Chapter 4

General Methodology
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4.1. Study population

A total of 1078 (535 boys and 543 girls) Santal children aged 5-12 years were examined in this cross-sectional study. Children were recruited from primary and secondary schools of Balarampur and Bagmundi areas of the Purulia district of West Bengal (Figure 4.1). Subjects were recruited by announcement and data were collected after obtaining informed consent from children, parents and school authorities. Children with disability, any systemic disease or with major surgical operations of neurological, sensory or motor problems that could influence their measurements were excluded from participating in the study. The protocol and procedures employed were in accordance with the human ethical guidelines of Helsinki Declaration (Touitou et al. 2004).

4.2. Socio-economic status

Socio-economic status (SES) of Santal children was measured using the updated Kuppusswami scale (Kumar et al. 2007). The scale is based on monthly family income, parental education and parental occupation. Structured questionnaires were used to collect information on socioeconomic characteristics of subjects’ families from their parents and/or school authorities. The scoring system considering above three criteria is given in Table 4.1.

4.3. Anthropometries

The anthropometric measurements like height, weight and circumference of mid-upper arm (MUAC), head (HC), mid-calf (MCC), and skinfold thickness of triceps (TRSF), biceps (BCSF), suprailliac (SPSF), subscapula (SBSF) and calf (CFSF) of each subject were measured using standard technique (Lee & Nieman, 2007). Body mass index (BMI) was calculated as the ratio of [weight (kg)/ height (m²)]. Skinfold thickness was measured using the Harpenden skinfold caliper (Galaxi Informatics, India) with a constant spring pressure of 10 g/mm² on the right side of the body. Mean of the three reading in single location was accepted.
Figure 4.1. Location of surveyed regions of Purulia district in the present study.
Table 4-1. Updated Kuppuswamy’s socioeconomic status scale.

(A) Education

<table>
<thead>
<tr>
<th>Education</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Professional or Honours</td>
<td>7</td>
</tr>
<tr>
<td>2. Graduate or Post-graduate</td>
<td>6</td>
</tr>
<tr>
<td>3. Intermediate or Post-high school diploma</td>
<td>5</td>
</tr>
<tr>
<td>4. High school certificate</td>
<td>4</td>
</tr>
<tr>
<td>5. Middle school certificate</td>
<td>3</td>
</tr>
<tr>
<td>6. Primary school or literate</td>
<td>2</td>
</tr>
<tr>
<td>7. Illiterate</td>
<td>1</td>
</tr>
</tbody>
</table>

(B) Occupation

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Profession</td>
<td>10</td>
</tr>
<tr>
<td>2. Semi-profession</td>
<td>6</td>
</tr>
<tr>
<td>3. Clerical, Shop owner, Farmer</td>
<td>5</td>
</tr>
<tr>
<td>4. Skilled worker</td>
<td>4</td>
</tr>
<tr>
<td>5. Semiskilled worker</td>
<td>3</td>
</tr>
<tr>
<td>6. Unskilled worker</td>
<td>2</td>
</tr>
<tr>
<td>7. Unemployed</td>
<td>1</td>
</tr>
</tbody>
</table>

(C) Family income per month for 2007 (Rs.)

<table>
<thead>
<tr>
<th>Income Range</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 19575</td>
<td>12</td>
</tr>
<tr>
<td>9788 – 19574</td>
<td>10</td>
</tr>
<tr>
<td>7323 – 9787</td>
<td>6</td>
</tr>
<tr>
<td>4894 – 7322</td>
<td>4</td>
</tr>
<tr>
<td>2936 – 4893</td>
<td>3</td>
</tr>
<tr>
<td>980 – 2935</td>
<td>2</td>
</tr>
<tr>
<td>&lt; 979</td>
<td>1</td>
</tr>
</tbody>
</table>

Total score

<table>
<thead>
<tr>
<th>Total score</th>
<th>Socioeconomic scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 – 29</td>
<td>Upper (I)</td>
</tr>
<tr>
<td>16 – 25</td>
<td>Upper-middle (II)</td>
</tr>
<tr>
<td>11 – 15 (Middle)</td>
<td>Lower-middle (III)</td>
</tr>
<tr>
<td>5 – 10 (Lower)</td>
<td>Upper-lower (IV)</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>Lower (V)</td>
</tr>
</tbody>
</table>

4.3.1. Standing height

Height was measured with the anthropometer. The subjects were asked to stand with heels together, arms to the side, legs straight, shoulder relaxed, and head in the Frankfort-horizontal plane. The subjects were barefooted and were in minimal clothing to facilitate correct positioning of the body. Heels, buttocks, scapulae, and back of the head were against the vertical surface of the anthropometric rod. Just before the measurement was taken, the subjects inhaled deeply, held the breath, and maintained the erect posture while the scale was lowered on the
highest point of the head with enough pressure to compress the hair. The measurement was taken to the nearest 0.1 cm and with the eye level with the scale to avoid errors caused by parallax.

4.3.2. Weight

The subjects were asked to stand in the middle of the weighing machine without touching anything and with the body weight equally distributed on both feet. The reading was taken to the nearest 100 gm and recorded.

4.3.3. Mid-upper arm circumference (MUAC)

A flexible, nonstretchable measuring tape was used for MUAC measurement. The measurement was taken to the 0.1 cm at the mid-point of the upper arm between the acromion process and the tip of the olecranon.

4.3.4. Head circumference (HC)

A flexible, nonstretchable measuring tape was used for HC measurement. The lower edge of the tape was positioned just above the eyebrows, above the ears, and around the back of the head, so that maximum circumference was measured. The reading was taken to the nearest 0.1 cm and recorded.

4.3.5. Mid-calf circumference (MCC)

A flexible, nonstretchable measuring tape was used for HC measurement. The subjects were in standing position and the weight spread evenly on both feet. The tape was passed around the calf in a plane perpendicular to the long axis of the limb, the maximum circumference was judged by the profile of the gastrocnemius.

4.3.6. Triceps skinfold (TRSF)

The subject’s arm hanged loosely at the side, with the palm of the hand facing anteriorly to properly determine the posterior midline. The skinfold site was marked along the posterior midline of the right upper arm, over the triceps muscle, midway between the lateral projection of
the acromion process of the scapula and the inferior margin of the olecranon process of the ulna. The measurer stood behind the subject, grasped the skinfold with the thumb and index finger of the measurer’s left hand about 1 cm proximal to the skinfold site, the caliper was kept perpendicular to the long axis of the skinfold and recorded.

4.3.7. Subscapular skinfold (SBSF)

The subscapular site of each subject was marked 1 cm below the lowest angle of the scapula. The site was located by gently feeling for the inferior angle of the scapula. The skinfold was measured with the subject standing with arms relaxed to the sides. The skin was grasped 1 cm, above and medial to the site along the axis.

4.3.8. Suprailiac skinfold (SPSF)

This skinfold was measured just above the iliac crest at the midaxillary line. The subject stood erect with feet together and arms hanging by the sides. The measurer grasped the skinfold about 1 cm posterior to the midaxillary line and the reading was recorded.

4.3.9. Medial calf skinfold (MCS)

The measurement was taken with the subject sitting, the right leg was flexed about 90 degrees at the knee with the sole of the foot flat on the floor. The point of the maximum calf circumference was marked at the medial aspect of the calf. A vertical skinfold grasped about 1 cm proximal to the marked site and the reading was recorded.

4.3.10. Biceps skinfold (BCSF)

The subjects faced the measurer with the arm held relaxed at the side and the palm was facing forward. The skinfold was picked up over the belly of the biceps and 1 cm above the line marked for MUAC and TRSF on a vertical line, joining the center of the antecubital fossa to the head of the humerus. The caliper jaws were applied at the marked level and the skinfold reading was recorded.
4.4. **Upper arm anthropometry**

Upper arm anthropometric measurements were based on the MUAC and TRSF (Frisancho 1990).

**4.4.1. Upper arm muscle circumference (UAMC), Upper arm muscle area (UAMA) and Upper arm fat area (UAFA)**

Upper arm muscle circumference, upper arm muscle area and upper arm fat area were calculated from the mid-upper arm circumference and triceps skinfold using the following formulas (Frisancho 1990):

\[
\text{UAMC} = \text{MUAC} - (\pi \times \text{TRSF})
\]

\[
\text{UAMA} = \left[\text{MUAC} - (\text{TRSF} \times \pi)\right]^2 / 4\pi
\]

\[
\text{UAFA} = \text{TUA} - \text{UAMA}
\]

4.5. **Body fat measurements**

**4.5.1. Percentage of body fat (BF %)**

The BF% of Santal children was estimated from skinfold thickness, the formulae developed by Slaughter et al. (1988) have been used. This equation uses the sum of TRSF and SBSF to predict the body fat:

For boys (BF %) = 1.21 (TRSF + SBSF) - 0.008 (TRSF + SBSF)^2 - 3.2

For girls (BF %) = 1.33 (TRSF + SBSF) - 0.013 (TRSF + SBSF)^2 - 2.5.

**4.5.2. Fat mass (FM) and fat-free mass (FFM)**

FM was derived from (BF% / 100) × Body weight (kg). FFM was calculated by subtracting FM from weight (Slaughter et al. 1988).

**4.5.3. Fat mass index (FMI) and fat-free mass index (FFMI)**

FM and FFM were divided by height-squared in meter to produce the FMI and FFMI respectively (Slaughter et al. 1988).
4.6. **Measurement of Nutritional status**

The chronic undernutrition such as stunting and wasting and the acute undernutrition such as underweight were calculated by Z-score using the age-specific reference values of height-for-age, weight-for-height and weight-for-age of World Health Organization (WHO 1983). The grade of undernutrition was assessed according to the classification of World Health Organization (WHO 1995). Children with a Z-score between -1 and -1.99 of height-for-age were considered to be mild undernourished, children with a Z-score below -2 and -2.99 of height-for-age were considered to be moderate undernourished and children with a Z-score below -3 were considered to be severely undernourished.

4.7. **Measurement of food intake**

For assessment of dietary intake, raw food weighment method was employed. The housewife or the elderly woman who cooked and served the food in the family was the respondent. The consumption of different foods of the family per day was calculated from the collected data. The quantity of different nutrients present in the raw foods consumed by the whole family per day was calculated by using the food consumption table from ‘Nutritive values of Indian foods’ (Gopalan et al. 1993). The ACU (adult consumption unit) of each family was calculated and the nutrients intake per ACU was computed. The intake of nutrients by Santal boys and girls according to age groups of 5-6 years, 7-9 years and 10-12 years were then computed by multiplying the age-specific ACU of children with the consumed amount of nutrients per ACU in that family. The consumed values of nutrients were compared with that of the recommended daily allowances (ICMR 2011).

The amount of nine food groups (cereals, pulses, green leafy vegetables, roots & tubers, other vegetables, flesh foods, milk and milk products, fats & oils, and sugar & jaggery) consumed by the whole family per day was calculated from the collected data of raw foods. The
quantity of different food groups per ACU (adult consumption unit) consumed by the whole family was then computed. The quality of different food groups consumed by Santal children of different age groups (5-6 years, 7-9 years and 10-12 years) was then derived by multiplying the age-specific ACU value of children with the consumed amount of food groups per ACU in that family.

4.7.1. Protein-calorie adequacy status

The categorization of children into protein and calorie adequacy and inadequacy was undertaken according to the method suggested by Indian Council of Medical Research Expert Committee (ICMR 1990). For defining the protein-energy adequacy status, 70% of requirements were used as cut-off points. Hence, an individual was considered to be consuming adequate calories or protein if the intakes were equal to or greater than 70% of the RDA for the corresponding age, sex and activity. An individual was considered to be consuming inadequate calories or proteins when the intakes were less than 70% of the RDA.

4.8. Hematological and Biochemical measurements

About 5ml of venous blood was obtained from each child for hematological and biochemical tests, following a 12 hour overnight fast. EDTA containing vacutainers were used for the collection of whole blood samples, from which 2 ml blood was taken for hematological examination. The remaining 3 ml blood was collected in vacutainers with no added anticoagulant and kept at room temperature for 30 minutes for serum separation. After separation of serum by centrifugation, 1ml aliquot was pipetted into plastic eppendorf tubes and stored at -80° C for biochemical assessments.

4.8.1. TC of RBC, PCV and Hb concentration

Total count of RBC was measured with the help of Neubaur chamber and packed cell volume (PCV) was calculated by the following technique (Jacobs & Wood 2003). Hemoglobin
concentration was determined by Cyanmethaemoglobin method (Dacie & Lewis 1991). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by the following formulae:

\[
\text{MCV (cubic µ)} = \frac{[\text{PCV} \times 10]}{\text{RBC in millions}}
\]

\[
\text{MCH (microgram)} = \frac{[\text{Hb} \times 10]}{\text{RBC in millions}}
\]

\[
\text{MCHC (％)} = \frac{[\text{Hb} \times 100]}{\text{PCV}}
\]

4.8.2. Serum iron and total iron binding capacity (TIBC)

Serum iron and TIBC were measured using Iron & TIBC kit (Crest Biosystems: IRT010 & IRT011, Germany) by Ferrozine method (Siedel et al. 1984) in fully automated analyzer (BS – 300; MINDRY, USA).

4.8.3. Serum ferritin, serum transferrin and transferrin saturation

Serum ferritin was measured using DiaMetra kit (Ref. DK0039, Italy) by immunoenzymatic determination (Ronald 1983) in fully automated analyzer (Alpha Prime; manufactured by SFRI Laboratories, France). Transferrin value was determined indirectly by multiplying TIBC with 0.7 (Johnson 2006). Transferrin saturation was calculated by dividing serum iron with TIBC and multiplied by 100 (Lee & Nieman 2007).

4.8.4. Stages of iron depletion

Stages of iron depletion were determined according to the method described by Lee and Nieman (2007) (Lee & Nieman 2007). The stage I of iron depletion, referred to as iron deficient stores, was recognized by a serum ferritin level below 12 µg/ L. The stage II of iron depletion, referred to as iron deficiency erythropoiesis, was recognized by serum ferritin level below 12 µg/ L and transferrin saturation level below 15%. The stage III of iron depletion, referred to as iron deficiency anemia, was recognized by serum ferritin level below 12 µg/L, transferrin saturation level below 15%, and hemoglobin level below 12 g/dl.
4.9. Test for Cognitive development

Raven's Coloured Progressive Matrices (RCPM) was selected for test of general intelligence. RCPM was a non-verbal test and measure the ability to reason and solve the problems. The test was selected for its non-reliance on language, worldwide acceptability and easy to use. The test was administered followed according to the RCPM manual (Raven et al. 1998). Instructions were explained to the children, and one practice question was administered to ensure understanding. The test is made up of 3 sets of 12 visual problems. The subject was shown a visual pattern with a missing section and was required to select 1 of 6 alternative sections to complete the overall pattern. As the test progressed, the problems became progressively more difficult. The test was not timed, and the subject continued until satisfied with the choice made. The score was the number of correct items selected.

4.10. Test for motor development

The Bruininks-Oseretsky Test of Motor Proficiency-Second Edition-Short Form (BOT-2) was used to assess children's motor ability. The BOT-2 is a standardized assessment of motor skill achievement commonly used in the assessment of motor abilities in children (Bruininks & Bruininks 2005). The short form has been validated against the full scale and consists of 14 items taken from the 8 subtests. The 8 subtests assessed gross motor development, including running speed and agility, balance, bilateral coordination, and strength; gross and fine motor development, including upper limb coordination; and fine motor development, including response speed, visual-motor control, and upper-limb speed and dexterity. A total standard score, adjusted for child age and sex, was used to interpret test performance.

4.11. Statistical analyses

Descriptive statistics were used to compute the mean, median and standard error of mean (SEM) for different parameters by age and sex. Student's t-test or one-way analysis of variance
(ANOVA) was applied to compare the data between or among the groups. Post hoc comparisons were made using LSD or Bonferroni test, with alpha set as 0.05. Separate ANCOVA were also used to test the effect of nutritional status on the total standardized score and on each of the fourteen BOT-2 short form items, adjusting for age and sex. Chi-square test was performed to establish the association between different parameters. Pearson’s correlation coefficient was used to evaluate the relationship between the anthropometric parameters as well as between anthropometric and other parameters. Stepwise forward regression was employed to find out the association of nutritional and socioeconomic status with other motor or biochemical parameters. The statistical analyses were performed using statistical package for social science (SPSS software, Version 10.0.).