Author’s response to reviews

Title: Reference intervals for thyroid stimulating hormone and free thyroxine derived from neonates undergoing routine screening for congenital hypothyroidism at a university teaching hospital in Nairobi, Kenya: A cross sectional study

Authors:

Geoffrey Omuse (g_omuse@yahoo.com)
Ali Kassim (ali.kassim@aku.edu)
Francis Kiigu (francis.kiigu@aku.edu)
Syeda Hussain (ra'ana.hussain@aku.edu)
Mary Limbe (mary.limbe@aku.edu)

Version: 1 Date: 19 Feb 2016

Author’s response to reviews:

REVIEWER 1

1. Are the authors able to provide a sense that their population cohort did not include premature patients? Given the inherent immaturity of the thyrotrope axis with increasing prematurity, with a correlate delayed TRH and TSH surge, inclusion of premature neonates could skew the TSH and Free T4 values towards lower values than would be expected with a non-premature cohort.

This has been clarified. Please see line 143-144 in the manuscript with track changes.

2. Were the "total of 1673 and 1359 non duplicate values for TSH and ft4 respectively" representative of all consecutive patients who had samples drawn? Were there patients sampled that were not included in the survey? It would be helpful to make this less ambiguous.

Please see track changes from line 146-154. Following reviewer feedback, we subsequently have excluded all neonates that had fT4 or TSH values not within the Roche reference
intervals and had an acute illness or both fT4 and TSH out of range. Subsequently, the values included in the re-analysis are 1639 and 1329 for TSH and fT4 respectively. See track changes, line 197-201

3. The authors make the interesting observation of a gender difference between ages 0-7 days. For the different age groups, are the authors able to provide a sense of the male/female representation (perhaps a percentage) for the other age groups, to provide a reassurance of adequate representation of each gender, given their interesting observation in the subgroup.

After excluding the neonates as explained above and re-analysing the data using non-parametric methods namely Mann-whitney and Kruskal Wallis instead of one-way ANOVA, the difference previously described was no longer seen. The change in statistical tests was informed by the fact that the distribution of fT4 and TSh values was not normal hence requiring non-parametric methods. However, TSH values for neonates aged 0-14 days subsequently showed a significant gender difference. Please see track changes in data analysis and results section. The number of males and females is shown in table 1.

4. Semantic changes may be helpful (line 262 - suggest to delete "called Tietz" and have Reference [5] enable the reader to refer to the Tietz textbook).

Done

REVIEWER 2

1. The methods section should include more information about the approach to collection of data. For example, were the laboratory databases examined first to identify infants, or were the clinical records used to identify them? Would like to know additional details about the infants included in the reference population. Were there any predetermined inclusion or exclusion criteria for data to be included? Additional information I would like know include, for example, what was the average gestational age of the infants? How many were preterm? 3) Along the same lines, would like to know more about chart reviews that were conducted. These were mentioned in the discussion section, but not in the methods. In particular, I would like to know the extent to which chart reviews were performed, e.g. how many chart reviews? And what were the aims of the chart reviews? 4) There is also mention of examination of the charts of the outliers, and some might have non thyroidal disease, but how many? What if infants had sick euthyroid syndrome, which is when non-thyroidal illness could certainly affect thyroid function transiently.

Please see track changes from line 146-154. Following reviewer feedback, we subsequently have excluded all neonates that had fT4 or TSH values not within the Roche reference intervals and had an acute illness or both fT4 and TSH out of range. Subsequently, the values included
in the re-analysis are 1639 and 1329 for TSH and fT4 respectively. See track changes, line 197-201. We do acknowledge that the study has limitations due to the study design. A prospective study with better defined inclusion and exclusion criteria would be ideal.

2. Also, the analysis was repeated in a "parametric analysis" without the outliers, and there was supposedly no change. This additional analysis should be discussed in the methods section.

Done. Please see data analysis section lines 189-192

3. Would suggest some discussion about how using the new reference range might impact clinical detection and screening for congenital hypothyroidism.

Done. Please see track changes in the discussion section lines 340-344

4. Background line 91, "commonest" should be changed to "most common."

Done. Please see track changes line 94

5. Line 110, "…population-specific and derived from…” (add a hyphen before "specific", and add "and.")

Done. Please see track changes line 113

6. Line 122, should read "This population was comprised of primarily…” (add "was" and "of")

Done. Please see track changes line 125-126

7. Line 123, should read"…which is largely comprised of black…”

Done. Please see track changes line 127

8. Line 135 -Please write out what ISO stands for

Done. Please see line 138

9. Line 141, "Newborn" is one word

Done. Please see track changes line 149-150


Done. Please see reference number 22

11. Results line 192. For neonates age 8-14 days, is the lower limit 7 or 8? Some places in the manuscript say 7, and some say 8.
For the purpose of comparing with the Roche reference intervals, the data was stratified into 2 groups i.e. 0-6 days and 7-30 days respectively in line with the age stratification provided by Roche. For deriving our own reference intervals, we chose to initially stratify our data into 4 groups namely, 0-7, 8-14, 15-22 and 23-30. Therefore, use of 7 or 8 as a lower limit in the discussion is dependent on the context.

12. Results line 201, instead of "not within," suggest saying "outside."

Done. See track changes line 232

13. Discussion, line 262 and 265, please clarify what RI is being discussed. I presume TSH.

Done. Please see track changes line 298 and 300

14. Discussion line 289, suggest adjusting sentence to "in the first week of life, which was probably not sufficient…"

The sentence has been deleted

15. The Conclusion also needs editing for missing articles

Done. Please see line 359 and 360

In addition to responding to the reviewers’ comments, re-analysis of data was done and different results were obtained necessitating a change in discussion points as seen in the track changes. The main reason for the change in results stems from exclusion of neonates who had acute illnesses, discrepancies in age and thyroid function test results suggestive of a thyroid disorder. In addition, whereas the initial data analyses was done using one way ANOVA to compare groups, we have used Mann Whitney and Kruskal wallis tests which are non-parametric methods in the re-analysis. These are more appropriate statistical methods given that the data was not normally distributed.

All authors have reviewed and approved the changes.