Impact of Fortified Malt-Based Food on Immunity Outcomes in School Children in India: Nutritional Epidemiology and Study Design for a Cluster Randomized Controlled Trial

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Abstract

Background: Nutritional inadequacy and consequent diminished immunity among school age children is a public health problem in India. Nutrition interventional studies using cluster randomized controlled trial (RCT) design can avoid ethical issues inherent with double-blind individual RCT in children involving daily administration of empty-calorie placebo.

Objective: We tested the hypothesis that daily administration of a fortified malt-based food (FMBF), a multi-nutrient supplement, would improve immunity outcomes against common infectious diseases, nutritional status and gut health in Indian school-age children, by utilizing cluster RCT design. This report presents the study design attributes and the baseline characteristics of the study population.

Methods: This was an open-label, two-arm, parallel-group, matched-pair cluster RCT, stratified by gender, in children aged ?7 to ?10 years old with height-for-age Z-scores (HAZ) of ?-3 to ?-1 and good general health. Four schools located in Pune city in India participated in the study. Each school was deemed as a cluster and was randomized to test group (FMBF + dietary counseling) or control group (dietary counseling alone). A total of 924 participants from the 4 randomized schools were enrolled in the study.

Results: Observed mean age ±SD was 8.0 years ±0.81 (range: min 7 years and max 10 years). There was no significant difference in mean age (p=.06), gender (p=.55), race (p=1), HAZ category (p=.051), HAZ (p=.17), BMI (p=.03). A very large proportion of children had micronutrient inadequacies in terms of vitamin D (97.51%), folate (79.18%), zinc (66.05%), and vitamin A (34.27%) at baseline. Design provided an operational ease of administration of study intervention at cluster level. Mean compliance with test product was 99.99% (95% CI) and retention in the study was observed 98% (95% CI).

Conclusions: The findings highlight the extent of nutritional inadequacies in Indian school-age children, reaffirming the need for nutritional strategies to optimize the nutritional status among these children. Cluster RCT design can be effectively used in nutritional intervention trial in children by maintaining high compliance and retention in the study. Clinical Trial: Clinicaltrials.gov NCT02542865, https://clinicaltrials.gov/study/NCT02542865

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Impact of Fortified Malt-Based Food on Immunity Outcomes in School Children in India: Nutritional Epidemiology and Study Design for a Cluster Randomized Controlled Trial

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Background: Nutritional inadequacy and consequent diminished immunity among school age children is a public health problem in India. Nutrition interventional studies using cluster randomized controlled trial (RCT) design can avoid ethical issues inherent with double-blind individual RCT in children involving daily administration of empty-calorie placebo.
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Results: Observed mean age ±SD was 8.0 years ±0.81 (range: min 7 years and max 10 years). There was no significant difference in mean age (p=.06), gender (p=.55), race (p=1), HAZ category (p=.051), HAZ (p=.17), BMI (p=.03). A very large proportion of children had micronutrient inadequacies in terms of vitamin D (97.51%), folate (79.18%), zinc (66.05%), and vitamin A (34.27%) at baseline. Design provided an operational ease of administration of study intervention at cluster level. Mean compliance with test product was 99.99% (95% CI) and retention in the study was observed 98% (95% CI).
Conclusions: The findings highlight the extent of nutritional inadequacies in Indian school-age children, reaffirming the need for nutritional strategies to optimize the nutritional status among these children. Cluster RCT design can be effectively used in nutritional intervention trial in children by maintaining high compliance and retention in the study.
Trial Registration: Clinicaltrials.gov NCT02542865,
https://clinicaltrials.gov/study/NCT02542865

Keywords: multi-nutrient supplement; study design; Indian children; immunity; fortified malt-based food; school-based

Introduction

Nutritional inadequacy has been the major and long-standing cause of nutritional/health concerns and developmental shortcomings in Indian children [1]. Despite numerous healthcare advances, the burden of major communicable diseases in children continues to be high in India compared to other countries [2,3]. As per the 2021 Global Burden of Disease study, diarrheal diseases and respiratory infections among children (5-14 years) were two of the five leading infectious causes of disability-adjusted life-years in the country [3]. Furthermore, child and maternal malnutrition (nutritional deficiencies, diarrhea, lower respiratory infections, and other common infectious diseases) were among the top five leading risk factors for the disability-adjusted life-years [3].

In terms of nutritional status, approximately 60% of the Indian pediatric population suffers from protein-energy malnutrition [4-8]. A recent nation-wide survey further reported deficiency of zinc (Zn), iron (Fe), folate as well as vitamins A, B12, and D in 14-32% of pre-school children (aged 14 years), in 17-28% of school-age children (aged 5-9 years), and in 16-37% of adolescents (aged 10–19 years) [9].

Nutritional inadequacy hampers the overall development of children as it adversely affects their immunity, normal physiological functions, physical growth, and cognitive development [10-14]. Such inadequacies often co-exist with infectious diseases and exhibit complex interactions, leading to a vicious cycle of malnutrition and infections [, 15-18]. This is evident by the fact that child undernutrition is responsible for 22% of the country's burden of disease [19].

As per the recent Global Nutrition Report, India performs poorly across standard child nutritional measures, accounting for nearly 39% of the stunting and 15% of wasting worldwide [20]. On national level for children under 5 years of age, data from the 2015–2016 National Family Health Survey show reduced prevalence of child stunting and underweight (38% and 36%) since 1992-1993 (52% and 53%) [21], however, it is still alarmingly high considering the multi-sectoral nutritional policies and initiatives [22]. Although there is sufficient information anthropometry related nutritional data for under-5-year age group, there is dearth of national level anthropometric data for school going children. The Comprehensive National Nutrition Survey (CNNS) was the first national survey to measure anthropometry in children aged 5 to 14 years. According to the CNNS 2016-2019, the prevalence of child stunting and underweight for children in the age group of 5-9 years were nearly 22% and 35% respectively. However, the prevalence of stunting and underweight varied with school status with a higher percentage of stunting among children who were out of school, compared to school-going children (38% vs. 20% and 45% vs. 34% respectively) [9].

Evidently, at individual level, appropriate nutrition actions in children can help bridge the nutritional gap, reduce the morbidity burden through improved immunity, and can also contribute to their overall health during the crucial formative and learning years [13, 23-31]. Nutritional supplementation is one of the widely accepted approaches out of several effective nutritional strategies available worldwide [32,33]. Of note, in the Indian scenario, nutritional supplements have been vastly studied for their effect on nutritional status and immunity

among children <5 years of age. However, since school-age children contribute to around one-fifth of the total Indian population [34], highlighting the significance of aforesaid aspects in this cohort is equally essential. Also, of the existing literature, none of the studies have comprehensively evaluated any nutritional supplement for its overall effect on the growth and immunity/health outcomes of Indian school-age children [35-38].

Thus, we conducted a study to provide a comprehensive overview of one such supplement, multi-nutrient fortified malt-based food (FMBF), for its effect on immunity as well as other health aspects, when combined with dietary counselling, in a large group (>900) of Indian school-age children (> 7 and <10 years). The current manuscript gives an overview of the study design and baseline characteristics of the study population.

Materials and Methods

Study design

This was a single-center, multiple-site, open-label, parallel-group, school-based matched-pair cluster randomized controlled trial (RCT) conducted in Indian school-age children aged ≥7 to ≤10 years (Clinicaltrial.gov: NCT02542865). The study was conducted in 10 visits with screening (visit 1), baseline assessment (visit 2), dietary counseling (visit 3 to 9), and end-of-study visit (visit 10). Nutritional intervention with FMBF to the test group was done every day between visit 3 to 9 for 9 months.

After obtaining written permission from management boards, four non-government schools from Pune, India, with co-education system and no history of outbreaks/cluster of infectious disease cases in the prior month consented to participate in the study. Schools were matched for size (with respect to the number of children aged from 7 to 10 years), socioeconomic profile of the students, and type of school (government subsidized or private) and two matched pairs were formed. Each school was deemed as cluster and assigned a randomization number in ascending numerical order. A randomization schedule with details on treatment assignment was prepared by an independent statistician from Biostatistics Department, GSK CH, using validated internal software. Details of the schedule was unknown to any of the researchers involved in the study. This was stored concealed in an internal electronic document management system (eDMS). Basis the randomization schedule and school list, each school within the matched pairs were randomized to receive either multi-nutrient FMBF along with dietary counseling (test group) or dietary counseling alone (control group) during the intervention phase. The study assessed the effect of FMBF on the overall development of these school-age children (immunity, nutritional status, gut health, anthropometry, dietary status), along with its safety profile.

The study was conducted in accordance with the International Council on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. The protocol and all the amendments were approved by an in dependent institutional ethics committee (Jehangir Clinical Development Centre Pvt Ltd., Pune, India) prior to study initiation. Written informed consent was obtained from one of the parents or a legally acceptable representative (LAR) of each participant and written assent was obtained from all these participants before any study-specific procedures were performed.

For quality control- all instruments/equipment were regularly calibrated, accredited lab had been used and tools used were validated. Staff were trained and retrained at regular intervals. To maintain the quality standards of data collection as per protocol and regulatory standards, data monitoring, data collection and analysis was done by individual Contract Research Organization. Study has been also audited by “Clinical Data Quality Assurance” (CDQA) team from GlaxoSmithKline USA and shared Audit certificate.

Study inclusion/exclusion criteria

The key study inclusion and exclusion criteria are summarized in Table 1.

Study interventions

FMBF was prepackaged in sachets, each containing 27 g of multi-nutrient beverage powder. The nutrition profile of this supplement is presented in Supplementary Table 1. Participants from the test group consumed 27 g of this powder mixed in 150 mL lukewarm water, twice a day for 9 months. To avoid stomach fullness as a deterrent against compliance, the interval between the two doses was maximized. One dose was administered as soon as the participants entered the school and the second dose just prior to school dismissal. During absence from school e.g., holidays, sickness etc., one dose was administered in the morning and the second dose in the evening, by parents/LARs at home.

Nutritional inadequacy and the resulting immunity outcomes could be influenced by dietetic interventions implemented to improve nutritional in-takes. Background diet is a major confounder and its imperative to keep a check on the dietary quality throughout the study. Thus, to keep the back-ground diets comparable between the two groups, the test group was administered the test product, while both test and control groups received dietary counseling. All the study participants, their teachers, and their parents/LARs received age-specific dietary counseling to optimize the nutritional intakes of the participants, based on the dietary guidelines for Indians, established by the Indian Council of Medical Research–- National Institute of Nutrition [39]. A total of 7 separate sessions of dietary counselling were administered to study participants and parents/LAR in both test and control groups. The first 2 sessions were kept for delivery of content and were mandatory to attend. Remaining 5 sessions were for follow up, reinforcement and problem solving of contents delivered in first 2 sessions and these sessions were not mandatory to attend. The quality, content, and duration of the dietary counseling were equivalent in both the study groups.

Table 1. Key Study Eligibility Criteria

Inclusion criteria	Exclusion criteria

▪ Children of either gender
▪ Children aged 7 to 10 years (inclusive) with height-for-age Z-scores (HAZ) between -3 to -1 (inclusive)
▪ Children with good general and mental health in the opinion of the investigator or medically qualified designee, defined as:
  ▪ No clinically significant/relevant abnormalities in medical history or upon physical examination
  ▪ Absence of any condition that could affect the child's safety/wellbeing or their ability to understand/follow the study aspects
	▪ Children in care
	▪ Children with allergy/intolerance to any ingredient of the FMBF
	▪ Children with severe anemia (Hemoglobin <8g/dL)
	▪ Children with history of use of immunosuppressive therapy (e.g., oral corticosteroids or chemotherapy) in 6 months prior to screening visit
	▪ Children with recent history (2 months) of serious infections, injuries and/or surgeries
	▪ Children with current or relevant history of any serious/ severe/ unstable physical or
	▪ Psychiatric illness or any medical disorder that could have made them unlikely to fully complete the study
	▪ Children who consumed nutritional supplements and/or health food drinks on a regular basis (>=3 times a week) in the last 3 months
	▪ Children with any other condition that presented an undue risk to them from the study interventions or procedures, in the opinion of the investigator or medically qualified designee

Study endpoints

The primary efficacy endpoint assessed the total number of ill days due to GI and respiratory illness episodes in the participants over 9 months. GI illness was defined as an acute illness that included any of following symptoms: 3 or more loose/liquid/watery stools and/or any vomiting in 24-hours [40]. Diarrhea or vomiting arising from non-infectious causes, such as irritable bowel syndrome (IBS) or medications, were excluded from the diagnosis. Respiratory illness was defined as an acute illness that included ≥1 of the following symptoms: runny nose, stuffy or blocked nose, cough, fever or chills, sore throat, or sneezing [41]. Symptoms arising due to non-infectious causes (e.g., allergy) were excluded from the diagnosis. The secondary efficacy endpoints evaluated over 9 months included assessment of frequency and severity of the GI and respiratory illness episodes, school absenteeism due to these episodes, change from baseline in serum micronutrients levels (vitamins A, B12, D, E, iron, folate, and trace elements Zn, Fe, copper, selenium), change from baseline in macronutrient status, change from baseline in the dietary diversity assessed using 24 hour individual dietary diversity score (IDDS), change from baseline in the gut integrity as measured by lactulose : mannitol test and urinary neopterin test, and change from baseline in body mass index (BMI). Details on evaluation of secondary endpoints are presented in Table 3. Safety assessments included evaluation of

adverse events (AEs), treatment-emergent AEs, serious AEs throughout the study. The laboratory assessments, vital signs, physical examinations, and hemoglobin measurement in these participants were conducted at baseline and end of the study.

Endpoint assessments

Study endpoint assessments were performed as per the study schedule presented in Table 2.

To evaluate primary and secondary immunity outcomes, the parents/LARs of the participants regularly updated the symptom checklist and compliance report forms for study interventions, which were collected and analyzed during each study physician weekly review (SPWR) throughout the study. During the SPWR, the start/end date of GI and respiratory illness episodes along with the frequency, severity (mild, moderate, severe), and school absenteeism due to these episodes were noted in Diagnosis Form by the study physicians based on details obtained from followings:

- Symptom checklist completed by Parents/LARs
- External clinical record if treated by an external physician
- Direct information by participants/parents/LARs
- Clinical history and examination by study physician

Details of evaluation methods and tools used for assessment of secondary immunity outcome endpoints, anthropometry, serum biochemistry and dietary assessment, are provided in Table 3.

During the study, the AEs and serious AEs were monitored in addition to those reported by participants/parents/LARs to the study physicians as and when observed or through the weekly symptom checklists. The AEs were regarded as treatment-emergent AEs if they occurred on/after the first administration of FMBF in test group or on/after the first dietary counselling session in the control group.

Compliance check of study interventions was assessed as per schedule presented in Table 2. Concomitant medications were recorded at each study visit. Except for the immunosuppressive therapies (which eventually was never used in the study), there were no restrictions on medication and medical treatment. However, the use of medications (e.g., antibiotics, antipyretics etc.) was standardized across all study sites.

Table 2 Study baseline and endpoint assessments schedule

Activity	Visit 1[a]	Visit 2[b]	Dietary counselling visits[c] (Visit 3 to Visit 9) [d]	SPWR & Parent/ LAR symptom checklist	Visit 10[e]
Informed consent and assent[f]	X			[g, h]	

Demographics	X				
Medical history	X				
Current/concomitant medication	X	X	X		X
General physical examination	X				X
Vital signs	X				X
Hb assessment using Pronto	X				
Anthropometric measurements (height, weight, BMI, and HAZ)	X				X
Inclusion/exclusion criteria evaluation	X				
Participant eligibility	X				
Continued eligibility criteria		X	X		X
24-hour dietary recall and dietary diversity survey	X				X
Parental/LAR training to complete 'symptom checklist'[i]	X				
Dispense blank Parent/LAR symptom checklist and product compliance report forms	X				
Sample collection for urinary neopterin test and lactulose:mannitol test		X			X
Sample collection for analysis of serum ferritin, sTfR, CRP, AGP, salivary IgA[j], and nutritional biochemistry		X			X
Dietary counselling			X		
Product administration (applicable to the test group only)[k]			X		
Product compliance check and collect empty product sachet (applicable to test group only)			X		
AE monitoring		X	X		X
Study completion and medical sign off					X

Abbreviations: AE = adverse event, AGP = alpha 1acid glycoprotein, BMI = body mass index, CRP = C-reactive protein, HAZ = Height-for-age Z-scores, Hb = hemoglobin, IgA = immunoglobulin A, LAR = legally acceptable representative, SPWR = study physician weekly review, sTfR = serum transferrin receptor.

a Visit 1: Screening Visit.

b Visit 2: Baseline Visit (+1 to 21 days post screening visit).

c Session numbers 3, 4, 5, 6, and 7 could be postponed in view of long school holidays and exams. Session number 7 was not to be conducted any later than the last day of Month 8 of the intervention period. A minimum of 4 weeks gap was maintained between Session numbers 2, 3, 4, 5, 6, and 7.

d Visit 3/Session 1: Day 1 to 3 of Week 1 from baseline visit; Visit 4/Session 2: Week 2 from baseline visit + up to 3 days; Visit 5/Session 3: Week 6 from baseline visit + up to 3 days; Visit 6/Session 4: Week 10 from baseline visit + up to 3 days; Visit 7/Session 5: Week 14 from baseline visit + up to 3 days; Visit 8/Session 6: Week 18 from baseline visit + up to 3 days; Visit 9/Session 7: Week 22 from baseline visit + up to 3 days.

e Visit 10: End of study visit (+1 to 7 days post end of 9 months from baseline visit).

f Obtaining informed consent and assent and performing screening activities could be on different dates but informed consent and assent were obtained prior to performing and applying any screening criteria or procedures. Screening activities were done within 60 days of obtaining consent and assent.

h Parent/LAR symptom checklist and product compliance report forms were collected and reviewed for each weekly review with the physician.

i Could be done anytime post obtaining consent and assent but prior to baseline visit.

j Saliva samples were collected for the assessment of salivary IgA at Visit 2 and Visit 10.

k Intervention began +1 to 3 days from baseline visit and continued for 9 months. Compliance check, test product empty sachets were collected, and AEs were recorded throughout the intervention period.

A non-invasive, spot-checking device from Masimo Corporation's Pronto® Pulse CO-Oximeter® was used to screen study participants in school setting based on their hemoglobin (Hb). This device uses transcutaneous spectrophotometry to measure Hb concentration in the blood, and the result is comparable to invasive laboratory-based Hemoglobin estimations in children [42]. Anthropometric measurements included assessment of height, weight, body mass index (BMI), and HAZ. HAZ was obtained using the WHO Anthroplus software [43].

Table 3 Secondary Endpoints Evaluation Methods and Tools

Secondary efficacy endpoint	Frequency of assessment	Evaluation tool	Evaluation/Test method
Frequency (number of episodes) of GI and respiratory illnesses	Weekly basis during scheduled SPWR visits.	Diagnosis Form	Calculated as total number of GI and respiratory illness episodes, divided by duration of intervention, where each episode is defined as each incidence of illness followed by at least 3 symptom free days [44,45,46].
Severity of an illness episode	Weekly basis during scheduled SPWR visits.	Diagnosis Form	Assessed as per severity grading of mild, moderate, and severe as outlined in evaluated and classified by study physician [47].
School absenteeism due to GI and respiratory illnesses	Weekly basis during scheduled SPWR visits.	Diagnosis Form	Calculated as number of days when a child failed to attend school because of GI and/or respiratory illnesses.
Change from baseline in BMI	Twice in the study; first at screening and second at end of the study visit.	▪ Portable stadio-meter (SECA 213) – For measuring participant's height. ▪ Standardized weighing scale (SECA 874) – For measuring participant's weight.	Calculated using formula: BMI = Weight [kg] / (Height[m])^2 All anthropometric measurements were performed using the guidelines adopted at the National Institute of Health sponsored Arlie Conference [48].
Change from baseline in gut integrity/health	Twice in the study; first at baseline and second at end of the study visit.	Urine testing	Urine lactulose:mannitol assessment by HPLC Urinary neopterin assessment by ELISA
Change from baseline in mucosal immunity	Twice in the study; first at baseline and second at end of the study visit.	Saliva testing	Salivary IgA assessment by ELISA
Change from baseline in levels of micronutrients vitamin A, B12, D (25-hydroxycholecalciferol), E, folate, and trace elements Se, Zn, Cu, and Fe	Twice in the study; first at baseline and second at end of the study visit.	Blood testing	▪ Serum vitamin A, B12, D, E, and folate assessment by ELISA ▪ Serum Cu assessment by 3, 5-Dibromo-PAESA ▪ Serum Zn assessment

Secondary efficacy endpoint	Frequency of assessment	Evaluation tool	Evaluation/Test method
			by 5-Br-PAPS ▪ Serum Fe assessment by TPTZ ▪ Serum Ferritin assessment by turbidimetry ▪ Serum Se assessment by ICP
Change from baseline levels of ferritin, sTfR, CRP, and AGP	Twice in the study; first at baseline and second at end of the study visit.	Blood testing	▪ Serum sTfR and CRP assessment by Immunoturbidimetry ▪ Serum AGP assessment by turbidimetry
Change from baseline in dietary diversity score	Twice in the study; first at baseline and second at end of the study visit.	▪ 24-hour dietary recall survey ▪ Dietary diversity questionnaire # [49]	Based on data of foods and beverages consumed in last 24-hour as captured by 24-hour dietary survey, appropriate food groups in questionnaire in were selected. Dietary diversity scores were calculated by adding the number of food groups consumed by the child over the 24-hour recall period.
Change from baseline in energy, protein, carbohydrates, and fat consumption	Twice in the study; first at baseline and second at end of the study visit.	24-hour dietary recall survey [50]	24-hour dietary recall survey

Abbreviations: BMI= Body Mass Index, IgA= Immunoglobulin A, HPLC= High-performance liquid chromatography; ELISA=Enzyme-linked immunosorbent assay, TPTZ = 2,4,6-Tripyridyl-S-triazine ICP= Inductively Coupled Plasma, sTfR= Soluble Transferrin Receptor CRP= c- Reactive Protein, AGP= Alpha (1) acid glycoprotein

Sample size

The sample size was based on the primary efficacy endpoint, the total number of ill days due to Gastrointestinal 9(GI) and respiratory illnesses. Based on the data from the Global Burden of Disease by World Health Organization [51], the Institute for Health Metrics and Evaluation [52], and the Census of India [34], approximately 7 ill days due to GI and respiratory illnesses per 9 months was assumed in this study population. Thus, the sample size was calculated based on the assumption of an average of 7 ill days in the control group with a difference of 10% (0.7 days) between the study groups.

No sample size considerations were taken into account for secondary objectives. The simulated data were also used based upon the assumption of a Poisson distribution per study treatment. On observation of these simulated data, it was considered that these Poisson distributions were not heavily skewed and, as a result, normal distributions could be assumed. Consequently, a normality assumption was adopted for both the

generation of sample size and the intended approach to the statistical analysis. Because of the assumption of Poisson distribution, estimates of variability were calculated as square root of mean. Mean of control was assumed to be 7.0 and standard deviation (SD) was 2.65, mean of the test group was 6.3 and SD was 2.51. In order to achieve 80% power, 215 participants per treatment arm (total = 430) were required to complete the study in case of individual randomization. This assumed a 5% level of significance (two tailed t-test). As this study used cluster randomization, to retain equivalent power to an individually randomized trial, a design effect (DE) which is a function of intra-cluster correlation coefficient (ICC), was considered for sample size calculations. The scientific literature suggested that DE due to cluster sampling strategy should be assumed to be 1.5 [53]. A similar assumption was made in a study, which used three-stage systematic cluster sampling [54]. Using the following formula [55]:

$N^* = N \times DE$

Where, N^* = Number of participants required for cluster RCT

N = Number of participants required for individual RCT

DE = Design effect.

Since DE was assumed to be 1.5, the number of evaluable participants required to complete the study was determined to be 323 per arm (total = 646). To allow for a 30% drop-out rate, a total of 924 participants were to be randomized. Approximately 1300 participants were to be screened to randomize approximately 924 participants in order to obtain 646 participants completing the study.

Statistical Analysis

Analysis population

Safety population was defined as participants who received ≥1 dose of the study interventions (only dietary counselling [control group] or both FMBF and dietary counselling [test group]) during the study. The intent-to-treat (ITT) population comprised of all participants in the safety population with any post-interventional assessment (i.e., SPWR), according to the study group assigned. The modified Intent-to-treat (mITT) population was defined as all participants in the safety population with any post-interventional assessment (SPWR) who completed the entire intervention phase and attended the end-of-study visit.

Analysis methods

Access to the treatment code was appropriately restricted during the process of blinded data review involving clinical research scientists and statisticians. The database with potentially unblinding information was separately reviewed for study population inclusion/exclusion by unblinded clinical research scientists. The database was later validated by a blinded reviewer prior to its release for analysis.

Analysis of primary efficacy endpoint was done on mITT population and the analysis of secondary and exploratory efficacy endpoints were done on both mITT and ITT population. They were analyzed using the analysis of covariance (ANCOVA) model (SAS Studio version 9.4 or higher). The ANCOVA had cluster (school) as a random effect;

study group and gender as fixed effects; and baseline assessments, baseline serum levels, and baseline IDDS as covariates. Unlike other study endpoints, frequency for severity (mild, moderate, severe) of GI/respiratory illness episodes as well as IDDS categories (low, moderate, high) were compared using the Chi-Square test (if frequencies were >5%). If frequencies were ≤5% in any study group, then these were compared using Fisher's exact test. Adjusted means for each study group, 95% confidence intervals, within-group p-values, difference between the study groups, 95% confidence intervals of the difference, and the between-group p-values were also presented based on the above statistical model. All statistical tests of hypotheses were two-sided and employed a level of significance of α=0.05.

Demographics, baseline characteristics and safety assessment (including laboratory tests) summaries are produced for the Safety, ITT and mITT population. For them, descriptive statistics n [%], mean, standard deviation [SD], minimum, and maximum (as applicable) were provided. Baseline characteristics were compared between the treatment groups using independent t-test for continuous variables and chi-square test for categorical variables. Two-sided significance level of 5% was used for the comparisons.

Intervention was targeted at the cluster level; however, outcomes were measured at the individual level. Thus, all ANCOVA analyses included cluster as a random effect to introduce correlation between children from the same school, which was estimated by the intracluster correlation coefficient (ICC). The assumptions of normality and homogeneity of variance was investigated. Violation of these assumptions were overcome using suitable transformation (e.g., log).

Results

Study was conducted between July 2017 to July 2018. Recruitment of all participants completed in a 4-month period. After screening and baseline assessment, each recruited participant remained in the study for 9 months to receive the interventions and undergo assessments.

The mean product compliance in the test group was 99.99% (SD 0.02, minimum 99.69% and maximum 100%). Mean compliance with both mandatory and follow up sessions of dietary counselling, with participants as well as parent/LAR was 100% in both the test and control groups.

Demographic and baseline characteristics of study participants

Figure 1 depicts the study design and disposition of the study participants. Of 958 screened participants, 924 participants were randomized to the test group and the control group in 1:1 proportion (n=462 each). Total 907 participants (98.2%) completed the study (test: n=445, control: n=462), whereas 17 (3.7%) participants did not complete the study, either due to withdrawal of consent (test: n=16 [3.5%]) or other un-specified reasons (test: n=1 [0.2%]).

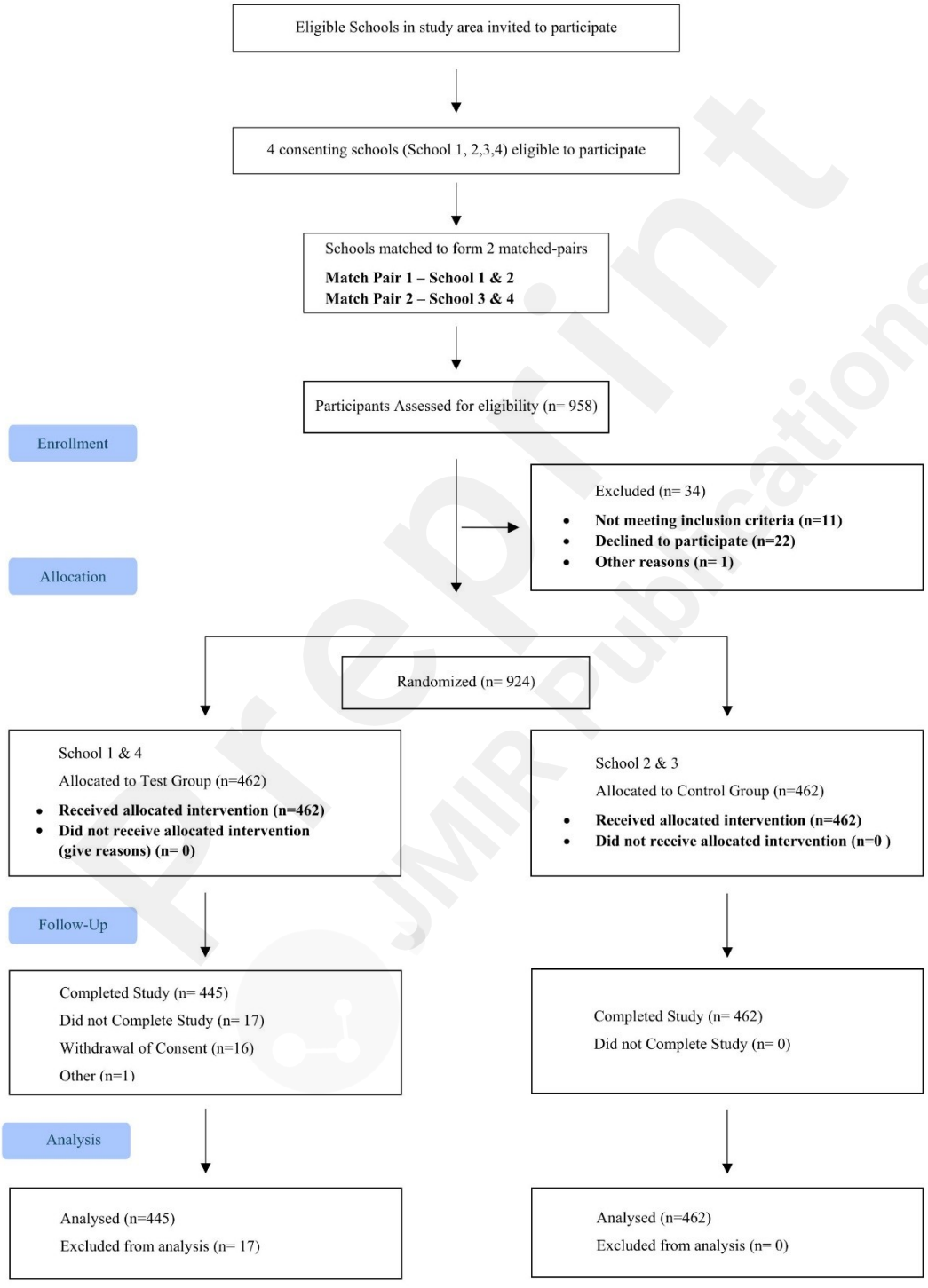
The demographic and baseline characteristics of the study participants are summarized in Table 4. All the participants had central/south Asian heritage. Both the study groups (test vs control) were comparable in terms of mean age of the participants (8.0 years vs 7.9 years), gender distribution (male: 57.4% vs 55.4%), mean HAZ (-1.52 vs -1.48), and mean BMI (15.47 kg/m^2 vs 15.12 kg/m^2).

Table 4 Demographic and baseline characteristics of study participants (safety population)

Parameters	Test group (N=460)	Control group (N=462)	Total (N=922)	*P*-value
Age, years				
Mean± SD	8.0±0.78	7.9±0.84	8.0±0.81	.06
Range	7, 10	7, 9	7, 10	
Gender, n (%)				
Female	196 (42.6)	206 (44.6)	402 (43.6)	.55
Male	264 (57.4)	256 (55.4)	520 (56.4)	
Race, n (%)				
Asian-Central/South Asian heritage	460 (100)	462 (100)	922 (100)	1.00
HAZ category				
≥ -3 to < -2	74 (16.1)	54 (11.7)	128 (13.9)	.051
≥ -2 to ≤ -1	385 (83.7)	408 (88.3)	793 (86.0)	
> -1 to ≤ 0	1 (0.2)	0	1 (0.1)	
HAZ				
Mean± SD	-1.52±0.48	-1.48±0.41	-1.50±0.44	.17
Range	-2.96, -0.45	-3.00, -1.00	-3.00, -0.45	
BMI, kg/m2				
Mean± SD	15.47±2.29	15.12±2.55	15.29±2.43	.03
Range	9.39, 23.68	10.14, 27.09	9.39, 27.09	

Abbreviations: BMI= body mass index, HAZ= height-for-age Z-scores, SD= standard deviation.

Figure 1 CONSORT flowchart of study design and participant disposition



Baseline nutrient status of study participants

Table 5 and Table 6 summarize the baseline micronutrient status of the study participants. The findings indicate a considerable burden of micronutrient inadequacy in these participants. Most frequently observed inadequacy was of vitamin D (97.51%), followed by folate (79.18%), zinc (66.05%), and vitamin A (34.27%) (Table 9). An overview of 24-hr. macronutrient intake status is depicted in Table 8. The nutritional intake status was similar between both the study groups (Table 5-Table 6).

Since the nutritional status is usually influenced by the gut health, the study participants were also evaluated for their gut integrity through the lactulose: mannitol test and urinary neopterin test. A total of 111 (12.04%) participants demonstrated abnormal lactulose: mannitol ratio (reference level: <0.035; test vs control, n [%]: 66 [14.3%] vs 45 [9.7%]), indicating malabsorption in these participants (Table 7) . On the other hand, the urinary neopterin test was normal for all the study participants across both groups (reference range: 0.10-5.00 mmol/mol creatinine).

Table 5 Summary of micronutrient status of the study participants at baseline (mITT population)

Parameters, mean±SD	Reference range[a]	Mean±SD			
		Test group (N=445)	Control group (N=462)	Total (N=907)	*P*-value
Serum vitamin A	26.00-49.00 µg/dL	29.52±6.30	28.14±5.41	28.82±5.89	<.001
Serum vitamin B12	312.00-1237.00 pg/mL	374.38±87.31	375.67±97.51	375.04±92.59	.83
Serum vitamin D	30.00-100.00 ng/mL	17.48±5.63	17.89±5.10	17.69±5.37	.25
Serum vitamin E	0.30-0.90 mg/dL	0.49±0.14	0.47±0.16	0.48±0.15	.046
Serum folate	5.00-21.00 ng/mL	4.51±0.73	4.42±0.71	4.46±0.72	.06
Serum selenium	55.00-134.00 µg/L	83.63±12.61	85.91±12.24	84.79±12.47	.01
Serum zinc	78.00-105.00 µg/dL (aged 7-9 years), 78.00-118.00 (females aged 10 years), 78.00-98.00 (males aged 10 years)	72.17±19.80	72.58±19.98	72.38±19.88	.76
Serum copper	51.00-121.00 µg/dL	117.89±15.92	117.77±16.08	117.83±15.99	.91
Serum iron	50.00-120.00 µg/dL	70.09±29.77	71.43±29.14	70.77±29.44	.49

Abbreviations: mITT= modified Intent-to-treat, SD= standard deviation[a] As per National Accreditation Board for Testing and Calibration Laboratories(NABL), India accredited lab, Intervein Lab, Ahmedabad, India.

Table 6 Summary of micronutrient inadequacy status in the study participants at baseline (safety population)

Parameter	Reference range	Status of micronutrient inadequacy, n (%)				
		Abnormality	Test group (N=460)	Control group (N=462)	Total (N=922)	*P*-value
Serum vitamin A	26.00-49.00 µg/dL	Low	146 (31.7)	170 (36.8)	316 (34.27)	.11
Serum vitamin B12	312.00-1237.00 pg/mL	Low	121 (26.3)	148 (32.0)	269 (29.18)	.06
Serum vitamin D	30.00-100.00 ng/mL	Low	449 (97.6)	450 (97.4)	899 (97.51)	1.00
Serum vitamin E	0.30-0.90 mg/dL	Low	32 (7.0)	53 (11.5)	85 (9.22)	.02
Serum folate	5.00-21.00 ng/mL	Low	354 (77.0)	376 (81.4)	730 (79.18)	.10
Serum selenium	55.00-134.00 µg/L	Low	1 (0.2)	0	1 (0.11)	.50
Serum zinc	78.00-105.00 µg/dL (aged 7-9 years), 78.00-118.00 (females aged 10 years), 78.00-98.00 (males aged 10 years)	Low	307 (66.7)	302 (65.4)	609 (66.05)	.68
Serum copper	51.00-121.00 µg/dL	Low	0	1 (0.2)	1 (0.11)	1.00
Serum iron	50.00-120.00 µg/dL	Low	133 (28.9)	121 (26.2)	254 (27.55)	.38

Table 7 Summary of Baseline Gut Integrity/Health using Lactulose:Mannitol Test and Urinary Neopterin Test (mITT Population)

Parameter	Mean value (SD) (Overall range min-max)		*P*-value
	Test group	Control group	
Lactulose Mannitol Test	0.0504 (0.19891) (0.000 - 2.200)	0.0218 (0.01150) (0.000 - 0.132)	.002
Urine Neopterin Test	0.419 (0.3967) (0.10 - 2.78)	0.388 (0.4410) (0.10 - 4.22)	.27

Table 8 Summary of macronutrient status of the study participants at baseline (mITT population)

Parameters, mean±SD	RDA (31)	Mean±SD					*P*-value
		Test group (N=445)	%RDA	Control group (N=462)	%RDA	Total (N=907)	

Energy (Kcal/day)	1690	1395.14±305.78	82.5%	1406.08± 352.75	83.2%	1400.71± 330.40	.62
Protein (g/day)	29.5	38.84±11.88	131.6%	42.41± 13.39	143.8%	40.66± 12.79	<.001
Carbohydrates (g/day)	NA	189.48±50.75		202.39± 55.08		196.05± 53.36	<.001
Fats (g/day)	30	47.52±16.57	158.4%	40.03± 16.41	133.4%	43.71±16.89	<.001

Abbreviations: RDA= recommended dietary allowance, mITT= modified Intent-to-treat, SD= standard deviation

Table 9 Prevalence of micronutrient inadequacy in Indian school-age children (current study vs literature findings)

Micronutrients	Prevalence of micronutrient inadequacies		
	Current study	Individual studies from literature	CNNS – 2019(8)
Serum vitamin A	34.27%	9.8%-43.9%[37, 56, 57]	22%
Serum vitamin B12	29.18%	7.4%-67.2% [37, 58,59]	17%
Serum vitamin D	97.51%	14.2%-93.0%[37, 57-63]	18%
Serum vitamin E	9.22%	No data found	Not reported
Serum folate	79.18%	1.5%-99%[37, 58, 64, 65]	28%
Serum selenium	0.11%	No data found	Not reported
Serum zinc	66.05%	0.7%-57.1% [37, 65, 66]	17%
Serum copper	0.11%	No data found	Not reported
Serum iron (serum ferritin)	27.55%	24.1%-54.5%[59, 65]	17%

Abbreviations: CNNS= Comprehensive National Nutrition Survey

Discussion

The current study revealed concerning facts about nutritional and growth parameters in the apparently healthy school-age children. The biochemical statuses of many micronutrients, namely vitamin A (34%), vitamin B12 (29%), vitamin D (97%), folate (79%), zinc (66%) and iron (28%) were observed to be inadequate in the children. In this study we also assessed gut health of the study participants. We observed abnormal lactulose: mannitol ratio in a substantial percentage of the (12.04%) study population. This indicates compromised intestinal permeability, thus affecting their nutrient absorption.

Current study recorded a high compliance of 99.99% with the test product, with a high retention rate of 98% (907 out of 924). Maintaining similar influence and motivation among children and parents, particularly in open-label individual RCT could have been very challenging. However, this being a cluster/school level intervention, peer influence could have helped improve compliance and adherence. Besides, strategical measures employed in the study including weekly compliance monitoring, regular meeting and briefing parents in school, random home visits, telephonic follow up with non-responders or during out-of-station travels helped make impact on retention.

Statistical implications of this study's cluster RCT design necessitated inflating the sample size by 1.5 times of the number needed for an individual RCT. Despite a relatively large sample size and a 9-month long duration, study was administratively more efficient because of more logistical convenience resultant because of operational ease of administration of study intervention at cluster level.

Magnitude of nutritional inadequacies observed in this study were in line with most of previous published literature (Table 9), re-affirming the nutritional concerns in Indian school-age children [9,37,56-66]. However, when compared with CNNS estimates, observed magnitude of vitamin D, folate and zinc deficiencies were relatively quite high. This may be due to, higher cut offs for serum vitamin D, folate and zinc used in the current study when compared to CNNS. Cut offs in current study vs CNNS are as follows: vitamin D (30 ng/ml vs 12 ng/ml), folate (5 ng/ml vs 4 ng/ml, equivalent to cut off 151 ng/ml of RBC folate used in CNNS) and zinc (78 mcg/ml vs 65 mcg/ml). As a result, a greater number of children were included under nutrient deficient group in the current study. Moreover, due to sedentary and indoor lifestyle, vitamin D deficiency is known to occur more commonly among children in urban setting [67,68], as in the current study conducted in participants residing in urban setting of Pune city.

In this study we also assessed gut health of the study participants. There is paucity of prevalence data on this parameter in apparently healthy children particularly those not suffering with diarrhea. We observed abnormal lactulose: mannitol ratio in a substantial (12.04%) study population. This indicates compromised intestinal permeability, thus affecting their nutrient absorption.

This was one of the largest nutritional intervention trials conducted with matched-pair cluster RCT design, on Indian school-age children. Cluster trials though are conceptually able to improve acceptability and adherence to an assigned intervention, but these qualities are rarely assessed and reported in cluster trial publications [69,70]. In a long nutritional intervention trial, consumption fatigue or boredom can set in, resulting in poor compliance and subsequently attrition from the study. Current study recorded a high compliance with the test product, with a high retention rate. Maintaining similar influence and motivation among children and parents, particularly in open-label individual RCT could have been very challenging. However, this being a cluster/school level intervention, peer influence could have helped improve compliance and adherence. Besides, strategic measures employed in the study including weekly compliance monitoring, regular meeting and briefing parents in school, random home visits, telephonic follow up with non-responders or during out-of-station travels helped make impact on retention.

Limitations

Open label design used in the study had a huge potential to pose threat on validity by introducing potential biases associated with such design. But this was curtailed significantly by using specific and objective criteria of diagnosing and recording morbidities by physicians. Besides, results based on biochemistry were also objective by nature. Additionally, access to the treatment code was appropriately restricted during the process of blinded data review involving clinical research scientists and statisticians. Study involved random allocation of clusters (i.e., schools), however participants' selection within school was not random as obvious with cluster RCT design. Prior

knowledge of treatment could still have led to recruitment bias which cluster RCT are usually vulnerable to [71]. However, the study did not seem to have been impacted by this bias, as shown by the high screening to enrolment ratio with a low proportion of participants refusing consent during screening in the both control group (1.3%) and test group (3.3%).

Although study groups were matched for overall socioeconomic status, the extent to which individual participants were matched was not ascertained. There was always a chance that potential socioeconomic status differences and associated factors e.g., access to healthcare, use of medications, hygiene, and sanitation practices had the potential to confound the results. This was minimized by harmonizing treatment of all morbidities by similarly trained study physicians in school itself as a part of adverse event management. It was known that the use of medications could impact study outcomes, particularly ill days, school absenteeism, and illness severity. Thus, the medications (e.g., antibiotics, antipyretics etc.) were standardized across all study sites, and were thus eventually similar across both study groups. Since there were different study physicians at all four sites, an inter-observer bias cannot be excluded. This was mitigated by making objective study endpoints and the physician's discretion was used only while ruling out the allergic etiology of the illnesses. Also, training of study physicians was harmonized with respect to quality, content, and duration.

Cluster randomization has statistical implications. The sample size was adjusted and planned to be increased 1.5 times that needed for an individual RCT. In the primary analysis, the observed ICC, which measures the relatedness of clustered responses, was higher than anticipated, corresponding to a design effect more than ten times higher than the protocol assumption. It is, however, comparable with estimates from the literature [72]. A higher number of clusters, with the same overall number of participants, could have increased statistical power. However, fewer clusters were used in the study due to practical reasons.

Conclusions

The study findings highlight the extent of nutritional inadequacies in Indian urban school-age children, re-affirming the need for nutritional strategies to optimize the nutritional status among these children.

This study suggested that cluster RCT design can be effectively used in nutritional intervention trial. The design mitigates the ethical issues inherent with double-blind individual RCT in children involving daily administration of empty-calorie placebo. The strategies employed to achieve a high recruitment and retention rates is valuable for the conduct of future studies in a similar population.

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USA; Global Medical Affairs Manager, Alliance Pharmaceuticals, Chippenham, UK; Advisor, Hindustan Unilever Limited, India respectively

Author Contributions: VR, PV and VG were mainly involved in the design of the study. AK was involved in investigation, methodology and review of manuscript. JB was involved in planning, quality check and supervising clinical study conduct. DC drafted the original manuscript. The final version of the manuscript was carefully reviewed and approved by all authors. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

All authors except AK were part of Funder GSK Consumer Healthcare (now Hindustan Unilever Limited) during this clinical study conduct phase. Dr Anuradha Khadilkar was Principal Investigator for this clinical study and part of clinical study center.

Abbreviations

AE – Adverse Event

BMI – Body Mass Index

CTCAE - Common Terminology Criteria for Adverse Events

CNNS - Comprehensive National Nutrition Survey

DE – Design Effect

eDMS - Electronic Document Management System

FMBF – Fortified Malt Based Food

GSK CH – GlaxoSmithKline Consumer Healthcare

GI - Gastro-Intestinal

HAZ - Height-for-Age Z-scores

Hb - Hemoglobin

ICC- Intracluster Correlation Coefficient

IDDS - Individual Dietary Diversity Score

ITT - Intent-To-Treat

LAR – Legally Acceptable Representative

mITT - Modified Intent-To-Treat

NABL- National Accreditation Board for Testing and Calibration Laboratories

RCT – Randomized Controlled Trial

SPWR – Study Physician Weekly Review

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Supplementary Files

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Figures

CONSORT flowchart of study design and participant disposition.