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Research Article

Indian Dahi as Immunonutrient- A Pilot Study

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Abstract

Aim: Indian Dahi was shown to improve immune recovery in children having protein energy malnutrition. Thus in this pilot study Indian Dahi has been compared against milk in WHO recommended 2 step diet.

Material and Methods: Study protocol used in earlier study by Dewan., *et al.* [1] was strictly followed. Severely malnourished children received WHO diet with Dahi for 22 children and 20 children received WHO diet with milk. The milk and Dahi were prepared and supplied by M/s Mother Dairy, Delhi in coded containers of 90 gm each under refrigerated conditions and maintained in the hospital. These 42 children were given diet for 15 days in the hospital and followed for 6 weeks at their homes. The cytokines (TNFα, IFNγ, IL-4 and IL-10) levels on 1, 15 days and 6 weeks and T-cell subpopulations (absolute lymphocyte counts, CD3, CD4, CD8, CD19 and CD 56) on 1 day and 6 weeks were estimated.

Results: Interleukin levels during treatment were much higher on WHO-Dahi diet as compared to WHO- milk diet after 15 days and 6 weeks. The absolute lymphocyte counts, CD3, CD4, CD8, CD19 and CD56 increased in children receiving Dahi in WHO diet for 6 weeks, in contrast, these counts decreased in children receiving WHO milk diet.

Conclusion: The improvement in lymphocyte counts using Dahi in WHO diet is a finding of interest and suggest that Dahi is an immunonutrient.

Keywords: Indian Dahi; Malnutrition; WHO-diet; Cytokines; T Cell Subpopulation; Immunonutrition

Introduction

In India and neighboring countries, Dahi (Fermented milk/curd-Indian yoghurt) has been used as a regular item of the diet. It has been mentioned in the Ayurvedic literature 600 AD for use in treatment of diarrhea. Indian physicians used fat free curd (Butter milk) orally as well as gave in enema to treat chronic gastrointestinal disorders e.g. colitis, chronic diarrhea etc. Dahi along with usual diet is known to reduce number of episodes as well as duration of diarrhea [2,3]. Researchers have shown that Dahi has total viable bacterial counts of 5 x 10^7 to 5 x 108/ml; of these 10^7 to 3.5 x to 108/ml are *Lactobacilli*. Common being *Lactobacillus bulgaricus* (83 - 86%), *Streptococcus thermophilus* (80 - 85%), and *Streptococcus lactis* in 74% varying from place to place [4,5].

Protein Energy Malnutrition (PEM) is the most frequent cause of secondary immune deficiency in children with significant impairment of cell-mediated and humoral immune responses. Treatment of malnutrition should reverse the acquired immune dysfunction(s). WHO recommends that children with severe acute malnutrition need to be treated with specialized therapeutic diets (F75 and F100 formula) alongside the diagnosis and management of complications during in-patient care. F-75 (contains 75 kcal of energy and 0.9 g protein per 100ml) and F-100 (contains 100 kcal of energy and 2.9g proteins per 100 ml). Recent studies [1,3,6] have shown that fermented milk (Indian dahi) in diet: a) prevented as well as controlled diarrhea [3] and b) in moderate to severe malnutrition- cytokine levels showed an increase in pro-inflammatory (TNFα, IFNγ), anti-inflammatory (IL-10) but a fall in IL-4, with an increase in CD4:CD8 ratio. These immunonutrient properties of dahi necessitated studies in severe protein energy malnutrition to evaluate if Dahi in WHO diet in place of milk, will enhance recovery and improvement in immune functions.

Material and Methods

The methods used by Dewan., *et al.* [1] for patient's recruitment, diet distribution, medicines, and techniques used for blood collection for cytokines were followed. After 15 days stay in the hospital patients were followed up to 6 weeks in outpatient clinic. Dahi and milk were prepared and supplied by M/s Mother Dairy, Delhi in coded containers of 90 gm each under refrigerated conditions and maintained in the hospital. However the difference in consistency remained. Dahi contained 10^8 colony forming unit(CFU) of *Lactobacillus bulgaricus* and 10^8 CFU of *Streptococcus thermophilus* per gm and other group received similar milk in diet. Each patient received 2 containers per day with lunch and dinner. Dietary advice was given to all patients. During the study period they continued to consume Dahi or milk supplied as before. 22 patients on WHO-Dahi diet (group-A) and 20 patients on WHO-milk diet (group-B) completed the study.

Cytokines

IL -1, IL-6, IL-10, TNF-alpha were estimated using kits based on principle of sandwich enzyme linked immunosorbent assay following the manufacturer protocol, using ELISA reader (Genzyme, Cambridge, Massachusetts, USA).

Lymphocyte counts

T and B cell populations were estimated by BD FACS caliber flow cytometers using four colour cytometry (Immunophenotyping). T and B cell subpopulations were enumerated using Monoclonal antibodies directed against cell surface antigens. Quantification of subpopulation is done using liquid counting beads. 1 to 1.5 ml of venous blood was collected using EDTA as anticoagulant, samples were transferred to M/s Vimta Labs, Cherlapally Hyderabad, A.P., India.

Results

Cytokines IL-1, IL-6 and IL-10 in age matched healthy children (n = 4 at 2 - 3 yr, n = 5 at 3 - 4 yr and n = 11 at 4 - 5 yr of age) means were 10.6 ± 5.6, 8.3 ± 5.7 and 11.4 ± 6.4 pg/dl respectively. The initial values for these interleukins were significantly higher in children suffering of protein energy malnutrition. On treatment IL-1, IL-6 levels increased significantly on day 15th and at 6 weeks ($p < 0.001$), in both the groups. IL-10 showed similar rise on day 15th and 6 weeks on WHO-Dahi diet, but there was fall on 15th day on WHO-milk diet with subsequent rise at 6 weeks. The rise in interleukin levels during treatment was much higher on WHO-dahi diet as compared to WHO- milk diet (Figure 1).

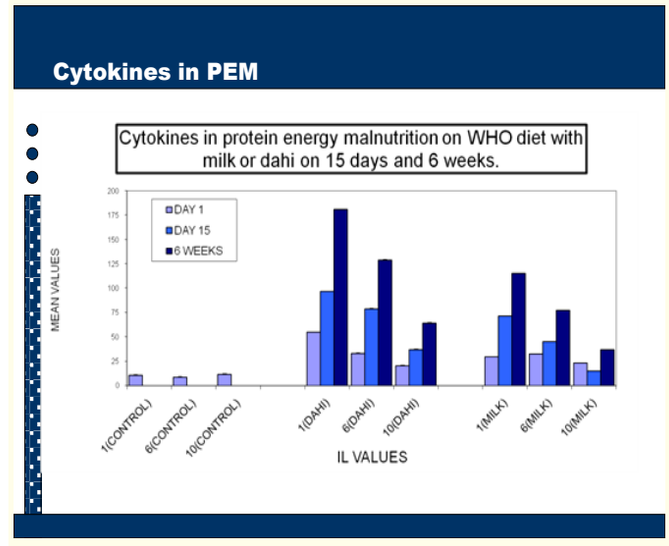

Figure 1: Cytokines in PEM.

Absolute Lymphocyte counts

The mean initial absolute lymphocyte counts were 3707 ± 1551 and 4553 ± 1776/µl on WHO-Dahi and WHO-milk diets, respectively, after 6 weeks of treatment the corresponding values were 6312 ± 1937 and 3493 ± 1418 µl. Thus WHO- diet with Dahi showed increase in contrast, WHO milk diet had decrease in counts ($p = 0.004$). Similar trend was observed for CD3+ CD4+ CD8+, CD19+ and CD56+ cells in two treatment groups. At 6 weeks differences in change for CD3+cells and CD4+ cells were significant p being ($p < 0.003$ and 0.001, respectively). The changes for CD+8, CD19+ and CD56+ were statistically not significant (Figure 2).

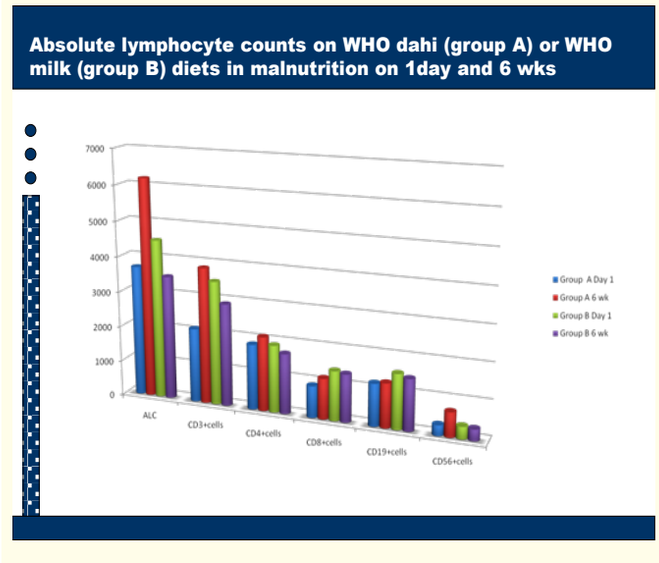

Figure 2: Lymphocyte response in PEM on dahi and milk in diet.

Discussion

Multiple abnormalities in the immune response, including T-cell number, ratio of T-cell subsets, NK cell activity, and cytokine production, have been described in connection with protein energy malnutrition.. Several studies on the effects of malnutrition at the immunological level have been carried out with humans and experimental animals. These studies indicate that malnutrition decreases T-cell function, cytokine production, and the ability of lymphocytes to respond appropriately to cytokines [7-9]. Rodrigues., *et al.* [9] showed that malnutrition decreased capacity of CD4 and CD8 cells to produce IL2 and IFN-Ý, while IL-4 and IL-10 production was increased. Malnutrition also showed decreased activation capacity of CD69+ and CD25+ cells as compared to well nourished(Infected/uninfected).The changes in protein energy malnutrition are specific as in patients of anorexia nervosa estimation of interleukins (IL-1, IL-2, IL-4, IL-6, IL-10), interferon (IFN-γ), tumor necrosis factor (TNF-α), and transforming growth factor (TGF-ß2) showed that only serum IL-2 and TGF-ß2 concentrations were significantly decreased in comparison to healthy subjects [10].

In the present study initial cytokine levels for IL-1, IL-6, IL-10 were increased in malnourished children; on treatment with WHO-Dahi diet these values further increased. The values for IL-1 and IL-6 on WHO milk diet also increased on 15th day and 6 weeks, however in case of IL-10 value fell on 15th day but subsequently increased at 6 weeks. The absolute lymphocyte counts, CD3, CD4, CD8, CD19 and CD56 markedly increased after 6 weeks on WHO-Dahi diet, while counts decreased in children receiving WHO milk diet. The differences observed between fermented and plain milk diets are of interest.

Conclusions

The findings of increased values of cytokines and lymphocytes on WHO-Dahi diet, suggest its role as immune nutrient and may be considered for use in place of milk in WHO –milk diet in management of severe acute malnutrition.

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