PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Iron deficiency, its epidemiological features and feeding practices among infants aged 12 months in Qatar, a cross-sectional study
AUTHORS	Zainel, A.Jaleel; Osman, Sherif; Al-Kohji, Sadriya; Selim, Nagah

VERSION 1 – REVIEW

REVIEWER	Claudio Sandoval New York Medical College, USA
REVIEW RETURNED	02-Nov-2017

GENERAL COMMENTS	Dear Authors- although you address a critically important public health issue there is really nothing new about your study. I gained no new insights into the incidence of iron deficiency and its resultant anemia. a better study would have been to prospectively follow these infants from birth and procure real time data from parents on feeding practices, infections and the effects of dietary counseling. Recall bias on feeding just leads to confusion and unreliability of data. I certainly do not remember what I eat yesterday not to mention several weeks/months ago. Moreover, the questionnaire is not provided and has not been validated. Lastly, the English needs serious polishing- it was at times difficult to read and understand your paper.

REVIEWER	Ekhard Ziegler University of Iowa Iowa City USA
REVIEW RETURNED	14-Nov-2017

GENERAL COMMENTS	This is a cross-sectional study of 12-month old infants living in Quatar. The findings are within what is expected and document a high prevalence of ID and IDA. The study is a welcome addition to the literature. 1. Study sample: It is not clear how the sample size of 350 was arrived at, although it seems reasonable. What is not clear is how the final sample of 306 was arrived at. Presumably 350 were invited and 306 agreed to participate. The 350 infants, were they all the 12 month-old infants that were seen during the study period? Or were eligible infants invited consecutively until 350 was reached? What is meant by "systematic random sampling technique" (line 45, p. 4)? 2. Subjects: The authors need to do more to convince the reader that the study sample of infants is representative of all or most infants living in Quatar.

	3. Subject age: the actual age of the study subjects should be given. 4. Was blood capillary or venous? And how was hemoglobin determined? How was ferritin determined? 5. The authors should provide information about what the official recommendation was, if any, for breastfed infants: To give iron drops? From what age? To feed iron-containing foods from a certain age? The authors should also provide information on the iron content of formulas available in Quatar. 6. If hospital information was utilized, this should be stated. 7. Food frequency score: Who administered it? And what does a "Full score" mean? 8. Was permission from mothers in writing or verbal? 9. Table 4: The legend of this table (and of all other tables) should be simplified to read: "Relation between ID and food frequency score." 10. Table 1 & 2: Not necessary to footnote p=0.029 or p=0.000 as "p<0.05"; ditto for Tables 3 and 4. 11. Discussion: Direct comparison of prevalence rates is useless if different cutoff values for Hb and SF were used. (The same is not true for factors associated with ID and IDA).

REVIEWER	Asher M. Moser M.D. Ben Gurion University of the Negev, Israel
REVIEW RETURNED	16-Nov-2017

GENERAL COMMENTS	This paper by Jaleel et al offers a unique opportunity to investigate the scope of iron stores in a capsule. The advantages that seem to be relevant are the capture of a well-defined population (12 months, no range offered, please add) over a relatively short time. From what the reader is to understand is that the Qatari health system provides free health care visits for the study population: "utilization of services for periodic health appraisal and scheduled vaccination at 12 month of age is as high as 100%". This needs to be stated clearly. Otherwise, the bias is uncontrollable. Nevertheless, there are some issues that one needs to consider before this paper can be evaluated for publication: <u>Major issues:</u> The first important issue that needs to be addressed is the choice to study ID rather than IDA. While the numbers are close (24 vs.28 toddlers) they reflect what is considered a more solid consensus of the ill-effects terminating from lack of iron. On the same theme: If, as the authors claim "Anemia early in life, with or without iron depletion, is known to seriously affect children's general health and immunity, retarding their growth and development by causing multiple disorders and abnormalities in different body stems.[3-5]" than the study should test the anemia vs the non-anemia infants. This paper's analysis, by the virtue of the

	definition, included a significant number of anemic infants (44/288). One is to wonder if this skews the results. The authors need to explain what the other reasons for non-ID anemia are (e.g. thalassemia will tip the scale due to inherent iron overload). The second issue is the statistical methodology: while the authors are able to point out single factors, my initial instinct is that most of what turned out to be significant will drop out when performing multiple regressions, and we might find out that lower income alone can provide a solid explanation of the results, a factor well known by multiple studies. Another weak area that needs to be addressed is the methodology. What is the size of population from which the study population was derived? How many 12 month-old-toddlers are in registered in Qatar (per year, during the study period?)? What was the randomization method for the 14 out of 23 centers evaluated? How many mothers (no fathers at all?) were approached? How many consented to the study? What were the reasons for refusal? Did the group of consented infants represent the overall population in Qatar (e.g. Hb, BMI). If the statistical evaluation called for a sample size of at least 350, are the results still valid under the same assumptions? Why hasn't the 350 number reached? <u>Minor issues that need to be answered:</u> From table 3 one is to understand that 63 infants were exclusively breastfed at 6 months while at 12 months there are 163 who answered yes to "All or almost all feeds". That means that an overwhelming amount of mothers switched to all or almost all breastfeeding between 6 and 12 months? What is the meaning of table 4: "Overall score of food consumption"? What units are used? What is the differential meaning of plant source? What is the significance of this value if this can only relate to the 42% (121/287) of infants that are not "All or almost all feeds" for breastfeeding Table 3 relates to 8 questions: The first four have a population of 287 responses the later 4 have 306. This needs to be corrected to include all answers or provide an explanation where we lost 19 in some items. Where are the BMI values? Why is the paragraph on overweight in the discussion relevant? Grammatical issue that need to corrected/addressed • "Blood tested against anemia" • Use of "p=0.00" • "Industrialized countries" or "developing countries" -lack of consistency • "a public health problem with defined impacts on the health

	of communities especially in developing countries" – no need to single the developing – problem is widespread everywhere • "Increase consistency, quantity and frequency of foods" One does not increase the frequency of foods as the infant grows • "prolonged exclusive breastfeeding (more than 6 months) causes decrease in dietary iron intake" – not true, in total mg the intake increases • "Many studies indicated that incidence of IDA has significantly decreased over time due to promotion of breastfeeding" grammatical error? If true, this stands against the paper • "The possibility of improvement in the figures**"** Do you mean Fraud? • "Anemia is a condition in which the number of red blood cells or their oxygen-carrying capacity decreased until insufficient level to meet physiologic needs" Can you provide a reference that claims that the Number of RBC by itself is relevant Paragraphs with missing or confusing contents "A study conducted in Raparin teaching hospital for children in Erbil - Iraq, 2007, among infants aged (12 - 24) months, showed that the prevalence of iron deficiency ranged between 51.9% and 48.1% respectively. It also showed that about 53% and 30 % of participants had anemia and iron deficiency anemia respectively" "In current study, iron deficiency was more prevalent among infants started solid, semi-solid or soft food at age of 6 months or later compared to whom started before the age of 6 months. This corresponds with the results of the Estonia study, where infants fed with solid food before 6 months had statistically higher Hb values than infants whom the solid food was introduced after 6 months of life (11.8 g/dl *vs.*11.4 g/dl) (*P*<0.05).[28**"** "Regarding iron supplementation, our findings are similar with previous studies, where iron deficiency was less among infants had history of iron supplementation. These infants also had higher Hb concentration and higher MCV." "A study conducted on 126 infants divided into 3 groups; 86 cases received iron supplementation irregularly, 27 cases regularly and 13 cases never received iron. Iron deficiency anemia in the first group was 26.7%, in the second group was 3.7% and in the third group was up to 69.2% and the difference between iron deficiency anemia and iron supplementation was statistically significant (*p*< 0.001).[42" randomized or deliberate? In addition, it is very important that the authors need to spell out clearly that the IRB allowed for the extra inconvenience of the additional blood drawing that served the purpose of this study

VERSION 1 – AUTHOR RESPONSE

Response to reviewer 1
Reviewer Name: Claudio Sandoval
New York Medical College, USA

- The current study re-confirms what is already known about the subject but also provides a much needed first snapshot of the extend of problem in the State of Qatar. Such information also lacks at regional level in the Gulf Cooperation Council (GCC) States. Therefore, the manuscript adds to gaps in knowledge necessary for the purposes of planning public health interventions at national as well as regional level. The manuscript has been revised to emphasize and make this point clearer.
- The authors agree that a prospective study design would have overcome potential recall bias. However, it was not feasible to undertake one at the time due to a shortage of resources. This limitation of the study has been acknowledged in the manuscript.
- The questionnaire used for the study was developed by authors after an extensive literature review to establish content and face validity. It was thoroughly discussed and assessed by experts in the field of pediatric hematology and community medicine. A pilot using the questionnaire was undertaken with 20 participants, conveniently sampled, to establish and confirm its suitability. Revisions were made to it based on findings from the pilot.
- Questionnaire has been provided as a supplement with the revised submission of the manuscript.
- The manuscript has been revised and edited with the assistance of native speaking colleagues.

Response to reviewer 2
Reviewer Name: Ekhard Ziegler
University of Iowa, Iowa City, USA

- To calculate the sample size required for the study, the authors used the below formula
n = [DEFF x Np(1-P)]/[d2/Z21-α/2 x (N-1)+p x (1-p)].
Where:
  - N is the target population (a total of 19782 registered children completed 12 months of age at time of the study)
  - n is calculated sample population
  - P is probability or prevalence of ID among a comparable similar population of children in a similar age conducted in the United Arab Emirates = 26% (it was derived from Miller CJ, Dunn EV, Abdouni SF, Shaheen HM, Ullah MS. Factors associated with iron depletion and iron deficiency anemia among Arabic preschool children of the United Arab Emirates. Saudi Med J. 2004 Jul; 25(7): 843-7) present as number 29 in the reference list.
  - d is the acceptable error rate = 5%
  - Z is statistic for α error of 0.05 corresponding to a 95% confidence level.
- Based on the calculation using the above formula, it was estimated that a sample size of 292 was required for the study. To compensate for non-responders, an additional 20% participants were added to the sample size calculation bringing the total to 350 participants.
- Of the 350 participants identified for the study, laboratory data was available for 306 participants. The sample size was therefore above the figure required based on the sample calculation.
- 'Systematic random sampling technique' is a sampling framework. The study utilized it as an approach to identify participants from the 'well baby' clinic electronic appointment list at fourteen primary healthcare centers that provided well baby services. From the daily appointment list, the participants were systematically identified, after selection of the first participant of the day on simple

random basis. The sampling selection technique gave each of them an equal opportunity to participate.

- In the State of Qatar, the governmental primary healthcare centers are the exclusive setting for vaccinations, which are mandatory by law. Therefore all children in Qatar are registered at the primary health centers and attend the well baby clinics to receive their vaccinations. Their count at time of the study was 19782. The sample was selected from this cohort, and hence, seemed considered to be representative of all or most infants living in Qatar at within this age group.
- Participants included in the study was individuals completed 12 months and under 13 months of age,
- The blood collected was venous using vacutainer push button butterfly needle.
- Hemoglobin was determined using Sysmex 500 I machines to determine the complete blood picture and count.
- Ferritin was determined using Cobas Integra 400 plus machine. The technique was particle enhanced immunoturbidimetric assay utilizing human ferritin agglutination with latex particles coated with anti-ferritin antibodies. The precipitate is determined turbidimetrically at 570/800 nm.
- "Clinical Practice Guideline for the Management of Anemia in Children" for the primary healthcare centers in Qatar recommend as the following:

o If IDA is suspected, oral iron therapy is recommended at dose of 3 to 6 mg/kg given once or twice daily for 1 month and the parent or guardian of the child should be counseled on proper nutrition.
o Oral iron therapy dose appropriate to the age elemental iron should be given 30 to 45 minutes before meals or two hours after meals, and only with juice or water, rather than with food or milk.
o For infants who are exclusively breastfed, iron supplementation of 2mg/kg per day is recommended starting at 4 months of age
o Children diagnosed with IDA should be referred to the general clinic within the same health center for a second complete blood picture and count after 4 weeks for re-evaluation.
o If hemoglobin improves, continue the treatment for 3 further months and repeat complete blood picture and count.
o Iron therapy should continue for 2 months after reaching the normal blood levels for age.
o If hemoglobin did not improve, serum ferritin and CRP level should be checked.
o If serum ferritin and CRP level were normal, thalassemia could be suspected, hemoglobin electrophoresis will be performed.
o If thalassemia is diagnosed, the child will be referred to secondary care.
o If ferritin or CRP level were abnormal (ferritin low, CRP is high and ferritin is high, ferritin is normal and CRP is high) refer to pediatric outpatient clinic in secondary care to rule out persistent and unrecognized blood loss, malabsorption of Iron, poor Iron utilization, chronic interaction and/or lead poisoning, in addition, IV iron therapy will be given.

- To the best of our knowledge, all commercially available baby formulae are fortified with iron. A supporting finding was seen in the current study - Children fed with formula milk showed lower levels of ID and IDA.
- There was no hospital information utilized in the study. Only information gathered using the questionnaires and relevant laboratory data of participants available on primary health care center information systems were included.
- The mothers interviewed in the study were asked about frequency of consumption of different food items rich in iron, which was used to calculate the food frequency score. Based on their responses, a score was constructed for each consumed food item according. A score of 1 for no previous consumption, 2 for 1-3 times/month, 3 for 1-6 times/week and 4 for daily consumption was assigned. Food items of animal origin were summed, as well as those of plant origin and the mean + SD was calculated for participants either with or without ID. Findings showed that infants suffered ID consumed less plants rich in iron compared to their counterparts without ID, and the difference was statistically significant (t test= 2.26 and p= 0.03). The difference was not significant statistically when studying the animal sources of iron and the overall score of food frequency. (it was derived from Al-

Mass M, Selim NA, Al-Kuwari M, Ismail MS. Assessment of Anemia, IDA and ID among Pregnants in Qatar: Cross Sectional Survey. SM J Public Health Epidemiol 2016; 2(3): 1035.)

- Permission was obtained from mothers in the form of written consent.
- Amendments to all tables have been made based on the feedback provided. Cut off points used within the primary healthcare centers in Qatar were applied in the study. These cut offs are sometimes different from those used elsewhere, it is mentioned as a limitation. The authors have revised the manuscript to address the comments in line with the authors response provided above.

Response to reviewer: 3
Reviewer Name: Asher M. Moser M.D.
Ben Gurion University of the Negev, Israel

- Participants included in the study was individuals completed 12 months and under 13 months. Based on the 12 months vaccination coverage rates calculated by the primary healthcare corporation which is responsible for all primary health care services in Qatar and confirmed by its head of child health section, vaccination coverage rates is over 97%. The service is completely free of charge to all nationalities.
- ID was used in this study rather than IDA, as ID is a fundamental cause in IDA development in this age group according to literature.
- Other reasons for non-ID anemia (e.g. thalassemia and sickle cell) were part of the exclusion criteria in this study.
- It is not possible to use multiple regression in the analysis due to fragmentation of groups.
- At the time of the study, a total number of 19782 children between 12 and 13 months were registered children in Qatar. This figure was used to calculate the sample size required for the study.
- All centers delivering well baby care service were included in the study (14 out of the total 21 centers on national basis on the time of the study).
- All mothers were consented to be included in the study. Usually fathers also attend well-baby clinics together with their spouse, but mothers (in most instances) do the active role, as they know better about feeding practices.
- Participation in the study was optional; mothers of participants were free to refuse participation without any adverse consequences with no obligation to give a reason. It was estimated that a sample size of 292 was required for the study. To compensate for non-responders, an additional 20% participant were added to the sample size calculation bringing the total to 350 participants.
- Of the 350 participants identified for the study, laboratory data was available for 306 participants. The sample size was therefore above the figure required based on the sample calculation.
- Regarding the comment on table 3: At age of 6 months, 63 of participants were on exclusive breastfeeding, and no other food was introduced to them at that time. On the other hand, the number of mothers depended mainly (but not exclusively) on breastfeeding at age of 6 months and continued on same manner until age of 12 months was higher (163).
- The mothers interviewed in the study were asked about frequency of consumption of different food items rich in iron, which was used to calculate the food frequency score. Based on their responses, a score was constructed for each consumed food item according. A score of 1 for no previous consumption, 2 for 1-3 times/month, 3 for 1-6 times/week and 4 for daily consumption was assigned. Food items of animal origin were summed, as well as those of plant origin and the mean + SD was calculated for participants either with or without ID. Findings showed that infants suffered ID consumed less plants rich in iron compared to their counterparts without ID, and the difference was statistically significant (t test= 2.26 and p= 0.03). The difference was not significant statistically when studying the animal sources of iron and the overall score of food frequency.
- Regarding population number in table 3: The 19 infants were not lost to follow up as the first 4 questions cover breastfeeding practice -only287 infants 'ever had breastfeeding' and the remaining 19

infants had no history of breastfeeding at all therefore no responses for them were recorded. However, other questions cover practices related to formula milk and other food items, in which all of the 306 infants had history with at the age of 12 months.

- References for BMI values are added to manuscript.
- The authors have revised the manuscript to address the comments in line with the authors response provided above including the grammatical issues that were requested to be addressed.

VERSION 2 – REVIEW

REVIEWER	Ekhard Ziegler University of Iowa
REVIEW RETURNED	21-Dec-2017

GENERAL COMMENTS	Sample selection: it has been clarified and it appears that the sample is representative of all children in Quatar. Most other questions have been answered satisfactorily. The author's response states that "there was no hospital information used in the study". However, on p. 5 line 17 the manuscript states that "laboratory results .... were retrieved from their electronic medical record". Table 4: It is still not clear what the overall score for food frequency is. The introduction is needlessly wordy and long P2 line47: remove the word "newborns" P 4 line 48: delete "randomly", line 49 change "systematically" to "randomly". P 4 line 53: Add methods used for hemoglobin and ferritin determination How many days were utilized? P 5 lines 19 & 20: What additional laboratory studies for ID detection" were performed? To Editor: The manuscript is definitely improved. But some of the requested information is presented only in the reply to the reviewer and not in the manuscript. For example, sample selection is explained reasonably well, but the manuscript does not reflect this. There are still numerous (I stopped counting at 15) small errors in the English diction, such as missing articles or a misplaced comma.

REVIEWER	Claudio Sandoval New York Medical College, USA
REVIEW RETURNED	03-Jan-2018

GENERAL COMMENTS	Dear Authors- the English remains subpar in some sections, though improved from the previous reviewed version. Stick to one version of the spelling of anemia- either anemia or anaemia but not both. From your study population it appears that 12 months of age is too late for any intervention to educate and perhaps reduce the incidence of anemia/ID/IDA.

VERSION 2 – AUTHOR RESPONSE

The authors would like to thank all the reviewers for their assistance and valuable comments. The Authors carefully considered the comments and have responded accordingly. Below are the point-by-point revision of the new comments that were received.

Editorial Requirements:

- Authors completed a full STORBE checklist as required. Any difference in stating specific page numbers was avoided.
- The quality of English throughout the manuscript was reviewed with the assistance of a second native English Speaker.
- Authors carefully included the previous responses to reviewer's comments in the main text of the manuscript.

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Claudio Sandoval

Institution and Country: New York Medical College, USA

- The quality of English was addressed throughout the manuscript.
- The spelling of anemia was unified all through the manuscript, except in the section of references where authors followed the original article titles.
- At the age of 12 months, it is may be late to interfere to reduce the incidence of anemia/ID/IDA, but further deterioration could be avoided through proper urgent treatment. Additionally, the study recommendations included an advice for decision makers to update their related guidelines.

Reviewer: 2

Reviewer Name: Ekhard Ziegler

Institution and Country: University of Iowa

- We agree that there was some confusion regarding the use of hospital information. Blood samples were collected in primary healthcare centers and tested in a hospital facility. Later, results were retrieved by accessing the electronic health record of the participants, which is held on a shared database between primary and secondary care.
- The calculation of the overall score for food frequency was described and added to 'Methods' section in the main manuscript.
- The introduction was reduced in size to be more relevant and less wordy.
- P2 line 47: the word "newborns" is removed.
- P4 line 48: the word "randomly" is deleted, in line 49 the word "systematically" is changed to "randomly".
- P4 line 53: the methods used for Hb and Ferritin determination is now described and added to 'Methods' section in the main manuscript.
- The additional laboratory studies for ID detection are the C-reactive protein and serum ferritin tests, as routinely both are not tested at the 12 months visit (at this age, routine testing is for Hb level only).
- Sample selection is explained within the 'Methods' section in the main manuscript with details similar to what was explained in the previous reply to the reviewers.

Reviewer: 3

Reviewer Name: Asher M. Moser M.D.

Institution and Country: Ben Gurion University of the Negev, Israel

- Authors did not find any additional comments than the previous ones.
- Authors reviewed the previous comments and addressed the issues appropriately.