PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prevalence of specific micronutrient deficiencies in urban school going children of India aged between 6-16 years: study protocol for a multicentric cross-sectional study
AUTHORS	Awasthi, Shally; Kumar, Divas; Singh, Shweta; Dixit, Swati; Agarwal, Girdhar; Mahdi, Abbas

VERSION 1 – REVIEW

REVIEWER	Raina, Sunil Kumar Dr Rajendra Prasad Government Medical College, Community Medicine
REVIEW RETURNED	14-Jan-2021

GENERAL COMMENTS	1. Whether the association of Calcium, Iron, Zinc, Selenium, Folic acid, Vitamin A, 25 Hydroxy Vitamin D and Vitamin B12 levels can be correlated with a three-day dietary intake assessed by 24 hours recall method, given their transient levels in the body. (Secondary objective D) 2. Role of physical activity on cognition; the cognitive assessment may vary not just due to diet but levels of physical activity, especially in children who are between age group 6-16 years. (Secondary objective C) 3. Will they consider government and private schools separately as the levels of micronutrients in children from that from the government schools might be partially attributable to schemes like mid-day meal, WIFS. 4. Limitation: Recall bias in 24-hour recall method

REVIEWER	Trilok-Kumar, Geeta Dehli University, Institute of Home Economics
REVIEW RETURNED	06-Feb-2021

GENERAL COMMENTS	The authors have submitted the study protocol of a cross sectional multicentric study on children aged 6-11 years and 12-16 years to collect data on anthropometry, diet, micronutrient status and cognition for publication. While the authors have planned a large study and such multicentre studies entail a lot of hard work, such a study is only

	useful if planned and implemented well and could be very useful. The protocol requires a lot of work and is far from being published. Hence my main comments are on the quality of protocol and relevance of the study that could not be addressed in the questions in the review pane. QUALITY OF PROTOCOL ***Background*** The references giving the brief rationale of the study in Background are old (2003, 2007, 2014) and a lot of data has been published after this period. ***Methods*** A clear hypothesis is completely missing. Methods are sketchy and lack clarity. However since the study is already ongoing since 2019 and expected to be completed in 2021 and none of this can be modified, I will refrain from detailed commenting on the study design and methods. Some important observations and errors are: It is unclear why the age group of 6-16 years is selected. No rationale for using this age group is seen and it is an unusual division and hence will lack comparability to any other study. The basis of selection of various micronutrients is also unclear especially the inclusion of Lead and Selenium in the list. Sample size calculations are not mentioned and statistical considerations are poorly covered. Table 1 is inserted but not referred to anywhere in the text. Hence it's unclear why it is there. Also the source of the table is unacknowledged and definition of obesity and thinness (not commonly used) are missing. Finally, the table divides children in three groups from 5-9, 10-14 and 15-19 years making it redundant in the context of this study. Distribution/selection of study sites is based on Figure 2 in the methods; however the figure is missing. Quality control in anthropometry (eg TEM) or in laboratory methods (external and internal QC) are missing. Details of lab methods to the extent given in the protocol are unnecessary. Bibliography Most references are old and outdated. RELEVANCE OF THE STUDY IN THE INDIAN CONTEXT

	The Comprehensive National Nutrition Survey (CNNS) data on children from birth to 19 years with all the outcome variables the authors mention is available on the Government of India website on public domain since 2018. The publications of this well conducted survey are out. This makes this study redundant and possibly reinventing the wheel.

REVIEWER	Engle-Stone, Reina University of California Davis
REVIEW RETURNED	29-Mar-2021

GENERAL COMMENTS	This manuscript describes the protocol for a cross-sectional study to assess nutritional status, dietary intake, anthropometry, socioeconomic status, and cognition among urban school-going children 6-16 years of age in 10 cities in India. Despite the importance of nutritional status for health, limited data are available on micronutrient status of children, particularly school-age children and adolescents. This study will help fill an important gap in knowledge on nutritional status of children in this age group in India. Interpretation of the data may be complicated by the COVID-19 pandemic, as data collection began in April 2019 and appears to still be ongoing 2 years later. It is not clear from the current manuscript whether data collection is taking place at the same rate in all sites, or if the dates of data collection are very different across sites. It will be useful to present this information on the timing of data collection at each site to aid in interpretation of the data. The authors are strongly encouraged to measure markers of inflammation (such as C-reactive protein and alpha-1-acid glycoprotein) in the collected blood samples to assist in interpretation of biomarkers of iron, vitamin A, and zinc (please see Thurnham et al, AJCN 2010 and publications from the BRINDA project: https://brinda-nutrition.org/). Without this information and appropriate statistical adjustment, the results could underestimate prevalence of iron deficiency and overestimate prevalence of zinc and vitamin A deficiency. Specific comments related to clarity of the protocol are provided below. In addition, some editing is needed for minor grammatical errors. Page 2, lines 11-13: Ferritin is an indicator of iron deficiency and so having both is redundant. To describe deficiency, it is more appropriate to refer to ‘folate’ rather than ‘folic acid’ (same applies throughout the paper). Page 2, abstract methods: It is not clear from the abstract if the sample will be representative of a specific geographic area. The introduction focuses on malnutrition and does not explain the rationale for measuring lead. The introduction would also be strengthened by including some discussion of existing literature on the relationships between nutritional status and cognition, as well as between nutritional status biomarkers and dietary intake, to illustrate how the comparisons in this study would build on this literature.

	Methodology Page 4, line 29: Are protocol numbers and committee names available from the ethical committees? Table 1. Please add data sources and variable definitions (for thinness and obesity) in footnote. Page 6, line 12-15: It is not clear why both iron and ferritin are mentioned, since ferritin is an indicator of iron status. Page 7 line 1 – Suggested additional information to address with regard to sample size description: Why was the overall sample size based on that estimated for folate deficiency? Were calculations performed for all other outcomes as well? The authors note in the introduction that anemia prevalence could range from 19-88%. If this is correct, the authors might consider including the precision for the "worst case scenario" of 50% prevalence. The authors also mention assessing the relationship between micronutrient status and other variables, such as cognition and dietary intake. How does the sample size relate to statistical power for those comparisons? Lastly, if stratified analyses will be conducted (e.g., by site) it would be useful to include estimates of precision for these stratified analyses. Page 7 line 16: Given that recruitment will be conducted in schools, in each city, what proportion of children 6-16 years of age attend school? Page 7, lines 18-21. The manuscript describes random selection of schools from a list, but also appears to describe having a minimum and maximum quota for private schools. More detail would be helpful to clarify how the selection will be done. Page 7, lines 24-28. Suggestions for additional detail to provide: Who will determine whether students are 'apparently healthy', and when and how? BMI < 12.5% is listed as an exclusion criterion – does this refer to BMI < 12.5 or the 12.5th percentile? When will BMI be measured? Will referrals be provided for medical evaluation for children who are excluded due to low BMI? Page 9, line 30 – Where will dietary recalls be conducted? Why have the authors selected the nutrient adequacy ratio to assess dietary nutrient adequacy, rather than other approaches to assess adequacy? Will statistical adjustment be conducted to estimate usual/habitual intake distributions? In the data analysis section, I do not see mention of how day-to-day, within-person variation in dietary intake will be considered in descriptive analyses as well as assessment of the relationship between dietary intake and nutritional status. Page 10, line 30. Will time of day of blood collection be standardized or recorded? Will IZiNCG precautions be followed to minimize risk of zinc contamination? Will aliquots for vitamin analysis be protected from exposure to light?

	What is the transit time from each site to the site of CBC measurement, and are there any concerns about the time required for transit? Regarding the laboratory methods, what (if any) standard reference materials will be analyzed to confirm accuracy of the methods? Page 12, line 27. Please confirm whether the ELISA for vitamin A or for retinol-binding protein (RBP) as a marker of vitamin A status? Typically, vitamin A would be measured by HPLC or spectrophotometry, and RBP by ELISA.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1			
Sr. No.	Comments	Response	Page no. Line no.
1	Whether the association of Calcium, Iron, Zinc, Selenium, Folic acid, Vitamin A, 25 Hydroxy Vitamin D and Vitamin B12 levels can be correlated with a three-day dietary intake assessed by 24 hours recall method, given their transient levels in the body. (Secondary objective D)	Various studies across the globe had tested the association between nutritional status biomarkers and dietary intake. Some significant association or role in progression of various malnutrition issues are already established. Plasma concentrations of vitamin B12 and folate are found to be associated with dietary intake provided that gender, age and energy intake are taken into account and this association is independent of physical activity of individual. A similar significant positive correlation is found between selenium intake and its blood level. Although some authors consider the nutritional assessment as a practical, noninvasive, and cost-effective tool for rapid nutritional evaluation, but others recommend concurrent collection of biological specimens to estimate levels of dietary biomarkers, so as to overcome possible sources of error, indigenously associated with every method of dietary intake assessment.	Page no.: 4 Line no.:10 -19

2	Role of physical activity on cognition; the cognitive assessment may vary not just due to diet but levels of physical activity, especially in children who are between age group 6-16 years. (Secondary objective C)	We agree with the reviewer. The cognitive performance may vary due to multiple factors like diet, physical activity, socialization etc. However, the current study is primarily focusing on specific micronutrients in detail and it is beyond the scope of this study to undertake other factors affecting cognitive performance. But this may, definitely be scaled up from here to plan future studies accordingly.	-
3	Will they consider government and private schools separately as the levels of micronutrients in children from that from the government schools might be partially attributable to schemes like mid-day meal, WIFS.	This is a very important suggestion by esteemed reviewer. Analysis of micronutrients level may be done between two groups i.e. participants from government and private schools.	-
4	Limitation: Recall bias in 24-hour recall method	Yes, as with other validated tools of nutritional assessment, the 24-hour Dietary Recall (24HR) also has some of its limitations like recall bias and dependency on interviewer's skills. To minimize these data will be collected by qualified and trained nutritionists, from the participants along with their primary care givers which will help in precision. Also, the data has to be collected for three days, so participants will be requested to maintain a record to overcome recall bias.	Page no.: 15 Line no.:18 -20

Reviewer 2			
Sr. No.	Comments	Response	Page no. Line no.
1	The references giving the brief rationale of the study in Background are old (2003, 2007, 2014) and a lot of data has been published after this period.	We have tried to update this section with recently published work.	Page no.: 4 Line no.:19
2	A clear hypothesis is completely missing.	This is a survey, so no hypothesis is drawn.	-

3	Methods are sketchy and lack clarity.	We have updated the section.	-
4	It is unclear why the age group of 6-16 years is selected. No rationale for using this age group is seen and it is an unusual division and hence will lack comparability to any other study.	6 to 16 years is the school age period which is nutritionally significant because this is the prime time to build up body stores of nutrients in preparation for rapid growth of adolescence. However, we will be capturing actual age of participants, based on which comparability with other studied may be established.	-
5	The basis of selection of various micronutrients is also unclear especially the inclusion of Lead and Selenium in the list.	Lead is a known toxic agent. Elevated blood lead level causes anemia as well as detrimental effect on cognitive performance which in turn effects school performance of the child. Selenium, a potent antioxidant, is known to influence the process of synaptogenesis, myelination, and neuronal cell differentiation by regulating thyroid hormones. Much data is not available on Lead and selenium in Indian children and adolescents, that is why we have included these two in our list.	-
6	Sample size calculations are not mentioned and statistical considerations are poorly covered.	Sample size calculations are mentioned on page 7. The formula used is the standard one " Sample size for prevalence with a given precision" as follows: $n = \frac{z^2_{1-\alpha/2}}{d^2}$ Here: p = 0.307, d= 0.02, and α = 0.05. Statistical considerations are given on page 13 and 14. Since here main objective is to find prevalence, we shall be calculating proportion along with their respective confidence intervals. We have made changes in section" Data Management and Statistical analysis".	Page no.: 7 Line no.:9-13 Page no.: 13 Line no.:30-32 and Page no.: 14 Line no.:1-10

7	Table 1 is inserted but not referred to anywhere in the text. Hence it's unclear why it is there. Also the source of the table is unacknowledged and definition of obesity and thinness (not commonly used) are missing. Finally, the table divides children in three groups from 5-9, 10-14 and 15-19 years making it redundant in the context of this study.	Table 1 shows demographic characteristics and key anthropometric indicators in urban areas of study site districts. It is referred in the text on by reference numbers 26 and 27. Obesity is defined as "BMI for age >+2 SD of the WHO Child Growth Standards" and severe thinness is defined as "BMI for age < –3 SD of the WHO Child Growth Standards". Both the definitions are given in table 2, but as advised by esteem reviewer they are also added as footnote in table 1. Three age groups as given in table are based on the source of table. As mentioned earlier we will be capturing actual age of participants, based on which comparability with other studied may be established.	Page no.: 5 Line no.:8
8	Distribution/selection of study sites is based on Figure 2 in the methods; however the figure is missing.	Figure 2 is given as a separate file named "Figure 2".	
9	Quality control in anthropometry (eg TEM) or in laboratory methods (external and internal QC) are missing.	Anthropometry data will be collected by trained and qualified nutritionists in accordance with Standard Operating Procedures developed specifically for the study. Routine Calibration of equipment along with the resampling of participants will be done. We have written this as "Quality of anthropometric measurements will be assured by routinely calibrating the equipment along with the resampling of participants." Biochemical analysis quality control is written as "Internal quality assurance of bio chemical analysis will be done by using calibrated instruments and analyzing test specific standards. For interlaboratory comparison of the test results, 10% of the total samples will be sent to peer laboratory."	Page no.: 13 Line no.:22-23 Page no.: 13 Line no.:26-29
10	Details of lab methods to the extent given in the protocol are unnecessary.	We have tried to revise this section as advised by esteemed reviewer.	-
11	Most references are old and outdated.	We have tried to include more of the latest work.	-

12	RELEVANCE OF THE STUDY IN THE INDIAN CONTEXT The Comprehensive National Nutrition Survey (CNNS) data on children from birth to 19 years with all the outcome variables the authors mention is available on the Government of India website on public domain since 2018. The publications of this well conducted survey are out. This makes this study redundant and possibly reinventing the wheel.	We understand that Comprehensive National Nutrition Survey (CNNS) was published in 2019. At the time of protocol development, it was not there. CNNS gives data on anemia, iron, folate, Vitamin B12, Vitamin A, Vitamin D, Zinc deficiencies. We are also collecting data on calcium, selenium and lead. In addition, we are seeing association of micronutrient deficiencies with cognitive performance and three-day dietary intake, which will be a knowledge addition to the existing data.	-

Reviewer 3			
Sr. No.	Comments	Response	Page no. Line no.
1	Interpretation of the data may be complicated by the COVID-19 pandemic, as data collection began in April 2019 and appears to still be ongoing 2 years later. It is not clear from the current manuscript whether data collection is taking place at the same rate in all sites, or if the dates of data collection are very different across sites. It will be useful to present this information on the timing of data collection at each site to aid in interpretation of the data.	India is a country with large geo-cultural diversity. We have selected the study sites in view of this diversity to get a nationally representative sample. School calendar of one state differs from that of others depending on geography, culture and climate. Data collection across the sites will be at different time points depending on school calendar of their respective state. As advised by esteemed reviewer we have added “Since, the school calendar of different states of India varies from another owing to their specific geography, culture and climate, time frame of data collection across the sites also varies accordingly.”	Page no.: 8 Line no.:7-9
2	The authors are strongly encouraged to measure markers of inflammation (such as C-reactive protein and alpha-1-acid glycoprotein) in the collected blood samples to assist in interpretation of biomarkers of iron, vitamin A, and zinc (please see Thurnham et al, AJCN 2010 and publications from the BRINDA project: https://brinda-nutrition.org/). Without this information and appropriate statistical adjustment, the results could underestimate prevalence of iron	We agree with the esteemed reviewer. But measurement of markers is beyond the scope of this study due to logistical issues.	-

	deficiency and overestimate prevalence of zinc and vitamin A deficiency.		
3	Page 2, lines 11-13: Ferritin is an indicator of iron deficiency and so having both is redundant.	We agree with the reviewer.	
4	To describe deficiency, it is more appropriate to refer to 'folate' rather than 'folic acid' (same applies throughout the paper).	Done as advised.	-
5	Page 2, abstract methods: It is not clear from the abstract if the sample will be representative of a specific geographic area.	We have now written this as "A multicentric cross sectional study will be conducted to recruit 2400 participants (240 per site) across India."	Page no.: 2 Line no.:12
6	The introduction focuses on malnutrition and does not explain the rationale for measuring lead. The introduction would also be strengthened by including some discussion of existing literature on the relationships between nutritional status and cognition, as well as between nutritional status biomarkers and dietary intake, to illustrate how the comparisons in this study would build on this literature.	This is now written as "There is a consensus on the fact that iron deficiency has a negative impact on cognition, behavior, and motor skills. It has been found that, hemoglobin though correlates to cognitive performance, iron supplementation improves cognitive functions regardless of the hemoglobin levels (9). Vitamin B12 deficiency is associated with poor cognitive development e.g. episodic memory and language ability (10) and growth in children (11). Zinc, Iron, folate, iodine, B12 and protein deficiency can also result in low IQ (12) and deficits in attention, learning, memory, and neuropsychological behavior (13,14). Lead, a well-known toxic heavy metal, though widely discontinued in many countries of world, is still a public health problem in developing countries like India. Worldwide every year 0.6 million cases of childhood intellectual disabilities are attributed to lead exposure. Iron deficiency, which is common in children can enhance lead absorption (15). Various studies across the globe had tested the association between nutritional status biomarkers and dietary intake (16-20). Some significant association or role in progression of various malnutrition issues are already established. Plasma	Page no.: 3 Line no.:30 -31 And Page no.: 4 Line no.:1-19

		concentrations of vitamin B12 and folate are found to be associated with dietary intake provided that gender, age and energy intake are taken into account and this association is independent of physical activity of individual (21). A similar significant positive correlation is found between selenium intake and its blood level (22). Although some authors consider the nutritional assessment as a practical, noninvasive, and cost-effective tool for rapid nutritional evaluation (23), but others recommend concurrent collection of biological specimens to estimate levels of dietary biomarkers, so as to overcome possible sources of error, indigenously associated with every method of dietary intake assessment (24)."	
7	Page 4, line 29: Are protocol numbers and committee names available from the ethical committees?	Yes. The names of all Ethics committees of all sites and their respective approval reference numbers are now given in Ethics and dissemination section. It is now written as "The study is approved by the Institutional Ethics Committee for MS Ramaiah Medical College and Hospital Bangalore (approval reference number (ARN): MSRMC/EC/AP-02/02-2019), Institutional Ethics Committee for Kalinga Institute of Medical Sciences Bhubaneswar (ARN: KIMS/KIIT/IEC/112/2016), Institutional Ethics Committee for PGIMER Chandigarh (ARN: PGI/IEC/2019/000152), Institutional Ethics Committee (H) Assam Medical College (ARN: AMC/EC/1430), Institutional Ethics Committee for All India Institute of Medical Sciences Jodhpur (ARN: AIIMS/IEC/2017/765), Institutional Ethics Committee for King Georges Medical University (ARN: 9334/Ethics/R.Cell-16), Institutional Ethics Committee for Kasturba Medical College (ARN: IEC:388/2019), Institutional Ethics Committee for All India Institute of Medical Sciences Patna (ARN:	Page no.: 14 Line no.:11 -27

		IEC/AIIMS/PAT/153/2017), Institutional Ethics Committee for Sher-i-Kashmir Institute of Medical Sciences (ARN: IEC/SKIMS Protocol # RP 175/2018) and Human Ethics Committee Medical College Thiruvananthapuram (ARN: HEC.No.04/34/2019/MCT). The study is registered prospectively with Clinical Trial Registry of India (registration number CTRI/2019/02/017783). Written informed consent will be obtained from parents of all study participants. Findings will be disseminated with stakeholders and will be presented in national and international conferences. Results will be published in a peer-reviewed journal".	
8	Table 1. Please add data sources and variable definitions (for thinness and obesity) in footnote.	Done as advised. Source of table added as reference number 26 and 27. Obesity is defined as "BMI for age >+2 SD of the WHO Child Growth Standards" and severe thinness is defined as "BMI for age < –3 SD of the WHO Child Growth Standards". Both the definitions are given in table 2, however as advised by esteem reviewer they are also added as footnote in table 1.	Page no.: 5 Line no.:8
9	Page 6, line 12-15: It is not clear why both iron and ferritin are mentioned, since ferritin is an indicator of iron status.	We agree with esteemed reviewer, However, we have mentioned ferritin as we have not included other inflammatory markers like C-reactive protein and alpha-1-acid glycoprotein, due to logistical issues.	
10	Page 7 line 1 – Suggested additional information to address with regard to sample size description: Why was the overall sample size based on that estimated for folate deficiency? Were calculations performed for all other outcomes as well? The authors note in the introduction that anemia prevalence could range from 19-88%. If this is correct, the authors might consider including the precision for the "worst case scenario" of 50% prevalence.	Here we have calculated the sample sizes for other micronutrient deficiencies also and have picked the one (corresponding to folate deficiency) with maximum sample size. We have to calculate the sample size corresponding to one of the micronutrient deficiencies, not anemia. If we calculate the sample size taking P= 0.5, d (precision) = 0.025, the sample size comes to be 1537, which is less than the one used by us.	

11	The authors also mention assessing the relationship between micronutrient status and other variables, such as cognition and dietary intake. How does the sample size relate to statistical power for those comparisons?.	As mentioned above, the relationship will be assessed by different kind of association and correlation coefficients. We shall calculate the precision for these coefficients when all the data shall be collected and analysis will be performed. Power shall be also calculated for different statistical tests with actual data.	
12	Lastly, if stratified analyses will be conducted (e.g., by site) it would be useful to include estimates of precision for these stratified analyses	Yes, we shall be doing the stratified (Site-wise) analysis to find pointwise estimates along with their confidence intervals. We have now stated this in Statistical analysis section as "Data will be entered in MS excel (Double data entry), matched electronically and discrepancies will be rectified by referring the source documents. Point estimates and confidence intervals of proportions of different micronutrient deficiencies shall be evaluated. These estimates shall be found for overall proportion as well as city-wise proportion. To assess the association of micronutrient deficiencies (continuous variables) with anthropometric measures (height and weight), Pearson's correlation coefficient shall be used along with their confidence intervals. To assess the association of micronutrient deficiencies (continuous variables) with cognitive assessments (categorical variables), analysis of variance shall be employed. The dietary intake, for each participant, is recorded for three days, which will be converted into nutritional value using DIETSOFT software. Using the three observations as repeated measurement for each participant, the appropriate "descriptive" as well as "inferential" analysis shall be done. The hierarchical (nested) linear model shall be used for analyzing repeated measures, or longitudinal data."	Page no.: 13 Line no.:30 -32 And Page no.: 14 Line no.:1-10
13	Page 7 line 16: Given that recruitment will be conducted in schools, in each city, what proportion of children 6-16 years of age attend school?	Assuming that children of this age group attain education in Class I to XII, 260 million* children were enrolled in school (2015-16), which is 89.7% of 294 million** children of age group 6-17 years. Reference: * ESAG-2018.pdf (education.gov.in), Page No. 8, Table no. 7. **ESAG-2018.pdf (education.gov.in), Page No. 4, Table no. 3.	

14	Page 7, lines 18-21. The manuscript describes random selection of schools from a list, but also appears to describe having a minimum and maximum quota for private schools. More detail would be helpful to clarify how the selection will be done.	This has now been written as “From this list, schools will be randomized repeatedly till we get a pool of six schools having at least one to three private schools and rest of the government schools.”	Page no.: 7 Line no.:19 -21
15	Page 7, lines 24-28. Suggestions for additional detail to provide: Who will determine whether students are ‘apparently healthy’, and when and how? BMI < 12.5% is listed as an exclusion criterion – does this refer to BMI < 12.5 or the 12.5th percentile? When will BMI be measured? Will referrals be provided for medical evaluation for children who are excluded due to low BMI?	An apparently healthy child is one without any history of illness or disease, as reported by coordinating teacher at the time, when study team in procuring the gender-wise list of students between 6 to 11 and 12 to 16 years of age in the school. It BMI less than 12.5. same has been updated in the manuscript. BMI will be measured after obtaining written informed consent and assent (where applicable). Parents of children having BMI less than 12.5, will be advised for a medical evaluation. This has been now written as “Participants having body mass index (BMI) below 12.5 will be excluded from the study and their parents will be advised to have a medical evaluation.” And “Any study specific assessment will be done after obtaining written informed consent”.	Page no.: 7 Line no.:28 -31
16	Page 9, line 30 – Where will dietary recalls be conducted? Why have the authors selected the nutrient adequacy ratio to assess dietary nutrient adequacy, rather than other approaches to assess adequacy? Will statistical adjustment be conducted to estimate usual/habitual intake distributions? In the data analysis section, I do not see mention of how day-to-day, within-person variation in dietary intake will be considered in descriptive analyses as well as assessment of the relationship between dietary intake and nutritional status.	Dietary recalls will be conducted at homes of the participants along with their primary care givers. We have now written this as “The intake will be recorded by interviewing participant along with his/her mother or primary caregiver, preferably at their home. We are using Nutrient Adequacy Ratio along with other tools like Food Frequency Ratio, Diet Diversity Score and 24 hour dietary recall method. Data derived from all the tools will be comprehended together. Existing literature showed the positive and significant correlation between Diet Diversity Scores and most of the Nutrient Adequacy Ratios. For each participant, food intake for three days is recorded. The dietary intake is converted into nutritional value using DIETSOFT software. Using the three observations as repeated measurement for each participant, the appropriate “descriptive” as well as “inferential” analysis shall be done. The hierarchical (nested) linear model shall be used for analyzing repeated measures, or longitudinal data.	Page no.: 9 Line no.:25 -27

17	Page 10, line 30. Will time of day of blood collection be standardized or recorded? Will IZiNCG precautions be followed to minimize risk of zinc contamination? Will aliquots for vitamin analysis be protected from exposure to light?	Blood samples will be collected in early school hours and collection date and time will be recorded. Yes definitely, we have employed all the precautions to minimize the risk of trace metal contamination and exposure to light. Section on Blood sample collection, processing and storage has now written as "Blood sampling will be done at school in presence of parent/s where available, during early school hours, by trained phlebotomists. Venous blood sample of 6 ml (4 ml in clot activator and 2 ml in EDTA) will be collected using vacuum-tube systems using a certified stainless steel hypodermic needle, preferably from cubital vein. Measures to prevent and counter any adverse event like syncope, hematoma or swelling will be adequately employed and recorded. Blood sample transportation from school to study sites will be done maintaining temperature of 2°C to 8°C. One ml of EDTA sample will be send to a centralized laboratory (National Accreditation Board for Testing and Calibration Laboratories (NABL) and The College of American Pathologists (CAP) accredited) for complete blood count (CBC) assessment, through a professional agency having experience in handling and air-transportation of blood samples. The CBC assessment will include estimation of hemoglobin, hematocrit, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, RDW-CV, platelet count, total leucocyte count, differential leucocyte count and absolute leucocyte count. Report of CBC assessment will be given to parents with relevant advice by site investigators. Rest of the samples will be processed at site to separate plasma, serum and packed cells, in a trace element free area. Plasma and serum will be stored in trace element-free cyro tubes, below -20°C and packed cells between 2°C to 8°C, at the study sites, with restricted access. Samples from study sites to CCU will be transported in two batches of 120 each, maintaining required temperatures. Sample transportation will be managed by professional agencies having expertise in handling and shipment of bio-medical samles. Samples will be	Page no.: 10 Line no.:25 -30 And Page no.: 11 Line no.:1-16

		prevented from exposure to light during this whole process.”	
18	What is the transit time from each site to the site of CBC measurement, and are there any concerns about the time required for transit?	As mentioned in point no. 17, we are looking to complete the sample collection activity by mid noon, so that they reach the central laboratory same day in controlled temperature. For this transportation, a professional agency having experience in handling and air-transportation of blood samples will be deployed. This has been now written as “One ml of EDTA sample will be send to a centralized laboratory (National Accreditation Board for Testing and Calibration Laboratories (NABL) and The College of American Pathologists (CAP) accredited) for complete blood count (CBC) assessment, through a professional agency having experience in handling and air-transportation of blood samples.”	Page no.: 11 Line no.:2-5

19	Regarding the laboratory methods, what (if any) standard reference materials will be analyzed to confirm accuracy of the methods?	The test specific standard reference material will be analyzed to ensure proper functioning of the instrument and accuracy of results. Any unexpected result during test run, will initiate the analysis of cause and its rectification. Batch of such samples will be analyzed again for accuracy of results.	
20	Page 12, line 27. Please confirm whether the ELISA for vitamin A or for retinol-binding protein (RBP) as a marker of vitamin A status? Typically, vitamin A would be measured by HPLC or spectrophotometry, and RBP by ELISA.	We are looking for the retinol concentration. Competitive enzyme-linked immuno-sorbent assay-based kit will be used for vitamin A measurement. An antibody is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the biotin-labelled Retinol and the unlabelled- Retinol on the pre-coated antibody. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient Retinol will produce a blue colored product, which then changes to yellow after adding the acidic stop solution. The intensity of the color yellow is inversely proportional to the Retinol amount bound on the plate. The OD is measured spectropotometrically at 450 nm in a microplate reader, from which the concentration of Retinol can be calculated.	