Author’s response to reviews

Title: A randomized controlled double blind trial comparing the effects of the prophylactic antibiotic, Cefazolin, administered at Caesarean delivery at two different timings (before skin incision and after cord clamping) on both the mother and newborn

Authors:
Chinta Jyothirmayi (dranniethomas97@gmail.com)
Ajay Halder (drajayhalder@yahoo.com)
Bijesh Yadav (bkyadav2007@yahoo.com)
Santosh Samuel (drsantosh97@gmail.com)
Anil Kuruvilla (anilkk@cmcvellore.ac.in)
Ruby Jose (rubyjose1@gmail.com)

Version: 2 Date: 03 Jan 2017

Author’s response to reviews:

Reviewer 1

Response to Reviewer Comments (Version 1):

Title: A randomized controlled double blind trial comparison of the effects of the prophylactic antibiotic Cefazolin, administered at Caesarean delivery at two different timings (before skin incision and after cord clamping) on both the mother and newborn.

Authors: Chinta Annie Jyothirmayi, Ajay Halder, Bijesh Yadav, Santosh Thomas Samuel, Anil Kuruvilla and Ruby Jose

Reference No: (PRCH-D-16-00087R1)

Reviewer reports:

Reviewer 1:
Thanks for giving me the chance to review the manuscript entitled "Comparison of the effects of the prophylactic antibiotic, intravenous Cefazolin, administered at Caesarean delivery at two different timings (before skin incision and after cord clamping) on both the mother and newborn.

Comment 1:

Conclusions and results are contradictory as regards hospital stay. (The pre-incision group had less post operative complications (SSI, endometritis, UTI, length of hospital stay) (p<0.001) and longer hospital stay (p = 0.008) when compared to the post incision group)

Response 1:

Sorry. That was a glaring oversight. The results have been corrected.

“The pre-incision group had significantly less febrile illness (RR=0.31, 95% CI: 0.16 – 0.62) and SSI (RR=0.07, 95% CI: 0.02 – 0.31) when compared for post operative complications, with the post-incision group. The pre-incision group had lower risk of hospital stay more than 7 days (RR=0.38, 95% CI: 0.19 – 0.76) as compared to the post-incision group”.

Comment 2:

Please change "materials and methods" into "patients and methods"

Response 2:

Thank you.” Materials and methods” has now been changed to “patients and methods”.

Comment 3:

How was sample size estimated?

Response 3:

Thank you for pointing this out. The sample size was calculated as follows:

The sample size to compare the effect of IV Cefazolin on the mother and infant on infections was found to be 600 in each arm with 90% power, at 5% level of significance, an anticipated difference of 9% in each group and with a 20% loss to follow-up.

Formula:

Details of the formula provided in supplementary file.
Reviewer 2

Response to Reviewer Comments (Version 1):

Title: A randomized controlled double blind trial comparison of the effects of the prophylactic antibiotic Cefazolin, administered at Caesarean delivery at two different timings (before skin incision and after cord clamping) on both the mother and newborn.

Authors: Chinta Annie Jyothirmayi, Ajay Halder, Bijesh Yadav, Santosh Thomas Samuel, Anil Kuruvilla and Ruby Jose

Reference No: (PRCH-D-16-00087R1)

Reviewer reports:

Reviewer 2:

Comment 1:
I don’t know if it was due to the conversion to a pdf, but there are numerous spacing errors throughout the manuscript. These should be corrected.

Response 1:
Thank you. All the spacing errors have now been corrected.

Comment 2:
In the abstract, the authors state that the pre-incision group has longer hospital stay. This is not supported by the data. In fact, the abstract conclusion says the pre-incision decreased hospital stay significantly.

Response 2:
Thank you. The glaring oversight has now been corrected to “The pre-incision group had significantly less febrile illness (RR=0.31, 95% CI: 0.16 – 0.62) and SSI (RR=0.07, 95% CI: 0.02 – 0.31) when compared for post operative complications, with the post-incision group. The pre-incision group had lower risk of hospital stay more than 7 days (RR=0.38, 95% CI: 0.19 – 0.76) as compared to the post-incision group.”
Comment 3:

Page 5, line 27: I am not sure what the authors mean by "The data supporting their findings can be found at Neonatal Department." I am not sure what findings they are talking about and what the Neonatal Department is located or what it has to do with anything.

Response 3:

Thank you. What was meant to be conveyed was that the dataset was preserved in the department where the study was conducted.

Now in page 5, line 27 has been deleted.

Comment 4:

Page 5, line 47. It is not necessary to exclude preterm babies since the inclusion criteria required mothers to be at least 37 weeks gestational age at delivery.

Response 4:

Sorry. This sentence has now been rewritten as “babies with known major congenital anomalies were excluded”.

Comment 5:

Page 6: Why did the authors decide to recruit 1200 participants? Was this based on a sample size calculation? If yes, then the details need to be provided. If not, the authors need to justify why this number was selected.

Response 5:

Sorry for not explaining. The sample size calculation has now been added under the “Patients and methods” section.

Details:

The sample size to compare the effect of IV Cefazolin on the mother and infant on infections was found to be 600 in each arm with 90% power, at 5% level of significance, an anticipated difference of 9% in each group and with a 20% loss to follow-up.

Formula:

Details of the formula provided in supplementary file.
Comment 6:

Page 6: The authors state block randomization was used, but do not describe what was blocked. In addition, they do not describe the randomization strategy used and how it was done.

Response 6:

Now the details of randomization have been provided in the manuscript in page 6.

“Permutated Block Randomization of sizes 2, 4 and 6 was done for treatment allocation using SAS 9.1.3. Pre numbered or coded identical containers for the antibiotics and the placebo prepared by pharmacy which are administered serially to patients”.

Comment 7:

The authors did not operationalize or justify many of the outcomes the reported in the results section. For example, What Apgar scores were considered "abnormal", what is meant by blood culture yes, what is "proven" sepsis, how were all the post op complications defined and diagnosed, etc. No where do the authors provide details on how these any of these outcomes were collected and determined. Who did the Apgar scores the nurse or neonatologist? Who diagnosed the post op complications; was it done by one individual? If not, how did then ensure the was consistency in the diagnoses?

Response 7:

Thank you. Now, operational definitions have been added in the Patients and methods section.

Surgical site infections (SSI): Evidence of clinical signs and symptoms of infection with or without microbiological evidence, based on the CDC definition that describes three levels of SSI.

Endometritis: Inflammation of the inner layer of the uterus, diagnosed clinically by the presence of pyrexia, uterine tenderness, sub involution of the uterus and foul smelling lochia along with or without microbiological evidence.

Urinary tract infections (UTI): Presence of clinical symptoms of dysuria, increased frequency of micturition and pyrexia along with or without microbiological evidence.

Early onset neonatal sepsis: Presence of any or a combination of features of sepsis namely hypo or hyperthermia, poor feeding, lethargy, dusky extremities, tachypnea or apnea within the first 72 hours of life.
Late onset neonatal sepsis: Presence of the above features after the first 72 hours of life until the first one week of life.

Oral thrush: Presence of white plaque like rash on buccal mucosa caused mainly by fungal organism giving rise to difficulty in feeding.

Necrotizing enterocolitis (NEC): Necrosis of the gut seen as temperature instability, poor feeding, lethargy, vomiting, abdominal distension, feed intolerance, sometimes with bloody stools.

Length of hospital stay: Defined as the number of days, the day mother is admitted into the hospital and for the neonate, the day neonate is born, until mother / neonate is discharged from hospital.

Maternal and neonatal readmission: Any admission to the same hospital or any other facility after first discharge from hospital.

Neonatal deaths due to Sepsis:

All maternal outcomes were assessed by the Consultant Obstetrician who is assigned to the post operative ward, who had a minimum of 4 years of clinical experience. All newborns were assessed by the Registrar /Neonatologist on duty, who assigned the APGAR score as well. If the Neonatologist or Registrar was not available on site in an emergency, the Labour room Registrar assessed the APGAR score. Sepsis in the neonate was assessed by a Consultant Neonatologist with a minimum of 4 years of clinical experience. All monitoring details were collected by the Principal Investigator.

Comment 8:

The statistical analysis conducted on this study is very weak. First they say means and standard deviations were calculated for normally distributed data, but do not say how they determined if the data were normally distributed. In addition, for all the outcomes they placed the continuous variables into categories for analysis. There is no justification for doing this. This type of analysis decreases the strength of the results. For example why would you compare hospital days between the pre-incision and post-incision groups using categories? Why not compare the actual number of days. If that was done, a much strong statistical test could be used. Also, the authors state they used chi-square tests for the analysis, but for many of the comparisons this is not appropriate because for several of the outcomes had less than give observations. The chi-square test is not appropriate in this situation. Instead, a Fisher's exact test should have been used. The authors also say a Levene's test for equality of variance was used, but this doesn't make sense because all the comparisons were conducted on categorical data so variance isn't an assumption for the statistical test.
Response 8:
Thank you very much. The statistical analysis has been modified based on your suggestions.

“Data entry was done into EpiData software in the Neonatal department. Analysis was done using SPSS 16 software. All categorical variables were summarized using frequencies and percentages and continuous variables were using mean and standard deviation. The association between risk variables and the primary outcome (Pre-incision and Post-incision) were tested using Fisher’s exact test. All the quantitative variables were compared using t test. For all comparisons that had more than two categories that were tested using Bonferroni correction test. Adverse effects with IV Cefazoline, though thought to be unlikely were monitored and all data were submitted to the institutional Data Safety Monitoring Board for review at the end of the trial”.

Comment 9:
Page 6, line 57: it should read, "...and all data were" not "...all data was".
Response 9:
Thank you very much for the correction. Now it is corrected.

Comment 10:
Page 7, line 3: The first sentence, 1200 what? were recruited?
Response 10:
Thank you very much. Now it has been corrected as 1200 mothers were recruited

Comment 11:
Page 8, line 3: The first sentence should be in the present tense.
Response 11:
Thank you very much for the correction. Now it is corrected as “are presented in Table 1”.

Comment 12:
Page 8, line 12 the second word in the sentence (Mean) should not be capitalized. The authors should provide the gestational age for both groups and give the results of the statistical test (p value) for the comparison. Did the authors look at maternal age? If yes, that information for both groups should be in the manuscript including the results of the statistical comparison.

Response 12:

Thank you very much for correction. Now the sentence Mean has been corrected as mean.

Now, Comparison of gestational age and maternal age provided in Table 2 as mean (SD).

Comment 13:

Page 8, lines 25-27: Who determined the non-reassuring fetal heart status and how was this diagnosed? What is meant by "abnormal lie"?

Response 13:

Fetal heart rate status is determined by the duty Consultant Obstetrician in Labour room and is based on the universally accepted standard classification of 3 categories by the American College of Obstetricians and Gynecologists.

Abnormal lie has been clarified in the text as” transverse or oblique lie of the fetus”.

Comment 14:

Again, neonatal weight should not be compared in categories. Instead use the continuous variables and compare it using a more powerful statistical test.

The authors state there were not differences between the groups for demographics, but none of the results are presented. The p values for each of these should be included in Table 1.

There is not a reference to Table 2 in the text.

Response 14:

Thank you very much. Now neonatal weight compared as a continuous variable.

The p values for each variables included in Table 1.

Reference to Table 2 provided in the result section.
Comment 15:
Page 9, line 12 (sentence starting "Mothers who received antibiotics..." This is an awkward sentence structure. Needs to be reworded.

Response 15:
Thank you. This sentence has now been reworded appropriately.

“When considering the primary maternal outcomes, the mothers who received prophylactic antibiotics pre incision, had less post operative complications such as febrile illness, SSI and total UTI”.

Comment 16:
Page 9, 27: The authors report the mean hospital days for the mothers, but then put the days into categories for the comparison between the groups, why?

Response 16:
Sorry. Now hospital days for the mothers have been provided as mean (SD).

Comment 17:
Page 9, line 32: I am not sure how the authors justify the statement, "...delay was probably due to some infectious morbidity..." What is meant by infectious morbidity?

Response 17:
Longer hospital stay could be assumed to be due to presence of post operative infections included in the primary outcomes.

Comment 18:
Page 9, line 34: The blood culture was positive for what?

Response 18:
Sorry. An explanatory line has been added.
“Blood culture reported significant growth in 23(4.2%) mothers in the Pre-incision and in 70(12.9%) mothers in the Post-incision group, signifying increased rate of infections in the post incision group.”

Comment 19:
Page 9, line 59: Give the non-significant p value.
Response 19:
Thank you. Now p-value (0.37) provided in the manuscript.

Comment 20:
For the comparison of post op complications, did the authors conduct post hoc tests to determine where the differences were among the different post op complications? It looks like the main difference is in SSI, but this needs to be verified with post hoc tests. In the discuss the authors state they had no difference in some of the post op complications as reported in other studies, but without the post hoc tests, they can't say that for sure. For all comparisons that had more than two categories that were statistically significant, post hoc tests should have been conducted.

Why did the authors not report the relative risk and confidence intervals for the results? These results should be provided.
Response 20:
Thank you very for the correction.

For all comparisons that had more than two categories that were tested using Bonferroni correction test. Relative Risk (RR) with 95% confidence intervals provided for the results. Also in Table 2.

Comment 21:
Page 11, line 12: "Lesser" is not a word.
Response 21:
Thank you. Now it has been changed as “decreased”.
Comment 22:

Nowhere in the discussion section do the authors discuss the limitations of their study. For example, is the study sufficiently for all the maternal and neonatal outcomes? It is likely, based on the incidence rates of some of the outcomes they looked at that the study does not have sufficient power to detect a difference. Without knowing this, they need to be careful making the conclusions they made. It is possible that a difference was not found between the groups because of a type II error.

Response 22:

The study was powered mainly for the maternal outcomes and not for the neonatal outcomes and therefore this has been acknowledged and the conclusion for the neonatal outcomes has been softened.

Limitations of the study: added in the last paragraph of discussion.

“The limitations of the study was that, though the sample size calculated was 1200 mothers, the actual numbers fell short. The study was not adequately powered for the neonatal outcomes. The operational definitions for some of the maternal and neonatal outcomes were based on clinical signs and symptoms, and not on any laboratory tests”.

Reviewer 3

Response to Reviewer Comments (Version 1):

Title: A randomized controlled double blind trial comparison of the effects of the prophylactic antibiotic Cefazolin, administered at Caesarean delivery at two different timings (before skin incision and after cord clamping) on both the mother and newborn.

Authors: ChintaAnnie Jyothirmayi, Ajay Halder, BijeshYadav, Santosh Thomas Samuel, AnilKuruvilla and Ruby Jose

Reference No: (PRCH-D-16-00087R1)

Reviewer reports:

Reviewer 3:

Comment 1:

Abstract.
I believe the authors should say "an important risk factor", rather than "most important risk factor" given other risks such as chorioamnionitis, PPROM, morbid obesity, etc. It's difficult to prove one is the single most important factor.

Response 1:
Thank you very much. Now abstract has been corrected as “an important risk factor”.

Comment 2:
There should be one primary outcome, not 7 as listed.

Response 2:
Thank you very much. Now the primary objective has been changed as
“To compare the effects of the prophylactic antibiotic, intravenous Cefazolin 1gm, administered at Caesarean delivery (CD) at two different timings (30 minutes to 1 hour) before skin incision and immediately after cord clamping) on both the mother and newborn”.

Comment 3:
Endometritis misspelled in Result sub-heading

Response 3:
Thank you. It is corrected now.

Comment 4:
Can the authors list RR as well as p-values?

Response 4:
Thank You. Now RR with p-values provided in the manuscript and the manuscript was modified based on RR.

Comment 5:
Background.
Line 19-21 is oddly worded and recommends edits. Wound infections still occur if strict aseptic precautions are taken.

Response 5:

Thank you. It is corrected.

Comment 6:

Materials and Methods.

What dose of Cefazolin was used

Response 6:

Thank you. The dose has been now added in the Patients and Methods section.

Comment 7:

Randomization misspelled

Response 7:

Thank you. It is corrected.

Comment 8:

Space between block and randomization

Space between added and sentence corrected

Response 8:

Thank you. It is corrected.

Comment 9:

What was the exact timing of pre-incision administration? 30 mins?

Response 9:
Thank you. The timing has now been added as 30 minutes to 1 hour.

Comment 10:

How were infections determined? What criteria?

Response 10:

Thank you. Operational definitions have now been added in Patients and Methods section.

Comment 11:

There is little detail on how cord blood was collected and analyzed?

Response 11:

Thank you for the question. The relevant details are now added to the Patients and methods.

“The cord blood (5ml) was collected soon after delivery of the baby in a blood collecting test tube, centrifuged and the serum was refrigerated for analysis. The drug level (Cefazolin) was analysed by a developed and validated High Pressure Liquid Chromatography (HPLC) with Ultra violet protection method in the clinical pharmacological unit”.

Comment 12:

Statistics.

What normality tests were used if any?

Response 12:

Thank you. Now Statistical methods section has been modified.

Comment 12:

Cefazolin misspelled

Response 12:

Thank You. It is corrected
Comment 13:

Results.

Demographics - several typos, spacing issues, strange capitalizations in this section. Please correct.

Response 13:

Thank You. They are corrected.

Comment 14:

The paragraph on birthweight can be shortened. Cefazolin should not affect.

Response 14

Thank you. The paragraph on birth weight has been shortened as means (SD).

Comment 15:

Again recommend relative risks for the outcomes, with confidence intervals

Response 15

Thank you. Now relative risk with confidence intervals has been provided.

Comment 16:

Is the "2-3%" of the drug based on a mean cord blood level? It was fairly variable

Response 16:

Yes, the mean cord level of the drug has been taken, though it was variable.

Comment 17:

Discussion.
Lines 27-39 is confusing, it isn't clear the authors are talking about Owens paper until the very end. Last sentence should be first.

Response 17:
Thank You. It is corrected as suggested.

Comment 18:
Conclusion

I don’t believe the study is powered to detect neonatal sepsis differences, so it is hard to say with confidence the final sentence. It is reassuring that no difference was seen in this and other studies, but should soften that conclusion.

Response 18:
Thank you. Now, the conclusion on this inference has been softened by adding that the study was not powered for this inference.

Comment 19:
References: #6 - why are the authors not listed?

Comment 19:
Thank You. It is corrected.

Comment 20:
Tables: Would list p-values in Table 1

Response 20:
Thank you very much. Now p-values have been listed in Table 1.