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

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Version: 2 Date: 03 Mar 2016

Author’s response to reviews:

REVIEWER 1

1. Regarding the response to Comment 2 - the authors state in the 1st Revision that they have excluded "fT4 and TSH" values that were "out of range", presumably of the Roche assay. Were these patients also ill, as if they were completely healthy, then, for this specific population, the reference range for Roche may not be relevant, as the authors are specifically developing a reference range for this specific population, and hence the reference range developed by the authors may wish to include these patients (if they were not ill or had some other reason to have a pertubed thyroid axis).

Indeed we excluded patients who had a TFT profile that suggested the possibility of a thyroid disorder based on the Roche cut offs. This included patients with a combination of results such as very low fT4 and high TSH, high TSH and high fT4, low TSH and low fT4, and low TSH and high fT4. One patient had a low fT4, low fT3 but no TSH available while another had a very high TSH but no fT4 or even fT3. Unfortunately for a number of these patients, we didn’t have medical records to confirm that the deranged TFTs were as a result of a thyroid disorder and as such had to make a decision purely based on the TFT profile. In situations where only one parameter was outside the Roche reference interval and the file review revealed no abnormality,
the values were included in the analysis. The exclusion of patients with 2 abnormal thyroid function test results based on the Roche cut offs can be considered a weakness of the study but we do believe that including them without conclusive evidence of the absence of a thyroid disorder would bias the results. A prospective study with better defined inclusion and exclusion criteria and stringent adherence to the same would be ideal. Being retrospective in nature, this study definitely has limitations such as what has been pointed out.

REVIEWER 2

1. Line 94. Need clarification, suggest revision to "where reduced function from dysgenesis or dyshormogenesis results in an increase….."

Done

2. Line 106. In the background section about detection, I recommend removing statements about subclinical hypothyroidism because the main goal of this paper is to establish references ranges to assist in the detection of congenital hypothyroidism so that infants may receive treatment. In adults and older children, subclinical hypothyroidism involves a TSH<10, but still above normal range, and normal thyroid hormone levels. These patients are sometimes treated, sometimes not, but the management is controversial. False positives for congenital hypothyroidism newborn screening do occur, but are uncommon. For this paper, I would recommend focusing on establishment of the reference range for the purpose of the detection of primary congenital hypothyroidism, and removing comments on "subclinical hypothyroidism."

Done

3. Line 108. Suggest changing "higher prevalence" to "higher diagnostic rate"

Done

4. Line 192. Need clarification. 34 TSH values and 30 fT4 values were excluded. Were TSH AND fT4 values in the same patient excluded? Did some patients only have TSH performed and not fT4?

Yes. Twenty nine patients had both fT4 and TSH values excluded. This has been clarified on line 195 in the track changes. One patient had low fT4, low fT3 but no TSH result. Five patients had TSH but no fT4.

5. Although Table 1 and Table 2 are noted to have some differences between groups, they are not noted by asterisks or other marker.
In the results section, it is explained that RIs were stratified based on the result of comparisons for age and gender. Where there were statistically significant differences, the results were stratified accordingly.

6. Figure 1. In the graph legend, there is "+ Suspect data." Are these the values that were excluded? Should they be removed from the graph?

No they shouldn’t. What is plotted is what was left after excluding neonates, with acute illness, age discrepancies and overtly abnormal TFTs. What is reflected as suspect and outlier data is based on the Refvals software for calculating RIs. Specifically, if Q1, Q2, Q3 respectively represent the first, second and third quartiles of the distribution and IQ=Q3-Q1 is the inter-quartile range, outliers are data smaller than Q1-3 IQ or greater than Q3+3*IQ and suspect data are data contained in the interval [Q1-3 IQ; Q1-1.5 IQ] or [Q3+1.5*IQ; Q3+3*IQ]. Please see line 186-188 in track changes.

7. There are quite a few grammatical, spelling or diction errors that need correction. Here are some examples below:

- Line 176. Grammar, verb should be past tense, ie,"Values were compared…"
  Done
- Line 177. Need a period after "as appropriate."
  Done
- Line 177. "Age wise" should be hyphenated.
  Done
- Line 209. Duplicate ss in "ranks."
  Done

All authors have reviewed and approved the changes.