Protein energy malnutrition (PEM) is the most frequent cause of secondary immune deficiency in children especially in developing countries. In PEM there is a significant impairment of cell-mediated and humoral immune responses. Following nutritional rehabilitation the immunological impairments take longer to recover than the anthropometric and some biochemical measurements. Therefore dietary supplements are needed to enhance immune recovery. Recently probiotics like curd and various micronutrients have been recognized for their immunomodulating effects. Incorporating them in the...
diet of malnourished children may play an important role in the recovery of the immunological impairments as well as diarrhoea. Though curd is an integral part of the Indian diet, micronutrient-rich leaf protein concentrate (LPC) may serve as a cheap alternative. Plant leaves are valuable source of protein, vitamins and minerals and the protein has been rated as good as egg protein. Composition of many common leaves has been studied. Aloe Vera, Olive leaf (Olea europaea) and tulsi (Ocimum sanctum) are known to have immunogenic properties. Berseem is rich in protein, β-carotene, B complex vitamins and minerals on feeding to young women, and showed significant improvement in haemoglobin level. Information on immunogenic effects to milk and dairy products, those suffering from severe infections, were excluded.

Of the 80 children, 68 could complete the study. These children were randomized into two groups using a computer-generated random number table. The children in group A (n=32) received curd as a supplement while children in group B (n=36) received LPC. The main reason for not having a control group was due to ethical reason. The follow up was restricted to a minimum of 15 days due to socio-economic reasons, as most patients’ attendants could not afford a prolonged loss of working days. Also, the bed occupancy in the turn over of patients is very high.

At admission a detailed history was taken and clinical examination, anthropometry and relevant biochemical investigations were done for each child. All children were given oral albendazole at admission to render them parasite free and oral chloroquine (25 mg/kg). Children received parenteral Ampicillin (100 mg/kg/day) and Gentamycin (5 mg/kg/day). Clinical non-improvement or deterioration, after 48 h of treatment warranted change to ceftriaxone (75 mg/kg/day) and amikacin (15 mg/kg/day). All children received a two-step mixed vegetarian diet from the hospital kitchen over 15 days, as per the recommended WHO guidelines.

Initial treatment phase (Day 1 to 7): Involved treatment of dehydration, correction of electrolyte imbalance, institution of appropriate antibiotics for associated acute infections and initiation of feeding using feeds providing about 80-100 kcal/kg/day.

Rehabilitation phase (Day 7 to 15): Involved increasing the calorie intake to 150-200 kcal/kg/day.

Of the total protein intake for each subject, 6 g were provided using either curd or LPC. The later was provided in the form of dried leaf powder in sachets such that three such sachets provided 6 g of protein. The subjects were required to consume three sachets of LPC per day by incorporating it in the dough used to make chapattis (bread) or in khichdi (a preparation of rice and pulse) or mixed with honey in subjects who could not consume chapattis.

LPC was prepared using leaves of Berseem by ultrafiltration and acid thermo coagulation (100g contains- 344 calories; fat 22.5g, CHO 3g, protein 3.1g, β-carotene 86700 μg, vitamin B complex- B₃ 0.5mg, B₄ 24.2mg, B₅ 1mg, B₆ 330mg, Pantothenic acid 4.3mg, vitamin C 2.2mg, vitamin K 2mg, Ca 187mg, P 604mg, Fe 1mg, Zn 9mg, Mg 384mg, Cu 2.1mg and K 713mg). Curd was set in the hospital kitchen using the starter provided by National Dairy Research Institute, Karnal. 1g of curd contained 10⁶ colony forming units (cfu), each of Lactobacillus bulgaricus and Streptococcus thermophilus.

Curd (100g contains- energy 60cal, protein 3.1g, fat 4g, CHO 3g, β-carotene 102 μg, B₃ 0.05mg, B₅ 0.16mg, B₆ 0.1mg, B₇ 12.5 μg, vitamin C 1mg, Ca 149mg, P 93mg, Fe 0.2mg, K 130mg, Na 32mg) was supplied to the subjects under refrigerated
conditions. 200 g of curd was provided in two containers of 100 g each, which were consumed by the subjects with meals. Parents of the enrolled children were advised to refrain from giving any other fermented product or any additional micronutrient supplements to their children during the period of the study.

All selected parameters were recorded before intervention and after 15 days of treatment. Anthropometric measurements were done by one of us (PD) to avoid observer bias and measurements were repeated three times and the mean of two closest values was recorded. Weight was recorded to the nearest 25 g using a balance scale. Haemoglobin was estimated using freshly collected venous blood on a coulter in the hospital laboratory, which was, standardized regularly. The patient’s serum were separated by centrifugation and stored at -20°C. Serum ferritin was estimated on stored sera using a kit (Spectro Ferritin Kit, Ramco Lab Inc., Huston, TX, USA) based on solid phase ELISA. The reference range of serum ferritin levels in children 6 months-15 yr is 7-140 ng/ml. The reference range of serum ferritin level below 12 ng/ml indicates depletion of body’s iron stores. In patients with chronic infection, inflammation or malignancy serum ferritin level below 50ng/ml is associated with reduced or absent iron stores. CRP was measured on stored sera using a kit (AVITEX-CRP Latex Test, Omega Diagnostic Ltd., Scotland, UK) based on slide agglutination. The upper level for normality was taken as 6 mg/dl. The T cell subpopulations were estimated on freshly collected venous blood using the fluorescent automated cell sorter technique, at the AIDS Reference Centre, National Institute of Communicable Diseases, New Delhi. Fluorescent-tagged antibodies directed against T cell subpopulations were used and estimation was done using a flow cytometer (Becton Dickinson, Netherlands).

The study groups were compared with regards to nutritional parameters, haematological parameters and immunological parameters before and after intervention. The statistical analysis was done using Pearson’s Chi-square test and student’s unpaired and paired t-test. The level of significance was at \( P<0.05 \) and 95 per cent confidence interval.

Results

Of the 80 children enrolled in the study, 12 were excluded (7 in group A and 5 in group B). Six (3 in each group) were lost to follow up as they either absconded or left against medical advice. Four (3 in group A and 1 in group B) were excluded as they refused to consume the food supplement and two patients suffering from bronchopneumonia (1 in each group) died during the study. Thus, 32 patients in group A and 36 in group B completed the study. Both the groups were comparable with respect to age, sex and malnutrition characteristics. At the time of enrollment 60 per cent had bronchopneumonia, 37 per cent had acute gastroenteritis and 3 per cent had pyoderma.

The changes in nutritional parameters viz. weight, haemoglobin and serum ferritin following nutritional supplementation at the end of the study are shown in Table I. While a significant average increase in weight \((P<0.001)\) was seen in both the groups, a mean fall in weight was seen in patients suffering from kwashiorkor. One patient in group A and 3 in group B were suffering from kwashiorkor. The mean fall in weight of these patients was -0.250 kg and -0.950 kg, respectively.

The haemoglobin rose significantly in both the groups after supplementation. There was a significant fall in serum ferritin levels in both the groups at the end of the study period \((P<0.001)\) (Table I).

| Table I. Weight, haemoglobin and serum ferritin before and after supplementation |
|-----------------------------|-----------------------------|-----------------------------|
| **Weight (kg)**             | **Hemoglobin (g/dL)**       | **Serum ferritin (ng/ml)**  |
| Curd group                  | LPC group                   | Curd group                  | LPC group                   |
| Before supplementation      | 7.46 ± 2.15                 | 6.42 ± 1.74                 | 9.33 ± 1.45                 | 8.07 ± 1.73                 | 195.56 ± 321.22                  | 173.86 ± 288.05                  |
| After supplementation       | 7.96 ± 2.43                 | 6.84 ± 1.75                 | 9.63 ± 1.48                 | 8.59 ± 1.36                 | 50.87 ± 82.88                    | 71.69 ± 154.94                   |
| Change                      | +0.50                       | +0.42                       | +0.29 ± 0.68                | +0.52 ± 0.92                | -144.69 ± 292.74                 | -102.17 ± 288.29                 |

Values are mean ± SD.

n=32 in curd group
n=36 in LPC group

The changes in nutritional parameters viz. weight, haemoglobin and serum ferritin following nutritional supplementation at the end of the study are shown in Table I. While a significant average increase in weight \((P<0.001)\) was seen in both the groups, a mean fall in weight was seen in patients suffering from kwashiorkor. One patient in group A and 3 in group B were suffering from kwashiorkor. The mean fall in weight of these patients was -0.250 kg and -0.950 kg, respectively.

The haemoglobin rose significantly in both the groups after supplementation. There was a significant fall in serum ferritin levels in both the groups at the end of the study period \((P<0.001)\) (Table I).
Though all patients had clinical or laboratory evidence of infection, only 22 (32%) patients were CRP positive, initially. Of these, 8 were in group A and 14 in group B. After supplementation, one in group A and 5 in group B remained CRP positive. Of these, 4 patients were not positive for CRP at the beginning of the study.

The mean T cell subpopulations in children in both the groups were within the normal reference range for age as depicted in Table II. The changes in mean CD3, CD4 and CD8 counts in both the groups after treatment were not significant, after supplementation. However, there was a significant increase in CD4:CD8 ratio in both the groups after treatment (Table II).

Discussion

After 15 days of intervention there was increase in weight, and haemoglobin, with decrease in serum ferritin in both the groups. Similar results have been reported earlier1,17,18. CRP was positive in one third of the patients only; CRP has been reported to have a sensitivity of 85 per cent and a specificity of 80 per cent as an acute phase protein in the presence of infection. Our findings suggest a possibility of blunting of the acute phase response in PEM, supporting earlier reports19,20.

Table II. T cell subpopulations before and after supplementation

<table>
<thead>
<tr>
<th></th>
<th>CD3 (cells/µl)</th>
<th>CD4 (cells/µl)</th>
<th>CD8 (cells/µl)</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Curd group</td>
<td>LPC group</td>
<td>Curd group</td>
<td>LPC group</td>
</tr>
<tr>
<td>Before supp.</td>
<td>2171.17 ± 2137.93 ±</td>
<td>1166.10 ± 1078.44 ±</td>
<td>913.14 ± 946.41 ±</td>
<td>1.42 ± 1.29 ±</td>
</tr>
<tr>
<td>lementation</td>
<td>938.28</td>
<td>454.07</td>
<td>462.06</td>
<td>0.54</td>
</tr>
<tr>
<td>After supp.</td>
<td>2171.07 ± 2146.56 ±</td>
<td>1220.17 ± 1209.97 ±</td>
<td>859.55 ± 1027.12 ±</td>
<td>1.79 ± 1.55 ±</td>
</tr>
<tr>
<td>lementation</td>
<td>956.69</td>
<td>490.43</td>
<td>521.11</td>
<td>0.92</td>
</tr>
<tr>
<td>Change</td>
<td>-46.10 ± +17.79 ±</td>
<td>+54.07 ± +82.16 ±</td>
<td>-53.59 ± +82.16 ±</td>
<td>+0.37 ± +0.33 ±</td>
</tr>
<tr>
<td></td>
<td>608.489</td>
<td>418.50</td>
<td>296.92</td>
<td>± 0.60 ± 0.51</td>
</tr>
</tbody>
</table>

P = 0.001

Values are mean ± SD.
n=32 in curd group and n=36 in LPC group

Though all patients had clinical or laboratory evidence of infection, only 22 (32%) patients were CRP positive, initially. Of these, 8 were in group A and 14 in group B. After supplementation, one in group A and 5 in group B remained CRP positive. Of these, 4 patients were not positive for CRP at the beginning of the study.

The mean T cell subpopulations in children in both the groups were within the normal reference range for age as depicted in Table II16. The changes in mean CD3, CD4 and CD8 counts in both the groups after treatment were not significant, after supplementation. However, there was a significant increase in CD4:CD8 ratio in both the groups after treatment (Table II).

Discussion

After 15 days of intervention there was increase in weight, and haemoglobin, with decrease in serum ferritin in both the groups. Similar results have been reported earlier1,17,18. CRP was positive in one third of the patients only; CRP has been reported to have a sensitivity of 85 per cent and a specificity of 80 per cent as an acute phase protein in the presence of infection. Our findings suggest a possibility of blunting of the acute phase response in PEM, supporting earlier reports19,20.

Though, PEM has been known to be associated with a decrease in absolute T lymphocyte count and a reduction in CD4: CD821,22, these values were in normal limit in the present study. The rise in the CD4: CD8 ratio after nutrition intervention supported findings of Chandra23. In the present study such changes were observed in a much shorter span of treatment in both the groups. This may be attributed to the immunoenhancing effects of curd and the micronutrient-rich LPC. Improvement in the total, CD4 and CD25 T lymphocyte counts have been reported by Gill et al24 in elderly volunteers following consumption of Bifidobacterium lactis HN019 for 3 wk. Devi et al25 also showed that lactobacillus supplementation along with a protein rich diet for fifteen days improved the immune status of malnourished preschool children indicating its therapeutic role in PEM. LPC is inexpensive, thus more studies are needed to explore full potential of this low cost vegetable source.

In conclusion, our results showed that supplementation of diet with curd or leaf protein concentrate for about two weeks to children with moderate to severe malnutrition produced significant improvement in weight and haemoglobin levels. Ferritin was not found to be a reliable marker of iron status in the malnourished children. Also the response of CRP to acute infection was blunted in PEM. Immune recovery as assessed by CD4:CD8 can be accelerated in PEM by supplementing the diet with curd or leaf protein concentrate indicating that curd or leaf protein concentrate could be an inexpensive and effective food therapy in malnourished children.

Acknowledgment

Authors thank National Dairy Research Institute, Karnal for providing the starter for setting curd and Shrimati Jasbir Kaur, Senior dietician and Shrimati Kamlesh Sethi, Dietician, Department of Dietetics, Guru Teg Bahadur Hospital for providing curd and adding LPC in dietary items for our patients. Authors also acknowledge help of Dr C.K. Katiyar, Dabur Research Foundation Ltd, Sahibabad, UP for providing leaf protein concentrate. Thanks are due to the India National Science Academy, New Delhi for financial support.

References

1. UNICEF. The state of the world’s children; Early childhood. New Delhi: UNICEF; 2003.


Reprint requests: Dr K.N. Agarwal, D-115, Sector 36, Noida, Uttar Pradesh 201301, India e-mail: adolcare@hotmail.com/kna_ped@yahoo.com