ORIGINAL RESEARCH ARTICLE

Multiple Micronutrient Deficiency Among Adolescent Girls with Normal Nutritional Status - Need for Fortified Nutritional Support in Rural Settings of South Tamil Nadu, India

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ABSTRACT

Introduction: Adolescents contribute to one fifth of the world's population and is a high priority lifecycle stage for nutrition needs and intervention. Nutritional deficiency, including micronutrients, hinders their normal growth and development. Improving adolescent girls' nutrition has reproduction-related benefits and serves as a unique opportunity to break the vicious cycle of intergenerational structural problems. The objective is to estimate the prevalence of anaemia, micronutrient deficiency and nutritional status among adolescent girls in rural India.

Methods: A community-based cross-sectional study was conducted among 241 adolescent girls in rural Tirunelveli, India with 220 blood samples.

Results: The mean age of 241 adolescent girls was 13.8±1.4 years and nutritional status was normal, overweight, obese, thin and stunted in 81.7%, 14.5%, 3.3%,0.4% and 4.6% respectively. The prevalence of anaemia, iron deficiency and iron-deficiency anaemia were 37.2%, 34.5% and 12.3%. Except for selenium, the estimated micronutrient deficiency levels were Vitamin B12 - 40.9%, Copper-30.9%, Zinc-13.6% and Iodine-11%. Only 19.1% girls had adequate levels of all micronutrients. Vitamin B12 deficiency was more in girls who were overweight ($p<0.05$).

Conclusion: Multiple micronutrient deficiency with normal nutritional status is high among adolescent girls with Vitamin B12 deficiency higher than Iron. Multipronged strategies, including introducing micronutrient fortified healthy snacks in schools, might bring greater acceptance and improvement in nutritional health among these girls.

Keywords: Adolescent girls, Nutritional status, Anaemia, Micronutrient's deficiency

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INTRODUCTION

The Global Strategy for Women's and Children's Health follows a life-cycle approach to achieve the highest attainable standards of health and well-being - physical, mental and social - at every age. Adolescents joining for the first time as key drivers of change to transform health and achieve sustainable development of women, children, and adolescents by 2030.[1] Globally there are about 1.2 billion adolescents in the age group between 10 to 19 years, of which 243 million (16%) are in India alone.[2]

The spectrum of malnutrition in adolescence ranges from mild nutritional deficiency to gross obesity. The interest in improving the nutrition status of adolescent females is evolving globally. Good nutrition (both macro and micronutrients) is critical not only to cover the past nutrition deficits experienced during childhood but also to meet the growing demand of rapid growth and development experienced during adolescence, build up reserves to provide adequate energy during illness, pregnancy, and to prevent the adult-onset of nutrition-related diseases.[3] Nutritional deficiency in adolescents increases the perinatal risks, including stillbirths, preterm births, small for gestational age, and low birth weight (LBW)babies. Adolescence obesity extends to adulthood with poor metabolic profile and is associated with chronic disease and increased risk of mortality later in life.[4-6] The prevalence of obesity has increased from <1% in 1975 to more than 5% in 5 to 19 years old girls, and 80% of obese adolescents have become obese adults.[7,8]

Micronutrient deficiency or "hidden hunger" results from inadequate intake or absorption of essential vitamins and minerals and directly affects growth and development in childhood and adolescence.[9] Multiple simultaneous nutrient deficiencies may limit linear growth. The Food and Agriculture Organization (2013) states globally, more than one-in-three suffer from micronutrient deficiencies, of which nearly 50% are in India alone.[10]

Iron deficiency anaemia (IDA) is among the top 10 causes of disability-adjusted life years lost in adolescents.[11] In 2013, the Government of India initiated a series of multipronged programme for targeted reduction of nutritional anaemia, worm infestation with weekly supplementation of Iron and Folic Acid tablets and Albendazole tablets.[12] National Family Health Survey (NFHS) - 5 reported that 57.2% of the non-pregnant women of reproductive age were still anaemic and suggested exploring anaemia control beyond iron deficiency.[13] Major micronutrients include Vitamin A, B6, B12, Selenium, Magnesium, Copper and Zinc. Micronutrients such as Ferritin and Iodine play a vital role in haematopoiesis.[14] Any deficiency of these nutrients in food or mal-absorption leads to different types of anaemia. These micronutrient deficiencies influence scholastic skills, the growth or occurrence of malformations in the fetus and altering course and outcomes of pregnancy.[15-24]

The present study was carried out to estimate the prevalence of nutritional, anaemic and micronutrient deficiency status among adolescent girls in rural Tirunelveli in Tamil Nadu state, India and assess the association between them. The findings shall help plan for specific interventions beyond IFA supplementation in improving the nutritional status of adolescent girls within the existing nutrition intervention programs.

METHODOLOGY

After obtaining the approval of the Institutional Ethics Committee, a community-based cross-sectional study conducted in the field practice area of the Model Rural Health Research Unit located in Kallur, a rural Govt Primary health Center, Tirunelveli District, Tamilnadu, India between the months of August 2015 to September 2016. Kallur village is located at a distance of 22 kms from Tirunelveli city with a total population of 39240 with agriculture as main occupation. Nearly 20% (7691) of the population are adolescents with 3838 girls. Based on NFHS-4 survey data reporting 64.2% anaemia prevalence in rural Tirunelveli with an allowable error of 10% and 95% confidence level, using the Epi-info software, the estimated sample size for the study was 241 adolescent girls. [25] We followed the cluster sampling method. A face-to-face interview was conducted with the adolescent girls in their household at a time convenient for them and the family by trained project technicians appointed for this purpose. Permission was obtained from the institutional ethics committee National Institute of Epidemiology before the start of the study on 29th June 2015 (NIE/IHEC/201504-04). The Written informed consent from the parent and assent from the participant was obtained. A semi-structured, pre-designed and pre-tested, data capture tool developed for the purpose in local language was used to collect information relating to the study.

Anthropometric measurements: Anthropometric measurements were recorded following World Health Organization guidelines and BMI-for-age Z-score (BAZ) was used for assessing nutritional status using WHO AnthroPlus software.[26] Based on BAZ scores, the nutritional status was classified as normal (− 2SD to +1SD), Thinness (< −2SD), Overweight (> +1SD) and obese (> +2 SD). Stunting was considered by Z score for height-for-age<−2SD.[27]

Micronutrient's estimation: 5ml of venous blood samples were collected from the forearm from 220 consented adolescent girls following standard aseptic precautions using single-use disposable syringes. 20µL of whole blood was pipetted onto Whatmann filter paper for haemoglobin estimation. The rest of the blood was collected in an acid-washed centrifuge tube, kept in an icebox, and transferred to the local lab, where serum was separated and stored at −20 degree Celsius until estimation of micronutrients such as Ferritin, Vitamin B12, Zinc, Copper and Selenium levels by the ICMR-Centre for Promotion of Nutrition Research & Training, New Delhi. (Annexure 1) Urine samples were collected and transported at 4˚C to estimate urinary iodine. The Haemoglobin levels were used to classify anaemia.[28] Deficient status was determined with cut-off values for serum Ferritin- ≤ 15 ng/ml[15], Vitamin B12≤ 203pg/ml[29], Zinc ≤700 µg/L[30], Copper ≤900 µg/L[31], Selenium ≤23µg/L[31] and urine Iodine -20 to 99µg/L[32].

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 26 for Windows (SPSS Inc., USA). Descriptive statistics such as mean, standard deviation, inter quartile range (IQR), frequencies and percentages were calculated. Odds ratio, Chi-square test, Fisher exact test, Mann Whitney U test and Kruskal Wallis test were used to assess the association with a statistical significance level, $p < 0.05$. Spearman correlation was computed to examine if micronutrients were related to the nutritional and anaemic status.

Results

Socio-demographic characteristics: The mean age of 241 adolescent girls was 13.8±1.4 (SD) years, with 118 (48.9%) in 10-14 years of age and 123 (51.1%) in 15-19 years of age. 164 (68%) have attained menarche with the mean age of attaining menarche 12.9±1.2 (SD) years. About 204 (84.6%) were from nuclear families, 137(56.9%) girls belonged to the upper socioeconomic class.[33] One girl was married. Out of 241, 224 (92.9%) girls were school-going, with 160 (66.4%) in high school and 81(33.6%) in middle school. Among the school going girls, 169 (70.4%) consumed lunch at the Government-sponsored school mid-day meal program, but only 12 girls (4.9%) were enrolled in the Integrated Child Development services (ICDS) programme and received nutrition and health education and referral services. (Table-1)

Table 1: Distribution of adolescent girls based on the nutritional status

Variables	Nutritional status (n=240)*		P value
	Normal (n=197) (%)	Overweight & Obese (n=43) (%)	
Adolescent stage			
10-14 yr (n=117)	93(79.5)	24(20.5)	
15-19 yr (n=123)	104(84.6)	19(15.4)	0.306
Family size			
≤5@ (n=174)	140(80.5)	34(19.5)	
>5@ (n=66)	57(86.4)	9(13.6)	0.287
Socio-economic status [33]			
Upper$ (n=137)	114(83.2)	23(16.8)	
Middle$ (n=103)	83 (80.6)	20 (19.4)	0.722
Community			
SC (n=66)	49(74.2)	17(25.8)	
Others (n=174)	148(85.1)	26(14.9)	0.051
Mid-Day Meal Enrolment			
Yes (n=169)	140(82.8)	29(17.2)	0.637
No (n=71)	57(80.3)	14(19.7)	
Availing ICDS services			
Yes (n=12)	10(83.3)	2(16.7)	1.000#
No (n=228)	187(82.0)	41(18.0)	

*One girl who was thin was excluded for analysis; chi-square test was used except; @ family members, $Socio-economic Class; # (Fisher exact test); Normal (− 2SD to +1SD), Overweight (> +1SD) and obese (> +2 SD) by Z score

Table 2: Distribution of factors associated with anaemic status

Variables	Anaemic status (n=220)*		Odds ratio	95% CI
	Anaemic (n=82) (%)	Normal (n=138) (%)		
Adolescent stage				
10-14 years (n=109)	48 (44.0)	61 (56.0)	**1.777**	1.022 - 3.11
15-19 years (n=111)	34 (30.6)	77 (69.4)	Ref	
Status of menarche				
Yes (n=149)	54 (36.2)	95 (63.8)	0.8735	0.4878 - 1.573
No (n=71)	28 (39.4)	43 (60.6)	Ref	
Family size				
> 5 members (n=46)	15(32.6)	31(67.4)	0.7736	0.3804 - 1.531
≤ 5 members (n=174)	67(38.5)	107(61.5)	Ref	
Socio-economic status				
Middle (n=91)	35 (38.5)	56 (61.5)	1.09	0.6236 - 1.901
Upper (n=129)	47(36.4)	82 (63.6)	Ref	
Community				
SC (n=59)	24 (40.7)	35 (59.3)	1.217	0.6546 - 2.244
Others (n=161)	58 (36.0)	103 (64.0)	Ref	
Mid-Day Meal Enrolment				
No (n=153)	56(36.6)	97(63.4)	0.9108	0.5039 - 1.659
Yes (n=67)	26(38.8)	41(61.2)	Ref	
IFA consumption (at least 50%)				
No (n=171)	64 (37.4)	107(62.6)	1.03	0.5341 - 2.02
Yes (n=49)	18 (36.7)	31(63.3)	Ref	
De-worming tablet consumption				
No (n=84)	35 (41.7)	49(58.3)	1.351	0.769 - 2.37
Yes (n=136)	47 (34.6)	89 (65.4)	Ref	

*21 girls did not consent for giving blood samples

Table 3: Prevalence of micronutrients deficiency among adolescent girls

Micro nutrient	Mean ± SD	IQR	Girls with Deficiency (n=220) (%)	Girls with co-existing anaemia (n=220) (%)
Ferritin (ng/ml)	27.4±25.0	8.95 - 36.2	76 (34.5)	27 (12.3)
Vitamin B12 (pg/ml)	274.6 ±139.	177 - 315.9l	90 (40.9)	37 (16.8)
Copper (µg/l)	1068.1±311.4	867.1 - 1202.8	68 (30.9)	24 (10.9)
Zinc (µg/l)	1256.6±617.4	790 -1561.30	30 (13.6)	11 (5.0)
Selenium (µg/l)	88.3±26.0	70.3 - 103.3	0	NA
Iodine (µg/L)	217.0±90.0	150.3 - 275.2	24 (11)	8 (3.6)

IQR - Inter-Quartile Range; NA- Not Applicable

Table 4: Mean values of micronutrients against the nutritional and anaemic status

Variables	Ferritin(ng/ml)	Vit B12(pg/ml)	Copper (µg/l)	Zinc (µg/l)	Selenium (µg/l)	Iodine (µg/l)
Nutritional status (n=219)*						
Normal (n = 181)	26.29±23.74	279.36 ± 134.78	1059.93± 323.7	1259.45 ± 614	87.86± 26.22	215.89 ± 91.62
Obesity** (n = 38)	29.18 ± 27.51	271.2 ± 177.56	1111.82 ± 217.75	1257.6 ± 692	93.56± 25.32	229.88± 134.78
P-value#	0.424	0.021	0.112	0.754	0.276	0.776
Anaemic status (n=220)						
No anaemia (n = 138)	26.09± 22.79	284.31± 146.19	1055.33 ± 313.55	1234.45± 590.57	88.01± 26.84	219.16 ± 96.82
Mild anaemia (n = 51)	27.37± 25.23	266.69± 133.96	1068.94± 257.01	1274.02± 675.32	87.14± 21.94	211.81± 76.76
Moderate anaemia (n = 31)	33.05± 32.61	241.74± 115.91	1119.07± 378.07	1328.67± 639.60	90.93± 28.90	215.90± 80.59
P-value##	0.554	0.138	0.766	0.725	0.798	0.882

All values in Mean ± Standard deviation; No anaemia -Hb>12gm/dl; Mild anaemia - Hb10-12gm/dl; Moderate anaemia -Hb 7-10gm/dl
*One underweight girl is not included in the table; ** does not include overweight
- Mann-Whitney U test (The BMI is not normally distributed, p=0.000)
##- Kruskal Wallis test (The Hb is not normally distributed, p=0.000)

Table 5: Distribution of adolescent girls based on micronutrient levels

	Ferritin (%) (ng/ml)		Vit B12 (%) (pg/ml)		Copper (%) (µg/l)		Zinc (%) (µg/l)		Selenium (%)(µg/l)	Iodine (%) (µg/l)**	
	IA	A	IA	A	IA	A	IA	A	A	IA	A
Nutritional status*# (n-219)											
Normal	62(34.25)	119(65.75)	66(36.46)	115(63.54)	59(32.6)	122(68.12)	26(13.77)	155(86.23)	181(100)	21(11.59)	55(30.43)
Obesity	14(36.84)	24(63.16)	23(60.53)	15(39.47)	9(23.68)	29(76.47)	4(17.65)	34(82.35)	38(100)	3(11.76)	12(27.45)
P-value	0.761		**0.010**		0.28		0.533#		-	0.770#	
Anaemic status (n-220)$											
Normal	49(35.51)	89(64.49)	53(38.41)	85(61.59)	44(31.88)	94(68.12)	19(13.77)	119(86.23)	138(100)	16(11.59)	42(30.43)
Mild	20(39.22)	31(60.78)	22(43.14)	29(56.86)	12(23.53)	39(76.47)	9(17.65)	42(82.35)	51(100)	6(11.76)	14(27.45)
Moderate	7(22.58)	24(77.42)	15(48.39)	16(51.61)	12(38.71)	19(61.29)	2(6.45)	29(93.55)	31(100)	2(6.45)	11(35.48)
P-value	0.285		0.554		0.321		0.357#		-	0.610#	

*One underweight girl is not included in the table;
** Girls with excess levels not included; IA- Inadequate; A-Adequate; P-value- Chi-square (#Fisher exact test)
#Nutritional Status - Normal (n=181) and Obesity (n=38)
$ Anemia status - Normal (n=138), Mild (n=51), and Moderate (n=31)

Table 6: Correlation between nutritional status, anaemia with micronutrients

Variables	Correlation coefficient	P- value
BMI*Hb	-0.058	0.388
BMI*Ferritin	0.082	0.226
BMI* Vitamin B12	-0.186	**0.006**
BMI*Zinc	-0.031	0.652
BMI*Copper	0.128	0.058
Hb*Ferritin	-0.013	0.852
Hb* Vitamin B12	0.068	0.312
Hb*Zinc	-0.042	0.539
Hb*Copper	-0.065	0.335

Nutritional status: The mean height of the participants was 149.6±7.6 cms with IQR from 145.5 to 154.5 cms, and the mean weight was 38.1±8.3 kgs with IQR from 32.5 to 42.6 kgs. Table 1 shows the distribution of adolescent girls based on the nutritional status.197 girls (81.7%) had a normal BAZ, 35 (14.5%) overweight, 8 (3.3%) obese, 1 (0.4%) thin, and 11 (4.6%) stunted. The prevalence of overweight & obese was higher among girls in early adolescence, lesser family size, upper socioeconomic status, but none of these variables was significantly associated (p>0.05).

Anaemia: The mean Hb level of 220 adolescent girls who consented to provide blood was 12.61±2.29 gm/dl, with the prevalence of anaemia being 37.2% (23.1% - mild anaemia and 14.1% -moderate anaemia and none had severe anaemia). Only 49 girls (22.3%) had consumed at least 50% of the IFA supplementation they received, and 61.8% consumed de-worming tablets. The distribution of adolescent girls based on anaemic status is presented in Table 2. Girls in the early adolescent stage were 1.7 times more likely to be anaemic. The prevalence of anaemia was higher among girls who attained menarche, girls with lesser family size and in better socioeconomic status.

Micronutrient status: Table3 shows the prevalence of various micronutrient deficiencies among 220 adolescent girls. 76 girls (34.5%) had Iron Deficiency (ID), while anaemia and ID coexisted in 27 girls, resulting in 12.3 % of Iron deficient Anaemia. Vitamin B12 deficient anaemia was found in 37(16.8%) girls. While with Vitamin B deficiency, 24 (29%) girls had copper-deficient anaemia. It was found that 67(30.5 %), 88(40 %) and 41(18.5%) girls had normal, above requirement and excess levels of urine Iodine respectively. 11% girls had insufficient Urinary Iodine Concentration (UIC).

Multiple micronutrient deficiencies: Only 42 (19.1%) girls were not at risk of deficiency in any of these micronutrients. While 43.6%, 24.1%, 11.8% and 1.4% girls suffered from at least one, two, three or four micronutrient deficiency respectively. Among the 55 anaemic girls with normal ferritin levels, 37(47%) and 24 (35%) had Vitamin B12 and Copper deficiency respectively.

Nutritional status, Anaemia and Micronutrient levels: The mean values of micronutrients against nutritional and anaemic status are presented in Table 4. The one girl who was thin was not anaemic and did not have any micronutrient deficiency. It was found that mean values of Ferritin, Copper, Selenium and Iodine levels were greater among overweight girls but the difference was not significant ($p>0.05$). Also, mean values of Ferritin, Copper and Zinc increased with the severity of anaemia though statistically not significant ($p>0.05$) However the mean Vitamin B12 value decreased with increasing severity of anaemia, indicating the temporal association of Vitamin B12 deficiency with anaemia in these girls.

Table 5 shows the distribution of adolescent girls based on micronutrient status with Vitamin B12 deficiency more in overweight girls and it was statistically significant. ($p< 0.05$). 55 girls with normal ferritin levels were found anaemic, strengthening the presence of anaemia other than Iron deficiency (Figure-1). Among the anaemic girls, 36.7% and 35.2% of girls had deficient zinc and copper levels too.

Table 6 shows the correlation between the nutritional, anaemic status and micronutrient levels. Nutritional status and Vitamin B12 levels show a negative correlation.

Discussion

The present study reports the nutritional, anaemic and micronutrient status of adolescent girls in this part of India. 81.7% of the adolescent girls had normal nutritional status, 0.4% thin, 14.5% overweight and 3.3% obese. This shows the transition in nutritional status of adolescent girls in a positive direction. This could be attributed to the better status of females in this region in terms of female literacy rate (75.9%) and a favourable sex ratio (1023) as showcased in the 2011 District census of Tirunelveli.[34] But unfortunately, the increased prevalence of overweight and obesity justifies that obesity is not merely a problem of urbanization and shall be the outcome of socio-economic development, changing food choices and physical activity habits among adolescents. Also, excess weight gain during this transitional period increases their risk of maintaining unhealthy body fat levels during pregnancy leading to poor pregnancy outcomes. Obese adolescents tend to remain obese as adults, with an increased risk of developing chronic diseases in later years of life. Thinness and stunting were found in 0.4% and 4.1% girls respectively, predicting that the stunting process could have occurred during their earlier childhood and their Height−for−age did not improve across adolescence. Also 9% of stunted girls, were obese depicting the coexistence of obesity and chronic malnutrition are interrelated and strengthens the similar finding described in countries undergoing nutrition transition.[35]

It was found that every third adolescent girl was anaemic (37.2%) with two third sufferings from mild anaemia and one third with moderate anaemia. This is lower than NFHS-4 data on anaemia prevalence among rural women at National (54.3%), State (56.8%) and District (64.2%) level. Also, the severity of anaemia is much lower when compared to Tamil Nadu NFHS-4 data with 40% mild, 14% moderate and 1% severe anaemia.[14] The study estimated 34.5% Iron Deficiency and 12.3 %. IDA was, indicating the latent stage of anaemia in these girls. The depleted iron stores will turn out into full blown anaemia when these girls enter motherhood perpetuating the burden of anaemia during pregnancy.

Also, the gap between prevalence of anaemia and IDA indicates the possibility of other causes of anaemia with every four out of 10 adolescent girls suffer Vitamin B12 deficiency and 41.1% of them being anaemic indicating a higher prevalence of Vitamin B12 deficiency than Iron Deficiency. To strengthen this fact, 11.8% anaemic girls had normal ferritin levels favouring macrocytic anaemia, but conclusive decisions couldn't be made as peripheral smear examination was not part of the study. Copper deficiency was 30.9% and can present as anaemia (microcytic, normocytic, or macrocytic). Copper and ferritin deficiency coexisted in 50% suggesting the major role of copper through various enzyme systems promoting enteric absorption and transport of iron in circulation which can be corrected by copper supplementation and are unresponsive to iron therapy. Zinc was deficient in 13.6% which may act as a limiting factor to growth and sexual maturation during adolescence.[36] 11% girls had insufficient UIC, warning an impending Iodine Deficiency Disorder in near future. Iron deficiency impairs thyroid hormone synthesis by reducing activity of heme-dependent thyroid per oxidase. However, 58.5% girls had iodine levels above requirement, which needs further evaluation. Selenium was the only micronutrient that presented with adequate levels among these girls. Adequate selenium helps normal thyroid functioning and also protects thyroid gland from excessive iodide exposure.[37] The beneficial role of selenium in thyroid function with 58.5% excess iodine levels needs further studies.

This study demonstrated that 80% adolescent girls living in rural Tirunelveli were at risk of one or more micronutrient deficiency even with normal nutritional status indicating the importance of widening the perspective of adolescent nutritional health beyond Iron deficiency. The prevalence of anaemia due solely due to iron deficiency is less certain and multiple micronutrient deficiency might be associated with the poor progress in improving adolescent anaemia under the WIFS programme which focuses on IFA supplementation alone, which was further worsened by poor adherence with 22.3% girls admitting consuming at least 50% of the received IFA supplements and 61.8% consuming de-worming tablets. Enrolment in Mid-day meal programme is high, indicating the usefulness of using schools as a focus point of any nutritional intervention. The study suggests that strategies for the prevention and control of micronutrient deficiencies and improving public health nutrition should be carried out with multi-pronged appropriate interventions that need immediate implementation as it reflects in the population for many generations with long-term impact on maternal health and outcomes.

The limitations of the study were that adolescent boys were not included, as the objective was specific towards betterment of maternal health. The dietary patterns of the adolescent girls, which would contribute additional inferences, were not collected. Peripheral blood smear, infections and other micronutrients such as folic acid, Vitamin A, calcium was not estimated in the study.

Conclusion

The study brings clear evidence of double burden of malnutrition among adolescent girls from under nutrition to overweight but with failing micronutrient levels. The higher prevalence of Vitamin B12 deficiency compared to iron highlights the need to look beyond iron deficiency anaemia. Also, micronutrient deficiency rarely occurs in isolation. To achieve long-term impact, interventions need to target multiple deficiencies with multiple appropriate strategies since promotion and prevention are more critical to adolescent nutritional health. With poor IFA compliance, food fortification shall be a useful alternative with school-based programme well suited for providing fortified foods to adolescents to improve the micronutrient status. With changing food preferences, the concept of fortified healthy snack inclusive of key micronutrients-Iron, Vitamin A, C, B complex, B12, Copper, zinc and calcium shall be more receptive among the beneficiaries. The Midday meal scheme shall consider food diversification in its meal plan.

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Annexure 1: Cut off values and Methods followed for quantification of Micronutrients with references

Test description	**Zn (μg/L)**	**Se (μg/L)**	**Cu (μg/L)**	**Ferritin (ng/ml)**	**Retinol (μg/dl)**	**To-coperol (mg/dl)**	**VitB12 (pg/ml)**	**Homocytein (μmol/L)**	**Hs-CRP (mg/L)**
Normal Values									
A	>700	23-190	900-1900	<15	≥20	>0.5	>203	Not done for ADL	
P	>700	23-190	900-1900	<15	≥20	>0.5	>203	5-15	< 3
Method followed for quantification of micronutrients	Mass Spectrometry	Mass Spectrometry	Mass Spectrometry	Solid Phase, two site Chemiluminescent Enzyme immunoassay	Bieri *et al* 1979	Driskell *et al* 1982	Solid Phase, Competitive Chemiluminscent Enzyme immunoassay	Solid Phase, Two site Chemi-luminscent Enzyme immunoassay	Solid Phase, Twosite Chemi-luminscent Enzyme immunoassay
References for cut of values	International Zinc nutrition-Consultative group (IZiNCG).Brown KH et al.Technical doccument 1.Assesment of the risk of zinc deficiency in populations and potions for its control.food Nutr Bull.2004;25op (1 Supp2)	Tiez clinical guide to laboratory test,4th edition,2006.alan H.B.Wu	Tiez clinical guide to laboratory test,4th edition,2006.alan H.B.Wu	WHO serum ferritin concentration for the asseement of iron status and vitamin and mineral nutrition information system.Geneva.WHO, 2011 (WHO/NMH/NHD/MNM/11.2)	Pee and Dary, 2002	CDC, 2008	WHO Technical Consultation,2008	Ueland et al,1993	American-Heart Association & CDC,2003