PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Congenital anomalies and associated risk factors in a Saudi population: a cohort study from pregnancy to age 2 years
AUTHORS	Kurdi, Ahmed; Majeed-Saidan, Muhammad Ali; Al Rakaf, Maha; AlHashem, Amal; Botto, Lorenzo; Baaqeel, Hassan; Ammari, Amer

VERSION 1 - REVIEW

REVIEWER	Lucia Borsari University of Modena and Reggio Emilia, Italy
REVIEW RETURNED	29-Oct-2018

GENERAL COMMENTS	Kurdi A and colleagues present the results of a longitudinal prospective study in a Saudi population evaluating prevalence of congenital anomalies in the offspring, associated risk factors and survival rates at 2 years' follow-up. The study includes a large number of cases (1,179). The topic is of great interest and relevance, however there are a number of limitations associated with the study. In particular, the objectives of the study, results on survival rates and results on risk factors associated with congenital anomalies should be more clearly reported in the manuscript. The manuscript needs language editing. The following comments are offered for the author's consideration: GENERAL COMMENTS: I suggest to substitute the term birth defects with congenital anomalies all around the manuscript (especially considering that the authors included in the analysis ETOPFA). It is important that the authors report all prevalence rates *1,000 or *10,000. The criterion should be unique all around the manuscript. ABSTRACT: I suggest to rename the "three key areas": congenital anomalies prevalence, associated risk factors and survival. It is not clear the meaning of "well-defined" longitudinal cohort. In the section "Participants", I suggest to write 28,646 eligible livebirths, stillbirths and ETOPFA (I found not correct defining ETOPFA as birth) It is important to maintain the same structure and sequence in the methods and in the results section. The authors evaluated prevalence of congenital anomalies in the whole cohort and then the association with socio-demographic and clinical characteristics of parents in the nested subpopulation. Therefore, I suggest to

	report first the congenital anomalies prevalence (412/10,000) and afterwards the association with risk factors and survival data. Moreover, I think it would be more interesting to report in the abstract which factors are associated with higher risk of congenital anomalies (instead of frequency of evaluated factors). For example: In multivariable analysis, factors associated with higher risk of CA were maternal diabetes (OR: … CI 95% …-…), etc Given the longitudinal design of the study, a survival analysis to compare mortality rate in affected and unaffected children could be interesting. MANUSCRIPT: Introduction Some sentences are not clear, ("As causes of early mortality…). Introduction section should include some sentences about recent literature on survival and risk factors in congenital anomalies. The objective of the study should be better defined. Patient and public involvement. The sentence "None of the parents were involved in the study" could be misinterpreted. Statistical analysis First, I suggest to describe that prevalence rates per 10,000 births and 95% confidence intervals were calculated. In the results section, I don't find any reported Chi-square or Fisher's exact test. The term "uncorrelated significant factors" is not entirely clear. Please, better specify. Results Figure 2 is not clear. I suggest some modifications as follow: - Highlight excluded subjects (i.e. grey boxes for born outside PSMMC and spontaneous abortions) - Cancel 58 cases and 221 controls born outside PSMMC to simplify the figure - Do not use the term "normal" (i.e. normal SB) - The total population consist of liveborn (n=28,376) + ETOPFA (n=18) + stillborn (n=252) = 28.646 eligible pregnancy outcomes. This number representing the total study population should stay in Figure 2. Page 13 line 36/37: Authors write "38 were stillbirths..". From figure 2, it seems 46 not 38. Table 1. It is not clear what columns 6 and 7 (505+38+18..) represent. In the text, I suggest to extend the descriptive analysis related to livebirths (i.e. congenital anomalies prevalence in this group), considering that the analysis on mortality includes only these infants. A figure representing mortality rates in the unaffected and affected group during the 2-year follow up could be added. Please, compare results in the two groups. In the 'risk factors' section, I suggest to briefly describe in the text the study population (n. of cases and related controls). Most important results (OR and 95% ci) should be included in the text. Table 3: - I'd like to understand more clearly which cofounders the authors used in the multivariable analysis. - Folic acid intake: It would be interesting to show results regular/irregular/no use (given the importance of this factor on the outcome)

	- Smoking: it would be interesting to have also the results on maternal smoking (I suppose that the reason why smoking results not significantly related to congenital anomalies is that it is predominantly paternal smoking. - Diabetes: it would be interesting to have also results on pregestational diabetes I suggest to give more detail in this section, eventually extending the text and not the table. It would be also interesting to have results for single congenital anomalies (at least for those most frequent)

REVIEWER	Dr. Godfrey Oakley Emory University Rollins School of PUblic Health Atlanta GA
REVIEW RETURNED	15-Feb-2019

GENERAL COMMENTS	Comments on Saudi cohort birth defects paper 2019 This is a timely and important paper. The pediatric and global health community concerned about child death and disability acts as if they do not understand the important role of birth defects in child death and disability nor are they preparing for the increasing proportion of these child deaths that will occur as there are further improvements in deaths from infection. This paper is an important contribution to global child health. I congratulate you on acquiring the resources and talent to conduct such a wonderful study. It is solid data from a cohort that removes under ascertainment of birth defects a serious problem of the Global Burden of Diseases project which has contributed to the world not understanding how important birth defects, preventable birth defects, too, are to causing child death. My main suggestion is to make these additions if they can be done with little efforts. 1. Include the proportion of all neonatal, infant and under 2 deaths attributed to birth defects. You do a nice job of showing that being born with a birth defect increases mortality by 15 fold, but I think the proportion of all infant deaths, etc. would be a very important addition. 2. You collected Hgb A1c levels. As you know these correlate with incidence of birth defects. I suggest projecting the reduction that would occur if the upper A1c would drop to say 6 rather than the likely 6 to 9 it is. It would make clear how important effective programs, hopefully screening you recommend would improve, but really it is the clinical care that lowers on a population base the A1c levels. As far as I know no health care system has yet to successfully lower the population A1c levels for insulin using women who become pregnant. 3. I do not recall seeing that Congenital Rubella Syndrome is mentioned. I suspect there is none. This should be noted and celebrated as a success in population care. 4. The proportion of births to women over 30, 35, 40 is alarmingly high. This a modifiable risk factor. Historically European rates were about 15 percent 35 and older producing a Down Syndrome rate of about 1.5 per 1000. The rates in the 1970s in Atlanta were much less, about 5 per cent above 35. You observed 7% above 40!!!!!. The rate of spina bifida in Atlanta in the early 1970s was about 1 per 1000. The current rate in your study is double this. I know reducing the proportion of births to women over 35 is a difficult topic to discuss. Reducing the

	maternal age proportions to something like the 1970s in Atlanta is possible if we identify effective strategies to nudge toward lower rates. In the USA it has in the last 40 years nudged itself to the levels you observed. It is a modifiable risk factor. 5. You identify consanguinity as a risk factor. Again, a difficult one to change but you rightly clearly put the opportunity on the table. 6. On the other hand, it should be straight forward to get folic acid fortification done correctly. Your results show that there is a problem. I suggest you suggest the government regulators determine folate levels in grain products to identify who is not fortifying As I understand it the wheat consumption is so high that it is very unlikely that people are not eating enough wheat to get enough folic acid if the fortification was being properly implemented. The government regulators should be doing this anyway. Yes, would be nice to get some blood folates from women of reproductive age. Others have used convenience samples of women at immunization clinics as a good place to do such studies. 7. I especially like the line listing of all cases rather than combining groups. Please keep this. It is especially important to see spina bifida and anencephaly alone as they are what of the neural tube defects (some include Trisomy 18 for example—not a good idea as folic acid does not prevent) that folic acid prevents. 8. I suggest you note that your study shows the value of the Triple Surveillance mentioned in ref 4. I think this study shows the value of including an ongoing pregnancy cohort study in a sample of the population as part of assessing prevention and care of children only with birth defects, but with other causes of infant mortality like prematurity.

VERSION 1 – AUTHOR RESPONSE

Response to reviewers

Reviewer 1 (Dr L. Borsari)	
1. The manuscript needs language editing	The grammar has been checked throughout the manuscript.
2. General comments 1. I suggest to substitute the term birth defects with congenital anomalies all around the manuscript (especially considering that the authors included in the analysis ETOPFA). 2. It is important that the authors report all prevalence rates *1,000 or *10,000. The criterion should be unique all around the manuscript.	“Birth defects (BDs)” has been replaced with “congenital anomalies (CAs)” throughout the manuscript. Prevalence has been changed to “as per 10,000” throughout the manuscript
Abstract:	
I suggest to rename the “three key areas”: congenital anomalies prevalence, associated risk factors and survival.	This has been reorganized as suggested. Page (P) 2, Line (L) 4-6.
1. It is not clear the meaning of “well-defined” longitudinal cohort.	The term “well-defined” has been deleted to avoid confusion. P2, L 9.
2. It is important to maintain the same structure and sequence in the methods and in the results section. The authors evaluated prevalence of	This section has been reorganized as suggested. P2, L, 21-26.

congenital anomalies in the whole cohort and then the association with socio-demographic and clinical characteristics of parents in the nested subpopulation. Therefore, I suggest to report first the congenital anomalies prevalence (412/10,000) and afterwards the association with risk factors and survival data.	
1. Moreover, I think it would be more interesting to report in the abstract which factors are associated with higher risk of congenital anomalies (instead of frequency of evaluated factors). For example: In multivariable analysis, factors associated with higher risk of CA were maternal diabetes (OR: … CI 95% …-…), etc	We wanted to emphasize how common risk factors are in this study population, and since the abstract has a 300-word limit, we were unable to add further results to the abstract.
Manuscript:	
Introduction	
1. Some sentences are not clear, ("As causes of early mortality…).	This sentence has been rephrased to "With better control of infections and other causes of early mortality". P4, L 11.
2. Introduction section should include some sentences about recent literature on survival and risk factors in congenital anomalies.	We included the WHO figures to illustrate the magnitude of the problem and the associated disability and mortality worldwide, ref. 3. P4, L 13 –18 A paragraph was also added to that effect. P 4. L. 18- 27.
3. The objective of the study should be better defined	The objective of the study is the triple chain-event (CA prevalence, associated risk factors and survival up to 2 years of age), which is stated in the abstract in the Objectives section and then repeated in the Introduction section. P4, L 33-35, and P. 5, Lines 1-3.
4. Patient and public involvement. The sentence "None of the parents were involved in the study" could be misinterpreted	We meant "none of the parents were involved in the study design". This has been amended in the text. P9, L 13..
Statistical Analysis:	
5. First, I suggest to describe that prevalence rates per 10,000 births and 95% confidence intervals were calculated. Second, In the results section, I don't find any reported Chi-square or Fisher's exact test. Third, The term "uncorrelated significant factors" is not entirely clear. Please, better specify.	This has been implemented. The text for statistical analysis has been rewritten with greater detail. Chi-square or Fisher's exact tests have been removed. P. 9, L. 24-33. The relevant text has been rephrased to clarify the meaning of "uncorrelated".
Results:	
1. Figure 2 Figure 2 is not clear. I suggest some modifications as follow: - Highlight excluded subjects (i.e. grey boxes for born	The suggested changes have been implemented. We kept the boxes for those born outside of our centre to give a more complete picture of the cohort. The

outside PSMMC and spontaneous abortions) - Cancel 58 cases and 221 controls born outside PSMMC to simplify the figure - Do not use the term “normal” (i.e. normal SB) - The total population consist of liveborn (n=28,376) + ETOPFA (n=18) + stillborn (n=252) = 28.646 eligible pregnancy outcomes. This number representing the total study population should stay in Figure 2. Page 13 line 36/37: Authors write “38 were stillbirths. From figure 2, it seems 46 not 38.	correct number of stillbirths among the cases is 38, as reported in the text and in Table 1. Figure 2 has been amended.
2. Table 1 It is not clear what columns 6 and 7 (505+38+18..) represent	 The numbers 505, 38 and 18 represent the prenatally detected CAs. The postnatally detected CAs have been added to table 1. P26, columns 7 - 10.
3. In the text, a) I suggest to extend the descriptive analysis related to livebirths (i.e. congenital anomalies prevalence in this group), considering that the analysis on mortality includes only these infants.	A new column has been added to Table 2, which shows the prevalence per 10,000 live births. Table 2, P. 27.
b) A figure representing mortality rates in the unaffected and affected group during the 2-year follow up could be added. Please, compare results in the two groups.	A detailed report about survival in the total cohort in relation to the various body systems involved with CAs is planned for a future report.
c) In the ‘risk factors’ section, I suggest to briefly describe in the text the study population (n. of cases and related controls). Most important results (OR and 95% ci) should be included in the text.,	We tried hard to avoid repetition in the manuscript, and since all the risk factors studied are in Tables 4 and 5, we did not elaborate on them in the text. ORs and 95% CIs have been added in the text for significant risk factors as shown in Table 5. P. 10, L. 2,3,7,9.
4. Table 3 a) I’d like to understand more clearly which cofounders the authors used in the multivariable analysis. b) Folic acid intake: It would be interesting to show results regular/irregular/no use (given the importance of this factor on the outcome).	 “The following confounders were considered in the multivariable analysis: consanguinity, maternal age, maternal education, diabetes mellitus, sibling with anomalies, parity, X-ray exposure during pregnancy and folic acid intake.” Details of FA intake were previously reported (Preventive Medicine Reports 2 (2015) 572–576) A paragraph has been added to the text summarizing the important results about FA and has also been referenced. P 14, L 23-27.
c) Smoking: it would be interesting to have also the results on maternal smoking (I suppose that the reason why smoking results not	Since smoking among women is considered socially unacceptable in Saudi Arabia, “although it’s rising

significantly related to congenital anomalies is that it is predominantly paternal smoking.	especially among the new generation", we asked about parental smoking (for both father and mother), and the questionnaire did not differentiate between them. Unfortunately, we cannot answer this question, although it is an important one.
d) Diabetes: it would be interesting to have also results on pregestational diabetes I suggest to give more detail in this section, eventually extending the text and not the table.	Further details have been added to the text regarding "Overt DM" P. 11, L. 1 – 6.
e) It would be also interesting to have results for single congenital anomalies (at least for those most frequent)	The following sentence has been added to the text " Two-thirds of all cases of CAs (773/1179, 65.6%) were isolated (e.g., they involved a single body system) P.10, L 11-12. A new "Table 3" for the most common single CA has been created. P. 29.

Reviewer 2 (Dr G. Oakley	
1. Include the proportion of all neonatal, infant and under 2 deaths attributed to birth defects. You do a nice job of showing that being born with a birth defect increases mortality by 15-fold, but I think the proportion of all infant deaths, etc. would be a very important addition.	We studied the annual reports from the ministry of health, Saudi Arabia over several years unfortunately it did not contain the percentage of death due to CA. For that reason, we cited a previous publication from our centre for the causes of deaths over 10 years period and we found "lethal malformations contributed to 36% of deaths" Ref. No. 34.
2. You collected Hgb A1c levels. As you know these correlate with incidence of birth defects. I suggest projecting the reduction that would occur if the upper A1c would drop to say6 rather than the likely 6 to 9 it is. It would make clear how important effective programs, hopefully screening you recommend would improve, but really it is the clinical care that lowers on a population base the A1c levels. As far as I know no health care system has yet to successfully lower the population A1c levels for insulin using women who become pregnant.	This is very interesting point. Unfortunately, regular screening for HbAic started after the first year of the study. With this alarmingly high level of DM in the Saudi society and the high maternal age, this needs a focused study on the topic.
3.I do not recall seeing that Congenital Rubella Syndrome is mentioned. I suspect there is none. This should be noted and celebrated as a success in population care.	A sentence added into that effect in the discussion. P14, L 18 - 21.

4. The proportion of births to women over 30, 35, 40 is alarmingly high. This a modifiable risk factor. Historically European rates were about 15 percent 35 and older producing a Down Syndrome rate of about 1.5 per 1000. The rates in the 1970s in Atlanta were much less, about 5 per cent above 35. You observed 7% above 40!!!!!. The rate of spina bifida in Atlanta in the early 1970s was about 1 per 1000. The current rate in your study is double this. I know reducing the proportion of births to women over 35 is a difficult topic to discuss. Reducing the maternal age proportions to something like the 1970s in Atlanta is possible if we identify effective strategies to nudge toward lower rates. In the USA it has in the last 40years nudged itself to the levels you observed. It is a modifiable risk factor.	The Saudi society is changing. Two important changes with regard to CA; the education of women is expanding all over the kingdom, not only in big cities, and the family size is getting smaller because of the education and increasing work force among women especially over the last decade. These may eventually lower the number of pregnancies at at 35 years and older. Health care planner in Saudi Arabia should take note of this type of studies for aggressive educational programmes, even about other risk factors like consanguinity and folic acid fortification.
5. You identify consanguinity as a risk factor. Again, a difficult one to change but you rightly clearly put the opportunity on the table.	We highlighted the importance of consanguinity as a risk factor for CA more aggressively in a recent publication (Birth Defects Research (Part A) 103:100–104, 2015.). This especially important when it comes to inborn errors of metabolism and other inherited conditions which are common in this society, ref. No 30.
6. On the other hand, it should be straight forward to get folic acid fortification done correctly. Your results show that there is a problem. I suggest you suggest the Government regulators determine folate levels in grain products to identify who is not fortifying As I understand it the wheat consumption is so high that it is very unlikely that people are not eating enough wheat to get enough folic acid if the fortification was being properly implemented. The government regulators should be doing this anyway. Yes, would be nice to get some blood folates from women of reproductive age. Others have used convenience samples of women at immunization clinics as a good place to do such studies.	Details of FA intake was previously published (Preventive Medicine Reports 2 (2015) 572–576) A paragraph was added to the text summarizing the important results about FA and a reference to the paper added. Since the Saudi's consume more rice than bread we suggested the fortification of rice too. P.23-27.
7. I especially like the line listing of all cases rather than combining groups. Please keep this. It is especially important to see spina bifida and anencephaly alone as they are what of the neural tube defects (some include Trisomy 18 for example—not a good idea as folic acid does not prevent) that folic acid prevents.	Thank you, will do.

8. Suggest you note that your study shows the value of the Triple Surveillance mentioned in ref 4. I think this study shows the value of including an ongoing pregnancy cohort study in a sample of the population as part of assessing prevention and care of children only with birth defects, but with other causes of infant mortality like prematurity	A paragraph was added to the discussion into that effect. P. 15, L. 4-9.

VERSION 2 – REVIEW

REVIEWER	Lucia Borsari University of Modena and Reggio Emilia, Italy
REVIEW RETURNED	03-Apr-2019

GENERAL COMMENTS	I found the new version of the mauscript well written. The manuscript is very interesting, in my opinion, it should be considered for publication in BMJ Open.