Author’s response to reviews

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

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Author’s response to reviews:

Dear BMC Pediatrics Editorial Office,

These are the response to reviewers comments in the first revision.

Reviewer reports:

Taddio Andrea (Reviewer 1): Although not innovative, the paper is well written and the methods are good.

However the discussion is a sort a summary of previous study on the field and schematic comparison to authors’ results: I would suggest to redefine to discussion session starting from authors’ results and thoughts about them. Then a comparison with others studies will be well accepted.

Reply

The discussion is totally revised and written again.
Eunice Adwoa Adei-Atiemo, FWACP, MPH, MBChB (Reviewer 2):

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?

Reply

The study clarified the role of iron in lymphocyte proliferation and the impact of iron deficiency on different lymphocyte subpopulation, an issue that still unclear in its explanation.

Have there been other similar studies conducted in Egypt? Does the study add any new information?

Reply

Only one study had conducted in Egypt and published after the submission of my article to your journal. But such study was done in another area. Moreover, this study was conducted in Upper Egypt, an area with low economic status and high level of illiteracy so the patients have much immunologic and nutritional challenges.

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?

Reply

there are much information regarding the clinical history of patients. We mentioned the effect of IDA on physical growth presented by the weight and height centiles which was differing significantly with controls. Other clinical finding will be away of the target of our research.
ABSTRACT

Line 47: suggest replace "promotes" with "may result in an" inadequate immune response
Reply
Had revised and corrected

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

Reply
Had revised and corrected

Line 49: The result presented did not really address any clinical correlations
Reply
Had revised and corrected. The word clinical is omitted

Line 50-52. Suggest rewording of sentence e.g. A complete history was obtained and clinical examination performed. Instead of "all were subjected to"

Reply
Had revised and reworded

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

Reply
Had revised and corrected

Line 57: Suggest "patients had significantly lower hemoglobin, serum iron … compared with controls"

Reply
Had revised and corrected
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.

Reply

Had revised and corrected

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…"

Reply

Had revised and corrected

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 Reply Had revised and corrected

INTRODUCTION

Line 73: close link… instead of closed link
Reply
Had revised and corrected

Line 75: mediated … instead of mediated

Reply
Had revised and corrected

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

Reply
Had revised and corrected

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

Reply
This was a reading error and the sentence is reworded

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"

Reply

I noted that the word does not present in this paragraph

Line 95: 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?

Reply

This could be another title for another research and sure well add more vision to the immunological status in a qualitative point of view.

PATIENTS

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

Reply

Had revised and mentioned in the methodology

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

Reply

yes due to cultural and orientation issues many children refuse to share in this group either patients or control. We are in a poor area in Upper Egypt where the ignorance and the nature of closed community are dominant.
What was the process for obtaining consent?

Reply

written consents were obtained from the caregivers of both patients and controls after explaining the nature and the aim of the research. These written consents were added to the patients recruitment file.

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

Reply

Had revised and corrected

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

Reply

Had revised and corrected

Line 107: 2-16 years.

Reply

Had revised and corrected

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."

Reply

Had revised and reworded
Line 119: parasitic infestation
Reply
Had revised and corrected

Line 120: remove "all"
Reply
Had revised and corrected

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?
Reply
A limitation section was added

Line 125: Details regarding sex of the controls should come in the results section.
Reply
Had revised and added 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
Reply
This was a writing mistake and corrected

Lines 132-133: Suggest this information be brought up into the section regarding eligibility criteria.
Reply
Had been added to the eligibility criteria
Line 137: Suggest "approximately" instead of "about"
Reply
Had revised and corrected

Line 153: suggest "used to calculate the neutrophil lymphocyte ratio"
Reply
Had revised and corrected

Line 176: Suggest removal of "On the other hand, description of"
Reply
Had revised and corrected

Lines 177-178: Use past tense
Reply
Lines 179-80: Suggest rewording of first half of sentence
Had revised and reworded

RESULTS
Line 186: Suggest "confirmed" instead of "proved"
Reply
Had revised and corrected

TABLE 1: If none of the controls had organomegaly, 0% should be used
Reply
Had revised and corrected
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.

Reply

Had revised and corrected

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.

Reply

Had revised and corrected

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.

Reply

Had revised and a limitation section was added included these points.

"while there was no significant difference between the two groups with regard to WBC…".

Reply

Had revised and corrected

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

Reply

Had revised and corrected
Line 199: remove "for both"

Reply

Had revised and corrected

Line 201: "lower" instead of "lowered"

Reply

Had revised and corrected

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

Reply

Had revised and median was used.

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

Reply

Had revised and corrected

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

Reply

Had revised and I found that they will not add much to our results explanation so, I omitted them.

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?

Reply

the absolute count but the percentage defined which subset is more affected

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?

Reply

Had been considered and explained in the re-formatted discussion.

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?

Reply

a limitation section had been added in the manuscript text.

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

Reply

had revised and the discussion is totally formatted and wrote again

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?

Reply

they indicate the chronicity of IDA which means more longstanding impact of the low iron status on phrephiral lymphocytes.
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"

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?

had revised and explained in the reformatted discussion section
Line 235: Sentence not complete
Reply
Had revised and corrected

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

Lines 250-251: Reword sentence
Reply
Had revised and corrected

Lines 253-255, 259: Correct spelling
Reply
Had revised and corrected