Reviewer's report

Title: Assessment of Peripheral Blood Lymphocyte Subsets in Children with Nutritional Iron Deficiency anemia (Two Egyptian Centers Study)

Version: 1 Date: 07 Nov 2017

Reviewer: Eunice Adei-Atiemo

Reviewer's report:

The authors noted that currently available research about the effect of IDA on immune function is confusing. Was this study able to help clarify any of the confusion? Have there been other similar studies conducted in Egypt? Does the study add any new information? They also aimed to "correlate the results with the clinical and laboratory criteria of the affected children." There wasn't much information regarding the effects of IDA on the clinical presentation or past clinical history of affected children. Are the findings likely to have any clinical significance?

ABSTRACT

Line 47: suggest replace "promotes" with "may result in an" inadequate immune response

Line 48: Sentence structure incomplete - there should be an "of" before "childhood iron deficiency anemia".

Line 49: The result presented did not really address any clinical correlations

Line 51-52. Suggest rewording of sentence e.g A complete history was obtained and clinical examination performed. Instead of "all were subjected to"

Line 53: Suggest "performed" instead of "all were done"

Line 57: Suggest "patients had significantly lower haemoglobin, serum iron … compared with controls"
Line 57-8: From the results presented patients had a significantly higher (3386) and not a lower total lymphocyte count than controls (2632) - Table 2. P value 0.03 and not 0.001 as noted in the text.

Line 61-63: Suggest restructuring of sentence. Sentence not complete. Ideally should not begin with "Significant reduction" - Should rather be "There was a significant reduction" or "CD4 counts etc were significantly lower in patients…"

Sentence structure should be corrected throughout the document"

Suggest "while there were no significant difference regarding CD8"… instead of "while no significant…"

Line 68-9: The study did not actually assess cell-mediated immune RESPONSE. It assessed specific immunological cell lines and parameters but these were not correlated with a specific response to any stimulus, illness etc. The study reported on the correlation between IDA and lymphocyte subgroups

INTRODUCTION

Line 73: close link… instead of closed link

Line 75: mediated … instead of mediats

Lines 77-81: Long and structurally incomplete sentence that is difficult to follow. Suggest separating into at least 2 sentences.

Line 83: what does "differentiation-convincing" properties mean?

Lines 91-93: Suggest removal of "However:" and sentence may read: There are 3 types of mature T cells based on their surface receptor expression…"

Line 94: "research" instead of "researchs"
What about the research regarding the effect of IDA on immune function makes it confusing? Are there any references to support this assertion? Which clinical criteria were the results correlated with?

PATIENTS

How many patients were recruited from each of the study sites?

Why was a 1:1 Case:control ratio not used? Where there challenges in recruiting controls?

What was the process for obtaining consent?

Line 103-4: Suggest "Patients were selected from the… Outpatient clinics of the Quena and Minia University Children's

Line 106: "proven" instead of proved (kindly correct in remainder of text). Suggest "included" instead of "involved"

Line 107: 2-16 years.

Lines 110-113: Suggest rewording this section and to maintain past tense. E.g Eligibility: Children aged ….. diagnosed with iron deficiency anaemia based on lab investigations of hypochromic…. and a history and examination correlating with anemia."

Line 119: parasitic infestation

Line 120: remove "all"

Line 124: May there have been a limitation of selection bias with regard to controls as all the controls were recruited from one of the study sites and not from both?

Line 125: Details regarding sex of the controls should come in the results section.
Lines 126-133. From how presented in the text, it appears that the examination and blood tests were conducted only on controls

Lines 132-133: Suggest this information be brought up into the section regarding eligibility criteria.

Line 137: Suggest "approximately" instead of "about"

Line 153: suggest "used to calculate the neutrophil lymphocyte ratio"

Line 176: Suggest removal of "On the other hand, description of"

Lines 177- 178: Use past tense

Lines 179-80: Suggest rewording of first half of sentence

RESULTS

Line 186: Suggest "confirmed" instead of "proved"

TABLE 1: If none of the controls had organomegaly, 0% should be used

Line 191: The use of terminology such as "a significant decrease in Hb concentration" or "patients showed a significant reduction in both count and percentage" gives the impression that there was a previously higher concentration or count in the same patient or group of patients. I would suggest something more along the lines of "Patients had significantly lower haemoglobin concentrations…. than controls" throughout the document.

Line 191: According to table 2, IDA patients had significantly higher (not lower) total lymphocyte counts (3386) than controls (2666), p value - 0.03 and not 0.001 as noted in the text.

Table 2: Median values instead of the mean were used, which according to the information provided in the methodology was because the data was not normally distributed. Why was the
data not normally distributed? Did the sample size contribute to this? This should be discussed as one of the limitations of the study.

Line 193 - 194: Suggest .."while there was no significant difference between the two groups with regard to WBC…."

Line 195: Can remove the p values from the text as they can be found in the table, particularly since they were not significant.

Line 199: remove "for both"

Line 201: "lower" instead of "lowered"

Table 3: Were mean values or median values presented? If mean values, it would be informative to provide the standard deviation also (mean ± SD)

Table 4: Note that "r" is the correlation co-efficient

Figure 1: The significance of the graphs shown in figure 1 isn't very clear.

DISCUSSION

A few inclusions required in the discussion include the following:

Is it likely that the difference in the CD counts and percentages would have any clinical significance?

Which has more clinical significance - the absolute count or the percent?

The finding that the total lymphocyte count among patients was significantly higher than controls was not discussed much. What lymphocyte subgroups accounted for this difference?
No limitations to the study were noted. Some limitations include the fact that the data was normally distributed and therefore medians instead of means were used. Would a large sample size have helped correct this?

A more detailed analysis of the immunological parameters may have been more informative.

Were other confounding variables taken into account? For example, patients with IDA had significantly lower median weight and height centiles than controls. Could that have contributed to the lymphocyte counts and percentages?

Lines 214-216: Correct sentence structure

According to the results presented, IDA patients were also found to have significantly lower absolute CD3 counts (328 compared to 523 in controls, p value 0.007). It's been noted here that they "showed insignificant change."

Line 217 - suggest "found" instead of "proved"

Line 219, 221 - spell check

Lines 223-224 - meaning of phrase unclear

Line 227: What may account for the difference in your findings compared to the reduced total lymphocyte numbers reported by Santos and Falcao?

Line 235: Sentence not complete

Line 236 - 238: Significance of this sentence and how it relates to the findings of this study is not very clear

Lines 250-251: Reword sentence

Lines 253-255, 259: Correct spelling
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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