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RESEARCH ARTICLE

Inconsistent Use of Condom in Italian HIV-Serodiscordant Heterosexual Couples as Revealed by the Detection of Y Chromosomal (Yc) DNA in Vaginal Swabs

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Abstract:

Introduction:

The prevention of transmission of HIV infection is based on the regular and correct use of condom and studies on transmission rates are generally based on the self-report of condom use.

However, consistent data on different population suggest that this often leads to overreporting possibly due to social desirability. In addition, self-report of condom use does not consider improper use or breakage.

Methods:

Vaginal biomarkers were proposed to detect exposure to semen and among these detection of chromosome Y DNA (Yc) appeared promising in different research settings.

Here, we searched for Yc in vaginal swabs of 33 Italian women, engaged in a regular heterosexual relationship with a HIV serodiscordant partner and reporting a regular use of condom during sexual intercourses.

Results & Discussion:

In 10 (30.3%) women Yc was detected, especially if the infected partner was male and if the couple did not have sons. This is confirmed in Italian heterosexual women and is already demonstrated in other populations: behavioural counselling is not always a valid tool and the self-reported use of condom is not fully reliable.

Conclusion:

Further studies could help in the future to individuate more effective preventive strategies for both HIV and sexually transmitted infections.

Keywords: HIV infection, Sexual transmission, Condom use, Behavioural counselling, Self-reported condom use, Chromosome Y DNA, Vaginal swab.

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1. INTRODUCTION

Despite the claimed efficacy of antiretroviral treatment and Pre-exposure Prophylaxis (PrEP) in preventing HIV transmission, a correct and consistent use of condom still

remains the most effective way to prevent HIV spread and is capable of also preventing the transmission of other Sexually Transmitted Diseases (STDs).

Evaluation of sexual behaviors, including condom use, is thus essential in HIV and STD prevention research and interventions; however, it mainly relies on patient's self-reported sexual behavior that may have questionable validity due to miscommunication, lack of understanding of questions

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asked, behavior report according to perceived expectations and social desirability and recall problems. In addition, it is frequently difficult to capture risk associated with improper condom use or accidental breakage.

Detection of semen in vaginal fluids has been proposed as an unbiased measure of unprotected sex. The most widely used biological marker for semen exposure has been Prostate Specific Antigen (PSA) [1, 2]. However, the detection of Y chromosomal (Yc) DNA has been demonstrated to be a better tool for this purpose, being detectable in vaginal swabs for a longer time, up to 15 days, after unprotected sexual intercourse, not detectable if condom is used and not being influenced by menses [3, 4].

Several studies, evaluating either one or the other marker, definitively demonstrated that the self-reported use of condom is generally over reported by interviewed individuals and that the detection of seminal markers is a more accurate measure of semen exposure of the woman and thus of high-risk sexual behavior [5 - 11]. Most of these studies, however, were conducted in North America and in developing countries and mainly on female sex workers.

The aim of our study was to compare the self-reported use of condom with results derived from the detection of seminal markers in vaginal swabs from a group of HIV uninfected or infected women from South East Italy, engaged in steady heterosexual relationship with an HIV serodiscordant partner.

2. MATERIALS AND METHODS

We enrolled in this partly prospective study a group of 33 steady, heterosexual, HIV-serodiscordant couples referring to the Section of Infectious Diseases, University Hospital of Foggia (Foggia, Italy).

Inclusion criteria were 1) to be engaged in a steady heterosexual relationship from at least six months and to report to be sexually active, 2) to be HIV serodiscordant, one of the partners is HIV positive (index case, IC) and the other one HIV negative, 3) to have reported in the previous visits a regular condom use during sexual intercourses, 4) to have IC treated with antiretrovirals and virally suppressed in plasma from at least six months.

Regular condom use was defined as the use, in all cases of penetrative intercourse, of a condom inserted before penetration and used until the end of the sexual act.

The seropositive individual in the couple (from now on IC, index case) was the man in 21 and the woman in 12 cases. The mean age was 34.3 years for men and 30.3 years for women.

None of the individuals enrolled in the study presented symptoms or signs related to sexually transmitted diseases.

During routine laboratory and/or gynaecological tests (for PAP-test and/or HPV-DNA detection), all couples have been interviewed by a training nurse about their sexual behavior. Each participant couple was asked how many times they had sexual intercourses in the last month and during how many of these encounters they used a condom. Moreover, women were asked to allow collecting a cervivovaginal swab for the detection of seminal fluid in the days after sexual intercourses

exclusively for research purposes, and they were told that the results, although they lack clinical relevance, would be discussed with their doctors at the next appointment. An informed consent was requested and collected for each patient participating in the study.

Vaginal samples were collected by a medical doctor or a trained nurse using a cotton-tipped swab inserted into the posterior fornix and rotated three times. Swabs were immediately sealed in individual transport tubes and transferred to the laboratory and stored frozen at -80°C until use.

At the time of use, swabs were thawed at room temperature, inserted into a 1.5 ml microcentrifuge with 1 ml of phosphate buffered saline and allowed to elute at room temperature for ten minutes. The samples were thus rotated and tubes centrifuged at 13000xg for five minutes.

Total nucleic acid was extracted from the pellets according to the manufacturer instructions by a commercially available DNA extraction kit (QIAmp DNA minikit, QIAGEN Ltd, Crawley, UK) and the eluted DNA was stored for further analysis.

Detection of y chromosomal DNA was performed by a commercially available kit (Quantifier Duo Kit, Applied Biosystem) according to the manufacturer instructions.

All uninfected individuals were subjected, as for routine evaluation, to HIV testing every six months.

3. RESULTS

Results are shown in Table 1.

Table 1. Yc DNA vaginal detection in 33 HIV serodiscordant heterosexual couples.

Index Case Sex	No	Yc Positive	Fisher Exact Test
Male	21	9 (42,8%)	$p < 0.05$ $r = 0.784$
Female	12	1 (8,8%)	–
Parenthood		–	–
Yes	18	4 (22,2%)	$P < 0.05$ $r = 0.4$
No	15	6 (40%)	–

All 33 studied couples reported a regular and proper use of condom during sexual intercourses, and no cases of condom breakage were reported. Nevertheless, in 10/33 women (30.3%) it was possible to detect y chromosome using PCR in vaginal swabs. This occurred significantly more frequently when the index case was a man (9/21 cases, 42.8%) than a woman (1/12 cases, 8.3%). Being already parents was also correlated with a lack of Yc detection in vaginal swabs, still significantly, but weakly. This occurred, in fact, in 4/18 (22.2%) women belonging to couples having sons but in 6/15 cases (40%) when couples had no sons.

At the subsequent appointment, the couples were informed about the results and their meaning.

Three couples (one with the men and two with the woman being the IC) still denied the lack of regular condom use advocating for laboratory technical mistakes or unrevealed breaks in the condoms. The remaining admitted the irregular

use of condom and the reason given for not telling the truth was the concern about disappointing the doctors and the nurses and to be blamed by them. Furthermore, couples had read on internet that HIV patients on highly active antiretroviral therapy (HAART) treatment and with undetectable blood viral load, do not transmit the infection sexually, thus the condom use was perceived as useless in spite of medical counseling.

After 6 and 12 months of enrollment in the study none of the seronegative individuals seroconverted for HIV.

4. DISCUSSION

Although the use of condom alone does not provide 100% protection from HIV [12] to date, the most efficient modalities for prevention of HIV sexual transmission are still the consistent use of condom along with blood viral suppression of infected individuals through HAART and pre- and postexposure antiretroviral prophylaxes for at risk individuals.

On the other hand, cases of HIV infections were reported in individuals on PrEP, due either to viruses resistant to antiretrovirals [13], a great number of unprotected sexual exposures [14] or low blood concentrations of antiretrovirals for heterosexual women [15].

This is not surprising: it is known that virological response in semen may differ with respect to peripheral blood [16, 17] and that undetectable in blood does not necessarily mean undetectable in semen or cervico-vaginal secretions. In addition, the emphasis given to viral suppression in infected individuals and protection acquired by the use of PrEP or post exposure prophylaxis may widely lead to increasing risk behaviours and neglecting condom use, that is concerning for other STD transmission as well, which as a matter of fact appears to increase in PrEP taking individuals [18]

These considerations may have important implications in the future scenario of HIV and STD transmission and lead us to the belief that to promote the use condom in at risk individuals is still important.

However, strategies are probably needed to estimate the efficacy of condom use counselling, since generally we do evaluate it by patient self-report. However, the self-reporting of condom use is considered a non-ideal tool to assess its efficacy. Studies on HIV prevention based entirely on self-reported sexual activity may be biased and difficult to interpret [19]. There are multiple types of self-reporting bias, such as over reporting [20], social desirability bias [21], under reporting, or recall problems. Also, individuals might be unaware of their HIV infection or exposure [22].

Biomarkers of vaginal exposure to semen, long used in forensic medicine, and later becoming important to evaluate the safety of physical barriers such as diaphragms or condoms, may provide information on unprotected intercourse [23]. Prostate-Specific Antigen (PSA) and Y chromosome DNA (Yc-DNA) have both been evaluated in the past as semen biomarkers and are the most widely used in this field. These markers of semen exposure are objective indicators in studying sexual behaviors and validation of self-reported condom use. While both are considered reliable for evaluating exposure to semen, each has unique characteristics [3], the PSA may be

more consistent as a marker of very recent exposure and the (Yc) DNA is more likely to be detected in the vagina after 12 h postexposure compared to PSA [24]. This assay is sensitive to five copies of Y chromosome for up to 14 days post exposure [25].

Our data indicate, in Italian steady heterosexual couples, that discrepancy of (Yc) DNA detection with self-reporting of condom use reached 30.3%, which was much less than what was already demonstrated by Jadack RA *et al.* (55.6%) [26] and comparable with the result of Rose E *et al.* (33.9%) [27]. Interpretation of our study results is in agreement with what was already revealed by other publications, the substantial discordance between self-reports and measurements of biologic markers of semen exposure in vaginal specimens indicates that self-reporting of sexual behavior cannot be assumed to be valid measures [28, 29].

The reason of self-reporting bias in our study was mainly (7/10 couples) the information they acquired on internet about HAART of infected individuals as the best and maybe single protection modality; *i.e.* the HIV patients on HAART and with undetectable blood viral load do not transmit the infection sexually. Therefore, they perceived the condom use as useless in spite of medical counselling.

Interpretation of the self-reporting bias of other 3/10 couples maybe more difficult, they denied the lack of regular condom use. The discrepancy between self-reporting of condom use and biological measures need to take in account the high sensitivity of (Yc) DNA detection and the possibility although rare of contamination by other biological fluids (such as saliva). (Yc) DNA detection may incorrectly suggest a poor behavioral outcome, improper condom use, micro-leakage of semen or unrevealed microbreaks in the condoms may lead to positive (Yc) DNA test.

None of the subjects enrolled in our study seroconverted over a one year follow up and this is in line with the known low rate of transmission per single sexual act of the virus. However, the study was not addressed to evaluate this point, that will need large cohort studies.

This study shared some limitations with other studies that considered to combine self-report and biological data as best approach, that is specific mechanism needed for combining the data from those two types of methods to improve HIV preventive strategies. In addition, the small study population allow us to provide suggestions but not to draw a firm conclusion about the effectiveness of (Yc) DNA detection as a sure biomarker for the study of sexual behavior.

However, this is according to our knowledge the first study performed in European heterosexual HIV serodiscordant couples and results are in line with the previously published studies in other populations [5 - 11].

In summary, despite many initiatives to implement condom use to prevent HIV infection, currently the condom meets with many resistances, justified by various motivations such as allergies, reduction of pleasure, limitation of intimacy or assumption of full protection of HAART and PrEP. The combination of different prevention methods, including pre- and post-exposure antiretroviral prophylaxes, condoms and

other risk-reducing behaviours, is a more adequate approach. Counselling and evaluation of the effectiveness of implementing protective strategies is still required, the use of (Yc) DNA detection as confirmatory biomarker will be a promising guide to improve sexual behaviors and developing more successful strategies to prevent HIV and STD transmission.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of Ferrara University, Ferrara, Italy, Prot.170394.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent was obtained by all study participants.

AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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