CHAPTER III
MATERIALS AND METHODS

The study was planned and the design of the research was formulated after reviewing a vast range of existing literature both national and international. Relevant literature was examined to look at how variables of interest were defined and measured, how the data were collected, and the possible ways adopted by eminent scholars of the field in analyzing the data. An effort was also made to discover not only what has been already done, but also what has not been done and what might be worth doing in the area of nutritional assessment of school children. The researcher has examined almost all the major studies and appropriate researches of consequence from the primary and secondary reports including Journals, scholarly texts, conference papers, textbooks, books, magazines, other mass media and Internet. Personal interactions with eminent scholars of the field were also done.

3.1 Locale of the Study

The present research study is carried out on school children aged 5 to 15 years in Aligarh city (Fig.1) of Uttar Pradesh. Uttar Pradesh is the most populous state of India with a population of over 150 million, 16 per cent of the country population. Aligarh is situated at a distance of 133 km of south east of Delhi. It comprises of 68.97 sq km and has a population of 6,58,165 (Census Department of Aligarh City Corporation, 2001). Aligarh is famous for its lock industries and Aligarh Muslim University. The population of Aligarh is of mixed nature representing people of different religion, occupation and socio-economic levels. Wheat and millets form the staple diet and the water sources for drinking and cooking purpose are from the suburbs water tables in wells and tube wells. The climate of Aligarh is of extreme nature very hot from April to August and very cold during December to February. It is well connected by roads and railways with its neighboring states. Data on the nutrition profile of this city with diverse geographical characteristic is virtually non-existent.
Fig. 1 Map of India indicating Aligarh City

Fig. 2 Location of Selected Schools in Aligarh City
The geographical boundaries of Aligarh district includes district Mathura in the Southwest, Mahamaya Nagar in South, Etah in Southeast, Badayun in Northeast, Bulandshahar in North and Faridabad (Haryana) in Northwest.

The major considerations for the selection of the locale are:

a) Studies have not been done to evaluate the nutritional status of school children in this region so far.
b) The city represents population of mixed nature comprising different socio-cultural and economic background.
c) Less variability in the dietary pattern, unlike in the metropolitan cities.
d) Easy accessibility and ease of approach.

3.2 Research Design

As per the information obtained from the office of the District Inspector of Schools, twenty-seven high schools were functioning in the city of Aligarh. The schools were selected for the purpose of the study by using stratified random sampling, by dividing the city of Aligarh into specific zones on geographical layout. Schools situated in these zones of the city catering to different income groups were identified. The schools from each zone were formerly contacted for seeking necessary permission from school authorities like principles, deputy principles, managers and directors. Based on the consent and active cooperation of the school authorities six schools catering to class I to X standard were selected to include in the study. (Fig: 2)

3.3 Population

The population comprised of the school children in the age group of 5 to 15 years. The reasons for the selection of this particular age group are as follows

1. This group forms a large percentage of the total population and thus provides high representativeness.
2. This group has very high vulnerability to nutritional deficiency diseases.
3. This is a period where in nutritional discrepancies can be remedied more easily than in later years.
4. It is easy to assess nutritional status and detect nutritional deficiency disease, at an early stage through clinical system and can be cured.
5. Early symptoms of nutritional deficiency are the first indication of the disease and if neglected is often the cause of several health problems in later life.

6. The need to evaluate the health needs of this group is currently felt as most studies have focused on preschool children and consequently health needs of this age group have been relatively neglected.

7. Due to their large proportion, children in school age group exercise a dominating influence on overall health status of population and highly deserve the priority attention of the health authorities.

3.3.1 Sample Selection

School children in the age group of 5 to 15 years studying in various classes ranging from class I to X standards in all the selected schools were included as population. Altogether there were 12898 children (Annexure-I A&B). Stratified sampling technique was used to select the sample from these school children, each stratum was identified as a class of the school and the common characteristic in each of the stratum was age of the student.

3.3.2 Criteria

The chronological age at the time of sampling was the criterion for inclusion in the sample. The age at the last birthday (i.e. age in complete year) was used for computation of data.

The other eligibility criteria for the study group were:

1. Apparent normal health of children.
2. Children in the age group of 5 to 15 years.
3. Who were resident of the city for at least five years.
4. Who do not have any chronic disease of any organ
5. Who have a regular attendance

3.3.3 Study Group

Using the above eligibility criteria a total number of 2582 eligible children (1218 boys and 1364 girls) were surveyed which is 20 per cent of the total population of selected schools. Children were interviewed based on pre-tested
questionnaire for age, sex, birth order, number of siblings, number of family members, type of family, religion, occupation of parents, family income, educational level of both the parents, dietary habits, medical history, school performance and general physical appearance. Anthropometric measurements were recorded with detail clinical examination for symptoms of various nutritional deficiency symptoms. Further in depth study was undertaken to identify the symptoms of Iodine deficiency. Clinical observation was done for each child to identify the symptoms of different grades of goitre according to Stanbury's classification (Annexure XI). The comprehensive data collected include enquires about type of salt consumed, brand of salt in case of iodized salt, source of water, type and frequency of goitrogenous food intake. Salt samples were also collected. From among the above sample of 2582 children, casual urine samples were collected from every fifth child included in the study irrespective of sex or thyroid size and analyzed for urinary Iodine excretion level using the standard laboratory method. Casual urine sample was collected from 517 children about 20 percent of the children studied. Iodine content of water and salt were also estimated.

3.4 Methodology

The present study was an intensive research to assess the overall nutritional status and the role of child family and socio-economic factors. It also examined the magnitude of nutritional diseases with special reference to iodine deficiency prevailing among school children aged 5 to 15 years. In order to get a realistic picture, the techniques adopted for obtaining data and information was intensive and time consuming. Multiple options were exercised to collect the required details from the subjects. It includes an oral questionnaire that comprised of an interview-cum physical examination, anthropometric measurements, and clinical observations. Urine sample were also collected for biochemical analysis. Standard procedure and techniques were employed for all investigations under taken.

The questionnaire was developed to collect the required information. It was prepared carefully in consultation with medical scientists and experts and
improved over several administrations. Each question was examined before finalizing for its relevance to the study and for its objectivity.

3.4.1 Pilot Study

Initial version of questionnaire and all the techniques for measurement of anthropometrical, clinical examination and biochemical test adopted were piloted on a set of five school children from all the age groups irrespective of gender under medical staff supervision. Consequently the content of the questionnaire was modified and minor changes were made in the method of gathering information on anthropometric measurements, clinical examinations and biochemical examinations. With these changes, tools were then used to study different sets of children and the results were found to be fairly satisfactory.

3.4.2 Data Collection

Examination/interviews based on the above pre-designed and pre-tested questionnaire (Annexure II) were carried out during the year 2001 to 2002. Contacts were established with all the selected schools for the study. Personal contact along with the permission and consent from the head of the institutions characterized the process of the rapport established and created no serious problem in data collection. The children of the study group were both physically examined and interviewed for family history background and other information in their respective schools. Care was taken to interview and examine the unit of class during games, C.C.A. or free periods. Timetables of all the classes section-wise were obtained from the authorities for easy approachability and to avoid any academic interruption to the particular class. Prior arrangements were also made with the teacher of that particular class unit before undertaking the interviews and the physical examinations in each class unit. The subjects under study group were assembled separately and were told about the purpose and objectives of the study. They were also explained about the procedures of the study. In all the schools children under study group were examined class wise and efforts were made to cover all the children of a particular class through repeated visits before shifting out to other class units. Each subject was called alone in a separate room, interviewed and examined personally by the investigator to avoid passing of
information to other members of the study group and to ensure privacy, as well as to avoid embracement situation to the respondent if asked in front of other children and to enable the child to give answer and respond freely to the investigator. All the work relating to data collection and physical examination were conducted by the investigator to maintain the uniformity. About 30 to 40 minutes were spent on each child for the physical examination during the school hours and for the children where urine samples were to be collected another about five minutes per child was taken. The process of data collection and physical examination was time intensive. But the efforts were worthwhile, as it secured reliability of data.

The questionnaire was based on issues having seven sections, they were:

1. General Personal information
2. Family background
3. School performance and general hygiene
4. Anthropometrical measurements
5. Clinical examination
6. Biochemical examination
7. Dietary information

While the first section dealt with serial number, date of study, school name, respondent’s name, class, gender, age, ordinal position, religion, place of residence etc. the second section deals with the complete family background to include name of the father, mother, number of sisters and brothers, their ordinal positions with age and date, parents educational level, their occupations (in case of mother whether working or house wife.) their income, (including the income of other family members, like brothers and sisters) total family income, per capita income, total family members, whether nuclear or extended family etc. in detail to ascertain the socio-economic status of the family.

The third section of the questionnaire includes information like child personal hygiene and general performance in the school etc. The child personal hygiene was assessed on the basis of cleanliness of hair, nails, eyes, ears, mouth and skin, nose, clothes, shoes and bath habits of the respondents. School performance was obtained from class attendance register and on the basis of
average percentage of total marks obtained in class examination. In this regard opinion of class teacher was sought.

The forth section deals with the anthropometric measurements like height, weight, mid arm circumference, skin fold thickness, which the researcher has to take for each child of the study group on the spot and noted down simultaneously on the space provided for it on the questionnaire.

The fifth section deals with in depth clinical examination of different parts of the body of the subjects for symptoms of various nutritional deficiencies including different levels of anemia and severity of iodine deficiency. The questions were set to include the symptoms for normal to various categories of particular nutritional deficiency disease. The various categories of symptoms found were noted down in the appropriate columns to compute later.

The sixth section of the questionnaire was of biochemical nature and involves taking of sample of urine for further analysis. The bottles for the urine sample collection were coded with the name of the child before sending it to the laboratories for analysis and the code number were noted down in the questionnaire of the particular child of the study group. The coded number of the questionnaire facilitate in writing down the results of the urinary iodine excretion level after analysis using standard laboratory methods for co-relating it with other variables. Salt samples were also collected, coded and analyzed and results noted down in the questionnaire in which code numbers for a particular sample were written already at the time of coding.

The seventh section elaborates on dietary habits (whether vegetarian or non-vegetarian), daily meal pattern with diet and intake of food items and their frequency with approximate quantity per day, type of salt consumed, brand of salt in case of iodised salt, type and frequency of goitrogen intake.

3.4.3 Filling up the Questionnaire

The questionnaire was prepared in English language and explained to the subject in Hindi also, to facilitate the subject’s response as per his choice either in Hindi or English. The questionnaire was given to the subjects and was asked to complete it in the presence of the researcher. It was never given away to the subject to filled in at any other time or left over night with the subjects. If the
subjects could not read or write either of the two languages, the researcher read out the questions and filled in the response given. The only exception was made in case of lower age groups subjects (subjects from 5 year to 9 years). Their responses in the questionnaires were filled in by the researcher irrespective of their reading abilities in order to maintain uniformity in the procedure with in that age group.

3.4.4 Measurement Procedure

Standard procedures and techniques were employed for all investigations undertaken to ensure the validity and reliability of the measurements, standardization exercises were carried out, before embarking up on the study.

Initial and base line information of the children under the study were collected on a pre-designed and pre-tested Performa.

3.4.5 Age and Other Variables

As mentioned earlier the chronological age at the time of sampling was the age criteria for inclusion in the sample. The age at last birthday i.e. age in completed years was used for computation of data. Hence 11 years refer to all those children, 11.00 through 11.99 years with an approximate mean of 11.50 year. Age was obtained from the child, identification card, and school register and was recorded in year and months: Attempts were made to verify the accuracy of age from birth certificates or school records. Ordinal position and number of siblings were recorded as per the information of the respondents.

Religion was recorded as per the information of the respondents and other available school records.

Information regarding education, occupation of the parents and financial sources of the family was collected from the respondents and verified with the teachers and when in doubt, data was even verified by visiting the respondent’s family. All sources of family incomes were taken into account to get per capita monthly income. The classification of income into various categories were determined according to modified B.G. Prasad’s classification with price index correction (Annexure-VI).
3.5 Anthropometrical Examination

For anthropometric measurements efforts were made to secure accuracy of high order by employing standardized equipment like weighing machine, and anthropometer, inch tapes, steal tape, fiber glass tape, calipers etc, to get information on all the anthropometric measurements. WHO recommends the use of vertical board with attached metric rule and a historical head bound for measurement of height and a leveled platform scale for the measurement of weight in adults. Standard techniques were used in respect of all the anthropometric measurements.

3.5.1 Weight

Weight was taken with the help of a Crown Victoria DX bathroom scale to the nearest of 0.5 kg reading. The instrument used was easily portable, sturdy, inexpensive and light to be carried for the field study. To ensure the accuracy, it was checked daily with standard weight and by taking repeated weight of the same individual. The subject was weighed preferably before the mid day meal in minimal clothing. The subject was asked to empty the bladder before weighing by a standard procedure as suggested by Jelliffe, (1966).

3.5.2 Height

Height was measured in centimeters nearest to 0.2 cm. The subject was asked to stand without shoes but with heels together and with the shoulder, buttocks, and heels, touching the scale. The subject looked straight and the head was held comfortably erect. The arms hung naturally on the sides. The subject was asked to take a deep breath, and stretch without raising the heels. The height was recorded when the anthropometer beam was brought down firmly on the vertex. In the case of girls it was necessary to remove any impediment like combs, ribbons, clips or bands etc.

3.5.3 Mid Upper Arm Circumference

The left mid-arm circumference was measured by a fiberglass tape with an accuracy of up to 1 mm (Jelliffe 1966). The subject was asked to stand or sit conformably with the arm relaxed at the side, with the fiberglass tape passed over
the arm at a point midway between the tip of elecranon process of ulna and the acromion process of the scapula.

3.5.4 Triceps Skin Fold Thickness

Subcutaneous fat can be measured easily by means of skin fold calipers (Guthrie, 1979; Robson, 1972). These instruments pinch a double thickness of skin and the intervening fat between two plates having a standard contact area and exerting a constant pressure. The Harpenden and Lange calipers are the two most commonly used. These instruments are capable of obtaining accurate, reproducible measurements of skin fold (fat thickness). Usually these are used over the back of the arm (triceps), the sub scapular region, and the abdomen region. Measurements from these sites can be used to estimate total body fat and the quantity of the calorie reserves. Skin folds are also being used as a measure of leanness and obesity.

The triceps skin fold thickness of each subject was recorded with the help of Harpender skin fold caliper. Readings were taken to the closet 0.2 mm with a least count of 1.00 mm. A vertical fold was raised midpoint of the triceps on the left side and its thickness was measured, with the arm slightly flexed at the elbow.

3.6 Grading Growth Status

There are several ways of combining weight and height data to construct indices of fatness (obesity) or thinness (under nutrition). The growth status of the children was assessed by expressing their weight and height as per ICMR (Annexure III) and NCHS (Annexure IV) standards and categorizing them into various grades of nutrition status according to Gomez classification. Low weight for age indicates levels of underweight and helps in instant monitoring of growth of children, where as height for age indices reflects long term nutritional status. Inadequate height for age indicates a chronic condition ‘stunting’. The third indices weight for height reflects ‘wasting’ an indicator of current nutritional state. Such combination of indices could be used to assess whether the child is fatter or thinner than is usual for a child of that sex, age and background. These were calculated separately for boys and girls for each year of age as the reference standards vary by age and sex. For assessing the nutritional status of Aligarh
children one of the most useful method that is the Gomez classification (India Nutritional Profile, 1998) was adopted along with equally famous Waterlow’s classification.

The children were grouped into different grades of nutrition status by both Gomez’s and Waterlow’s classifications (Annexure - V). Children were assigned to normal, grade I, grade II and grade III group of malnutrition using Gomez’s classification and normal, mild, moderate and severe stunted and wasted using Waterlow’s classification. In the present study children were considered to be normal if they were more than 90 per cent of normal standard, suffering from grade I malnutrition (mild) if they show 75-89 per cent of the normal standard and grade II malnutrition (moderate) if they show 60-74 per cent and grade III malnutrition (severe) if they were below 60 per cent of normal standard after calculations. Using height for age as the indicator, children were classified as being normal if they are at 95 per cent or above, mildly stunted (87.5-95 per cent) moderately stunted (80-87.5 per cent) and severely stunted (below 80 per cent) of standards. For weight for height as indicator, children were classified as normal, if they were more than 90 per cent of the standards, mildly wasted (80-90 per cent) moderately wasted (70-80 per cent) and severely wasted (below 70 per cent) of standards.

3.7 Dietary Assessment

Diet survey was conducted to elicit information regarding the diet and food habits of children. Every subject was questioned in detail about his or her daily food intake employing three-day recall method. The subjects were enquired about their dietary pattern, frequency of consumption of food to confirm about the changes in dietary habits. It gives almost a true representation of the usual intake of food over a period of time. It also minimizes the problems of intra-variability found in the 24-hour recall method. The method chosen overcome the problem of memory flogging found in 7 days recall. In order to help in quantifying the amount of food consumed, the respondents were emphasized to express the consumption in terms of katories (bowls) size (large A, medium B, small C) and of chapatti size (large A, Medium B and small C) which were based on pre-standard cups, katories (bowls), and card board cutouts. To ensure the
reproducibility and reliability of the measurement standardization exercises were carried out (Annexure X-A&B), before embarking on the study itself. This helped in quantifying the portion of food consumed by the subjects. In the present study mean daily intake of different food groups and food nutrients were examined in different age groups 5 to 6, 7 to 9, 10 to 12 and 13 to 15 years. According to Indian Council of Medical Research (1985; 1989) recommended daily allowances (Annexure – VIII and IX), the daily requirement of food groups as well as food nutrient were different for these age groups. So to ascertain the exact figures of intake of food groups and food nutrients were computed age group wise as per the standards determined by ICMR for both boys and girls separately.

The reasons for not adopting other methods are briefly mentioned below:

**Weighment method:** Though one of the most accurate methods, it could not be selected as it is expensive and difficult to use in the field. Beside, Todd et al (1983) found it to be difficult to use with children as subjects expressed a dislike to weigh foods.

**24-hour recall:** This method has been widely used in cross-cultural epidemiological studies, Block, (1982) and Beaton, et al (1983) have pointed out the shortcomings of single day diet. One-day recall may not be representative of the used intake since individual diets vary greatly from day to day. The method is found less accurate. Studies conducted by this method have not revealed significant relationship between nutrient intake and the disease variability of nutrient intake, within individuals, which is not reflected by the one day method could be reasonable for this observation.

**7-day recall:** the method is similar to the 24-hour recall. It gives a better representativeness than the 24-hour recall but its accuracy is lowered due to memory flogging.

**Diet history:** The method measures about 50 per cent nutrients reasonability. It can be used for usual intake of food groups, but not for nutrients consumed (Beaten et al. 1983).

In view of the above shortcomings, the 3-day recall method was considered the most feasible combination methods to be adopted. The 3 day recall method with quantifying pre-standard weights and measurements, it was felt, would be reasonably accurate, and would give a reasonable meaning, besides
being practical to use and inexpensive, since the method was to be used in young, literate motivated children, problems of poor recording and co-operation were also not envisaged. Persson (1984) and Beaton et al (1983) after reviewing methodologies for collection of dietary data has also concluded that there is no ideal method but a preferred method to suit the purpose of dietary assessment must be used.

3.8 Clinical Examination

Detailed clinical examinations of the subject were carried out. Clinical signs suggesting various nutrient deficiencies were examined by a standard procedure documented by Jelliffe (1966). The children were examined particularly for conjunctival xeroxis, keratomelacia, angular stomatitis, glossitis, dental caries, anemia, pallor and Iodine deficiency disease. Clinical history was taken for prevalence of IDD, which include any swelling in front of neck, features of hypothyroidism like weight gain, increased intolerance to cold, hoarseness of voice, menstrual irregularities, puffiness of face, hair loss, lethargy, easy fatigability, retarded growth and development.

A detailed clinical examination was done for prevalence of different stage of goitre. Grading of goitre was done according to stanbury's classification (Annexure-XI). The examination of goitre was done according to ICMR (1989) recommendation. The subject was asked to start walking towards the examiners to have a look at his/her gait, body proportion and the enlarged gland if any i.e. Grade III and IV.

If goitre was not visible, the patient was asked to sit and face the examiner at a close distance with the front of the neck well exposed to light. If the presence of goitre was detected straight away with the neck in normal position it was grade II. If goitre was not detected by this method, the subject was requested to extend his/her neck and asked to swallow that helped to note the visible enlargement of the thyroid gland i.e. Grade I. The examiner then palpates the thyroid gland with the subject turning his back to the examiner, either in a standing or a sitting position. The palpitation of the thyroid gland confirmed and supplements the result of inspection. The consistency and modularity of the gland were assessed by the four fingers with hand along with the trachea.
3.9 Biochemical Analyses

The casual urine samples were collected in wide mouthed screw capped glass bottles, rinsed with deionized benzine treated water (one drop of toluene was added to each sample to inhibit bacterial growth and to minimize bad odour). Bottles were coded to facilitate identification of sample with subject. Urinary iodine was estimated by standard laboratory method. First of all standard graph was drawn. After that 100 µ liter of urine sample was centrifuged at 2500 rpm/minute for 20 minutes. 2.65 ml of double distilled water along with 0.25 ml arsenious acid was added in the tube. After that 0.5 ml ceric ammonium sulphate was added in the tube. The tube was incubated at 40° C water bath for 30 minutes. 0.1 ml of 0.5% ‘O’ phenyldiamine was added. The tube was thoroughly mixed and kept for 15 minutes at room temperature. Reading by colorimetric at 470 nm against double distilled water was taken. A blank tube and a standard tube were put to check the reading. Actual reading was calculated by subtracting test reading from blank reading. The urinary iodine level of ≥ 10 µg/dl was taken as normal (Annexure XII). The estimation was done in the laboratory in the department of Preventive and Community Medicine, J.N.M.C. A.M.U. Aligarh.

In case of water iodine estimation, water sample were drawn from different localities of the city and laboratory analysis was made to determine the iodine content in the water. Iodine content of water was low if it was < 5 µg/l.

Iodine content of salt was estimated by Spot testing kit (STK) method. The spot testing kit contained vials with chemical solutions. One drop of chemical solution turned a salt sample light blue to violet in colour depending upon the content of iodine in the salt sample. The colour was compared with the colour given on strip of spot testing kit, indicating level of iodine concentration.

3.10 Analysis of Data

All the data obtained through questionnaire, observation and biochemical analysis were coded and entered into computer taking into consideration all the parameters of the relevance and importance of the study. Mean, standard deviation and percentiles were calculated for various anthropometric parameters
for each age group for boys and girls separately as well as combined for analysis and comparison. The mean and percentile for various parameters were utilized for analyzing the relationship with nutritional status and for comparison with other standard percentiles and studies. Normal ‘Z’ tests and student ‘t’ test were used for comparing observed mean for various anthropometric parameters with those of national, international and other studies. The Gomez classification and Waterlow classification for distribution of nutritional status was calculated from measured weight and height to classify children into two groups i.e., normal and Grade I + Grade II + Grade III category of malnutrition. Chi-square test was applied to find out the differences in the distribution of various parameters among different age groups and sex and their intra and inter relationships. The child, family and social factors associated with better nutritional status, higher prevalence of malnutrition and nutritional deficiency of children using chi-square test are depicted as probability levels from P< 0.001 to P< 0.05 indicating positive or negative association. Step wise multiple regressions were utilized to yield a combination of factors with greatest explanatory power predicting nutritional status. All the analysis was done using Statistical Package for Social Sciences.