CERVICAL IMMATURE AS A MARKER FOR INCREASED RISK FOR STI

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Disclosures

- I have no conflicts of interest.
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Background: STIs in adolescence

- Girls aged 15-24 comprise 25% of sexually active women in the US but account for nearly half of all new sexually transmitted infections (STIs) diagnosed each year.
- Globally, young women aged 15-24, are most vulnerable to HIV with infection accounting for 22% of all new HIV infections.

Is this due to an increased likelihood of engaging in riskier sexual behaviors or a biologic predisposition of the immature adolescent cervix?
Background: STIs in adolescence

- Early age of coitarche is an independent risk factor for developing invasive cervical cancer
- Susceptibility to HIV has also been shown to be higher during certain periods such as pregnancy and the secretory phase of the menstrual cycle

Is this due to behavioral, hormonal or age-related factors changing the innate immune milieu within the female reproductive tract?

Background: Innate Immunity

- The innate immune system of the female reproductive tract includes the cervical epithelium and mucus
- Levels of antigen-specific antibodies (Abs) and immunoglobulins in the cervical mucus of women vary with the menstrual cycle

Does this innate immunity vary with age or sexual activity?

Background: The Adolescent Cervix

- Cervical ectopy is the occurrence of single-layered secreting columnar epithelium from the endocervix beyond the external os
- Ectopy is thought to increase risk by exposing a larger surface area of the target columnar epithelium to potential infections
- Ectopy then is thought to be more common in adolescence and becomes more rare as one approaches menopause
Are younger women are at increased risk of numerous sexually transmitted infections simply because of their age and immature local immunity?

Study Summary and Objectives

- **This pilot proposal aims to determine the feasibility of evaluating the local immune system in the adolescent cervix.**
- **Hypothesis:** An immature cervix and an altered immune milieu in the genital tract increase the risk of HIV and other STIs in adolescent girls.

**Aim 1:** To examine levels of soluble immune mediators in genital tract secretions from sexually active and sexually inactive adolescent girls.

**Aim 2:** To determine the relationship of cervical ectopy to the presence of pro-inflammatory and protective soluble immune mediators in genital tract secretions.

**Aim 3:** To compare biomarker levels in sexually active and inactive adolescents.
### Inclusion and Exclusion Criteria

**Inclusion Criteria:**
- Adolescent females aged 12-19 years
- Post-menarchal
- Having an IUD placement

**Exclusion Criteria:**
- HIV positive status
- Abnormal vaginal discharge (indicating STI)
- Pregnant/breastfeeding
- On hormonal contraception
- On immunomodulatory medications
- Smoking

### Design and Methods

- IRB approval
- Consent/assent obtained
- Assent waived in girls with developmental delay
- Parental consent waived in cases of confidential services.
- Plan for 10 sexually active and 10 non-sexually active girls
- Patients identified prior to scheduled placement of intrauterine device (IUD).

- Cervicovaginal lavage (CVL) was performed using 10ml of saline just prior to insertion of the IUD.
- 3% acetic acid applied to the cervix
- Digital photographs taken
- Extent of ectopy quantified as a percentage of the cervical surface using planimetry techniques in Photoshop™ CS3
Design and Methods

- CVL samples tested in triplicates for soluble immune biomarkers according to manufacturer's protocol (R&D Systems and Peprotech).
- Levels of pro-inflammatory and anti-inflammatory factors determined for both groups.

<table>
<thead>
<tr>
<th>Pro-Inflammatory Cytokines</th>
<th>Anti-Inflammatory/Anti-HIV Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8</td>
<td>Elafin</td>
</tr>
<tr>
<td>ThfA</td>
<td>MIP3α</td>
</tr>
<tr>
<td>IL6</td>
<td>Human Beta Defensin 2 (HBD2)</td>
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</tbody>
</table>

- Associations between the extent of cervical ectopy and the levels of pro-inflammatory and protective immune mediators were estimated and compared.

Results: Demographics

- From 09/2014 to 03/2015, 29 patients were scheduled for IUD placement.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Age</th>
<th>LMP</th>
<th>Menarche</th>
<th>Sexually Active</th>
<th>Date of Last Sexual Activity</th>
<th>Menstrual Cycle</th>
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</thead>
<tbody>
<tr>
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<td>14</td>
<td>4/24/2014</td>
<td>Y</td>
<td>Aug-13</td>
<td>Regular</td>
<td>Regular</td>
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<tr>
<td>003</td>
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<td>7/18/2014</td>
<td>13 yrs old</td>
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<td>Regular</td>
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<tr>
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<td>Y</td>
<td>8/6/2014</td>
<td>Regular</td>
</tr>
<tr>
<td>006</td>
<td>17</td>
<td>8/2/2014</td>
<td>Unknown</td>
<td>Y</td>
<td>Aug-14</td>
<td>Regular</td>
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<tr>
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<td>17</td>
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<tr>
<td>010</td>
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<td>8/25</td>
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<tr>
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<td>16</td>
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<tr>
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<td>N/A</td>
<td>Irregular</td>
</tr>
</tbody>
</table>

Exclusion Set (n=13)

- Current Hormonal Use (n=8)
- Immunodeficiency (n=4)
- Refused to participate (n=1)
Results: Ectopy

Sexually Active Participants

<table>
<thead>
<tr>
<th>Patient's ID</th>
<th>Area of ectopy</th>
<th>Total cervical Area</th>
<th>Ratio (In percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>002</td>
<td>53805</td>
<td>300954</td>
<td>1.793</td>
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<td>005</td>
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Non-Sexually Active Participants

<table>
<thead>
<tr>
<th>Patient's ID</th>
<th>Area of ectopy</th>
<th>Total cervical Area</th>
<th>Ratio (In percentage)</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<tr>
<td>007</td>
<td>667773</td>
<td>1268222</td>
<td>52.654</td>
</tr>
</tbody>
</table>

The area of ectopy was variable within each group, with no statistically significant differences noted.

Results: Immune Mediators

- Anti-inflammatory mediators Elafin and HBD-2 as well as chemokine IL-8 were increased in the sexually active subjects (p>0.05)
  - Elafin: broad antiprotease and immunomodulator with Antimicrobial and anti-HIV1 activity
  - HBD-2 (Human beta-defensin 2): microbicidal and cytokine peptide with potent antimicrobial activity against Gram-negative bacteria and against HIV through modulation of the CXCR4 co-receptor
  - Interleukin-8 (neutrophil chemotactic factor): induces chemotaxis of neutrophils and granulocytes as well as subsequent phagocytosis of infectious agents
  - Chemotactic cytokine MIP3α and IL-6 were found to be increased in the non-sexually active group (p>0.05)
  - MIP3α: small cytokine strongly chemotactic for lymphocytes (including CD4+ T cells) which may fuel obligate HIV expansion following initial infection
  - Interleukin-6: pro-inflammatory cytokine
  - TNFα: a pro-inflammatory cytokine, was similar among sexually active and inactive adolescents
Conclusions

- It is feasible to evaluate the cervical innate immunity in adolescent girls, both sexually active and non-sexually active.
- Area of ectopy was variable, but no statistically significant difference was seen between sexually active and non-sexually active groups.
- Biomarker trends were seen between groups, albeit not statistically significant:
  - Increased in sexually active: Elafin, HBD2
  - Increased in non-sexually active: MIP3α
  - Same levels: TNFa, IL8, IL6

Future Directions

- Expand into larger cohorts of adolescents.
- Delineate immune mechanisms in the adolescent female reproductive tract relevant to HIV and STI acquisition/transmission.
- Measure the cervical ectopy and understand the effects of cervical ectopy on pro-inflammatory and protective soluble immune mediators in genital tract secretions from sexually active and sexually inactive adolescent girls.

References