CHAPTER 2

METHODOLOGY
Sampling:

Purposive sampling was considered, as it comprised of all the children - Nursery to 4th standard for the pilot study and nursery to 9th standard, for the main research study, attending Saifi Boy’s High School. However in the Follow up study the sample consisted of only those children from Udayachal Primary School from 1st to 4th standard, who volunteered to be a part of the study.

Purposive sampling is a form of non-probability sampling. With this type, the sample is "hand-picked" for the research. (Polit and Hunglar, 1999).

Dane, (1990) has pointed out the advantage of purposive sampling, which allows the researcher to home in on people or events, they believe, will be critical for the research. Instead of going for the typical instances, a cross-section or a balanced choice, the researcher will be able to concentrate on instances which display a wide variety. It also provided the possibility to focus on extreme cases, to illuminate the research question at hand.
Prior to start of the study, the following inclusion/exclusion criteria were taken into consideration:

a. Willingness of the School Management and Parents to participate in the study.

b. The School Timings include a short break.

c. No Lunch is provided by the school. The School has the facility of a makeshift canteen providing limited fare.

d. Lunch was carried as a dry lunch sent with the child in the morning or parents sent servants or dabbawala’s with hot lunch.

All children participated in the Nutrition Intervention Program, However for research purposes children were categorized based on the Mentzer Index and statistical analysis was performed for the group with Mentzer index >= 13.

The **Mentzer index** is used to differentiate iron deficiency anemia from beta thalassemia (Ntaios, et al., 2007) If a CBC indicates microcytic anemia, these are two of the most likely causes, making it necessary to distinguish between them. It is calculated from the results of a complete blood count. If the quotient of the mean corpuscular volume divided by the red blood cell count is less than 13, thalassemia is more likely. If the result is greater than 13, then iron-deficiency
anemia is more likely (Mazza, 2002). The quotient was characterized in by Mentzer in 1973.

A pilot nutrition intervention strategy was used in an initial study conducted in 2006-07. Where the children were initially screened for hematological evidence of anemia based on the hemoglobin status, which was followed by an interactive education program to motivate the parents to adopt better eating & hygiene habits. This was later followed by a second screening to prove to the parents that if they followed the advice given, the child will shift to a normal hemoglobin status. The duration between the first blood test and the second was 10 months.

In the Pilot Study conducted on 234 children (boys) of Saifi Preschool and Primary, the students were screened in July 2006, 96 anemic children were identified in Phase 1 (41%) whose Hemoglobin was <12gm%. In Phase 3 – April 2007 Blood screening was conducted on 89 children of the 96 identified with anemia.

In Phase 2, of the pilot study, which was from August‘06- March’07, a Nutrition Improvement Program was implemented, which included the following:

1. Three individual counseling sessions which was completed in a period of 6 months
2. Three Group Diet Counseling sessions which was completed in a period of 6 months.

3. One Large group General Awareness Talks to all Parents and Teachers on:
   a. The Need for Good Nutrition
   b. Health Assessment Awareness and Attitude
   c. Hygiene and Worm Infestation

The Main Study conducted at Saifi (Boys) High School included 471 Preschool and School children. The duration between the first blood test and the second was 6 months on the request of the school so as not to disturb the normal school schedule of examinations, co curricular and extracurricular activities., The categorization of the children was based on:-

Mentzer index which is a ratio of Mean Corpuscular volume and the total of red blood cell (in lac) (MCV/RBC). Based on such calculation, the total sample was categorized into 2 groups (N= 471).

**Group 1** Mentzer index <13, probable hemolytic anemia’s; n= 141 (29.93%) had Mentzer index <13 in Phase 1 and 3.

**Group 2** Mentzer index >=13, iron deficiency anemia; n=330 (70.06%) had Mentzer index >13 in Phase 1 and 3.
Statistical analysis was undertaken only for group 2 (N=330) However while enrolling individual age of 4 children was not recorded hence only 326 children were considered for the study of Hematological and Biochemical parameters. The total number of children varied for other parameters also because of non participation due to absenteeism while assessing and recording them.

The purpose of the follow up study was to implement the same Plan of Action of the nutrition intervention program in a mixed, upper middle class primary school population. Follow up study (2009-10) comprising of 94 children (Boys and Girls) from 1st to 4th standards from Udayachal (Godrej) Primary school was undertaken using the same protocol; the duration between the first blood test and the second was 6 months. The categorization of the children was based on:-

<table>
<thead>
<tr>
<th>MCV/RBC</th>
<th>Mentzer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;13</td>
<td>probable hemolytic anemia’s</td>
</tr>
<tr>
<td>n= 6</td>
<td>6.38% had Mentzer index &lt;13 in Phase 1 &amp; 3</td>
</tr>
<tr>
<td>&gt;=13</td>
<td>iron deficiency anemia</td>
</tr>
<tr>
<td>n=88</td>
<td>93.62% had Mentzer index &gt;=13 in Phase 1 &amp; 3</td>
</tr>
</tbody>
</table>

Based on the pilot study and Guidelines for Communications for a Behavioral Change on nutrition as has been derived from various articles and reviews
reported by Allen and Gillespie, in ACC/SCN, (2001) a Plan of Action was designed for the current study. This plan has been elaborated in Figure 2.1 & 2.2.

The Triple A” Process or cycle, pioneered by UNICEF in 1990 as described by Allen and Gillespie in ACC/SCN, (2001), is a participatory decision making process where in the problem is Assessed, its causes Analyzed, and along with the available resources and capacity to combat it, followed by a decision on appropriate mix of Actions. This process is cyclic and iterative in that once the actions have been initiated; they are subsequently monitored and evaluated. (Reassessment).Figure 2.1 Assessment, Action, & Analysis initiated in the pilot study, was continued during the present study.

Figure 2.1 the Triple A” Process adapted for pilot study.
Assessment of the children with reference to hematological, biochemical, anthropometric parameters was done in Phase 1 & 3 which was considered as the Before and After the Nutrition Intervention Program. The total duration of the study was only 6 months; this was on the request of the school, so it did not disturb the regular school exams and other activities.

Action based on the analysis and available resources in Phase 2 which was the Nutrition Intervention Phase (duration 4 months).

The Analysis of the hematological, biochemical, and dietary parameters was shared with the parents and the causes of the problems faced while implementing the nutrition programme were discussed on the basis of a face to face and also group interaction with the parents. The solutions arrived at were reviewed at subsequent meetings during the Nutrition intervention program. In Phase 4 a School Report was prepared and submitted to the school authorities.
Figure 2.2 CBC (Communication for Behavioral Change) outlines the guidelines elaborated in Chapter 1.

Taking this CBC guideline (Figure 2.2) into consideration the Nutrition Intervention Strategy for the main study design as depicted as a flow chart in Figure 2.3 was divided into 4 phases.
Figure 2.3 The Main Study Design.

- **Phase 1** only
  - Orientation and Motivational Talk to all parents of preschool & school children

- **Phase 1 & 3**
  - Blood screening was conducted for all preschool & school children
  - Clinical examination was followed by recording of anemia related physical ailments
  - Assessment of Anthropometric Status
  - Implementation of Attitudes and Practices Questionnaire (AP) on healthy eating and personal hygiene of the child and parents

- **Phase 3** only
  - Academic and Attendance data collection and recording

For research purposes only all the children were placed in 2 groups, based on their Mentzer Index, and statistical analysis was performed for the group with Mentzer index $\geq 13$. 
Phase 4
Statistical analyses & report writing.

Time Duration of the Main Study Design.

Phase 1 = 1 month (September 2008).
Phase 2 = 4 months (October 2008 - January 2009).
Phase 3 = 1 month (February 2009).
Phase 4 = 2 months (March-April 2009).
1. Orientation and Motivational Talk.

After seeking permission from the School trustees an orientation and motivational talk was organized for all parents, school teachers in the presence of trustees stressing the importance of good nutrition, the need for blood screening and the consequences of anemia in children with the help of power point presentation. This was conducted by the researcher, very good support was received from the trustees and school principal who emphasized the need for the good health for their children, they made a strong effort to convince all parents, to come and participate in this program and more important, make the changes in the food habits and lifestyle not only for their child but also for themselves also. An outline of the nutrition intervention program plan was explained and distributed to all parents. They were motivated to remain present for the various sessions.
2. Background Data and Registration Card.

This provided information about Name, Age, Occupation, and Gross family Income range of the parents of the child who was enrolled in the program, as well as address and phone numbers. This information helped to identify those parents who would fail to turn up for the sessions especially the individual counseling and small group sessions. Such parents were telephonically contacted and urged to attend and special extra sessions were organized for them.

The Parents had to carry the registration card for each session they attended and had to get it signed by the nutritionist besides signing on an attendance sheet given to them. This enabled the nutritionist to keep a track of all the parents.
3. Hematological Parameters Assessment.

Tools, Materials & Methodology, used in Blood screening.

Blood collection and investigation was conducted in Phase 1 and 3 by Medical Laboratory Technicians.

Collection of Venous Blood:

Collection volume:

2 ml. The most commonly used sites for venipuncture were the veins inside the bend of the elbow (the antecubital fossa).

 Technique:

Apply a tourniquet to the upper arm sufficiently tight to restrict the venous flow and make the veins stand out. The student was asked to keep the arm straight and clench the fist.

Usually the veins become obvious by this time. It is advisable to feel the veins so that the most suitable one can be selected. A little tapping or gently massaging the arm from the wrist to the elbow helps in dilation of the veins. Surface sterilize the
arm by swabbing the selected vein and site with 75% alcohol & allowing it to dry.

Prepare the syringe & appropriate containers. Usually a 21-gauge needle is appropriate for very fine veins, 22 or 23 gauge needles may be used only if necessary. Using the left thumb, press just below the puncture site to anchor the vein. Insert the needle smoothly with the bevel facing upwards, at angle of 20 to 30 degree to surface of the arm, and in a direct line with the vein. When the needle has entered the vein, blood is withdrawn into the syringe and tourniquet released. When a sufficient quantity of blood is collected, tourniquet is loosened and a wad of cotton wool at the puncture site was placed while withdrawing the needle gently. The puncture sight should be kept pressed to stop the flow of blood. The student was asked to release the clenched fist. The needle was detached and discarded in an appropriate disposal container. The blood was dispensed in the sample tubes as required. A strip dressing was applied at the puncture site.

Note: Disposable syringes & needles were used throughout the present study.
**Complete Blood Count:**

The blood samples were analyzed using a fully automated analyzer (MYTHIC 18) performing hematological analysis on whole blood collected in EDTA tubes.

Reference source: User’s Manual cod.M18/UM-EN/003
( http://www.C2diagnostics.com)

Name of the Model: MYTHIC 18

Instrument Make: Orphee

Sr. No: 100506-001120

Manufacturer: C2 Diagnostics

2214, Bd de la Lironde

F-34397 Montpellier cedex5, FRANCE

Sample volume 9.8µl

Throughput: 60samples/hour

18 analysis parameters

The Parameters considered for the study included under the title ‘complete blood count (CBC) were as follows:
**Erythrocyte Parameters.**

HGB     Hemoglobin
RBC     Red Blood Cells
PCV     Packed Cell Volume
MCV     Mean Corpuscular Volume
MCHC    Mean Corpuscular Hemoglobin Concentration
RDW     Red Cell Distribution Width

**Leucocyte Parameters.**

WBC     White Blood Cells

**Thrombocyte Parameters.**

PLT     Platelets

**Detection Principle: For Detection of WBC, RBC, PLT counting:**

The counting of the cellular elements in a blood sample is done with the impedancemetry technique. This technique is based on the modification of the
impedance of a calibrated aperture soaking in an electrolyte and going through a constant course delivered by two electrodes located on both sides of the aperture.

A vacuum applied on a side of the aperture allows the cells passage. They oppose their physical volume to the course passage. A voltage impulse is registered at the electrodes terminal. The height of this impulse is proportional to the cell volume.

Detection Principle for Hemoglobin measurement:

The hemoglobin measurement is directly done in the WBC chamber, by spectrophotometry at 555 nm. Hemoglobin is detected by formation of a chromogen cyanmethemoglobin type, for lytic solution with cyanide and oxyhemoglobin for lytic solution without cyanide.

A measurement of the blank of hemoglobin is done for each analytic cycle and during the start up rinsing step.

An automatic offset circuit for the LED 555 nm allows maintaining the blank level at the same range. It is not necessary to adjust this range with a potentiometer.
Detection Principle for Leucocytes Analysis:

The leukocyte analysis is done by impedancemetry in the WBC counting chamber, seven parameters are obtained such as WBC, Lymphocytes %, Lymphocytes number, Monocytes %, Monocytes number, Granulocytes %, Granulocytes number.

The formula approach is obtained by WBC distribution curve analysis after action of the lytic reagent. This reagent destroys the RBC, their stromas and acts on the cytoplasmic walls of white blood cell.

Detection Principle for Erythrocyte Analysis:

The erythrocyte analysis is done by impedancemetry in the RBC counting chamber and by analysis of the hemoglobin inside WBC chamber as previously described. Seven parameters are such as RBC, HGB, HCT, MCV, MCH, MCHC, and RDW, are estimated.

Detection Principle for Platelets Analysis:

Platelet analysis is carried out by impedancemetry in the RBC counting chamber at the same time with red blood cells.
4. Biochemical Parameter Assessment - Iron Studies included the following parameters.

**Determination of Serum Iron (SI) Total Iron Binding Capacity (TIBC) and % Iron Saturation** (source: Bichile, 1985)

Determination of Serum Iron (SI)

Total Iron Binding Capacity (TIBC)

% Saturation

**Reagents**

1. 0.2% 2-2 Dipyridyl in 3% glacial acetic acid V/V. Add 7.5ml glacial acetic to 250ml glass D/W ,add 0.5 gm dipyridylin to 250 ml of glacial acetic acid.

2. Sodium Sulphite (Na2so37H2O) Add 2.52gm of Na2So37h2Oin 100ml of glass D/W.

3. Ferric Chloride Solution (StockFeCl3) 145mg of ferric chloride in 100ml of 0.05 N HCL solution.

4. Working FeCl3 Solution. Dilute 1ml of stock solution to 100 ml with glass D/W.

5. Stock Standard and FeSO4 Solution Standard Solution containing 100 ug of iron /ml. Dissolve 0.498gm of ferrous sulphate 7H2O in D/W and add 1 ml of conc. H2SO4 and make it to 1 L
6. Working FeSO₄ Standard Solution – Take 3ml of Stock Solution; dilute it to 100 ml with D/W ie.3mg/ml.

**Procedure** - This method was scaled down and validated for 0.5ml serum sample

1. Take 2 ml of serum, Standard and Blank
2. Add 2ml of glass D/W to each
3. Add 1 ml of dipyridyl to each
4. Add 1ml of Sodium Sulphite to each
5. Mix well and heat it for 5 minutes in a boiling water bath.
6. Cool it and add 1ml of chloroform and mix well and centrifuge
7. Filter the supernatant through filter paper No. 1
8. Take the reading on Spectronic 20 at 520nm Wave length

**TIBC (Total Iron Binding Capacity)**

1. Take 2 ml of serum
2. Add 4ml of working Ferric Chloride Solution
3. Add 200 mg Mg- carbonate light powder
4. Mix well and Keep for half an hour, (Mix with interval of 5 minutes)
5. Centrifuge till clear supernatant is obtained
6. Take 4ml of clear supernatant
7. Add 1ml of Sodium Sulphite solution

8. Add 1 ml of dipyridyl

9. Mix well and heat it for 5 minutes in a boiling water bath.

10. Cool it and add 1ml of chloroform and mix well and centrifuge

11. Filter the supernatant through filter paper No. 1

12. Take the reading on Spectronic 20 at 520nm Wave length

**Calculation**

Serum Iron $\mu$g/dl = \frac{\text{OD of TEST}}{\text{OD of STD}} \times 300

TIBC $\mu$g/dl = \frac{\text{OD of TEST}}{\text{OD of STD}} \times 450

\%
Iron Saturation = \frac{\text{Serum Iron}}{\text{TIBC}} \times 100

5. Assessment of Nutritional Status by Anthropometry.
Anthropometry is the measurement of the human body. It is a quantitative method and is highly sensitive to nutritional status, especially among children.

Anthropometry was carried out as per protocol adapted from the following reference sources:-

National Center for Health Statistics (NCHS):-
http://www.cdc.gov/growthcharts/html_charts/wtage.htm (for children 3- 18 years);
(http://krpcds.org/report/Aravindan.pdf);

Type of measurements.

1. Weight.

Is an indicator of present nutritional status and is influenced by environmental factors, hence, while considering underweight; it is necessary to consider all these factors to attain a standard definition for underweight This parameter provides information with regard to total body mass, it is simple and widely used. It is sensitive to small changes in nutrition.

Technique:
1. Weight of a child was recorded using a bathroom scale, which was calibrated using standard weights.

2. Take a weighing scale. Keep it on a flat floor. Adjust to zero.

3. Ask the individual to remove any heavy clothing.

4. Ask the individual to stand bare feet at the center of the weighing scale with his hands on the side and head held straight.

5. Record the body weight to the nearest decimal.

2. Height.

Height is genetically determined, however it is environmentally influenced. Stunting reflects long duration of under nutrition. The subject is made to stand erect looking straight on a level surface with heels together and toes apart, without shoes. A height meter is used to measure height.

Technique:

1. Ask the individual to stand straight with bare feet on the floor against the wall.

2. The feet should be parallel with heels, buttocks, shoulders and back of the head touching the wall.

3. The head should be erect with eyes looking straight in front.
4. The flat horizontal portion of the Height meter is pulled downwards till it touched the top of the head.

5. Height was read to the nearest of 0.5 cm. An average of three measurements was taken as the final measurement.

**a. Height for Age:**

Low height-for-age index identifies past under nutrition or chronic malnutrition. It cannot measure short-term changes in malnutrition. For children below 2 years of age, the term is length-for-age; above 2 years of age, the index is referred to as height-for-age. Deficit in length-for-age or height-for-age is referred to as stunting.

**b. Body Mass Index for Age (BMI):**

Is calculated by the formula

\[ \text{BMI} = \frac{\text{Weight [in kilograms]}}{(\text{Height in meters})^2} \]

In children and teens, body mass index is used to assess underweight, overweight, and obesity. Children's body fatness changes over the years as they grow. Also, girls and boys differ in their body fatness as they mature. (Hammer, *et al.*, 1991; Pietrobelli, *et al.*, 1998). This is why BMI for children, also referred to as BMI-for-age, is gender and age specific. BMI-for-age is plotted on gender specific growth charts. These charts are used for children and teens 2 – 20 years of age.
c. Weight for Age:

Low weight-for-age index identifies the condition of being underweight, for a specific age. The advantage of this index is that it reflects both past (chronic) and/or present (acute) under nutrition, although it is unable to distinguish between the two.

A comparison of anthropometric data to reference standards was based on, the NCHS/WHO reference standards are available for children up to 18 years old along with, Indian standards published by Khadilkar, et al., (2009) (for 5 – 18 years).

References used to evaluate a child's measurement was carried out by comparing them with the percentile measure for children at the same age and sex. The percentile is the rank position of an individual on a given reference distribution, stated in terms of what percentage of the group the individual equals or exceeds.

The use of a cut-off enabled the different individual measurements to be converted into prevalence statistics. Cut-off is also used for identifying those children suffering from or at a higher risk of adverse outcomes. To assess stunting during adolescence, the indicator and cutoff are the same as used in early childhood, i.e., <3rd percentile of height-for-age. Body mass index (BMI) in relation to age, BMI (kg/m²) or BMI-for-age, was recommended as the best anthropometric indicator of thinness and overweight during adolescence. The recommended cutoffs are < 5th and ≥85th percentile for thinness and overweight, respectively. The Malnutrition Classification System used was Vishveshwara Rao
Classification (Table 2.1) for height for age, and Gomez system (Table 2.2) for weight for age percentiles of NCHS standards (Gopaldas and Seshadri, 1987).

Table 2.1 Vishveshwara Rao Classification. - Height for Age. Source : (Gopaldas and Seshadri, 1987).

<table>
<thead>
<tr>
<th>Nutritional Grade (% of NCHS Standards)</th>
<th>Nutritional Grade (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;80%</td>
<td>Poor</td>
</tr>
<tr>
<td>80-90%</td>
<td>Mild Retardation</td>
</tr>
<tr>
<td>100%</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Table 2.2 Gomez Classification - Weight for Age. Source: (Gopaldas and Seshadri, 1987).

<table>
<thead>
<tr>
<th>Nutritional Grade (% of NCHS Standards)</th>
<th>Nutritional Grade (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90%</td>
<td>Normal</td>
</tr>
<tr>
<td>75-89.9%</td>
<td>Grade I (Mild Under nutrition)</td>
</tr>
<tr>
<td>60-74.9%</td>
<td>Grade II (Moderate Under nutrition)</td>
</tr>
<tr>
<td>&lt;60%</td>
<td>Grade III (Severe Under nutrition)</td>
</tr>
</tbody>
</table>
6. Clinical examination & recording for anemia related physical ailments.

The anemia related physical ailments were examined in each child before and after the Nutrition Intervention Program and recorded by a panel of doctors. The data were reported in the following format (Table 2.3) which was specially designed for this research:

Table 2.3 Clinical Examination Format.

<table>
<thead>
<tr>
<th>Observation with regard to your Child’s General Health at Home</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of Child___________ Class______________ Code no.__________</td>
</tr>
<tr>
<td>a) Feels Weakness a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>b) Feels Fatigue a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>c) Feels Dizziness a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>d) Feels like Fainting a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>e) Feels Lethargic a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>f) Feels Breathlessness a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>g) Has Headaches a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>h) Has Body aches a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
<tr>
<td>i) Has leg pain a) Always b) Sometimes c) Rarely d) Never _______</td>
</tr>
</tbody>
</table>


7. Dietary and Nutrient Intake Assessment.

During the course of their development young children interact with their environment and learn culturally determined behaviors from constituents of their micro-environment, which include family’s belief systems, attitudes, traditions, and food likes and dislikes. It is well known that food behavior, nutritional status, growth and development are influenced by each other. These factors are synergistically modulated by the socio-economic factors that include the literacy status, income and occupation of parents/caregivers, demographic features of the home, access to quality foods and healthcare, exposure to newer information, and the resultant child care practices. Thus, attempts directed towards improvement of the consumption of nutritious foods by young children, requires multi pronged interventions based on strong formative research data that can be developed into specific behavior change strategies. It is therefore important that the conventional nutritional intervention efforts are examined critically and modified on the basis of the dynamics of socio-cultural realities and perceptions of communities. Community participation throughout the processes of planning, implementation and evaluation is necessary for the sustainability of community nutrition intervention.(ICMR Bulletin, 2003).