Investigating the impact of using intravaginal products on cervical lesions, genital inflammation, and curable sexually transmitted infections/ genital infections in adolescent girls and young women in KwaZulu Natal

By

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Of

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PREFACE

All experimental work carried out in the description in this thesis was performed at the Centre of the AIDS Programme of Research of South Africa, Masiphumelele clinic, Medical school University of Cape Town, University of KwaZulu Natal, Pietermaritzburg, South Africa, under the supervision of Dr. Pamela Gumbi and co-supervision of Prof. Jo-Ann Passmore and Dr. Singeziwe Sibeko. The research was financially supported by the Poliomyelitis Research Foundation (PRF).

This research has never been submitted to any other tertiary institution, where the work of any other person might have been of use has been acknowledged in the text. All of the results reported are due to an investigation by the candidate.

05 / 02 /2024 _____

Phumla Londeka Radebe

As the candidate's supervisor, I agree to the submission of this dissertation.

05 / 02 /2024 _____

Dr Pamela Gumbi

DECLARATION

I, Phumla Londeka Radebe declare that the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work. This work has not been submitted for any degree or examination at any other university. It does not contain other person's data, pictures, graphs, or another person's information unless specifically acknowledged as being sourced from another person. No other person's writing is contained in this thesis unless specifically acknowledged as being sourced from other researchers. Where written sources have been quoted and where precise words have been used, the writings are placed within quotes and referenced.

05 / 02 /2024 _____

Phumla Londeka Radebe

DEDICATION

I dedicate this thesis to my newly born son, Oyinkosi Kamvalethu Magwaza. Mommy did it.

ACKNOWLEDGEMENTS

I am utterly grateful to my supervisor Dr. Pamela Phumelele Gumbi for the support you have given me since that day you welcomed me into your lab, this was rather a very weird setup (us working remotely 99% of the time) but thank you for the long nights, devoting your time to me even when I least deserved it. Thank you for always picking up my chin when I doubted myself, thank you for being the best reflection of everything I want to be and more. Thank you for opening doors I never in my wildest dreams thought I'd ever step into. To Prof Jo-Ann Passmore, when it comes to you words fail me. My biggest secret ever since I came into your lab is that I find you so intimidating, you hold so much velour that humbles me all the time, thank you for welcoming me with warm hands and a kind spirit, and for always making me feel more than just 'a part' of the team. Thank you for always pushing me toward greatness. I could never be grateful enough.

To Ramla, Zanenhlanhla, Mona, Hoyam, and the rest of the MIG group thank you guys for holding me up and assisting in many ways in making sure this study is a success.

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And everyone else who worked in the background to ensure the success of this study. Izandla zidlula ekhanda!

ETHICS DECLARATION

The study protocol was approved by the BREC of the University of KwaZulu-Natal, ref no: BREC/00003306/2021. Good Clinical Practice (GCP) and Human Subjects Protection (HSP) guidelines were followed and written informed consent was obtained from all participants before enrolment. The identity of participants was kept confidential.

ABSTRACT

Background:

Vaginally inserted products (VIPs) are used to cleanse, enhance sexual pleasure or modify the female genital tract to a desired state by young South African adolescent girls and young women (AGYW), in regions of the country that practice “dry sex”. This study hypothesized that sexual immaturity in adolescent females in conjunction with VIP use would exacerbate injury to the cervicovaginal mucosa and increase vaginal inflammatory responses, which may influence the risk of acquiring human immunodeficiency virus (HIV) and other sexually transmitted infections (STIs).

Methods:

Sexually active and HIV-uninfected cisgender adolescent girls (n=188, 14-19-years old) and adult women (n=64, 25-35 years old) were enrolled in an HIV endemic rural Vulindlela area, in KwaZulu-Natal (KZN), South Africa. A detailed questionnaire was used to collect demographic, sexual behaviour, and vaginal practices data. Cervical ectopy and other cervical abnormalities were identified by colposcopy. Luminex assay was used to measure the concentrations of various inflammatory cytokines from cervicovaginal secretions. Human papillomavirus (HPV) genotyping was done using a DNA Flow hybridization system. Participants were also tested for the presence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), bacterial vaginosis (BV), yeast and fungal hyphae.

Results:

This study found that adult women were more likely to be involved in risky sexual behaviour compared to adolescents, although the prevalence of STIs was higher in adolescents compared to adults, particularly CT ($p<0.001$). Colposcopic observations showed that adults were more likely to have signs of cervical injury and trauma compared to adolescents ($p<0.0001$), and significantly higher cervicovaginal concentrations of interleukin (IL-)8 ($p<0.0001$), granulocyte-colony stimulating factor (G-CSF) ($p<0.0001$) and IL-10 ($p<0.0001$) compared to adolescents. However, tumour necrosis factor alpha (TNF-α) concentrations were significantly higher in adolescents compared to adults ($p<0.0001$). The use of vagina stimulating products was common in this cohort, with adolescents reporting a wider range than adults. These products were either traditionally made or available commercially, taken orally, or applied internally or externally in the vagina. According to the adolescents and women, the products were used for different reasons and the preferences by age group were different:

Adolescents most commonly used alum (a combination of aluminum sulphate and potassium sulphate) while adults most commonly used “ibhodwe labafazi” (translated from Zulu to mean “ladies pot”; a commercially available scented petroleum jelly with unknown ingredients). Adolescents who used alum were likely to have injury signs compared to adolescents who did not use any products (p=0.002). In addition, adult women who used “ibhodwe labafazi” were 20 times more likely to have cervical ectopy compared to adult nonusers (p=0.004). Among adolescents, the users of “ibhodwe labafazi” had significantly elevated cytokine concentrations, remarkably so for IL-1α (p=0.0223), vascular endothelial growth factor (VEGF) (p=0.0198) and IL-17 (p=0.0150) compared to nonusers. However, in adults, there were no remarkable changes in genital tract cytokine concentrations in users compared to nonusers. However, the use of the products did not appear to have a direct link with the prevalence of infections.

Conclusion:

In adolescents, the use of alum and “ibhodwe labafazi” may cause injury and inflammation, respectively, which are known to increase the risk of HIV acquisition. In addition, in adult women, using “ibhodwe labafazi” were more likely to have ectopy, which is associated with STIs and HIV risk. These findings need to be confirmed by more extensive cohort studies and suggest that the use of some of the vaginal enhancing products may partly contribute to differences in biological risk profiles that influence HIV susceptibility.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

ºC	Degrees Celsius
µl	Microlitres
%	Percentage
AGYW	Adolescent Girls and Young Women
BREC	Biomedical Research Ethics Committee
BV	Bacterial vaginosis
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CCR5	C-C chemokine receptor type 5
CD	Cluster of Differentiation
CIN	Cervical Intraepithelial Neoplasia
CVS	Cervicovaginal secretions
CT	*Chlamydia trachomatis*
CXCR-4	C-X-C Chemokine receptor type 4
DCs	Dendritic cells
Ectopy	Cervical ectopy
Env	Envelope glycoprotein
GBV	Gender-Based Violence
G-CSF	Granulocyte-Colony Stimulating Factor
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
gp	Glycoprotein
GSVP	Gender, Sexuality and Vaginal Practices
H_2O_2	Hydrogen peroxide
HIV	Human Immunodeficiency Virus
HLA-DR	Human Leucocyte Antigen-DR
HPO	Hypothalamus–Pituitary– Ovary
HPV	Human Papillomavirus
HR-HPV	High-Risk HPV
IFN	Interferon
IL	Interleukin
IL-1Ra	IL-1 Receptor antagonist
IP-10	Inducible Protein-10
IQR	Inter-Quartile Range
KZN	KwaZulu-Natal

LCs	Langerhans cells
L. crispatus	*Lactobacillus crispatus*
L. jensenii	*Lactobacillus jensenii*
L. vaginalis	*Lactobacillus vaginalis*
LR-HPV	Low-Risk HPV
MCLRs	Mannose-dependent C-type Lectin Receptors
MCP-1	Monocyte chemoattractant Protein-1
Min	Minutes
MIP	Macrophage Inflammatory Protein
MIST	Mucosal Injury from Sexual Trauma
ml	Millilitre
NG	*Neisseria gonorrhea*
PBS	Phosphate buffer saline
PID	Pelvic Inflammatory Disease
PLHIV	People Living with HIV
PSA	Prostate-Specific Antigen (PSA)
py	Person years
RANTES	Regulated upon Activation, Normal T cell Expressed and Secreted
SRH	Sexual and Reproductive Health
STI	Sexually Transmitted Infection
SSA	Sub-Saharan Africa
Tat	Transactivator of transcription
TNF-α	Tumour Necrosis Factor alpha
TRAIL	TNF-Related Apoptosis-Inducing Ligand
TV	*Trichomonas vaginalis*
T-zone	Transformation zone
RT	Room temperature
UNAIDS	United Nations Programme on HIV/AIDS
VEGF	Vascular Endothelial Growth Factor
VIPs	Vaginal Inserted Products
WHO	World Health Organisation

Chapter 1

Literature Review

1.1 Introduction

Despite the remarkable progress made in the last ten years to prevent HIV infections, new HIV cases are still on the surge (Cervantes and Atta, 2023). According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), there are 4000 new HIV infections daily, with 7800 young people (aged 15 to 24 years) becoming infected every week (UNAIDS, 2022). East and southern Africa continue to be the centre of the HIV epidemic, with 20.6 million people (18.9 million–23.0 million) infected, representing 54% of all people living with HIV in the world (Govender *et al*., 2023, UNAIDS, 2022, Figure 1.1). Women remain disproportionately affected by the HIV/AIDS pandemic, accounting for 63% of the region's new HIV infections in 2021. There were approximately 250,000 [150,000–360,000] adolescent girls and young women who became newly infected with HIV in 2021, and this is three times more than among male peers (UNAIDS, 2022).

The HIV pandemic has increased interest in mechanisms that facilitate the risk to sexual transmission of infections, HIV, and other genital infections. One of the most reported mechanisms in the sub-Saharan Africa is vaginal practices and vaginal product usage. This practice comprise of a variety of behavioral factors that have been identified to be used by women to positively manage their hygiene and sexual health (dry vaginal state) which include but not limited to, the insertion of vaginal products, the ingestion of herbs, modification of genitalia, washing or cleansing the vagina with a variety of products besides just water alone. Globally, there is a growing market for over-the-counter vaginal cleansing products that are portrayed as a necessity for women (Jenkins *et al*., 2021,Chersich *et al.*, 2009; Humphries *et al.*, 2019; Jenkins *et al.*, 2021; Low et al., 2011; Nsereko *et al*., 2021). Several studies have found an association between HIV risk and VIPs (Chersich *et al.,* 2009; Hesham *et al*., 2021; Hilber *et al.,* 2010; Humphries *et al*., 2019; Karim et al., 2011; Andrasik et al., 2019; Low *et al.,* 2011; Ramjee & Daniels, 2013). However, few studies have looked at the effect of VIP use on the female cervix.

Adolescent girls and young women (AGYW) are exposed to multiple HIV risk factors that are interrelated, including harmful social norms and practices, and social, economic, and gender inequalities (UNAIDS, 2022). Several biological factors that contribute to HIV susceptibility are

known, however, remain poorly characterized, especially during the adolescent stage which comes with puberty. Puberty and changes in female hormones are associated with cervical ectopy and lesions and possibly increased acquisition risk for HIV and other STIs. Cervical lesions have been linked to traumatic or mechanical etiologies, including the use of intrauterine devices or other foreign products introduced into the vagina (Petre et al., 2023).

Critical to developmental changes in the reproductive system during puberty, the young adolescent immune system must also be able to distinguish between semi-allogeneic male semen from sexual partners which must be tolerated to ensure fertility (Jewanraj *et al.*, 2020; Mngomezulu *et al.,* 2021) and potential pathogens that require an inflammatory attack to avoid infections. Although several studies have shown that inflammatory responses in the female reproductive tract are linked to HIV risk (Cromarty & Archary, 2020; Masson et al., 2015; Mtshali *et al*., 2021; Nkwanyana *et al.,* 2009; Passmore *et al*., 2016), few have looked at the impact of vaginal sex and use of vaginal products on the female genital tract, cervical changes measured by colposcopy, and inflammatory responses.

This study will test the hypothesis that sexual activity in adolescent females in conjunction with VIP use is likely to exacerbate injury to the cervicovaginal mucosa and increase vaginal inflammatory responses, which may influence their risk of acquiring HIV and other infections.

1.2 HIV epidemic and disproportionate risk in adolescent girls and young women

HIV remains a global challenge, even after three decades of intensive interventions to avert HIV-related deaths. In 2022 alone, there were approximately 1.3 million (1 million–1.7 million) people who became newly infected with HIV globally, with 29.8 million accessing HIV treatment, giving a total of 39.0 million (33.1 million–45.7 million) people living with HIV (PLHIV) at the end of 2022 (UNAIDS, 2022). There were 630,000 (480,000–880,000) reported cases of people who died from AIDS-related illnesses in 2022, despite excellent antiretroviral drugs to prevent HIV-disease progression (UNAIDS, 2022). South Africa has the highest number of PLHIV, with approximately 20% of the global number of PLHIV reported to be coming from South Africa in December 2022 (MacLean and Wetherall, 2021**;** UNAIDS, 2022).

Globally, mostly young women and adolescent girls are burdened by HIV (Birdthistle *et al*., 2019; Karim and Baxter 2019; Mannell *et a*l., 2019**)**. In 2022, there were approximately, 4000 adolescent girls and young women aged 15–24 years who became infected with HIV every week globally, and the majority (77.5%) were in sub-Saharan Africa (SSA) (Birdthistle *et al.*,

2019; Stover *et al.,* 2021; UNAIDS, 2022). In a recent study which enrolled women in the ECHO trial in 2015 - 2017 in nine South African sites, the incident of HIV was >3 per 100 woman-years at all sites, with KZN showing the highest prevalence of 6.80 per 100 woman-years (95% CI 5.14 – 8.84). Moreover, HIV incidence was higher among women of the ages 21-30 years (5.03 (95% CI 4.1 – 6.12) compared to women of the ages 31-35 years (4.72 (95% CI 4.13–5.36) (Palanee-Phillips et al., 2022). The high levels of HIV prevalence among young people have led to a focus on adolescent girls as a target population for HIV prevention. Therefore, many studies have focused on understanding the drivers of HIV infection in this group.

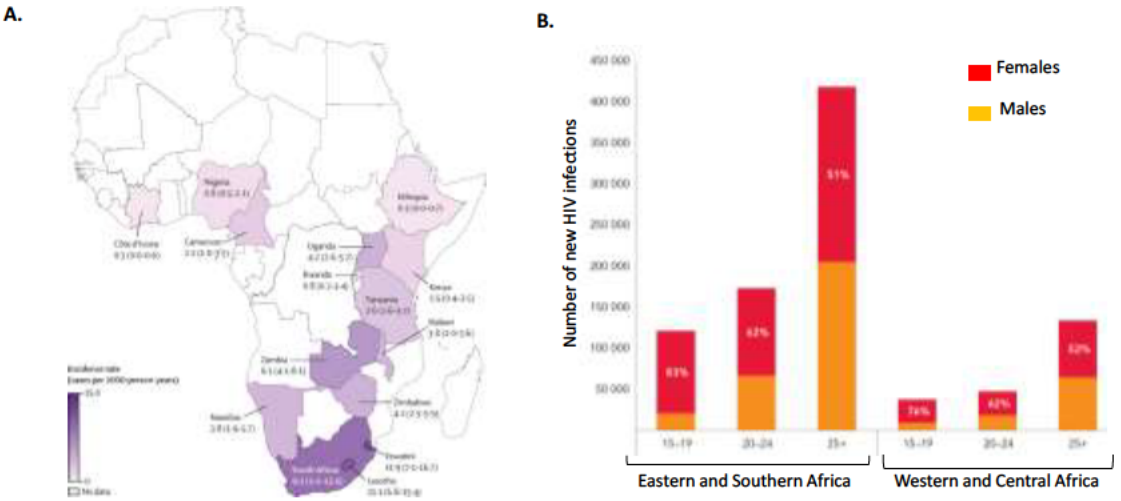


Figure 1.1 HIV burden in Sub-Saharan Africa. A. HIV incidence across 15 countries in Sub-Saharan Africa with high burden of HIV. HIV incidence rate is expressed as 1000 person-years (Source: Rosenberg *et al*., 2023). B. Number of new HIV infections in Eastern and Southern Africa and Western and Central Africa regions (in females and males, by age, Source: UNAIDS, 2019).

1.3 Social and psychological factors associated with HIV and STI risk in AGYW

The HIV risk factors in AGYW are numerous and complex and require multi-faceted approaches to prevent infection. The social and psychological factors linked to HIV can be broadly categorized into the following: behavioral, socioeconomic, structural, gender-based violence (GBV), gender inequality, and mental health factors (Mabaso *et al*., 2018; Duby *et al*., 2021; Ferguson *et al.,* 2021; Lewis *et al.,* 2022).

1.3.1 Behavioural factors

Many previous studies have been published on behavioral factors associated with HIV and STI risk in AGYW, including inconsistent use of condoms, number of sexual partners and age-disparate relationships), early sexual debut, and pregnancy during adolescence (Mabaso *et al.*, 2018; Jonas *et al.,* 2023; Rudgard *et al*., 2023). The use of alcohol and drugs also contributes to promoting riskier sexual behaviors. Promoting sexual well-being and linkage to sexual and reproductive health (SRH) education and counseling services are key interventions that may mitigate risky sexual behaviors (Dietrich *et al*., 2023).

1.3.2 Socio-economic factors

It is widely acknowledged that family structures play an important role in health outcomes. A recent study by Lewis *et al.* (2022), reported that young women who reported absence of family support were more likely to have more than one partner than those with family support. In addition, AGYW who reported having not completed their secondary school education had higher HIV incidence than those that had (Lewis *et al*., 2022), and education enrolment was associated with a lower probability of an age-disparate sexual relationship (Gumede *et al.,* 2023). Another recent study showed that women are more impacted by food insecurity than men, and that unemployment was one of the main factors associated with food insecurity in South Africa (Wet-Billings, 2023). Among adolescent girls, family support and having enough food at home, were each associated with a lower probability of HIV risk behaviors (Gumede *et al*., 2023).

1.3.3 Structural factors

Inadequate or lack of access to SRH services and sexual education has been associated with increased sexual risk behaviors in South African women (Mushwana *et al*., 2015). As a result of lack of access to SRH care and low rates of HIV testing, there is poor HIV status disclosure between AGYW and their sexual partners and poor ART adherence (Dietrich *et al*., 2023; Rudgard *et al.,* 2023).

1.3.4 GBV and gender inequality

The links between GBV, gender inequality, and HIV are complex and culturally specific (Strebel *et al.,* 2006). In Africa, a culture that promotes the development of stereotypically masculine and feminine behaviors and strong peer pressure to conform can encourage aggressive sexual behavior from males (Leach, 20022). Young women exposed to GBV were likely to experience depression, post-traumatic stress, suicidal thoughts as well as riskier sexual behavior (Pérez-Martínez *et al*., 2023).

1.3.5 Mental Health

Young people living in SSA are affected by poor SRH and mental health (Musindo *et al.,* 2023). A recent study showed that two-thirds of AGYW with depressive symptoms had high HIV incidence, suggesting that interventions addressing mental health are also likely to reduce the risk of acquiring HIV among AGYW (Goin *et al*., 2020).

1.4 VIPs and adverse health outcomes

Intravaginal practice is a term that broadly defines a collection of behaviours that women use to alter the environment and structure of the vagina, for an enhanced sexual experience (dry sex) or hygiene purposes (Table 2.1; Hilber *et al.,* 2010). The most frequently described practice includes intravaginal use of liquids, considered douching (with or without an applicator). Women practice various methods to intravaginally cleanse themselves to possibly self-manage their vaginal health and possibly "control" their marital roles and sexual life (Hilber et al., 2010; Low et al., 2011). Globally, over 10,000 over-the-counter vaginal cleansing products are available and are a part of a growing market (Jenkins *et al.,* 2021). These products are advertised to make these vaginal products and vaginal cleansing practices desirable for women. One of the most concerning factors is that intravaginal cleansing is being marketed as an absolute necessity for vaginal hygiene (Chersich *et al*., 2009; Humphries *et al*., 2019; Jenkins *et a*l., 2021; Low *et al*., 2011; Nsereko *et al*., 2021), capitalizing on cultural messaging that women's vaginal environment is unclean, inadequate, and requires an intervention by the use of VIPs that will improve their vaginal state (Jenkins et al., 2021).

In some regions, reasons for using VIPs include, but are not limited to: (i) retaining (to please) male partners and spouses; (ii) maintaining social norms such as sexual pleasure/morality; and (iii) to support and enforce gender roles and power dynamics (Humphries *et al*.,2019; Mngomezulu *et al.,* 2021; WHO, 2012). In these settings, it is paramount for women to have a tight, hot/warm, and a dry vagina to be regarded as wholesome (an ideal women).

Several important studies have raised concerns that vaginal products may adversely affect reproductive health, including increasing the risk of HIV infection (Chisembele *et al*., 2018; Hilber *et al*., 2010; Low *et al.,* 2011). The stratified squamous epithelium that lines the lower reproductive tract of women provides an important physical barrier to pathogens. Any physical or chemical effects of VIP use could therefore allow HIV to infect the intraepithelial T cells. For example, the insertion or application of products (including traditional herbs) to prepare the

vagina for sexual intercourse (for dry sex) or wiping (with a cloth, cotton wool, or paper) during sex or after intravaginal cleansing could cause physical abrasions to this protective mucosal barrier. To support this, previous studies have shown that women who performed vaginal washing with soap (or other domestic substances) were at a higher risk for HIV-1 than those who used water alone (Low *et al*., 2011). Therefore, in a population where vaginal washing and VIP use are common, community engagement should aim to modify intravaginal practices as an HIV prevention strategy. In AGYW, the lower reproductive tract may present a naïve reactive state that is anatomically immature, and tolerant to the trauma caused by VIPs. This lack of tolerance could manifest as increased genital inflammatory responses that may increase the chance of AGYW acquiring HIV.

Another mechanism by which some vaginal cleansing products may increase sexual health risks is by disrupting the optimal vaginal microbiome, which in turn would predispose young women to lower reproductive tract infections and other gynecological problems. Intravaginal cleansing products were shown to disrupt optimal vaginal microbiota by removing/thinning healthy cervical mucus and commensal bacteria from the lower reproductive tract (Paramel Jayaprakash *et al.,* 2016). A study of three common vaginal douching products (including Benzalkonium chloride [which has a pH of 5]; Dynaphytols burdock 1.2% pH of 8; and tartaric acid [which has a pH of 2.7]) (Aslan & Bechelaghem 2018), altered the cervicovaginal mucosal barrier by interfering with the survival of optimal Lactobacilli strains and harming squamous epithelial cell integrity (Aslan & Bechelaghem 2018; Fashemi *et al*., 2013). Hilber *et al.* (2010) and McClelland *et al.* (2006) observed that some soaps, detergents, and anti-septics included in feminine vaginal washes (intended to be used inside the vagina) caused chemical damage and increased the vaginal pH to >4.5), which encourages the growth of microorganisms associated with BV. Some other adverse health effects associated with VIP uses include an increased risk of acquiring HPV infections and cervical cancer, and urinary tract infections been associated with this practice (Bautista *et al.,* 2016; Hilber *et al*., 2010; Hwang and Moscicki, 2014; Jenkins *et al*., 2021; Moscicki *et al.,* 2001).

In women with cervical ectopy, intravaginal practices or VIP use could be damaging due to exposure of sensitive endocervical columnar epithelial cells, that are more likely to be damaged. However, there is a lack of information about the physical, chemical, or biological factors associated with the use of vaginal products with cervical ectopy. The cervical area outside the cervix where the glandular cells encounter squamous epithelial cells, is an important gateway to infection. Therefore, studies are needed to understand the VIP use in women with ectopy that influences their STI acquisition risk.

Table 1.1. Definitions of intravaginal practices, described in this study

Intravaginal practice[b]	Definition[a]
Cleaning with water	Cleaning the inside of the vagina, beyond the introitus with water as the only product. Can be with or without the use of fingers, other material, or douching devices to introduce water inside the vagina.
Cleaning with soap	Cleaning the inside of the vagina beyond the introitus with generic soap or household soap or named proprietary bath soaps. Can be with or without the use of fingers, other material, or douching devices to introduce water inside the vagina.
Cleaning with other household products	Cleaning the inside of the vagina beyond the introitus generic household cleaning products such as "Omo" (clothing detergent), antiseptic solutions, vinegar, or lemon juice. Can be with or without the use of fingers, other material, or douching devices to introduce water inside the vagina.
Use of a cloth	Using material such as a cloth, tissue, paper, cotton wool to wipe the inside of the vagina beyond the introitus. This excludes the use of tampons, or the use of inserted medications.
Insertion of products to dry or tighten the vagina	Pushing or placing non-liquid products inside the vagina (including powders, creams, herbs, tablets, sticks, stones, leaves, or other "traditional products"). For the intention to dry or tighten the vagina.

[a] Definitions of intravaginal practice and insertion adapted from classification developed by the World Health Organisation (WHO) Gender, Sexuality and Vaginal Practices Study group (GSVP Study group) (Palanee-Phillips et al., 2022). Additional definitions adapted from (Low *et al*., 2011). [b] Commonly cited vaginal practices used by women in the Sub-Saharan Africa.

1.5 Transmission of HIV in the female genital tract

The vaginal, ectocervical, and endocervical mucosa can all be infected and penetrated by HIV (Hladik and Hope, 2009). Although the relative contribution of these three sites to successful HIV transmission is unknown, both the vaginal and ectocervical mucosa have a multi-layered squamous epithelial barrier which offers better protection against HIV invasion when intact compared to a monolayered endocervical mucosa (Hladik and Hope, 2009). Several biological and mechanical factors can determine the risk of HIV infection these include the epithelial integrity, micro lacerations of the mucosal surface caused by sexual intercourse, cervical lesions, and the presence of anti-HIV factors and host immune factors such as activation status of immune cells, level of inflammation, hormonal levels (due to use of hormonal contraceptives, menstrual cycle, pregnancy, puberty, and menopause), the composition of the commensal microbiota, and co-infections with STIs (Balle *et al*., 2020; Bayigga *et al.,* 2019; Dabee *et al*., 2019; Gonzalez *et al.,* 2019; Morrison *et al*., 2014; Newbern *et al.,* 2013).

HIV can effectively enter the epithelium through micro-abrasions via either paracellular passage (by passing through the intercellular space between the cells) or by transcytosis (transported across the interior of an infected cell) (Gonzalez *et al.,* 2019; Yasen *et al*., 2017; Figure 1.2). In addition, HIV- proteins (such as transactivator of transcription (Tat) and envelope glycoprotein, gp120), are associated with the disruption of epithelial tight junctions (Tugizov *et al*., 2013). Treatment of polarized epithelial cells with purified HIV-1 envelope protein gp120 disrupts tight junctions, implying that this protein may play a critical role in the paracellular passage of HIV (Tugizov *et al*., 2013; Tugizov, 2021).

HIV encounters a variety of mucosal cell types, such as Langerhans cells (LCs), dendritic cells (DC), tissue macrophages, and more recently fibroblasts (Gonzalez *et al*., 2019; Shaw and Hunter, 2012). Several previous studies suggest that vaginal epithelial DCs also known as LCs are the abundant cells in the cervix and vaginal epithelium to first encounter and capture the HIV and deliver to underlying susceptible cells in the first few hours of infection. Although LCs do not express C-C chemokine receptor type 5 (CCR5), they express other receptors such as human leucocyte antigen (HLA)-DR, cluster of differentiation (CD-)1a, and mannose-dependent C-type lectin receptors (MCLRs) that can be used by HIV for attachment (Xu *et al.,* 2013). Once the virus has been captured, the DCs migrate to the lamina propria and encounter other abundant cells such as CD4+ CCR5+ T cells, macrophages, and other cells that may support viral amplification (Xu *et al.,* 2013). HIV-1 envelope glycoprotein [Env; trimeric (gp160)3 cleaved to (gp120/gp41)3] attaches the virus to a susceptible cell and induces fusion of viral and cell membranes to initiate infection (Chen, 2019). The successful establishment of

HIV infection is highly dependent on the abundance and activation status of CD4 T cells, which when they express CCR5 and C-X-C chemokine receptor type 4 (CXCR-4) receptors can easily be infected by CCR5-tropic or dual tropic (CCR5/CXCR4) viruses (Shaw and Hunter, 2012**)**. In addition, preferential infection of T helper 17 (Th17) CD4 T cells has been reported (Stieh *et al*., 2016).

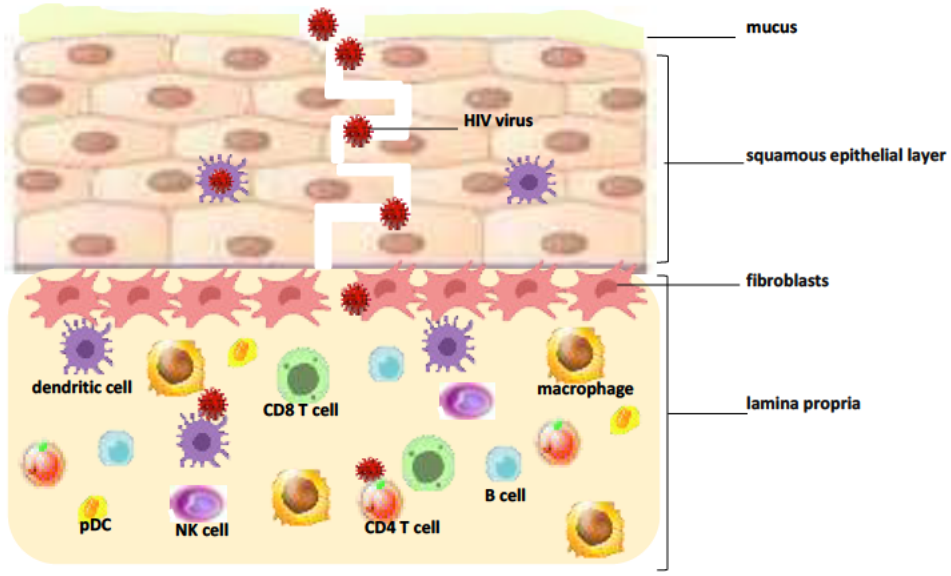


Figure 1.2 Early interactions of immune cells in the female genital tract with HIV virus. The virus can induce mucin secretion, which can lead to the inhibition of mucus production. In addition, ovulation leads to hydration and alkalinization of the mucus plug, possibly diminishing its barrier function. Both free HIV or cell associated HIV, can penetrate the epithelium. Microabrasions of the mucosal surface induced by sexual intercourse may allow HIV to directly access target cells (paracellular passage) or by transcytosis across epithelial or dendritic cells (DCs). Alternately HIV can gain access via HIV-induced epithelial disruption by HIV soluble proteins that lead to the formation of gaps in the epithelial layer. Through these gaps, the virus may reach the submucosa and target cells, such as DCs, fibroblasts, T cells, and macrophages, at the basal epithelium. pDC = plasma dendritic cells, NK cell = Natural Killer cell.

1.6 Prevalence of cervical ectopy and other genital tract lesions in adolescents and adult women

Anatomical and physiological maturation of the genital tracts of adolescents (particularly in girls) may partly explain the age-associated risk of HIV infection (Porter *et al.,* 2016). The female genital tract of an adolescent is quite different from that of an adult, and the transition from puberty and adolescence to adulthood is gradual. During puberty, the hypothalamus–pituitary– ovary (HPO) axis, controls the maturation of the female genital tract and function and during this phase, there are significantly more fluctuations in estrogen levels than in adulthood (Porter *et al.,* 2016). In addition, cervical ectopy is one of the most commonly found

gynecological conditions. The prevalence of cervical ectropion decreases with age 35 and above (Garg, 2020). Cervical ectopy, also referred to as “cervical erosion” or “cervical ectropion” is a lesion that is considered a benign condition that occurs not only during puberty but also under the stimulation of reproductive hormones during late gestation, prolonged use of hormonal contraceptives and pregnancy (Jacobson *et al*., 2000a). In cervical ectopy, the columnar epithelium of the endocervical canal extends onto the endocervix. It appears as an inflamed and erythemic layer of glandular cells. In puberty, ectopy is observed because of the newly secreted ovarian hormones that cause an increase in intracervical edema (Jacobson *et al*., 2000a; Mitchell *et al*., 2017). Cervical ectopy has been described as one of the potential driving factors leading to susceptibility to STIs including HIV (Bautista *et al.,* 2016; Bradshaw *et al*., 2014; Dimian *et al.,* 1992; Kleppa *et al*., 2015; Low *et al.,* 2011; Moench *et al.,* 2001). The relationship between cervical ectopy and STIs, changes in the vaginal microbiome, inflammation, and VIP use will be described in more detail in Section 1.7.

The ectopic tissue is thin and vascularized and therefore, very fragile and sensitive to trauma and infection (Venkatesh & Cu-Uvin, 2013). Given the immature state of the female genital tract in adolescents, it is not surprising that several signs of injury and trauma have been reported in this group, these include, redness (erythema), bruising (ecchymosis), scrapes (abrasions), tears (lacerations) and other lesions of the reproductive mucosa (Porter *et al.,* 2016). However, clinical definitions of anogenital injury have not been well defined and it is not always conclusively associated with sexual assault (Porter *et al.,* 2016).

There is a knowledge gap when it comes to anogenital injuries, particularly in adolescents. Whether the injuries result from exposure to semen, sexually transmitted organisms, or other factors is not clear and this requires further investigation. In a previous study, anogenital trauma was documented in 73% of adolescent females after consensual sexual intercourse versus 85% of victims of sexual assault (Jones *et al.,* 2003). Although a previous study has reported higher cases of mucosal injuries in rape survivors who were adolescents compared to adults (Baker and Sommers, 2008), age is not the only determining factor. Adolescents and reproductively mature women may both experience genital injury in their genital tracts whether this is the result of sexual assault or consensual sex and the degree and frequency of injuries can be influenced by hormonal status (Porter *et al*., 2016). In literature there are noticeable inconsistencies in how authors interpret and label the various genital injuries, making it difficult to accurately gauge the prevalence of genital injuries in adolescents and adult women. A recent retrospective descriptive chart review of the sexual assault forensic records covering a period of 4 years in Canada, reported that most injuries were redness (41%) followed by

bruises, abrasions, and tears which accounted for 4%, 15%, and 14% of the injuries, respectively (McNair and Boisvert, 2021).

Some other immunological differences have been reported between adolescent and adult genital tracts. Wound healing in the genital tract is also associated with age, with young adults' wounds healing more rapidly than older adults' wounds (Engeland *et al*., 2009; Porter *et al*., 2016). Another previous study showed that women with immature epithelium demonstrated significantly higher levels of IL-1α, IL-1β, IL-6, IL-8, macrophage inflammatory protein-1 alpha (MIP-1α), regulated upon activation, normal T cell expressed and secreted chemokine (RANTES), TNFα, IL-10, IL-12 and interferon (IFN)-γ, compared to women with mature epithelium (Hwang *et al.,* 2014).

1.7 Cervical ectopy and lesions: relationship with HIV, STIs, BV and inflammation

1.7.1 Association between cervical ectopy and HIV

The female genital tract is the main site of HIV infection for women following penovaginal sex in heterosexuals and contributes to more HIV incident cases (Hladik and Doncel, 2010). Cervical ectopy has been hypothesized to facilitate HIV acquisition, although the mechanism by which cervical ectopy increases the risk of HIV acquisition is not entirely understood. Studies suggest that the single layer of the exposed columnar epithelial cells leads to compromised mucosal barrier integrity that allows for easy access to STIs, including HIV (Critchlow *et al*., 1995). Furthermore, the exposed columnar epithelial layer has been shown to have significantly higher frequencies of HIV target cells compared to the squamous epithelial layer (Hwang *et al.,* 2011; Venkatesh and Cu-Uvin, 2013; Kleppa *et al.,* 2015).

Different studies have shown that the cervix is a vital infection site for acquiring STIs, particularly HIV. A review by Moench *et al*.(2001); hypothesized the cervix as an essential primary site most susceptible to HIV infections (Moench *et al.,* 2001). This was suggested to be attributed to its fragility, HIV receptor sites, and the number of cervical lesions that expose the cervix to STIs. An abundance of CD4+ T-cells was identified in the ectocervix and endocervix in young and postmenopausal women (Trifonova *et al*., 2014). Similarly, another study showed that simian immunodeficiency virus (SIV) could be detected in the cervical cells on day three post-infection (Zhang *et al*, 1999) In contrast, infection in the vaginal mucosa could only be detected on day 12. These studies suggested that the columnar epithelium of the upper genital tract might be a preferential site for HIV infection. Although it has been established that HIV can infect both the lower and upper genital tracts (i.e., the labia, vaginal,

ectocervical, endocervical mucosa, uterus, and ovaries), the relative contribution of each site to the development of the initial infection remains controversial (Hladik and McElrath, 2008). Moreover, how cervical ectopy impacts this relative contribution remains unknown.

In both the univariate and multivariate analysis of the cross-sectional cohort study, cervical ectopy was identified as a possible risk factor that stimulates HIV infection in seropositive women (Moss *et al*., 1991; Myer *et al.,* 2006). However, this study did not evaluate the effect of cervical ectopy on the differences in seroconversion in these women (Moss *et al*., 1991). A nested case-control study suggested that HIV seroconversion is directly linked to the size of cervical ectopy and other cervical lesions that rendered women susceptible to HIV infection. In this study, it was concluded that cervical ectopy covering >20% of the cervix was associated with HIV seroconversion. The resulting analysis was observed to be 2-fold higher in women younger than 35 years (Myer *et al.,* 2006). These studies confirm that cervical ectopy should be a focal point in sexual transmission and acquisition of HIV in young women.

On the other hand, other studies have found independent links between cervical ectopy and HIV (Moench *et al.,* 2001; Mosciki *et al.,*2001). A study that examined the association between HIV and ectopy in HIV-positive and HIV-negative adolescents (12-20 years), provided evidence that adolescents with 10% or more cervical ectopy were independently linked to HIV infection and cervical ectopy did not influence the acquisition of HIV (Mosciki *et al.,*2001). Although the negative evidence observed demonstrates that cervical ectopy is not associated with the transmission of STIs, it does not disprove that the cervix is a site that is vulnerable to HIV acquisition in women (Moench *et al.,* 2001).

One of the main limitations of the studies analyzed was assessing and measuring cervical ectopy after the patient had already acquired HIV. In addition, evaluating ectopy in women with a high prevalence of other STIs, including genital ulcers, may independently impact the mucosal barrier, resulting in unreliable data. Ideally, studies need to evaluate and verify the presence of cervical ectopy at the time of infection and possibly, study patients with low to no prevalence of any STIs to reduce overestimation of the frequency of cervical ectopy. Furthermore, other confounders associated with both ectopy and HIV should include age, parity, and gynecological pathology.

1.7.2 Cervical ectopy and other lesions as gateway to Human papillomavirus (HPV) infection in women

Human papillomavirus (HPV) is a common viral STI of the reproductive tract that is prevalent in approximately 44% to 85% of sexually active adolescents and young women in South Africa

(Forman *et al.,* 2012; Kury *et al*., 2022; Mbulawa *et al*., 2021; Zayats *et al.,* 2022). Despite the availability of effective HPV vaccines, this STI remains a major healthcare burden due to its etiological impact to high-grade lesions that increase the risk of cervical cancer (Bonab *et al.,* 2021). HPV has a unique mode of action, where HPV viral cells bind to the basal epithelial cells of the stratified squamous epithelial (same site as cervical ectopy). Since these epithelial cells are thin and fragile even during HPV latency, any form of lesion or abrasion provides easy entry of HPV to the genital tract.

(i) Human Papillomavirus and cervical ectopy

The prevalence of cervical ectopy is considered a potential risk for several STIs (Clemetson *et al.,* 1993; Moench *et al.,* 2001; Moscicki *et al.,* 1999, 2001; Myer *et al.,* 2006; Venkatesh & Cu-Uvin, 2013). Several studies show cervical ectopy to predispose women to a higher risk of HPV. High-risk (HR)-HPV genotypes were 2.2 times more frequent in cervical ectopy than in the normal endocervix (Monroy *et al.,* 2010). A cross-sectional study that identified factors that induced inflammation in cervical smears of asymptomatic women found a significantly higher frequency of HPV in biopsies from women with cervical ectopy and cervical intraepithelial neoplasia (CIN) (Toon *et al.,* 1986). In a study examining the association of cervical ectopy and CIN in 100 women who attended a gynaecological clinic for treatment of cervical ectopy, 19% of the women were diagnosed with CIN and 5% of this population had higher grade lesions (Sarkar & Steele, 1996). The study provided some evidence that CIN prevalence is higher in women with cervical ectopy. The association was further confirmed by another study where it was shown that a high degree of metaplastic activity was likely to develop into a cervical squamous intraepithelial lesion for every 10% of the area of an immature cervix (larger area of ectopy) (Moscicki *et al.,* 1999). In an unstable vaginal environment, HPV infection is likely to delay the recovery of the cervical epithelium in women with cervical ectopy (López-Filloy *et al.,* 2022). Arguably, any prolonged exposure to the regions of metaplasia in the ectocervix exposes the cervix to susceptibility to carcinogenic lesions and recurrent infection by HPV (Zayats *et al.,* 2022). Additionally, 60% to 80% of case studies of CIN were associated with persistent risk of HPV infection (Insinga *et al.,*2004). However, fewer studies have not observed an association between HPV and cervical ectopy. A study done in healthy adolescent and young women could not find an association between an extent of cervical ectopy and HPV acquisition, and they speculated that other biological factors such as immune function or other characteristics of the cervical epithelium may lead to HPV risk (Hwang *et al.,* 2012). Fernandes *et al.* (2019), could also not observe an association between HR-HPV and ectopic size.

(ii) Human Papillomavirus and leukoplakia

According to the WHO, leukoplakia is described as “a white patch of questionable risk that is visible on the cervical epithelium even before the application of acetic acid” which often appears on the transformation zone (Kramer *et al*., 1978). Three decades of literature support the possible notion that these white patches could be induced by HPV infection (Feller & Lemmer, 2012; Gravitt & Winer, 2017; Kramer *et al.*, 1978; Saghravanian *et al.,* 2015; Shang *et al*., 2020; Sundberg *et al.,* 2021). These studies have explored the relationship between HPV and oral leukoplakia. Although HPV is a common STI, it is also transmitted through non-sexual contact such as skin to skin, skin to mucosa (induced by low risk types - HPV-6, 11, 13, and 32), and even through mucosa-mucosa (high risk types - HPV-16, 18, 31, 33, and 35). Thus, there is a need to evaluate HPV infections in the cervix and oral cavity in relation to developing leukoplakia.

A study, identified the number of oral and vaginal sexual exposure and partners as risk factors for the high prevalence of oral and anogenital condylomata (Walboomers *et al*., 1999). Human Papillomavirus infection found in the oral cavity has been linked to a similar prevalence of infection in the cervix, and this arises from oral sex or open mouth kissing which have been previously described as risk factors for HPV infection. Cervical infection of HPV occurs where infected cells differentiate from basal/basilar cells to keratinocytes (peripheral cells). HPV infection progresses as it accesses the infected basilar epithelial stem cells through micro abrasions or trauma, or in the epithelia of the squamo-columnar junction of the mouth or cervix, depending on the genotype.

A review by (Peltecu *et al*., 2009) identified oral leukoplakia as the common cause of HPV infection and interestingly, low risk HPV subtypes especially- 6 and 11 have been associated more commonly with benign leukoplakia, including *Condyloma Acuminatum.* This type of leukoplakia presents itself as an anogenital wart with a cauliflower-like surface. Conversely, high risk HPV is moderately associated with leukoplakia, this was shown in a study conducted in Sweden, Brazil, and Romania that analysed women with leukoplakia. The study showed that 3% of the Brazilian cohorts tested positive for HR-HPV-16, 31, 33 and LR-HPV-11. However, women with leukoplakia cases that transformed to cancer forming leukoplakia, were HR-HPV-16 negative (Sundberg *et al*., 2021) which was previously associated with oral leukoplakia and proliferative verrucous leukoplakia (Palefsky *et al*., 1995).

Most review analysis showed that there might be slight correlation between HPV and the prevalence of leukoplakia, however, there is limited evidence. It is still questionable whether or how HPV infection affects leukoplakia growth and imbalance, and whether HPV can initiate

leukoplakia occurrence or whether the lesion, leukoplakia, stimulates HPV infection and its persistence.

1.7.3 The association between cervical ectopy, cervical abnormalities, and sexually transmitted infections, including *Chlamydia trachomatis*, *Trichomoniasis vaginalis*, and *Neisseria gonorrhea*

It is estimated that annually there are 376 million new cases of curable STIs among young people between the ages of 15-49 years, these include *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (NG), syphilis, and *Trichomoniasis vaginalis* (TV) (Rowley *et al*., 2019). The prevalence of these STIs varies by region and gender. Syphilis and TV are a prominent and common cause of cervicitis. However, CT and NG, have been reported as the most common STIs associated with cervical ectopy in young women. Therefore, for the purpose of this review focus will be given to the association between cervical ectopy and the two STIs, CT and NG.

i) Chlamydia and cervical ectopy

Chlamydia trachomatis is the main cause of chlamydia which can progress and manifest to cause broad human infections such as reactive arthritis, prostate gland infection, and epididymitis. This STI has a global infection rate of approximately 131 million people annually (Rowley *et al*., 2019). In South Africa, the rate of transmission and infection of CT was 9.9 – 21% in young women between the ages of 15 – 24 years in 2017(Kularatne *et al.*, 2018). More recently in a study of AGYW from South Africa and Zimbabwe, CT incidence was reported to be 27.8 per 100 person-years (95% CI 23.1, 33.2) (Delany-Moretlwe *et al.,* 2023). About 15 – 40% of these infections when left untreated tend to ascend to the upper genital tract where they represent reservoirs for ongoing transmission of the organism. Currently, no data shows how rapid the spread of the infection is from the lower genital tract (Carey *et al.,* 2009). Infection with CT in asymptomatic women leads to serious sequelae including pelvic inflammatory disease (PID), infertility, and ectopic pregnancy (Darville & Hiltke, 2010; Filipp et *al*., 2005; Geisler *et al.,* 2007).

A previous study on the pelvic examination of CT in asymptomatic young women suggested cervical ectopy to increase the risk of the infection by exposing the columnar epithelium to the potential infectious inoculum (Geisler *et al.,* 2007; Harrison *et al.,* 1985). *Chlamydia trachomatis* was shown to preferentially infect columnar epithelium cells, and in the presence of ectopy, these cells are more susceptible to infection. The edges of the site of ectopy contain active metaplastic cells that also appeared to have increased susceptibility to infection; probably because microbes may easily invade the single-layered columnar epithelium than

the multi-layered epithelium in the stratified squamous epithelium (Geisler *et al.,* 2007). While the columnar epithelium of the cervix transforms into squamous epithelium i.e., metaplasia, this process does not occur until puberty. Hence, adolescents are more likely to have immature epithelium or large areas of ectopy that could facilitate the acquisition of STIs (Hwang *et al.,* 2014).

Association between cervical ectopy and CT has been reported by 19 of the 24 identified published studies (Figure 1.3). Several cross-sectional studies using univariate analysis reported an association (Arya *et al*., 1981; Blythe *et al.,* 1988; Harrison *et al.,* 1985; Kleppa *et al*., 2015; Lee *et al*., 2006; Paavonen *et al.,* 1989; Rahm *et al.,* 1991). In one of the studies, women between the ages of 17 – 45 years (with a mean age of 23.3 years) participated in the study which determined the association between ectopy and CT. This study found that 13 of the 162 women infected with CT had cervical ectopy. It was concluded that cervical ectopy is the principal determinant of CT and the infection is associated with any degree of ectopy (Harrison *et al.,* 1985). However, the small sample size of women who were infected with CT limited the depth of the analysis and the strength of the conclusion drawn.

The association between cervical ectopy and CT was further confirmed by another cross-sectional study that used a larger population of 700 sexually active young women in SA. The univariate analysis demonstrated that 35% of women who had a 40% coverage of cervical ectopy, also tested positive for CT (Kleppa *et al.,* 2015). It was, therefore, concluded that an increase in the degree of ectopy, increased the risk of infection, suggesting a stronger positive correlation of cervical ectopy and CT (Kleppa *et al.,* 2015). A prospective cohort study investigating the prevalence of CT infections in adolescent girls in one year confirmed a 20% infection rate in sexually active adolescents who had ectopy as opposed to an 11% rate in those without ectopy, thereby showing a strong tendency towards the association of ectopy with CT (Rahm *et al*., 1991). The study showed that a spread of ectopy creates reservoirs for the infective agent suggesting ectopy to be of importance in the prevention and treatment of CT.

In a multivariate analysis of women separated into two groups (using oral contraceptives and not using oral contraceptives), CT seemed to be more related to the women's lifestyle than to clinical signs (Rahm *et al*., 1991). They observed that previous history of CT infection, multiple sexual partners, and smoking were also predisposing factors for contracting a CT infection. However, the results were not significant. The reason given for statistical insignificance was that the two groups were analyzed at different apparent sampling intervals.

Other studies that looked at confounding factors related to CT and cervical ectopy such as number of sexual partners, smoking, contraception (i.e., oral contraceptives), and age at sexual debut found this association to disappear in a multivariate analysis (Jacobson *et al.,* 2000a; Moscicki *et al.,* 2001). A study by Jacobson *et al.* (2000a), determined if CT was more prevalent in adolescents with greater cervical ectopy and those who were using hormonal contraceptives. They did not observe elevated CT infections in adolescents who had cervical ectopy. Instead, they observed a higher risk of CT infections in adolescents who were using depot medroxyprogesterone acetate compared to those who were using estrogen and progestin oral contraceptives. The study was cross-sectional which limited the ability to study the differences in infection rates between the two groups and imprecise estimation methods were used to measure cervical ectopy. A study by Moscicki *et al.* (2001), found oral contraceptives to be associated with increased ectopy but not CT. None of the cervical or vaginal infections namely CT and gonorrhea, were found to be associated with cervical ectopy since the rate of infection of CT was still high in women with less ectopy (14% of women with less than 10% ectopy had CT). The interrelationship between hormonal or oral contraception, CT, and cervical ectopy is important to understand in young women. Given that young women with ectopy have a high prevalence of CT, there is a need to reduce this infection by possibly introducing routine screening for cervical ectopy and CT and follow-up treatment with their partners.

ii) *Neisseria gonorrhea* and cervical ectopy

Neisseria gonorrhea (NG) is a common STI that is a public health issue among women in the sub-Saharan Africa (Buve *et al.,* 2001). The most recent study reported NG incidence to be 11.4 per 100 persons years (95% CI 8.5, 15.0) in AGYW (Delany-Moretlwe *et al.,* 2023), with prevalence of between 7.1% in women aged 15 to 49 years in 2020/2021 (Kassa *et al.,* 2020). The Gram-negative bacterium, NG that results in gonorrhea is asymptomatic in most women, and when left untreated it results in infertility, PID, and ectopic pregnancy. The sites of infection of NG have been reported to be the endocervix, urethra, and the anal canal (Brunham *et al*., 1982).

Few studies have looked at the association between cervical ectopy and NG (Barnes et al., 1990; Brunham *et al.,* 1982; Kleppa *et al*., 2015; Morrison *et al.,* 2004; Moscicki *et al*., 2001). Of the five studies analyzed, only one study found a correlation between cervical ectopy and NG (Kleppa *et al.,* 2015). A cross sectional-study reported that NG was common in 24.4% of women (with the average age of 19.1 years) who have cervical ectopy compared to 17% of the women without ectopy (Figure 1.3). Due to the relatively low rate of infection in the study, the data analyzed for CT and NG was combined and their association was examined in a

univariate analysis. It was observed that both CT and NG infections were associated with ectopy suggested that CT shedding might increase with concurrent NG infection. However, when adjusting for other STIs such as HIV, the significance of this association was lost when using the multivariate regression model (Kleppa *et al*., 2015).

Other previous studies found correlating data to that of Kleppa *et al*. (2015) where they observed that concurrent infection with CT and NG was highly prevalent in women with cervical ectopy (Barnes *et al*. (1990), Brunham *et al*. (1982)). When examining the relationship between epidemiological variables such as age, race, and oral contraceptive use, and the incidence of CT infection, CT occurred more frequently in young women with NG compared to those without NG. These findings were upheld in a multivariate analysis. It was suggested that infection by NG may reactivate dormant CT (Barnes *et al*. (1990)). In a cohort study, 89 women with NG were treated with ampicillin and of those women, 56 had NG alone and 23 had coexisting CT and NG infections Brunham *et al*. (1982). It was observed that women with a large area of exposed columnar epithelium (ectopy) who had NG were more likely to be infected with CT. This further suggests that concurrent infection of CT and NG is prevalent in women with cervical ectopy.

Two prospective studies contradicted the results of Kleppa *et al*. (2015), where the association between NG and ectopy was not statistically significant (Morrison *et al.,* 2004; Moscicki *et al*., 2001). Due to the nature of the study (cross-sectional) focus was shifted to other STIs and no further analysis was carried out to fully elucidate the association between NG and ectopy. In the current literature very few studies have showed a significant association between NG and cervical ectopy. However, the concurrent infection of NG and cervical ectopy is paramount to examine and understand in order to bridge treatment innovations in asymptomatic women.

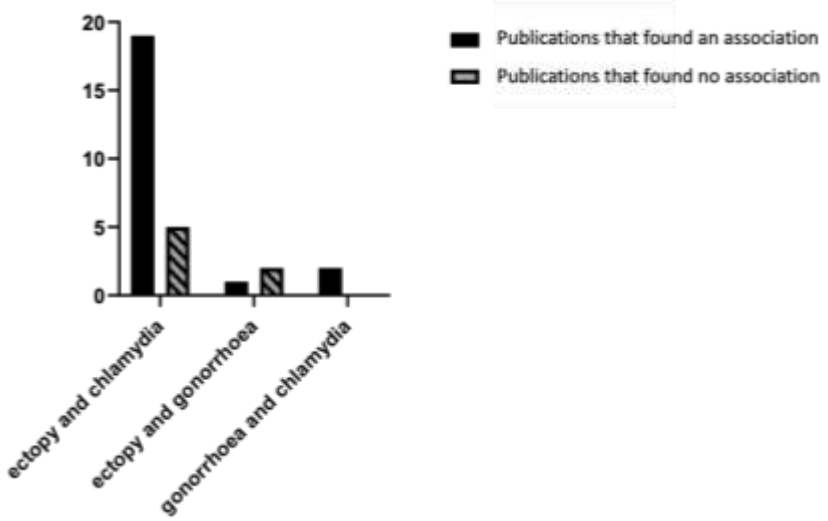


Figure 1.3 Bar graph depicting number of publications over the years on the association of cervical ectopy and *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoea* (NG). PubMed was utilized to obtain data on the correlation of cervical ectopy and the acquisition of sexually transmitted infections (STIs) between time periods 1980-2021. Two STIs, CT and NG were filtered for the analysis and a total of 25 and 5 publications were found, respectively. Chlamydia showed the highest number of publications, with 19 journal articles verifying the association of the infection to cervical ectopy. The least reported association was seen on ectopy and gonorrhoea with only one publication showing positive relation. A concurrent infection of CT and NG was associated with cervical ectopy in two publications.

1.7.4 Genital inflammation and cervical ectopy

Inflammation is an immune and physiological response to tissue damage or the presence of an infection. While inflammatory responses are somewhat beneficial in probing immunity, genital inflammation has been identified as the major predisposing factor for HIV acquisition in sub-Saharan African women (Feller & Lemmer, 2012; Kramer *et al*., 1978; Kury *et al*., n.d.; Saghravanian *et al.*, 2015; Shang *et al.*, 2020; Sundberg *et al.*, 2021). The exact etiology of genital inflammation is unknown, however, it has been hypothesized that its causes are multifactorial. These include factors such as STIs, the use of hormonal contraception, allergic reactions in response to either a contraceptive spermicide, latex in condoms, feminine hygiene products use (douches, feminine soaps or deodorants), semen and bacterial overgrowth as a result of dysbiosis, or BV (Blair *et al*., 2020; Coudray & Madhivanan, 2020; Paavonen & Brunham, 2018; Redelinghuys *et al.*, 2020;).

Higher levels of cervicovaginal inflammatory and regulatory cytokines (MIP-1α, MIP-1β and inducible protein-10 [IP-10]), and chemokines in healthy young women were also associated with immature cervical epithelium (Hwang *et al*., 2011). These findings suggest differential immune responses when columnar and metaplastic epithelia are abundant, a change linked to cervical ectopy. Cervical ectopy, which is found in the glandular endocervix comprises

delicate and fragile cells. Therefore, the exposure of the glandular tissue to the vaginal cavity is expected to create an area of low resistance to all types of trauma and infectious agents that attack the cervix (Kleppa *et al.,* 2015). A cross-sectional study defining factors associated with dense inflammation on the standard clinical readings of the Pap smear observed an association between cervical ectopy and dense genital inflammation in the Pap smear in both high risk and low risk sexually active women. This association was maintained in the logistic regression model, which showed the combination of cervical ectopy with NG, CT, and herpes strongly correlated with dense genital inflammation (Eckert *et al*., 1995).

Singh *et al.* validated these findings by identifying the etiology and biological factors of inflammatory smears in 257 women attending maternal and child health clinics (Singh *et al.*, 1999). Significantly higher proportions of women with inflammatory Pap smears had cervical ectopy (28.5% had ectopy compared to 10.2% without ectopy) (Singh *et al.,* 1999). In another study where conventional cytology was used, it was observed that cervical ectopy was higher in women with inflammatory smears, which was noted in 43% of the women compared with 27% present in women with non-inflammatory smears (Dimian *et al*., 1992). Furthermore, atypical or inflamed Pap smears were frequent in the ectopy group and were not dependent on the extension of ectopy (Junior *et al*., 2014). The link between inflammation and ectopy can be attributed to the easy penetration of inflammatory cells through the basement membrane of columnar epithelium in patients with larger areas of ectopy.

Although the symptoms of cervical ectopy such as erythema, increased vaginal discharge, pain during intercourse and postcoital bleeding overlap with the symptoms of genital tract inflammation, some studies have not found an association between cervical ectopy and genital inflammation (Dimian *et al*., 1992; Junior *et al.*, 2014; Moscick*i et al.*, 2001; Singh *et al*., 1999). The link between cervical ectopy, the presence of STIs and genital inflammation is a limitation to the interpretation of the findings. Therefore, in future prospective studies, treatment should be performed to not only identify whether the etiology of inflammation is directed by infection by microorganisms associated with cervical ectopy but also to provide proper management of patients with inflammation. Another limitation is that most of the studies are cross-sectional and lack follow-up visits to assess the progress of the abnormality of inflammation.

1.7.5 Bacterial vaginosis and cervical ectopy

Bacterial vaginosis (BV) is a common vaginal infection that affects the vaginal microbiota in approximately 30% of the global female population (Bautista *et al*., 2016). It is associated with non-optimal vaginal microbiota and the presence of diverse facultative and strict anaerobes,

including *Gardnerella vaginalis*, *Prevotella, Atopobium vaginae*, and *Sneathia*. The optimal vaginal microbiota is characterized by *Lactobacillus* species that maintain a healthy vaginal environment by acidifying the vagina to pH 3.8 – 4.5 and producing antimicrobial molecules that protect the vagina against pathogens. Such antimicrobial molecules include hydrogen peroxide (H2O2), an antimicrobial product that protects the vagina from harmful pathogens, lactic acid, which maintains normal pH, and bacteriocins, a home-grown antibiotic that prevents overgrowth of harmful pathogens in the vagina (Bautista *et al.*, 2016).

Numerous risk factors are associated with BV, such as contraceptive use, menstrual cycle, antibiotic use, age, race, and genital inflammation (Passmore *et al.,* 2016). In addition, women are more likely to report BV if they have had the following: i) a number of lifetime sexual partners; ii) are not married; iii) have engaged in their first sexual debut at a very young age; iv) have engaged in commercial sex work; and v) practiced regular douching (Coudray & Madhivanan, 2020). However, data on individual risk factors of BV and its recurrence in many women are lacking. Other factors include a high frequency of unprotected heterosexual or vaginal sexual intercourse. Exposure to semen as evidenced by the presence of prostate-specific antigen (PSA) in menstrual cup supernatants of women was highly associated with BV prevalence (Mngomezulu *et al.* 2021; Jespers *et al.* 2014). This finding emphasizes previous literature finding that in high-risk populations, male semen and vaginal penetration influence the development of BV (Bautista *et al.,* 2016).

Many STIs, including HIV, herpes simplex virus type-2 (HSV-2), CT, and NG, are associated with BV (Moscicki *et al.,* 2001). The hypothesis that BV is sexually transmitted is still controversial, and with no known etiologic agent, it is rather difficult to classify BV as an STI and current literature is lacking in studies that have concluded to such a finding (Rosca *et al.,* 2021).

A few studies have found an association between BV and cervical ectopy. Junior and colleagues conducted a cross-sectional study in 193 women with ectopy to examine an association between cervical ectopy and other epithelial infections, they found a stronger association between cervical ectopy and BV, concluding that the presence of BV in these women increases the size of ectopy in the cervix and causes a shift in the normal vaginal microflora (Junior *et al*., 2014). This finding suggests that a causal relationship but without follow-up, it is unclear whether BV causes ectopy or ectopy facilitates the development of BV. Furthermore, in a review by Soares *et al*. (2019), the association between cervical ectopy and STIs, a positive association between cervical ectopy and BV was observed (Soares *et al*.,

2019). However, they pointed out that BV was a possible confounding factor that was not taken into consideration and thus diminished the strength of the analysis.

Contrary to the findings above, a prospective cohort study by Mosciki and colleagues evaluating ectopy data from 291 women and its association with BV, reported no statistically significant association (Moscicki *et al*., 2001). The findings from different studies suggest that the association between BV and cervical ectopy is inconclusive and should be viewed with caution. In addition, confounding factors that influence the association between BV and ectopy need to be explored.

1.8 Conclusion

Young women continue to be at risk for HIV in Sub-Saharan Africa. Contributors of risk are complex and multi-factorial (socio-cultural and biological) and not fully elucidated. VIP use may be linked to biological changes that contribute to HIV risk, however, data showing a direct causal pathway link with HIV acquisition (and other STIs) are lacking. Changes in cervical anatomy are linked to several adverse health outcomes, including HIV risk. Although vaginal products are commonly used by South African women, it is not known whether they are linked to changes in the cervical anatomy and immune-biological factors in the female genital tract.

1.9 Study Rationale

Cervical ectopy is widely known as a benign condition that is prevalent during adolescence and in women of reproductive age and as yet, there is no treatment for the adverse effect as a result of cervical ectopy. Literature over the years has suggested that cervical ectopy is associated with increased susceptibility to STIs including HIV. The size of cervical ectopy on the cervix has been shown to be a risk factor for HIV acquisition in young women and a contributing factor to seroconversion in women. Moreover, the ectopic tissue compromises the mucosal barrier integrity that, in the event of trauma contributes to an increased risk to HIV infection. The benign condition has been associated with the concurrent infection of CT and NG, especially in asymptomatic women. In addition, while there is evidence that cervical ectopy influences inflammation, the contribution of VIP use to the risk of cervical ectopy and other cervical lesions is yet to be investigated. Research is needed to investigate the temporal relationship between cervical lesions, genital inflammation, and STIs and to also generate information about the clinical significance of the lesions in adolescent girls with unique socio-behavioral and biological characteristics closer to sexual debut. This study hypothesized that

sexual immaturity in adolescent females in conjunction with VIP use would exacerbate injury to the cervicovaginal mucosa and increase vaginal inflammatory responses, which may in turn influence the risk of HIV acquisition and other infections.

1.10 Study aims and objectives.

The aim of the current study was to investigate the impact of products that potentially impact the cervix and vagina following intravaginal insertion or oral ingestion on cervical inflammatory cytokine responses and colposcopic changes in the female genital tract that may be important in determining HIV risk in AGYW from the rural KZN area.

The objectives of the study were as follows:

- To evaluate and compare the prevalence of VIP use among adolescents and adult women.
- To compare colposcopic changes (such as cervical ectopy, leukoplakia, injury, genital warts, and vaginal discharge) among adolescents and adults that use vaginal enhancing products and non-users.
- To compare cytokine levels associated with genital inflammation among adolescents and adult women that use vaginal enhancing products and non-users.
- To investigate the impact of sex and/or product use on genital tract inflammatory cytokine responses in adolescents and adults by comparing cytokine responses at baseline (at least two weeks of no sex and no product use) versus exposure visits (within 48 hours of sex only or sex and product use).
- To investigate the association between the use of sexual enhancing products and prevalence of STIs (CT, NG, TV and HPV) and other infections (BV and vulvovaginal candidiasis).

Chapter 2.

Methods and Materials

2.1 Study design

The participants were recruited in 2018-2022 from a Mucosal Injury from Sexual Trauma (MIST) cohort study at the Centre for the AIDS Programme of Research in South Africa in rural Vulindlela, KZN, South Africa. Of the women (n=353) attending the research clinic during this time period, all with complete demographic data (n=252), sexual and VIP data (n=251), and STI testing (n=239) were included to be analysed at baseline (Table 2.1). Participants (n=101, Table 2.1) were lost to follow-up or had incomplete data set. All included young women (n=63) and adolescent girls (n=188) were sexually active, HIV-uninfected, and using VIPs. The study participants were interviewed by a trained nurse. The questionnaire was written in English and translated to a local language (IsiZulu) to participants and included questions on behavioural factors (including sexual behaviour, VIP use), menstruation, and contraception use. Furthermore, participants had to undergo cervical examinations that were conducted in the clinic setting by a professional and trained nurse, and a gynecologist. Colposcopy images were stored for cervical lesions; a total of 176 adolescents and 50 adults had full colposcopy records appropriate for analysis.

Table 2.1 List of questionnaires# and data completeness record

Questionnaire	Overall population response (N=)	Adolescents (N=)	Adults (N=)
Demographics N=252			
Age/Age group	252	188	63
Education level	252	188	63
Employment status	252	188	63
Sexual and socio-behavioural data N=251			
Age at first sex debut	251	188	63
Number of lifetime sexual partners	251	188	63
Age differences with sex partner	250	187	63
Condom use at last vaginal sex	242	181	61
Type of relationship	239	180	59
Sexually transmitted infections and genital infections N=239			
Bacterial vaginosis	237	175	62
Neisseria gonnorhoea	238	177	61
Chlamydia trachomatis	239	177	62
Trichomonas vaginilis	217	155	62
Candida vulvovaginilis	233	171	62
HPV	234	173	61
Intravaginal product usage N = 251			
Sex enhancers/vaginal hygiene/ side effects	251	188	63
Menstruation and contraception N=252			
Menstruation	252	188	64
contraception	251	187	64
Colposcopy examinations N=226			
Pelvic/ cervix diagnosis (gynaecology)	226	176	50

2.2 General Reagents

Phosphate Buffered Saline (PBS) and other common reagents were obtained from Sigma-Aldrich (St. Louis, Missouri, USA) and ThermoFisher Scientific (Waltham, MA, USA).

2.3 Study setting

This study was an ancillary of the MIST longitudinal cohort study. Mucosal Injury from Sexual Trauma (MIST) study investigated the socio-behavioural, the female reproductive tract anatomical and biological characteristics following both consensual vaginal sex and intravaginal product use among female adolescents aged 14 – 19 years old around sexual debut in response to early sexual exposure and mucosal trauma compared to older women aged 25 – 35 years old. The study took place between July 2017 and June 2021 and was approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee (Ref: BF504/17). All participants provided written informed consent before enrolment, and for adolescents between the ages of 14-17 years, both assent and parental informed consent were sought. Volunteers interested in participating in the study came to the CAPRISA Vulindlela Research Site situated in rural KZN for their study visits. Participants came for study visits at baseline (no use of vaginal products and no sexual intercourse for at least 2 weeks), within 48 hours following product use and sexual intercourse, and at 7 to 10 days post-exposure. Participants meeting the following criteria were enrolled in the study: HIV seronegative, no cervical disease history, were not pregnant, not taken antibiotics four weeks before the enrollment visit, able to provide full consent or assent, were sexually active, and had no intentions of relocating in the next 12 months. Ethics approval for this ancillary study was granted by the Biomedical Research Ethics Committee (BREC) sub-committee at the University of KwaZulu-Natal (BREC/00003306/ 2021).

2.4 Demographic and socio-behavioural characteristics

Data were obtained at the clinic using interview-administered case report forms. The following information was collected: demographic data, sexual behaviour (including detailed information about the use of vaginal products), and partner characteristics (Table 2.1).

2.5 Colposcopy examination and assessment of mucosal trauma

Econo™ Sterile Graves vaginal speculum (Sklar, Pennsylvania, USA) was inserted. Eva System colposcopy (MobileODT, Tel Aviv, Israel) was used to magnify the cervix, and identify, and objectively grade the presence of cervical lesions, including cervical ectopy. According to the area of the cervical ectopy on the cervix, the size was classified as 12 o'clock – (100%) of the cervical region was affected, 6 O'clock – not more than 1/2 (50%) of the cervical area was affected, 3 o'clock – not more than 1/4 (25%) of the cervical region was affected (Prendiville & Sankaranarayanan, 2017). In addition, other cervical abnormalities were assessed including leukoplakia (grossly white findings), trauma (ecchymosis, laceration, and cervical abrasion), inflammation (erythema, peeling, and edema), and discharge. A colposcopy was performed at the clinic by a trained study nurse. Colposcopy images were labelled with the participant ID and sent to the study gynaecologist, where the colposcopy findings were confirmed.

2.6 Specimens' collection and STI testing

Urine specimens were collected to test for pregnancy before any genital specimen collection. The remainder of the urine sample was used to test CT and NG infections by pre-optimized Cepheid Xpert CT/NG assay performed on the GeneXpert® Instrument Systems (Cepheid; Sao Paulo-SP, USA) at Vulindlela clinical site laboratory. A lateral vaginal wall/posterior fornix swab was also collected, this was used to test for TV antigen testing, combined with wet mount analysis. In addition, the collected lateral wall swab was used to test BV by Nugent score. Microscopic potassium hydroxide preparation was used to detect fungal hyphae or yeast in collected lateral wall swabs. TV, BV, and yeast presence were tested at Neuberg Global Laboratories, Amanzimtoti, South Africa. The vaginal pH was measured at the CAPRISA clinic by clinical staff; this was done by placing the collected vaginal swab on a pH test paper (Sigma-Aldrich, Maulide Cane *et al*., 2021) and pH was recorded by using the pH reference chart.

Other genital specimens collected at baseline included: (1) a Softcup (Evofem Biosciences, San Diego, California, USA) for cervicovaginal secretions (CVS) collection; and (2) endocervical swabs for HPV testing. The Softcup was inserted after colposcopy examination and followed by the collection of all other genital specimens. The Softcup was inserted for one hour (minimum 30 minutes – maximum 1.5 hours). Upon completion, the Softcup was immediately placed into a sterile 50-ml Falcon tube and transported on dry ice to the laboratory within 6 hours of collection. At the laboratory, the tube was centrifuged (1500 x g;10 min; at 4°C), after which the Softcup was carefully removed and discarded. The tube containing the secretion was weighed, and the volume of the secreted fluid was calculated (weight of tube with secretion − weight of empty tube). The secretion was diluted 5-fold with sterile PBS and stored at -80°C until use for the measurement of cytokines (Section 2.7). Endocervical swabs were also collected; these were immediately stored at 2–8°C until transport to the laboratory, where they were placed in 1 ml tubes and kept at -80°C until use for HPV testing (Section 2.8).

2.7 Measurements of cytokines

The CVS samples were assayed by Luminex using Human XL cytokine 20-plex premixed kit (R&D Systems, Inc., Minneapolis, USA, catalog number FCSTM18), to measure the concentrations of 20 cytokines, including inflammatory cytokines (IL-1β, TNF-α, Monocyte chemoattractant Protein-1 [MCP-1], IFN- β, IL-6); chemokines (IL-8,, IP-10, MIP-1α, MIP-3 α, MIP-1β, RANTES, TNF-related apoptosis-inducing ligand [TRAIL]); adaptive cytokines (IL-17A, IFN-γ); growth factors (G-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF], VEGF); and regulatory cytokines (IL-1 receptor agonist [IL-1RA], IL-10), according to the manufacturer's protocol. Samples with values below the lower detection limit were assigned half the value of the lowest extrapolated concentration and values above the detection limit were assigned the median of the highest standard concentration.

2.7.1 Sample processing and plate preparation

Stored CVS samples were thawed and filtered using 0.2-µm Costar® Spin-X® centrifuge tube filters (Corning Incorporated, - Life Sciences, Salt Lake City, USA). A total of 332 CVS samples were run using 13 different plates. To control for inter- and intraplate variation, 3 CVS samples were run in duplicate on each plate (intraplate controls), and a panel of 5 samples was run on all plates (interplate controls) to enable comparison across plates (Figure 2.1).

Figure 2.1 Representative Plate layout used for all cytokine Luminex assay. A total of 65 thawed CVS (cervicovaginal secretions samples) of individual participants per clinic visit were used. Seven duplicated sets of the standard were prepared and plated, the BL (blank) used was PBS (Phosphate buffered saline), and the high and low control (HC and LC, respectively) were provided by the manufacturer (R&D systems, Minneapolis, USA, MN 55413).

2.7.2 Beads preparation and validation

A 1x concentrated human XL panel microparticle cocktail consisting of 0.6 ml preservative was prepared fresh at the time of the assay (30 min before use, RT). The microparticle cocktail vial was centrifuged (1000 x g, 30 s, RT) before cap removal and then gently vortexed to resuspend the microparticles, avoiding inverting the vial. The microparticles (0.5 ml) were then diluted in 5.0 ml PBS in an amber plastic mixing bottle. Microparticle RANTES and IL-17E were prepared by adding 55 mL into the previously diluted microparticle cocktail. Fifty microliters (50 ml) of the microparticle solution were then plated in 96 wells (R&D Systems, Inc., McKinley Place, Minneapolis, USA) and incubated in a shaker (800 x g, 2 hr, RT).

2.7.3. Preparation of detection antibody

A lyophilized and concentrated biotinylated antibody cocktail was freshly prepared at the time of the assay. It was reconstituted in 650 ml of PBS 20 min prior to dilutions. The reconstituted Biotin-antibody (500 mL) was then diluted in 5 ml PBS, gently mixed, and then plated to 96 wells and incubated on a shaker (800 x g, 1 hr, RT).

2.7.4. Preparation of fluorochrome conjugate

Streptavidin-Peroxidase (Streptavidin-PE) was prepared in a polypropylene amber bottle (R&D systems, Minneapolis, USA, MN 55413). The Streptavidin-PE vial was centrifuged (1000 x g; 30 s, RT) prior to removing the cap and then gently vortexed as per the manufacturing manual. The concentrated Streptavidin-PE (220 ml) was diluted in 5.35 ml PBS and plated into 96 wells. The plate was incubated on a shaker (800 x g; 1 hr, RT).

2.7.5. Preparation of standards

The human panel standard cocktail consisted of a buffered protein base with a preservative (2 lyospheres; white and blue). The standard lyospheres were diluted in 950 ml PBS and were left at RT for a minimum of 20 min before diluting. Using seven labelled polypropylene tubes (ThermoFisher Scientific, Waltham, MA, USA), 300 ml of the reconstituted standard was pipetted into the standard 1 tube. Standard 2 to 7 tubes were filled with 200 ml PBS, respectively. Using a standard 1 tube, a 3-fold dilution series was produced in the remaining tubes, with thorough mixing between transfers.

2.7.6. Cytokine data acquisition

Data were collected using Bio-Rad's Bio-Plex® suspension array Reader (Bio-Rad Laboratories, California, USA). A Bio-Plex® manager software (version 4, Bio-Rad Laboratories, California, USA) was used to analyze the data, and a 5-parameter logistic regression formula was used to calculate cytokine concentrations from standard curves. The lower limit of detection for cytokines ranged between 5.09 – 1542 pg/ml and the upper limit of detection for cytokines ranged between 128 – 48 201 pg/ml.

2.8 Screening for HPV by reverse dot blot hybridization

2.8.1 Sample preparation

Cervical swabs were prepared in 2 ml Eppendorf tubes containing 400 μl of 1X PBS buffer (repeated for every sample). The tip of the swab was dipped into the 1X PBS solution and squeezed against the wall of the tube to detach the cells. The solution was centrifuged (2000 x g, 1 min) and the supernatant was discarded. Depending on the size of the pellet, the cell pellet was suspended in 200 μl of 1X PBS buffer, and 30 μl of this cell suspension was used as the DNA template for the PCR reaction. The remaining volume was stored at 4 ^{o}C for 1 week.

2.8.2 Multiplex PCR reaction

The multiplex PCR mix (HPV Direct flow CHIP kit (HS12), Master Diagnóstica, Ciencias de la Salud Granada) was thawed on ice for 1 hour, then mixed by inversion. The PCR mix was then added to the whole volume of Hot start DNA polymerase and centrifuged for 20 seconds.

The PCR denaturation and amplification reaction was conducted according to the manufacturing manual under a set PCR program (Table 2.2).

Table 2.2. PCR programming

Number of cycles	Temperature (ºC)	Time
1 cycle	25	10 min
1 cycle	94	3 min
15 cycles	94	30 s
	42	30 s
	72	30 s
35 cycles	94	30 s
	60	30 s
	72	30 s
1 cycle	72	5 min
	8	infinity

2.8.3 Flow-through reverse hybridization

Samples were hybridized immediately after the PCR reaction was completed. The PCR products were denatured (95 ^{o}C, 10 min) in a thermocycler and then cooled on ice for 2 min. All hybridization reagents were supplied in a “ready to use” format. Reagent A (hybridization solution) was pre-warmed at 41 ^{o}C. The HPV Chip was placed in the chamber (hybriSpot12 device, Vitro group). A mixture of Reagent A (270 μl) and 30 μl of the denatured product were added to a pre-heated chamber (41 ^{o}C) and incubated for 8 min. The solution was removed by vacuum and 3 washes with 300 μl Reagent A were performed. The chamber temperature was then set to 29 ^{o}C. Then 300 μl of Reagent B (Blocking solution) was dispensed into each chip and incubated for 5 min. When the chamber reached 29 ^{o}C 300 μl of Reagent C (Streptavidin-Alkaline Phosphatase) was dispensed into each chip. The temperature was increased to 36 ^{o}C. The chamber was washed with Reagent D (Washing buffer I). When the chamber reached 36 ^{o}C, Reagent E (Developing solution) was dispensed into each chip and incubated for 8 min. The reagent was removed by vacuum. Two washes were performed using Reagent F (Washing buffer II, 300 μl). The reagent was removed and image capture was performed according to the manufacturer's manual.

2.9 Statistical analysis

All patient raw data at baseline was recorded and analyzed in MS Excel (Microsoft, USA). The data was separated into age groups to identify risk factors associated with age. A chi-squared test was used to calculate associations of categorical data, a Wilcoxon-sum rank test was used to calculate numerical data. The association of STIs and genital infections, cervical abnormalities, and product use with age group was identified using univariant analysis, however, due to no significant differences identified, a multivariant analysis and p-value adjustment was not performed. STI and product use analysis at baseline were evaluated using Fisher’s exact test and χ^2; while differences in cytokine levels were determined using the Mann Whitney U test for unpaired samples and the Wilcoxon signed rank test for matched pair samples. All cytokines were log10 transformed. Cytokines detectable at less than 40% frequency were analyzed as binary variables (IFN-β, Rantes, IL-25), and the remainder of the cytokines were analyzed as continuous variables. Intraplate correlations were determined by nonparametric Spearman rank correlation test. P-values <0.05 were considered significant. GraphPad Prism versions 8.4.3 and 9.4.1 were used for statistical analyses and data visualisation.

Chapter 3

Results

3.1 Demographic and socio-behavioural characteristics of study participants

To investigate the impact of the vaginal product use (intravaginally inserted or ingested) on cervical lesions and inflammatory responses linked to HIV risk in adolescents, analysis was completed in 252 sexually active female participants (188 adolescent females, 64 adult women, Table 3.1). The median age of adolescent females was 17.0 years [Inter-quartile range (IQR) 16-18], and the median age of adult women was 28.0 years (IQR 27-31). More than 90% of the study cohort (adolescents and adults) completed secondary education ($p=0.034$), and surprisingly both adolescents and adults were equally unemployed with more than 80% unemployed in the cohort ($p=0.464$). Furthermore, 90% of the study cohort lived separately from their partners and described their relationships as stable. A small portion of adolescents (28.2%) reported ever being pregnant compared to adults (89%). More than half of adolescents (73.4%) reported having regular menstrual cycles compared to 50% of adults. Adults were more likely to use contraception compared to adolescents (71.9% of adults compared to 57.2% of adolescents ($p<0.001$)), with more than 40% of adults reporting using the injectable contraceptive Depo-Provera ($p=0.002$). At baseline, adolescent females had lower levels of parity, a higher rate of amenorrhea, and higher preference for Nuristerate as a contraceptive method as compared to adult women, although the use of contraception in the group was relatively lower when compared to adult women.

Table 3.1. Demographic and reproductive health characteristics of participants in the study

Characteristics from questionnaire [n (%)]			
Characteristics	Adolescents	Adults	p-value
N	188	64	
Age (years)*	17 [16.0 – 18.0]	28 [27 – 31]	**<0.001**
$BMI ($kg/m^2$)*	23.4 [21.1 – 27.6]	32.1 [27.5 – 37.5]	**<0.001**
Highest educational level			
Primary school	3/188 (1.6%)	1/64 (1.6%)	0.667
Secondary school	184/188 (97.9%)	59/64 (93.6%)	**0.034**
Tertiary school	1/188 (0.5%)	4/64 (6.4%)	**0.005**
Employment status			
Employed	27/188 (14.4%)	5/64 (7.8%)	0.174
Unemployed	160/188 (85.1%)	52/64 (81.3%)	0.464
School drop out	1/188 (0.5%)	3/64 (4.7%)	**0.022**
Relationship status			0.539
Living with partner ±	2/187 (1.1%)	1/48 (2.1%)	
Living separately with a partner±	184/187 (98.4%)	47/48 (89.7%)	
Ever pregnant#			**<0.0001**
Yes	53/188 (28.2%)	56/63 (88.9%)	
No	135/188 (71.8%)	7/63 (11.1%)	
Regular menstrual cycle			**0.046**
Yes	154/188 (81.9%)	36/64 (56.3%)	
No	34/188 (18.1%)	16/64 (25.0%)	
Hormone Contraception use~			**0.023**
Yes	107/187 (57.2%)	46/64 (71.9%)	
No	81/187 (43.3%)	17/64 (26.6%)	
Nuristerate	30/187 (16.0%)	6/64 (9.4%)	0.189
Depot	51/187 (27.3%)	31/64 (48.4%)	**0.002**

$Body mass index
*Median and the interquartile range; n: sample size
n=1 missing adult participants' responses
± n=1 missing adolescent participant response; n=16 adult participant response

To investigate sexual health risk factors in the study cohort, social and sexual behavioural characteristics were collected at baseline (Table 3.2). Adolescent females reported a slightly earlier age of sexual debut than adult women, the average age reported to be 16 and 17 years in adolescents and adults, respectively ($p<0.001$). Furthermore, adolescent females were more likely to report condom use during vaginal sex (85.6%; $p<0.001$) compared to adult women (21.3%, $p=<0.0001$). However, adult women reported a higher number of lifetime sexual partners than adolescent females (adults had a median of 3 compared to adolescents who had a median of 2, $p<0.001$). Sexual partners of adults were reported to be significantly older, with a median age difference of approximately 5 years compared to 3 years in adolescents ($p<0.001$). Adolescents reported higher preference of VIP use (41.5%, $p=0.0244$), whereas adults were more likely to report ingested product use (44.4%, $p=0.0065$) for the purposes of sexual enhancement. Transactional sex within the cohort was common in both age groups, with more than 90% of women reporting to have received some form of a “gift” in exchange for sexual intercourse.

Table 3.2. Baseline sexual and socio-behavioural and sexual risk characteristics of participants in the study

Socio-behavioural and sexual risk data at baseline [n/N (%)]			
Characteristics	Adolescents	Adults	p-value
Sexual risk behaviors [n (%)]			
Condom use at the last vaginal sex act	161/188 (85.6%)	13/64 (21.3%)	**<0.0001**
Age at sex debut*	16 [15.0 – 17.0]	17 [15.5 - 18.0]	**<0.0001**
Sexual intercourse during menstruation			**0.047**
Yes	8/188 (4.3%)	7/63 (11.1%)	
No	180/188 (95.7%)	56/63 (88.9%)	
Use vagina enhancing products			
User Ingested/ Oral	47/188 (25.0%)	28/63 (44.4%)	**0.0065**
User Vaginally inserted	78/188 (41.5%)	16/63 (25.4%)	**0.0244**
Number of lifetime sexual partners*	2 [1.0 – 2.0]	3 [2.0 – 5.0]	**<0.0001**
Age difference with sexual partner*	3 [2.0 – 5.0]	5 [3.0 – 9.0]	**<0.0001**
Ever had transactional sex			0.411
Yes	186/188 (99.6%)	63/63 (100.0%)	
No	2/188 (0.4%)	0/63 (0.0%)	

*Median and 25th and 75th percentile; n: sample size
P-values were calculated using [a] chi-square test (categorical),
[b] Wilcoxon-sum rank test (numerical); p-value <0.05 is considered significant

3.2 Prevalence of sexually transmitted infections and other infections

To evaluate the prevalence of STIs in the study cohort, the presence of CT, NG, TV, HPV, BV and vulvovaginal candidiasis was compared between adolescents and adult women. It was observed that adolescent females had higher prevalence of most infections compared to adult women. The prevalence of CT was 33.9% in adolescent girls compared to a prevalence of 9.7% in adult women (p<0.001, Table 3.3). The prevalence of NG was 7.9% in adolescent girls compared to 3.3% in adult women. Overall, the prevalence of HPV was higher in

adolescents (85.6%) compared to adults (77.1%) although this was not significant. When HPV was stratified according to high risk (HR) and low risk (LR), adolescents had significantly higher prevalence of HR-HPV (p=0.048) and LR-HPV (p=0.0003) compared to adults. In addition, the prevalence of vulvovaginal candidiasis was slightly higher in adolescent females than in adult women, with a prevalence of 8.2% in adolescents versus 6.5% in adult women. TV was less common in both age groups, with more than 90% of the sample population testing negative for the infection. The prevalence of BV was similar in adult women (40.3%) and adolescents (38.9%), and there was no difference in vaginal pH between adults and adolescents (median 4.7).

Table 3.3. Prevalence of sexually transmitted infections, other genital infections, and vaginal pH amongst adolescent girls and adult women

	Overall prevalence [n/N (%)]	Adolescents [n/N (%)]	Adults [n/N (%)]	p-value
CT	66/239 (27.6%)	60/177 (33.9%)	6/62 (9.7%)	[a]**0.008**
TV	15/217 (6.9%)	11/155 (7.1%)	4/62 (6.5%)	0.866
NG	16/238 (6.7%)	14/177 (7.9%)	2/61 (3.3%)	0.213
Bacterial vaginosis§				0.411
Negative (0-3)	88/237 (36.7%)	62/175 (35.4%)	26/62 (41.9%)	
Intermediate (4-6)	56/237 (23.6%)	45/175 (25.7%)	11/62 (17.7%)	0.691
Positive (7-10)	93/237 (39.2%)	68/175 (38.9%)	25/62 (40.3%)	0.185
*__Vaginal pH__		4.7 [4.4 – 5.3]	4.7 [4.7 – 5.3]	<0.999
Vulvovaginal candidiasis	18/233 (7.7%)	14/171 (8.2%)	4/62 (6.5%)	0.661
Human papillomavirus	195/234 (83.3%)	148/173 (85.6%)	47/61 (77.1%)	[b]0.161
#HR-HPV	151/234 (64.5%)	118/173 (68.2%)	33/61 (54.1%)	[a]**0.048**
HPV-16	36/234 (15.4%)	30/173 (17.3%)	6/61 (9.8%)	0.162
HPV-18	15/234 (6.4%)	13/173 (7.5%)	2/61 (3.3%)	0.246
^LR-HPV	155/234 (66.2%)	126/173 (72.8%)	29/61 (47.5%)	[a]**0.0003**

*Median and 1st and 3rd quartile; n: sample size
p-value <0.05 is considered significant and p-values were calculated using the [a]Chi-squared test or [b]Fisher's exact test
§ Nugent scoring used for bacterial vaginosis
^Low-risk HPV genotypes studied- HPV type 6,11,40,42,43,47,54,61,67,69,70,71,72,84,44/45,44/55, and 62/81.
#High-risk HPV types included- HPV types 16,18,31,33,35,39,45,51,52,53,56,58,59,66,68,73, and 82

3.3 Intravaginal practices amongst adolescents and adult women

Intravaginal practices amongst adolescents and adult women were investigated and compared. Twenty-seven (27) different vaginal products (either applied externally or internally in the vagina) and nineteen (19) orally ingested products were reported by users (Figure 3.1 and Appendix I). The products reported come in the forms of powder, crystals, gel, liquid, and paper. These products are used for several reasons, including but not limited to 1) vaginal heating (snuff, Ibhodwe labafazi, traditional herbs, holy ash/incense, and herbal vaseline), 2) vaginal tightening (alum, snuff, ibhodwe labafazi, blue stone, traditional herbs, and newspaper), 3) sexual stimulation (snuff, ibhodwe labafazi, and holy ash/incense), or 4) hygiene/ detoxification (ibhodwe labafazi, traditional herbs, and feminine washes) (Appendix I).

Adults were more likely to ingest products than adolescents (25.0% adolescents versus 44.4% adults, p=0.0065), while adolescents were more likely to use products intravaginally (41.5% adolescents versus 25.4% adults, p=0.0244). However, adolescents reported a wider range of products than adults. While adolescents reported using 27 different vaginal products and 19 oral products adults only reported using 9 vaginal products and 12 oral products (Figure 3.1). The following vaginal products were reported: alum (11.2%), ibhodwe labafazi (7.2%), snuff (2.8%), black halls (2.4%), indlovukazi Vaseline (0.8%), ugogotshitshi (0.8%), traditional herbs (0.8%), ice cubes (0.4%), holy ash (1.2%), tartaric acid (0.4%), vutha cream (0.8%), herbal mixture (0.8%), newspaper (0.8%), Indian ash (0.4%), blue stone (0.8%), unspecified traditional petroleum jelly (0.4%), vaseline powder (0.8%), dispirin tablets (1.6%), umxovo (0.4%), vinegar (1.2%), iyashisa (0.4%), itshe lomgodi (0.4%), water and vinegar (1.2%), awema (0.4%), and impukane (0.4%). Orally ingested products reported were: cinnamon spice and milk (2.4%), umchamo wemfene (3.6%), stoney ginger beer (10.0%), ibhodwe labafazi with milk (4.0%), holy ash with milk (2.4%), stoney and dispirin (aspirin)/halls (mentholyptus lozenges) (1.6%), imbawula (1.6%), amoeba tea (0.4%), boiled rice water (1.6%), and green pepper (0.8%). The detailed descriptions of the majority of the aforementioned products are shown in Appendix I.

Alum [$KAl(SO_4)_2$)], reported to be the most prominently used product by adolescents [(33.3%) of adolescents vs (6.3%) of adults; p=0.056], constitutes a combination of aluminium sulfate and potassium sulfate and comes in the form of white crystals, powder or solid rock that is soluble in water (Appendix I). The product is used as a douching agent, as well as a steaming agent; for the purpose of vaginal tightening. Other reported products that were preferred by adolescents included ibhodwe labafazi (direct translation: ‘the women’s pot’) (5.0%), and

newspaper (5.1%). Conversely, ibhodwe labafazi, which is a pink-scented Vaseline with unknown contents, was commonly preferred by adults [(56.3%) of adults compared to adolescent females (5.0%), p=0.027]. This product was used for a number of different reasons which include, detoxification, sexual stimulation, vaginal drying, tightening, and heating. Other selected common products were used at a similar frequency amongst adolescent girls and adult women. A common usage of ingested stoney ginger beer was noted within the two groups. The remaining 30% (75/251) in the cohort were not using any products (Figure 3.1).

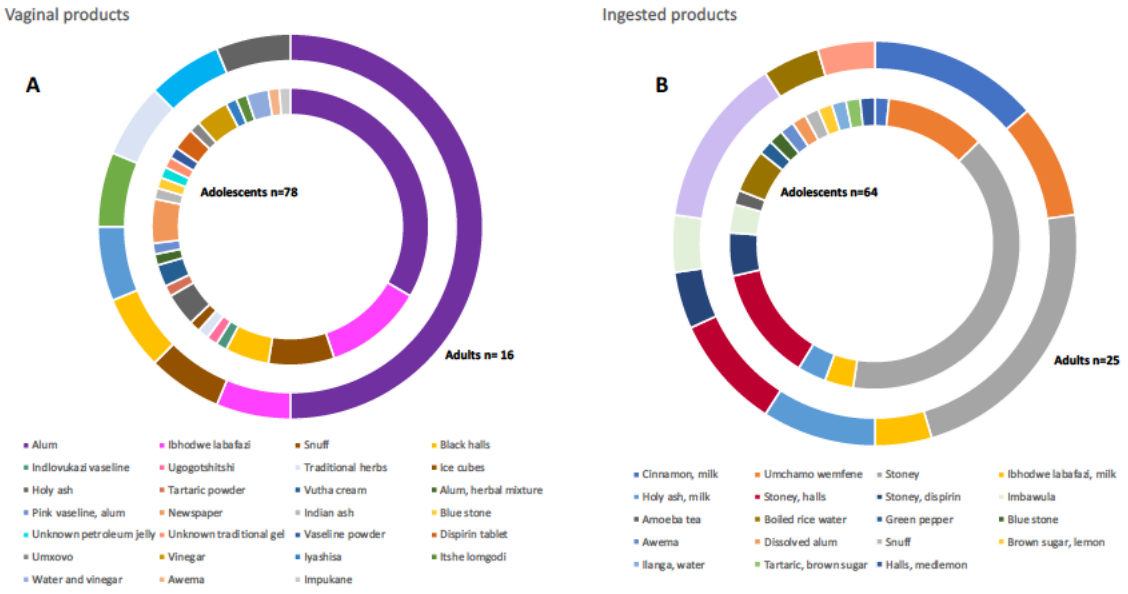


Figure 3.1 Overall vaginal stimulating products used by adolescent females and adult women in rural KwaZulu-Natal. A questionnaire was administered to evaluate product usage within the cohort. A) Vaginal products, adolescent females reported using twenty-seven (27) different vaginally applied or inserted products, and adult women reported using nine (9) of these products. B) Oral products, adolescent females and adult women used nineteen (19) and twelve (12) different ingested or masticated products, respectively. The stacked donut depicts the distribution of different products used by study participants taken orally or intravaginally.

The most preferred vaginal products in adolescents and adults were evaluated further. Adolescents and adults had preferences for similar products; however, adolescents reported to use a combination of these products more than adults. In adolescents' alum was widely used in conjunction with ibhodwe labafazi. Ibhodwe labafazi was most favored by adults over alum or snuff in this group (Figure 3.2).

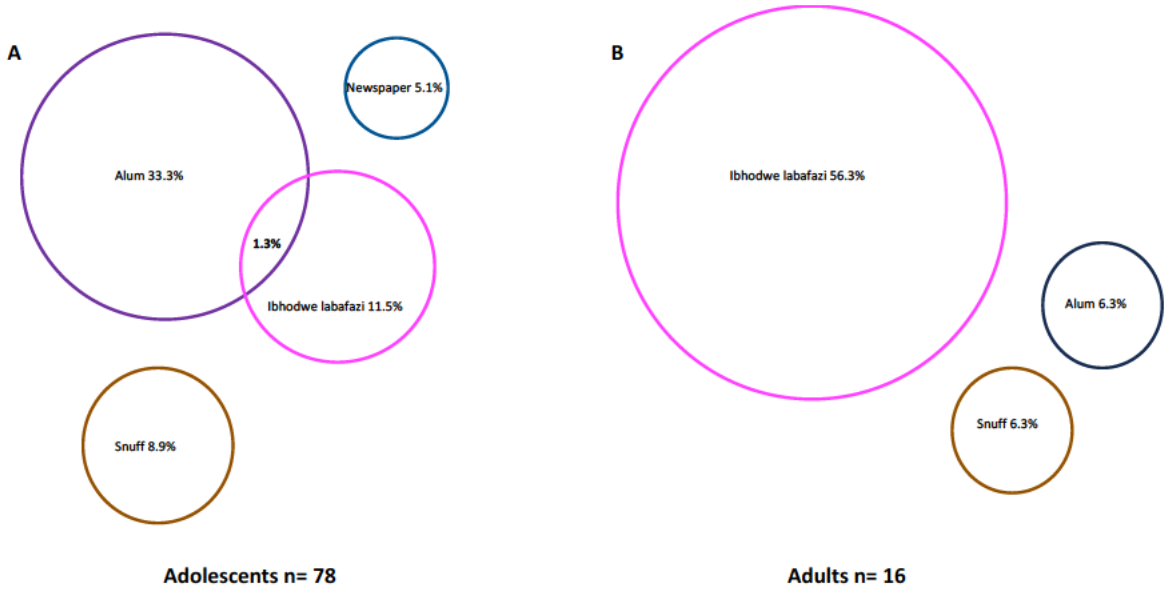


Figure 3.2 Shared product use by adolescent female vs adult women. The area proportional Venn diagram presents the proportions of the most exclusive intravaginal products used by women in KZN- A) Adolescents alum (purple), ibhodwe labafazi (pink), snuff (brown), newspaper (light blue); B) Adults ibhodwe labafazi (pink), alum (purple), snuff (brown).

3.3 Cervical abnormalities overall and by age category

To compare cervical changes and abnormalities among adolescents and adults that use intravaginally inserted products, colposcopy was used to visualize and grade the cervix for the appearance of cervical abnormalities (Figure 3.3). The cervical abnormalities investigated in the study include cervical ectopy (Figure 3.3B) which is the extension of the columnar epithelium out onto the ectocervix; injury (Figure 3.3C and Figure 3.3D)- any form of petechiae, a bleeding or blood clot under the cervix (Figure 3.3C), or any form of redness and swelling- clinically evident inflammation (Figure 3.3D); leukoplakia or vaguely termed 'grossly white findings' (Figure 3.3E) which are flat white plaque with slight ridges/ grooves (corrugation); genital warts - condyloma acuminatum; and any form of vaginal discharge (frothy, white, cream, brownish, clear, greenish, with or without odour).

A total of 226 colposcopy images were reviewed at the baseline visit (Figure 3.3). Adult women (n=50) depicted more cervical abnormalities than adolescent females (n=176) as shown in Table 3.4. The most prevalent cervical abnormality in adolescents was determined to be leukoplakia, with a total of 86/176 (48.9%) adolescents presenting the abnormality. Clinically evident inflammation and trauma (signs of injury) was present in 10/176 (5.7%) adolescents in the form of erythema or edema, cervical ectopy was present in 8/176 (4.5%) adolescents. Adults showed slightly different proportions of cervical abnormalities as compared to

adolescents. There were statistically more significant signs of injury observed in adults compared to adolescents, [14/50 (28%) compared to 10/176 (5.7%), p<0,0001]). There was significantly more leukoplakia in adolescents compared to adults [86/176 (48.9%), and 15/50 (30.0%), respectively, p=0.018]. However, the prevalence of cervical ectopy was similar between adult women and adolescents (Table 3.4).

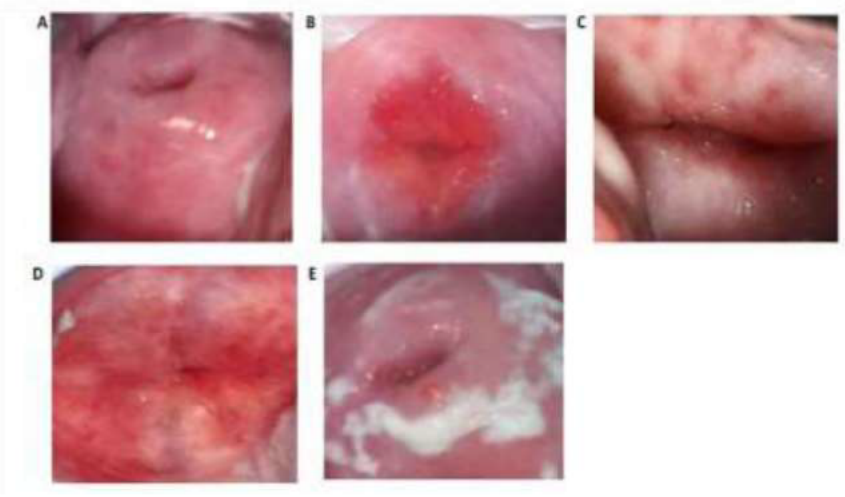


Figure 3.3 Classification of cervical abnormalities included in the study. Colposcopy photographs of the cervix using EVA system. The studied cervical abnormalities were classified as, A) Normal cervix; B) Cervical ectopy; C) and D) which were signs of injury indicated by: C) petechiae and D) erythema, or clinically evident inflammation; E) Grossly white findings – Leukoplakia. Genital warts and vaginal discharge were also analysed (images not shown).

Table 3.4. The prevalence of cervical abnormalities in adolescent females and adult women

	[b]Adolescents N= 176	[b]Adults N= 50	[=]p-value
Cervical ectopy	8/176 (4.5%)	6/50 (12.0%)	0.054
Leukoplakia (Grossly white findings)	86/176 (48.9%)	15/50 (30.0%)	**0.018**
Injury	10/176 (5.7%)	14/50 (28.0%)	**<0.0001**
Genital warts	3/176 (1.7%)	0/50 (0.0%)	0.353
Vaginal discharge	31/176 (17.6%)	7/50 (14.0%)	0.547

[=]p-value <0.05 is considered significant and p-values were calculated using the [a]Chi-squared test
[b] Proportions of participants with visibly identified cervical lesions

3.4 The prevalence of genital inflammation in adolescents compared with adult women

To compare cytokine levels associated with genital inflammation between adolescents and adult women, a total of 134 study participants with CVS samples available at baseline and followed up for exposure visit (V1.1) were included. A total of 22 cytokines were quantified using the Luminex assay, however, for the benefit of this analysis focus was given to 18 pro-

inflammatory, chemotactic, growth factors, adaptive, and regulatory cytokines (IL-1α, IL-6, TNF-α, TRAIL, IL-1β, IL-8, IP-10, MCP-1, MIP-1α, IFN-α, MIP-1β, , MIP-3α; G-CSF, GM-CSF, VEGF; IL-17; IL-10, and IL-1ra, respectively). At baseline visit, cytokine levels were mostly similar between the two age groups, with fewer cytokines showing some significant differences. Adolescent females had a significantly higher median concentrations for pro-inflammatory TNF-α compared to adults (median 2.887 vs 1.827, respectively, p<0.0001) (Figure 3.4), whereas adults had significantly higher median concentrations for chemokine IL-8 compared to adolescents (median 3.848 vs 2.618, respectively, p<0.0001) (Figure 3.4). Moreover, both growth factor G-CSF and regulatory IL-10 were significantly higher in adults compared to adolescents, G-CSF (median 3.173 vs 2.443, respectively, p<0.000) and IL-10 (median 3.193 vs 2.362, respectively, p<0.0001) (Figure 3.4).

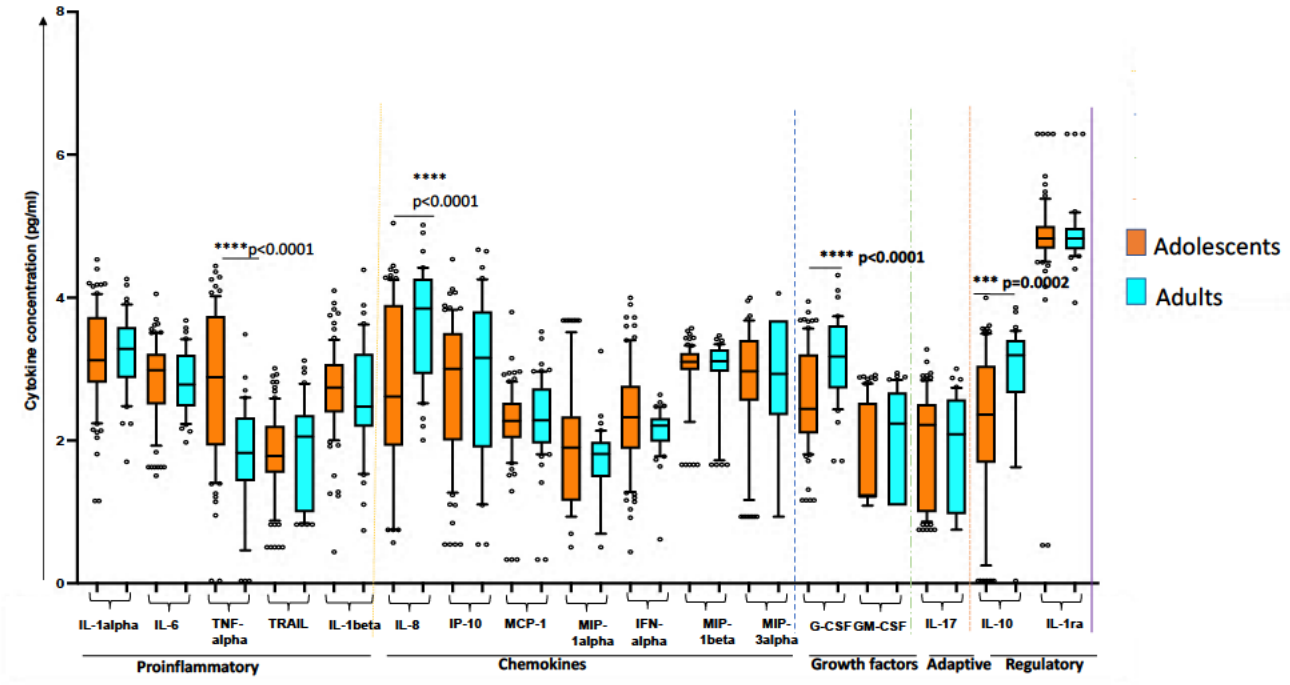


Figure 3.4 Boxplot of the overall distribution of the genital tract cytokine concentrations in adolescent and adult females at baseline. Luminex assay was used to quantify cytokine concentrations at baseline visit in adolescents (n=78) indicated by orange boxes; and in adult women (n=56) indicated by blue boxes. Horizontal lines indicate medians. Statistical differences (P < .05) were calculated using the Mann–Whitney U test. Abbreviations: IL-, Interleukin-; TRAIL-Tumor necrosis factor-related apoptosis inducing ligand, IP-10, interferon-γ inducible protein-10; MCP-1, Monocyte chemoattractant Protein-1; MIP-, Macrophage Inflammatory Protein-, IFN-, Interferon G-CSF-Granulocyte macrophage colony, GM-CSF- Granulocyte macrophage colony stimulating factor.

3.6 Evaluation of colposcopic changes associated with vaginal practices

To determine whether colposcopic changes (cervical ectopy, leukoplakia, injury, genital warts, and vaginal discharge) were linked to vaginal practices, cervicovaginal images collected by colposcope were examined among adolescents and adults product users (vaginal/oral) compared to nonusers. Overall, the colposcopy findings were mostly similar when comparing product users and nonusers in both adolescents and adults although at baseline, cervical ectopy was found to be more prevalent in adults who used oral (p=0.023) and vaginal products (P=0.032) compared to nonusers (Table 3.5).

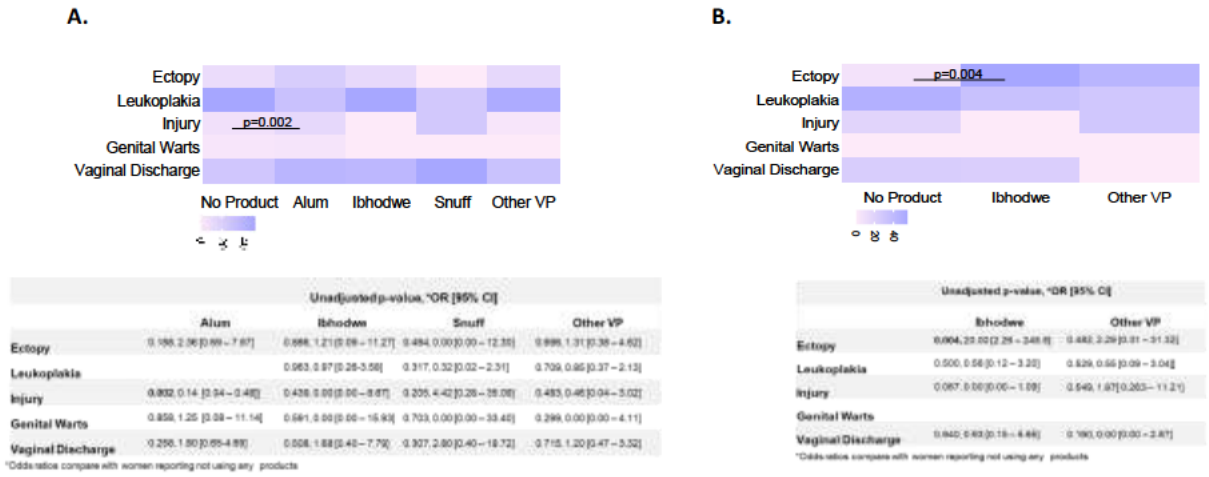
Table 3.5. Comparison of cervico vaginal colposcopic findings amongst vaginal product users and non-users in KZN

Variable	No product(s) [%(n/N)]	Vaginal product user [%(n/N)]	*Unadjusted P-value	Oral product user [%(n/N)]	*Unadjust P-value
Baseline visit Adolescents (N=192)					
Cervical ectopy	10.3% (6/58)	15.4% (10/65)	0.407	12.1% (4/33)	0.795
Leukoplakia	51.7% (30/58)	40.0% 26/65	0.479	42.4% (14/33)	0.211
Injury	8.6% (5/58)	7.7% (5/65)	0.765	18.2% (6/33)	0.179
Genital warts	3.4% (2/58)	1.5% (1/65)	0.456	0.0% (0/33)	0.281
Vaginal discharge	25.9% (15/58)	35.4% (23/65)	0.234	27.3% (9/33)	0.883
Exposure visit Adolescents (N= 180)					
Cervical ectopy	0.0% (0/23)	13.6% (3/22)	0.067	10.0% (2/20)	0.120
Leukoplakia	8.7% (2/23)	22.7% (5/22)	0.194	20.0% (4/20)	0.286
Injury	4.3% (1/23)	18.2% (4/22)	0.140	10.0% (2/20)	0.468
Genital warts	4.3% (1/23)	0.0% (0/22)	0.323	0.0% (0/20)	0.322
Vaginal discharge	17.4% (4/23)	22.7% (5/22)	0.655	25.0% (5/20)	0.541
Baseline visit Adults (N=59)					
Cervical ectopy	5.3% (1/19)	35.3% (6/17)	**0.023**	31.8% (7/22)	**0.032**
Leukoplakia	47.4% (9/19)	23.5% (4/17)	0.137	27.3% (6/22)	0.183
Injury	26.3% (5/19)	23.5% (4/17)	0.847	36.4% (8/22)	0.491
Genital warts	-	-		-	-
Vaginal discharge	21.1% (4/19)	17.6% (3/17)	0.865	4.5% (1/22)	0.107
Exposure visit Adults (N=58)					
Cervical ectopy	-	-	-	-	-
Leukoplakia	11.1% (1/9)	25.0% (2/8)	0.453	13.3% (2/15)	0.873
Injury	33.3% (3/9)	12.5% (1/8)	0.312	20.0% (3/15)	0.465
Genital warts	-	-	-	-	-
Vaginal discharge	44.4% (4/9)	12.5% (1/8)	0.149	46.7% (7/15)	0.916

p-value <0.05 is considered significant and unadjusted p-values were calculated using the *Chi-squared test Cervicovaginal colposcopic findings were grouped into: Leukoplakia; Injury- erythema, peeling, edema, ecchymosis, laceration, and abrasion.

To further examine colposcopic changes associated with the use of vaginal products, a sub-analysis focusing on the most used vaginal products (alum, ibhodwe labafazi, snuff and other

vaginal products) versus nonusers was done (Figure 3.5). During the exposure visits, in most cases, no striking differences were observed in participants who were using vaginal products compared to those who were not using any products in both age groups. However, adolescent females who used alum (the most common product among adolescents) were likely to have injury signs compared to adolescents who did not use any products (p=0.002, Figure 3.5A). In addition, adult women who used ibhodwe labafazi (the most common product among adults) were 20 times more likely to have cervical ectopy compared to adult nonusers (p=0.004, Figure 3.5B).



Unadjusted p-value, *OR [95% CI]

	Alum	Ibhodwe	Snuff	Other VP
Ectopy	0.168, 2.06 [0.69 – 7.67]	0.666, 1.21 [0.09 – 11.27]	0.494, 0.00 [0.00 – 12.35]	0.999, 1.31 [0.38 – 4.62]
Leukoplakia		0.963, 0.97 [0.26-3.58]	0.317, 0.32 [0.02 – 2.31]	0.709, 0.85 [0.37 – 2.13]
Injury	0.002, 0.14 [0.04 – 0.48]	0.439, 0.00 [0.00 – 8.67]	0.205, 4.42 [0.28 – 35.08]	0.483, 0.46 [0.04 – 3.02]
Genital Warts	0.959, 1.25 [0.08 – 11.14]	0.991, 0.00 [0.00 – 15.93]	0.703, 0.00 [0.00 – 33.46]	0.299, 0.00 [0.00 – 4.11]
Vaginal Discharge	0.256, 1.80 [0.65-4.89]	0.508, 1.68 [0.48 – 7.79]	0.307, 2.80 [0.40 – 19.72]	0.715, 1.20 [0.47 – 3.32]

*Odds ratios compare with women reporting not using any products

Unadjusted p-value, *OR [95% CI]

	Ibhodwe	Other VP
Ectopy	0.004, 23.00 [2.29 – 248.8]	0.482, 3.29 [0.01 – 31.32]
Leukoplakia	0.500, 0.56 [0.12 – 3.22]	0.829, 0.66 [0.09 – 3.04]
Injury	0.067, 0.00 [0.00 – 1.09]	0.549, 1.67 [0.263 – 11.21]
Genital Warts		
Vaginal Discharge	0.640, 0.63 [0.15 – 4.66]	0.190, 0.00 [0.00 – 2.87]

*Odds ratios compare with women reporting not using any products

Figure 3.5 Comparison of cervicovaginal colposcopy findings between vaginal product users and nonusers in adolescent and adult women. A. Top panel: Heat map showing frequencies of cervicovaginal colposcopic findings in adolescents who reported not using any products (n=63), vaginally inserting or applying Alum (n=27), Ibhodwe labafazi (indicated as Ibhodwe, n=9), Snuff (n=6) and any other vaginal products (indicated as Other VP, n=36).Bottom panel: Tabulated p values, odds ratios and 95 percent confidence interval (95% CI) calculated by comparing users of vaginal products (Alum, Ibhodwe Labafazi, Snuff and Other Vaginal Products) with nonusers. B. Top panel: Heat map showing frequencies of cervicovaginal colposcopic findings in adults who reported not using any products (n=19), vaginally inserting Ibhodwe labafazi (indicated as Ibhodwe, n=9), and any other vaginal products (indicated as Other VP, n=7). Bottom panel: Tabulated p values, odds ratios and 95 percent confidence interval (95% CI) calculated by comparing users of vaginal products (ibhodwe labafazi, and Other Vaginal Products) with nonusers. Heatmaps were generated in GraphPad Prism version 8.4.3. Percentages of cervicovaginal colposcopic findings were expressed as color scales and depicted according to a color scale, where blue represents high frequencies and pink represents low frequencies. p-value <0.05 were considered significant and unadjusted p-values were calculated using the Chi-squared test.

3.7 Impact of sexual exposure and product use on genital tract inflammatory responses

To determine the potential impact of sexual exposure or product use on inflammatory responses in the female genital tracts of adolescents and adults, an analysis of baseline (at least two weeks of no sex and no product use) versus exposure visits (within 48 hours of sex only or sex and product use) was conducted in 62 adolescent girls and 40 adult women from whom genital cytokine concentrations were measured at both visits. The concentrations of chemokines (IL-8, IP-10, MIP1-α, IFN-α, MIP1-β, MIP3-α), proinflammatory (IL-1α, IL-6, TRAIL, IL-1β, TNF-α), growth factors (G-CSF, GM-CSF, VEG-F), adaptive (IL-17) and regulatory (IL-10, IL-1ra) in genital secretions were compared in matching baseline and exposure visits from adolescents (Figure 3.6) and adult women (Figure 3.7). Sexual exposure and or product use did not dramatically alter cytokine concentrations in either group, with the exception of genital IFN-α concentrations in adolescents showing a 0.8 fold decrease at exposure visit ($p=0.0199$, Figure 3.6).

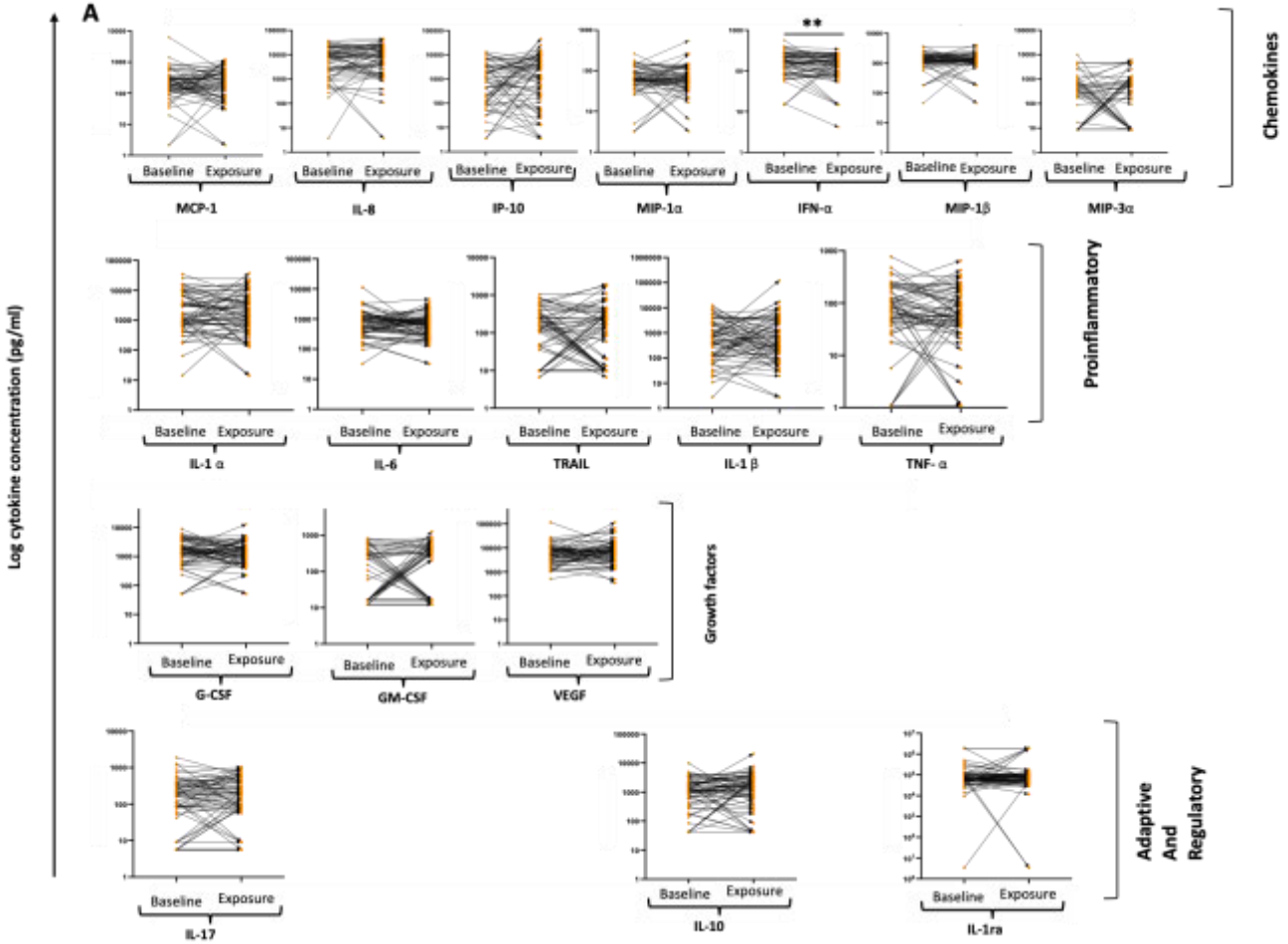


Figure 3.6 Comparison of chemokines, proinflammatory, growth factors, adaptive and regulatory genital tract cytokine concentrations between baseline and exposure visits in adolescents. Cytokine concentrations were tested at both baseline (at least two weeks of no sex and no product use) and exposure (indicated as Exposure, within 48 hours of either sex only in the case of nonusers or sex and product use in the case of product users) visits. Wilcoxon matched pairs signed rank test was used to compare groups and. ** represents p-value $p \leq 0.01$ and was considered significant.

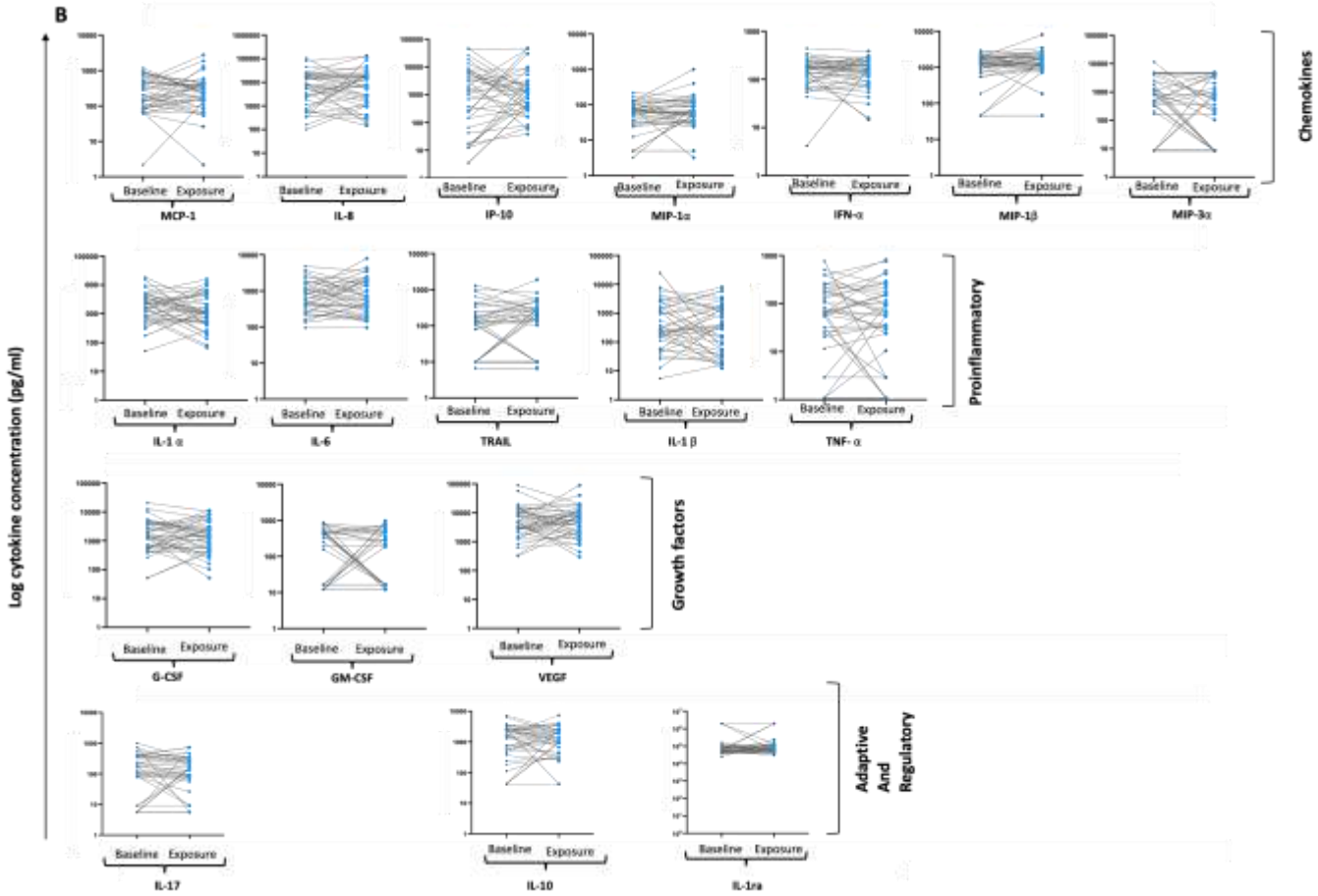


Figure 3.7 Comparison of chemokines, proinflammatory, growth factors, adaptive and regulatory genital tract cytokine concentrations between baseline and exposure visits in adults. Cytokine concentrations were tested at both baseline (at least two weeks of no sex and no product use) and exposure (indicated as Exposure, within 48 hours of either sex only in the case of nonusers or sex and product use in the case of product users) visits. Wilcoxon matched pairs signed rank test was used to compare groups.

A further subanalysis was done to examine the inflammatory cytokine responses associated with the use of selected vaginal and ingested products common in the study cohort (Figure 3.8). In adolescents, the focus was given to the following groups: (i) those who reported not using any products, those who vaginally inserted or applied (ii) alum, (iii) ibhodwe labafazi, (iv) snuff, (v) any other vaginal product, those who drank (vi) stoney, (vii) umchamo wemfeme and (viii) ingested any other product. Among adolescents, the median cytokine concentrations were mostly higher in product users compared to nonusers, specifically MCP-1, IL-8, IP-10, IL-1α, IL-1β, and VEGF, although most did not reach statistical significance. Interestingly, the users of ibhodwe labafazi had significantly elevated cytokines, remarkably so for IL-1α ($p=0.0223$), VEGF ($p=0.0198$) and IL-17 ($p=0.0150$) compared to nonusers. In addition, adolescents who reported drinking stoney had significantly elevated levels of G-CSF ($p=0.0452$) compared to nonusers (Figure 3.8A). In adult women, the focus was given to the following groups: (i) those who reported not using any products, those who vaginally inserted or applied (ii) ibhodwe labafazi, (iii) any other vaginal product, those who drank (vi) stoney, (vii) umchamo wemfeme and (viii) ingested any other product. Contrary to what was observed in adolescents, there were no remarkable changes in genital tract cytokine concentrations in users compared to nonusers. This was the case for all the categories analysed (Figure 3.8B).

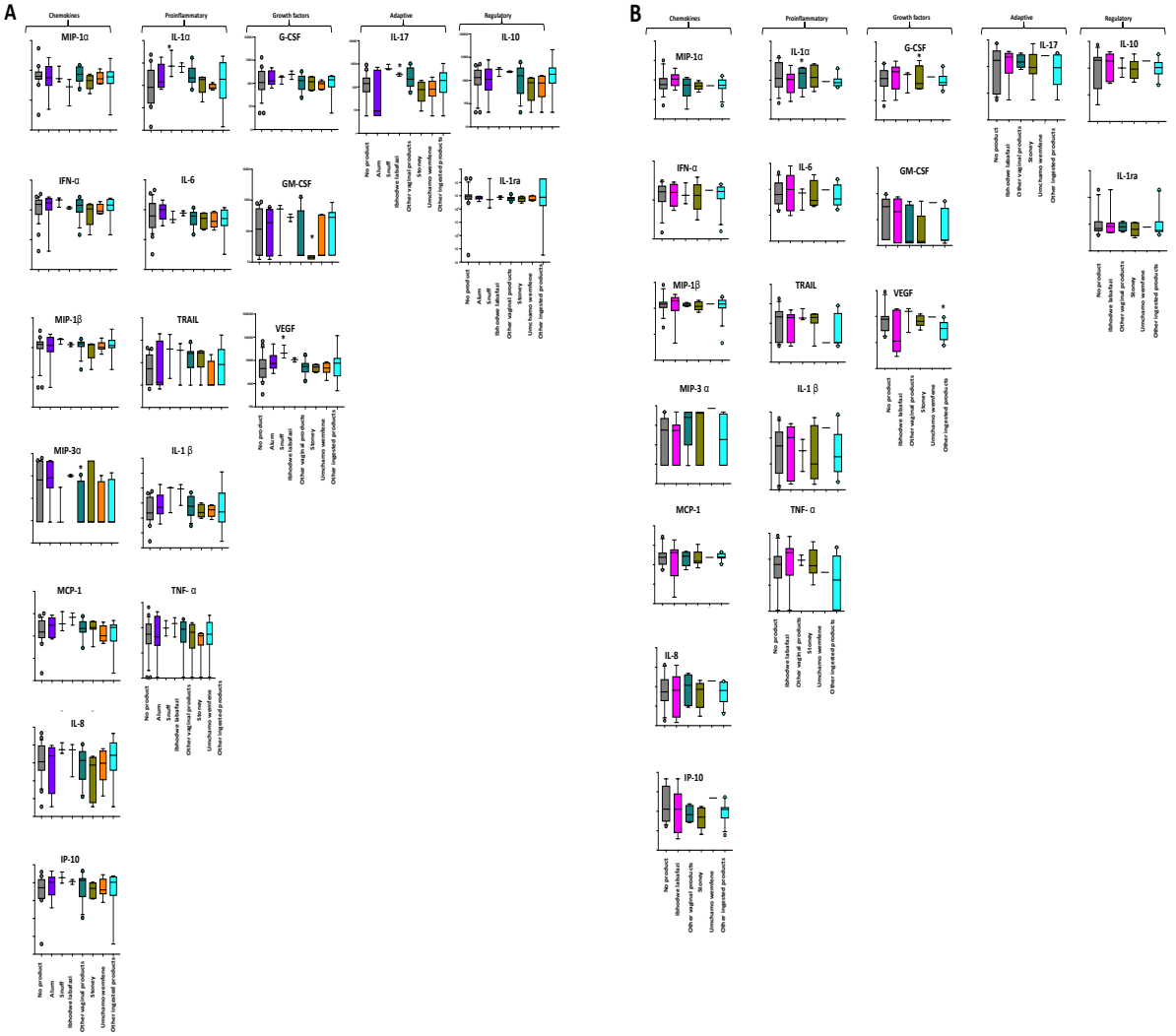


Figure 3.8. Comparison between chemokines, proinflammatory, growth factors, adaptive and regulatory genital tract cytokine concentrations in adolescents(A) and adult women (B) using various sexual enhancing products to those not using any products at exposure visit. A. Genital tract cytokine concentrations in adolescents not using any products (indicated as 'No product', n=24) are shown in grey boxes, these were compared with the cytokine concentrations of adolescents vaginally inserting or applying Alum (n=10), Ibhodwe labafazi (indicated as Ibhodwe, n=3), Snuff (n=2), any other vaginal products (indicated as Other VP, n=11), drinking Stoney ginger beer (indicated as Stoney, n=4), Umchamo Wemfene (indicated as Umchamo, n=05) and any other ingested products (indicated as Other Ingested, n=7). **B**. Genital tract cytokine concentrations in adult women not using any products (indicated as No product, n=13) are shown in grey boxes, these were compared with the cytokine concentrations of adult women vaginally inserting or applying Ibhodwe labafazi (indicated as Ibhodwe, n=7), any other vaginal products (indicated as Other VP, n=4), drinking Stoney ginger beer (indicated as Stoney, n=6), Umchamo Wemfene (indicated as Umchamo, n=1) and any other ingested products (indicated as Other Ingested, n=10). The Mann Whitney U-test was used to compare those not using any products to each of the groups and a p-value of ≤0.05 (*) was considered statistically significant

3.8 Association between infections and vaginal practices

Vaginal practices may increase vaginal inflammatory responses that may in turn influence the risk of HIV acquisition, STIs and other infections, therefore the association between the use of sexual enhancing products and the likelihood of having sexually transmitted infections and other infections was investigated. The use of vaginal and ingested products did not appear to have a direct link with the prevalence of infections in adolescents. Among nonusers and those who orally ingested products, the likelihood of testing negative and positive for infections was not significantly different. Among vaginal product users, the proportion testing positive for STIs was similar compared to the proportion testing negative. Although vaginal product users were more likely to have intermediate BV, this did not reach statistical significance (Figure 3.9A). A significantly higher percentage among users of ingested products in adult women tested positive for any STIs (p=0.0019), however significance was lost when stratifying for each STI (CT, TV, NG). The likelihood of testing negative or positive when using vaginal products did not reach significance in this age group (Figure 3.9B and Figure 3.9D). A significantly higher proportion of adolescents who ingested products tested positive for high-risk HPV types (p=0.0304) and this was more significant for HPV16 (p=0.0072). In addition, a significantly higher proportion of adolescents who inserted vaginal products tested positive for HPV-16 (p=0.0056; Figure 3.9C).

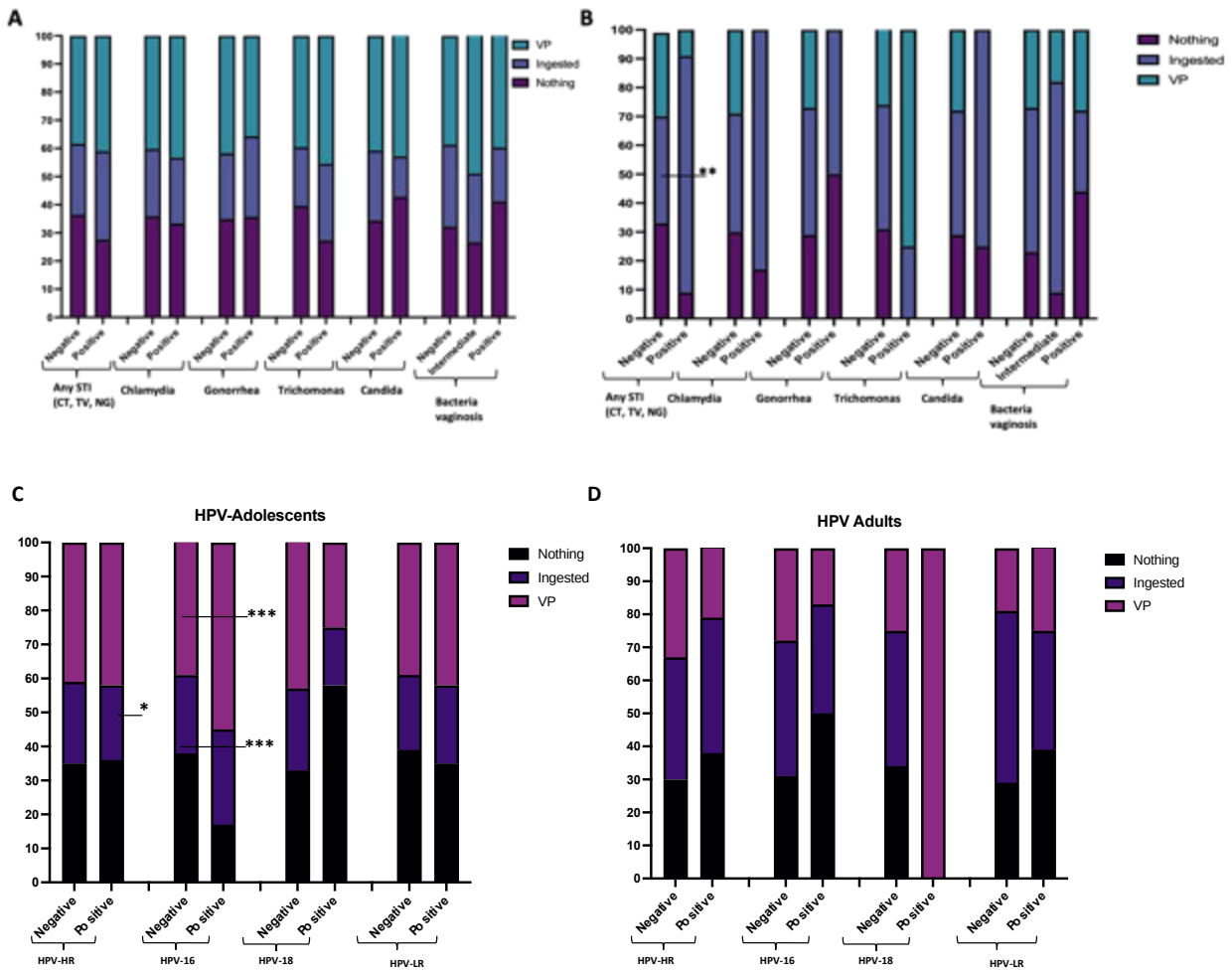


Figure 3.9 Stacked bar plot of the prevalence of sexually transmitted infections and genital infections amongst product users. The prevalence of STIs (CT, TV, NG), BV, and Candida was measured amongst A) Adolescents. B) Adults. The prevalence of different classes of HPV was measured in C) Adolescents and D) Adults using vaginal/oral product. Fisher's exact test was used to compare the prevalence of infection among product users (vaginally inserted (blue-top panel; pink-bottom panel) / oral (dark blue-top panel; purple-bottom panel) in comparison to participants reporting to not use any on the products in the cohort and a p-value of ≤ 0.05 was indicated by * and $p \leq 0.001$ was indicated by *** and were considered statistically significant.

Chapter 4

Discussion

The study aimed to evaluate anatomical, behavioural, and immunological factors that have previously been linked to increased risk of HIV and STI acquisition, and compare underlying risk contexts for adolescents and adult women in South Africa. While other cross-sectional studies have examined behavioural or immunological risk factors (separately) for HIV infection in the region, no comprehensive evaluation of these in young adolescent girls has been conducted specifically. The study compared STIs and genital infections, cervicovaginal abnormalities, and vagina-stimulating product usage as risk factors for HIV acquisition in young adolescent girls and adult women residing in a high HIV-burden rural setting in KZN, where the practice of “dry sex” is common, identifying several differences between the two groups.

Adult women were found to report higher sexual risk behaviours in comparison to adolescent girls, including a greater number of lifetime sexual partners, more significant age differences between them and their sexual partners, sex during menstruation, use of hormonal contraceptives, and low condom usage in their last vaginal sex; all of which has been previously associated with increased risk for HIV acquisition (Assi et *al.*, 2019; Kleppa *et al*., 2015; Kteily-Hawa *et al*., 2022; Stoner *et al.*, 2019). The most prevalent HIV risk factors identified in adolescents in this cohort were the early sexual debut and use of VIPs, as previously described (Dine *et al*., 2023).

The cohort described in this study highlight broader socioeconomic factors impacting women in South Africa as most adolescents and adults were unemployed and involved in transactional sex, characterized by the exchange of gifts for sexual intercourse. This could influence the dynamics of sexual behaviour and health choices in this population.

Some important differences between adult women and adolescents were evident. Adult women were markedly more likely to use hormonal contraception compared to adolescents, with injectable contraceptive Depo-Provera being the more prevalent. In contrast, adolescents reported a higher prevalence of condom use at the last sex act than adults, possibly reflecting variation in knowledge, attitudes, and accessibility to contraceptives by age group. Adolescent females also exhibited a significantly higher prevalence of CT, NG, and HPV (particularly HR HPV types) compared to adult women. BV prevalence was, however, higher in adult women

compared to adolescents, although not significantly. Given the significant health burden of STIs and their potential to cause cancers (especially HPV-related cancers), these findings highlight areas where prevention efforts could be significantly impactful.

Previous studies from multiple African countries have reported that products are used by women for a variety of reasons including genital hygiene, sex preparation (sex enhancement or sexual stimulation), and vaginal treatment of infections and symptoms (Hilber *et al*., 2010; Low *et al*., 2011), which was similar to some of the motivations reported in women living in the same community that this cohort was recruited from (Humphries *et al.*, 2019). Humphries *et al*. (2019) found that vaginal products were motivated by a strong focus on vaginal hygiene, and ensuring that the enjoyment of sex is enhanced (for their partners) by the drying, tightening or raising of the vaginal temperature.

More than half of the women in this cohort, irrespective of age, reported using VIPs, which supports previous reports from sub-Saharan Africa (Myer *et al*., 2005), although some important differences in the prevalence and types of products used were evident between adolescents and adult women. Adults were more likely to ingest products, while adolescents were more likely to use products intravaginally, and adolescents were more likely to report use of a wider range of products, both vaginal and oral. Alum was the most commonly used product by adolescents, while Ibhodwe labafazi ('the women's pot') was more commonly preferred by adults. Alum is known for its astringent properties (Zeenat *et al*., 2018) and is used for douching and steaming, primarily for vaginal tightening. Although alum has been described to act as a therapeutic agent in diseases such as leukorrhea, haematuria, haemorrhages, ecchymosis, and discharge, it is also a well-known agent used in vaccine preparations to induce inflammatory cytokine production and immune cell recruitment to the injection site (Zeenat *et al.*, 2018), which could indicate the mechanism by which it is linked to vaginal tightening, by causing inflammation in the vagina. Interestingly, stoney ginger beer emerged as a commonly ingested product among both adolescents and adults, indicating a shared cultural or traditional practice within the cohort. Understanding the cultural and contextual factors influencing the use of certain products is crucial for developing culturally sensitive and effective health interventions.

A range of cervical conditions was evident from colposcopy assessment in this cohort, including cervical ectopy, injury, clinically evident inflammation, leukoplakia, genital warts (condyloma acuminatum), and various forms of vaginal discharge, with some noteworthy differences between the age groups, and adult women having a higher prevalence of cervical abnormalities overall than AGYW. This suggests that age and potentially increased sexual

activity may be factors in the development of such conditions. There is limited published data that compares cervical abnormalities between adolescents and adult women. In adolescents, leukoplakia was the most prevalent cervical abnormality, characterized by flat white plaques with slight ridges or grooves. This could be indicative of HPV infection, which is a known risk factor for cervical cancer. Indeed, the prevalence of HPV in adolescents was high (85.6%). In contrast, adults exhibited more prevalent signs of cervical injury than adolescents. The frequency of injuries can be influenced by hormonal status (Porter *et al.,* 2016). Interestingly, the prevalence of cervical ectopy, hypothesized to be more common in adolescents than adult women, was similar between age groups. This is similar to a study performed by (Jacobson, *et al*., 2000b) where they measured areas of ectopy and the T-zone of adolescent women and concluded no differences in the size and prevalence of ectopy in adolescent and adult women. However, contrary to studies by Hwang *et al*. (2014), Jacobson *et al.* (2000), Morrison *et al*. (2004), Moscicki *et al*. (2001) found the prevalence of ectopy to be higher in adolescent females and the maturity of ectopy decreased with age and sexual experience. However, the sample size in this current study was smaller compared to the study population in the research done by Moscicki *et al*. (2001). Therefore, the difference in this finding could be due to the limited sample size in this study.

For adolescents, commonly using alum as a VIP, cervical injury signs were also commonly identified, suggesting alum as a possible cause of injury in this study cohort. Adult women who used ibhodwe labafazi were 20 times more likely to have cervical ectopy compared to adult non-users. This can also possibly explain the higher prevalence of ectopy in the adult population in this study compared to what was shown by Moscicki *et al*. (2001). In addition, ibhodwe labafazi was found to increase genital inflammatory responses in adolescents, which could increase susceptibility to HIV/STIs.

At baseline, the majority of cervicovaginal cytokine concentrations were similar between adolescents and adult women, with few significant differences. Notably, AGYW exhibited higher median concentrations of the pro-inflammatory cytokine TNF-α compared to adults, potentially suggesting heightened inflammatory responses in younger women. The elevated levels of TNF- α may signify an active immune response working to control and reduce the progression of a potential infection, however STI data analysis was not included to verify this, therefore it remains uncertain whether this elevated response is due to an existing infection. Conversely, adults showed significantly higher concentrations of the chemokine IL-8 compared to adolescents. IL-8 is a key mediator of acute inflammatory responses and is involved in the recruitment of neutrophils to sites of infection or injury (Brennan & Zheng, 2007). Furthermore, the growth factor G-CSF and regulatory cytokine IL-10 exhibited

significantly higher concentrations in adults compared to adolescents. G-CSF plays a role in stimulating the production of granulocytes, while IL-10 is an anti-inflammatory cytokine with immunosuppressive properties (Sullivan *et al.*, 2023) The elevated levels of G-CSF and IL-10 in adults could also indicate a more balanced and regulated immune response, possibly reflecting a mature and well-established immune system compared to adolescents.

Contrary to baseline observations, this study found that sexual exposure and product use did not dramatically alter genital cytokine concentrations in either the adolescent or adult groups, with one important exception: concentrations of type I IFN (IFN-α) were moderately decreased in adolescents following sexual exposure and VIP use, suggesting a potential suppressive effect on IFN-α associated with sexual exposure or product use in the adolescent group. This potent cytokine is known for inhibiting virus replication by activating multiple antiviral mechanisms and pathways to block both the early and late stages of HIV-1 replication (Goujon & Malim, 2010). However, the specific decrease in IFN-α in adolescents raises intriguing questions about the nuanced impact of these factors on immune responses, potentially indicating a more complex interplay in this specific cytokine during sexual immaturity. The overall observation of limited alterations in cytokine concentrations may suggest that the inflammatory responses in the female genital tracts of both adolescents and adults are relatively resilient to the immediate effects of sexual exposure or product use.

In most cases, no striking differences were observed in participants who were using other vaginal products compared to those who were not using any products during the exposure visits in both age groups. This suggests that the impact of various products on colposcopic and inflammatory changes may be product-specific, highlighting the importance of differentiating between specific products in research and health interventions. This information about common VIP practices in KZN emphasize the importance of promoting awareness and education about safe intravaginal practices, particularly among adolescents who may be more prone to using products associated with adverse colposcopic changes. The implications of these findings extend beyond the study population and underscore the need for broader public health initiatives to address the potential risks associated with certain intravaginal products.

Previous studies from South Africa hypothesised that women develop immunity against STIs, like chlamydia, as they mature and gain sexual experience (Esra & Johnson, 2020; Omori *et al*., 2019). Adolescent females in this cohort also had higher prevalence of chlamydia as compared to adults, regardless of product usage, possibly linked to the higher fragility of the lower genital tract mucosa of adolescents than adults, which influences their susceptibility to infection (Miller, 2000; Monteiro *et al.*, 2023). However, causality is difficult to infer because

several of the socio-behavioural factors found to be associated with the prevalence of STIs (BV, body mass index, cervical injury, and visible signs of vaginal discharge) may also have been caused by STIs rather than being risk factors of STIs. The association between STIs and VIP use was not clear although a higher proportion of adolescents (and not adults) who inserted vaginal products tested positive for HPV-16. This is the first study to show this association. These findings need to be confirmed by more extensive cohort studies and suggest that the use of vaginal enhancing products may partly contribute to differences in biological risk profiles that influence HIV susceptibility.

Limitation of the study

Because of fear of stigma, some of the adolescents and adult women enrolled in this study may have underreported information about their VIP use to avoid shame. Women in this study, however, did report a wide array of VIPs with differing properties, so broad categorization of VIP characteristics was not possible and sample size for individual product groupings was limited. The presence of male partner semen (determined by PSA and the Y chromosome tests) was not available at the time of submitting this dissertation, so analysis depended on condom use data, for which reporting is also unreliable. The MIST study took place during coronavirus disease, so a loss to follow up was higher than anticipated. Only a limited number of colposcopy images were captured, which limited statistical power for interpreting colposcopy findings.

Conclusion

In conclusion, this study showed the possible impact of VIP use on the cervical and genital anatomy of adolescent girls and young women residing in rural KZN. VIP preferences differed by age group in this study. The most common product used by adolescents - alum - caused cervical injury and higher risk of HR HPV infections, linked to cervical cancer risk. In adults, “ibhodwe labafazi” use was associated with higher prevalence of cervical ectopy as well as heightened levels of IL-1α, VEGF, and IL-17. These findings suggest that use of VIPs differs by age of the women, which may contribute to differences in biological risk profiles that influence HIV and cervical cancer susceptibility suggesting that women should be warned about the potential dangers of using VIPs. This study also highlights the importance of health communication in communities such as these, where development of health messages about sexual health for women needs to sensitively consider different cultural practices and the tendency in this community to accept vaginal product use.

Chapter 5

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APPENDICES

Appendix I: Common vagina-stimulating products used by adolescents and adults and their purpose for the use

[a]Name of product used	Composition description	Purpose	[b]Overall usage [% (n/N)]
Applied internally or externally in the vagina			
  Alum	A combination of aluminum sulphate and potassium sulfate	Vaginal tightening	11.2% (28/251)
 Snuff/Idoshi/I-Ntsu/Smokeless tobacco	Dried tobacco leaves, polonium 210, formaldehyde, cadmium, lead, nitrosamines, arsenic, cyanide, water	Sexual stimulation believed to cause vaginal heating and tightness	2.8% (7/251)
Ibhodwe labafazi (women's pot)	'Pink Vaseline'- unknown contents	Detoxification; sexual stimulant; vaginal drying, tightening, and heating	7.2% (18/251)
	Copper sulphate, powder	Vaginal tightening	0.8% (2/251)

 **Traditional herbs (incl. isiwasho, Tshitshi powder)**	**A variety of herbs**	**Detoxification, vaginal tightening, and heat**	**0.8% (2/251)**
 Holy ash/Incense	Burnt cow dung, burnt dried wood	Sexual stimulation, drying and heat	2.4% (6/251)
 Herbal petroleum jelly	Paraffin oil, paraffin wax, petroleum, fragrance, vitamin E	Vaginal heating	1.6% (4/251)
 Newspaper[§]	Any plain print newspaper (preferably soft paper, e.g., telephone directory paper)	Removing moisture from the vagina-drying, tightening	0.8% (2/251)

 **Feminine washes (Gynae guard)**	**Aqua, sodium laureth sulfate, glycerin, sodium chloride, lactic acid, dichlorobenzyl alcohol**	**Intimate cleansing, removal of odour causing germs**	**0.8% (2/251)**
Ingested, masticated			
 Stoney ginger beer	Carbonated water, sugar, citric acid, stabilisers, preservatives, flavouring, non-nutritive sweeteners (sodium cyclamate, sodium saccharin, acesulfame-K), and trisodium citrate	Tightening and causes heat	10.0% (25/251)
 Halls losenges	Sugar, glucose syrup, flavouring acids 9lactic acid, citric acid), acidity regulators E325,E332	Tightening	3.2% (8/251)
 **Tartaric acid**	**Potassium hydrogen tartrate**	**Tightening vaginal walls**	**1.6% (4/251)**

[a] Over-the-counter name of the product/ vaguely used term for the product
[b] Proportions calculated from the questionnaire responses of adolescents and adults in the cohort

Appendix II: Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 September 2021

Miss Phumla Londeka Radebe (216011668)
School of Life Sciences
Pietermaritzburg

Dear Miss Radebe,

Protocol reference number: BREC/00003306/2021
Project title: Investigating cervical ectopy, genital inflammation, and the frequency of sexually transmitted infections in adolescent girls using intravaginal products.
Degree: MSc

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 28 September 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Amended_level_2_Lockdown_Guidlines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 28 September 2021. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 October 2021.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

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