Chapter 10

UNDERNUTRITION IN SANTAL CHILDREN: A BIOCHEMICAL AND HEMATOLOGICAL STUDY
10.1. INTRODUCTION

The most objective and quantitative data of nutritional status is provided by biochemical and hematological tests. It has been reported that nutritional insufficiency is first indicated by biochemical changes followed by impairments in cells or organs and finally clinical malnutrition is established (Lee & Nieman 2007). A number of parameters such as RBC count, hemoglobin concentration (Hb%) and packed cell volume are generally used as early indicators for describing different types of nutritional anemias (Lee & Nieman 2007, Anumundu et al. 2008). Measurement of iron status has been opined as a stronger biochemical tool for nutritional assessment. It is used to detect the nutritional anemia, most prevalent undernutritional problem among worldwide preschool and school aged children (Anumundu et al. 2008, Dallman & Reeves 1984). Most commonly the serum ferritin, total iron binding capacity (TIBC), serum transferrin and transferrin saturation (TS) have been assessed as confirmatory tests for diagnosis of iron-deficiency and iron-deficiency anemia (Milman 1996, Monkeberg et al. 1962). In addition, serum albumin has been used as an important parameter for identifying protein nutritional status (Yoder et al. 1987). Relationship between undernutrition and above mentioned biochemical parameters has been reported in children of general communities (Awasthi et al. 2003). In this regard, the data from tribal community is scanty and scarce. Except a small study (Rao & Vijay 2006), the nutritional status of Santal children has not been assessed by using biochemical and hematological parameters. Rao & Vijay. (2006) reported that anemia was found to be a significant health problem among Oraon, Santal and Munda children in Purnia district of Bihar (Rao & Vijay 2006). Therefore, the purpose of the study presented in the present chapter is to evaluate the cause of the undernutrition of surveyed Santal children by measuring some hematological and biochemical parameters.
Chapter 10: Biochemical & Hematological study

10.2. METHODS

10.2.1. Hematological and Biochemical measurements

About 5 ml 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, 1 ml aliquot was pipetted into plastic eppendorf tubes and stored at -80°C for biochemical assessments.

10.2.1.1. 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:

\[ MCV = \left( \frac{PCV \times 10}{RBC \text{ in millions}} \right) \text{ (cubic } \mu \text{)} \]
\[ MCH = \left( \frac{Hb \times 10}{RBC \text{ in millions}} \right) \text{ (microgram) } \]
\[ MCHC = \left( \frac{Hb \times 100}{PCV} \right) \% \]

10.2.1.2. 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. 1994) in fully automated analyzer (BS-300; MINDRY, USA).
10.2.1.3. Serum ferritin, serum transferrin and transferrin saturation

Serum ferritin was measured using DiaMetra kit (DK0039, Italy) by immunoenzymatic determination (Ronald et al. 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).

10.2.2. Stages of iron depletion

Stages of iron depletion were determined according to the method described by Lee and 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.

10.2.3. Statistical analysis

Descriptive statistics of hematological and biochemical parameters were expressed as mean, standard error of mean (SEM). One-way analysis of variance (ANOVA) was conducted to test for differences in biochemical and hematological parameters between normal stunted and stunted groups. Post-hoc comparisons were made using Bonferroni test, with alpha set as 0.05. Student’s t-test was performed separately to find out the difference in serum albumin between normal stunted (> -1 z-score) and stunted (< -1 z-score) children. Multiple forward regression analysis was used to examine the association between nutritional status (measured by height-for-age z-score) and different biochemical parameters using five incremental models, where the nutritional status was considered as the outcome variable. Pearson’s correlation was performed
to evaluate the relationship among biochemical and hematological parameters, and between socioeconomic parameters and biochemical parameters. The statistical analyses were performed using the SPSS 10.0 statistical software package for Windows.

10.3. RESULTS

10.3.1. Hematological parameters

Hemoglobin, PCV, MCV, and MCH of severe stunted children are significantly lower (p<0.001) than that of normal statured children. Values of MCHC are significantly lower (p<0.01) in moderate and severe grades of stunted girls than that of normal statured girls. RBC count and PCV are significantly higher (p<0.05) in boys than that of girls. In contrast, MCV and MCH are significantly higher (p<0.05) in girls than that of boys (Table 10.1).

10.3.2. Biochemical parameters

Mean values of serum iron, serum ferritin and TS of stunted children are significantly lower (p<0.05) than that of normal statured children. In contrast, mean values of TIBC and transferrin are significantly higher (p<0.05) in stunted children than that of normal statured children. There is no significant difference in measured biochemical parameters between the sexes (Table 10.2).

10.3.3. Stages of iron depletion

About 18.82%, 4.71% and 5.88% of Santal children are suffering from stage-I, stage-II and stage-III of iron depletion, respectively (Table 10.3).

10.3.4. Serum albumin

The serum albumin level of moderate and severe grades of stunted children is significantly lower (p<0.05) than that of normal statured children (Figure 10.1).
Table 10.1. Total count of RBC, hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in normal and different grades of stunted Santal children. (n = Number of subject, Value: Mean ± SE, * p<0.05  ** p<0.01  ***p<0.001)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total (n=45)</th>
<th>Normal (n=10)</th>
<th>Mild (n=10)</th>
<th>Moderate (n=15)</th>
<th>Severe (n=10)</th>
<th>Girls (n=40)</th>
<th>Normal (n=10)</th>
<th>Mild (n=10)</th>
<th>Moderate (n=10)</th>
<th>Severe (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (million/mm³)</td>
<td>4.63 ± 0.11</td>
<td>4.78 ± 0.11</td>
<td>4.73 ± 0.11</td>
<td>4.45 ± 0.11</td>
<td>4.56 ± 0.12</td>
<td>4.12 ± 0.09</td>
<td>4.14 ± 0.13</td>
<td>4.13 ± 0.12</td>
<td>4.15 ± 0.12</td>
<td>4.08 ± 0.12</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>12.26 ± 0.31</td>
<td>13.51 ± 0.31</td>
<td>13.47 ± 0.31</td>
<td>12.42 ± 0.43</td>
<td>9.64 ± 0.41 **</td>
<td>12.32 ± 0.31</td>
<td>13.44 ± 0.18</td>
<td>13.41 ± 0.21</td>
<td>12.62 ± 0.38</td>
<td>9.81 ± 0.49 ***</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>39.38 ± 1.01***</td>
<td>42.5 ± 0.77</td>
<td>41.7 ± 1.03</td>
<td>40.9 ± 1.29</td>
<td>32.4 ± 0.91 ***</td>
<td>29.87 ± 0.86</td>
<td>40.3 ± 0.52</td>
<td>40.6 ± 0.85</td>
<td>40.7 ± 0.74</td>
<td>33.9 ± 1.34***</td>
</tr>
<tr>
<td>MCV (cubic μ)</td>
<td>85.36 ± 2.29</td>
<td>89.39 ± 2.27</td>
<td>88.19 ± 1.62</td>
<td>92.27 ± 2.31</td>
<td>71.58 ± 2.98 ***</td>
<td>94.37 ± 1.97</td>
<td>97.62 ± 1.59</td>
<td>98.49 ± 2.62</td>
<td>98.61 ± 2.73 **</td>
<td>82.77 ± 1.73 **</td>
</tr>
<tr>
<td>MCH (microgm)</td>
<td>26.58 ± 0.93</td>
<td>28.48 ± 0.92</td>
<td>28.52 ± 0.83</td>
<td>28.01 ± 0.89</td>
<td>21.33 ± 1.11 ***</td>
<td>29.86 ± 0.55</td>
<td>32.55 ± 0.62</td>
<td>32.54 ± 0.27</td>
<td>30.48 ± 0.69 *</td>
<td>23.88 ± 0.64 ***</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>31.11 ± 0.74</td>
<td>31.89 ± 0.81</td>
<td>32.43 ± 0.67</td>
<td>30.37 ± 0.89</td>
<td>29.74 ± 0.89</td>
<td>31.59 ± 0.58</td>
<td>33.39 ± 0.49</td>
<td>33.11 ± 0.61</td>
<td>31.01 ± 0.69 **</td>
<td>28.86 ± 0.53 ***</td>
</tr>
</tbody>
</table>

Table 10.2. Serum iron, total iron binding capacity (TIBC), serum ferritin, serum transferrin and transferring saturation in normal and different grades of stunted Santal children. (n = Number of subject, Value: Mean ± SE, * p<0.05  ** p<0.01  ***p<0.001)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total (n=45)</th>
<th>Normal (n=10)</th>
<th>Mild (n=10)</th>
<th>Moderate (n=15)</th>
<th>Severe (n=10)</th>
<th>Girls (n=40)</th>
<th>Normal (n=10)</th>
<th>Mild (n=10)</th>
<th>Moderate (n=10)</th>
<th>Severe (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Iron (µg/dl)</td>
<td>85.88 ± 4.53</td>
<td>98.88 ± 4.39</td>
<td>88.68 ± 3.67</td>
<td>81.19 ± 4.02**</td>
<td>74.79 ± 6.05***</td>
<td>80.51 ± 4.95</td>
<td>94.05 ± 3.04</td>
<td>80.48 ± 5.57</td>
<td>76.72 ± 5.88**</td>
<td>70.76 ± 5.33***</td>
</tr>
<tr>
<td>TIBC (µg/dl)</td>
<td>314.03 ± 6.36</td>
<td>299.94 ± 4.51</td>
<td>302.78 ± 4.81</td>
<td>312.97 ± 6.38</td>
<td>340.44 ± 9.76**</td>
<td>328.43 ± 9.07</td>
<td>299.14 ± 5.34</td>
<td>325.11 ± 11.94</td>
<td>344.27 ± 11.69**</td>
<td>345.22 ± 7.34 **</td>
</tr>
<tr>
<td>S. Ferritin (ng/ml)</td>
<td>19.17 ± 2.03</td>
<td>26.47 ± 2.39</td>
<td>19.97 ± 2.18*</td>
<td>15.29 ± 1.75***</td>
<td>14.95 ± 1.82 ***</td>
<td>18.28 ± 2.62</td>
<td>24.89 ± 2.49</td>
<td>18.59 ± 3.07*</td>
<td>15.86 ± 2.79**</td>
<td>13.79 ± 2.15***</td>
</tr>
<tr>
<td>Transferrin (gm/L)</td>
<td>251.66 ± 5.21</td>
<td>241.72 ± 4.09</td>
<td>242.23 ± 3.85</td>
<td>250.37 ± 5.09</td>
<td>272.35 ± 7.81**</td>
<td>262.49 ± 7.26</td>
<td>239.31 ± 4.27</td>
<td>260.08 ± 9.55</td>
<td>275.42 ± 9.36 **</td>
<td>275.18 ± 5.88 **</td>
</tr>
<tr>
<td>TS (%)</td>
<td>27.8 ± 4.09</td>
<td>32.77 ± 3.85</td>
<td>29.53 ± 5.09</td>
<td>26.37 ± 7.81**</td>
<td>22.53 ± 25.25</td>
<td>31.65</td>
<td>25.58</td>
<td>23.03</td>
<td>20.76</td>
<td>1.87***</td>
</tr>
</tbody>
</table>
Table 10.3. Distribution of Santal children in different stages of iron deficiency.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Normal stunted (&gt;2SD)</th>
<th>Stunted (&lt;-2SD)</th>
<th>Normal stunted (&gt;2SD)</th>
<th>Stunted (&lt;-2SD)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19 (42.23)</td>
<td>14 (31.12)</td>
<td>17 (42.5)</td>
<td>10 (25)</td>
<td>60 (70.59)</td>
</tr>
<tr>
<td>Stage I</td>
<td>1 (2.23)</td>
<td>5 (11.12)</td>
<td>2 (5)</td>
<td>8 (20)</td>
<td>16 (18.82)</td>
</tr>
<tr>
<td>Stage II</td>
<td>-</td>
<td>3 (6.67)</td>
<td>1 (2.5)</td>
<td>-</td>
<td>4 (4.71)</td>
</tr>
<tr>
<td>Stage III</td>
<td>3 (6.67)</td>
<td>-</td>
<td>2 (5)</td>
<td>5 (5.88)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 10.1. Serum albumin (gm/L) in different nutritional groups in Santal boys and girls (*p<0.05. **p<0.01 and ***p<0.001).

10.3.5. Regression analyses

It has been found that hemoglobin concentration has strong significant association (p<0.001) to the nutritional status (Table 10.4).

10.3.6. Correlation analysis

All biochemical and hematological parameters are significantly (p<0.01) correlated to each other (Table 10.5).

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Hemoglobin concentration, serum iron and serum albumin are significantly (p<0.01) correlated with parental education and monthly family income (Table 10.6).

**Table 10.4.** Stepwise regression models of the association between nutritional status (Dependent variable) and different biochemical parameters.

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta (95% CI)</th>
<th>p-value</th>
<th>R²</th>
<th>Change in R² from previous model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Hb conc. .712 0.506</td>
<td>0.001</td>
<td>0.506</td>
<td>0.506</td>
</tr>
<tr>
<td>Model 2</td>
<td>Hb concen. .643 0.001</td>
<td>0.513</td>
<td>0.011</td>
<td>0.007</td>
</tr>
<tr>
<td>Model 3</td>
<td>Hb concen. .715 0.001</td>
<td>0.525</td>
<td>0.011</td>
<td>0.008</td>
</tr>
<tr>
<td>Model 4</td>
<td>Hb concen. .697 0.001</td>
<td>0.532</td>
<td>0.015</td>
<td>0.008</td>
</tr>
<tr>
<td>Model 5</td>
<td>Hb concen. .581 0.001</td>
<td>0.548</td>
<td>0.015</td>
<td>0.008</td>
</tr>
</tbody>
</table>

TS% = Transferrin saturation, Hb conc. = Hemoglobin concentration

**Table 10.5.** Correlation analyses among biochemical and hematological parameters in Santal children.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>S. Iron</th>
<th>S. Ferritin</th>
<th>TIBC</th>
<th>Hemoglobin</th>
<th>RBC count</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Albumin</td>
<td>0.63**</td>
<td>0.56**</td>
<td>-0.62**</td>
<td>0.75**</td>
<td>0.43*</td>
</tr>
<tr>
<td>S. Iron</td>
<td>-</td>
<td>0.74**</td>
<td>-0.77**</td>
<td>0.71**</td>
<td>0.51*</td>
</tr>
<tr>
<td>S. Ferritin</td>
<td>-</td>
<td>-</td>
<td>-0.57**</td>
<td>0.63**</td>
<td>0.38*</td>
</tr>
<tr>
<td>TIBC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.61**</td>
<td>-0.41*</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.28*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
Table 10.6. Correlation study between socioeconomic parameters and Hb concentration, serum iron and serum albumin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Parental Education</th>
<th>Family income</th>
<th>Parental Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Conc.</td>
<td>0.52**</td>
<td>0.41**</td>
<td>0.12</td>
</tr>
<tr>
<td>S. Iron</td>
<td>0.51**</td>
<td>0.36**</td>
<td>0.13</td>
</tr>
<tr>
<td>S. Albumin</td>
<td>0.32**</td>
<td>0.31**</td>
<td>0.21</td>
</tr>
</tbody>
</table>

**p<0.01

10.4. DISCUSSION

The significant difference of all the biochemical and hematological (except total RBC count) parameters in stunted children compared to normal statured children indicates the undernutrition in Santal children is due to deficiency of some essential nutrients, especially iron. The Hb concentration of stunted children is found to be significantly lower than that of normal statured children, which indicates that a moderate grade of anemia is prevalent in stunted children. Regression study also suggests that Hb concentration in surveyed children is significantly associated with their nutritional status as determined by z-score values of height-for-age. In the present study, there is no significant difference in the RBC count between normal statured and stunted children. However, the MCV is decreased in severely stunted children compared to normal statured children suggesting that a low level of Hb in stunted children is probably due to the deficiency of iron among surveyed children.

In some cases, PCV has also been considered as a useful parameter along with Hb level for defining anemia and iron-deficiency anemia (Lee & Nieman 2007). According to the WHO criteria (Latham 1997), a child having Hb level below 12 gm/dl along with PCV value below 32% should be considered as anemic. The present investigation does not find any child as anemic according to these criteria. Though Hb and PCV values are useful for defining anemia, these are
changed at the last stage of iron deficiency and hence, are not considered as good indicators of early iron deficiency (Lee & Nieman 2007).

The risk of iron deficiency increases as the body’s iron stores are depleted. Stages of iron depletion lead to the progression of magnitude and severity of undernutrition in a subject. In the present study, serum ferritin, transferrin saturation and Hb are used to identify the iron deficiency status. In stunted children, Hb, serum ferritin and transferrin saturation levels are decreased compared to the surveyed children having normal stature. Although the serum ferritin level and transferrin saturation in stunted children are in the normal range (serum ferritin: 18-250 ng/ml and transferrin saturation: 20-50%), it is in the lower border of the normal range. Moreover, the levels are significantly lower than that of normal statured children. This finding suggests that surveyed children have mild iron deficiency to maintain the acceptable levels of either Hb or serum ferritin. The dietary intake of Santal children has been measured and found that they had insufficient dietary intake of iron (Chapter 9). Therefore, it appears that the iron deficiency of these children may be due to the insufficient dietary intake of iron.

The mild iron deficiency of the stunted children is also supported by higher serum transferrin and TIBC level. Probably, these changes are indicating a regulatory mechanism to maintain the iron homeostasis in the body. The low serum ferritin is a marker of depleted iron storage in the body. A mild iron deficiency in stunted children is evident from biochemical measurements. Rao and Vijay (2006) found that about 78.1% Oraon and Santal tribal children had been suffering from iron deficiency anemia in the Purnia district of Bihar. Recently, Sharma et al. (2007) reported that prevalence of iron deficiency was 100% in Gaddi tribal girls of Himachal Pradesh. The prevalence of iron-deficiency anemia in the present study is not severe like that of other studies.
As the protein-calorie malnutrition is found to be the most widespread nutritional problem in developing countries, the assessment of protein status is essential to the diagnosis and treatment of undernutrition. In this regard, measurement of albumin, the most familiar, abundant and readily available clinically serum protein, gives an indication about the overall serum protein status (Young et al. 1990). A significant lower serum albumin concentration for undernourished children compared to well-nourished children was reported in a number of studies (Kumar 1986, Clark et al. 2006). The present study shows a significant reduction of serum albumin concentration in moderate and severe stunted children compared to normal statured children. It has already been established that serum albumin is a poor indicator of early protein depletion as it responds slowly to undernutrition due to its relatively long half-life (14-20 days) and large body pool (4-5 gm/kg of body weight) (Young et al. 1990, Benjamin 1989). About 60% of total body protein is found outside the bloodstream. During early undernutrition, when the serum concentration is beginning to fall, this extravascular albumin moves into the bloodstream helping to maintain the normal serum concentration despite protein and energy deficit. The serum albumin concentration may not decline at earlier stages of undernutrition, i.e. in mild stunted children.

Some earlier studies revealed that lower Hb level was associated with hypoproteinemia in undernourished children (Shahidi et al. 1961, Nnakwe 1995). As the present study shows a strong correlation between serum albumin level and Hb concentration, it can be said that lower protein level of stunted children can be one of causative factors for lower Hb concentration. Dietary intake of the surveyed children also reveals that protein consumption by surveyed children is low in comparison to recommended values (Chapter 9). Lower protein status of stunted children, which is reflected from their lower serum albumin level, may be due to their
lower protein intake in their diet. The parasitic infection of surveyed children has not been investigated in the present study, several studies reported that parasitic infection caused low Hb concentration in children (Aini et al. 2007, Rahman 1998).

In the present study, it has been observed that Hb concentration, serum iron and serum albumin are significantly associated with parental education and monthly family income suggesting that socioeconomic status is likely to be an important determinant of the above nutritional parameters and hence iron deficiency. Similar observation was reported by other researchers in preschool children of Morocco (Hioui et al. 2010, Schwartz et al. 1986).

The data of the present study indicate that the stunting may be associated with the deficiency of specific nutrients like iron and protein in surveyed Santal children. The results of this study may be utilized to design preventive measure by supplementing the deficient micronutrients or by awareness program in the community to select the required food items. The interpretation of the present chapter also cannot be generalized for the Santal children as the study has been carried out on small number of subjects considering the total number of children in Purulia district.