SEXUAL VIOLENCE AND HIV: A BIOMEDICAL APPROACH

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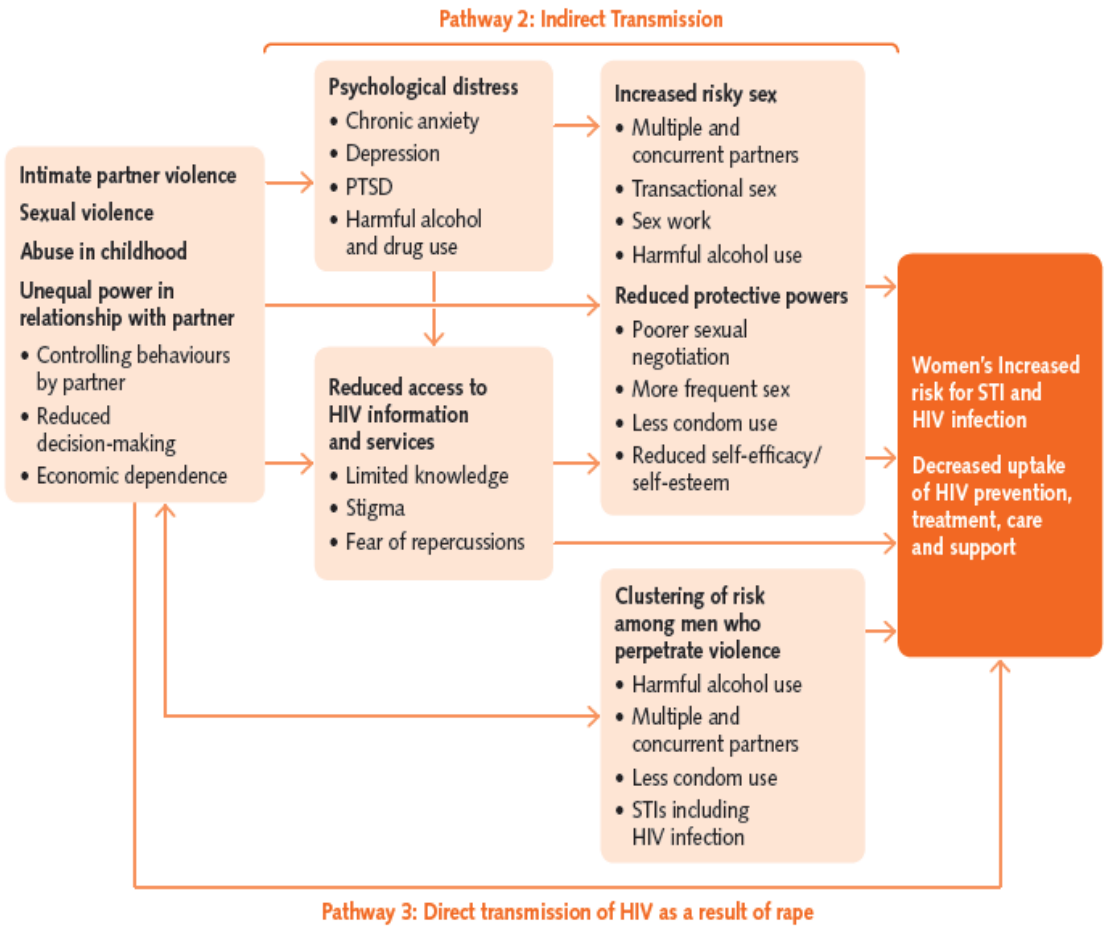
Outline

- Why it is important to look at the mucosal effects of sexual violence for HIV transmission
- Hypothesis
- Aims
- Experimental Approach
  - Feasibility
- Challenges
- Successes
- Where we are
- Significance

Figure 2: Indirect and direct links between violence against women, HIV risk and uptake of services (Pathways 2 and 3)



- The risk of HIV transmission from a single incident of rape is small, but the risk may be elevated in cases of **genital injury** (e.g. degree of trauma, lacerations or tears in the vagina resulting from the use of force or objects), penetration by multiple perpetrators
- A woman's individual risk of HIV from forced sex may be elevated when it occurs **repeatedly** within intimate (**IPV**).
- **Sex workers** may experience sexual violence **repeatedly**.
- Girls may be at elevated risk of transmission because of **ectopic** cervix
- Older women at elevated risk due to **friable** vagina
- Those who suffer **anal rape** are also considerably more susceptible to HIV since anal tissues can be easily damaged
- If the **perpetrator is infected**, HIV transmission may occur during the rape.

http://www.who.int/reproductivehealth/publications/violence/vaw_hiv_epidemic/en/

Sexual violence and immune response

- **HIV infection associated with inflammation and immune activation**
  - **Genital injury and exposure to HIV/STI as a result of sexual violence can induce inflammation, immune activation**
- Higher rates of depression and lower T-cell function in women who experience chronic abuse
- Link between immune compromise and both physical and mental abuse by measuring antibody levels of HSV-1
  - lowest virus neutralization capabilities in women who were physically abused, intermediate levels in women who were verbally abused compared to women without abuse history: linking immune status with stress associated with both physical and psychological trauma.
- A longitudinal research study from South Africa indicated that women who experienced IPV were at increased risk of acquiring HIV with increasingly severe violence associated with increased risk of infection
- PTSD associated with dysregulation of cortisol pathways, fight or flight responses

The Impact of Sexual Violence on the Systemic and Female Genital Tract Immune Responses to HIV Exposure

DC-D-CFAR Supplement (PI Greenberg, PD Ghosh)

➢ Hypothesis

- ➢ Sexual trauma alters the systemic and vaginal immunologic environment which may predispose women to an increased risk of infection following HIV exposure.

- ➢ **Aim 1:** To assess the impact of sexual violence (SV) on cellular and soluble immune biomarkers in blood and genital tract secretions.

- ➢ **Aim 2:** To evaluate the functional loss of anti-HIV immunity in genital tract secretions in women experiencing sexual violence.

Inclusion/Exclusion Criteria

- **Inclusion:** premenopausal women 18-45 years old, be able and willing to provide informed consent for behavioral and clinical procedures, be able to complete the survey in English, HIV negative.

- **Exclusion**: Women who are pregnant or breast feeding, women with STI

- A total of 20 women who report SV within the past 4-12 weeks will be recruited along with 20 age-matched controls with no history of sexual violence

- Incentives:
  - $20 CASI+$30 blood+$50 CVL AND up to 5 eligible referrals @ $10 each.
  - Pregnancy, HIV and STI testing

Experimental Approach (1)

- **Blood and Cervical Lavage Cell Immunophenotyping**:
  - Blood and CVL leukocytes will be characterized by flow cytometry
    - **Leukocytes CD45, neutrophil (CD13+, CD16+, CD14-), monocytes (CD14+), activated cells HLA-DR, T-cells CD3+, T-cell subsets CD4, CD8, CCR5, CD45RA, CD45R0**

- **Soluble pro-inflammatory and anti-inflammatory/anti-HIV biomarker analysis**:
  - **Pro-inflammatory biomarkers IL-8, IL-6, TNF- α, IL1-α, IL1- β (**Luminex)
  - **Anti-inflammatory/anti-HIV biomarkers, SLPI, Elafin, MIP3α/CCL20, and HBD2** (ELISA)

Experimental approach (2)

- **Assessing the impact of CVL on HIV infectivity in TZM-bl cell-line**
  - TZM cells will be exposed to HIV in the presence of CVL from SV women and control women to determine whether CVL in SV women would have lost their intrinsic anti-HIV activity.
- **Assessing the impact of CVL on HIV infectivity in PBMCs**
  - PBMC from Control women will be exposed to HIV in presence of CVL from SV women to determine how factors in CVL from SV women affect HIV infection.

- Data obtained from Aim 2, i.e. the change in anti-HIV activity in CVL of SV women will be **correlated** with results from Aim 1, i.e. alterations in biomarkers in CVL to postulate mechanisms of immune dysfunction in SV women.

Sexual Violence in postmenopausal women

- SV occurs in all ages: “invisible” in older women
  - The aging process severely impairs wound healing abilities thereby putting postmenopausal women with genital tract injuries at significantly higher risks of immune compromise and HIV susceptibility.
  - Vaginal friability, higher baseline inflammation, loss of estradiol controlled protective immune mediators (e.g. SLPI), loss of lactobacilli

Impact of menopause and sexual trauma on HIV acquisition in women

1R56AI111933-01 (NIAID) (Ghosh)

- **Hypothesis:** We hypothesize that genital tract injuries in women experiencing sexual violence will modify the mucosa in a manner that will increase susceptibility to HIV, a risk further enhanced in postmenopausal women due to vaginal friability, higher baseline inflammation and compromised wound healing.

- In **Aim 1** we will test the effects of recent sexual trauma on the genital tract immune microenvironment in women who report being assaulted in the previous four days.
  - **Recruited from District of Columbia-Sexual Assault Nurse Examiners (DC-SANE)**

- In **Aim2** we will investigate the duration, extent, and effects of menstrual cycle or menopause on the genital tract immune microenvironment in women reporting sexual assault in the past 4-12 weeks, by following them longitudinally 2 months post-assault.

Impact of menopause and sexual trauma on HIV acquisition in women

- In **Aim 3** we will use plasma and CVL samples from the Women's Interagency HIV Study (WIHS) repository:
  - Since history of depression strongly associates with history of sexual abuse and trauma, we will study the following 8 groups:
  - HIV-negative
    - Control (no depression, no abuse), Depression only, Abuse only, Depression plus abuse
  - HIV-positive
    - Control (no depression, no abuse), Depression only, Abuse only, Depression plus abuse
  - Abuse defined as cumulative through lifetime including childhood sexual abuse, adult sexual abuse, IPV, transactional sex.
  - Depression defined as >16 on the CES-D scale at the time of sample collection

Inclusion/exclusion criteria

- **Inclusion:** Premenopausal women 21-45 years old, postmenopausal women >50 years old with no menses for at least 1 year, be able and willing to provide informed consent for behavioral and clinical procedures, be able to complete the survey in English.

- **Exclusion:** Women who are pregnant, breast feeding and on HRT , HIV and STI positive will be excluded.

- Non-pregnant status , menstrual staging and menopause, HIV and STI status will be confirmed.

- **Incentives:**
  - $20 CASI+$30 blood+$50 CVL AND up to 5 eligible referrals @ $10 each
  - Pregnancy, HIV and STI testing
  - 20 premenopausal, 20 postmenopausal
  - Women recruited from community (Cases and Controls) will have 5 visits, baseline and four follow-ups (corresponding to 2 cycles of proliferative and secretory stages).

Experimental approach (1)

- **CVL cells and blood cells will be analyzed by Flow cytometry for:**
  - CD45, neutrophil (CD13+, CD16+,CD14-), monocytes (CD14+), activated monocytes (HLA-DRhi, CD16+) and T lymphocyte (CD3+): CD4 and CD8CD45RA, CD45R0 subsets, CCR5, HLA-DR

- **Cervical and vaginal swabs, CVL, blood analyzed for soluble mediators**
  - Pro-inflammatory biomarkers **IL-8, IL-6, TNF-α, IL1-α, IL1-β** will be quantified using Luminex
  - Anti-inflammatory/anti-HIV biomarkers, **SLPI, Elafin, HNP1-3, and HBD2**, **MIP3α/CCL20** will be measured by ELISA

- Functional loss of anti-HIV activity in genital tract fluid will be determined using the **TZM-bl assay** for HIV infectivity

Experimental approach (2)

- Since SV results in varying degrees of vaginal wounds we will measure a panel of wound/wound healing biomarkers to assess the condition of female reproductive tract microenvironment. We hypothesize that in women experiencing severe violence or repeated violence, normal wound healing pathways will be impaired resulting in a "chronic" wound microenvironment with a distinct immune profile that can potentially enhance risks of HIV acquisition and transmission . Further, it is known that healing abilities of the body slows down considerably with age and postmenopausal women are particularly poor healers following mucosal injury . Therefore, postmenopausal women are likely to heal significantly slower than younger women following SV related genital tract injuries.

- The major regulators of wound healing and tissue repair are growth factors **(IGF, EGF, KGF, TGF-β)**, inflammatory cytokines (**IL-8, IL-6, TNF-α, IL1-α, IL1-β**), proteases (Matrix metallo-proteases (**MMP) 1, 2, 3, 8, 9,** and anti-proteases (**TIMP, SLPI, Elafin**).
  - ELISA and Luminex

Challenges

- Lab challenges
  - Isolating cells from CVL
    - *Why are we using CVL?*
  - Blood, semen "contamination"
- Identification of appropriate window for sampling following assault
  - New field, minimal preliminary data
  - How long can you wait to see immunological signature of genital tract trauma?
  - How long is the woman at enhanced risk?
  - How does natural hormonal changes affect trauma microenvironment?

- Recruitment of cases
  - Timing to recruit and sample following trauma challenging
  - Stress and burden on women following sexual assault
  - Suitability of research
  - Ability to provide informed consent
  - Ensuring linkage
  - Barriers to enrollment
  - IRB challenges: Vulnerable population
  - Appropriate compensation

Successes

- Coordination of multiple units feasible
  - Recruiters, clinical staff, lab staff
- Sampling effectively yielding necessary data for soluble mediators
  - Therefore collection and processing protocols are mostly effective
- Outstanding partnerships with District of Columbia-Sexual Assault Nurse Examiners
- Recruitment of controls
- Despite small n, relatively diverse sample
- Use of CASI acceptable and eliciting candid responses on sensitive questions
- Preparation of myriad resources for linkage of cases when identified

Where we are

- Study 1
  - 18 Controls
  - 2 Cases (possibly 3)
    - *Recruitment at GWU*
- Study 2
  - Beginning to recruit

Significance

- Extend our understanding of the role sexual violence plays in HIV transmission and acquisition.
- Understanding the local vaginal immune response as well as its systemic correlates may lead to the development of **novel interventions** that provide greater benefit within this altered environment.
- Since SV is rarely associated with condom use, the role of **reducing local inflammation** may become an important consideration for individuals who are at risk to suffer SV.
- Similarly, the use of **pre-exposure prophylaxis** may become an important adjunct in this population.
- **New algorithms for prevention**

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