Original Article

Semen Washing and Intrauterine Insemination for Reducing the Risk of Human Immunodeficiency Virus Transmission in Serodiscordant Couples: A Cross-sectional Study

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Abstract

Background: Antiretroviral therapy has helped human immunodeficiency virus (HIV)-infected people live an enhanced quality of life and attempt for a pregnancy, without placing their partner at risk. Although periconceptional pre-exposure prophylaxis for the uninfected partner and consistent antiretroviral therapy for the HIV-infected partner are important to prevent HIV transmission, semen washing could be a great option to further reduce the semen viral load. **Aim:** The aim of this study were as follows: to determine if semen washing with intrauterine insemination provides an added safety net to HIV-serodiscordant couples when the male partner is HIV-infected and virally suppressed and to determine if the U = U concept (undetectable = untransmittable) holds true in virally suppressed HIV-infected males. **Settings and Design:** This was an observational study conducted in seropositive HIV men under treatment with highly active antiretroviral therapy (HAART) in collaboration with Metropolis Laboratory, a CAP recognised private Healthcare Laboratory in Mumbai, India. **Materials and Methods:** Blood and semen samples were collected from a total of 110 adult HIV-1-infected males virally suppressed on HAART. These samples were processed to assess the viral load in plasma as well as raw and processed semen fractions. **Statistical Analysis Used:** Descriptive statistics were used to analyse the data. **Results:** Only men with plasma viral loads < 1000 copies were selected in our study. Out of the 110 HIV-infected individuals, 102 (92.73%) patients had undetectable (<20 copies/ml) plasma viral load while 8 (7.27%) patients had a detectable (>20 copies/ml) viral load, who were excluded from the study. In the virally suppressed 102 men, the raw semen samples of 100 men showed an undetectable viral load, while 2 samples showed detectable contamination, even though their plasma samples from the blood showed a viral load of <20 copies/ml. The semen was then separated into the sperm and the seminal plasma samples. The seminal plasma had <20 copies/ml in 95 samples (93.14%) but a detectable viral load in 7 (6.86%) samples. After subjecting all the 102 processed (post-wash) sperm samples to quantitative analysis, an undetectable viral load of <20 copies/ml was found in all the samples. Thus, the raw sample (prewashed),seminal plasma

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and processed (postwash) samples were evaluated. The post-wash sperm sample showing zero contamination was frozen for intrauterine insemination (IUI) in the uninfected female partner. **Conclusions:** Semen washing with IUI should be advocated as a safe, efficacious way to increase the safety net and to further reduce the minimal risk of HIV transmission in serodiscordant couples in addition to the U = U concept.

Keywords: *Human immunodeficiency virus, intrauterine insemination, semen washing, serodiscordant couples, U = U concept, HIV and fertility*

Introduction

Human immunodeficiency virus (HIV), a retrovirus, is recognised as one of the world's biggest health challenges. In spite of huge strides to curb its spread, there are approximately 39 million people currently living with HIV and >90% of HIV-infected individuals are of childbearing age.[1] The 2023 HIV estimates indicate that around 2.5 million people are living with HIV in India, with an adult HIV prevalence of 0.7%.[2] Clearly, India has the third highest burden of HIV in the world, with nearly 44% being in the reproductive age group.[3]

HIV-1 and HIV-2 are the two subtypes of this virus, of which HIV-1 is the leading cause of 95% of infections worldwide.[4] The sub-Saharan African region is home to two-thirds of the global prevalence, followed by the Asia and Pacific regions.[5]

In 1988, the Centers for Disease Control and Prevention (CDC) recommended against the use of intrauterine insemination (IUI) in HIV-infected men, making donor insemination or adoption as the only way to achieve a pregnancy.[6] By 2030, the Sustainable Development Goals aim to eradicate HIV using a robust approach both at the global and the national levels.[7] With the breakthrough advent of antiretrovirals, there was a substantial decrease in the plasma viral load usually below the level of detection, which sustained for longer periods.[8] The advanced highly active antiretroviral therapy (HAART) significantly enhanced both life expectancy and quality of life for HIV-infected people. Thus, HIV infection changed from a death sentence to a manageable chronic disease. Today, 28 years and a multitude of studies later, the CDC revised its recommendations stating that limiting condomless sex during the ovulation period with viral suppression by HAART in the positive male and pre-exposure prophylaxis (PrEP) for the uninfected woman can drastically decrease the sexual transmission.[6]

As a result of these advances, HIV-serodiscordant (SD) couples struggling with infertility issues can fulfill their unmet family goals. Globally, it has been reported that approximately 20%–50% of HIV-infected people desire children due to improved prognosis.[9] Keeping this in mind, the Indian Society of Assisted Reproduction developed ethical guidelines in 2018 and stated that HIV-positive couples should not be denied access to fertility-based therapies.[10]

The Prevention Access Campaign of 2016 launched the undetectable = untransmittable (U = U) concept with the aim of ending the HIV-related stigma. U = U signifies that individuals with HIV who receive HAART and achieve and maintain an undetectable viral load for 3–6 months cannot sexually transmit the virus to others.[11] It suggests that the viral load in blood is less than 200 copies/ml and should be considered undetectable. Although a large body of clinical evidence supports HAART with PrEP and condomless sex during the fertility window as one of the safest and least expensive strategies for conception amongst HIV-SD couples desiring children,[6,9] offering SW as a standard clinical practice is a great option to prevent HIV transmission.

With this first-of-its-kind study in Indian HIV-SD couples, the researchers hope to show that even if the HIV-infected male partner is virally suppressed, the seminal viral load varies and often may not be correlated to plasma viral load.[12]

Materials and Methods

This cross-sectional study was conducted over a duration of 6 months in seropositive HIV men under treatment with HAART. The study was done in collaboration with Metropolis Laboratory, a CAP recognised private Healthcare Laboratory in Mumbai, India. Informed written consent was obtained from all the study subjects after explaining the study details. The study was approved by the institutional ethics committee.

Ethical approval

The study was approved by the Inter System Biomedica Ethics Committee, Mumbai, with the Approval Number ISBEC/NR-1/DD-SU/2019 as of 2 January 2019. Written informed consent was obtained for participation in the study and for using the data for research purposes. The procedures follow the guidelines laid down in the Declaration of Helsinki 2013.

Subjects

A total of 110 adult HIV-1-infected males attending an HIV clinic and receiving HAART with a virally suppressed load of <1000 copies/ml were selected for the study in the stipulated 6-month duration. One hundred and two men with a plasma viral load of <20 copies/ml were further selected for semen evaluation after due consent for the same. The sequence of the study is elaborated in Figure 1, as shown below.

Methods

Blood

The blood samples were collected and tested for plasma viral load and CD4 count.

Semen

Those with viral load <20 copies/ml in plasma (men well-controlled on HAART) were included for detection of virus in semen. The sample was collected after a minimum of 2 days and a maximum of 5 days of sexual abstinence.

Semen processing

The semen sample was divided into three aliquots.

- Aliquot 1: Approximately 1 ml of raw (pre-washed) semen sample was tested for viral load
- Aliquot 2: Approximately 1–3 ml of semen sample was subjected to density gradient centrifugation to obtain the seminal plasma and tested for viral load
- Aliquot 3: Post-wash semen sample was obtained through the swim-up technique from the pellet formed in aliquot 2.

All the above aliquots were processed for RNA extraction, and real-time polymerase chain reaction (PCR) to detect the HIV-1 virus. This was done using the Quantitative analysis technique, COBAS AmpliPrep/COBAS Taqman HIV-1 test version 2.0 kit. The detection limit of the kit ranged from 20- 10,000,000 copies/ml. The aliquot 3 post-wash (processed) sample was frozen for future IUI in the uninfected female partner.

CD4 counts were measured in the peripheral blood using the flow cytometry single-platform technology (BD FACSCanto II) with the help of Canto clinical software.

Statistical analysis

Data recording was done in MS Excel. Descriptive statistics was used to analyse the data. Continuous variables are reported as mean + standard deviation, median interquartile range and range. Categorical variables are summarised in terms of frequencies and percentages. Since it was a pilot study, a sample size of 110 men was included in the study. Due to the sudden COVID-19 pandemic lockdown, we were unable to extend our sample size.

Results

Amongst the total 110 HIV-1 infected males tested, 60% were in the age group of 31–45 years, followed by those between 46 and 60 years (24.55%) and 18–30 years (15.45%), respectively, as represented in Table 1.

Majority of the patients in this study were on a nucleoside reverse transcriptase inhibitor- and integrase strand transfer inhibitor-based regimen including tenofovir alafenamide or tenofovir disoproxil fumarate/emtricitabine or lamivudine with dolutegravir or bictegravir or raltegravir. All 110 patients were tested for viral load in the plasma samples.

As represented in Table 2, out of the 110 HIV-infected individuals, 102 (92.73%) patients had undetectable plasma viral load while 8 (7.27%) patients had a detectable viral load. These eight samples were excluded from the study.

The semen analysis for the viral load was done in the remaining 102 samples. Out of 102 samples, the viral load in raw (pre-washed) semen sample, i.e., 100 samples, was low (<20 copies/ml), while the viral load was above 20 copies/ml in 2 samples.

The seminal plasma showed <20 copies/ml in 95 samples (93.14%), whereas 7 (6.86%) samples still showed a detectable viral load (>20 copies/ml). All 102 semen samples were further subjected to processing with density gradient followed by swim-up technique, as shown in Figure 2. The post-wash (processed) sperm sample showed an undetectable viral load (<20 copies/ml) in all 102 samples.

Table 3 shows the different plasma and semen viral load, highlighting the discordancy between both, even if virally suppressed.

Table 1: Overall demographics

Age group (years)	Frequency, *n* (%)
18–30	17 (15.45)
31–45	66 (60.00)
46–60	27 (24.55)

Table 2: Viral load in plasma and semen fractions

HIV-1 virus	Total	Below 20 (copies/mL), *n* (%)	Above 20 (copies/mL), *n* (%)
Viral load plasma (blood)	110	102 (92.73)	8 (7.27)
Viral load raw semen (pre-wash)	102	100 (98)	2 (2)
Viral load seminal plasma	102	95 (93.14)	7 (6.86)
Viral load sperm (processed/post-wash)	102	102 (100)	0

HIV=Human immunodeficiency virus

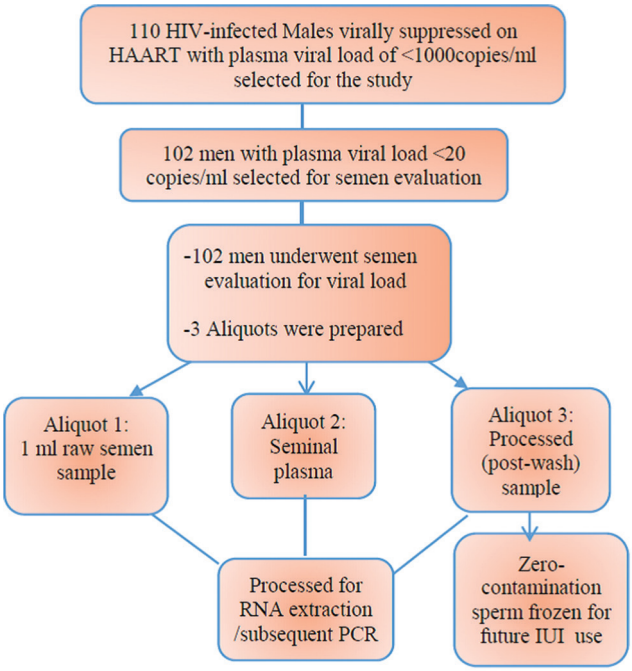

Figure 1: Flow chart elaborating the study sequence

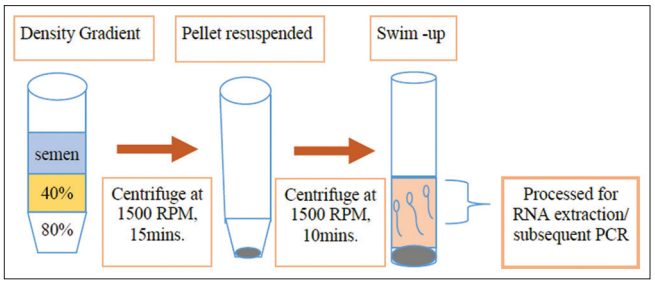

Figure 2: Diagram showing the process of semen processing

Table 3: Discordant plasma and semen viral load

Patient ID	Viral load (copies/mL)		
	Plasma (blood)	Seminal plasma	Post-wash sample
SMC-003	<20	107	<20
SSM-001	<20	125	<20
VCC-011	<20	340	<20
AKM-016	<20	935	<20
NMK-040	<20	176	<20
VRC-002	<20	731	<20
HVN-012	<20	434	<20

The post-wash sample was then cryogenically stored for future IUI use. The entire process was carried out in adequate personal protective equipment suits within a biosafety cabinet and an aseptic environment.

To confirm the immunity status of the virally suppressed men, CD4 counts were performed in the peripheral blood sample. Both CD4% and absolute CD4 counts were analysed. The absolute CD4 count can vary depending on the presence of any immunological and inflammatory disorders, but the CD4% is considered a stable parameter with minimal biological variation. In our study, the absolute CD4 count amongst 102 samples showed an average of 642.04 ± 33, while CD4% showed a mean value of 29.683 ± 15.02, as represented in Table 4. This value suggested a good immune status, putting these men at a lower risk of concurrent infections and hence fit to plan a pregnancy.

Discussion

Transmission of HIV through unprotected intercourse amounts to a relatively low probability of transmission[13] (approximately 1 per 1000 acts of unprotected intercourse). Various risk factors such as genital tract infections or an uncircumcised male partner can contribute to an increased risk of transmission. The rate of transmission is correlated with the peripheral blood viral load and is lowest in individuals with undetectable viral loads.[14]

The past four decades have seen immense progress in the diagnosis and management of HIV. Significant advances in HIV treatment and preventive options like PrEP have further contributed to many HIV-infected individuals seeking parenthood. It is safe to say that HIV is now a manageable chronic condition enabling one to lead a normal life. The HIV Prevention Trials Network (HPTN)-052 study including 1762 HIV-SD couples found that if the HIV-positive person is virally suppressed, the transmission rate estimate per 100 couple years is 0.00![15] This is similar to the PARTNER study done amongst 1163 couples, wherein the positive partner on HAART had a viral load of <200 copies/ml.[16] The final arm of the HPTN study also showed a 96.4% reduction in HIV transmission in the early HAART group versus the delayed start group.[15] A recent 2019 review article combining over 4000 couple years of follow-up reported a transmission estimate risk of 0.00–0.07 per 100 couple years when the HIV-positive partner was virally suppressed. Furthermore, there were no instances of seroconversion amongst uninfected women or any evidence of newborn transmission.[17] The Lancet recently published a review article eliminating all doubts of transmission in cases of low-level viraemia, i.e., <1000 copies/ml. They found no transmission at <600 copies/ml, but a rare occurrence between 600 and 1000 copies/ml, thus asserting the importance of consistent HAART.[18] Hence, the US Department of Health and Human Services recommends that HIV transmission can be reduced by advising consistent HAART use by the infected partner, prior PrEP intake by the uninfected partner and limiting condomless sex to only the fertile period.[19] The PrEP intake can be advised about 20 days before ovulation.[19]

Table 4: CD4 count status

	n	Mean±SD (cells/mm^3)	Median (IQR)	Range (cells/mm^3)
CD 4 (T-cells) absolute	102	642.039±336.9550	589 (400–818)	448–1611
CD 4% (T-cells)*	102	29.683±15.027	29.00 (21.420–35.190)	27–51

*A CD4% of 12%–15% is about the same as a count of 200–350 cells/mm^3. SD=Standard deviation, IQR=Interquartile range

Our pilot study, done in an Indian population evaluated seminal viral loads in the raw semen sample, seminal plasma and processed semen sample in HIV-infected men who were virally suppressed. Few (6.86%) of the patients in this study had higher viral load in the semen despite a suppressed plasma viral load, thus matching with previous studies that viral load can be different in blood versus semen due to viral shedding in the semen.[12] Few studies claim that even highly effective immune responses with undetectable blood viral loads can intermittently shed the virus into the seminal plasma, despite being on long-term HAART.[20,21] Even the HPTN study showed a small risk (1.2 per 100 person-years, confidence interval = 0.9–1.7) for transmitting HIV-1 to their female partner in the face of an undetectable viral load.[15]

Moreover, certain disadvantages such as (a) multiple exposure to the virus in the semen till conception is achieved, (b) PrEP medication every time the woman is ovulating and (c) exposure to anxiety and stress every month are important points to be considered. Initial studies found that the HIV virus receptors do not attach themselves to the spermatozoa, but are present in the seminal fluid,[22] but this fact is still under debate.

The discovery of SW technique, first reported in 1992 in Milan, has transformed the face of assisted reproduction in SD couples.[23] Since then, the concept of SW for HIV-SD couples has gained momentum. In 2014, a published systematic review and meta-analysis of 24 studies including HIV-positive men or women undergoing IUI and *in vitro* fertilisation (IVF) treatment had a 17%, 30%, 14% and 16% per cycle fecundability, respectively. No HIV transmission was observed in 8212 IUI and 1254 IVF cycles, leading to a true rate of only 4.5 transmissions per 10,000 IUI cycles or less.[24] An another comprehensive review of 11,585 cycles confirmed that SW is a highly effective method which can be used in virally suppressed men to achieve a pregnancy, with no cases of vertical transmission.[25] Its use is especially noteworthy in men who are non-compliant to consistent HAART or who cannot gain access to it. On the basis of these highly reassuring data, in 2017, the CDC recommends the usage of appropriate risk-reduction strategies to prevent HIV transmission to the uninfected female partner.[6] Consequently, the American Society of Reproductive Medicine (ASRM) released the ethics committee opinion for HIV and infertility and has recommended SW as a standard of care practice after counselling of the seeking couple.[9]

The HIV titre is known to decrease almost 1000 folds post a double gradient centrifugation, washing and a final swim-up technique, post which the sample is checked for any HIV RNA detection. In our study, we could not find any remnant RNA copies in the processed samples. Recent studies involve various modified protocols such as extended centrifugation and continuous density gradient with swim-up technique.[26,27]

Given the rise of infertility cases worldwide, many such couples would need to resort to IUI or antiretroviral therapy (ART) services. However, ASRM guidelines for reducing the risk of viral transmission recommends that viraemia in the peripheral blood of the infected partner should be minimised to <40 copies/ml through the use of HAART to reduce levels of HIV in semen.[28] Our study along with existing clinical evidence also demonstrates that SW can provide an additional layer of safety to HIV-SD couples in conjunction with taking HAART and PrEP. Thus, although the potential risk of transmission with HAART and PrEP is low, even when the partner is virally suppressed, there is still a possibility of contamination in their semen sample. This suggests that including semen washing in addition to HAART and PrEP can further eliminate even the negligible transmission risk to the uninfected partner.

Prior evidence suggests decreased fertility rates amongst both infected men and women secondary to suppressed immune function or co-infection with other sexually transmitted diseases (STDs).[29] HIV-infected or virally suppressed men on HAART show impaired semen parameters in terms of volume, motility and morphology.[30,31] Moreover, previous studies observed that CD4+ T-cell counts first decline in the male genital tract before they decrease in the peripheral blood circulation.[32] This shows that genital tract T-cell functions are impaired in HIV-infected men, making them more prone to other STDs that can further multiply HIV transmission rates. In our study, we found normal CD4 values in the peripheral blood circulation as our subjects were virally suppressed with HAART.

Since IUI involves millions of sperms, there is a possibility that microscopic possible remnants of HIV

from the seminal fluid could come into contact with the female partner. Hence, the use of IVF/intracytoplasmic sperm injection looks more appealing due to higher success rates than IUI and technically only the least sperm/seminal fluid–oocyte interaction. A 2018 American study showed that almost 40% of clinics in the USA offer ART services to HIV-SD couples.[33] Unfortunately, the sub-Saharan region, which bears the maximum brunt of these couples, faces issues of inadequate funds and poor infrastructure. Yet, affordability and accessibility to ART clinics managing HIV-SD couples remain a dream in developing countries.

Our study is an Indian study, wherein many clinicians have registered Level 1 ART clinics which need minimum investment and skills. This enables them to perform IUIs conveniently even in low socioeconomic areas. As IUI will only help expedite the time to pregnancy as compared to natural intercourse, it is a wiser option to reap the benefits of semen washing in serodiscordant couples by reducing the viral load and achieving a pregnancy sooner. In the past, serodiscordant couples were advised donor insemination which most couples were hesitant to opt for. Others were advised IVF, which is an expensive option as compared to IUI. As IUI cycles can be done either in a natural cycle or with minimal oral ovulogens, the added costs of gonadotropins can be avoided. Hence, the use of U = U concept along with semen washing and IUI on the day of ovulation seems an inexpensive way to treat HIV-SD infertile couples.

Our pilot study consists of a small sample size, showing almost 6.8% of samples with contamination, which increases the numbers needed to test. Even though the sample size is small, it brings out the fact that although the U = U concept exists, there is a potential risk of infected sperm still transmitting HIV to the partner and eventually to the foetus. More studies are needed on a larger sample population. Second, as the post-wash sample was subjected to subsequent processing to check the viral load, fresh IUIs could not be performed. The second stage of the study was to perform IUIs with the frozen samples, but unfortunately, we could not go ahead with the same due to the COVID-19 pandemic lockdown. Furthermore, the transmission rate of HIV to the seronegative partner or the newborn was not studied as the washed samples were stored for IUI in the future. This is because this was only a proof-of-concept study to evaluate if U = U concept prevails in infertile HIV-SD couples.

The COBAS AmpliPrep/COBAS Taqman HIV-1 test version 2.0 kit used to detect the HIV-1 RNA virus depends on the number of the virus particles present in the specimen. This may be affected by specimen collection methods and patient factors, (i.e., age, presence of symptoms, and/or stage of the infection). Furthermore, this test has been validated for use with only human plasma collected in EDTA anticoagulant, hence results for calculating semen contamination can be slightly different.

Conclusions

This first-of-its-kind study in an Indian population demonstrates that even if the HIV-infected male partner is clinically suppressed on the HAART regimen, the viral load in the semen varies from that of plasma and hence there is a potential risk of transmission to the uninfected woman and the unborn foetus. Hence, semen washing provides an additional safety net to help reduce the risk of transmission. Although treatable, HIV is yet not curable. Hence, a complete safe conception package option should be offered to HIV-SD couples, including HAART and PrEP for both partners along with SW instead of condomless sex on the fertile day of the uninfected woman. If infertile, the couple may be counselled for IUI or ART services, depending on their diagnosis.

Hence, timely referrals of HIV-SD couples to fertility specialists with ensuring simultaneous HAART regimen are mandatory to decrease prolonged transmission risks and guide them through the journey of parenthood.

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Authors' contributions

DS – Concept, design, manuscript preparation, data acquisition and analysis, editing and review; RH – Design and data acquisition; and VY – literature search, manuscript preparation and editing.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data supporting the results can be made available on an E-mail request to durushah@gmail.com.

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