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Background:

- The phenomenon of condom migration remains a major concern whenever a new HIV prevention approach is being considered to strengthen the existing preventive arsenal.
- In the context of a demonstration project on the implementation of a program on HIV treatment as prevention (TasP) and pre-exposure prophylaxis (PrEP) among female sex workers (FSWs) in Cotonou, Benin, we are using a combination of biomarkers for sexual exposure to assess condom migration and the trends in condom use over time.

Objective:

- Preliminary assessment of the validity of recent self-reported unprotected sex, compared to a gold standard comprising several biomarkers, based on recruitment visits carried out between October 2014 and June 2015 in a TasP/PrEP demonstration project among FSWs in Cotonou, Benin.

Methods:

- Details on the overall study are provided in another poster presented at this conference
- At their recruitment visit in the study, two weeks following their screening visit where HIV status and other eligibility criteria are assessed, a questionnaire on socio-demographic characteristics and sexual behaviour is administered to the participants. They also undergo a physical examination, where blood and genital samples are collected.
- Recent unprotected sex, based on questionnaire data, is defined as not having used a condom for at least one sexual intercourse or having had at least one episode of condom breakage or slippage in the last 2 days. To build this variable, we used questions that were specific to clients, regular partners and other non-paying partners.
- The biomarkers used included the detection of:
 - Prostate-specific antigens (PSA) on vaginal samples
 - Y chromosome DNA (Yc-DNA) on vaginal samples
 - Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) on cervical samples
 - Human chorionic Gonadotropin (hCG) on urine samples
- PSA was detected using the commercially available ABACard p30 PSA assay (Abacus Diagnostics, West Hills, CA, USA).
- Yc-DNA was detected using an in-house PCR assay using the primer set SRY3F (5'-CGC ATT CAT CGT GTG GTC TCG-3') and SRY3R (5'-ATT CTT CGG CAG CAT CTT CGC-3'), specific for a 229-bp region in the sex-determining region, a gene located on the short arm of the Y chromosome.
- NG and CT were detected using the NG/CT Probetec® assay from Becton Dickinson (Cockeysville, MD, USA).
- The pregnancy tests used a simple urine assay from Intec Laboratories (Xiamen, China).
- Recent unprotected sex based on biomarkers was defined as the presence of at least one positive test among the biomarkers tested for. This was considered as the gold standard for recent semen exposure.
- We assessed the sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV) of self-reported recent unprotected sex in comparison to the gold standard.
- The difference in frequency of unprotected sex between self-report and gold standard was assessed using the McNemar chi-square test.
- Written informed consent was obtained from all participants. This study and all its procedures were approved by the ethics committee of the CHU de Québec, Québec, Canada, and by the National Ethics Committee for Health Research in Benin.

Results:

- 198 FSWs (55 for TasP and 143 for PrEP) were recruited in the study between October 1st, 2014 and June 30, 2015.
- Median age of all participants was 32.5 years and 44% of them were from Benin, whereas the others were from surrounding countries.
- During the last 2 days, 159 (80.3%), 38 (19.2%) and 0 participants had sex with clients, regular (boyfriends or husbands) and other non-paying partners, respectively. Corresponding figures were 188 (94.9%), 80 (40.4%) and 4 (2.0%) in the last 14 days.
- Consistent condom use (CCU) in the last 2 (14) days was reported at 98.3% (93.0%) with clients and 13.3% (8.8%) with regular partners. CCU was 75% with other non-paying partners in the last 14 days.
- 10.5% of FSWs reported at least one episode of condom breakage or slippage when having sex with clients in the last 2 days (20.3% in the last 14 days). No such episode occurred with other partners.
- Results for combined biomarkers were available for 190 subjects.
- Table 1 shows recent self-reported unprotected intercourse (UI) with any type of partner (including condom breakage or slippage), PSA and Yc-DNA results for PrEP and TasP subjects separately. There was not much difference between the different methods of assessing recent UI, except for HIV-infected women where self-reported UI was less frequent than positive PSA and Yc-DNA PCR.

Results (continued):

- Table 2 shows NG and CT prevalence for PrEP and TasP subjects separately. Overall prevalence of infection by either NG or CT was 13.2%.
- Only one subject was pregnant (positive for hCG on urine samples) at recruitment (also positive for both PSA and Yc-DNA).
- Table 3 shows the comparison between recent self-reported UI and the PSA results. Although the proportion of self-reported UI is slightly lower than that of positive PSA results, the difference is not statistically significant.
- As the Yc-DNA PCR can remain positive for up to 14 days following UI, table 4 shows a comparison between self-reported UI over the last 14 days and the Yc-DNA PCR results. Surprisingly, the proportion of self-reported UI over the last 14 days is significantly higher than the proportion of positive Yc-DNA PCR results.
- Table 5 shows a comparison between PSA and Yc-DNA PCR results. Concordance between the results is 83.6%. The kappa coefficient is 0.60.
- 13 out of the 25 FSWs (52%) with infection by either NG or CT were positive for PSA or Yc-DNA vs. 58/165 (35.2%) of the uninfected women ($p < 0.001$, McNemar test).
- Finally, table 6 shows the comparison between recent self-reported unprotected sex and our gold standard of recent unprotected sex based on a combination of biomarkers. Recent UI is significantly lower according to self-report (23.7%) than according to biomarkers (43.7%). Only 87 of the 190 women (45.8%) have negative biomarkers and reported only protected sex, compared to an estimation of 76.3% if protected sex were assessed on self-report only.

Table 1. Distribution of recent self-reported unprotected intercourse, PSA and Yc-DNA results among FSWs recruited in a TasP/PrEP demonstration study in Cotonou, Benin

| | Arms | | | | | | Total | | |
|-------------------|-------------------|-------------------|-----------------|------------------|------------------|-------------------|-------------------|-------------------|---------|
| | PrEP | | | TasP | | | Self-reported | PSA+ | Yc-DNA+ |
| Self-reported | PSA+ | Yc-DNA+ | Self-reported | PSA+ | Yc-DNA+ | Self-reported | | | |
| 36/139 (25.9%) | 35/139 (25.2%) | 36/138 (26.1%) | 9/51 (17.6%) | 20/51 (39.2%) | 19/51 (37.3%) | 45/190 (23.7%) | 55/190 (28.9%) | 55/189 (29.1%) | |

Table 2. NG and CT prevalence among FSWs recruited in a TasP/PrEP demonstration study in Cotonou, Benin

| | Arms | | Total (N=190) |
|-----------------------|--------------|-------------|---------------|
| | PrEP (N=139) | TasP (N=51) | |
| NG + (%) | 11 (7.9) | 3 (5.9) | 14 (7.4) |
| CT + (%) | 10 (7.2) | 3 (5.9) | 13 (6.8) |
| Either NG or CT + (%) | 20 (14.4) | 5 (9.8) | 25 (13.2) |

Table 3. Comparison between recent self-reported unprotected sex and PSA results among FSWs recruited in a TasP/PrEP demonstration study in Cotonou, Benin

| Recent self-reported unprotected intercourse | PSA results | | Total (%) | p-value* |
|--|-------------|------------|------------|----------|
| | Positive | Negative | | |
| Yes | 19 | 26 | 45 (23.7) | 0.253 |
| No | 36 | 109 | 145 (76.3) | |
| Total (%) | 55 (28.9) | 135 (71.1) | 190 | |

*According to McNemar chi-square test
For the comparison between self-report and PSA:
Se: 34.5%; Sp: 80.7%; PPV: 42.2%; NPV: 75.2%

Table 4. Comparison between self-reported unprotected sex in the last 14 days and Yc-DNA PCR results among FSWs recruited in a TasP/PrEP demonstration study in Cotonou, Benin

| Self-reported unprotected intercourse (last 14 days) | Yc-DNA PCR results | | Total (%) | p-value* |
|--|--------------------|------------|-----------|----------|
| | Positive | Negative | | |
| Yes | 26 | 71 | 97 (51.3) | <0.001 |
| No | 29 | 63 | 92 (48.7) | |
| Total (%) | 55 (29.1) | 134 (70.9) | 189 | |

*According to McNemar chi-square test
For the comparison between self-report and biomarkers:
Se: 47.3%; Sp: 47.0%; PPV: 26.8%; NPV: 68.5%

Presented at the World STI & HIV 2015 Conference
Brisbane, AUSTRALIA

Table 5. Comparison between PSA and Yc-DNA PCR results among FSWs recruited in a TasP/PrEP demonstration study in Cotonou, Benin

| PSA results | Yc-DNA PCR results | | Total (%) | p-value* |
|-------------|--------------------|------------|------------|----------|
| | Positive | Negative | | |
| Positive | 39 | 15 | 54 (28.6) | p=1.0 |
| Negative | 16 | 119 | 135 (71.4) | |
| Total (%) | 55 (29.1) | 134 (70.9) | 189 | |

*According to McNemar chi-square test

Table 6. Comparison between recent self-reported unprotected sex and recent unprotected sex based on a combination of biomarkers among FSWs recruited in a TasP/PrEP demonstration study in Cotonou, Benin

| Recent self-reported unprotected intercourse | Presence of biomarkers of recent unprotected intercourse | | Total (%) | p-value* |
|--|--|------------|------------|----------|
| | Yes | No | | |
| Yes | 25 | 20 | 45 (23.7) | <0.001 |
| No | 58 | 87 | 145 (76.3) | |
| Total (%) | 83 (43.7) | 107 (56.3) | 190 | |

*According to McNemar chi-square test
For the comparison between self-report and biomarkers:
Se: 30.1%; Sp: 81.3%; PPV: 55.6%; NPV: 60.0%

Discussion:

- Despite very high levels of self-reported condom use with clients, the proportion of FSWs admitting unprotected sex in the last 2 days is >20% and >50% in the last 14 days, largely because of very low CCU with regular partners and relatively high rates of condom breakage or slippage.
- When using PSA only as a measure of recent UI leading to semen exposure, we did not find much difference with self-reported UI in the last 2 days. This could be partly due to the fact that 2 days is the upper limit of time for a positive PSA following semen exposure. Indeed, there is indication that a majority of women with a positive PSA result reported protected sex only, but also that the majority of women reporting UI in the last 2 days had a negative PSA.
- Results from the literature suggest that Yc-DNA PCR may remain positive for up to 14 days after semen exposure. Our results suggest that in most cases, the time limit for detection is much shorter, as self-reported UI in the last 14 days was much higher than the proportion of women with a positive Yc-DNA PCR.
- There was a relatively good correlation between PSA and Yc-DNA PCR results and almost identical positivity rates. One could have expected the PCR to be more sensitive.
- Results when using a combination of biomarkers and comparing with self-reported UI in the last 2 days are closer to our expectations, with a relatively large proportion (69.9%) of women reporting protected sex only while having positive biomarkers and a lower proportion (44.4%) of all negative biomarkers among those reporting recent unprotected sex.
- Among the latter 20 women, all reported CCU with their clients, but 14 admitted regular partners with whom they did not always use condoms and 6 reported condom breakage or slippage.
- Despite careful implementation of PSA and Yc-DNA PCR testing in our laboratory, validity issues are still a possibility and these tests are not also intrinsically perfect (as all tests). External quality control is planned over the next few months.

Conclusion:

- Our results support the need to supplement self-reported data by biomarkers when assessing condom migration as there is also a possibility of an increase in social desirability biases among FSWs participating in an intervention with close follow-up and regular exposure to condom promotion activities.
- The safest baseline to use for completely protected sex in our study, assuming a good validity of our biomarker tests, is the proportion of FSWs reporting protected sex only in the last 2 days and having all biomarkers negative (87/190: 45.8%).
- Similar questionnaires and biomarker testing will be used during follow-up visits to assess condom migration in the course of this TasP/PrEP demonstration study.

Disclosure of interest statement:

This study is funded by the Bill and Melinda Gates Foundation, and Truvada® for pre-exposure prophylaxis is provided free of charge by Gilead Sciences, Inc.