Short Communication

Vitamin B12 Status in Rural Adolescent School Girls in Mysuru, India: A Community-Based Cross-Sectional Study

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Abstract

Almost 56% of Indian adolescent girls aged 15–19 years suffer from anemia. Adolescent age is a period of many significant physiological changes that increase nutritional demand, and they remain at risk for nutritional deficiencies. Our aim is to assess the prevalence of Vitamin B12 deficiency among school-going adolescent girls aged 13–16 years of rural Mysore. This study was a cross-sectional study, conducted in two high schools in rural Mysuru. Adolescent girls aged 13–16 years were included. The chosen subjects underwent complete hemogram, peripheral smear examination, and vitamin B12 level estimation. Out of 98 subjects enrolled, 40.81% were found to be deficient in vitamin B12. Macrocytes and hyper-segmented neutrophils were found to have statistically significant (P value < 0.001) relationship with vitamin B12 deficiency. The relationship between vitamin B12 deficiency with either type of diet and anemia was statically insignificant ($P > 0.05$). The prevalence of vitamin B12 deficiency among rural adolescent girls in Mysuru is high. Other causes of nutritional anemia apart from iron deficiency, such as vitamin B12 deficiency, must be considered in the etiology of anemia and optimally treated. It is recommended to further strengthen the adolescent national health programs and food fortification programs.

Keywords: Adolescent girls, anemia, rural, vitamin B12

Introduction

Nutritional anemia is the most common form of anemia in India. According to the National Family Health Survey-III, almost 56% of adolescent girls aged 15–19 years suffer from anemia.[1] Adolescents account for about 21% of Indian population.[2] Hence, more focus is needed in the areas of adolescent health, more specifically that of females. The adolescent age group is the vulnerable period in the human life cycle for the development of nutritional anemia. Adolescence is an age group of significant changes for girls, including menarche, physical, psycho-social, and mental changes, which increase the nutritional demand, hence placing them at a higher risk of developing nutritional anemia. Screening for nutritional deficiency and early intervention makes this transition easier. Nutritional anemia has many causes, which include iron deficiency, folic acid deficiency, and vitamin B12 deficiency. The Government of India implemented the Weekly Iron and Folic Acid (IFA) Supplementation (WIFS) Programme under the Adolescent Girls Anaemia Control Program but not vitamin B12 supplementation.[3] Pawlak *et al.*[4] observed vitamin B12 deficiency rates of 25–86% among children, 21–41% among adolescents, and 62% among pregnant women. Vegans are at the highest risk of developing vitamin B12 deficiency.[4] Pregnant vegetarian/vegan women with a low vitamin B12 status may result in vitamin B12-deficienct infants.[4] Sub-clinical cobalamin deficiency is more common than symptomatic vitamin B_{12} deficiency.[5] Out of 382 rural adolescent girls from North India, vitamin B12 deficiency (58%) was more common than iron deficiency (11%), which clarifies the need for treating the other causes of nutritional anemia rather than iron deficiency alone.[6] The data on the prevalence of vitamin B12 deficiency among rural adolescent girls in South India are limited. Hence, this study was undertaken to throw light on the prevalence of vitamin B12 deficiency among rural adolescent girls in Mysuru.

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Materials and Methods

Study design and setting

This cross-sectional study was carried out over a period of 18 months from March 2020 to October 2021.

Study participants

Adolescent girls aged between 13 and 16 years studying in rural Mysore were included. Adolescent girls receiving frequent blood transfusions, hematinics, multi-vitamins, anti-convulsants, chemotherapeutic agents, and anti-microbial agents were excluded.

Sample size and sampling method

The sample size was calculated based on the prevalence of vitamin B12 deficiency in rural adolescents to be 43.9%[7] with 10% absolute allowable error and the alpha of 5% to be 94.61, which was rounded off to 95 in our study. Two schools in the rural areas in Mysuru district were randomly chosen. Simple random sampling was used to select adolescent girls for the study. The study was conducted among the schools in the rural field practice area of JSS Medical College, Mysore. There are 12 schools across three adapted primary health care jurisdictions, out of which two schools were selected by simple random sampling by lottery method. The number of children included in each school was decided by probability proportionate to size technique. After visiting the schools, girl children between 13 and 16 years were line-listed using the attendance register. A unique serial number is given to each of these children. The serial number was subjected to Microsoft Excel to generate 101 random numbers. The students with these random serial numbers selected through this method were selected for the study.

Study tool

Vitamin B12 level was analyzed using CLIA (chemiluminescence immunoassay analyzer) in the fully automated hormone analyzer Beckman Coulter Access 2.

Data collection

Details of history and examination were entered in the proforma. Data were collected and entered by the investigators. JSS Institutional Ethical Committee clearance and necessary permission from the school authority were taken. Written informed consent/assent was taken from the parents of the children/children themselves.

Statistical analysis

Data collection was entered in MS Excel and analyzed using SPSS version 21. Descriptive statistical measures like percentage, mean, and standard deviation were applied. Inferential statistical tests like Chi-square test and Pearson correlation at ANOVA were applied. Data were interpreted as statistically significant at a *P* value less than 0.05.

Results

A total of 101 girls were included for the study. Among them, two received multi-vitamins and one child did not give assent for the blood sampling. The remaining 98 subjects were enrolled and subjected to complete hemogram, peripheral smear examination, and vitamin B12 level estimation. The demographic profile of adolescent rural girls is depicted in Table 1. The majority of them were 15 years of age. In our study, 40 (41.8%) children were found to be deficient in vitamin B12 levels (serum vitamin B12 < 120 pg/mL). A comparison of parameters with respect to vitamin B12 levels is shown in Table 2. The relationship between type of diet, nutrition status, and anemia with vitamin B12 deficiency was found to be statistically insignificant ($P = 0.581$, 0.358, and 0.286, respectively). Out of 98 girls, 21 (21.4%) had macrocytes on their peripheral smears. Among 21 of them, 20 girls (95.2%) were found to have vitamin B12 deficiency. Peripheral smear showed hyper-segmented neutrophils in 31 (31.6%) of the study group. Among 31 of them, 29 (93.5%) had vitamin B12 deficiency. Macrocytes and hyper-segmented neutrophils were found to have statistically significant (P value < 0.001) relationship with vitamin B12 deficiency. Despite 40.8% of study subjects being deficient in vitamin B12, only two subjects had mean corpuscular values (MCVs) of 101 fL and 100.4 fL, and both were deficient in vitamin B12. In this study, four subjects showed knuckle hyperpigmentation, out of which three were vitamin B12-deficient. However, the relation between them was statistically insignificant ($P = 0.155$).

Discussion

Adolescent age is a period of many significant physiological changes that increase nutritional demand, and as they are the future mothers, they remain at risk for nutritional deficiencies. In the current study, the prevalence of vitamin B12 deficiency

Table 1: Demographic profile of adolescent rural girls (*n*=98)

Parameter	*n* (%)
Age (years)	
13	11 (11.2)
14	21 (21.4)
15	49 (50)
16	17 (17.5)
Anemia status	
Anemia	65 (66.3)
Non-anemia	33 (33.6)
Menarche	
Attained	92 (93.8)
Not attained	6 (6.1)
Diet	
Vegetarian	83 (84.6)
Mixed	15 (15.3)
Calorie intake	
Deficient	17 (17.3)
Adequate	81 (82.6)
Protein intake	
Deficient	75 (76.5)
Adequate	23 (23.4)

Table 2: Comparison of parameters with respect to vitamin B12 deficiency©

Parameter	Vitamin B12 levels		Total number *n*=98	*P*
	Deficient=40 (40.8%)	Normal=58 (59.1%)		
Diet				
Vegetarian	35 (42.2%)	48 (57.8)	83	0.58
Mixed	5 (33.3%)	10 (66.7%)	15	
Calorie deficit				
Adequate	35 (43.2%)	46 (56.8%)	81	0.41
Deficit	5 (29.4%)	12 (70.6%)	17	
Protein deficit				
Adequate	11 (47.8%)	12 (52.2%)	23	0.47
Deficit	29 (38.7%)	46 (61.3%)	75	
Menarche attained				
Yes	39 (42.4%)	53 (57.6%)	92	0.36
No	1 (16.7%)	5 (83.3%)	6	
Anemia status				
Anemic	24	41	65	0.28
Non-anemic	16	17	33	
Macrocytes				
Present	20 (95.2%)	1 (4.8%)	21	<0.001
Absent	20 (26%)	57 (74%)	77	
Hypersegmented neutrophils				
Present	29 (93.5%)	2 (6.5%)	31	<0.001
Absent	11 (16.4%)	56 (83.6%)	67	
Knuckle hyperpigmentation				
Present	3	1	4	0.155
Absent	37 (16.7%)	57 (83.3%)	94	

©Vitamin B12 deficiency was defined as values <120 pg/mL as per the lab reference range (120–914 pg/mL) of the lab used for the processing of the samples

among rural adolescent girls was found to be 40.81%. Among 382 rural adolescent school girls aged 10–18 years at Raipur district, 58% had vitamin B12 deficiency.[6] In a study from Delhi and rural areas of Haryana, among school-going adolescents (11–17 years), the prevalence of vitamin B12 deficiency was found to be 32.4% and it was more common in rural adolescents [rural 43.9% versus urban 30.1% ($P < 0.001$)]. Serum vitamin B12 levels were found to be lower in rural females when compared to males ($P = 0.030$).[7] Kapil *et al*.[8] observed that the prevalence of deficiency of vitamin B12 in HIG, MIG, and LIG categories of adolescents was 47.1, 80.7, and 87.5%, respectively, in Delhi. In a study by Surana *et al*.[9] done in an urban Indian adolescent population, the prevalence of B12 deficiency was identified to be 49.76% among 211 adolescents with anemia. A study from Loni city revealed vitamin B12 deficiency in 72.7% among the adolescents aged between 11 and 18 years.[10]

There is wide variation in the prevalence of vitamin B12 deficiency among children and adolescents from other developing nations. In a study from Turkey, the prevalence of vitamin B12 deficiency among adolescents was found to be 24.5% in the low-income groups of the Aegean Region when compared to 69.2% when assessed in Ankara.[11,12] Htet *et al*.[13] observed less than 1% prevalence of vitamin B12 deficiency among 391 adolescent anemic schoolgirls living in the delta region of Myanmar. The prevalence of vitamin B12 deficiencies in pregnant women was found to be 61.34% in Venezuela.[14]

Variations in vitamin B12 deficiency in different geographical areas may be due to multiple reasons like differences in diet, differences in genetic predisposition to various malabsorption syndromes, and available amenities. Factors like financial background, health education regarding good dietary practices, different nutritional programs, and food fortification plans may also have contributed.

Megaloblastic anemias are characterized by the presence of large red blood cell precursors called megaloblasts in the bone marrow. Peripheral smear findings of macrocytic RBC [mean corpuscular volume (MCV) greater than 100 fL] or hyper-segmented neutrophils (>5% of neutrophils with ≥5 lobes) are characteristic of megaloblastic anemia.[15] Macrocytosis and hyper-segmented neutrophils precede the development of anemia and may be present as isolated findings.[15]

In the current study, among 98 subjects, despite 40.8% being deficient in vitamin B12, only two subjects had MCV more than 100 fL and both were deficient in Vitamin B12. Peripheral smear examination, however, showed a statistically significant correlation between the presence of macrocytes and vitamin B12 deficiency ($P = 0.001$). Surana *et al*.[9] observed significant correlations between vitamin B12 deficiency and macrocytosis. Patel S *et al*.[6] identified 58.58% of rural school-going adolescent girls to be deficient in vitamin B12; however, only <1% had increased MCV values. In a study of 111 children aged 1–18 years with anemia in Rishikesh,

64.8% had vitamin B12 deficiency, and among them, 37.5% had macrocytosis.[16] These observations show that peripheral smear macrocytes were a better indicator of vitamin B12 deficiency than MCV values.

In our study, hyper-segmented neutrophils were observed in 31 (31.6%) of study subjects. Out of 31 subjects showing hyper-segmented neutrophils, 29 (93.5%) had vitamin B12 deficiency, which was statistically significant (P = 0.001), which suggests that presence of hyper-segmented neutrophils is a reliable indicator of vitamin B12 deficiency. In a study done by Thompson *et al.*,[17] neutrophil hyper-segmentation was more sensitive (91%) than MCV >95 fL (62%) in identifying of vitamin B12 deficiency. The presence of macrocytes and hyper-segmented neutrophils on the peripheral smear is reliable evidence of vitamin B12 deficiency, rather than relying on blood indices. Peripheral blood smear is inexpensive and accessible and can be done widely across all areas of the country, in contrast to performing vitamin B12 serum levels, which is expensive and not available in all areas.

Knuckle pigmentation was found in four children, and three among them had vitamin B12 deficiency. Knuckle pigmentation was noted in 25% of children with vitamin B12 deficiency in a study from Rishikesh.[16]

Limitations

The study could have included adolescent urban girls also.

Conclusions

It can be reiterated that vitamin B12 deficiency is an important health problem among rural adolescent girls in India. As the prevalence of vitamin B12 deficiency is high and the consequences of deficiency in adolescent females can be detrimental, we recommend that along with iron and folic acid, vitamin B12 should also be supplemented regularly in the adolescent nutrition programs.

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Conflicts of interest

There are no conflicts of interest.

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