38.09%) had microcytic anaemia, while in group B maximum number of patients had microcytic anaemia 65 (61.32%). Vitamin B<sub>12</sub> deficiency was more likely to be associated with macrocytosis (p=0.000).

Table 2: Hematological characteristic.

| Severity of anaemia | Group A (n=105) | Group B (n=106) | p value |
|---------------------|-----------------|-----------------|---------|
| Mild                | 34 (32.38%)     | 55 (51.86%)     | 0.016   |
| Moderate            | 58 (55.23%)     | 42 (39.62%)     |         |
| Severe              | 13 (12.38%)     | 9 (8.4%)        |         |
| MCV                 |                 |                 |         |
| <80                 | 40 (38.09%)     | 65 (61.32%)     | 0.000   |
| 80 – 100            | 48 (45.71%)     | 41 (38.67%)     |         |
| >100                | 17 (16.19%)     | 0.00            |         |
| Platelet count      |                 |                 |         |
| < 1.5 lac           | 44 (41.95%)     | 26 (24.52%)     | 0.007   |
| >1.5 lac            | 61 (58.09%)     | 80 (75.47%)     |         |
In Table 3, Mean values of various parameters were compared with different levels of vitamin B12, which confirmed the earlier findings of age and BMI not related with vitamin B12 level.

| Table 3: Comparison of vitamin B12 level with mean values. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Vitamin B12 level (pg/ml) | p value |
|                 | <60              | 60 - 100         | 100 - 160        | >160            |
| Mean age        | n=13             | n=36             | n=56             | n=106           |
|                 | 13.77 ± 1.83     | 14.28 ± 2.17     | 14.32 ± 2.02     | 14.28 ± 2.15    | 0.8578          |
| Mean BMI        | 15.97 ± 2.58     | 17.56 ± 2.40     | 17.20 ± 2.88     | 17.21 ± 2.99    | 0.3932          |
| Mean Hb         | 6.55 ± 3.21      | 8.72 ± 2.62      | 9.36 ± 1.53      | 9.75 ± 1.65     | 4.1E-07         |
| Mean platelet count | 1.69,846.2 ± 2,21,459.5 | 1.76,535.7 ± 2,22,358.5 | 91,068.4 | 1,23,521.7 | 0.99918 |
| Mean MCV        | 98.84 ± 13.90    | 78.75 ± 16.58    | 78.82 ± 13.83    | 73.33 ± 11.60   | < 0.000001 |

There was no statistical significant difference noted in mean platelet count and severity of vitamin B12 deficiency. Patients with vitamin B12 level of <60 pg/ml had severe degree of anemia (mean hemoglobin level <7 gm/dl). Statistically significant fall in mean hemoglobin level was found with increasing severity of vitamin B12 deficiency (p =0.000000413). We also observed that mean MCV values were higher with more severe vitamin B12 deficiency (p <0.000001). Hematological changes like severe anemia and macrocytosis were late findings, more likely to be seen with severely low vitamin B12 levels.

DISCUSSION

We found 46.66% prevalence of anaemia in our study. Studies in prevalence of anaemia from different states of rural India, reported high prevalence of anaemia from 46-98%. However, most of the study has target population of adolescent girls. As per WHO classification prevalence rate of anaemia >40% is considered as severe health problem. Considering this, adolescent group should be considered as risk group for anaemia and special attention should be given on the strengthening of existing “package” of services for adolescents.

Studies from India reported up to 36% of infants and 47% of adults to have low serum vitamin B12 concentrations. Studies from other countries have reported the prevalence of 50%, 41% (Turkish infant & adolescent), 40% (Latin Americans). Our study also showed high prevalence of vitamin B12 deficiency of 49.76% among anaemic adolescents.

In study done in Colombian school age children, low vitamin B12 concentration was strongly associated with age >9 year, male gender, increased maternal parity status, the amount of money spent on food at home and lower SES. However such association with SES or education level of mother or patient was not seen in Guatemalan and Venezuelan study. We also did not find age, socio economic class, maternal education level or patient’s education level to be associated with B12 deficiency. We observed statistical significance between male gender and vitamin B12 deficiency which is similar to observation made by Velez et al. This male predominance could be due to higher requirement of micronutrients in males than females to sustain their rapid growth.

The source of vitamin B12 is mainly from foods of animal origin and synthesis by microorganism in body. Various studies have found association between vegetarianism and low vitamin B12 level. In our study also patient having vegetarian dietary habits were more affected with vitamin B12 deficiency suggesting that diet has major influence on vitamin B12 status of individual.

Various hematological findings in patient with Vitamin B12 deficiency are anaemia, neutropenia, macrocytosis, macrocytic anaemia, thrombocytopenia or pancytopenia. Ineffective DNA synthesis accounts for majority of hematological changes seen in vitamin B12 deficiency.

In our study, in vitamin B12 deficiency group 55.23% subjects had moderate anaemia and 12.38% had severe anaemia as compared to 39.62% & 8.4% subjects in vitamin B12 non deficient group. Atay et al reported 13% adolescents and 26% infants and Andres et al reported 37% elderly patients having anaemia with vitamin B12 deficiency. Our study population was anaemic adolescents, so we had higher number of patients having anaemia. Statistical significant difference was noted between mean haemoglobin values of B12 deficient as compared to non B12 deficient subjects, with greater fall in haemoglobin seen with severity of B12 deficiency.

Neutropenia was reported less frequently in vitamin B12 deficiency. In our study we did not find any patient having neutropenia. Thrombocytopenia was observed in 41.95% of subjects; however no difference in mean platelet count was seen in relation to different vitamin B12 level. Andres et al reported 9.9% incidence of thrombocytopenia in elderly patients with B12 deficiency. Obeid et al reported 3% incidence of thrombocytopenia in his study, however he found elevated mean platelet volume in 54% of subjects with vitamin B12 deficiency.
Finding of macrocytes on peripheral smear or MCV value (>100fl) has been suggested as one of the indicator of vitamin B12 deficiency. In our study, we found macrocytosis in only 17% of patients. Comparing the mean MCV values with different level of vitamin B12 showed that chances of getting macrocytosis (MCV >100 fl) were more with severe degree of Vitamin B12 deficiency.

In literature, macrocytosis in subjects with vitamin B12 deficiency has been reported at different rates, 36%, 23% and 16.1%. The MCV levels maybe normal in the one third of patients with Vitamin B12 Deficiency. Iron deficiency anaemia complicates the condition by masking macrocytosis. Also significant megaloblastosis could be seen in bone marrow of Vitamin B12 deficient subject, hematological finding in peripheral blood is not so common, a finding making it difficult to suspect vitamin B12 deficiency.

**CONCLUSION**

Vitamin B12 deficiency is an important cause of nutritional anaemia in adolescents especially in males and vegetarian population. Hematological finding of macrocytosis is a late occurrence. Severe degree of anaemia was more seen with severe vitamin B12 deficiency, thus measurement of vitamin B12 level of children in regions with low consumption of animal foods should not await for occurrence of hematological findings. National program should address vitamin B12 deficiency problem along with iron & folic acid supplementation.

**Limitation**

This is a hospital based study. Study population was anemic adolescents. Follow up after vitamin B12 therapy was not done so we could not document improvement in hematological parameters after treatment.

**Funding:** No funding sources  
**Conflict of interest:** None declared  
**Ethical approval:** The study was approved by the Institutional Ethics Committee

**REFERENCES**

1. Saratha A, Singh Z, Datta SS, Boratne AV, Senthivel V, Joice S. Prevalence of Anaemia among Young Adult Female Students in Medical teaching Institution in Pondicherry. Indian J Maternal Child Health. 2010;12(4):1-8.
2. Basu S, Hazarika R, Parmar V. Prevalence of Anaemia among School going Adolescents of Chandigarh. Indian Pediatr. 2007;42:593.
3. Premalatha T, Valarmathi S, Srijayanth P, Sundar JS, Kalpana S. Prevalence of Anaemia and associated Factors among Adolescent School Girls in Chennai, Tamil Nadu, India. Epidemiol. 2012;2:118.
4. Dugdale M. Anaemia. Obstet Gynecol Clin North Am. 2001;28(2):363-81.
5. Bhardwaj A, Kumar D, Raina SK, Bansal P, Bhushan S, Chander V. Rapid Assessment for Coexistence of Vitamin B12 and Iron Deficiency Anaemia among Adolescent Males and Females in Northern Himalayan State of India. Anaemia. 2013;2013:959605.
6. Fishman SM, Christian P, West KP. The Role of Vitamins in the Prevention and Control of Anaemia. Public Health Nutrition. 2000;3(2):125 -50.
7. Stabler S. Vitamin B12 Deficiency. N Engl J Med. 2013;368:149-60.
8. Molloy AM, Kirke PN, Brody LC, Scott JM, Mills JL. Effects of Folate and Vitamin B12 Deficiencies during Pregnancy on Fetal, Infant and Child Development. Food Nutr Bull. 2008;29(2):101-11.
9. Atay E, Akin M, Ozman B, Oztekin O, Karakus YT, Erdogan F. Frequency of Hematological Findings Associated with Severe Plasma Vitamin B12 deficiency in Infants and Adolescents. Clin Lab. 2014;60:659-62.
10. Kanani S. Combating Anaemia in Adolescent Girls: a report from India. Mothers Child. 1994;13:1-3.
11. Kumar S, Raharatam A. Prevalence of Anaemia and Hookworm infestation among Adolescent girls in one rural block of Tamilnadu. Indian J matern Child Health. 1997;8:73-5.
12. Muzammil K, Kishore, Semwal J. Common Nutritional deficiencies of Adolescents in Dehradun. Indian J Sci Res. 2010;1(1):77-80.
13. Villamor E, Mora-Plazas M, Forero Y, Lopez-Arana S, Baylin A. Vitamin B12 Status is Associated with Socioeconomic Level and Adherence to an Animal Food Dietary Pattern in Colombian School Children. J Nutr. 2008;138(7):1391-8.
14. Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. Am J Clin Nutr. 2007;86:1302–9.
15. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Orning L, et al. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr. 2001;74:233–41.
16. Thomas D, Chandra J, Sharma S, Jain A, Pemde KH. Determinants of Nutritional Anaemia in Adolescents. Indian Pediatr. 2015;52:867-69.
17. Koc A, Kocyigit A, Soran M, Demir N, Sevinc E, Erel O, et al. High Frequency of Maternal Vitamin B12 deficiency as an Important Cause of Infantile Vitamin B12 Deficiency in Sanlinurfa Province of Turkey. Eur J Nutr. 2006;45:291-7.
18. Isik Balci Y, Karabulut A, Gurses D, Ethem Covut I. Prevalence and Risk Factors of Anaemia among Adolescents in Denizli, Turkey. Iran J Pediatri 2012;22:77-81.
19. Allen LH. Folate and vitamin B12 status in the Americas. Nutr Rev. 2004;62:29–33.
20. McLean ED, Allen LH, Neumann CG, Peerson JM, Siekmann JH, Murphy SP, et al. Low plasma vitamin B-12 in Kenyan school children is highly prevalent and improved by supplemental animal source foods. J Nutr. 2007;137:676–82.
21. Garcia-Casal MN, Osorio C, Landaeta M, Leets I, Matus P, Fazzino F, et al. High prevalence of folic acid and vitamin B12 deficiencies in infants, children, adolescents and pregnant women in Venezuela. Eur J Clin Nutr. 2005;59:1064–70.
22. Ramirez Velez R, Correa-Bautista JE, Martinez-Torres J, Meneses-Echavez JF, Lobelo F. Vitamin B12 concentration and its Association with Sociodemographic Factors in Colombian Children: Findings from the 2010 National Nutrition Survey. Nutr. 2016;32(2):255-9.
23. Verma M, Chhatwal J, Kaur G. Prevalence of Anaemia among Urban School Children of Punjab. Indian Pediatr. 1998;35:1181-6.
24. Ajuria-Morentin I, Mar-Medina C. Determination of Reference Values for Serum Folate and Vitamin B12 Using Three Different Immunoassays: Is it Worth Making an Effort to Produce them in Our Laboratory? Clin Lab. 2014;60:1135-43.
25. Andrès E, Affenberger S, Zimmer J, Vinzio S, Grosu D, Pistol G, et al. Current Hematological Findings in Cobalamin Deficiency. A study of 201 consecutive patients with documented cobalamin deficiency. Clin Lab Haematol. 2006;28:50-6.
26. Obeid R, Geisel J, Schorr H, Hbner U, Herrman W. The Impact of Vegetarianism on Some Hematological parameters. Eur J Haematol. 2002;69:275-9.
27. Stabler SP, Allen RH, Savage DG, Lindenbaum J. Clinical spectrum and diagnosis of cobalamin deficiency. Blood. 1990;76:871-81.
28. Stott DJ, Langhorne P, Hendry A, McKay PJ, Holyoake T, Macdonald J, et al. Prevalence and hematopoietic effects of low serum vitamin B12 levels in geriatric patients. Br J Nutr. 1997;78:57-63.
29. Yüksel S, Uslan, Acartürk G, Çölbaý M, Karaman O, Maralcan M, et al. A Retrospective Evaluation of Patients with Vitamin B12 Deficiency. Med J Bakırköy. 2006;2(4):126-9.

Cite this article as: Surana A, Tilwani S, Patel S, Prajapati H, Prasad R. Vitamin B12 status among anaemic adolescents. Int J Community Med Public Health 2017;4:1780-5.