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**SDI Review Form 1.6**

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| 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** |
| Type of the Article | |

## General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty'**, provided the manuscript is scientifically robust and technically sound.
To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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**SDI Review Form 1.6**

**PART 1: Review Comments**

| | **Reviewer's comment** | **Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)* |
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| **Compulsory** REVISION comments | **2. MATERIAL AND METHODS**<br><br>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.<br>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?<br><br>**3.0 RESULTS**<br><br>**3.1 HIV-1 *pol* Subtyping**<br><br>Lines 137-138: Phylogenetic analyses of the partial *pol* gene revealed heterogeneous 138 distribution of four non B HIV-1 strains at different prevalence: CRF02_AG (48%), G (41%), CRF06_cpx (6%) and A (5%).<br><br>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 | |

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| | 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. Above all, please differentiate between mutations and polymorphisms in all sections of this manuscript.**<br><br>3.3 Frequency of HIV-1 Subtype G Specific Protease Inhibitor Mutations<br>Line 149: One samples from non-B subtype G harbored a major drug resistance mutation (Q56E) to protease inhibitors.<br>Comment: 'Q56E' mutation cannot be found on Figure 2. This mutation is neither on the Stanford University list of drug resistance mutations, nor on the IAS-USA 2013 guidelines. Did you mean to mention "Q58E', which according to Stanford is a non polymorphic accessory mutation?<br><br>Comment: Figures 1-4 which are used to indicate the mutations appear very crowded and make no meaning. They should be summarized accordingly and polymorphisms should be left out. | |

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| **Minor** REVISION comments | Line 1 (Topic): Comment1: Please, delete s from subtypes<br><br>Abstract (Design) :The study prospectively recruited HIV-1 po<br>sitive drug-naïve patients …..<br><br>Comment 2: The authors should be consistent. Since you started using 'individuals' in the topic, continue using individuals through out. So change 'patients' to 'individuals'.<br><br>Abstract (Methodology): ……the unrooted tree estimating the evolutionary distances between the sequence isolates.<br><br>Comment3: The tree and mean genetic distances have not been captured in the results section of the text.<br><br>Abstract (Conclusion): Such mutations define the subtype diversity which may dictate virulence and drug responses, thus further studies are needed to evaluate clinical implications of these mutations.<br><br>Comment 4: The word dictate seems too crude. Please, consider using 'impact on', instead of 'dictate'. Use semicolon immediately after 'responses'<br><br>**IINTRODUCTION**<br><br>Line 17:…. variants are alternative lifelines HIV-1 uses to evade sustained drug pressure and host immune responses eventually<br><br>Comment: Change to …. variants are alternative lifelines that HIV-1 uses to evade sustained drug pressure and host immune responses eventually;<br><br>Comment (Line 18): Please, abbreviate 'antiretroviral drugs' and then use this abbreviation (ARV) throughout the text. This should be done for all other words that you wish to abbreviate. Give them an abbreviation when you use them for the first time in your write up. | |

Line 20: Protease inhibitors (PIs) are one of the recommended second-line antiretroviral (ARV) drugs in Nigeria.

Comment: Delete 'ARV', state the drugs that are used in this line of therapy and include the reference.

Line 23: Resistance to antiretroviral (ARV) drugs is one of the major threats……..
Comment: Delete 'antiretroviral'

Line 35: ……in Nigeria [20-23], a region with the second highest proportion of people living with HIV.

Comment: Nigeria is a country and not a 'region'.
Comment: complete that sentence using HIV/AIDS and then abbreviate (PLWHA).

Specific Comment on the Introduction as a whole: This study is conducted in Nigeria on HIV-1 infected individuals. Please, include data on the prevalence rate of HIV in Nigeria and the study community – Jos.

**2. MATERIAL AND METHOD**

**2.1 Settings, patient recruitment and sample collection**

Line 48: ……University Teaching Hospital (JUTH) in Jos, Nigeria.
Comment: Please, give a brief description of the study area.

Line 50: ……of 230 HIV-1 infected treatment-naïve

Comment: Be consistent in the way you write the word 'naive' in line 50 and 'naïve' in the topic. Choose one version and apply it throughout the manuscript.

Line 52:……Questionnaires were used to collect demographic data from study

participants.

Comment: How many questionnaires did you use in total? If just one, then delete the 's' at the end of the word 'questionnaires'.

Line 57: CD4+ lymphocyte count was measured same day……

Comment: Edit the grammar used here and thereafter in the entire manuscript. For example, this sentence should read as follows: 'CD4+ lymphocyte count was measured on the same day of the blood draw using Partec CyFlow Counter® (Partec GmbH, Munster Germany) according to manufacturer's instructions and as previously described [24]; while HIV-1 RNA viral load was measured using the Roche Cobas Amplicor HIV-1 Monitor, version 1.5 (Roche Diagnostics GmbH, Mannheim, Germany).

Line 62: …… the Kenya Medical Research Institute (KEMRI) HIV-R Laboratory
63 Kisian, Kisumu Kenya, where genotypic testing using in-house Genotyping assay.

Comment: What is the meaning of 'R' as written above?

Comment: That sentence on line 62 is incomplete

**2.2 HIV-1 RNA Extraction, Amplification and Detection**

Line 72: …… step reverse transcription (RT) PCR

Comment: Delete the brackets and use RT-PCR

Lines 74-75:…… included 65 ºC for 10 min, 50 ºC for 45min, 94 ºC for 2 min,
75 94 ºC for 15 sec, 50 ºC for 20 sec, 72 ºC for 2min, 72 ºC for 10min and 4 ºC until removal,

Comment: How many reaction cycles were used altogether? What was the final reaction volume?

Lines 77-79: For RT-PCR mixture (primers Prt-M-F1,RT-R1 8μM each, RT-PCR mixture and the SuperScript III one-step RT-PCR system with Platinum *Taq* DNA polymerase high fidelity, following the manufacturer's protocol (Invitrogen, Carlsbad, CA).

Comment: This sentence is not well written and leaves the reader unable to understand this RT-PCR step.

Line 79: ……. For nested PCR, the product of RT-PCR was added to primers (Prt-F2 and RT-R2, each 8 μM), dNTPs, GeneAmp gold buffer II, 2 mM MgCl2, AmpliTaq gold LD DNA polymerase mixture (Applied Biosystems, Foster City, CA).

Comment: how many microlitres of first round product did you use in your nested PCR, and what was the final volume of your reaction?

Lines 83-85: The products from nested PCR were verified by visually comparing the intensity of each sample's band to that of the DNA mass ladder's bands of known DNA quantity. This was performed on 1% agarose gel electrophoresis……

Comment: Capture this sentence as follows: The products from nested PCR were verified for desired size and specificity by visually comparing the intensity of each sample's band to that of the DNA mass ladder's bands of known DNA quantity. This was performed by running a 1% agarose gel electrophoresis……

Comment: what DNA molecular weight marker was used?

**2.3 Sequence Interpretation**

Line 104: …… the International Antiviral Society (IAS)-USA 2013 guidelines.

Comment: It should be 'International AIDS Society'

Comment: where is the reference for IAS-USA 2013 guidelines?

Lines 104-16: These guidelines recommend the following list of mutations as minor in relation to PI drug-specifics: atazanavir/ritonavir (L10I/F/V/C, G16E, K20R/M/I/T/V, L24I, V32I, L33I/F/V, F34Q, M36I/L/V, M46I/L, G48V, F53L/Y, I54L/V/M/T/A, D60E, I62V, I64I/M/V, A71V/I/T/L, G73C/S/T/A, V82A/T/F/I, I85V, L90M, and I93L/M); darunavir/ritonavir (V11I, V32I, L33F, T74P, and I89V); fosamprenavir/ritonavir (L10F/I/R/V, V32I, M46I/L, I47V, I54L/V/M, G73S, L76V, V82A/F/S/T, and L90M); indinavir/ritonavir (L10I/R/R/V, K20M/R, L24I, V32I, M36I, I54V, A71V/T, G73S/A, L76V, V77I, and L90M); lopinavir/ritonavir (L10F/I/R/V, K20M/R, L24I, L33F, M46I/L, I50V, F53L, I54V/L/A/M/T/S, L63P, A71V/T, G73S, I84V, and L90M); nelfinavir (L10F/I, M36I, M46I/L, A71V/T, V77I, V82A/F/T/S, I84V, and N88D/S); saquinavir/ritonavir (L10I/R/V, L24I, I54V/L, A71V/T, G73S, V77I, V82A/F/T/S, and I84V); tipranavir/ritonavir (L10V, L33F, M36I/L/V, K43T, M46L, I54A/M/V, H69K/R, and L89I/M/V). Major mutations were defined as atazanavir /ritonavir (I50L, I84V, and N88S); darunavir/ritonavir (I47V, I50V, I54M/L, L76V, and I84V); fosamprenavir/ritonavir (I50V and I84V); indinavir/ritonavir (M46I/L, V82A/F/T, and I84V); lopinavir/ritonavir (V32I, I47V/A, I76V, and V82A/F/T/S), nelfinavir (D30N and L90M); saquinavir/ritonavir (G48V and L90M); tipranavir/ritonavir (Q58E, T74P, V82L/T, N83D, and I84V) [27].

Comment: Please, delete all of this because after citing the IAS-USA guidelines, this becomes unnecessary.

Lines 117-118:…….. 5% of sequences while subtype-difference mutations were those mutations that were more prevalent in a given subtype.

Comment: Where did you get this classification scheme? Please, justify with a reference.

**2.4 Statistical Analysis**

Lines 122-126:

The data for all 100 patients were entered into Microsoft office excel work sheet (Microsoft office system, 2007) and then exported into the Stata software version 10.0

(Stata Corporation, College Station, Texas, USA) for analysis. Categorical variables (sex, marital status and mode of transmission) were summarized as percentages, while continuous variables (age, CD4+ T cell count and viral load) which were not normally distributed had their medians (IQR) determined. The graphs were plotted using Microsoft office excel.

Comment: Please, edit the grammar in this section. Follow my edits highlighted in blue.

3. RESULTS AND DISCUSSION

Line 132: …median CD4+ T-cell count of the patients at baseline was low - 141 cells/mm3 with their viral load ranging from 22, 202 to…….

Comment: it should be corrected to: …..median CD4+ T-cell count of the patients at baseline was low - 141 cells/$mm^3$, while their viral load ranged from 22, 202 to……..

**3.1 HIV-1 *pol* Subtyping**

Line 139:…. HIV-1 subtypes CRF02_AG and G accounted for majority of the infections(89.0).

Comment: Is 89.0 an absolute figure or a percentage? Please, indicate accordingly.

Lines 206-210: Comment: Please, rephrase sentences here and correct all the grammatical errors.

Line 211: ….through heterosexual mode of transmission, with women being the majority (55%) (Table I).

Comment: This value (55%) contradicts what is seen on table 1 for females (56%). Please, explain this.

Lines 221-226: Comment: please delete the fist two sentences in this paragrapgh and

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| | then re-write the entire section, citing references where appropriate (the entire results and discussion section needs to be re-written).<br><br>**4. CONCLUSION:**<br><br>Lines 274-275: Although our study is a cross-sectional study, the heterogeneous genotypes derived from the patients in Nigeria<br><br>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.<br><br>**REFERENCES:**<br><br>Lines 317-434.<br><br>Comment: Please, follow the guidelines prescribed by the Journal. The first reference is different from this ' Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. Clin Exp Allergy. 2002;32(4):489-98'.<br><br>The year of publication should appear before the Volume. | |
| **Optional/General** comments | | |

**Reviewer Details:**

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