Folate status and intake of tribal Indian adolescents aged 10 to 17 years

Rati Jani, Nisha Salian, Shobha Udipi, Padmini Ghugre, Neha Lohia, Jere Haas, and Erick Boy

Abstract

Background. *Adequate folate intake and levels are advisable throughout life but are of particular importance during adolescence, a period of rapid growth. However, folate insufficiency in economically deprived Indian adolescents is understudied.*

Objective. *This cross-sectional study examined the prevalence of folate deficiency and adequacy of folate intake of 224 tribal Indian adolescents (10 to 17 years of age). The secondary aim was to study the association between anemia status and folate status.*

Methods. *Radioimmunoassay, multiple-pass 24-hour dietary recall, and HemoCue were used to measure red blood cell (RBC) folate, folate intake, and anemia status, respectively.*

Results. *The geometric mean (95% CI) RBC folate concentration (nmol/L) was 360.2 (329.7 to 393.6), and the mean ± SD folate intake (μg/day) and hemoglobin level (g/L) were 159.9 ± 44.7 and 125.4 ± 13.0, respectively. Almost half of boys and girls aged 10 to 12 and 13 to 15 years and 66.7% of girls aged 16 to 17 years were deficient in RBC folate (< 340 nmol/L). The mean ± SD folate intake (μg/day) of girls (139.4 ± 34.5) was lower than that of boys (173.8 ± 45.5) ($p < .001$). With respect to adequacy of folate intake, a greater proportion of girls in the age group of 13–15 years (78.5% vs 38.6%, $p < 0.001$) and 16–17 years (100.0% vs 76.9%, $p = 0.04$) had intakes below their Recommended Dietary Allowance (RDA). No association was observed between folate intake and RBC folate deficiency or between anemia status and RBC folate deficiency.*

Conclusions. *Folate insufficiency was widespread in tribal Indian adolescents. There is an urgent need to develop culturally sensitive strategies for improvement.*

Key words: Adolescents, anemia, folate intake, Indian, red blood cell folate

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Introduction

In India, nearly half (47%) of adolescent girls and 58% of adolescent boys aged 15 to 19 years are underweight (body mass index [BMI] < 18.5 kg/m^2) [1]. Underweight status could reflect inadequate nutrient intake and thereby increased susceptibility to nutritional deficiencies [2]. In India, the prevalence of micronutrient deficiencies is high, given that the diets are predominantly cereal based. According to national Indian data for 2009, all girls and 92% of boys aged 10 to 17 years consumed the suggested amounts of cereals and millets (240 to 330 g/day for girls and 300 to 450 g/day for boys) [3]. In contrast, none of the boys or girls consumed the suggested amounts of green leafy vegetables (100 g/day) and fruits (100 g/day) [3]. This is of particular interest in this paper, as green leafy vegetables and citrus fruits are among the important natural sources of folate in Indian diets, which are predominantly vegetarian [2, 4].

Folate is obtained from the diet, as it cannot be synthesized by humans [5]. As a coenzyme it plays an integral role in amino acid and nucleotide metabolism. Specifically, folic acid is required for the synthesis of methionine from homocysteine [6]. High levels of homocysteine in childhood are an independent risk factor for cardiovascular and cerebrovascular diseases in adulthood [5, 7]. Folic acid is also essential for the biosynthesis of DNA and RNA and therefore is vital for erythrocyte production. Folate deficiency is manifested clinically as megaloblastic anemia [6]. When the rate of cell synthesis increases, folate requirement increases. Adolescence, the stage of the second-highest

growth rate after infancy, is a crucial stage that requires an adequate intake of folate [8]. Consuming the recommended amounts of folic acid and maintaining adequate levels is of particular importance for females, especially during pregnancy due to associated birth defects, specifically neural tube defects [8, 9]. A recent meta-analysis of 19 studies conducted in India reported a total of 308,387 births, among which neural tube defects were identified in 4.1 of 1,000 births (95% CI, 3.1 to 5.4) [9]. Nearly all studies examined in the meta-analysis were conducted in government hospitals, which mainly cater to the lower socioeconomic strata [9]. This is in line with previous research, which has indicated that nonaffluent and rural people may be at greater risk for folate deficiency than affluent and urban people [4, 10].

There is a dearth of literature examining the prevalence of folate deficiency and adequacy of folate intake in economically deprived, healthy (without chronic diseases) Indian adolescents. Only two studies were identified that examined red blood cell (RBC) [11] and serum [12] folate deficiency in 869 and 1,037 urban, affluent, healthy Indian children aged 5 to 18 years, respectively. Sivakumar et al. [11] showed that all children were deficient in RBC folate (< 550 nmol/L), and Kapil and Sareen [12] reported that 38% of children 12 to 18 years of age had serum folate deficiency (< 6 nmol/L). In addition, there is a scarcity of research investigating the association between folate intake and folate status in the Indian scenario. The present study examined the prevalence of folate deficiency and the adequacy of folate intake in tribal adolescents aged 10 to 17 years. The study also investigated the relationship between dietary folate and RBC folate status of the participants.

In India, the national data report the prevalence of anemia in adolescents aged 15 to 19 years [1], but no data are available examining the relationship between anemia status and folate status. A narrative review by Jack [13] suggested that micronutrient (folate and vitamin B_{12}) deficiencies may be prevalent irrespective of anemia status. The secondary aim of the study was to report the prevalence of anemia and test the hypothesis that nonanemic tribal adolescents aged 10 to 17 years may be folate deficient.

Methods

Characteristics of participants

This cross-sectional research was part of a longitudinal project entitled "Efficacy of Iron Biofortified Pearl Millet in Improving the Iron Status of Adolescents in India" conducted from August 2011 to April 2012 by the Department of Food Science and Nutrition, S.N.D.T. Women's University, Mumbai, India, in collaboration with Cornell University, USA, and HarvestPlus, USA. The present study was conducted in March 2012 in the economically disadvantaged tribal district (Sangamner Taluka, Ahmednagar District) of Maharashtra. Ethical clearance was obtained from the Inter Systems Bio-Medical Ethics Committee, Mumbai. Subjects were excluded if they were not between 10 and 17 years of age, were consuming multivitamin supplements, had physical disabilities, were medically diagnosed with chronic malabsorption issues (e.g., inflammatory bowel disorders) or health disorders (e.g., diabetes), or were severely undernourished (≤ –3 SD BMI z-scores) [14]. The children were residents of one of three selected boarding schools. The schools were sampled based on convenience. Written approval was sought from the school heads before commencing the study. During school hours, the homeroom teacher, accompanied by the research assistant, provided information about the study to the attending students. Students willing to participate were requested to submit the written informed consent form approved by their parents or guardians. Initially 262 students agreed to participate in the study. However, on the dates designated for data collection, taking blood samples, and recording sociodemographic information, a total of 224 students were present, and 204 students were present to provide dietary data. In summary, 224 tribal adolescents aged between 10 and 17 years participated.

Sociodemographic characteristics

A trained research assistant recorded the age and sex of the participants. Heights and weights were measured three times and averaged to assess whether the participant met the inclusion criteria (> –3 SD BMI z-scores) [14]. Weight was measured with a digital weighing scale (Equinox model EB6171) and recorded to the nearest 0.1 kg; height was measured against a vertical wall with a fiberglass measuring tape (Shenzhen Weiye glass-fiber measuring tape model B-0020) calibrated against a stadiometer and recorded to the nearest 0.1 cm. Weight was measured with the subject wearing light clothing without shoes, and height with the subject wearing no hair accessories or shoes.

Dietary assessment

Dietary data were collected every month as part of the longitudinal study conducted from August 2011 to April 2012. For the present study, the dietary data collected at the time of blood collection in March 2012 were used. Dietary data were collected by 13 research assistants who were trained for 15 days prior to the study. First, a 24-hour weighing method was conducted followed by a single multiple-pass 24-hour dietary recall. The multiple-pass 24-hour dietary recall was then validated against the 24-hour weighing method.

In the first step of the 24-hour weighing method, weighed food records were obtained 15 days prior to the beginning of the study in August 2011. Each research assistant was provided with two standardized and well-calibrated digital weighing scales (1-kg capacity, Eagle make, model no. EL01200; 7-kg capacity, Metro make, model no. SF-400), which recorded weights to the nearest 1 g. A standardized measuring tape (Shenzhen Weiye glass-fiber measuring tape model B-0020) was used to record the length, thickness, and diameter of food items to the nearest millimeter. Lastly, a set of calibrated pans, standardized glasses, cups, serving spoons, ladles, teaspoons, and serving dishes was provided. Data collection by the weighing method was conducted at two levels: the entire hostel level and the individual level.

Hostel level

In all three hostels, a cyclic menu was used, consisting of three standardized, uniform meals (breakfast, lunch, and dinner) in a day. The menus were monotonous, consisting of pearl millet flat bread, rice, pulses, less than 30 g of vegetables, and no fruits. For each recipe, all the raw ingredients used for the entire hostel were first weighed, and the volume was measured followed by measurement of the cooked yields in terms of weight as well as volume, numbers, and size/dimensions, including diameter, thickness, and length, wherever applicable, using standardized measuring equipment. Since the participants' diets were predominantly millet-based (pearl millet flat bread [*bhakri*]), the preparation of bhakri was monitored by research assistants specifically trained for bhakri assessment (i.e., recording the method of bhakri preparation and intake). The assistants periodically (every third day) weighed and recorded the flour used for bhakri preparation, and three randomly selected bhakris were weighed each day and their length, thickness, and diameter were measured in order to estimate actual intakes by the participants.

Individual level

The research assistant followed a randomly selected subsample of the participants ($n = 40$) over a 24-hour period. All foods and beverages consumed by the participants were weighed and/or measured using the calibrated digital weighing scales and standardized equipment.

In the single multiple-pass 24-hour dietary recall (second step), each participant was asked to recall all food items and beverages consumed on the previous day from the time he or she woke up in the morning to the last food item or beverage consumed at night. The time of consumption of each item was noted. Thereafter, for each item, the amount consumed was recorded in standard measures. Portion sizes were quantified using standardized food models and actual food items. The intake of *bhakri* was monitored daily by the specially trained research assistants by recording the number of *bhakris* consumed (to the nearest 0.25 unit) by each participant. Although a multiple-pass single 24-hour dietary recall conducted at the time of blood collection (March 2012) was used for the present study, it is important to note that the coefficient of variation) observed for specific nutrients (energy 2.2%, protein 2.4%, carbohydrate 2.2%, fat 2.9%, and folate 3.3%) was low for all time points in the longitudinal data (August 2011 to April 2012), as dietary data were collected every month. This indicated the monotonous and standardized nature of the diets.

Standardization of all food items (third step)

The data obtained from the 24-hour weighing method at the hostel level were cross-checked by preparing four portions of all recipes served to the participants during the entire study period in the nutrition laboratory of the Food and Nutrition Department at S.N.D.T Women's University. The yields of the prepared recipes were measured with standardized equipment. The prepared recipes were also analyzed for specific nutrients (energy, carbohydrate, protein, fat, and folate), and compared with the nutrient intakes obtained from the multiple-pass 24-hour dietary recall. A strong coefficient of correlation (r) was observed between both dietary methods for the selected nutrients ($r = 0.70$ for energy, 0.76 for protein, 0.65 for carbohydrate, 0.72 for fat, and 0.79 for folate). Therefore, for the present study, the single multiple-pass 24-hour dietary recall method could be considered valid.

CS Dietary software (version 1.11, Serpro S.A & HarvestPlus) was used to calculate nutrient intakes. The nutritive values of 479 raw foods given by the National Institute of Nutrition, Hyderabad, India, were entered [15]. Since these values are mainly for raw food items, a database for all the cooked foods was created for 105 standardized preparations that were commonly consumed. The software accounted for nutrient losses during cooking and processing. Intakes of total energy (kilocalories), carbohydrate (grams), protein (grams), fat (grams), and folate (micrograms) were calculated daily.

Supplement intake

Although consumption of multivitamin or mineral supplements was considered an exclusion criterion, the participants prior to data collection (blood serum and diet) were asked about any form of supplement usage. None of the participants reported consumption of nutritional supplements, and they had access to only three standardized meals per day provided by the school hostels.

Blood analysis

RBC folate was measured at Hinduja Hospital, Mumbai. using the Dual Count Solid Phase No Boil radioimmunoassay (Siemens Medical Solutions Diagnostics), which has been used in previous research [11] and has been shown to be comparable to the immunofluorescence method [16] and automated immunoenzymatic assay [17]. Fasting blood (7 mL) was collected by a trained phlebotomist from the antecubital vein into a single heparinized vacutainer tube (Beckman Dickinson). The red blood cells were separated from the plasma by centrifugation, stabilized with 1% ascorbic acid in a vacutainer, incubated for 60 minutes at room temperature, and frozen at –20°C. The radioactive iodine (I^{125}) count in the precipitate was measured with a Perkin Elmer automatic gamma counter, and folate concentrations were read from the calibration curve. Quality control was carried out using a number of tests involving lyophilized protein-based controls (control 1, 2.6 to 3.8 ng/mL; control 2, 6.9 to 9.3 ng/mL; control 3, 10.9 to 14.1 ng/mL; tracer reagent, radioactive I^{125}-labeled folic acid) with folate concentrations in the deficient range.

Hemoglobin was measured with the HemoCue Hb 201^+ (A Quest Diagnostic Company), which has been shown to be comparable to the cyanmethemoglobin method and the automated Sysmex KX21N Hematology Analyzer [18]. Whole blood was analyzed for hemoglobin within 6 hours. The HemoCue Hb 201 consists of a single-use, disposable microcuvette that contains reagents in a dry form that convert hemoglobin into methemoglobinazide. Hemoglobin content is determined by a portable photometer, which measures the absorbance of methemoglobinazide at 570 and 880 nm to ensure automatic compensation for turbidity.

Statistical analysis

Logarithmic transformations were conducted to normalize the distribution of folate status, with RBC folate (nmol/L) reported as geometric mean (95% CI). Folate intake (μg/day) and anemia status (g/L hemoglobin) followed the normal distribution and are reported as mean ± SD. Median (25th, 75th interquartile range [IQR]) values are reported for all these variables. Folate status was categorized according to the World Health Organization (WHO) [19] recommendations as RBC folate deficiency levels < 340 nmol/L (< 151 ng/mL). Folate intakes were categorized according to the Recommended Dietary Allowances (RDAs) for folate by the Indian Council of Medical Research (ICMR) [20]: >140, > 150, and > 200 μg/day for the age groups 10 to 12, 13 to 15, and 16 to 17 years, respectively. The WHO cutoff points for nonanemia are ≥ 115 g/L hemoglobin for children aged 5 to 11 years, ≥ 120 g/L for children aged 12 to 14 years, ≥130 g/L for boys aged ≥ 15 years, and ≥ 120 g/L for girls aged ≥ 15 years. For mild anemia, the cutoff points are 110 to 114 g/L hemoglobin for children aged 5 to 11 years, 110 to 119 g/L for children aged 12 to 14 years, 110 to 129 g/L for boys aged ≥ 15 years, and ≥ 110 to 119 g/L for girls aged ≥ 15 years [21]. For all age groups and both sexes, moderate and severe anemia are defined as hemoglobin levels of 80 to 109 g/L and < 80 g/L, respectively [21]. For each adolescent, according to his or her age and sex, the anemia status (g/L hemoglobin) was coded as nonanemic, mildly anemic, moderately anemic, or severely anemic based on the WHO [21] age- and sex-specific categories. The participants were classified into age groups (10 to 12, 13 to 15, and 16 to 17 years) according to the categories given by the ICMR [20] to establish RDAs for adolescents. RBC folate status, folate intake, and anemia status are reported by sex and age group. ANOVA/independent-samples *t*-test and Pearson's chi-squared test were used for continuous and categorical variables, as appropriate. Pearson's chi-square test was used to examine the association between adequacy of folate intake and RBC folate deficiency and between anemia status and RBC folate deficiency across age groups and sexes. A p-value < .05 was considered to indicate statistical significance. The analyses were performed with SPSS, version 21.

Ethical approval

The study was approved by the Inter Systems BioMedical Ethics Committee, Mumbai.

Results

Characteristics of the participants are presented in **table 1**. The highest proportion of the participants were 13 to 15 years of age (68.3%, $n = 153$), followed by those aged 16 to 17 (18.3%, $n = 41$) and 10 to 12 years (13.4%, $n = 30$). Forty-one percent of the participants were girls.

Overall, there was no significant difference in mean RBC folate concentration between girls (152.0 nmol/L; 95% CI, 133.0 to 173.7) and boys (164.2 nmol/L; 95% CI, 145.6 to 185.1; $F(1) = 0.71$, $p = .39$). RBC folate status by age and sex is reported in **table 2**. Among children aged 16 to 17 years, girls were 3.8 times more likely than boys to be deficient in RBC folate (< 340 nmol/L) (OR = 0.27; 95% CI, 0.07 to 1.02; $p = .04$).

Overall, mean ± SD folate intake was significantly lower in girls than in boys (139.4 ± 34.5 vs. 173.8 ± 45.5 μg/day; $t(202) = 5.8$, $p < .001$). **Table 2** highlights folate intakes of the participants by age category and sex. For the age group 13 to 15 years, girls had significantly lower mean folate intake than boys ($t(139) = 5.9$,

TABLE 1. Summary of participants' characteristics (n = 224)

Characteristic	Mean ± SD	Median (25th–75th interquartile range)
Age (yr)	14.0 ± 1.3	14.0 (13.0, 15.0)
RBC folate status (nmol/L)	360.2 (329.7–393.6)[a]	365.3 (254.3, 551.3)
Folate intake (μg/day)	159.9 ± 44.7	152.6 (131.7, 154.8)
Hemoglobin status (g/L)	125.4 ± 13.0	125.0 (117.3, 134.0)
Energy (kcal/day)	1938.4 ± 391.4	1946.4 (1696.5, 2168.0)
Carbohydrate (g/day)	330.6 ± 71.5	334.3 (277.1, 367.1)
Protein (g/day)	59.7 ± 23.4	54.9 (45.6, 69.1)
Fat (g/day)	45.8 ± 12.8	43.3 (36.9, 53.4)
Cell morphology	%	*n*
Normocytic	82.1	184
Normochromic	82.1	184
Mild microcytosis	11.6	26
Moderate microcytosis	0.9	2
Mild hypochromasia	14.3	32
Moderate hypochromasia	1.8	4
Mild macrocytosis	0.9	2
Moderate macrocytosis	0.4	1

a. Geometric mean (95% CI) is reported.

TABLE 2. RBC folate (n = 224), folate intake (n = 204), and hemoglobin status (n = 224) of participants by age and sex

Measurement	Boys	Girls	*p*
RBC folate status (nmol/L)—mean (95% CI)[a]			
10–12 yr	447.4 (306.9–652.2)	365.7 (222.2–601.7)	.49
13–15 yr	366.5 (316.2–424.8)	353.5 (301.3–414.7)	.75
16–17 yr	346.9 (265.8–452.7)	291.3 (223.3–379.9)	.38
RBC folate deficiency (< 340 nmol/L)—no. (%)[b]			
10–12 yr	7 (41.2)	6 (46.2)	.78
13–15 yr	38 (43.2)	28 (43.1)	.99
16–17 yr	9 (34.6)	10 (66.7)	.04
Folate intake (μg/day)—mean ± SD[c]			
10–12 yr	171.0 ± 51.4	159.7 ± 44.6	.56
13–15 yr	174.4 ± 46.7	133.1 ± 31.2	< .001
16–17 yr	172.3 ± 39.4	151.3 ± 30.6	.13
Inadequate folate intake—no. (%)[b]			
10–12 yr (< 140 μg/day)	5 (29.4)	7 (53.8)	.11
13–15 yr (< 150 μg/day)	34 (38.6)	51 (78.5)	< .001
16–17 yr (< 200 μg/day)	20 (76.9)	15 (100.0)	.04
Hemoglobin status (g/L)—mean ± SD[c]			
10–12 yr	126.1 ± 11.8	120.7 ± 10.4	.20
13–15 yr	129.8 ± 11.1	118.0 ± 13.0	< .001
16–17 yr	130.1 ± 11.3	127.5 ± 15.3	.53

a. ANOVA.
b. Pearson's chi-square.
c. Independent-samples *t*-test.

$p < .001$) and were 5.8 times (OR = 0.17; 95% CI, 0.08 to 0.36; $p < .001$) more likely to have an inadequate intake of folate (< 150 μg/day). For the age group 16 to 17 years, all girls and 76.9% of boys did not meet their daily RDA of folate intake (200 μg/day) ($p = .04$) (**table 1**).

Overall, girls had significantly lower mean hemoglobin concentrations than boys (119.9 ± 13.4 vs. 129.4 ± 11.3 g/L; $t(222) = 5.7$; $p < .001$). Mean ± SD hemoglobin concentrations by age and sex are reported in **table 2**. Girls aged 13 to 15 years had significantly lower mean hemoglobin levels than boys ($p < .001$). Anemia status by age and sex is reported in **table 3**. Overall, the greatest proportion (63.8%) of the sample were nonanemic; 27.7%, 7.6%, and 0.9% were mildly, moderately, and severely anemic, respectively. With increase in age, the proportion of nonanemic boys decreased; in contrast, the proportion of nonanemic girls increased with age.

The associations between adequacy of folate intake and RBC folate deficiency (< 340 nmol/L), and between anemia status and RBC folate deficiency across age groups and sex were nonsignificant (**table 4**). For both sexes, the linear, nonsignificant association between dietary folate and RBC folate status is represented in **figures 1** and **2**.

Discussion

The study aimed to examine the prevalence of folate deficiency, report the adequacy of folate intake, and study the association between folate intake and RBC folate status in adolescents aged 10 to 17 years. The secondary aim of the study was to investigate the association between anemia and RBC folate status. In brief, the principal findings showed that folate deficiency was widely prevalent in the study sample. Among both boys and girls aged 10 to 12 and 13 to 15 years, almost half were folate deficient (RBC folate < 340 nmol/L). In the older age group (16 to 17 years), two-thirds of girls were folate deficient. In all age groups, a greater proportion of girls (at least 50%) than boys did not meet their RDA for folate. Nearly two-thirds of the adolescents were nonanemic. No significant associations were observed between folate intake and RBC folate

TABLE 3. Anemia status of adolescents by sex and age ($n = 224$)

Sex and age group (yr)	Anemia status—no. (%)			
	Nonanemic	Mildly anemic	Moderately anemic	Severely anemic
Boys				
10–12	13 (76.5)	—	4 (23.5)	—
13–15	65 (73.9)	20 (22.7)	3 (3.4)	—
16–17	12 (46.2)	14 (53.8)	—	—
Total	90 (68.7)	34 (26.0)	7 (5.3)	0 (0)
Girls				
10–12	8 (61.5)	3 (23.1)	2 (15.4)	—
13–15	33 (50.8)	22 (33.8)	8 (12.3)	2 (3.1)
16–17	12 (80.0)	3 (20.0)	—	—
Total	53 (57.0)	28 (30.1)	10 (10.8)	2 (2.2)

TABLE 4. Association between folate intake and RBC folate deficiency (< 340 nmol/L) and between anemia status and RBC folate deficiency by sex and age (boys, $n = 122$; girls, $n = 113$)

RBC folate-deficient adolescents by sex and age group (yr)	Folate intake—no. (%)			Anemia status—no. (%)		
	Inadequate[a]	Adequate	p^b	Nonanemic	Anemic	p^b
Boys						
10–12	3 (42.9)	4 (57.1)	.31	7 (100.0)	0 (0.0)	.06
13–15	17 (44.7)	21 (55.3)	.31	26 (68.4)	12 (31.6)	.31
16–17	6 (66.7)	3 (33.3)	.33	3 (33.3)	6 (66.7)	.34
Girls						
10–12	4 (66.7)	2 (33.3)	.39	2 (33.3)	4 (66.7)	.05
13–15	22 (78.6)	6 (21.4)	.99	13 (46.4)	15 (53.6)	.54
16–17	10 (100.0)	0 (0.0)	—	8 (80.0)	2 (20.0)	1.00

a. Inadequate folate intake: 10 to 12 years, < 140 μg/day; 13 to 15 years, < 150 μg/day; 16 to 17 years, < 200 μg/day.
b. Pearson's chi-square test.

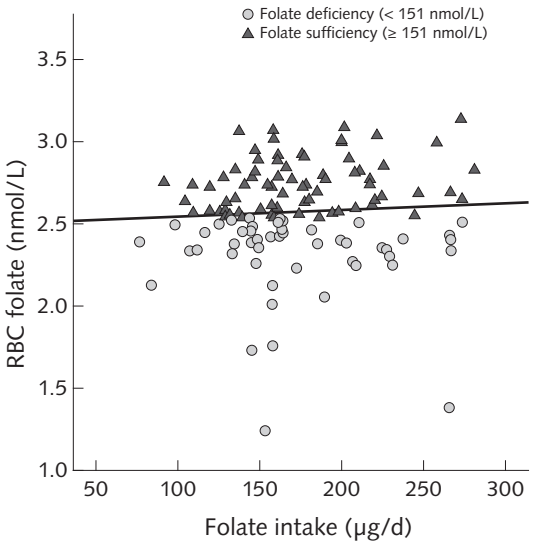

FIG. 1. Lack of significant association between folate intake and red blood cell (RBC) folate status for boys (n = 131, r = 0.06, p = .48)

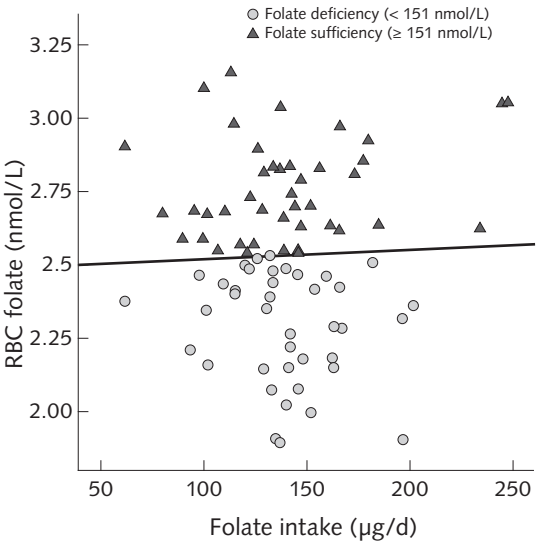

FIG. 2. Lack of significant association between folate intake and red blood cell (RBC) folate status for girls (n = 82, r = 0.04, p = .76)

deficiency or between anemia status and RBC folate deficiency.

Research has predominantly examined folate status using serum folate [8, 22–25]. Serum folate levels reflect recent folate intake, whereas RBC folate is a better indicator of long-term intake, because it has a half-life of approximately 100 days and may not easily be manipulated by short-term dietary changes [6, 26]. There are limited studies reporting RBC folate levels for adolescents. The mean RBC folate levels for 890 Taiwanese children [6] were markedly higher than those of Indian children in the present study (645 vs. 181 nmol/L for ages 10 to 12 years). Similarly, the median (721 to 518 vs. 365 nmol/L) and mean (543 vs. 360 nmol/L) RBC folate concentrations for European (n = 1,051, 12.5 to 17.5 years) [7], British (n = 485, 11 to 18 years) [5], and American (n = 4,050, 12 to 19 years) [27] adolescents were distinctly higher than the RBC folate values noted for the tribal Indian adolescents from a comparable age group (10 to 17 years).

Several factors may explain the lower mean RBC folate levels of the tribal adolescents compared with those reported in the wider literature. Previous studies were carried out in affluent nations; in contrast, our study was conducted in tribal India. None of the tribal Indian adolescents consumed folate-fortified products or nutritional supplements. In contrast, mandatory and voluntary (e.g., fortified breakfast cereals) folate fortification programs are operated in the United States [27] and the United Kingdom [5], respectively (**table 4**). Consumption of nutritional supplements was reported among 9% of Taiwanese children [6] and 11% of European adolescents [7]. However, RBC folate status was not reported separately for adolescents of the comparable age group (10 to 17 years) consuming vs. not consuming nutritional supplements. Therefore, RBC folate status as per supplement consumption vs non-consumption was not compared.

Furthermore, in contrast to the predominantly cereal-based diets consumed by the studied Indian adolescents (pearl millet flat bread, pulses, less than 30 g of vegetables [potatoes, gourds, occasionally leafy vegetables], and no fruits), Taiwanese diets [6] are rich in fruits and vegetables that are important sources of dietary folate [6]. Thus, these factors may have contributed to higher RBC folate concentrations observed in previous research compared with the present study.

Our findings showed that, in comparison with 890 Taiwanese children, the mean folate intake for boys (171 vs. 322 μg/day) and girls (159 vs. 287 μg/day) was lower for a similar age category (10 to 12 years) [6]. For a comparable age group (10 to 17 years), the mean folate intake of Indian boys (173 vs. 331 μg/day) and girls (139 vs. 227 μg/day) was lower than that of American adolescents (n = 3,121, 12 to 19 years) during the prefortification period from 1988 to 1994 [28]. In contrast, for a similar age range (10 to 17 years) the mean folate intake of tribal Indian boys (162 vs. 173 μg/day) and girls (133 vs. 139 μg/day) was higher than that of adolescents from a low socioeconomic background in Brazil (10 to 19 years, n = 722), a developing nation similar to India [4] (**table 4**). Similarly, the mean folate intakes observed in the present study were considerably higher compared with the national Indian data for all age groups and both sexes (10 to 12 years, 39 to 41 μg/day; 13 to 15 years, 43 to 47 μg/day; 16 to 17 years, 47 to 53 μg/day) [3]. The National Nutrition Monitoring Bureau [3] provides the latest and the only data on

the folate intakes of Indians at a population level. The data examine the rural but not the tribal populations from nine states across India. Both the national data (rural adolescents) [3] and the present study (tribal adolescents) reflect intakes of economically deprived sections; however, the differences between the study findings could be partly due to the populations studied and the accuracy of the methods used. The national data [3] estimated folate levels (μg/day) from foods consumed in home settings recorded using a 24-hour dietary recall; therefore, the findings will show more dietary variation and also could be subjected to greater measurement (recall) bias. In contrast, the present study had a relatively controlled environment, since the school hostels ensured three meals from standardized menus. Therefore, the quantity and quality of the food and the number of meals available in rural homes versus school hostels could partly explain the higher mean folate intake by tribal adolescents in the present study.

The results showed that the mean folate intake of girls was significantly lower (by 20 percentage points) than that of boys. Similarly, in previous studies, girls had a lower folate intake than boys [3, 6, 28]; this difference reached statistical significance in the study by Vitolo et al. [4]. In light of the standardized menus served to all participants, significant differences in physiological capacities (amount of food consumed) between boys (2088.2 kcal) and girls (1713.6 kcal) may partly account for the lower folate intake in girls. With respect to adequacy of folate intake, the US dietary guidelines [29] recommend that females consume a minimum of 400 μg of folate per day. In India, it would be advisable to commence consuming the recommended 400 μg/day of folate during the adolescent years, as the median age of Indian women at the birth of their first child is 19 years [1]. However, none of the tribal adolescent girls in our study met the guidelines (the maximum intake was 247 μg/day).

Finally, no association was observed between folate intake and RBC folate status. This tendency is consistent with previous cross-sectional research [6]. In the present study, folate intake may reflect current and long-term dietary patterns, as all participants were served standardized and uniform meals by the school hostels. Therefore, folate intake is suggested to be constant, only affected by the amount of food consumed, which varied by sex. This low disparity in folate intake may account for minimal variance in RBC folate status.

In comparison with the Indian national data [1], a lower proportion of girls aged 15 years or more in the present study were diagnosed with anemia (hemoglobin < 120 g/L) (38.5% vs. 55.8%). In contrast, a higher proportion of boys aged 15 years or more were anemic (hemoglobin < 130 g/L) in comparison with the national data (46.4% vs 30.2%) [1]. The results showed that girls aged 13 to 15 years had significantly lower mean hemoglobin levels than boys; this may partly reflect blood loss during the onset of menstruation, which occurs on average at the age of 12 or 13 years [30]. Secondary findings also revealed that 60.2% of all adolescents who were folate deficient were nonanemic. Therefore, the hypothesis was accepted that nonanemic adolescents could still be folate deficient. This emphasizes that micronutrient deficiencies need due attention at the national level. In April 2012, the Government of India announced the Weekly Iron (100 mg) and Folic Acid (500 μg) Supplementation Programme (WIFS) for urban and rural adolescents (aged 10 to 19 years) for 52 weeks annually [31]. However, implementation of the project throughout the nation is rather slow, and the inclusion of tribal adolescents is unclear.

The main strength of our study is that it is the first to investigate folate status, folate intake, and the associations between folate intake and folate deficiency and between anemia status and folate deficiency in nonaffluent tribal Indian adolescents. The findings are also strengthened by the use of validated procedures to measure folate and hemoglobin status and record dietary intake data. Unlike other published studies, our study was conducted in a relatively controlled setting (school hostels) where meals were prepared from standardized menus. Therefore, comparison of the present study findings with those of other studies should be done with care. The cross-sectional design of the study cannot examine changes over time. Furthermore, the convenience sampling technique limits the findings to tribal adolescents residing in school hostels in Ahmednagar District. The relatively small sample size limited the amount of analysis that could be done on subgroups of participants, and therefore the findings need to be replicated in studies with larger samples. Considerable internal validity of the dietary intake data was assured because of the systematic data collection approach (i.e., validation of the multiple-pass 24-hour dietary recall against the weighing method). Furthermore, all research assistants were sufficiently trained (15 days) to consistently and accurately record the dietary data. However, errors secondary to multiple researchers' bias cannot be ignored. In addition, due to pragmatic concerns (time, funds), only a single multiple-pass 24-hour dietary recall was conducted. However, it is important to consider that the participants' diets were monotonous, as previously discussed. Folate intakes were categorized according to the age- and sex-specific RDAs suggested by the ICMR [20]. In comparison with the RDA, the Estimated Average Requirement (EAR) represents the preferred dietary reference value for nutrient intakes [32]. The RDA is two standard deviations above the EAR and therefore may overrepresent folate intake inadequacy. Presently, in India the ICMR only provides the RDA for folate intake for Indians, which was used to study inadequacy of folate intake. In addition, the EARs suggested by the

US dietary guidelines [33] for comparable age groups are higher than the RDAs recommended for Indians by the ICMR [20], which further emphasizes the folate-deficient diets of the participants.

An important take-home message from the study would be to consider potential strategies to address the issue of folate deficiency and dietary folate inadequacy among economically deprived Indian adolescents. In the United States, the mandatory folic acid fortification program resulted in an increase in RBC folate status and folic acid intake by 33 and 25 percentage points, respectively, from the prefortification period (1988 to 1994) [28] to the postfortification period (2003 to 2006) [34]. A 14-month, randomized, controlled study examining a micronutrient-rich-beverage in 869 affluent Indian children 6 to 18 years of age observed a significant increase in mean RBC folate status from baseline (300 nmol/L) to the end of the trial (600 nmol/L) [11]. In addition to the existing measures implemented in India (WIFS Programme) and overseas (mandatory folate fortification in the United States), novel approaches are required that are not only economically feasible but also culturally acceptable to improve folate status and intake, especially among tribal Indian adolescents. Increasing awareness of indigenous fruits and leafy vegetables, which are abundantly available in the tribal districts, may be a pragmatic strategy to improve folate intake. Finally, cell morphology indicative of deficiencies of iron (e.g., microcytosis, hypochromasia) and vitamin B_{12} (macrocytosis) were examined, but actual blood parameters were not, due to insufficient serum samples and funding. Future research can examine folate status in relation to specific biochemical parameters for vitamin B_{12} (e.g., serum holotranscobalamin level) and iron (serum ferritin) status, as deficiencies of these nutrients can lead to abnormal hemopoiesis and anemia status [20].

Conclusions

Folate deficiency was widespread among tribal Indian adolescents aged 10 to 17 years. In addition, girls had poorer folate intakes than boys. These findings are of concern because insufficiency of folate may have detrimental health consequences (e.g., cardiovascular diseases) for the individual that may be carried forward into adulthood. Therefore, there is an urgent need to develop strategies (e.g., increase dietary diversity using locally available fruits and leafy vegetables) that may address the issue of folate insufficiency in this potentially vulnerable group.

Conflicts of interest

All authors declare that they have no conflict of interest.

Authors' contributions

Rati Jani critiqued the published literature, performed the data analyses, and drafted the manuscript. Nisha Salian, Shobha A. Udipi, and Padmini S. Ghugre managed the biochemical, dietary, and anthropometric assessments. Shobha A. Udipi, Padmini S. Ghugre, Jere Haas, and Erick Boy served as principal investigators responsible for the design and implementation of the project. Shobha A. Udipi, Padmini S. Ghugre, and Jere Haas also provided their expert feedback in revising the manuscript. Neha Lohia contributed to the preliminary data analyses. All authors gave their final approval for submission.

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