



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	British Journal of Medicine and Medical Research
Manuscript Number:	2013_BJMMR_7449
Title of the Manuscript:	High frequency of non-B HIV-1 subtypes specific mutations at the protease gene among treatment-naïve HIV-1 infected individuals in Jos, Nigeria

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
<p>Line 60: Due to the high cost of HIV-1 genotyping, 105 randomly selected (from computer-generated random numbers) samples out of 230 were assayed.</p> <p>Comment: This is a prospective study. 230-105 samples= 125 samples not analyzed. Why did you collect 230 samples from the onset, only to end up using less than half of what you initially programmed in your research proposal? This is a major deviation from the protocol. Was this reported to the JUTH ethics committee? If no, why? If yes, what was the outcome?</p> <p>THIS QUESTION HAS NOT BEEN ANSWERED. WAS THE DEVIATION REPORTED TO THE JHU ETHICS COMMITTEE?</p> <p>Lines 137-138: Phylogenetic analyses of the partial <i>pol</i> gene revealed heterogeneous</p> <p>138 distribution of four non B HIV-1 strains at different prevalence: CRF02_AG (48%), G (41%), CRF06_cpx (6%) and A (5%).</p> <p>Comment: The Protease gene makes up only a small fragment (297bp) of the entire HIV-1 Pol gene. Other gene regions (RT, IN, etc) do influence subtype assignment. Don't you think that subtype assignment using only the PR gene is very unreliable? Infact, this should be included in this manuscript as a limitation of the study. I suggest that the authors should just mention the subtypes obtained based on the PR gene and then make only the mutations observed as the primary focus of this manuscript. This implies a modification of the topic, results and other sections of the manuscript.</p> <p>YOUR RESPONSE: In subtype assignment pol gene (RT, PR) were analysed, this gives a different percentages of RT and PR gene. The obtained result is subject to software for bootscanning analysis using recombination identification program (RIP) of the Stanford sequence HIVDB analysis program. The Stanford mutation analysis differentiates the mutations based on the RT and PR gene and you can actually any of them as long as the recommended interpretation algorithms are used. This can be verified from many other studies on the web</p>	<p>Thanks for the comment, although we did not report back the reanalysis of the data segment as earlier mentioned to the ethics committee on the sample size but the change did not impact on the study or patients care and treatment.</p> <p>We agree with the reviewer that the larger pol gene is more adequate for genetic diversity analysis than single protease or other minor gene regions. We would like to state that this consideration was put in place in our analysis and appropriately reported in the manuscript on page 4, from line 101, where the primers covering the whole protease and part of RT regions are specified. Therefore the subtyping was based on both protease and partial RT genes as suggested by the reviewer while the resistance mutations analysis focuses on the protease gene.</p>



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My new comment: IN YOUR WRITE UP, YOU HAVE NEVER MENTIONED THAT YOU ALSO SEQUENCED THE RT GENE!

Abstract. Please remove s from aim
Abstract. Conclusion. Use semicolon after responses please. Ie
drug **responses**; thus further studies are needed to evaluate **the** clinical implications of these mutations.
Methods: line 100: please, there is no end for the brackets. You opened it as(Prt-M-F1 andbut you did not close it.
Line 108.....You r response is that :The essence of mass ladder weight is for quantification of amplicons that permits further analysis (sequencing) Invitrogen corporation company fragment of 100-2000 (1062bp).
Please, THIS IS NOT TRUE. Markers help us to know if we amplify the correct gene – in this case, the protease gene. State the DNA molecular weight marker that was used.

Lines 274-275 (of 1st draft): Although our study is a cross-sectional study, the heterogeneous genotypes derived from the patients in Nigeria

My previous comment: This conclusion may not be very reliable because other gene regions were not examined. The PR gene is very short and cannot be reliable used to make meaningful conclusions about HIV-1 subtypes.

This comment has not been addressed at all.

We agree with the sentiments of the reviewer. As a standard procedure, a gel electrophoresis step was carried out following the nested PCR, primarily to determine the success of the RT and nested PCR procedures. In addition, the electrophoresis step was used both to gain basic idea if the targeted gene is amplified (protease and partial RT gene) and also tell the quantity of the amplicons with reference to the 100-2000bp standard molecular weight marker (Invitrogen Corporation, CA, USA).
We are grateful for the views of the reviewer; we have made this clearer in the write-up.

As mentioned earlier, we have made reference to the methodology we used for sequencing where we covered both the protease and partial RT gene, page 4 of the write-up. Therefore, the genetic diversity was based on both genes and not only on the protease gene.