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Immune biomarkers and anti-HIV activity in the reproductive tract of sexually active and sexually inactive adolescent girls

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Abstract

PROBLEM—Adolescent girls are disproportionately affected by the HIV/AIDS pandemic, accounting for 22% of all new HIV infections globally. Yet little is known regarding the immune microenvironment of the adolescent female reproductive tract, especially regarding differences among sexually active and inactive girls, a critical parameter to evaluate HIV susceptibility associated with young age and sexual debut.

METHODS—Cervico-vaginal lavage (CVL) was collected from sexually active (10) and inactive (8) girls aged 11-19 years and analyzed by ELISA for inflammation-associated biomarkers IL-6, IL-8, TNF- α , MIP-3 α , IL-1 α , IL-1 β , matrix-metalloproteases (MMP) 1, 2, 7, 8, 9, as well as anti-HIV mediators, Elafin, SLPI, human beta-defensin-2 and tissue-inhibitor-of-matrix-metalloproteases (TIMP) 1 and 2. Cervical ectopy was analyzed using Volocity. Anti-HIV activity was determined by TZM-bl assay. Statistical analyses were performed using GraphPad Prism and R.

RESULTS—Sexually inactive girls had significantly higher levels of TNF- α ($p = 0.029$) in CVL compared to sexually active girls. In contrast, sexually active girls showed a trend toward higher levels of IL-1 α ($p = 0.051$) compared to the sexually inactive girls. Heat-map correlations between cervical ectopy and immune biomarkers were also distinct between the two populations with significant positive associations between % ectopy and inflammation-associated biomarkers

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IL-6, IL-1 β , IL-8, MIP-3 α , MMP-8, and MMP-9 observed in the sexually inactive but not sexually active group.

CONCLUSIONS—Higher pro-inflammatory biomarker TNF- α , as well as a distinct inflammation-associated immune clustering in sexually inactive girls can potentially increase risk for infections including HIV upon sexual debut. Future studies with larger sample sizes are needed to characterize the immune parameters associated with sexual activity.

Keywords

Adolescent girls; HIV; sexual activity; female reproductive tract; TNF- α

Introduction

The HIV pandemic affects women disproportionately, particularly girls and young women¹. Even though young people aged 15–24 years represent only 25% of the sexually active population, data from Centers for Disease Control and Prevention (CDC) shows that this population acquires nearly half of all new sexually transmitted infections (STI). Further, young girls are significantly more affected than young boys². Research in this area has mostly focused on behavioral risk factors whereas immunobiological risk factors in this population are poorly characterized.

One of the critical areas of knowledge gap is how early sexual debut relates to immunological risks of acquiring STI including HIV. Whereas young age of debut is a consensual phenomenon in some cultures, in many others, this event is one of coercion that can be mediated through widely practiced customs of child marriage^{3,4}. World-wide, prevalence of forced sexual initiation ranges from approximately 5% to 46%, with rates in the USA at about 12.5%. Forced sexual initiation has been linked to sexual risk-taking behavior as well as risk of HIV and other STI⁵. Additionally, age of sexual debut has been linked to risk of HIV infection⁶ and to the risk of contracting other STI including Chlamydia, Gonorrhea, and Trichomonas later in life⁷.

Biologically, the adolescent cervix is immature, characterized by ectopy. Ectopy is a condition where the ectocervix is composed of a fragile single layer of columnar epithelial cells as opposed to adult ectocervix which consists of multiple layers of squamous epithelial layers that are less fragile and therefore more protective against pathogen entry⁸. Ectopy is influenced by a number of factors, including age, hormonal levels, pregnancy, and oral contraceptive usage⁸. It has also been associated with time since sexual debut⁸, number of sexual partners⁹ and local inflammation^{10,11}. The role of ectopy in infection however, is debated, with some studies supporting a role in enhancing reproductive tract infections^{12–14}, while others showing no such link¹⁵. One reason for the differences in study findings could be attributed to the variability in quantification of ectopy¹⁵.

Data regarding genital biomarkers in adolescent girls is sparse. A study by Madan et. al.¹⁶, comparing adolescent and adult CVL reported increased anti-HSV activity, decreased E. Coli activity and distinct vaginal microbiome in the adolescent group. However, this study did not take into account the effect of cervical ectopy. Another study by Hwang et. al.¹⁰

compared genital biomarkers in adolescent girls with and without ectopy and demonstrated significant correlation of ectopy with higher inflammatory biomarkers^{9,15,16}.

Interestingly, studies suggest sexual activity can alter immune microenvironment of the female reproductive tract (FRT). It is known that seminal fluid contains a number of cytokines and chemokines and triggers an immune response in the female reproductive tract¹⁷. However, studies disagree on whether seminal fluid has an enhancing or an inhibitory effect on HIV¹⁸⁻²⁰. Fichorova *et al.* have demonstrated increased IL-8 and IL-RA in genital secretions of adult sexually active women compared to adult sexually abstinent women²¹. Recent unprotected sex in adult women has also been associated with increased vaginal pH and TGF- β levels (present in high amounts in seminal fluid) and decrease in protective factors such as defensins²². In adolescent girls, sexual activity has been positively associated with inflammatory immune factors such as IL-6 whereas other immune factors showed negative associations, possibly due to the effect of seminal fluid²³. A recent study has associated sexual debut with genital inflammatory biomarkers in adolescent girls²⁴. However, in this case, the inflammatory biomarkers were strongly correlated with high Nugent scores indicating presence of bacterial vaginosis²⁴.

Immuno-biological research is infrequent in adolescent girls as this population is considered vulnerable and therefore difficult to access and recruit. Considerable uncertainty in the field exists about what constitutes appropriate levels of protection for adolescents as research participants and whether parental permission is needed²⁵. In this study we demonstrate feasibility of recruitment and biological sample collection in sexually active and sexually inactive adolescent girls from the Washington DC metro area. We analyzed cervical vaginal lavage for inflammation-associated biomarkers IL-6, IL-8, TNF- α , MIP-3 α , IL-1 α , IL-1 β , as well as HIV inhibitory/protective biomarkers, Elafin, SLPI, human beta defensin 2 (HBD2). These biomarkers were selected based on our previous publications regarding associations of genital immune mediators and anti-HIV activity^{26,27}. We also analyzed levels of matrix-metalloproteases (MMP) 1, 2, 7, 8, 9 and tissue inhibitor of matrix metalloproteases (TIMP) 1 and 2, which are critical to menstrual cycle and pregnancy associated tissue repair, remodeling and wound-healing²⁸. These biomarkers have not previously been analyzed in context of either adolescent genital immunity or HIV associated immune responses in the FRT. Our data from this exploratory study indicates distinct genital tract immune profile in sexually active and inactive adolescent girls, which may impact their susceptibility to STI including HIV.

Materials and Methods

Studies were conducted according to the principles expressed in the Declaration of Helsinki. This study was approved by Medstar Washington Hospital Center (MWHC), Washington DC, Institutional Review Board (IRB) on February 19, 2014, protocol number 2013-232, and Children's National Medical Center (CNMC), Washington DC, IRB on November 7, 2013, protocol number 4213. A reliance agreement was executed between George Washington University, MWHC and CNMC.

All patients provided written informed consent for the collection of samples and subsequent analysis. In sexually active patients waiver of parental consent was obtained given the confidential nature of the clinical care. All patients required pelvic exam for clinical care and thus the study procedures were considered minimal risk.

Subject recruitment and study participants

We recruited 10 sexually active and 8 sexually inactive post-menarche adolescent girls aged 11-19 from the pediatric gynecology practice at Children's National and MedStar in Washington DC. As per routine care in the pediatric and adolescent gynecology clinic, a confidential sexual history was collected from all patients followed by appropriate counseling based on best practices in adolescent care. Sexually active status was based on self-report during a confidential examination detailing sexual experiences. The recruitment target population consisted of girls who were scheduled with the pediatric and adolescent gynecology team to have an IUD insertion for contraception or menstrual suppression. All sexually active girls had IUD placed for contraception. Indication for IUD insertion in the non-sexually active girls was for menstrual suppression due to developmental delay, transgender status or heavy menstrual bleeding. Only those who were assigned female sex at birth and had not received puberty suppression or cross sex hormones were included in the study. Girls were excluded if they were known to be HIV-infected, had abnormal vaginal discharge indicative of genital infection at the time of visit, pregnant/breast-feeding, on hormonal contraceptives and/or immunomodulatory medications for last 3 months and if currently smoking. As per standard of care, sexually active girls underwent testing for Chlamydia and Gonorrhea. All girls in this group were offered HIV testing and those who opted for testing were negative. Girls who declined testing (n=5) were considered very low risk for HIV infection based on demographic and behavior data²⁹. Non-sexually active patients were not screened for STI. As girls in this age group often do not have regular periods, menstrual cycle status (follicular versus luteal) was roughly estimated from time of last menstrual period. Following informed consent, participants were asked questions about medical, sexual and reproductive history. Cervico-vaginal lavage (CVL) collection was performed prior to IUD insertion.

CVL collection and processing

CVL was collected by gently rinsing the cervical-vaginal area with 10 mL of normal saline (BD Biosciences, Franklin Lake, NJ) and transported to the laboratory on ice within 2 hours of collection. CVL was spun at 1500×g for 10 min, supernatant removed, and re-spun at 2200×g for 10 min. Supernatants were aliquoted and stored frozen at -80° C.

Measurement of cytokines, chemokines, and antimicrobials in cervico-vaginal lavage

CVL supernatants were assayed for IL-6, IL-8, TNF- α , MIP-3 α , IL-1 α , IL-1 β , matrix-metalloproteases (MMP) 1, 2, 7, 8, 9, Elafin, SLPI, and tissue inhibitor of matrix metalloproteases (TIMP) 1 and 2 using ELISA kits from R&D Systems (Minneapolis, MN) per manufacturer's protocols. HBD2 was assayed using ELISA kit from PeproTech (Rocky Hill, NJ). Quantification was based on standard curves obtained using a Microplate Reader (Biotek, Winooski, VT). Samples with values below the lowest limit of detection were assigned a value of half of the lowest reading for a particular assay.

Quantitation of total protein concentration in CVL

Total protein concentration in CVL samples was determined using the Pierce BCA protein assay kit (Thermo-Fisher Scientific), following manufacturer's instructions.

HIV viral stocks

HIV strains IIIB (CXCR4 tropic) and BaL (CCR5 tropic) were kindly provided by Dr. P. Gupta (University of Pittsburgh, PA). Virus stocks were propagated in PHA-stimulated human PBMC and stored frozen at -80°C . Virus titers were determined on TZM-bl cells.

Measurement of anti-HIV activity in CVL

Anti-HIV activity in CVL was determined using TZM-bl indicator cell-line (AIDS Reagent Repository, NIH) essentially as previously described³⁰. Briefly, Cells were seeded at 2×10^4 cells per well in a 96-well plate, and allowed to adhere overnight at 37°C . CVL were diluted 1:4 in TZM-bl media (phenol red-free DMEM (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% defined FBS (HyClone, Logan, UT), 2 mM L-glutamine (Invitrogen Life Technologies), and 50 $\mu\text{g/mL}$ Primocin (Invivogen, San Diego, CA) and incubated with IIIB and BaL at 250 tissue culture infectious dose (TCID_{50}). The dose was selected to minimize virus-induced cytopathic effects, while maintaining an ability to measure >1 log reduction in virus infectivity. Samples were incubated with virus for 1 hour at 37°C and added to TZM-bl cells. Luciferase activity was measured in 48h upon application of substrate beta-Glo (Promega, Madison, WI). Uninfected cells and cells treated with CVL alone were used to determine background luminescence expressed as relative light units (RLU). All conditions were tested in triplicates and repeated twice. To calculate percent inhibition, RLU values of "virus only" wells were averaged and set to 100%. Viability of cells upon treatment with CVL was quantified using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega) according to manufacturer's instructions. Briefly, reagent was added directly to cell cultures and incubated for 30 min at 37°C followed by reading the plate in a plate reader at OD 490 nm.

Cervical Imaging and quantification of ectopy

Photographs of the cervix were used to quantify ectopy. Images were captured after applying 3% acetic acid to the cervix using a Nikon Coolpix L830 prior to IUD insertion. The camera was positioned at a distance of 15-20 cm from the cervix and each image was taken in a way that the cervix occupied 75% of the viewing area. Cervical ectopy was quantified using computerized planimetry on the digital images. The area of ectopy and the total cervical area was marked by the clinical staff and quantified in pixels using Volocity software (Perkin-Elmer, Waltham, MA). The percent ectopy (ratio of area of ectopy to the total cervical area) was calculated using Microsoft Excel. Total cervical area and the percent ectopy were compared between the sexually active and inactive groups.

Statistical Analyses

Statistical analyses were performed using R-studio (version 1.0.136). Biomarkers, percent ectopy and HIV inhibition against strains IIIB and BaL were compared using box-and-whiskers plots and p-values were calculated using Mann-Whitney-Wilcoxon test.

Associations between variables were calculated using Spearman's correlation coefficient and depicted as heat-maps.

Results

Characteristics of study samples

Sexually active and inactive girls were compared by age, race, age of menarche, cycle phase and cycle regularity as shown in Table 1. The groups differed significantly only in terms of age with the median for sexually inactive girls at 14 while median for sexually active girls was 16.5 ($p=0.003$) (Table 1).

Since research involving vulnerable population such as young girls is inherently challenging, we gathered feasibility data as part of our study. Of the 21 girls approached over a period of approximately two years, 19 consented, of which 17 were eligible for the study. The two that did not consent were apprehensive about the procedure. One patient was not eligible due to active Chlamydia infection and the other was menstruating on the date of the procedure.

Ectopy

Since cervical ectopy has been linked to inflammation and HIV acquisition, we measured area and percent ectopy in the cervix of sexually active and inactive girls. Median percent ectopy in sexually inactive girls (14.44) was not significantly different from that in sexually active girls (12.01) (Table 2).

Cervico-vaginal immune mediators

To determine whether levels of immune mediators were altered in genital tract secretions of sexually active vs sexually inactive girls, we analyzed CVL samples for inflammation-associated biomarkers IL-6, IL-8, TNF- α , MIP-3 α , IL-1 α , IL-1 β , matrix-metalloproteases (MMP) 1, 2, 7, 8, 9 as well as HIV inhibitory/protective biomarkers, Elafin, SLPI, human beta defensin 2 (HBD2) and tissue inhibitor of matrix metalloproteases (TIMP) 1 and 2.

We found significantly higher levels of TNF- α (median 21.42 vs. 14.9 pg/mL, $p = 0.029$) in CVL from sexually inactive girls compared to sexually active girls (Figure 1, Table 2). In contrast, IL-1 α levels were almost significant ($p=0.051$) with higher values in CVL from sexually active girls compared to sexually inactive girls (median 16.79 vs 67.34 pg/mL) (Table 2). All other biomarkers showed detectable values (except MMP1, which was mostly undetectable) for both groups but no statistically significant differences were observed between groups (Table 2).

As total protein content in CVL samples can be variable^{31,32}, we normalized levels of each biomarker in each sample to its corresponding total protein content. We did not find differences in total protein concentration comparing sexually active vs sexually inactive groups (median 222.46 ug/mL vs 298.48 ug/mL). TNF- α remained significantly different among groups following protein adjustment (Table 2). Significance level of other biomarkers did not change (Table 2).

For the MMP and TIMP genes, we analyzed the data both by levels (pg/ml) and by molar ratio of MMPs to TIMPs, individually and in combination. This is a well-characterized method used to determine whether an immune environment is more activated or inflamed (MMPs will be higher than TIMPs) or whether inflammation is controlled by TIMPs (TIMPs will be higher than MMPs)^{33,34}. For this data set, neither levels nor molar ratios were significantly different between the two groups (Table 2).

Anti-HIV activity

Intrinsic anti-microbial activity in CVL has been previously demonstrated in adult women and adolescent girls^{16,27}. To determine whether sexually active and sexually inactive girls differ in terms of genital anti-HIV activity, we tested CVL against HIV-1 BaL strain using the TZM-bl indicator cell line. A range of anti-HIV activity was detected in both groups. However, no significant differences were noted between the groups (median % inhibition BaL 52.7% in sexually inactive vs 39.4 in sexually active; median % inhibition IIIB, 97.8% in sexually inactive vs 97.2% in sexually active) (Table 2).

Correlations among immune mediators (cytokine/chemokine/antimicrobial)

Distinct immune clusters have been described previously in the context of HIV infection and susceptibility³⁵. Associations between immune mediators are important to consider as they can interact synergistically or antagonistically and be clinically predictive³⁶⁻³⁹. Even though our sample size was small, we observed distinct clustering patterns in sexually active vs sexually inactive adolescents. Specifically, significant positive associations between % ectopy and inflammation associated biomarkers IL-6, IL-1 β , IL-8, MIP-3 α , MMP-8, and MMP-9, was observed in the sexually inactive but not sexually active group (Fig 2). In contrast, in the sexually active group, % ectopy was negatively correlated with IL-1 β and Elafin. Other significant correlations observed between inflammatory biomarkers were similar in both groups (Fig 2).

Discussion

Our study indicates that immune-biological research in adolescent girls is feasible. Since this population is highly impacted by HIV epidemic, it is critical to involve them in HIV prevention and intervention research.

We observed higher levels of pro-inflammatory biomarker TNF- α in sexually inactive girls. TNF- α has been associated with enhanced HIV infection, both directly and indirectly⁴⁰. Therefore a potential negative consequence of higher TNF- α can be increased susceptibility to HIV and STI at sexual debut. Interestingly, CVL TNF- α levels were significantly higher in both of our adolescent groups compared to our other studies in adult premenopausal and postmenopausal women (Ghosh, unpublished observations). It is unclear whether the lower levels of TNF- α in sexually active adolescents is driven by increase in age or exposure to semen-associated antigens following sexual debut. We did not find other reports of age-associated changes in TNF- α levels in the FRT in adolescents versus adult women. However, increase in CVL levels of TNF- α in postmenopausal women compared to premenopausal women have been previously reported³⁰. An intriguing possibility is that

semen exposure following sexual debut results in the reduction of FRT inflammatory mediators, perhaps, from an evolutionary perspective to facilitate reproduction and perpetuation of species. Studies conducted in highly exposed seronegative women (HESN) who do not seroconvert even after repeated unprotected sexual encounters, suggest that chronic exposure to semen-associated antigens can result in a tolerant, immune quiescent phenotype⁴¹. In the reproductive health literature, chronic semen exposure has been described to elicit immune hypo-responsiveness, reduce conditions such as preeclampsia and result in successful birth outcomes⁴².

Although other biomarkers were not significantly different between groups, we did observe a trend toward higher levels of majority of biomarkers we measured (IL-1 α , IL-1 β , IL-6, IL-8, HBD2, SLPI, Elafin, MMP8, MMP9, TIMP1) in the sexually active group. In particular, the higher levels of IL-1 α in sexually active girls reached a p-value very close to significant. This is in agreement with a report by Jespers et. al.²⁴ where significantly higher levels of inflammatory mediators including IL-1 α , were detected in vaginal swabs collected from girls who had recently experienced sexual debut.

Ectopy can be highly variable among women and impacted by sex hormones⁴³. In adolescent girls, prevalence of ectopy increases following menarche. Ectopy gradually decreases with increasing squamous metaplasia stimulated by estrogen, decreased vaginal pH, and sexual activity^{8,44}. We observed a large amount of variability but no significant differences in ectopy among sexually active and inactive girls, possibly due to our limited sample size. Future studies with larger sample sizes might help us to delineate relationship between sexual activity and cervical ectopy. Ectopy in adolescent girls has also been consistently associated with higher rates of STI and higher genital inflammation^{10,16,45}. Results from our heat maps analyses showed strong positive correlations between ectopy and multiple immune factors associated with inflammation, in samples from the sexually inactive girls. These associations did not exist in the samples from the sexually active girls, indicating differential immune clustering and network in these two populations. As with higher levels of inflammation, a strong clustering of inflammation associated immune mediators can potentially be predictive of increased susceptibility to HIV and other STI.

We did not observe any differences in anti-HIV activity in CVL of sexually active versus sexually inactive girls against two laboratory strains of HIV, IIIB and BaL. It is possible that we might have observed differences against clinical strains or transmitted/founder (T/F) strains of HIV. Our previous studies have demonstrated significant differences in anti-HIV activity in CVL of adult women against laboratory strains vs T/F strains²⁶.

To our knowledge, anti-HIV activity has not been previously described in sexually active versus inactive adolescent girls. However, genital secretions in adolescent girls have been reported to have distinct functional inhibitory activity against *E. coli* and Herpes simplex virus, when compared to adult women, and may be attributed to reduced concentration of specific lactobacilli species¹⁶. Our future studies will incorporate T/F strains as well as vaginal microbiome analyses.

To our knowledge, this is the first study that compares genital immune biomarkers in sexually active versus sexually inactive girls. Although designed as a pilot study, we show that it is indeed feasible to recruit this vulnerable population. We also find distinct patterns of biomarkers in these two groups. The major limitation is the small sample size. However, given that we find changes in biomarkers even with the small sample size indicates the importance of future research in this area. Some of the data, such as the heat-maps, must be interpreted with caution because of the small sample sizes and age difference between the two groups. Thus data confirmation in larger cohorts are recommended. Our samples were also collected in absence of sexually transmitted infections, hormonal contraception, recent sexual activity and recent use of vaginal products. Although these exclusions are standard in female genital biomarker studies, this does limit real-life implications of our findings. Future studies are needed to understand the impact of sexual activity in the adolescent genital tract immune microenvironment.

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Figure 1. Sexually inactive girls have significantly higher levels of pro-inflammatory mediator, TNF- α
CVL from sexually active (n=10) and sexually inactive (n=8) girls were tested for levels of TNF- α by standard ELISA assays. Data is presented as pg/mL. Bars depict medians.

Figure 2. Heat-map showing distinct correlations between cervico-vaginal biomarkers and HIV BaL inhibition, for (A) Sexually active and (B) Sexually inactive girls

Distinct network and clustering patterns between immune mediators were observed in (A) sexually active and (B) sexually inactive girls. Each cell of the heat map denotes the Spearman correlation coefficients. Cells highlighted in shades of RED indicates a positive association with darker shades representing stronger associations. Similarly, cells highlighted in shades of BLUE indicates a negative association with darker shades representing stronger associations. Black borders indicate notable clustering patterns in the two groups which are similar in cases of the inflammatory biomarkers (IL-6, IL-8, IL-1 α ,

IL-1 β , MIP3- α , MMP-7, MMP-8, MMP-9) but distinct in associations with % ectopy.
Statistically significant ($p < 0.05$) associations are denoted in **BOLD**.

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Table 1

Participant characteristics among sexually active and sexually inactive girls

Sexual Activity	No	Yes	p value
n	8	10	
Age (median [IQR])	14.00 [12.75, 15.00]	16.50 [16.00, 17.00]	0.003
Race (%)			0.316
African-American	5 (62.5)	3 (30.0)	
Caucasian	3 (37.5)	6 (60.0)	
Hispanic	0 (0.0)	1 (10.0)	
Menarche age (%)			0.236
11-12 yrs old	5 (83.3)	4 (50.0)	
13 yrs old	1 (16.7)	1 (12.5)	
9-10 yrs old	0 (0.0)	3 (37.5)	
Cycle Phase (%)			0.108
Follicular	4 (50.0)	7 (70.0)	
Luteal	4 (50.0)	1 (10.0)	
Unknown	0 (0.0)	2 (20.0)	
Regular cycle (%)	6 (75.0)	8 (80.0)	1
Last sexual activity (%)			
<1 month	N/A	2 (20.0)	
>3 Months	N/A	4 (40.0)	
1-3 Months	N/A	4 (40.0)	

Table 2

Levels of immune biomarkers in CVL from sexually active and sexually inactive girls

	Sexually Active	Sexually Inactive	p value	p-value after protein adjustment
Variables (pg/mL)	Median [IQR] (n=10)	Median [IQR] (n=8)		
TNF- α	14.93 [5.67, 16.74]	21.42 [18.30, 40.15]	0.029	0.006
IL-6	11.54 [1.81, 87.61]	8.44 [4.15, 108.51]	0.719	0.076
IL-1 α	67.34 [36.70, 87.20]	16.79 [11.02, 33.00]	0.051	1
IL-1 β	20.72 [3.61, 65.78]	5.42 [3.61, 39.75]	0.642	0.155
IL-8	497.32 [125.48, 731.81]	221.48 [69.80, 1005.05]	0.79	1
MIP-3 α	100.49 [95.65, 105.87]	108.78 [95.31, 225.66]	0.657	0.214
HBD-2	3976.99 [2923.22, 17292.50]	1778.94 [1366.82, 11084.62]	0.214	0.477
SLPI	130360.56 [70871.34, 571690.73]	194001.41 [100383.74, 380311.31]	0.563	0.534
Elafin	584875.05 [162476.19, 834774.15]	274624.47 [116701.71, 487942.25]	0.183	0.286
MMP-1	UD	UD	N/A	N/A
MMP-2	4577.02 [1869.16, 7090.10]	6519.21 [4101.47, 8910.55]	0.328	0.131
MMP-7	127.70 [51.02, 542.09]	514.10 [204.05, 1378.56]	0.228	0.131
MMP-8	30259.81 [5699.98, 78717.55]	3875.06 [348.93, 58823.34]	0.248	0.155
MMP-9	34551.71 [4299.78, 91855.07]	7017.64 [2091.13, 119386.61]	0.821	0.859
TIMP-1	5481.35 [2573.42, 7235.10]	4274.63 [2223.06, 17720.91]	0.894	0.091
TIMP-2	5406.89 [3546.32, 8990.47]	12330.70 [5300.81, 19281.53]	0.286	0.091
MMP/TIMP Ratio	6.35 [1.82, 12.03]	3.22 [1.46, 5.16]	0.374	
Total Protein (μ g/mL)	298.48 [227.96, 343.43]	222.46 [95.30, 305.30]	0.286	
HIV BaL Inhibition (%)	39.39 [31.89, 54.60]	52.70 [34.96, 59.10]	0.594	
HIV IIIB Inhibition (%)	97.15 [93.50, 105.10]	97.79 [93.23, 101.82]	0.696	
Cervical Ectopy (%)	12.01 [5.46, 43.09]	14.44 [9.47, 43.21]	0.626	

Data shows medians and IQR for each group and presented in pg/mL. Significant p-values are denoted in **BOLD**. UD, undetectable values.