Author's response to reviews

Title: Seroprevalence and risk factors of herpes simplex virus type-2 infection among pregnant women in Northeast India.

Authors:

Dipankar Biswas (biswas_dip@yahoo.com)
Biswajyoti Borkakoty (biswaborkakoty@rediffmail.com)
Jagadish Mahanta (jmahanta@gmail.com)
Kamini Walia (waliakamini@yahoo.com)
Lahari Saikia (lahari.saikia@yahoo.com)
Akoijam Brogen S (brogen@rediffmail.com)
Lobsang Jampa (ljampa2003@yahoo.com)
Alia Kharkonggor (akharkongor@bsnl.com)
Eric Zomawia (ezomawia@yahoo.com)

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Author's response to reviews: see over
**Reply to Reviewers Queries**

**Reviewer's report**

**Title:** Seroprevalence and risk factors of herpes simplex virus type-2 infection among pregnant women in Northeast India.

**Version:** 2 **Date:** 10 June 2011

**Reviewer:** Joshua Schiffer

**Reviewer's report:**

Thank you for your many revisions. The multi-variate analysis is much improved & I continue to think that this is a very useful data set with important implications for the region.

**Major compulsory revisions:**


**Reply:** As per suggestion, the sentence “Also, HSV is thought to enhance HIV acquisition due to dense inflammatory infiltrates of CD4 / CD8 / dendritic cells in the genital tract associated with HSV-2 shedding” has been added (in background, Para 2, line 9-11) and cited the reference ‘Zhu J et al, Nat Med, 2010’ (Ref No. 15).

2. The vast majority of HSV2 seropositive people shed virus whether or not they are aware of recurrent genital lesions. Genital tract inflammation is evident in "asymptomatic shedders". These are key concepts in the field. The end of this discussion in its current form completely misses this point. The use of ACV to prevent HIV acquisition or transmission is a dead concept & will not be tested again in a large clinical trial. The studies by Celum et al were in fact quite "relevant" 7 addressed the relevant scientific question completely.

If the authors wish to argue that suppressing HSV is still a valid strategy for HIV prevention, then they need to advocate for a new drug that eliminates viral shedding and genital tract inflammation to a greater extent than acyclovir.

**Reply:** As per suggestion, the key concepts have been added in the discussion (6th para, line 6-9) as “Also, it is known that majority of HSV2 seropositive people shed virus whether or not they are aware of recurrent genital lesions. Genital tract inflammation is evident in "asymptomatic shedders” and also in (7th Para, line 29-31) as “Therapeutic options that provide a complete eradication of HSV-2 including suppression of genital inflammation may be more relevant for decreasing HIV transmission in high HSV-2 prevalent regions”

3. In reference to above, my opinion is that the value of rising HSV
seroprevalence in a pattern that differs from more typical age cohorts probably implies high HSV incidence among the young in certain regions within NE India. These data argue for targeted public health efforts (condoms, education campaigns etc...) to alter risk behaviors for HIV. In a sense, these data provide a useful epidemiologic signal for a possible impending spike in HIV incidence.

**Reply:** As suggested by the reviewer, the targeted public health efforts **have been added** in the conclusion (6th para, line 3-8) as “The data provides a useful epidemiologic signal for a possible impending spike in HIV incidence among the young age group (22-25 years) as is indicated by a higher HSV-2 prevalence in this age group. Targeted public health awareness campaigns directed at high risk groups having multiple sexual partners, condom non-users, ‘early coitarchal age, Christian community etc. are needed to be advocated for initiating successful intervention strategies to control HSV-2 and indirectly limiting HIV epidemic in northeast India”.

4. It should be stated in the methods that these ANCs are representative for the region as mentioned by another reviewer.

**Reply:** As per suggestion, **it has been stated in the method** (1st Para, line 5-6) “A total of 1640 pregnant woman attending antenatal clinics (ANCs) in five tertiary referral hospitals representing the general population of each of the five northeastern states”.

5. While I agree with the selection of statistical test, this does not obscure the fact that all cross-sectional studies can at best only assess correlations, and are prone to residual confounding to a greater extent than case control studies. This needs to be mentioned as a limitation.

**Reply:** As suggested by the reviewer, the **limitation is mentioned in the discussion** (1st Para, line 17-20) as “We acknowledge the limitations of statistical test used in this cross-sectional study which may be prone to residual confounding effect in comparison to case control studies”.

6. The manuscript still needs substantial technical editing.

**Reply:** As suggested the manuscript has been edited.

**Level of interest:** An article whose findings are important to those with closely related research interests

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

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interest
Reviewer’s report
Title: Seroprevalence and risk factors of herpes simplex virus type-2 infection among pregnant women in Northeast India.

Version: 2 Date: 3 June 2011

Reviewer: Christine Johnston

Reviewer’s report:
The authors have revised the paper substantially, making it much stronger. They have included additional important information on the demographics of the study population, highlighted the HIV/HSV-2 connection with more up-to-date references, and have built a multivariate model which more completely describes risk factors for the HSV-2 infection in northeastern India. Importantly, sexual risk taking behaviors (>1 sex partner, early age of first sex, and lack of condom use) were significantly associated with HSV-2 seropositivity, as has been found in multiple other populations throughout the world.

Minor essential revisions:
Please include the methods of building the multivariate model in the methods section, as you did in the response to reviewers section (“We used the Wald Backward elimination method with probability for stepwise entry at p=0.05 and removal at p=0.1”)

Reply: As suggested, it has been stated in the method (1st Para, line 5-6) “The multivariate analysis was performed by binary logistic regression using Wald Backward elimination method with probability for stepwise entry at p=0.05 and removal at p=0.1, to find the association between categorical variables with HSV-2 seroprevalence”.

Minor discretionary revisions:
Paragraph 2 of the background is repetitive and could be more succinctly written. For instance, it is mentioned twice that HSV-2 doubles the risk of HIV acquisition.

Reply: As per suggestion the repeat sentence has been removed from the background.

The final sentence of paragraph 2 could be disputed (“HIV-1 replication can be reduced with antiviral therapy directed solely at HSV-2.”) as acyclovir has been found to have anti-HIV activity in vitro (1-3).

Reply: As suggested by the reviewer, the sentence has been modified in the background (para 2, line 20-22) as “suggesting that elimination of HSV-2 viral shedding and genital tract inflammation can reduce the transmission and acquisition of HIV infection” instead of “This finding has direct clinical implications, suggesting that HIV-1 replication can be reduced with antiviral therapy directed solely at HSV-2.”
The sentences in the final paragraph are not helpful to understanding the populations in the study-I think it is adequate to say that the states are very heterogeneous. I would consider excluding these sentences “Assam is the most populous northeastern state of India with a population of 31.11 million in 2011. Assam acts as a mixing cauldron for many ethno-cultural groups including convergence of Mongoloids and Caucasoid. Whereas the other four states has predominantly a uniform distribution of population mostly of local tribes”

Reply: As per suggestion, the sentences are removed from the background.

Results:

In the results (page 10), you state: “However, due to confounding interactions (with State), there was loss of the significance on multivariate analysis.” Did you look for any interaction terms? If so, these may be described here.

Reply: The apparent interaction between variables was analyzed separately. After careful examination of our data revealed that partitioning of HSV-2 seroprevalence across the religion and state was not uniform, because in some states Christian population was almost negligible e.g. Assam and Manipur and on the other hand in some states Hindu and Muslim population was negligible. During multivariate analysis by binary logistic regression using Wald Backward elimination method the religion was eliminated, therefore the variable “Religion” is removed from Table-2,. A sentence also been added in method (Statistical analyses section) as “The variables showing significant interaction were analyzed separately”.

Multivariate model: I think that age would still be best described as a continuous variable. The authors state that the categories were selected to result in equal distribution of people in each category. However, there are nearly twice as many women in the age 22-25 group as compared to the other groups. I do think that the seroprevalence of 10.5 (in 22-25 year olds) and 9.4 (in >29 year olds) is significantly different; the outlier is likely the 26-29 year old group. The abstract continues to state that there is a significant peak in the age 22-25 group, however, in the new multivariate model this is not the case. If you elect to keep age as a categorical variable, you could state in the abstract that HSV-2 seroprevalence increases with age (aOR=1.9 for 22-25 yr old, aOR=2.29 for>29 yr old).

Reply: As per suggestion, the AOR for age groups have been added in the abstract (result section, line no. 4)

Discussion, page 12: The statement: “Studies from United State reported 33% HSV-2 prevalence in inner-city communities.” seems irrelevant and could be removed. This population is not at all comparable to that found in NE India. Table 2. Why are p-values listed for the reference groups?
Reply: As suggested, the reference of United State has been removed from discussion. In Table 2, the $p$-values listed in the reference groups are removed.

References


Reply: Two references, Lisco A, et al., Cell Host Microbe. 2008; 4:260-270 and McMahon MA, et al., and J Biol Chem. 2008; 283:31289-31293 have been added (Ref No. 46 & 47).

Level of interest: An article whose findings are important to those with closely related research interests

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

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I DECLARE THAT I HAVE NO COMPETING INTERESTS
Substantial technical correction:

1. Abstract (conclusion section): This sentence “The role of vaccination against HSV-2 in near future and suppression of HSV-2 by antivirals in high HIV/HSV-2 areas may be an option for initiating successful intervention strategies to control HSV-2 and indirectly limiting HIV epidemic in northeast India” is replaced with “The role of vaccination against HSV-2 in near future and elimination of HSV-2 viral shedding and genital tract inflammation in high HIV/HSV-2 areas may be an option for initiating successful intervention strategies to reduce the transmission and acquisition of HIV infection in northeast India”.

2. In Result (Risk factors section): This sentence “Subjects from Christians community were at higher risk (OR, 3.5; 95% CI, 1.2-10.1, p=0.02) of getting infection with reference to Muslims. However, due to confounding interactions (with State), there was loss of the significance on multivariate analysis” is replaced with “Subjects from Christians community were at higher risk (OR, 3.5; 95% CI, 1.2-10.1, p=0.02) of getting infection with reference to Muslims (analyzed separately because of significant interaction with ‘state’).

3. Discussion (Para 6, line 10-13): This sentence “This difference in prevalence with religion (3.8% in Muslim compared to 12.6% among Christians), may be due to practice of male circumcision at infancy or early childhood which is common in Muslims” is replaced with “This difference in prevalence with religion (3.8% in Muslim compared to 12.6% among Christians), may be due to practice of male circumcision at infancy or early childhood by the spouses of the pregnant women among Muslims”.

4. Discussion (last Para): This sentences “However, in the study by Watson-Jones D et al., had enrolled female workers who were HIV-seronegative and HSV-2–seropositive in a randomized, double-blind, placebo-controlled trial of suppressive treatment with acyclovir. The primary outcome was the incidence of infection with HIV. We think that the design of the controlled trial of enrolling HSV-2 seropositives for suppressive therapy may not yield the true outcome from such intervention as seropositives mostly may not have active disease process nor do they have GUD or virus shedding regularly. Instead, the study would have been more relevant if they would have enrolled subjects based on genital viral shedding also apart from seropositives. It will be difficult to discard the role of HSV-2 suppressive therapy, until more conclusive data are available on this” is replaced with “This landmark study fail to demonstrate the role of acyclovir in decreasing the transmission of HIV among HSV-2 seropositives. This is in contrary to the fact that acyclovir is activated into a human herpes virus (HHV) DNA polymerase inhibitor exclusively by HHV kinases. It suppresses HIV-1 in HHV coinfected human tissues but not in HHV free tissue or cell cultures. Acyclovir treatment in patients coinfected with HSV/HIV has been observed to alter the disease course and decrease HIV viral load which attributed to indirect effects of HSV suppression on HIV replication. Also, if we accept the fact that HSV-2 replaced with increases the risk of HIV acquisition by 2-3 folds, than suppression of HSV-2 by ACV which results in decrease genial ulceration and inflammation would be expected to influence HIV acquisition. Use of antivirals for
suppression of HSV-2 for lowering the risk of HIV transmission cannot be overlooked until more light is shed on this topic. Therapeutic options that provide a complete eradication of HSV-2 including suppression of genital inflammation may be more relevant for decreasing HIV transmission in high HSV-2 prevalent regions.

5. **In conclusions:** This sentence “Along with role of advocating HSV-2 awareness towards high risk groups such as subjects in the age group ‘22-25 years’, having multiple sexual partners, condom non-users, ‘early coitarchal age, Christians community etc., the role of HSV-2 vaccination in near future and role of suppression by antiviral drugs in high HIVHSV-2 areas may be some of the options in future” is replaced with “The data provides a useful epidemiologic signal for a possible impending spike in HIV incidence among the young age group (22-25 years) as is indicated by a higher HSV-2 prevalence in this age group. Targeted public health awareness campaigns directed at high risk groups having multiple sexual partners, condom non-users, ‘early coitarchal age, Christian community etc. are needed to be advocated”

6. **In Result:** This sentence “whereas Hinduism followed by Islam was found to be the major religion of the enrolled subjects in Assam (90.3% and 8.7%) and Manipur (86.1% and 13.9%) respectively (see Table 1)” is replaced with “whereas Hinduism followed by Islam was found to be the major religion of the enrolled subjects in Assam (90.3% and 8.7%) and Manipur (86.1% and 13.9%) respectively”

7. **In Result:** This sentence “Overall 11.7% (192/1640) enrolled subjects were migrants with highest number of migrants in Arunachal Pradesh (19.9%) followed by Meghalaya (19.5%) and Mizoram (10.8%)” is replaced with “Overall 11.7% (192/1640) enrolled subjects were migrants with highest number in Arunachal Pradesh (19.9%) followed by Meghalaya (19.5%) and Mizoram (10.8%).”

8. In background: This sentence “Highest prevalence of HSV-2 has been found in some parts of Africa, America and the lowest prevalence has been observed in Asia” is replaced with “Highest prevalence of HSV-2 has been found in some parts of Africa, America and the lowest in Asia”.

9. In Table 2: The variable ‘Religion’ is removed because of interaction with ‘State’ and analyzed separately