Reviewer’s report

Title: Examining Plasmodium falciparum and P. vivax clearance subsequent to antimalarial drug treatment in the Myanmar-China border area based on Quantitative Real-Time Polymerase Chain Reaction

Version: 0 Date: 10 Dec 2015

Reviewer: Khalid Beshir

Reviewer's report:

COMMENT

There is a clear need to monitor parasite clearance and molecular markers of antimalarials particularly the relatively well established genes pfmdr1 and pfcr as well as Pfmrp1 and pfatp6 and pfk13. This is an interesting study which is certainly of significance for monitoring the spread of drug resistance and adds value to the ongoing establishment of the roles of different genotypes in the efficacy of many antimalarial drugs. The manuscript confirms results previously shown by several researches in Africa ie the selection of certain genotypes after many years use of ACT or genetic association between parasite clearance and various genes of interest. The strength of this study lies in the fact that the authors used qPCR to detect low level parasitaemia, which are otherwise missed by microscopy and generated more phenotypic data in order to get meaningful association study. Below are comments for improvements in order to strengthen the value of the study.

MINOR COMMENTS

1. Line 88 - delete "with"

2. Line 262 - Please use "patients clearing slowly" rather than "slow clearance samples" in all the manuscript.
3. The authors used day 3 positivity as a cut-off value to decide whether or not a patient is slow clearing - Patient who cleared parasites on day 2 or 3 was classified as fast clearer while patient who cleared parasites after day 3 was classified as slow clearer. The authors have not justified why they used such a cut-off value. Parasite reduction ratio (prr48) can give a better estimation of clearance rate and it would strength their finding if they can include the data for prr48 (ratio of day 0 parasitemia divided by parasitaemia 48 hrs later). Though day 3 positivity is a simple parameter to use, its association with prr48 should be evaluated as prr48 is the better parameter to measure clearance rate as it takes into account initial parasitaemia.

4. The authors described mutations in the genes they looked at and classified them as mutant or wild type. They classified Y184 as wild type and 184F as mutant. However, the pfmdr1 184 mostly is found in both Y184 and 184F form and is difficult to decide which one is mutant. Even in the current study, their prevalence is similar (~ 50% each for both slow and fast clearers), which reflect the prevalence observed in many endemic countries. Therefore, I recommend the authors avoid using mutant/wild type description for codon 184.

5. Some researchers reported that sub-microscopic residual parasites on day 3 are more likely to carry the CQ-sensitive haplotypes in western Kenya (76K and pfmdr1 NFD haplotype etc) (Beshir et al, 2014 and Henriques et al 2014). It would be interesting if the authors can look particularly at pfmdr1 184, pfmrp1, pfatp6 and pfk13 on day 3 and verify if the findings by the above researchers can also be observed. If there are any molecular marker data on day 3 or day 28 after treatment, is there a selection of a particular allele on those days?

6. Line 307 - "… qPCR analysis could detect both living as well as remnant or dead parasite DNA of an infected sample". Please provide the reference for this. Dead parasites are known to be cleared quickly by spleen and the evidence is in the patients that cleared parasite by day 1. Had there been dead parasite, which are not quickly cleared by spleen we would have detected them in all patients regardless of parasitaemia.

7. Line 344 - Please revise the statement. Reference 60 doesn't report the association of 184F with slow parasite clearance when stratified by site, which is the relevant part for this study.
8. Line 360 - "Our data presents evidence of strong selection of pfmdr1 N86 allele, as well as increased pfmdr1 gene copy number in post-ACT parasites". Pfmdr1 CNV is not part of the current study and the authors refer to a previously published data (Ngala et al. 201) and presented it as if it was part of the current findings. Please review discussion about pfmdr1 copy number.

9. Please report in the method section which antimalarial was used. If ACT, please discuss how the artemisinin and the partner drug affect parasite clearance.

10. The title of the paper is somehow misleading. Though the authors used qPCR to detect parasites, the parasitaemia was not used for the analysis - a simple presence or absence of parasite in different days was employed to decide clearance time. If parasite positivity is used for analysis then avoid using parasitaemia in the title. Alternatively, include the parasitaemia in the results section and discuss clearance rate based on prr48 and then keep the title as is.

11. Please report the PCR efficiency of the qPCR assay.

12. Since the authors have not used internal negative control how did are false negative rule out for both DNA extraction and PCR? Primers which amplify human DNA are usually used for internal control purposes for both. If this was not done please discuss them as drawbacks of the study.

13. The authors have not included detailed information about clinical outcome including initial parasitaemia, haemoglobin level, patient age as well as treatment. Initial parasitaemia and age are widely accepted to affect parasite clearance. The authors should report this in the result section and normalize the clearance data using initial parasitaemia and stratify the data by age to see whether age significantly change the findings.

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.

No
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

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 recommend additional statistical review

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal