Author's response to reviews

Title: Association of characteristics of delivery and medical conditions during the first month of life with developmental defects of enamel

Authors:

mahtab Memarpour (memarpour@sums.ac.ir)
Ali Golkari (golkari@gmail.com)
Reihaneh Ahmadian (ahmadianR@sums.ac.ir)

Version: 4 Date: 2 August 2014

Author's response to reviews: see over
Dear Ms Nolasco, Dear Doctor Do,

Thank-you for your mail of 2014-07-14. Our manuscript (no. 5770904691298118) titled “Relationship of birth and first month of life in children and developmental defects of enamel” has been revised according to the reviewers’ comments. Please note that we have revised the title for accuracy to read “Association of characteristics of delivery and medical conditions during the first month of life with developmental defects of enamel”.

If there is anything else we can do, please do not hesitate to contact us.

With best wishes,

Dr M. Memarpour

Replies to the reviewers’ comments

Reviewer's report
Title: Relationship of birth and first month of life in children and developmental defects of enamel
Version:3Date: 11 July 2014
Reviewer: Peter Arrow
Reviewer's report:

1. Abstract - The authors stated in the background section that, “the aim of the study was to identify …… conditions during the first month of life that can lead to DDE in permanent teeth. Lead implies a causal relationship, which the design of this study (cross-sectional study) is unable to do and the sentence should be rephrased accordingly.

Answer: This sentence in the Abstract has been rewritten and now reads:
The aim of the study was to identify the main characteristics of birth (delivery) and adverse medical conditions during the first month of life that may be related to DDE in permanent teeth.

2. Background — The authors have presented and reviewed the literature outlining what is known of the condition of interest. It would have been useful to delineate in some manner the different types of enamel defects that are discussed in the literature because the causal factors for different types of defects are likely to be different; for example some of the studies refers to molar incisor hypomineralisations, while others refer to hypoplasias. And also to focus on studies which discussed DDE in permanent teeth, if the focus of this study is to be on DDE in permanent teeth, as suggested by the background comments in the abstract.

Answer: Because DDE includes hypoplasia and hypomineralization we used the general term (DDE) to report the results of studies. However, as the reviewer recommends, we have explained the types of defects in the revised manuscript.

3. Methods —
The authors reported that 1000 children were sampled; this is a result and should be reported in that section.

Answer: This information has been moved to the Results section.

4. The authors selected girls’ school and boys’ school, some context for the reader to explain this is warranted, such as, are there only separate schools for girls and boys and no co-educational primary schools in Shiraz?

Answer: Yes. Schools are segregated by gender in Iran. Added as requested.

5. From the description provided; 4 schools (2 boys’ and two girls’ schools) were selected from each of the 4 zones, which equates to 16 schools, and each school had 80 children sampled, which equates to 1280 children being sampled, which does not agree with the 1000 reported by the authors. An explanation is required for this difference.

Answer: Added as requested. If the total number of fourth and fifth graders at a given school was less than 80, all students were included, and if the total number was more than 80, students were selected randomly by using simple randomization.

6. The reason/s for separation into the 4 geographic zones should also be provided.

Answer: Added as requested. The city of Shiraz is divided into four zones by the Educational Head Office.

7. Sample size estimation (how determined) and the estimated number should be reported.

Answer: The required sample size based on a prevalence of 50% for having a DDE in permanent teeth, accuracy of 5% and confidence level of 95% was 385. This was doubled to 770 because multistage randomization was used in this study. The final sample size was calculated as 1027 based on a 75% estimated response rate. Considering the fact that 16 schools were included in this study, 65 pupils should have been selected in each school. However, to compensate for the possibility of having fewer than 80 eligible pupils at a given school (i.e. within the study age range), especially at private schools, up to 80 were selected at schools with higher numbers of pupils.

8. The reasoning behind the criteria applied to exclude some children should be explained more fully, especially when some of the criteria may be associated with the occurrence of the outcome of interest, such as atypical restorations or fissure sealant applications, which are likely to be associated with the occurrence of severe hypomineralisations.

Answer: Cases of full-coverage restoration, filling or fissure sealant at the buccal or palatal surfaces were excluded from the study to focus on the diagnosis of DDE. (Added as requested). It should be mentioned that in Iran there is no free public dental care and some preventive measures such as the use of fissure sealant are rarely seen in children.
9. The authors mentioned that fluoride level was the same throughout the city, the level should be reported, and clarified that it was the same across the geographical zones.

Answer: Added as requested.

10. The authors reported institutional ethical approval, and information on whether informed consent was sought and/or obtained by parents/carers of the children should also be reported.

Answer: Parents who provided their consent for their child to participate were asked to complete the questionnaire (page 4, lines 135 to 136)

11. The section on collection of exposure factors needs clearer explanation. For example, were all information provided by the parent; as it is written, it seems to suggest that the information contained on the mandatorily completed “Health Status Evaluation Form” was obtained by the researchers “derived”, Line 129.

Answer: Yes. All neonates receive a health status evaluation form which must be completed by the doctor or midwife at birth, and parents are instructed to keep the form. Therefore access to the data was possible.

12. Line 130, and “the parents were asked to refer to their children’s birth card when answering these questions”, further explanation of what questions and what the birth card is should be included. What information was sought by the questionnaire, how many questions and who was to complete the questionnaire should be included in the explanation of data collection methods. Do parents and carers have information relating to APGAR scores?

Answer: This information is now explained in Table 2. The parents completed the questionnaires and all questionnaires were checked by the examiners. Yes, the parents knew the Apgar score had been documented in the health status evaluation form.

13. The information on examiner training should be reported in a separate section and more information should be provided including how the training was undertaken. The authors reported an “alpha” of 0.73, this is unclear and given that there were 2 examiners both between and within examiner reliabilities should be reported.

Answer: Added as requested.

14. The authors reported using the modified DDE index. The modified DDE index used index teeth, and the age range of this study’s participants may not have all the index teeth present (an explanation what the index was, how it was scored etc. should be included). The authors need to explain how this was handled.

Answer: The modified DDE Index for epidemiological studies was developed by Clarkson and O’Mullane in 1989. It does not use indexed teeth, but each tooth is assessed independently. The results can be reported on three levels: individual level (does each subject have at least one DDE?), tooth level (how many of each subject's teeth have at least one DDE?), and defect level
(how many DDEs exist in each tooth and how many defects does each subject have?). The two first levels were used in the present study. Therefore, the percentage of children with a DDE and the average number of DDEs per child were calculated. In all children in this study, all eight permanent incisors and four permanent first molars had erupted.

15. On line 155, the authors mentioned that “each of the characteristics of birth……… chi-squared test.” What the characteristics are should be reported.

Answer: Added as requested.

16. On line 157 the authors mentioned “seven factors …”, these seven factors should be reported.

Answer: Added in the information provided in response to the previous comment.

Results —
17. The authors reported the number who presumably consented (1000) in the methods section, some information on consent rate as well as number of returned questionnaires, how many were used etc. should also be provided. There should also be report on the number of teeth by type of teeth present, given that the age groups of children selected would have both primary and permanent teeth present (refer to comments in methods section regarding the DDE index used).

Answer: The sample size calculation and the inclusion criteria are explained in our replies to comments number 3 to 7 above.

18. The age distribution of the study participants should be reported.

Answer: Children were between 9 and 11 years of age. Average age ± standard deviation was 10.23±0.66 years.

19. Table 1 as presented, showing the proportion with DDE with the factors as measured, is a bit confusing and the authors should reconsider presenting the information in an alternative form. Similar to Table 2, with the factors in one column and the outcome in the other two columns and then the statistical test findings in another column, but with minimal amount of borders to enable an uncluttered view of the table. Also in general it is preferable to present as n and %.

Answer: Corrected as recommended.

20. Line 170 appears to evaluate a mean score; the statistical approach adopted for that should be included in the Methods section.

Answer: The percentage of children with at least one DDE in the permanent teeth and the average number of permanent teeth with a DDE per child were calculated.

21. The results should also present the prevalence of DDE by types of DDE; hypomin, hypoplasia, diffuse opacities or demarcated opacities etc., and types of teeth affected with DDE;
permanent/primary teeth. Along with that, consideration then should be given to undertaking appropriate analyses.

Answer: In the current study we identified associations between birth conditions and DDE indirectly (not causal relationships). To determine the causal relationships, the time when defects were caused and the kinds of defects need to be known. Therefore different kinds of DDE may be involved.

22. Line 191 presumably is referring to Spearman’s correlation, this should be made clear and the rho also reported.

Answer: Answer: added as requested. $r=0.423$

23. Overall the authors should make clear throughout the manuscript when they are referring to primary or permanent dentition because in some sections it is unclear, for example line 188 “81 children had at least one DDE” presumably is referring to at least one tooth affected. And also the number of excluded children with permanent tooth restorations should be reported.

Answer: Added as requested. The current study analyzed the frequency of DDE only in permanent teeth, as mentioned in the Methods. In addition 14 restored permanent teeth were excluded from the study. These children were not excluded from the study because only one tooth per child had to be excluded. However, 10 children were excluded because their parents did not provide their informed consent, six were excluded because some items on their returned questionnaires were left blank, and four children were absent on the days of both visits to their schools.

24. In Table 2 the authors presented the results from the logistic regression analysis; it would be useful to have the estimates derived from the coefficients and their interpretation as well as the p values presented.

Answer: Added in the manuscript text and Tables 2 and 3.

25. The authors should more clearly explain the methodology adopted in the multiple regression and the rationale behind their model development. Usually when multiple regressions are undertaken the results from the bivariate analyses are used to consider inclusion and exclusion from the overall regression model. Then the model is fitted and variables are retained or excluded from the model based on the contribution of the variable to the overall fit of the model or some theoretical understanding of the impact of the variable to the outcome under consideration (usually a well known confounder). The authors need to explain why they have retained the variables they have given the bivariate findings and what criteria, if any, were used in the fitting of the model to determine retention in the model. The model should be the most parsimonious, consistent with the prevailing understanding of the subject matter.

Answer: Added in the manuscript text and Tables 2 and 3.

Discussion —
26. There should be some discussion on response rates, and potential for bias as a result of the response rates that as well as bias from exclusion/inclusion criteria.

Answer: The response rate was about 98%, which was relatively high considering the nature of the study. The results of this study can be assumed to be essentially unaffected by the data we excluded from analysis.

27. The authors’ commentary in the second paragraph is unclear to this reviewer. The developmental defects are, by their very definition defects which arise at a time when enamel development is taking place and, therefore the risk factors need to be present at those critical periods of enamel development to affect the enamel. Exposure to risk factors outside of this window of critical period is unlikely to lead to developmental enamel defects. Hence, the authors’ should expand on their use of the term “direct effects” and “indirect effects” in an epidemiological context and link it more clearly to the findings of their study.

• As outlined earlier it would be more useful if the discussion involved the effects of the measured factors on the types of DDE and also in relation to the types of teeth so that biological plausibility for the critical period can be evaluated, given that health conditions around the time of birth and within the first month of birth were primary factors of interest.

Answer: In the current study we identified association between birth conditions and DDE indirectly (not causal relationship). To determine the causal relationships, the time when defects were caused and the kinds of defects need to be known. Therefore different kinds of DDE may be involved.

Also most of the studies investigated the relationship between DDE in primary teeth with birth condition factors (direct effect). However, to determine the associations between different kinds of defects and specific birth-related factors, other studies such as cohort studies are needed.

28. The limitations of the study, outlined in the last paragraph Line 251 (parental recall) is critical to the study’s findings and more information should be provided in the methods section on how the information was collected and what, if any, measures were used to determine reliability of parental recall.

Answer: Added as requested. The birth conditions were recorded in the children’s health status evaluation form. This form is mandatory in Iran, so the parents’ responses to these items can be assumed to be accurate.

29. Table 1 also suggests possibly significant differences in DDE occurrence among boys and girls from areas 3 and 4, which should be discussed.

Answer: Added as requested. Yes. The percentage of boys and girls with DDE may have differed between zones 3 and 4. But the two zones offset each other and therefore no significant difference was seen in the data for all boys versus all girls.
General Comments —

30. The authors mentioned in the introduction that DDE may cause problems for children and hence the need to understand the causes of such conditions. The impact of DDE is dependent on the type and severity of the defect and the authors have not presented differentiated information on the types of defects which is necessary for the reader to form an opinion on the likely impact of the presence of enamel defects on the health of the study participants. Grouping the defects under the umbrella of DDE is insufficient.

Answer: Added as requested in the Introduction. These defects are divided in two main groups: hypomineralization (change in the quality) and hypoplasia (change in the quantity of enamel). In hypomineralization, changes in enamel transparency are visible as staining or opacity.

31. There are some minor typographical and syntax errors within the manuscript, which should be corrected, some of which are listed (not exhaustive). For example, Line 71 “latter group of factors cause disturbances ……”, and Line 74 ,“DDE to range………”, Line 87 there should be a space after the full stop and before Apgar, and Line 89, spelling for parenteral ? or perinatal?

Answer: Corrected as recommended.

32. Line 76 “DDE may cause problems ……… lack of oral hygiene” should be rephrased, because it seems to suggest that DDE causes lack of oral hygiene, which is not the case.

Answer: Corrected as recommended.

33. The sentence in Line 158-159 is unclear and should be rephrased.

Answer: Corrected as recommended.

34. Line 167 should perhaps be “at least one tooth (or permanent tooth) ……….

Answer: Corrected as recommended.

35. Line 181, the authors used the term “rate” when referring to a proportion, which is not strictly correct and the wording should be changed.

Answer: Corrected as recommended.

36. Line 218, the sentence is unclear; what is the consistency with other studies on the importance of the infants health in relation to what? The reference is in relation to Apgar score to enamel defects in the primary dentition and the discussion should expand on how that finding relates to the findings in this study. As indicated before, the authors also should make clear whether they are referring to DDE in permanent teeth only, primary teeth only or both.

Answer: Corrected as recommended.
37. The authors found no significant association with birth weight while there was an association with the Apgar score. Is it possible that the Apgar score may have subsumed the birthweight relationship given the likelihood of Apgar score’s association with birthweight? Some commentary on this would be appropriate.

Answer: Apgar score is not always related to birth weight. These two variables might be related only in very-low-birth-weight or preterm children. However, the number of children with these two conditions (very LBW and preterm) was very small in our study. We used the factors in a regression model which showed that Apgar score was independently associated with having DDE.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being Published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing in
**Reviewer's report**

**Title:** Relationship of birth and first month of life in children and developmental defects of enamel  
**Version:** 3  
**Date:** 20 June 2014  
**Reviewer:** Patrícia Corrêa-Faria

**Reviewer's report:**
Minor essential revisions:

1. **Title:** Replace the term "relationship" by "association"

   **Answer:** Corrected as requested.

2. **Standardize the use of DDE sogla throughout the text**

   **Answer:** Corrected as requested.

3. **Page 3, line 92:** Avoid citing authors' names throughout the text. It’s better to put the reference just down the sentence. For example, factors associated with DDE can be categorized into ...

   **Answer:** Corrected as requested.

4. **Methods: Sample size was calculated?**

   **Answer:** Yes. The required sample size based on a prevalence of 50% for having a DDE in permanent teeth, accuracy of 5% and confidence level of 95% was 385. This was doubled to 770 because multistage randomization was used in this study. The final sample size was calculated as 1027 based on a 75% estimated response rate. Considering the fact that 16 schools were included in this study, 65 pupils should have been selected in each school. However, to compensate for the possibility of having fewer than 80 eligible pupils at a given school (i.e. within the study age range), especially at private schools, up to 80 were selected at schools with higher numbers of pupils.

5. **As the differential diagnosis between dental caries and DDE was performed?**

   **Answer:** Yes. The examiners’ scores were calibrated by having them examine 20 patients under supervision by the professor.

6. **As the oral exam was performed?**

   **Answer:** Yes. This is reported in the Methods.

7. **There was pre-cleaning of teeth?**
Answer: Yes. The examiner first cleaned the teeth with sterile gauze, then examined all the permanent teeth with a disposable mirror, explorer and head light.

8. How many examiners?
Answer: Two dentists.

9. The examination was performed in school?
Answer: Yes.

10. The teeth were examined under natural or artificial light?
Answer: Natural light enhanced with a head light.

11. In these cases, we chose to exclude the child or just by deleting the tooth statistical analysis?
Answer: This is explained at the end of method, page 5, lines 165 to 167.

12. In cases where the prevalence is higher than 20%, some researchers choose to perform Poisson regression. There is possibility that the measure of OR obtained from logistic regression superetime “overestimate” results. The authors considered perform Poisson regression?
Answer: You are right. However, considering that most dental indices are based on frequency counts, many dental studies should use Poisson regression, although they usually do not do this. This is mostly because there is little or no difference between the two regression models, especially when the sample size is large.

13. Results: What were the reasons for the losses?
Answer: Some parents were unwilling to allow their child to participate in the study. Also some children did not meet the inclusion criteria (page 6, lines 186 to 188).

14. As examiners differed DDE and MHI?
Answer: The examiners were trained as described on page 5, paragraph 3. They were trained in identifying MIH. However, MIH defects are also developmental enamel defects. So there is no reason to separate them from DDE. MIH is a subtype of DDE. MIH defects can differ from other types of DDE, for example generalized defects that affect all the teeth. Considering that we used the Modified DDE Index, and that the Modified DDE Index does not have a separate code for MIH, this type of defect was included in the study using codes based on their appearance.

15. The term risk factor is more appropriate for longitudinal studies in which patients are monitored to determine whether those with particular characteristics at higher risk of developing
the disease. The present study is cross-sectional design, which allows checking only association between the variables. The term needs to be revised throughout the text.

Answer: Corrected as requested.

16. The regression results need to be further described. Which OR values? Explore these results more

Answer: Table 3 added.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.