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Anemia prevalence, its determinants, and profile of micronutrient status among rural school adolescent girls aged 14-19 years: A cross-sectional study in Nagpur district, Maharashtra, India"

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Short title: Anaemia and its predictors in adolescent girls

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Abstract

Objective: The objective of our study was to determine the prevalence of anemia among 14-19 years school going girls, risk factors for it and profile of micronutrient status among rural girls from western state of India.

Design: Using a cross-sectional design, we obtained information on socio-demography, menstruation, dietary habits, knowledge and daily consumption of the government recommended iron and folic acid (IFA) tablets, and anthropometry. Blood was collected to assess haemoglobin, red blood cell indices, serum iron, folate and vitamin B_{12} levels.

Settings: Nagpur district, Maharashtra, India

Participants: A total of 221 girls aged 14-19 years studying in 24 government institutes included.

Results: 57% girls were anaemic, 84% had deficiency of one or more micronutrients and 60% were malnourished based on Body Mass Index (BMI). The prevalence of iron, vitamin B_{12} and folate deficiency was 37.7%, 69.8% and 1.4% respectively. Among anaemic girls, iron and vitamin B_{12} deficiency was observed in 45.5% and 67.5% respectively, vs. among non-anaemic girls it was 27% and 73% respectively. Iron deficiency was a predictor of anaemia and its severity. Girls residing in non-nuclear family were more likely to have anaemia. The consumption of daily non-vegetarian food and green leafy vegetables was 3% and 3.6% respectively. Only 9% consumed IFA tablets in the past two weeks.

Conclusions: Anemia is common in adolescent girls, particularly associated with iron and vitamin B12 deficiency. There is need to reconsider the approach to prevention of anaemia in adolescent girls, particularly before they become pregnant.

Keywords*:* Adolescent, anemia, iron, vitaminB_{12}

Introduction

Anaemia among young women has remained an arduous health challenge in India as 59% of adolescent girls aged 15-19 years were anaemic in the National Family Health Survey (NFHS) conducted between 2019-2021[(1)]. The growth rate during adolescence is rapid and pubertal changes like onset of menarche occurring among girls during this phase expose them at greater risk to develop anaemia. Evidence suggest that even mild forms of anaemia can have deleterious impact on physical capacity and cognitive ability among adolescents [(2)]. Hence, detecting anaemia at its earlier stages or prior to the advancement to its clinical manifestation is crucial.

In view of the multifactorial aetiology of anaemia, it is equally imperative to identify its specific drivers. Systematic reviews of nationally representative surveys from India reported 37% of anaemia to be associated with iron deficiency among non-pregnant women of reproductive age [(3)]. A recent study from rural Haryana reports a prevalence of 29.6% of iron deficiency anaemia and 28.1% of anaemia due to folate or vitamin B_{12} among adolescent girls [(4)]. Another study from South India observed that half of the women of child bearing age were deficient in folate and vitamin B_{12} [(5)]. These variations firstly, underscore the need to study region specific pattern of micronutrient deficiencies. Secondly, it also emphasises the need to assess the challenges in the implementation of national Weekly Iron Folic acid Supplementation (WIFS) program. The program aims to provide free iron and folic acid (IFA) tablets (containing 100 mg of elemental iron and 500 μg of folate) to adolescents on a weekly basis[(6)], but issues of awareness and compliance may affect its effectiveness. In this study, our aim was to determine the prevalence of anaemia and its severity, the prevalence of iron, folate, and vitamin B_{12} deficiency and determinants of anaemia in rural school adolescent girls from Nagpur district.

Methods

Study design and settings

The study employed cross-sectional design. Data collection commenced from December 2019 to March 2020 in the rural areas of Nagpur district.

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Study Participants

The study covered adolescent girls aged 14-19 year studying at educational institutes aided by the state government. The government aided educational institutes were selected as these are covered under national WIFS program.

Sample Size and sampling

Assuming the prevalence of anemia among rural adolescent girls as 87%[7], precision of 5%, 95% confidence interval (CI) and 20% non-response rate the minimum required sample size was 214.

A multistage purposive random sampling was used (Fig.1). We selected the rural areas of Nagpur district from an ongoing Maternal Newborn Health Registry (MNHR) site. The MNHR has been collecting prospective data on maternal, fetal and neonatal outcome since 2009. Since its inception, the MNHR has registered more than 750,000 pregnant women and their babies in rural and semi-urban communities from various sites in Africa, Asia and Central America. Each site comprises between 6 and 24 distinct geographic locations (clusters, i.e. Primary Health Centres (PHC)). The rapport established with the public health department was helpful for the smooth conduct of the study. We first selected four PHC that had villages with government aided educational institutes. The institutes that consented to participate provided a complete list of girls aged 14-19 years. We used population proportionate sampling of participants who were randomly chosen using online random number generator program.

Inclusion criteria

We included those girls who were apparently healthy, had not received any blood transfusion in last three months, willing to provide consent for participating in the study and to withdraw blood for estimation.

Data Collection and measurements

Trained data collectors captured data from 225 girls on socio-demographics (total family size, type of family, parent's education and occupation), menstruation (menarche status, age at menarche, number of days of bleeding due to menstruation per month), dietary habits (type of diet consumed (vegetarian or non-vegetarian), consumption frequency of green leafy vegetables, citrus fruits during the season and frequency of non-vegetarian food consumption- daily , weekly/monthly or never), knowledge, and consumption of IFA tablets.

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Specific questions covered participants' understanding of IFA supplementation, history of receiving IFA tablets, timing of the last receipt, reasons for any non-receipt, actual consumption of received tablets, and reasons for any non-consumption in the past 14 days. Additionally, we inquired about a diagnosis of sickle cell disease, given its prevalence in the Nagpur district.

Data were captured electronically using Android tablets with a pre-tested questionnaire developed in Epi-collect 5. The data collectors were trained in anthropometric techniques. Weight (nearest 0.5 kg) of the subject was recorded using portable digital weighing scale (OMRON HN-286). Height (nearest 0.1 cm) was measured using portable stadiometer (Easy Care Stature Meter EC 1080) using standard procedures [(8)]. The household standard of living index (SLI) was calculated using NFHS-2 [(9)].

A trained, experienced laboratory technician collected venous blood samples from the study subjects. Total 5 ml blood was drawn from each subject in two separate vacutainers- one with EDTA and other in plain. After blood draw, the samples were transferred to ice box within 30 minutes. The blood samples were sent for analysis to the Super Religare Laboratory (SRL) on the same day for estimation. The SRL laboratory is accredited by National Accreditation Board for Testing & Calibration. Vacutainers containing EDTA were used for estimation of haemoglobin (Hb) (Cyanmethemoglobin, Photometric, Horiba Micros ES 60). Plain vacutainers with blood were used for estimation of serum ferritin (Electrochemiluminescence competitive immunoassay, Roche Cobas e411), serum folate, serum vitamin- B_{12} (Electrochemiluminescence competitive immunoassay, Roche Cobas e411), and C-reactive protein (CRP) (Immunoturbidimetry, Siemens, Dade dimension Xpand plus).

Data compilation and analysis

Data from the multiple android tablets were synced to the server and exported to Microsoft excel. After coding the file was exported into Epi-Info 7 and STATA ®. NFHS-2 conducted in the year 1998-99 provided an index reflecting economic well-being of a rural household based on number of variables such as household type, toilet facility, source of lighting in the house, type of fuel used for cooking, source of drinking water, presence of kitchen, ownership of house, agriculture land (irrigated/ non- irrigated), livestock and ownership of durable goods like mattress, cooker, chair, clock, cot, fan, cycle, radio, phone, refrigerator, television, scooter, bullock cart, crop thresher, tractor/car. Each variable is given a score and sum of these scores is calculated. Scores of 0 to 14, 15 to 24, and 25 to 67 are defined as low, medium, and high respectively. The nutritional status of adolescent girls was evaluated using body mass index (BMI) (kg/m^2). The BMI was derived by dividing the weight of girls (in kg) by the square of their height (in meters). The following BMI (kg/m^2) categories were used to classify girls: <16 severely undernourished, 16-17 moderately undernourished, 17.1-18.4 mild undernourished, 18.5 – 22.9 normal, 23-24.9 overweight, >25 obese. BMI for age Z score (BAZ) and height for age Z score (HAZ) was calculated using WHO anthroplus software (2009). Z-score less than -2SD was regarded as wasted in case of BAZ and stunted in case of HAZ.

World Health Organization (WHO) cut offs were used to define anaemia (Hb < 12 g/dL) and its grades (mild :11.0–11.9 g/dL, moderate :8.0–10.9 g/dL, and severe: <8.0 g/dL)[(10)] and deficiencies of iron (serum ferritin<15µg/L)[(11)], folate (< 4 ng/mL) and vitamin B_{12} (< 203 pg/mL) [(12)]. Additionally inflammation was assessed with C-reactive protein (CRP) levels > 5 mg/L[(13)].

Frequencies, prevalence statistic were derived using EPI-info7. Normality of all the continuous variables was assessed using Shapiro Wilk's test. For non- normally distributed continuous variables median (25^{th} ,75^{th} percentile) and frequencies (%) for categorical variables were reported. Statistical analyses including uni-variate, bi-variate and multinomial logistic regression analysis were performed using STATA®. For all statistical tests, a two sided p- value <0.05 was considered significant. Linear regression was performed to assess the association of factors with haemoglobin level as the outcome variable. Multiple logistic regression was used to examine the factors associated with anaemia. Multinomial logistic regression was used to examine factors associated with the severity of anaemia among adolescent girls. We adjusted for SLI (High versus Low-medium), type of family (Nuclear versus Non-nuclear), family size (<4 versus $\geq$ 4), type of diet consumed(vegetarian versus egg/ non-vegetarian), body mass index for-age z score and height for age z score (Normal versus BAz<-2sd, HAz <-2sd respectively), serum ferritin and vitamin B_{12} (Normal versus Deficiency) status in our analysis.

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Results

There were 1514 eligible adolescent girls (14-19 year) from 18 villages and 27 educational institutes. Of these 304 girls were randomly selected from 24 consenting institutes of which 225 were eligible and consented. We were able to estimate the haemoglobin in 221 and micronutrients in 212 girls (Fig.2).

Socio-demographic characteristics and menstrual history of the study population (n=221)

The majority (84%) of the girls was aged 14-17-year (Table 1). The SLI as described in NFHS-2 was calculated. Majority of these girls belonged to high SLI. More than half of the girls (58%) belonged to nuclear family and most of the girls (93%) had four or more members in the family. Above 80% of the girls' parents were literate and the mothers of 68% of the girls were working. Only 2% of the girls had not attained their menarche. The median (25^{th}, 75^{th} percentile) age at menarche was 14 (13,15) years. Of the girls who had attained their menarche, 72% reported to have four or more days of bleeding during menstruation.

Sickle cell disease status

Five girls of the 221 had history of being diagnosed with sickle cell disease.

Knowledge, duration of receipt and consumption of IFA tablets

Although most (94%) of the girls could identify IFA tablets (packets of tablets shown to them) having received in the past, only 56% reported understanding its purpose to prevent anaemia (Table 1). Almost half (47%) of the girls reported to have received IFA tablets more than four months ago and the girls those who had received IFA tablets, majority had consumed the tablets but only 9 % of the girls reported to have consumed the tablets in the past two weeks.

Dietary habits and nutritional status

Almost one third of the study population was vegetarian (i.e. did not consume meat) but their daily diet was devoid of green leafy vegetables (Table 2). Daily citrus fruit consumption was found in one fifth of the girls and daily consumption of non-vegetarian food was found in only 3% of the girls despite 72% of the girls reported that they consumed eggs/non-

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vegetarian food. Almost 50% of the girls were undernourished (BMI<18.5kg/m^2) and of this, half were in the severe to moderate category (BMI<17kg/m^2). The BAZ and the HAZ scores (calculated till 19 years of age) showed that 11% were wasted and a third was stunted.

Anaemia prevalence

Anaemia was observed in 57% (95% CI = 50.8 - 63.8) of the girls. Mild, moderate and severe anaemia was present in 24%, 29% and 4% respectively (Fig. 3).

Micronutrient status in anaemic and non-anaemic girls (n=212)

Deficiency of any micronutrient was observed in 88% (108/123) of anaemic and 80% (71/89) of the non-anaemic girls. The presence of iron, vitamin B_{12} and folate deficiency was observed in 37.7%, 69.8% and 1.4% girls respectively regardless of their anaemic status (Table 3). In anaemic girls, the presence of iron, vitamin B_{12} and folate deficiency was 45.5%, 67.5% and 0.8%. The distribution of mild, moderate and severe anaemia in those with iron deficiency was 28.5%, 59% and 12.5%. The distribution of mild, moderate and severe anaemia in those with vitaminB_{12} deficiency was 48.2%, 49.4% and 2.4% respectively. Only one girl had folate deficiency and anaemia was mild. Anaemia due to other causes was observed in 15 (12%) girls (Fig. 4-a).

Exclusive iron deficiency was observed in 20% of anaemic girls whereas exclusive vitamin B_{12} deficiency was found in 42% of anaemic girls. Among anaemic girls with exclusive iron deficiency (n=24), the distribution of mild, moderate and severe anaemia was 16.7%, 62.5% and 20.8% respectively (Fig. 4-a). Girls having exclusive vitamin B_{12} deficiency had only mild (55.8%) and moderate (44.2%) forms of anaemia. Girls who did not have any deficiency (n=15) but anaemia due to some other reasons had mild (46.7%) and moderate (53.3%) anaemia.

Among non-anaemic girls, 45 girls (51%) had only vitamin B_{12} deficiency, 6 (7%) had only iron deficiency and concomitant deficiency was observed in 18 (20%) girls (Fig. 4-b). Folate deficiency existed in combination with vitamin B_{12} deficiency among only 2 (2%) girls.

The multivariate analysis for anaemia showed that girls belonging to non-nuclear family tended to be at a higher risk for anaemia (AOR=1.7, CI=0.98 –3.2; p=0.057). However, the strongest predictor of anaemia was iron deficiency (AOR=2.38, CI=1.29 – 4.4; p=0.005) (Table 4). The multinomial logistic regression showed that iron deficiency was the only predictor of moderate-severe form of anaemia (AOR=3.58, CI= 1.79-7.2) (Table 5).

Discussion

We found 57% of adolescent rural girls in Nagpur district were anaemic. Our finding is in agreement with current national anaemia estimate [1] and the reported studies [14] from rural Maharashtra conducted 15 years ago. This indicates that anaemia among adolescent girls remains a major public health problem [15] in this region.

In our study we observed overall 38% of the girls had iron deficiency, which is moderate public health problem [11]. Few other studies conducted in rural communities from Maharashtra have reported 41% iron deficiency (ferritin < 15μg/L) among 12-16 year adolescents from boarding school from Ahmednagar district [16], which is consistent with our result. A study from Wardha district reported 67% iron deficiency (ferritin < 12μg/L), 50% iron deficiency anaemia among 12-15year girl students [17]. We observed about 46% iron deficiency anaemia (Table 3) which is in consonance with this study and the generally postulated opinion that iron deficiency accounts for almost half of anaemia among children, adolescents, and women of reproductive age [18]. Almost one third of the non-anaemic girls in our study had iron deficiency. Studies reporting iron deficiencies among non-anaemics are few [4,17] and the prevalence range between 11-17%. The variations in prevalence could be due to variations in regional dietary patterns, wealth index, rates of infections, compliance to WIFS program and also difference in the cut offs of serum ferritin used to define iron deficiency.

The median (25th, 75th percentile) levels of serum ferritin among anaemic iron deficient girls in our study was 6.4 μg/L (4.6, 8.4) and 10.3μg/L (6.8, 13.1) among non-anaemic iron deficiency which is also known as stage I (storage depletion) / stage II (mild functional deficiency stage) of iron deficiency [18]. Our result thus presents functional lower reference limit of serum ferritin which is associated with transition from deficiency into anemic stage. Thus our study indicates threshold levels of serum ferritin (10.3μg/L) below which iron deficient erythropoiesis occurs manifesting into anemia (19).

Iron deficiency was not only a predictor of anaemia but also of its severity. Among several socio-demographic factors it was found that girls residing in non-nuclear family had higher

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prevalence (66%) of anemia (p value= 0.057) as compared to girls residing into nuclear family (50.4%) (Table 4). Similar findings have been reported by other studies [20]. This could be because the foods like lentils, nuts, non-vegetarian foods are rich in proteins but are expensive. So, in non-nuclear family set up it is less likely to be consumed which could be cause of anaemia among girls. Secondly, it is common to find that males in patriarchal Indian rural families may have significant advantages in terms of quality and quantity of food consumed. Although majority (72%) of the girls belonged to families that consumed non-vegetarian food, the frequency of its daily consumption among the girls was observed in only 3%. Similarly, daily green leafy vegetable consumption was observed in only 3.6%. This indicates that their diet was predominantly of cereals and lentils. This could be attributed to the finding that in our study one third (33%) of the girls had chronic malnutrition (HAZ < -2SD) and the percentage of girls having acute malnutrition (BMI<18.5) was almost 50%. Integrating iron –fortified cereals like rice into school mid-day meals can be an effective strategy for improving iron status and anaemia in the region where iron deficiency is prevalent(21).

Serum ferritin concentrations are elevated during inflammation. CRP and a-1-acid glycoprotein are the two commonly used markers to detect underlying inflammation [11]. We have estimated only CRP and found that only four girls had elevated (> 5 mg/L) CRP. Excluding these girls, the prevalence of iron deficiency (80/208, i.e. 38.5%) did not differ. The observed iron deficiency in our study could be attributed to failure of regular consumption of IFA tablets as nearly half the girls had not received IFA tablet in the last 4 months and only 9% had received it in the two weeks prior to the interview (Table 1). A recent study has shown low coverage in receiving IFA supplements among adolescents in preceding year of the survey conducted during 2015-2016 [22]. Inadequate supply of IFA tablets has remained one of the bottlenecks in the WIFS implementation and the extent of which vary widely across the districts [23].

A more comprehensive analysis of adolescent girls' knowledge about IFA tablets, the obstacles to their consumption, and the supply issues will be covered in separate article.

Majority of the girls (69.8%) in our study had deficiency of vitamin B_{12}. The predominance of vitamin B_{12} deficiency among adolescents has also been reported from north and eastern India [24,25] with the prevalence in the range of 50-90%. The studies examining vitamin B_{12} status among adolescents from central India are scarce. In our study only 3% girls consumed non-vegetarian foods daily which could be the cause of high prevalence of vitamin B_{12}

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deficiency among them. Out of total non-anaemic girls, 51% were vitamin B_{12} deficient (Fig 4-b). Very few studies [4] in India report vitamin B_{12} status among non-anaemic girls. The high rate of vitamin B_{12} deficiency without hematologic changes may go unnoticed [26]. Deficiency of vitamin B_{12} may affect reproduction and cause frequent abortions [12]. Hence, it needs attention earlier during adolescence as the symptoms of deficiency can be reversed with early diagnosis and treatment [27]. To find out the extent of deficiency among individuals to help prioritize and plan future interventions, screening like National Health and Nutrition Examination Survey (NHANES) is needed.

We found only 1.4% folate deficiency in our study population. This may be due to adequacy of dietary folate. Only 3.6% girls reported to consume green leafy vegetables daily. This could be perhaps because the question was framed to ask consumption of specifically green leafy vegetables as a vegetable preparation. The respondents may have failed to report or overlooked addition of spinach or other green leafy vegetables in lentils which is staple food in this region. The study from Himachal Pradesh [24] also did not find folate deficiency among adolescent boys and girls. We observed 12% of the adolescent girls had anaemia without iron, folate or vitamin B_{12} deficiency. Anaemia may also be due to multiple other micronutrients deficiency and estimations of these were beyond the scope of our study. Similarly, possibility of anaemia due to non-nutritional factors like bleeding due to menstruation, worm infestations, peptic ulcers, gastritis or other haemoglobinopathies could not be ruled out.

The major strengths of our study are that it provided regional estimates of anaemia and common concomitant micronutrient deficiencies in rural adolescent girl population that was being served by the national WIFS program. We not only used high quality standardized laboratory methods to estimate hematologic parameters and micronutrient levels in the blood but also assessed their demographics, dietary habits and caveats in the regional implementation of the WIFS program. The current findings are specific to the study area, characterized by high malnutrition rates, and may not be applicable to different settings. Further, the limitations are that we did not assess dietary intake of micronutrients which would have provided better insights into dietary patterns among these girls. We selected rural clusters with education institutes where WIFS program was being implemented so the generalizability of this study to other rural regions where educational institutes are not present will be limited.

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Conclusions

Our study fills a gap on micronutrient deficiencies among rural adolescent girls from central India. Majority of the girls from our study were anaemic, 84% had deficiency of one or more micronutrients. While vitamin B_{12} deficiency remained the predominant deficiency, it was iron deficiency that was predictive of anaemia and its severity. We conclude that iron deficiency remains a moderate public health problem [9]. Our study highlights the need of strengthening the WIFS program and underscores the need for screening to unveil micronutrient deficiencies to inform preventive public health action.

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Conflict of Interest

None of the authors declares a conflict of interest with regard to this manuscript. All authors have read and approved the submitted manuscript.

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Authorship

VSD, ABP, LML and PLH: Designed the study and formulated research questions. VSD and LML : Formulated data collection tools. VSD supervised data collection. VSD and ABP analyzed the data and the findings were interpreted by VSD, ABP and PLH. VSD and ABP wrote the manuscript. PLH reviewed the manuscript, edited and approved the final version submitted for publication. All authors read and approved the final manuscript.

Ethics of human subject participation

The authors declare that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving research study participants were approved by the ethical committee constituted by the Institutional Review Board of Lata medical research foundation, Nagpur. Written informed consent was obtained from the parents of the girls who were below 18 years of age. Those who were 18 years of age and older provided written informed consent. All the consent forms were coded with unique identifier prior to the data collection.

This clinical trial was registered with Indian Council of Medical Research, (**CTRI/2020/01/023035).**

Data sharing statement

The data used for this study are available from corresponding author on reasonable request.

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Table 1: Socio-demography, menstrual history, sickle cell disease status and knowledge of iron folic acid (IFA) tablets, duration of receipt and consumption of IFA tablets of the population

Variables		**Frequency (n)**	**Percentage (%)**
Age group (years)	14-17	185	84.0
	18-19	36	16.0
Standard of living index (SLI)	(0-14) Low	2	1.0
	(15-24) Medium	19	9.0
	(25- 67) High	200	90.0
Family Type	Nuclear	129	58.0
	Non-nuclear	92	42.0
Family size	<4	16	7.0
	≥ 4	205	93.0
Mother's education	Illiterate	23	10.0
	Literate	188	85.0
	Do not know	10	5.0
Mother's occupation	Housewife	64	29.0
	Working	151	68.0
	Do not know	6	3.0
Father's education	Illiterate	17	8.0
	Literate	186	84.0
	Do not know	18	8.0
Father's occupation	Non-working	2	1.0
	Working	205	93.0
	Do not know	14	6.0
Menarche status	Attained	216	98.0
	Not attained	5	2.0
Number of days of bleeding during menstruation	<4	61	28.0

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	>=4	155	72.0
Sickle cell disease status	Yes	5	2.0
	No	183	83.0
	Do not know	33	15.0
Identification and having previously received IFA tablets	Yes	208	94.0
	No	13	6.0
Knowledge regarding use of IFA tablets	Having knowledge of anemia	123	56.0
	No knowledge of anemia	98	44.0
Consumption of IFA tablets ever received	Yes	194	93.0
	No/ Do not know	14	7.0
Period of last receipt of IFA tablets	$\leq$ 2 months	80	38.0
	>2 and $\leq$ 4 month	31	15.0
	> 4 months	97	47.0
Consumption of IFA tablets in last 14 days	Yes	20	9.0
	No	201	91.0

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Table 2: Dietary habits and nutritional status of population

Variables	Frequency (n)	Percentage (%)
Type of diet consumed		
[illegible]	[illegible]	[illegible]
Consume eggs/ non-vegetarian	158	72.0
Frequency of green leafy vegetables consumption		
Daily		
Weekly/ monthly	212	96
Never	1	0.4
Frequency of citrus fruit consumption		
[illegible]	[illegible]	[illegible]
Weekly/monthly	169	76.0
Never	9	4.0
Frequency of eggs/ non-vegetarian consumption		
Daily	4	
Weekly/monthly	154	97.0
Body Mass Index (kg/m^2)	22	
< 16 – Severe		
16-17 -Moderate	24	11.0
17.1- 18.4 - Mild	59	27.0
18.5-22.9 - Normal	89	40.0
23-24.9 - Overweight	12	5.0
>25 – Obese	15	7.0
Body mass index-for-age Z-score (BAZ)*	24	
Normal	190	
Height-for-age Z score (HAZ)*	71	
[illegible]		[illegible]
Normal	143	67.0

***:** n=214 Data for seven cases could not be calculated as their age was >228 months (19years)

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Table 3: Distribution of girls by micro-nutrient and anaemia status (n=212)

Micro-nutrient Status n (%)		Non-anaemic n (%) 89 (42.0)	Total anaemic n (%) 123 (58.0)	Anaemia grades n (%) Mild 52 (42.3)	Moderate 64 (52)	Severe 7 (5.7)
Iron*	Deficiency 80 (37.7)	24 (27.0)	56 (45.5)	16 (28.5)	33 (59.0)	7 (12.5)
	Normal 132 (62.3)	65 (73.0)	67 (54.5)	36 (53.7)	31 (46.3)	0.0
Vitamin-B_{12}#	Deficiency 148 (69.8)	65 (73.0)	83 (67.5)	40 (48.2)	41 (49.4)	2 (2.4)
	Normal 64 (30.2)	24 (27.0)	40 (32.5)	12 (30.0)	23 (57.5)	5 (12.5)
Folate$	Deficiency 3 (1.4)	2 (2.3)	1 (0.81)	1(100)	0.0	0.0
	Normal 209 (98.6)	87 (97.7)	122 (99.2)	51 (41.8)	64 (52.5)	7 (5.7)

*: This may include vitamin B_{12} or folate deficiency also

: This may include iron or folate deficiency

$: This may include iron or vitamin B_{12} deficiency

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Table 4: Multivariate logistic regression for anaemia (n=209)$^{\$}$

Characteristics	N	Anaemia (n=119) n(%)	No anaemia (n=90) n(%)	COR (95% CI)	p-value	AOR(95% CI)	p-value
Standard of Living Index (SLI)							
High SLI	**189**	110 (58.2)	79 (41.8)	1.7 (0.67-4.3)	0.37	1.4 (0.51-3.6)	0.53
Low-Medium SLI	**20**	9 (45.0)	11 (55.0)				
Family Type							
Non-nuclear	**88**	58 (66.0)	30 (34.0)	1.9 (1.1– 3.4)	0.03*	1.7 (0.98-3.2)	0.057
Nuclear	**121**	61 (50.4)	60 (49.6)				
Family Size							
< 4	**14**	7 (50.0)	7 (50.0)	1.34 (0.45-3.9)	0.79	0.98 (0.31-3.1)	0.97
>4	**195**	112 (57.4)	83 (42.6)				
Type of diet							
Vegetarian	**60**	33 (55.0)	27 (45.0)	1.1 (0.61-2.0)	0.83	1.2 (0.6-2.2)	0.65
Eggs/non-vegetarian	**149**	86 (57.7)	63 (42.3)				
Body mass index-for-age Z-score (BAZ)							
Normal	**186**	105 (56.5)	81(43.5)	1.2 (0.49-2.91)	0.85	1.1 (0.43-2.9)	0.77
BAZ<-2 SD	**23**	14 (60.9)	9 (39.1)				
Height-for-age Z score (HAZ)							

Normal	**138**	75 (54.3)	63(45.7)	1.36 (0.76-2.45)	0.36	1.5 (0.79-2.8)	0.21
HAZ <-2 SD	**71**	44 (62.0)	27 (38.0)				
Serum ferritin							
Normal	**129**	63(48.8)	66 (51.2)	2.44 (1.35 – 4.4)	0.004**	2.38 (1.29-4.4)	0.005**
Iron deficient	**80**	56 (70.0)	24 (30.0)				
Serum vitamin B_{12}							
Normal	**63**	38 (60.3)	25 (39.7)	0.82 (0.44-1.5)	0.62	0.93 (0.49-1.76)	0.83
B_{12} deficient	**146**	81 (55.5)	65 (44.5)				

$^{\$}$n= 209, data for three participants for HAZ and BAZ could not be calculated as their age was greater than 228 months (19 years); COR : Crude odds ratio; AOR : Adjusted odds ratio; *: p <0.05, **: p<0.01

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Table 5: Multinomial logistic regression analysis results on anaemia severity among adolescent girls by socio-demographic and nutritional characteristics

Characteristics	N	Anaemia status by Hemoglobin levels n (%) $				
		No anaemia –90 (43.1) (Ref) n (%)	Mild anaemia-50 (23.9) n (%)	AOR (95% CI)	Moderate-severe anaemia-69 (33) n (%)	AOR (95% CI)
Standard of Living Index (SLI)						
High SLI	**189**	79 (41.8)	46 (24.3)	0.66 (0.19 - 2.3)	64 (33.9)	0.78 (0.24 - 2.6)
Low-Medium SLI	**20**	11 (55.0)	4 (20.0)		5 (25)	
Family Type						
Non-nuclear	**88**	30 (34.1)	25 (28.4)	1.91 (0.92 - 3.9)	33 (37.5)	1.69 (0.85 - 3.4)
Nuclear	**121**	60 (49.6)	25 (20.7)		36 (29.7)	
Family Size						
<4	**14**	7 (50.0)	3 (21.4)	1.01 (0.24 - 4.4)	4 (28.6)	1.03 (0.26 - 4.1)
>4	**195**	83 (42.6)	47 (24.1)		65 (33.3)	
Type of diet						
					[	
Vegetarian	**60**	27 (45.0)	14 (23.3)	0.83 (0.38 - 1.8)	19 (31.7)	0.87 (0.42 - 1.8)
Eggs/non veg	**149**	63 (42.3)	36 (24.2)		50 (33.5)	
Body mass index-for-age Z-score (BAZ)						

Normal	**186**	81 (43.5)	45 (24.2)	1.04 (0.32 - 3.4)	60 (32.3)	1.2 (0.42 - 3.5)
BAZ<-2 SD	**23**	9 (39.1)	5 (21.8)		9 (39.1)	
Height-for-age Z score (HAZ)						
Normal	**138**	63 (45.7)	29 (21)	1.7 (0.79 - 3.6)	46 (33.3)	1.33 (0.64 - 2.7)
HAZ<-2 SD	**71**	27 (38.0)	21 (29.6)		23 (32.4)	
Serum ferritin						
Normal	**129**	66 (51.1)	34 (26.4)	1.29 (0.59 - 2.8)	29 (22.5)	3.58(1.79 - 7.2) **
Iron deficient	**80**	24 (30.0)	16 (20.0)		40 (50)	
Serum vitamin B_{12}						
Normal	**63**	25 (39.7)	12 (19.0)	1.26 (0.55 - 2.9)	26 (41.3)	0.76 (0.37 - 1.6)
B_{12} deficient	**146**	65 (44.5)	38 (26.0)		43 (29.5)	

[$] n= 209 data for three participants for HAZ and BAZ could not be calculated as their age was greater than 228 months (19years). AOR : adjusted odds ratio; *: p <0.05, **: p<0.01

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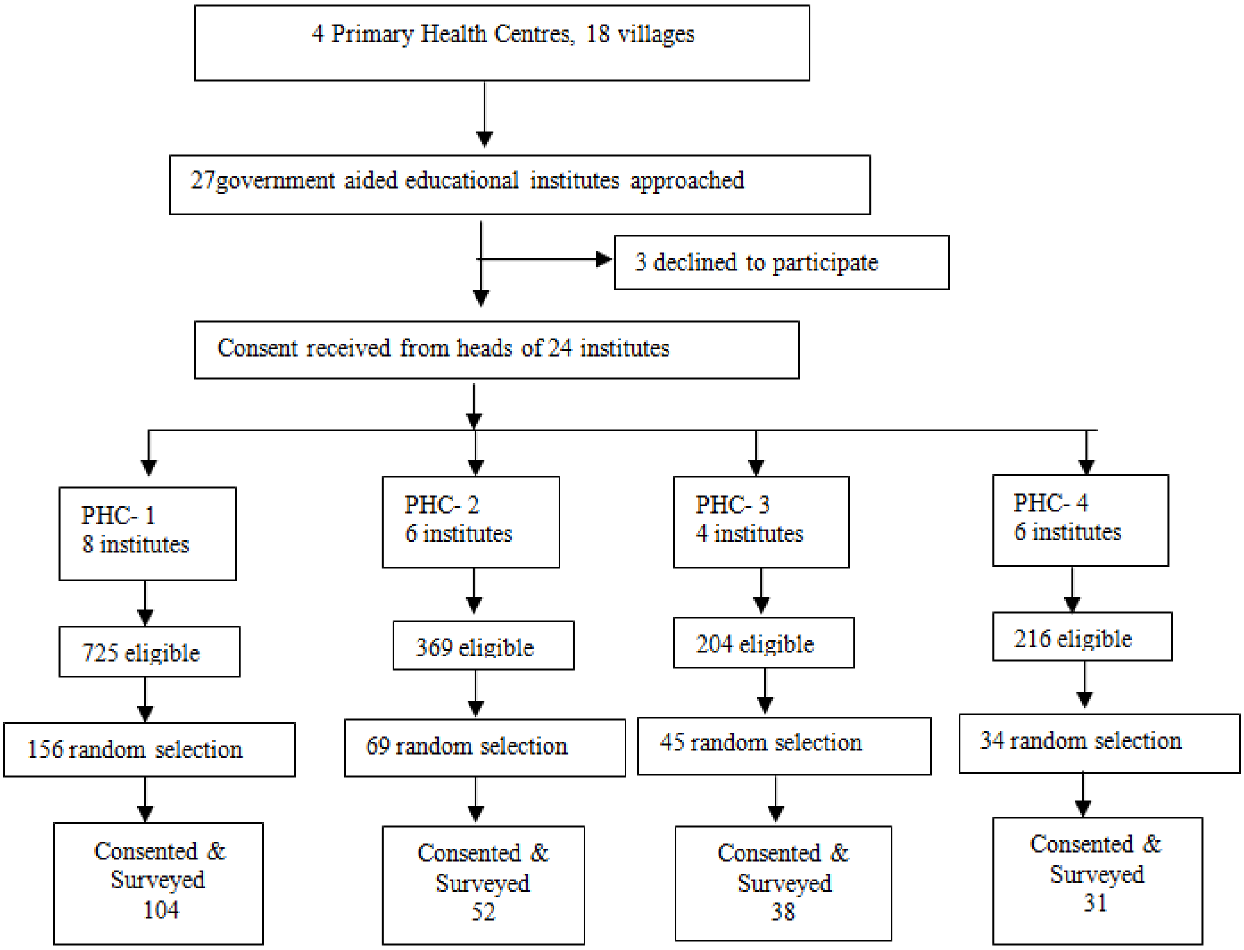

Figure 1 : Sampling frame of the study

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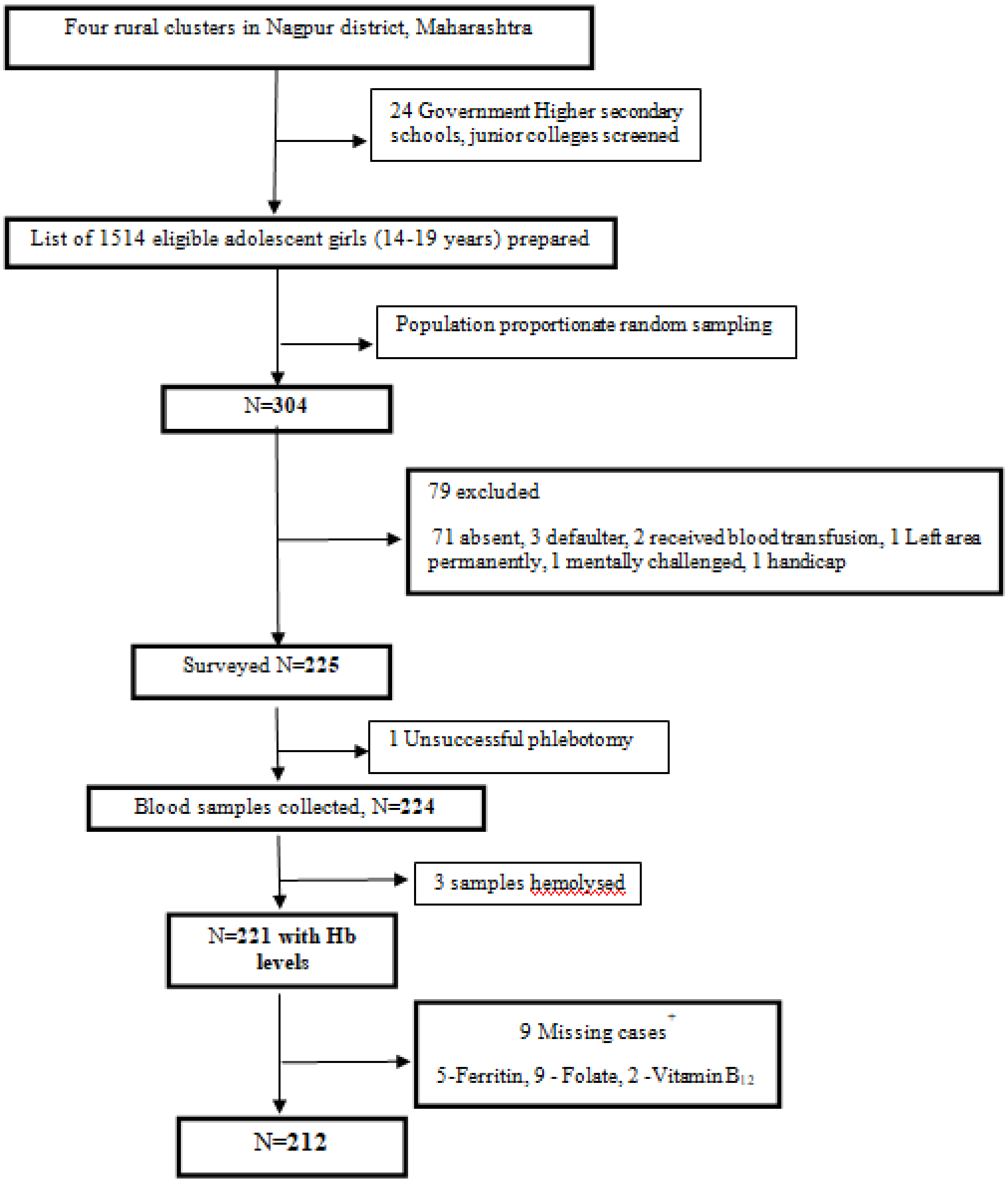

Figure 2: Analytical sample included all participants with required variables. Data for cases missing if the sample quantity was not sufficient or if the sample was spoilt. + Not mutually exclusive

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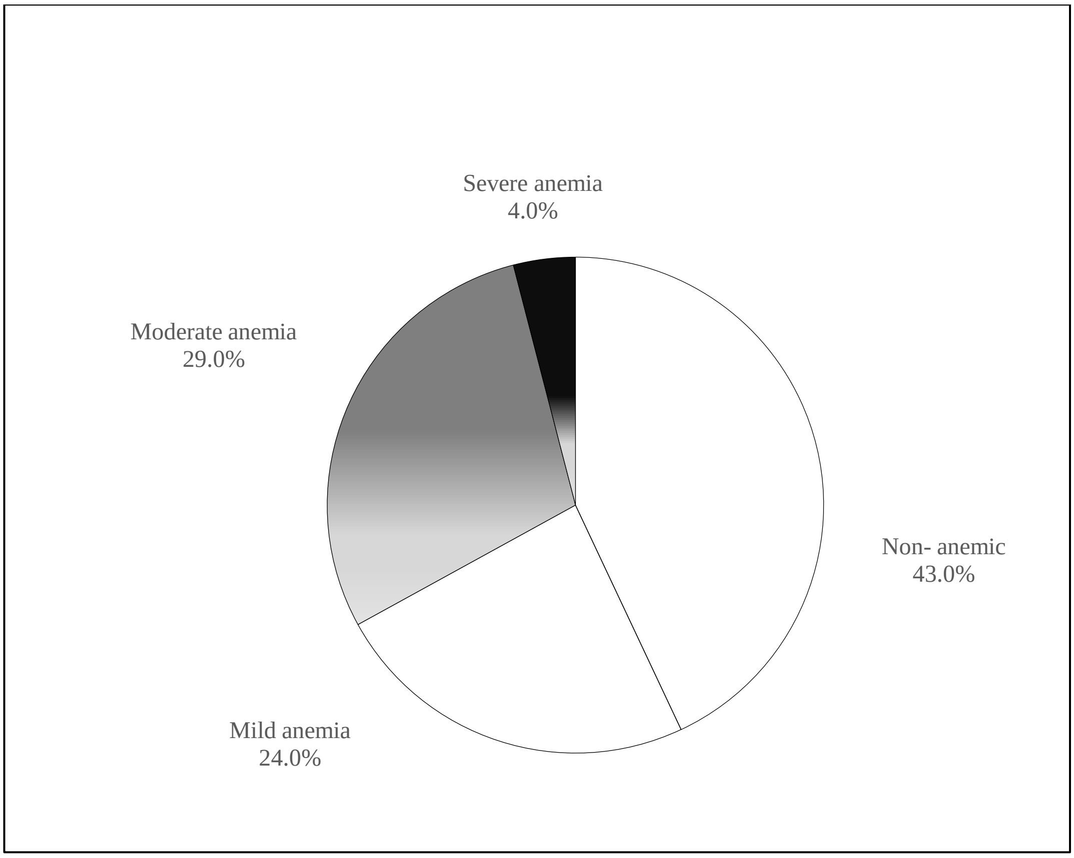

Figure 3 : Prevalence and severity of anemia in the study population (n=221)

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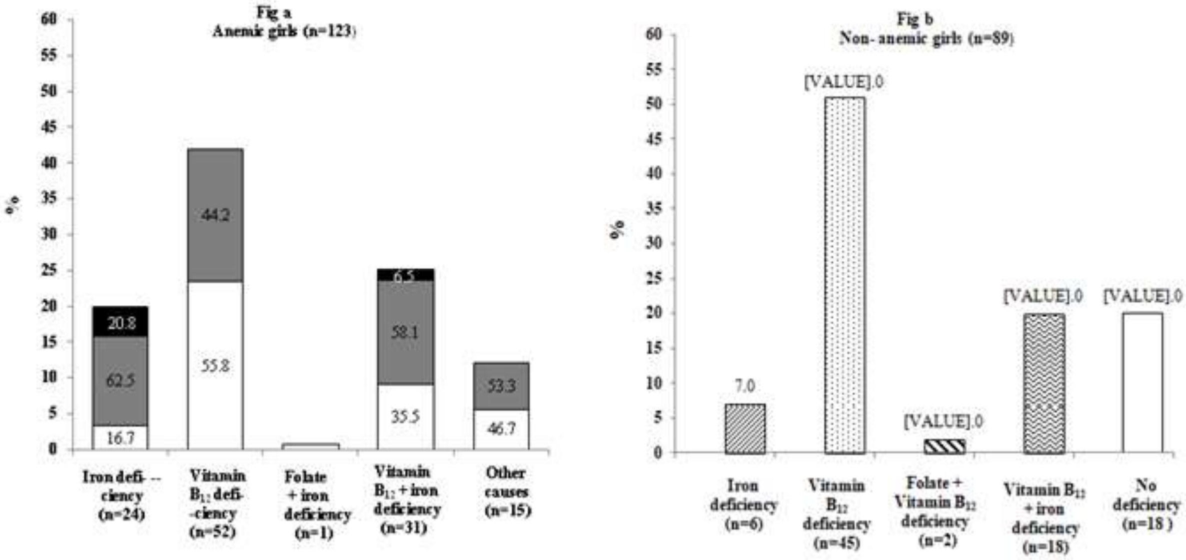

Figure 4 : Status of micro-nutrients in study population by anemia status

Legends :

□ Mild anemia ■ Moderate anemia ■ Severe anemia