Reviewer's report

Title: Detection and molecular characterisation of human adenovirus in diarrhoeic children of Dar es Salaam, Tanzania; a case control study

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Reviewer's report:

Manuscript Title: Detection and molecular characterisation of human adenovirus in diarrhoeic children of Dar es Salaam Tanzania; a case control study.

The authors described the prevalence rate and genotyping of Human Adenovirus (HAdV) that the one of pathogenic agent responsible for acute diarrhea especially in children in Tanzania. The virus had been detected in fecal samples that collected from two group of peoples who present and absent of diarrhea. The result shows that the virus could be detected in those two groups of samples, but in different detection rate. And HAdV genotype 40 and 41 responsible for the majority genotype detected in both groups of sample. The result is very interesting, however, the critical concerns in the manuscript had been shown in all along the text. The following are my comments for this article.

Major Compulsory Revisions:
Overall: The contents in the text were confusing, did not well organizing, and fragmented. The main problem was the objective in the study was not focusing along the text. The authors should keep in mind all the time when building up the manuscript. What was the main objective(s) in this manuscript? Did the objective(s) was/were The prevalence study?, The molecular epidemiology study?, and/or The association study? And what was a proposing for HIV patient that including in the study?

Title of manuscript: Could change to relate the texts that write in the article. The authors should be deiced which point of the research that want to pointing out. It will be the same criticize as the objective that mention above. If the objective is clears, the rest of the manuscript, even the title, would be better.

Abstract and Key word: If the authors want to address HIV patient in the manuscript, the stronger information, reference, and objective would be more specific too. (Personal opinion I still not quit understand the propose for adding HIV patient into this study)

Introduction: The authors should provide more details especially the information that related to the study. The aim of introduction part is to provide reader of basic and relate knowledge to this study. Therefore, if the authors had been already decided what story that going to tell the reader, the introduction should relate to the following story. The un-necessary information should be omitted.
Material and Methods:

1. Even the diarrhea patient was used as the previously study; the brief detail should be show in the text. Moreover, another group of samples had been collected for this study, for clearly and easily understanding, all details of samples MUST BE shown. It should be great if compare the details in two groups of samples in table.

2. What were the inclusion and exclusion criteria of diarrhea and none-diarrhea patient? Or did the patients were out- or in- patient?

3. For the non-diarrhea group, “…admitted to hospital due to diseases other than diarrhoea (n=235)”, what was a disease that identified in this group of patient? Because it should be affect the result interpretation and discussion.

4. In Data collection “…some of the clinical information…” What data(s) was collected? These SOME data(s) could be collected in all patients. Or just some of patients? And the quality of data is homogeneous in all of individual. And why collect only SOME data?

5. In Data collection “… HIV status was obtained from patient files.” Why this information was so importance? And was it ethically right?

6. For Adenovirus detection, Why using ELISA for detection method? As I mention before now a day more than 51 genotypes of HAdV had been classified. The ELISA kits that used in this study were designed to detect only 51 genotypes. As my experience, the ELISA is very limit to detected HAdV.

7. For Real time PCR for adenovirus genotyping, Why did not used this technique for virus detection in all samples? Because this technique is more sensitive and specific for HAdV.

8. For Real time PCR for adenovirus genotyping, “…In some cases when a larger PCR-product (322 bp) was needed…” Why the longer PCR-product was needed in some cases?

9. For Sequencing of PCR amplicons, The region for HAdV genotyping is fiber gene. Why the author did not sequence the fiber gene instead of hexon gene for genotyping?

Result

1. The data in “Prevalence of adenovirus” and “Distribution of adenovirus infection by age” should be summarize and put in the table. And these two parts should be combined into only one part. Then summarized the table into text that will make this part is less confusing.

2. For “Association between demographic/clinical characteristics…” why HIV so importance? And what about the common clinical signs of HAdV infection like watery diarrhea, vomit, etc.?

3. For “Seasonality of adenovirus infection”, “We divided the months of the study according to the season of the year…” What were the criteria that used for your season divided? Rain level? Temperature? Humidity? And the reference(s) was needed.
4. The phylogenetic tree analysis of HAdV should be constructed. Because the authors would be more understanding and discussing about molecular distribution of this virus.

Discussion

The following are the comment base on contents in the manuscript, however, the overall of details in this discussion part is fragmented mis-pointed.

1. “...Enzyme Immune Assay (EIA) able to detect 51 types...” Please discuss more about sensitivity, specificity, and limitation of this kit.

2. “...Comparing adenovirus prevalence in this study with reports from other African countries, we observe large variations. Studies detecting HAdV by PCR, which is known to be more sensitive, have reported higher prevalences [18, 19, 24] compared to studies employing EIA [15, 25]...” WHAT is a point that the authors going to tell from this sentence? AND WHY PCR base method did not used for the detection in this study?

3. “...prolonged shedding of adenovirus in stool after previous infection of more than one month prior to the study. Alternatively, it could be asymptomatic adenovirus infections in children who may have acquired immunity from previous infections.” WHAT is the evidence(s) and reference(s) that make the authors conclude like this? IS it reasonable?

4. “...We found that the majority of HAdV infected diarrhoeic children were dehydrated. This concurs with reports from other developing countries [26] suggesting that adenovirus can cause severe diarrhoea...” There are so many studies that already proof that HAdV can cause acute watery diarrhea. Please search more details and re-write this paragraph.

5. “Sequence analysis showed... This observation is consistent with...adenovirus type 40 has been observed” This paragraph is totally confusing. Please re-arrange and re-write this paragraph.

6. “...Hence findings of this study partly support the theory of prolonged shedding of these human adenovirus species in faeces.” THIS is totally mis-conclusion.

7. For HIV point, it is still not clear what the point that the authors want to mention is? It make HIV is another story inside your manuscript. Therefore if the authors want to incorporate HIV into the study, the authors should make the clear objective at the beginning.

Conclusion

“...indicate prolonged excretion of adenovirus in stool, or acquired immunity from previous exposure from non-diarrhoeic children.” This is over conclusion.

Minor Essential Revisions:

Abstract:

1. Too long and too much information. The abstract should be summarize and highlight the study.
2. “...prolonged viral shedding in stool, or acquired immunity from previous exposure for the non-diarrhoeic children.” Is it over conclusion?

3. “This first report on molecular epidemiology of HAdV in Tanzania observed diversity of HAdV types that circulate in the study setting.” Are the authors sure about this sentence?

Introduction:
1. Please provided the reference for “Human adenovirus (HAdV) causes acute diarrhoea sporadically, as well as in outbreaks”.
2. “To date there are 52 types of adenoviruses identified...” Please check the up to date data. I think it more than 52 type of HAdV had been classified already.

Material and Methods:
1. “...(UNG activation),... (polymerase activation),... (denaturation) ,... (annealing) and ...(extension)....” Could be deleted if desired.
2. In real time PCR condition, the fluorescent detection step should be addressed. (Normally the detection should be after each of extension step.)

Result:
1. All P-value in the text “P” should be italic “P”
2. For “Molecular epidemiology”, “...BLAST...” should be also mention in material and method section.
3. For “Molecular epidemiology”, “...The proportions of enteric adenoviruses (type 40 and 41) were not significantly different in diarrhoeic and non-diarrhoeic children (50%, 12/24 vs. 46%, 6/13, P=0.82).” How about other genotypes result rather than F40,41?

Discussion:
1. “The molecular epidemiology of human adenovirus species and types from Tanzania is described for the first time in the present study.” Be careful about over claim.
2. “...EIA method specific to enteric adenovirus 40/41 which was used in the previous study.” Pleases add the reference.
3. “...In order to detect serotype drift in the study setting, future studies are needed over a prolonged period of time, as reported elsewhere [9,10, 29].” What is the point of this sentence? Please re-write.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:

I declare that I have no competing interests