

Chapter III

METHODOLOGY



In view of the inadequate research on various functional benefits of intermittent iron-folate supplementation on young adolescents, like growth, physical work capacity and cognition, the present research, an experimental-control semi-longitudinal study, was undertaken with the following **broad objective**:

To study the impact of daily and. intermittent (once and twice weekly) iron folic acid supplementation on hemoglobin levels, pubertal growth, food and nutrient intake, cognitive function and physical work capacity among underprivileged primary school going girls in early adolescence (9-13 years) of Vadodara.

The null hypothesis was stated as below:

Among girls in early adolescence from deprived communities and studying in government schools, whether supplemented with iron folic acid (IFA) tablets daily or twice a week or once a week,

- a) there is no significant impact (when compared to controls) on hemoglobin levels, growth (in terms of BMI), food and nutrient intake, cognitive function test scores and physical work capacity (in terms of steps climbed and recovery time).
- b) there is further, no significant difference in impact on the above indicators within the intervened groups, i.e. IFA Daily, IFA twice weekly and IFA once weekly.

The specific objectives are mentioned with each phase of the study.

Site Of The Study And Sample Selection

This study was conducted in Vadodara Municipal Primary Schools catering to the girls from low-income group families (LIG) studying in standard V and VI. To select the universe for sampling, a list of all the schools under the Vadodara Municipal Corporation was obtained. All the schools matching the following criteria were selected as the universe for purpose of sampling:

- The school should have a primary section (I to VII standards), as the age group of the study was girls in early adolescence;
- The study focused on girls, therefore listed schools should have only girl students.
- Morning school timings: To be able to compare and pool data, schools with similar timings i.e. morning timings, were selected.

Based on these criteria, seventeen schools were short-listed, which formed the universe for the sample selection for the present study.

Under the Vadodara Municipal Corporation, Gujarat, the primary school comprises students studying in standard I to VII. The study focused on the age group of 9-13 years, thus, standards V and VI were selected for this study. Standard VII was excluded as this was a semi-longitudinal study and girls of standard VII would leave the primary school, and hence would not be available in the school in the following year for post data collection.

From the short-listed seventeen schools, four schools were randomly selected using random number table. All the girls studying in standard V and VI were enrolled in the study. The sample thus available when the study was initiated was 358 girls in the age group of 9 - 13 years after excluding eighteen girls who had attained menarche¹. Further details on sample size of the various indicators are given later.

¹ To remove the effect of menarche as a confounding variable, only pre-menarcheal girls were included in the study.

In addition to the LIG schools, one morning school catering to girls from highincome (HIG) families was purposively selected to be able to compare the cognitive functions and physical work capacity of the LIG girls with that of the girls from the well-to-do families of Vadodara. In the HIG school all the girls studying in standards V, VI and VII were enrolled (N=69) to match the age group in LIG schools i.e. 9 to 13 years. As explained for LIG schools, similar data were also collected in HIG school twice, with a gap of one year

As seen in next section on experimental design, this was an experimental vs. control intervention trial where the impact of three types of intervention (given over one year) was compared.

- 1. Iron-folate supplementation given once a week
- 2. Iron-folate supplementation given twice a week
- 3. Iron-folate supplementation given daily

The fourth group was the control group.

Estimation of Required Sample Size

Sample size calculation was done to estimate how many schools would be required for the study assuming that there are 100 girls in standard V and VI in each school (based on our exploratory visits). Using standard formulae for estimating sample size (Fisher et al 1991) for a study such as this, where subgroups are compared pre and post intervention, the desired sample size came to 46 subjects per group (Annexure 1). This would make possible inter-group comparison pre and post intervention, assuming the proportion girls with anemia was 60% at baseline, a difference of 20% after the intervention and significance at 0.05 level.

Allowing for dropouts, it was decided that each study group (representing one school) would have about 60 subjects. Since one school on an average had approximately 60-100 girls available in standards V and VI, it was clear that one school would give enough sample size for one study group. As there were four

groups (three experimental and one control), it was decided that four schools would be randomly sampled for this study.

Informed Consent

Prior permission and approval for the study was obtained from the Primary School Board, Vadodara (Annexure 2) and the schools were explained the purpose of the study. Informed consent was taken from the students and their parents (Annexure 3). The students were free to opt out of the study anytime they wanted. Girls who had attained their menarche prior to, or, during the study, though were excluded from the analysis, they did receive IFA supplements.

Experimental Design Of The Study

The study was designed as an experimental-control, semi longitudinal study, as shown in Figure 3.1.

In the three experimental schools, intervention was carried out for one year. These schools were supplemented with IFA tablets (100 mg elemental iron + 0.5 mg folic acid) as indicated below.

- School E1: IFA-1Wkly, girls received IFA tablets once a week
- School E2: IFA-2Wkly, girls received IFA tablets twice per week
- School ED: IFA-Daily, girls received IFA tablets daily

The control school (CS: No-IFA) did not receive any intervention but participated in the baseline and post intervention data collection. After a period of one year post intervention data was collected.

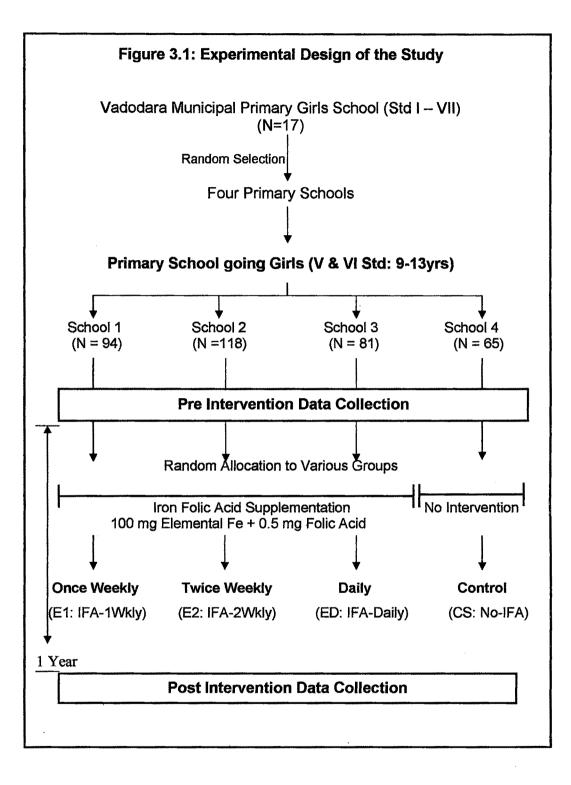
The study was divided into three main phases:

Phase A: Baseline Survey - Pre Intervention Data Collection

Phase B: The Intervention with Ongoing Process Evaluation

Phase C: Impact Evaluation - Post Intervention Data Collection

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Iron Folic acid Tablets (100 mg Fe plus 0.5 mg Folic Acid) Supplemented to the School Girls

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Phase A: Baseline Survey – Pre Intervention Data Collection

The specific objectives of this phase were:

- To assess the present nutritional status of schoolgirls in early adolescence (9-13 years) with respect to the prevalence of undernutrition, anemia, morbidity and food intake.
- > To measure specific aspects of physical work capacity and cognitive function (concentration, attention, and memory) in the study girls.
- To determine the perceptions of the girls regarding anemia, its causes, adverse effects and prevention.

Indicators and Methods of Data Collection

The study indicators and tools for data collection are summarized in **Table 3.1**. All the methods were pre-tested in a different school with a similar setting.

Sr. No	Indicators	Tools	Reference	Sample size
1	Socio-Economic Status	Structured questionnaire	Bernard (1991)	358
2	Morbidity	Structured questionnaire	Bernard (1991)	358
3	Weight and Height	Standard methods	Gibson (1989)	358
4	HB estimation	Cyanmet-hemoglobin method	INACG (1985)	334
5	Food and	Dietary recall method	Thompson and	160
	Nutrient Intake*	Food frequency	Byers (1994)	
6.	Cognitive abilities*	 WISC tests, modified and adopted for this group. Clerical Task Maze Visual Memory Test Digit Span 	Bhatt (1973) Department studies Kanani, Singh and Zutshi (1999)	240
7.	Physical Work Capacity*	Modified Harvard's Step Test, modified and adopted for this group	Skubie and Hodgkins (1964)	240
8	Perception of girls *	Semi structured interview	Bernard (1991)	80

Table 3.1: Study Indicators and Tools for Data Collection

* Random sample

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Since all data collection and intervention had to be completed within the school days available, it was decided that for the methods* involving considerable time inputs, a random sub-sample will be selected, such that even if there were dropouts post intervention, statistical calculations would be possible (**Table 3.1**).

Methods of Data Collection

1. Structured Questionnaire on Socioeconomic Status and Morbidity

Background information was obtained from each student, including child and parents' names, age, education, occupation and other relevant information. Since morbidity which is not treated by visiting a healthcare provider (like mild fever or cold) is often poorly recalled, the girls were asked to recall any illness suffered during last 15 days for which they had to go to a doctor (Annexure 4).

2. Assessment of Nutritional Status

2.1 Anthropometry

2.1.1 Weight-for-age

Weighing is a key anthropometric measurement of body mass. Weight deficiency is the best indicator for the detection of protein energy malnutrition and growth failure in children (Gibson 1989).

Procedure: The girls were weighed barefoot on a standardized portable digital 'SECA' balance (manufactured by UNICEF). The girls were asked to stand straight on the scale without touching anything and look straight ahead. The weight was recorded to the nearest 0.1 Kg.

2.1.2 Height-for-age

Height is a linear measurement of the body. Height-for-age is an indicator of long term growth deficit. It is associated with chronic insufficient food intake and frequent infections.

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Procedure: A polyvinyl-coated fiberglass tape was fixed perpendicular to the floor on the wall and the girls were made to stand against it. The head, shoulder and heels touched the wall and the girl was asked to look straight ahead. A thin scale was kept straight on the head, perpendicular to the wall, so as to slightly press the hair. Height was read off from the markings on the tape to the nearest 0.1 cm.

2.1.3 Body Mass Index (BMI)

BMI has been recommended by WHO (1995) as an indicator of choice for measuring undernutrition among adolescents. Among adolescents, BMI slowly increases with age. BMI is calculated as follows:

$$BMI = \frac{Weight (in Kg)}{Height (in m^2)}$$

2.2 Blood Hemoglobin Estimation

Blood hemoglobin estimation is used as a biochemical parameter for detection of anemia using cyanmethemoglobin method. The principle behind this method is that hemoglobin in the blood sample reacts with ferricyanide present in Drabkin reagent to form methamoglobin which is reduced by cyanide to form cyanmethamoglobin which is a rust coloured complex measured at 540 nm (Oser 1979).

Procedure: The tip of the middle finger was cleaned with a cotton swab dipped in ethanol and the finger was allowed to dry. The fingertip was pricked using a sterile lancet. The first drop of blood was wiped away with a cotton swab. Using a microhaemometer pipette, $20 \ \mu$ l of free flowing blood was collected from the finger tip. The pipette was cleaned from outside and blood was transferred into vials containing 5 ml of the Drabkin reagent. The blood sample and the reagent were mixed carefully and properly. During blood collection care was taken to get free flowing blood and not to press hard or squeeze the fingertip. The blood sample was analyzed at 540 nm using clinical autoanalyzer (Metro Lab q600 plus, version 3.05 I).



Primary School Girls – All Are 9 Year Old – Is Their Growth Satisfactory



Blood Sample Collection from Finger Prick for Cyanmethamoglobin Method

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2.3 Dietary Intake

Food and nutrient intake is an important component of nutritional status assessment. The 24 - hour dietary recall and food frequency methods were used to record the dietary intake of girls. Both pre and post intervention data regarding dietary intake was collected during winter season.

2.3.1 24-Hour Dietary Intake Method

This method is based on the process of recall of food consumption over a specified period of time (24 hours), prior to the survey. The ingredients recalled by the respondents are measured using standard cups and spoons. From the cooked amount, the raw ingredients as well as their nutritive value are calculated using recipes of the conventional cooked foods.

Procedure: The food intake of the subjects was noted for the previous day through individual interviews. Amount of cooked food eaten in each meal was recorded using standard measures (Annexure 5). Foods eaten by the subject outside home was also recorded. The nutrient content of food eaten was calculated using the food composition tables (ICMR 1991).

2.3.2 Food Frequency Method (FFQ)

Food frequency method is usually used to assess the habitual food intake of the girls, qualitatively. The girls were asked to report their usual frequency of consumption of each food from a list of foods, rich in iron and vitamin C (Annexure 6). The frequencies asked for were daily, 1-2 times per week, 3-5 times per week, occasionally and rarely/never. It is an accepted method to estimate usual dietary intake (Thompson and Byers 1994). In addition, the appropriate quantity eaten (standard cups) was also asked and recorded.

3. Cognitive Functions Tests

Iron deficiency adversely affects the ability of the school children to learn. The cognitive functions of the girls were assessed using selected tests from the Gujarati version of Wechsler Intelligence Scale for Children (WISC) (Bhatt 1973), which were pre-tested and suitably modified for the present study groups. These cognitive function tests have been previously used in the department (Kanani, Singh and Zutshi 1999). The WISC is a battery of tests for 6 - 17 years olds that evaluates intellectual abilities. The various tests used were Digit Span, Maze test, Clerical task and Visual Memory test.

3.1 Digit Span

This test measures short-term auditory memory for non-meaningful information; concentration; and ability to remember a sequence of numbers (both backwards and forwards).

Procedure: In this test a series of numbers were read out by the investigator (Annexure 7), which gradually increased in length. Each list of numbers was presented only once and the girls were asked to recall and write each set of the numbers in the same sequence called out: the forward recall. Another set of numbers were read and the girls were then asked to recall and write the numbers in the reverse sequence; the backward recall. Different series of numbers were presented for forward recall and backward recall. The girls were instructed to be attentive as each set of numbers were read only once, clearly and loudly. They were instructed to write the numbers only after the whole set of the numbers was read out completely.

Scoring: Whether the series of numbers were written in the correct order was carefully checked. Scores were given separately for forward recall and backward recall as per the method description. Both the scores were added and a total score was given. Seventeen was the maximum score.

3.2 Maze Test

Mazes are used to measure psychomotor coordination: planning, visual-motor coordination and speed; and fine motor coordination, planning, following directions. Performance is also based on time.

Procedure: The maze tests have complex pathways having blind alleys. Papers containing seven mazes were given and the girls were asked to find their way out of the mazes using a pencil, without making any errors within specific time given for completion of each maze. There were seven mazes increasing in complexity (Annexure 8). The girls were instructed to find their way outside, and were not allowed to draw lines approaching from outside to inside. The girls were asked not to lift their pencils.

Scoring: Each maze was checked for the number of errors and accordingly scores were given (Table 3.2).

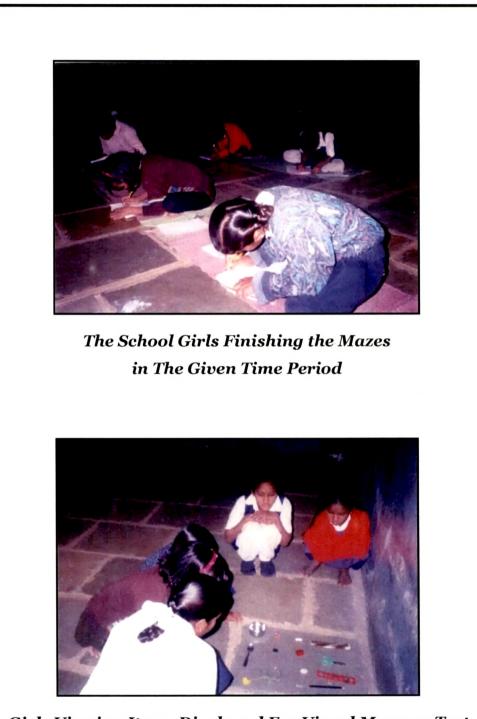
Error:

- If the line of the maze was touched by the girl, it was counted as one error.
- Crossing an imaginary line into a major blind alley is one error.
- Crossing line (walls of the maze) is one error. A line is crossed if any white space can be seen between the printed line and pencil line on wrong side of the printed line.

Maze	Errors	rors Time Limit		Points		
	Allowed		0 error	1 error	$\geq 2 \text{ error}$	
Maze A	2	30 sec	2	1	0	
Maze B	2	30 sec	2	1	0	
Maze C	2	30 sec	2	1	0	
Maze 1	3	30 sec	3	2	1	
Maze 2	3	45 sec	3	2	1	
Maze 3	5	60 sec	3	2	1	
Maze 4	6	120 sec	3	2	1	

Table 3.2 Errors Allowed, Time Limit And Scoring For The Seven Mazes

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Girls Viewing Items Displayed For Visual Memory Test

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3.3 Visual Memory Test (VMT)

Visual memory test is used to assess the short term memory of girls.

Procedure: Fifteen commonly used items were placed on a table such as pen, pencil, rubber etc. (Annexure 9). The girls were allowed to observe the objects for one minute, and then the objects on display were covered with a cloth. The girls were asked to recall and list down the items which they had seen within 2 minutes. One point was given for each item listed correctly.

Scoring: The scores were calculated as the ratio of the total number of items correctly listed to the total number of items, i.e. 15. The highest score was one.

3.4 Clerical Task (CT)

Clerical task assesses the ability of the girls to concentrate and discriminate.

Procedure: The girls were given a typed page of Gujarati text pre-tested in another school on the same age group. They were asked to encircle all the "matras" of "a" and "o" in Gujarati within 2 minutes (Annexure 10). The girls were instructed not to encircle any other matras of 'e', 'ra', 'au'. The investigator motivated the girls to encircle as many matras as they could in two minutes.

Scoring: Higher the number of matras they encircled more was the points they scored. In all there were 108 matras in the text, which the girls had to encircle. The final score was calculated as the ratio of the number of matras encircled by the girls to the total number of matras in the text. The highest score was one.

4. Physical Work Capacity (PWC)

Iron deficiency impairs the physical work capacity and work output. The physical work capacity of the school girls were assessed using Modified Harvard's Step test (Skubie and Hodgkins 1964). The tests for physical work capacity (PWC) were modified and pre-tested to make them appropriate for this age group.



A Girl Concentrating On the Clerical Task Test



A Girl Performing the Modified Harvard Step Test

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Procedure: A set of five steps was selected in the school premises. Before the test was started, the resting pulse rate of the girls was recorded for minimum one minute. The girls were then asked to climb up and down the set of five steps for a period of three minutes as fast as they could. The girls were asked to keep both feet on one step and then only proceed to the next step. The girls were instructed not to take support of the walls or the railing while climbing the steps. The investigator motivated the girls to climb as many steps as they could, since more the number of steps they climbed, higher will they score. Care was taken to see that step height was similar in all schools (within 16-19 cms).

The total number of steps climbed up and down was counted. Immediate postexercise pulse rate was then counted within the first 10 seconds after the exercise for a minimum of 30 seconds. The pulse rates were counted after every one minute for a minimum of 30 seconds, until the girls recovered their basal pulse rate. Finally the pulse recovery time was calculated in minutes.

5. Perceptions of Girls Regarding Anemia, Its Causes and Its Treatment

In this study perception of school going girls regarding anemia, its causes, consequences and treatment were assessed prior to the intervention. Semi structured interview method was used to collect information about perception, among girls about anemia. Semi structured interviews are based on the use of an interview guide, which is a written list of questions, and topics that need to be covered in some order. The investigator's queries follow a pre-determined order but their substantive focus shifts according to the responses of the informants (Bernard 1991).

Procedure: Each girl in the sub-sample was interviewed regarding anemia, its causes, consequences and treatment individually (Annexure 11).

Phase B: Intervention with Ongoing Process Evaluation

The specific objectives of this phase were:

- To distribute iron folic acid tablets to the girls in the different experimental schools (daily, once weekly and twice weekly) over a period of one year, as shown in the experimental design.
- > To study the compliance of various intervention schedules in the schools.
- To study and compare the benefits and side effects experienced by the girls following the various iron folic acid supplementation regimens.

The Intervention

Following the baseline survey each school was randomly assigned to either daily or twice weekly or once a week iron-folate treatment group, or no treatment group (control). Baseline data analysis indicated that the four groups were comparable with regard to key indicators i.e. hemoglobin, body mass index, cognition and PWC.

Orientation Session to Sensitize the Girls and Class Teachers to the Study

Before the initiation of the intervention, in each school, one session was conducted for the girls as well as the teachers regarding the causes, consequences and treatment of anemia. All the girls were gathered at one place in the school premises along with the class teachers and were explained the importance of iron during adolescence and the rich dietary sources of iron and vitamin C.

Further in the iron-folate treatment groups, importance of iron folic acid supplementation was explained. They were sensitized to the benefits of IFA tablets and were assured that side effects usually occur in a small percentage of girls. And if at all side effects occur, they were explained how to manage them. They were motivated and encouraged to actively participate in the program.

The girls were clearly advised to take the tablet after having food and not on empty stomach.

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The IFA Supplementation in The Classes

After consulting the class teacher, two girls (preferably class monitors) were identified from each class who would actively assist the investigator in the distribution of iron folate tablets. They would also regularly maintain compliance records under the guidance of the investigator. The investigator regularly visited and supervised the distribution and recording of compliance in all the schools.

Distribution of IFA tablets

To ensure that girls do not take the tablets on empty stomach, IFA tablets were distributed after the lunch break. As soon as all the girls assembled in the class, in the presence of the girls, the class monitors (sometimes along with the class teachers) called out each girl's name according to the attendance register. Each girl was given water and the tablet, and she consumed the tablet in front of the monitor and the investigator. As drinking water facility was not available in all schools, a bottle was kept especially for the purpose and the monitors filled the bottle with water while coming to class after lunch break.

Recording of compliance

Once the tablet was consumed a tick mark was put in the compliance register corresponding to the girl's name and date (Annexure 12 a, b, c). Each page of the compliance register was filled in duplicate using a carbon.

Taking Care of the Absentees and the Vacations

In the once weekly and twice weekly schools, those girls who were absent on the day of the supplementation were given the tablet the next day when they attended the school and the compliance was recorded in the register. During the *Dipawali* and winter break each girl was given separate poly-pack containing the number of IFA tablets they should be consuming during the vacations along with a compliance card to be filled by them after consuming the tablets. The girls were asked to return the compliance card as well as the left over IFA tablets, if any, to the investigator after the school reopens.

Storage of IFA Tablets

As per the requirement, the IFA tablets were stored in cool, dark place, away from sunlight, in each school, either in the teacher's cupboards or in a trunk. The compliance register was kept safely along with the tablets.

Process Evaluation

The feasibility of the intervention in terms of compliance, side effects and teacher cooperation was assessed.

Calculation of Compliance with IFA

From the compliance registers, the investigator calculated the number of tablets consumed by each girl. From the vacations compliance cards, the compliance was calculated for that period. At the end of the supplementation program, the total number of tablets consumed for each month was added up and percentage compliance was calculated for each girl.

Considering the total expected number of tablets to be consumed as per the dose regimen, the percentage compliance for each girl was calculated as

= <u>Number of tablets consumed</u> x 100 Number of tablets expected to be consumed

The expected number of tablets to be consumed was:

- a) 51 IFA tablets for once weekly IFA regimen
- b) 100 IFA tablets for twice weekly IFA regimen
- c) 305 IFA tablets for Daily IFA regimen

The compliance with IFA for each supplementation group was compared with another. A cutoff level of 70% dose consumed was used to separate girls with good and poor compliance in all the three IFA regimens.

Recording Of Benefits And Side Effects Experienced By The Girls: After Taking IFA Tablets

The benefits as well as the side effects experienced during the phase of the intervention were recorded by the investigator during school visits. In each visit the girls were asked to explain the changes (benefits as well as side effects), if any, they experienced after consuming the iron tablets. Their comments were recorded in the field notes maintained by the investigator.

The benefits and the side effect reported after consumption of IFA tablets were compiled and compared between the three treatment groups.

Interest Of The Students And Teachers

The field notes regarding the interest and co-operation of the teachers and the girls were maintained and studied by the investigator.

Phase C: Impact Evaluation – Post Intervention Data Collection

The specific objectives of this phase were:

- To study the impact of various IFA interventions given to the young school girls
 - a) on the prevalence of anemia
 - b) on growth in terms of weight-for-age, height-for-age and BMI
 - c) on food and nutrient intake
 - d) on cognitive function
 - e) on physical work capacity
- To compare the relative impact of IFA supplementation among the daily, once weekly and twice weekly groups.

Specifically impact was compared

- a) of each intervened group with control group,
- b) within each intervened group

Impact was also compared in relation to different age groups, anemic and nonanemic status, level of compliance and impact in relation to extent of Hb gains.

Impact evaluation was conducted after a year of supplementation. Impact data was collected with respect to change in hemoglobin levels, growth, dietary intake, cognitive function and physical work capacity. The indicators used were the same as those in the baseline survey. The data analysis section explains this further.

Data Analysis

All the data was coded, entered and analyzed in Epi Info, Version 6.04-d (2001) and some aspects were also analyzed in Microsoft Excel 2000, Windows XP. The study data was analyzed as follows:

Baseline Data

Percentage values for different indicators were calculated for the following

Indicators	Standards / Cut off levels used		
Socio-economic Status	Percentage of total girls studied		
 Percentage experiencing morbidity 	Percentage of total girls studied		
Percentage anemic	Hb <12 g/dl		
Percentage Stunted	Height-for-age <5 th percentile of CDC Standards (200 b)		
• Percentage undernourished	BMI <5 th percentile of Must et al standards (1991)		
Intake of Various			
• Food Groups	Percentage of RDA (NIN 1998)		
 Nutrients (Calories, protein, fat, iron, vitamin C, β carotene) 	Percentage of RDA (ICMR 1991).		
• Percentage responses – consumption of iron and vitamin C rich foods	Percentage of total girls studied		
• Percentage responses – perceptions regarding anemia, its causes, consequences and treatment.	Percentage of total girls studied		

WHO growth standards have now been recommended as the reference standard for children. However at the time when this study was carried out, analyzed and written, the norms for adolescent children were not available. Hence, CDC growth standards given by National Health and Nutrition Examination Survey, United States (2006) were used to compare height-for-age and weight-for-age, and Must et al standards (1991) for BMI.

Mean, median and standard deviations were calculated for indicators such as:

- Hemoglobin (Hb)
- Height-for-age, Weight-for-age, Body Mass Index (BMI)
- Food intake and intake of selected nutrients (i.e. Energy, Protein, Fat, Iron, Vitamin C, and β Carotene)
- Cognitive Function test scores: Digit Span, Visual Memory, Maze and Clerical Task
- Physical work capacity: Steps Climbed and Recovery Time.

Comparison with HIG girls: To obtain a local reference standard for cognition and PWC, girls from one HIG school were also measured using the same cognition and PWC tests as for the LIG. As expected the HIG girls had high cognitive function test scores than LIG girls (see results section). However, for PWC it was found that among the LIG and HIG girls the time taken to revert back to basal pulse rate i.e., recovery time and the number of steps climbed by wellnourished HIG girls (BMI within 18 to 22 kg/m^2) were comparable perhaps because LIG girls are more used to household work and other high calorie expenditure activities as compared to HIG girls. Therefore eventually the PWC data of HIG girls was not used as standard for LIG girls.

Measuring The Impact Of Intervention

All analysis presented in this section was done on the final data set comprising of only those girls for whom data was available at both the pre-intervention and postintervention phases.

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The changes in each indicator of impact (pre to post) in each group (ES and CS) were measured and compared. For example, increments in

- Hemoglobin levels
- Growth in terms of body mass index,
- Cognitive function: improvement in cognitive function test scores
- Physical work capacity: improvement in number of steps climbed and reduction in recovery time to basal pulse rate.

Further, the following groups were compared:

- Experimental schools (ES) vs Control School (CS)
- Within the intervened groups: IFA-1Wkly vs. IFA-2Wkly vs. IFA-Daily
- Younger girls vs. older girls
- Initially anemics vs. initially non-anemics
- Good compliance vs. poor compliance with IFA.

As appropriate, either F test, Students 't' test or Chi-square test was used to compare various intervention groups for statistical significance of impact.