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Do human immunodeficiency virus-positive postmenopausal women on highly active antiretroviral therapy show reduced cervical atrophy

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DO HUMAN IMMUNODEFICIENCY VIRUS-POSITIVE POSTMENOPAUSAL WOMEN ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY SHOW REDUCED CERVICAL ATROPHY?

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the requirements for the degree

of

Master of Medicine in the branch of Anatomical Pathology

Johannesburg, 2015

Declaration

I, Gillian Elaine Davies declare that this research report is my own work. It is submitted for the admission to the degree of Master of Medicine by the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature:

Signed at: University of the Witwatersrand, Johannesburg

Date: 27 June 2015

Abstract

Background

It has been anecdotally observed that postmenopausal Human Immunodeficiency Virus (HIV) infected women on Highly Active Antiretroviral Therapy / combined antiretroviral therapy (cART) show a lesser degree of cervical epithelial atrophy on cervical Papanicolaou (Pap) smears than is expected in women of this age group. There is currently no literature to support or discredit this observation.

Aim

The aim of this study is to determine whether postmenopausal HIV positive females on cART show reduced cervical atrophy than expected in the postmenopausal state.

Materials and methods

Routine Pap smears from postmenopausal HIV positive women submitted to the NHLS Braamfontein Cytology department from Helen Joseph Themba Lethu Clinic, over a four year period, were assessed by use of the Maturation Index to determine whether the epithelium was predominantly atrophic or mature.

Results

Of a total of 135 smears, 50% were predominantly mature, which is a significantly larger proportion to what is expected in postmenopausal women. Although not statistically significant, 52% on cART were predominantly mature while 38% not on cART were predominantly immature. There was a significant difference in maturation between the women who started cART before the onset of menopause compared to those who were initiated after menopause. There was no statistically significant trend with CD4 count, duration of cART use, cART regimen, and duration of menopause.

Conclusion

With the advent of cART, HIV-infected individuals are expected to live longer and the number of HIV-infected postmenopausal women will increase. Thus it is important to determine the effects of HIV and cART on the cervix. This study reveals less than expected atrophy in HIV infected women, especially those who were started on cART before menopause.

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CHAPTER 1

1.0 Introduction and background

This chapter serves to provide an introduction to the study and a background to the topic in order to orientate the reader.

1.1 Introduction

It has been anecdotally observed that postmenopausal Human Immunodeficiency Virus (HIV) infected women on Highly Active Antiretroviral Therapy/ combined antiretroviral therapy (cART) show a lesser degree of cervical epithelial atrophy on cervical Papanicolaou (Pap) smears than is expected in women of this age group. There is currently no literature to support or discredit this observation.

1.2 Background

With the advent of cART, HIV positive individuals are expected to live longer [1,2]. Thus it is expected that larger numbers of older patients with the disease will be encountered. Most studies have not shown a reduction in cervical pathology following the use of cART [1]. As a result, we can expect to see more Pap smears from HIV positive postmenopausal women on cART and thus it is important to determine what the expected 'normal' is in order to more readily recognise abnormalities.

Cervical Squamous Intraepithelial Lesion (SIL) is more frequently encountered on Pap smears of HIV positive than negative women, as a result of co-infection with Human Papillomavirus (HPV) and particularly with high risk types [1-3]. At times, the difficulty of distinguishing atrophic change from dysplasia means either that patients are over-treated, with attendant complications such as infection, haemorrhage and a waste of precious resources, or undertreated, allowing lesions to progress. HPV testing may in future, help to reduce this problem, however, this is not currently routinely available in the South African public health sector [4].

Atrophy of the cervix is a normal physiological phenomenon which can be seen on cervical Pap smears in postmenopausal women due to reduced oestrogen levels [5,6]. The features of atrophy can cause diagnostic difficulty as they are not unique. In some cases it can be difficult to distinguish atrophic change from squamous intra-epithelial lesion (SIL) [5,6]. The Pap smear is a screening tool used to detect cervical epithelial abnormalities and advise an appropriate course of action in order to reduce the incidence and mortality of cervical cancer [4,6,7]. It is important to know what background changes are expected in order to detect abnormalities.

Loss of atrophy, or increased cervical maturation, can be caused by increased oestrogenic activity or androgenic antagonism [8]. cART is known to cause gynaecomastia [9]. The aetiopathology behind this side effect is thought to be, at least partly, as a result of anti-androgenic action [9]. Thus it seems logical that cervical maturation may be affected similarly, resulting in loss of the expected atrophy (or increased maturity).

Cervical maturation can be assessed by applying the Maturation Index, which is a ratio of the three cell types present on Pap smears (viz. superficial, intermediate and parabasal

squamous cells) [6]. A predominance of immature basal cells over mature superficial cells characterises atrophy [6]. If it can be shown reliably that this group of women are indeed not expected to be atrophic, then equivocal changes on Pap smear could be more readily attributed to dysplasia and appropriate management advised.

The above chapter serves to provide an introduction to the study and a background to the topic in order to orientate the reader. It aids the reader in providing understanding of the topic, the thoughts behind the study and why the study is felt to be important.

In this research report, the aims, objectives, literature review, materials and methods utilised to investigate the relationship between the use of ART and cervical maturation on Pap smears in HIV positive postmenopausal women will be provided. This will be followed by the results, discussion and conclusion thereof.

CHAPTER 2

2.0 Aims and Objectives

This chapter provides details of the aim of the study and the objectives used to fulfil this aim.

2.1 Aim

The aim of this study is to determine whether postmenopausal HIV positive females on cART show greater cervical maturation (or reduced atrophy) than expected in the postmenopausal state.

2.2 Objectives

The objectives of this study are to determine:

- Whether HIV positive women on cART are likely to have a different maturation index than expected for the postmenopausal state.
- If there is a significant difference in cervical epithelial maturation between women on cART and those not yet on cART.
- If there is a relationship between cervical maturation and duration of cART use.
- Whether there is a difference in maturation with the specific cART regimen used.

- Whether there is a trend in maturation in relation to number of years postmenopausal.

- If there is a difference in maturation according to when cART was initiated, with regards to when menopause occurred.

- If there is a relationship between CD4 count and cervical maturation.

The above chapter states the aim of the study and the list of study objectives. This provides the reader with the various headings under which the data was examined.

CHAPTER 3

3.0 Literature review

This chapter provides a summary of the current available literature on important matters related to this study.

3.1 Introduction

With the continued spread of Human Immunodeficiency Virus (HIV) and increased use of Combined / Highly Active Antiretroviral Therapy (cART / HAART), the demographics of HIV continue to change and infected individuals live longer[1-3,10-14]. In women, the effects of the interaction of Human Papilloma Virus (HPV) and HIV on the incidence of cervical disease are well recognised[2-4,14,15]. Thus it is likely that these women will undergo more frequent cervical Papaniculaou smears (Pap smears) than their HIV negative counterparts and into older ages[2-4,15,16].

It has been anecdotally noted in day to day practice that cervical smears in postmenopausal HIV positive females on HAART seem to show a lesser degree of physiological atrophy than would be expected in the postmenopausal period. There is very limited data on the subject of cervical maturation and atrophy in HIV infection and almost nothing regarding the effects of cART on cervical maturation. Establishment of the 'new normal' for this population is important so that abnormalities are more readily recognised and appropriate intervention is undertaken.

3.2 Background

As of midyear 2013 data, 10% of South Africans were HIV positive (approximately 5.26 million people) and over 1 million were reported to be on HAART [7]. The worldwide trend is to report HIV statistics up to the age of 49, thus there is very limited data on the proportion of older individuals with the disease [7,17,18]. The prevalence of HIV in people over the age of 50 years is 7.1%, according to a large scale survey conducted by the Human Sciences Research Council. This value is similar to that of the group aged 15-24 years [19]. According to projections, by 2030, South Africa will have up to 7.3 million people living with HIV, a larger proportion of older people and three times the current number on HAART [7,10,14,17-19].

The demographics of HIV positive individuals in South Africa are continuing to change [1,2,7]. The use of cART has improved survival in HIV infection, thus HIV positive individuals on cART are expected to survive to older ages [1-3,11-13].

Although this is not yet practiced, it is proposed that all HIV positive patients over the age of 50 be started on cART, regardless of CD4 count [20]. This is because it is thought that the immune response to cART in this group may be reduced and the probability of complications from non-AIDS related conditions increased [20]. In addition, these patients are likely to require closer follow up and monitoring than younger counterparts as cART related complications are expected to be encountered more often [20].

The current cART regimen in South Africa includes the use of Tenofovir, Stavudine (d4T), Lamivudine (3TC), Efavirenz (EFV), Nevirapine (NVP), Zidovudine (AZT), Didanosine (ddI), emtricitabine, lopinavir (LPV) and ritonavir [21-23]. Most patients are initiated on regimen 1, which consists of TDF or AZT with 3TC and EFV or NVP. Regimen 2, used for treatment

failure or in cases where regimen 1 is contraindicated, usually comprises AZT, 3TC or ddI, LPV and ropinavir [24].

The Papanicolaou test (Pap smear) is a screening test which entails sampling of epithelial cells from the cervix (ectocervical epithelium, endocervical epithelium and the zone of transition between the two), placing them on a slide and examination of these cells under a microscope, in order to detect abnormalities which require further medical management. The main purpose of the test is to detect changes indicative of risk of cervical cancer development and initiate management to prevent this cancer [25].

Current South African national policy advises three Pap smears to be done in a woman's lifetime, at ten year intervals starting at the age of 30, provided each is normal [23]. At ground level, however, many healthcare professionals practice opportunistic Pap smear screening and will perform smears outside of these guidelines, particularly with regards to HIV infection [23]. The recommendation in developed countries, such as the USA, is to undergo Pap smears every three years from the point of coitarchy (initiation of sexual activity), until the age of 65, provided no abnormalities are detected [25]. Any woman who develops symptoms which may indicate development of cervical cancer, such as irregular bleeding, discharge or pain, should undergo examination and sampling (and potentially formal biopsy) even if outside of the recommended guideline intervals [25] .

When abnormalities are detected on Pap smear screening, further recommendations for management are made. This may be a repeat Pap smear or referral for colposcopic examination and cervical biopsy [23].

Postmenopausal women remain at risk for cervical cancer development. Even women who have undergone hysterectomy should continue screening, with sampling of the upper vagina (for total hysterectomy) or remaining cervix (if subtotal hysterectomy was performed), if there is a history of SIL [25,26].

With prolonged survival, HIV positive women will have an increased likelihood of acquiring HPV and more time for progression of cervical disease [1-3]. HPV infection and HPV related pathology of the cervix have been shown in several studies, to be more frequent in HIV infected women. They have been shown to have an increased incidence of high risk HPV serogroups, increased incidence of intraepithelial lesions with less regression of these lesions and greater risk of progression to carcinoma [2,3,11,12,27]. In addition, earlier menopause has been linked to increased progression of squamous intraepithelial lesions in HIV positive women [28]. Most studies have failed to show a reduction in the incidence of cervical carcinoma with the use of cART [1, 2, 27,28].

Recommendation of when to initiate cervical screening and frequency of screening in both HIV-infected and non-infected patients vary from country to country, depending on available resources. Cervical screening programs have been successful in developed countries with regards to cervical cancer prevention. Unfortunately, in developing countries, this is not always the case [29,30]. There are examples of successful, cytology-based screening programmes in low and middle resource countries. In 1995 the Viet/American Cervical Cancer Prevention Project facilitated the implementation of Pap smear screening in southern Vietnam. The cervical cancer rate was reduced by 50% between 1998 and 2003 [29]. For a screening program to be successful, it requires human resources, sufficient financing and facilities to cope with the medical management required thereafter [31]. The

infrastructure required for successful implementation of widespread, monitored, easily accessible and free Pap smear programs is not established in many developing countries [30]. Problems encountered in some developing countries include underdeveloped healthcare systems with high patient numbers, numerous competing medical needs, political unrest, poverty, limited education and acceptability of the testing procedure by the community [30].

However, where Pap smear screening programs are in place, HIV positive women are likely to have more Pap smears in their lifetime as a result of more frequent access of health care facilities with opportunistic screening, and because more abnormalities may be detected and quite possibly well into the postmenopausal period [2,3,4,15]. It is recommended that women who have had a diagnosis of HSIL or worse continue Pap test screening over the age of 65 for at least 20 years [4,8]. It thus can be expected that in South Africa we can expect to see a greater number of Pap smears from older, postmenopausal women on cART in the future.

3.3 Menopause and HIV infection

The average age of menopause in South Africa is in the region of 49 years [5]. There have been many studies looking at the age of menopause in HIV positive women. While the results are varied, the trend appears to be that these women experience earlier menopause and possibly more severe symptoms of menopause. A greater proportion of HIV positive women experience premature menopause than the general population [8,11-14]. Early onset of menopause has been linked to increased progression of squamous intraepithelial lesions in HIV positive women [28].

The age of onset of menopause can be modified by multiple factors, including smoking, substance abuse, physical stress, socioeconomic status and body mass index. In addition, some studies have shown a correlation between lower CD4 count in HIV positive women and earlier onset of menopause [8,11-13]. These women are also less likely to undergo hormone replacement therapy. The reason for this is unclear [8].

3.4 Normal physiological atrophy and the importance of differentiation from dysplasia

Atrophy of the cervical and vaginal epithelia occurs normally in the postmenopausal stage as a result of sex hormone, particularly oestrogen, depletion [32]. Oestrogens (oestriol, oestradiol and oestrone) increase proliferation, growth and maturation of tissues in the female genital tract [6,32]. With menopause, reduced oestrogen results in thinning and loss of maturation (atrophy) of the cervical and vaginal epithelium [6,32]. Additional factors which affect cervical maturation include smoking and oral contraceptive use, which both cause maturation [33]. Maturation can be observed on cytological examination of cervical Pap smears [6,32].

There are three cell types present on cervical smears, parabasal (least mature), intermediate and superficial (most mature) cells [6]. With atrophic change, the proportion of mature cells present diminishes, and that of less mature, parabasal cells, increases [6].

Other features of atrophy include the presence of crowded parabasal-like cells with nuclear enlargement and an increased nuclear: cytoplasmic ratio (a larger nucleus relative to cytoplasmic content), as well as hyperchromasia (darker nuclear colour), elongation of

nuclei, autolysis with 'naked' nuclei (nuclei that have lost their cytoplasm), a granular background reminiscent of tumour diathesis and degeneration with nuclear pyknosis [26,33] . These features are not unique to atrophy, and there is considerable overlap with dysplasia and malignancy [26,27] . In addition, with menopause the transformation zone moves upward toward the lower uterine segment, which makes sampling thereof more difficult [25].

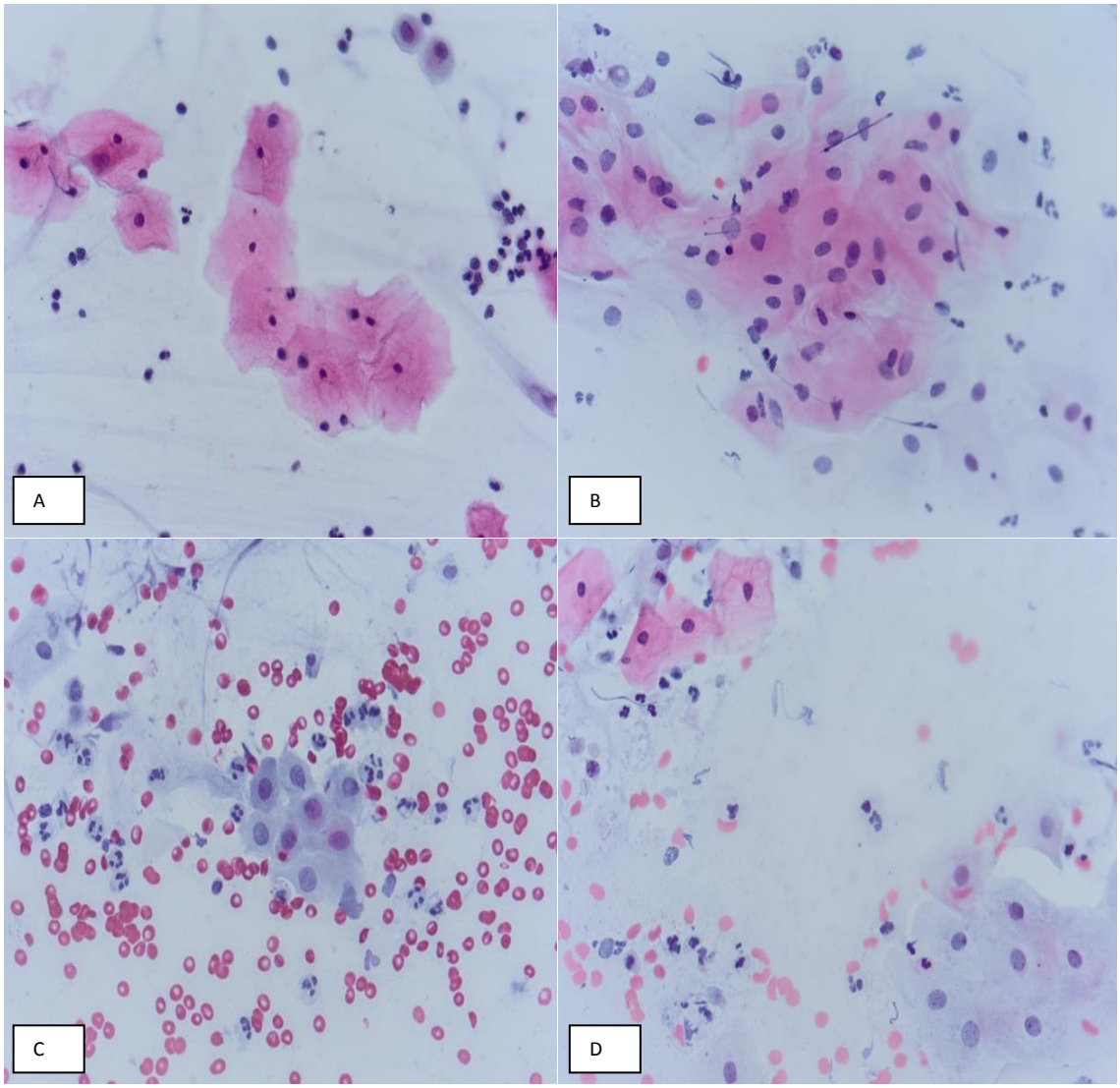


Image1:10x40 photomicrograph images of normal ectocervical squamous cells with background blood and inflammatory cells. A – Mature cells, B – Intermediate cells, C – Parabasal cells, D – Mature (top left) and intermediate cells (bottom right).

Atrophy may be difficult to differentiate from atypical squamous cells of uncertain significance (ASCUS), high grade squamous intraepithelial lesion (HSIL), and even invasive carcinoma, increasing the risk of false positive results [26,32,34,35]. It is important to accurately differentiate between atrophy and more sinister lesions as the management of these differs. Pure atrophy is a normal physiological finding and does not require further intervention as far as cervical screening is concerned.

Where there is doubt as to whether there is pure atrophy or an additional underlying lesion, the recommendation is to apply topical oestrogen therapy and repeat the smear [6,36-39]. The use of topical or systemic hormone replacement therapy (HRT), results in the reduction of cervical and vaginal atrophy and its symptoms. In addition, it renders the transformation zone more accessible, allowing more successful sampling [25]. Topical oestrogen therapy will induce maturation of immature cells, but will not affect dysplastic cells, thus helping to differentiate between a physiological process and more sinister lesions and avoiding false positive results on Pap smear [6,9,25].

Topical oestrogens are unfortunately not available in most of our primary health care facilities and many women need to be referred to larger centres. The resultant risk is that some of these women will be lost to follow up, running the risk of progression of an as yet undiagnosed premalignant or malignant lesion [32].

There is literature regarding the use of proliferation markers, such as Ki67, to assist in the differentiation of atrophy and SIL. This is not currently practiced in our setting [36].

HSIL requires referral to higher centres for colposcopic examination and histological biopsy. Invasive carcinomas are treated in a similar manner, but with greater urgency.

It has been shown that the majority of patients with atrophic change who had a diagnosis of ASCUS, had few abnormalities on follow up colposcopy [26,32]. Atypia identified on Pap smear in postmenopausal women is thus not a reliable indicator of histologically demonstrable SIL (poor cyto-histological correlation) [26]. However, these patients do have a risk of dysplasia, warranting further investigation [32]. There is currently a long waiting list for colposcopy in South Africa, with limited facilities and expertise. Thus it is important to differentiate these lesions with accuracy to avoid inappropriate referrals and save bookings for those who need them most.

An additional important consideration is that greater cervical epithelial maturation may result in greater susceptibility to HPV infection [33]. This will thus increase the risk of dysplastic lesions and malignancy.

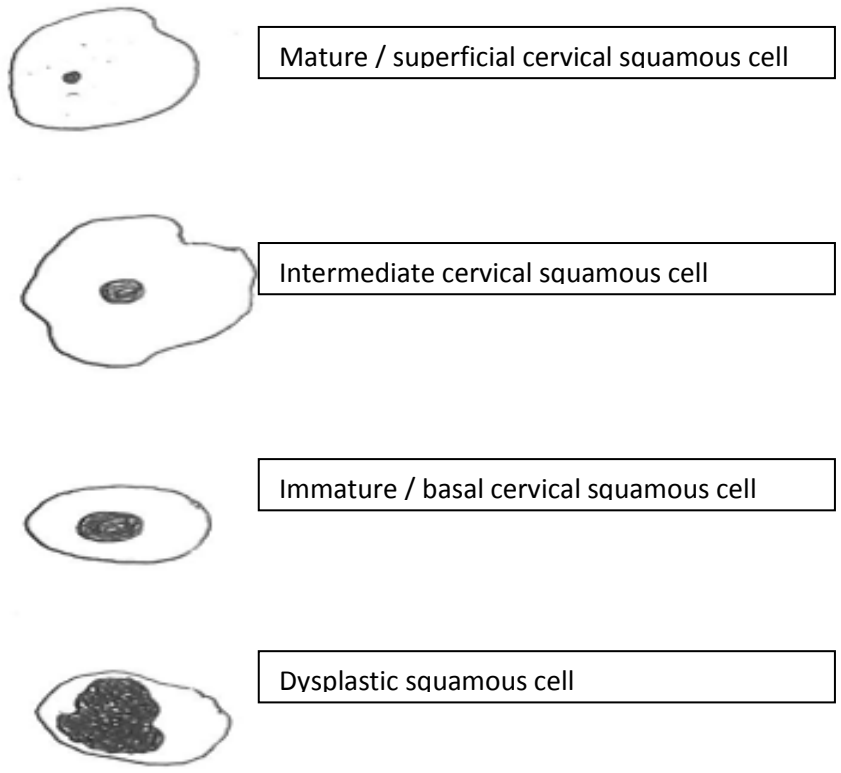
If it can be reliably determined that HIV positive postmenopausal women on cART are expected to show greater cervical epithelial maturity than expected in the normal physiological state, this could be helpful in difficult cases. In instances where doubt exists as to whether the features mentioned previously should be attributed to atrophy or dysplasia, one could be advised that the atypia should be more readily attributed to dysplastic change as these women are not expected to be atrophic, and thus colposcopic examination advised sooner. In addition, if these women are at increased risk for HPV infection as a result of increased maturation, then this would further highlight the need and importance of continued cervical Pap smear screening.

3.5 Measurement of epithelial maturation

Atrophic change of cervical and vaginal epithelium is measured with the Maturation Index (MI) [6,37]. This test was traditionally performed on vaginal smears to determine hormonal status (oestrogenic effect) [6,37].

Determining the MI involves counting nondysplastic cell types (parabasal, intermediate and superficial) in a random area and reporting their percentages as a ratio (parabasal% : intermediate% : superficial%) [6,37]. While vaginal and cervical epithelial maturation can vary from day to day, ultimately, a predominance of superficial cells indicates maturity, while a predominance of parabasal cells indicates atrophy [6,37]. Diagram 1 is an illustration of these cells.

Diagram 1: Schematic diagram of cervical squamous cells



Typical values for the MI in non-atrophic, oestrogenised females are around 0:40:60 – 0:70:30, with marked variation. In menopause, this value approaches 0:100:0, with a fully atrophic smear reaching 100:0:0 [6,37] .

Whilst the MI is a relatively outdated method to assess hormone state and may not be perfectly scientifically reliable, this was the only tool available to test the hypothesis that postmenopausal HIV-infected women have reduced cervical atrophy in this retrospective, Pap smear based study. This study was performed in a public sector, urban clinic in Johannesburg, South Africa and may not be applicable to women in better resourced or rural communities. Ideally serological tests should be performed to assess hormonal status but these are not performed as a routine at primary health care clinics in South Africa [38].

3.6 Effects of antiretroviral therapy

If the observation is correct and there is indeed a trend toward increased cervical epithelial maturation in postmenopausal HIV positive females on cART, it is thought that the underlying aetiopathology may be similar to that of the development of gynaecomastia. Gynaecomastia is a well described side effect of cART. While some cases can be attributed to fat redistribution, others show proliferation of glandular epithelium which is in keeping with true gynaecomastia [9,40].

In general, drugs that cause gynaecomastia do so by one of three mechanisms. The first is by centrally increasing prolactin, which is frequently accompanied by lactation. The second is to act peripherally by oestrogen or prolactin agonism and the third, by androgen antagonism [9,40]. Particular antiretroviral drugs associated with gynaecomastia include

Efavirenz and Didanosine [40]. An antiandrogen effect is favoured in the aetiopathology [40]. This antiandrogen effect could explain, at least in part, the observed increased maturation proposed.

There is also some work to show that serum retinoid concentrations are altered by both HIV infection and antiretroviral therapy [41]. Retinoids play a role in epithelial maturation and hormonal processes, amongst others [41]. Thus alterations in retinoid levels may also explain alterations in epithelial maturation.

3.7 Conclusion

As the demographic of our HIV positive population changes, it is important to keep up with trends in effects of the treatment of this disease. It has been anecdotally noted that there may be a general lack of physiological atrophy (or increased maturation) in HIV positive postmenopausal women on cART. There is no available data to support this observation at this time, but should this observation be proven correct, the postulated mechanism that may explain this is by oestrogenic or antiandrogenic effects of cART in a similar way to the aetiopathology of the development of gynaecomastia and hypermastia, or by retinoid concentration alterations.

As the features of atrophy and dysplasia have considerable overlap, it is important to be aware of the likelihood of dysplasia over atrophic change so as to risk stratify patients and recommend appropriate management.

The above chapter provides a summary of the current available literature on important matters related to this study. It serves to educate the reader on the available literature on the subject studied.

CHAPTER 4

4.0 Materials and Methods

This chapter provides details regarding the materials and methods used to conduct the study, including the research design, ethical considerations, study population, study sample, data collection and data analysis.

4.1 Research design

The study was contextual, retrospective and semiquantitative.

The study was contextual as it was conducted on Pap smears received by the NHLS Braamfontein Cytology department from Helen Joseph Themba Lethu Clinic, taken from postmenopausal HIV positive women.

It was retrospective as archived slides were examined and recorded data from previous clinic visits used.

The study was semiquantitative because it involved assessing a qualitative trait (maturation) using a numerical system (maturation index), to determine the proportion of cases that fell into maturation groups (predominantly immature or predominantly mature).

4.2 Ethical considerations

Application to the Human Research Ethics Committee (Medical) and Postgraduate Office of the Faculty of Health Science, University of the Witwatersrand was submitted for the clearance of this study. This was approved. (Appendix 1).

This was a retrospective study. No new procedures were performed. This study was conducted on archived departmental slides. The patients remained anonymous as patient details (name and patient file number) were not associated with the material and was only available to the primary investigator. The patients included in the study are not known to the investigator, there was no communication with these patients. The information was discussed with the statisticians in a completely anonymous form. Patient names were not divulged at any time during or after the study. This study did not influence patient care nor did it hold any financial implications for the patient. This was a retrospective study and individual patient consent, although ideal, was not thought to be necessary to obtain for the above reasons.

Permission was obtained to undertake this study in the Cytology Unit, Department of Anatomical Pathology, University of the Witwatersrand and National Health Laboratory Service (NHLS).

4.3 Study population

The study population consisted of HIV positive postmenopausal women attending the Helen Joseph Themba Lethu Clinic who had a Pap smear submitted to the NHLS Braamfontein cytology department, as a routine diagnostic procedure, from January 2009 to December 2012 (4 years).

4.4 Study sample

4.4.1 Sample size

After ethics approval was obtained, a DISA system database search was conducted to compile a list of HIV-positive postmenopausal women who had pap smears performed in the given time period. 523 cases were initially identified. Figure 1 illustrates the process of case selection.

The DISA record for each patient was checked to ensure that they were appropriately captured. 338 cases were excluded at this point as they did not fit the inclusion criteria of this study. Women were deemed postmenopausal if they were over the age of 50 years and had not menstruated for a year before the cervical smear was taken. Women between the ages of 40 and 49 were included if they were deemed clinically, at the time of routine clinical examination, to be postmenopausal viz, cessation of menses for a year prior to the cervical smear being performed and not on hormonal contraception. Biochemical analysis of hormonal levels is a more accurate method of determining hormonal status. However, biochemical analysis of hormonal levels, e.g. Follicle stimulating hormone (FSH) levels, are

not standard of care within a public sector, resource-constrained South African HIV clinic and could not therefore be used to determine hormonal status, nor confirm cessation of menses as being due to menopause. In addition, women were excluded if the test performed was not in fact a Pap smear (incorrectly coded), if the Pap smear was performed outside of the time period decided upon or if there was a discrepancy in the information (eg. patient recorded with two different ages). At this point, 185 cases remained.

Thereafter, the Themba Lethu patient database was accessed by a designated research assistant familiar with the database, to retrieve relevant information required for the study. The research assistant was given the list of the 185 cases with hospital numbers and Pap smear reference number to record each patient's cART initiation date, cART regimen at the time of the Pap smear and closest CD4 count to the time of the Pap smear. 23 cases were then excluded as essential information for the purpose of this study was not recorded, or there was no record of these patients on the clinical database. At this point, 162 cases remained.

Once a list of 162 appropriate cases was identified, the corresponding slides were retrieved from archives, with the help of the file clerk, using the storage numbers. 14 slides could not be found, and thus these cases had to be excluded.

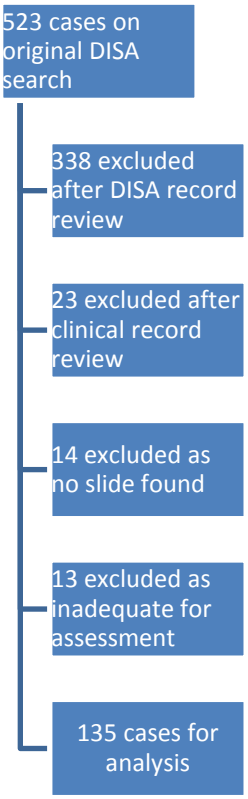
The 148 slides retrieved were renumbered and distributed amongst the viewers. Renumbering was necessary to cover the reference numbers corresponding to reports on file, in order to prevent viewers accessing formal reports, thus limiting bias.

At the microscopy stage, 13 cases were excluded as there were fewer than 100 normal cells for assessment, i.e. adequacy criteria could not be met - seven due to extensive

inflammation, two due to extensive dysplasia, three due to an inadequate number of ectocervical cells and one as a result of degenerate material which could not be accurately analysed.

Thus 135 cases could be used for the purposes of this study (Figure 1).

Figure 1: Flow chart of case exclusion process



4.4.2 Sampling method

The DISA data base of the Cytology Unit, Department of Anatomical Pathology, University of the Witwatersrand and National Health Lab Service (NHLS) was searched to find all women from the Helen Joseph Themba Lethu Clinic who were over 50 years or stated to be postmenopausal and had pap smears performed between January 2009 and December 2012. These were recorded by an allocated number, with a separate key corresponding to the case number. The key was kept separately, to ensure anonymity.

The filing clerk assisted the Primary Investigator in retrieving the slides from the departmental archives according to the identified case number. These were then distributed for examination after renumbering. Four cytotechnologists assisted with the study and cases were distributed between the Primary Investigator, Supervisor and cytotechnologists for examination. The number of cases allocated to each was determined by the participants themselves according to their time availability, with the bulk of the cases being viewed, at least once, by the Primary Investigator.

4.4.3 Inclusion and exclusion criteria

Cases included in this study were those of postmenopausal women (stated on record to be postmenopausal or over the age of 50), attending the Themba Lethu clinic and known to be HIV positive, who had a Pap smear in the time period from January 2009 to December 2012 submitted to the Braamfontein Cytology department for which the slide could be retrieved.

Cases were excluded if:

- The patient was younger than 50 and not clearly stated to be postmenopausal.
- The patient was older than 50 but not menopausal (last menstrual period within one year of the Pap smear).
- The HIV status was recorded as negative on the Pap smear report history.
- The procedure was not a Pap smear.
- There was no record of the patient on the Themba Lethu database.
- There was a discrepancy with recorded age between the Pap smear report and Themba Lethu database.
- The Pap smear slide could not be retrieved in the cytology unit slide archives.
- There were too few visible normal cells for assessment (i.e the non-dysplastic ectocervical component was inadequate to determine the maturation index (<100 cells), because of sampling problems, or if it was degenerate, obscured by blood, inflammation, or extensive dysplasia).

Pap smears from HIV negative women were not included in the study for two reasons viz. (1) this study utilised Pap smears from an HIV clinic thus all women are HIV positive and (2) in a South African Department primary health care or family planning clinic, the HIV status of many women is unknown and or if it is known, this information is often not recorded on the Pap smear laboratory request form. Thus for comparison, existing accepted literature was used with regards to expected atrophy in postmenopausal women. Data presented in a

similar manner to that of this project was not found, thus comparison was made by considering what one would expect on a case by case basis in postmenopausal women.

4.5 Data collection

The slides were examined by the primary investigator, supervisor and three cytotechnologists.

The maturation index was conducted with light microscopy on a single Pap smear slide for each case and recorded as per the data capture sheet. Each case was examined twice, by different viewers and when the results were discrepant, they were viewed a third time by the Primary Investigator and/or supervisor. Cases already viewed by the Primary Investigator that were discrepant when examined by a Cytotechnologist, were viewed by the supervisor independently of the Primary Investigator.

The data sheets distributed had only the slide reference number at this point, without additional information (age, cART use, etc.) to limit bias.

4.6 Data analysis

Once the maturation index was recorded for each case according to the data capture sheet, the form was returned to the Primary Investigator. At this point, the primary investigator married the case numbers to the retrieved patient information (age, years menopausal, cART status, cART regimen, duration of cART, use of hormone replacement therapy, and

CD4 count at time Pap smear was performed) for analysis. This information was not made available to the viewers at the time of slide examination in order to limit bias.

The maturation index (basal cells:intermediate cells:superficial cells) was used to place each case into one of two categories – 'Predominantly mature' and 'Predominantly immature'. A case was called 'Predominantly mature' if the number of superficial cells was greater than the number of basal cells (eg. 20:40:40), and 'Predominantly immature' if the number of basal cells was greater than the number of superficial cells (eg. 30:60:10) or if the cells were all intermediate (i.e 0:100:0). In cases where there was a discrepant result (eg. the maturation index performed by each observer resulted in a different maturation category), the case was viewed by the supervisor and the maturation index from the supervisor was used.

Descriptive statistics were used to analyse demographic data (means and ranges). Data was recorded in contingency tables for ease of statistical analysis. Fisher's exact test was used to analyse data when two variables were considered (eg. cART use and maturation) with in only two categories each (eg. on cART/not on cART and mature/immature). Fisher's exact test is used for categorical data (eg. on cART or not on cART) which can be displayed in a two row by two column contingency table. It is similar to the Chi squared test, but calculates the P value more accurately and is better suited to small numbers. Chi squared test for trend was used when a variable had more than two categories (eg. CD4 count ranges). The Chi squared test looks for a trend in values of one variable according to changes in another (eg. maturation changes according to duration of cART use). It is used for categorical data that can be displayed in a contingency table with more than two rows or columns, where

the columns are continuous and equally spaced (eg. 1-2 years, 2-3 years). P values were considered significant when less than 0.05 ($P<0.05$).

Interobserver reliability was assessed by showing each slide to two different observers and the rate of agreement recorded. Intraobserver reliability was analysed by showing a group of slides to the same observer at two points in time and was assessed as the percentage agreement. The percentage agreement is considered acceptable at greater than 85%.

The Primary Investigator was responsible for the data search, identification of appropriate cases according to inclusion and exclusion criteria, assisting in case retrieval, numbering of cases, and distribution of cases amongst observers, examination of a proportion of slides, data collection and analysis and then write up of the findings.

The above chapter provides details regarding the materials and methods used to conduct the study, including the research design, ethical considerations, study population, study sample, data collection and data analysis. It provides the reader with the details of how, where, when and on whom/what the study was conducted and how the information collected was analysed to provide a result.

CHAPTER 5

5.0 Results

This chapter provides the results obtained from the data collected and analysed, with accompanying graphs and figures.

5.1 Demographics

A total of 135 cases were included in the study. The patients were of a mean age of 55 (range 41 to 69 years). 111 were on cART, and 24 were not on cART.

106 patients had a recorded duration of menopause (years since last menstrual period). The mean number of years postmenopausal was 6.5 (range 1 to 20 years). The mean age of menopause was 47.2 years (range 30 to 68 years).

No patients were recorded to be on HRT.

Once the maturation index was performed on each case, 67 (50%) were predominantly mature, and 68 (50%) predominantly immature (Figure 2). If one assumes, from available literature [6,26], that all postmenopausal women should have a predominantly immature cervical epithelium (0% predominantly mature and 100% predominantly immature), then this result is extremely significant (P=0.0001) with Fisher's exact test.

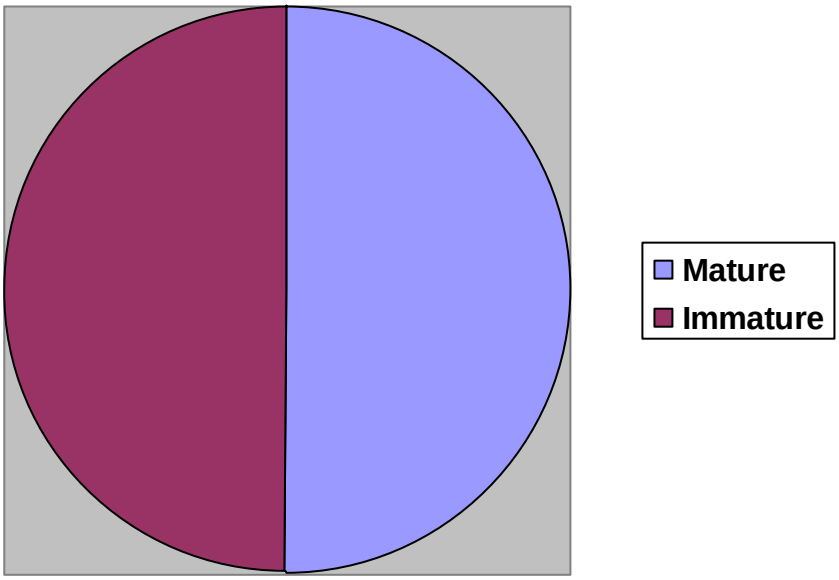


Figure 2: Proportion (%) of total included cases in each maturation group

5.2 Determining whether HIV positive women on cART have a significantly different maturation index than expected for the postmenopausal state.

111 patients were on cART at the time the Pap smear was performed. Of these, 58 (52%) were predominantly mature, and 53 (48%) predominantly immature (Figure 3). If one assumes, from available literature, that all postmenopausal women should have a predominantly immature cervical epithelium (0% predominantly mature and 100% predominantly immature), then this result is extremely significant (P=0.0001) with Fisher's exact test.

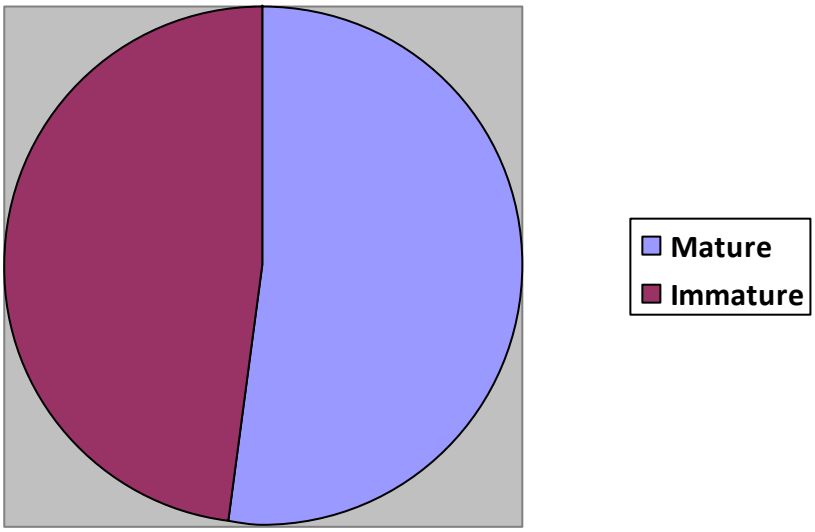


Figure 3: Proportion (%) of patients on cART in each maturation group

5.3 Determining if there is an association between maturation and cART use.

- 111 patients were on cART, 58 (52%) were predominantly mature, and 53 (48%) predominantly immature. (Figure 4).
- 24 patients were not on cART, 9 (38%) were predominantly mature, and 15 (62%) predominantly immature. (Figure 4).

Fisher's exact test revealed no significant statistical difference in maturation according to cART use (P=0.2605).

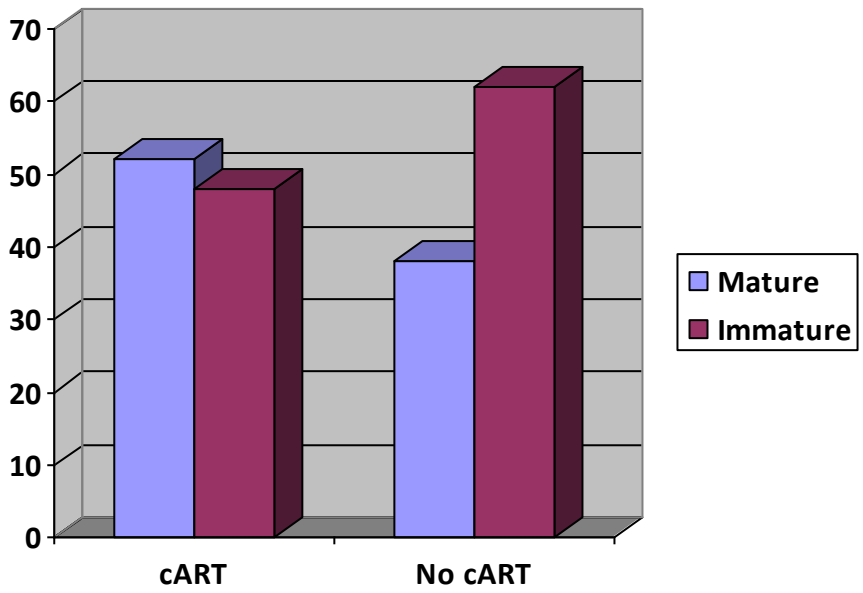


Figure 4 : Proportion (%) of cases in each maturation group according to cART use

5.4 Determining if there is a trend in maturation with duration of cART use.

Duration of cART use was recorded for 103 patients. The mean duration of cART use at the time of the Pap smear was 4 years (range <1 to 8 years). 54 women used cART for less than 4 years, and 49 for greater than 4 years. Figure 5 illustrates the following results.

- 54 used cART for no more than 4 years, 28 (52%) were predominantly mature, and 26 (48%) predominantly immature.
- 49 used cART for longer than 4 years, 22 (45%) were predominantly mature, and 27 (55%) predominantly immature.

Fisher's exact test shows no significant correlation between maturation and duration of cART use for less or greater than 4 years (P=0.5554).

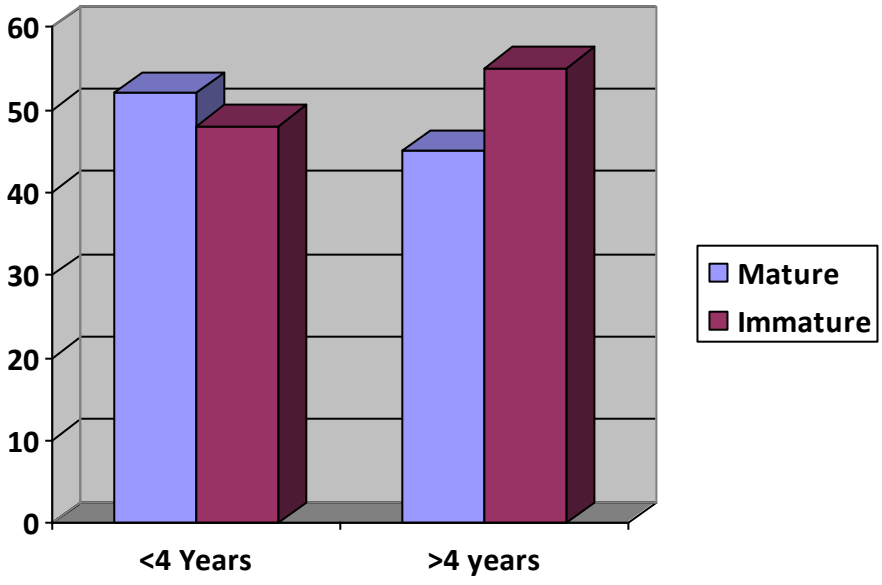


Figure 5: Proportion (%) of cases in each maturation group according to duration of cART use

5.5 Determining whether there is an association between cervical maturation the specific cART regimen used.

Of the 111 patients on cART, 77 had a record of the regimen used at the time of the Pap smear. Figure 6 displays the following results.

- 66 were on regimen "1", 32 (48%) predominantly mature, and 34 (52%) predominantly immature.
- 11 were on regimen "2", 7 (64%) predominantly mature, and 4 (36%) predominantly immature.

Fisher's exact test shows no statistically significant correlation between regimen used and maturation (P=0.5170).

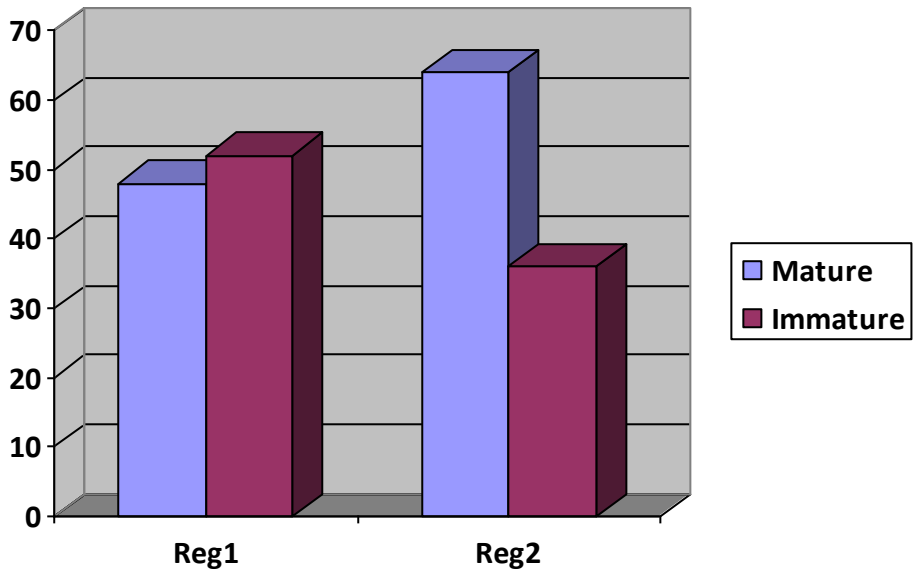


Figure 6: Proportion (%) of cases in each maturation group according to regimen used

5.6 Determining if there is a trend in maturation in relation to number of years postmenopausal.

The duration of postmenopausal period (number of years since last menstrual period) was available for 106 patients. The mean number of years postmenopausal was 6.5 (range 1 to 20 years). Figure 7 illustrates the following results. 40 women were postmenopausal for less than 5 years, 30 for between 5 and 10 years, and 26 for longer than 10 years.

- 40 were postmenopausal for <5 years, 26 (65%) predominantly mature, and 14 (35%) predominantly immature.
- 30 were postmenopausal for 5 – 10 years, 15 (50%) predominantly mature, and 15 (50%) predominantly immature.
- 26 were postmenopausal for >10 years, 11 (42%) predominantly mature, and 15 (58%) predominantly immature.

Chi squared test for trend shows no significant statistical correlation between duration of menopause and cervical maturation (P=0.0627).

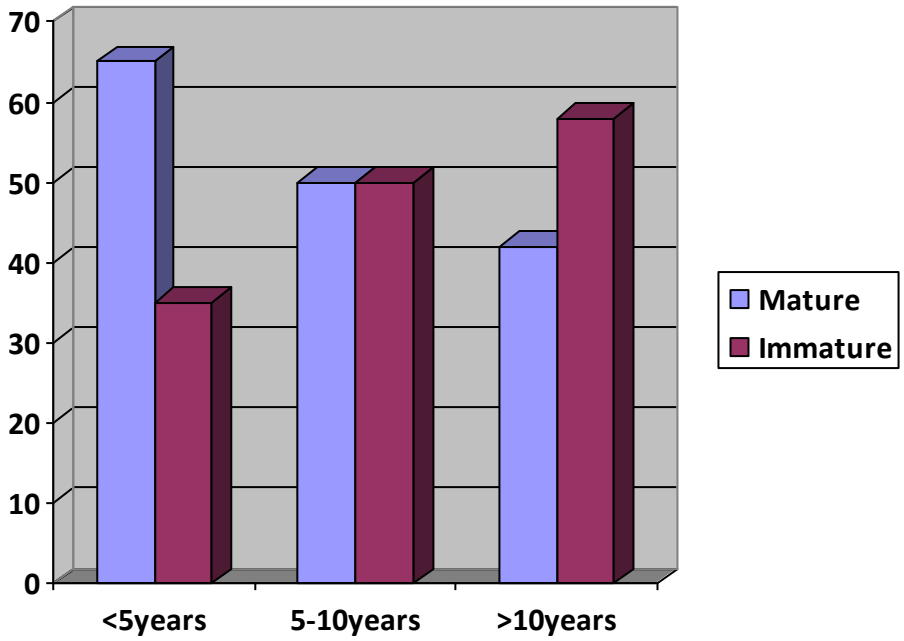


Figure 7: Proportion (%) of cases in each maturation group according to duration since menopause

5.7 Determining if there is a relationship between CD4 count and cervical maturation.

126 patients had a recorded CD4 count. The mean count was 508 $x10^6$/L (107 to 1306 $x10^6$/L). Figure 8 illustrates the following results.

- 19 had a CD4 count less than 250 $x10^6$/L, 8 (42%) predominantly mature, and 11 (58%) predominantly immature.
- 19 had a CD4 count between 250 and 350 $x10^6$/L, 7 (37%) predominantly mature, and 12 (63%) predominantly immature.
- 21 had a CD4 count between 350 and 450 $x10^6$/L, 13 (62%) predominantly mature, and 8 (38%) predominantly immature.
- 21 had a CD4 count between 450 and 550 $x10^6$/L, 10 (48%) predominantly mature, and 11 (52%) predominantly immature.
- 17 had a CD4 count between 550 and 650 $x10^6$/L, 4 (24%) predominantly mature, and 13 (76%) predominantly immature.
- 29 had a CD4 count greater than 650 $x10^6$/L, 15 (52%) predominantly mature, and 14 (48%) predominantly immature.

Chi squared test for trend shows no statistically significant association between CD4 count and maturation (P=0.8745).

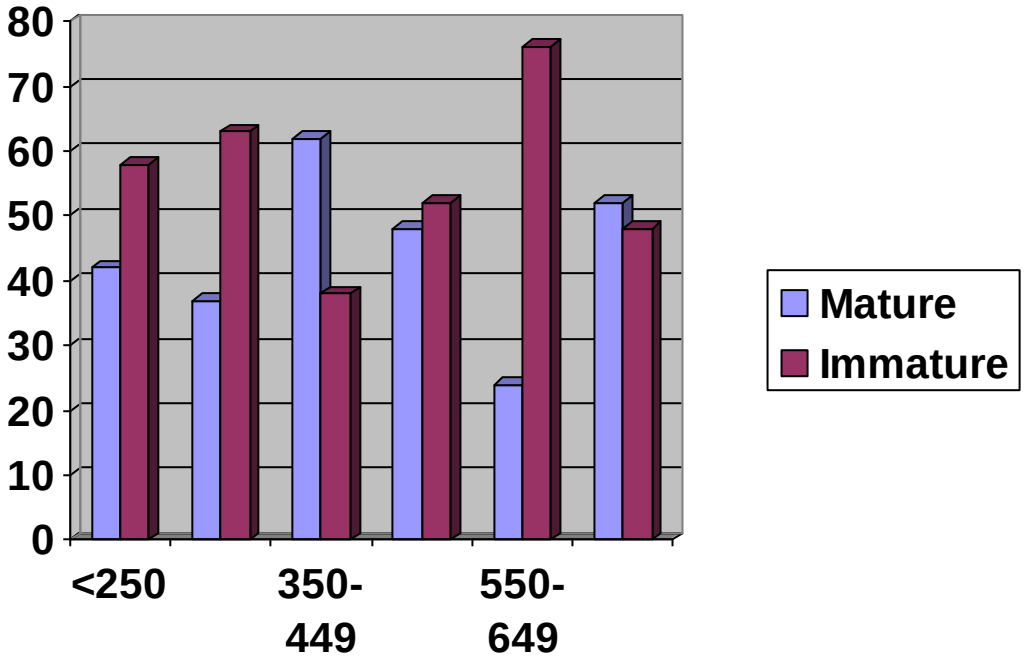


Figure 8: Proportion (%) of cases in each maturation group according to CD4 count (x10^6/L)

When combined, 38 patients had a CD4 count of less than 350 x10^6/L and 88 of greater than 350 x10^6/L. Figure 9 illustrates the following results.

- 38 patients had a CD4 count less than 350 x10^6/L, 15 (39%) predominantly mature, and 23 (61%) predominantly immature.
- 88 patients had a CD4 count greater than 350 x10^6/L, 42 (48%) predominantly mature, and 46 (52%) predominantly immature.

Fisher's exact test shows no statistically significant correlation with CD4 count below or above 350 x10^6/L and maturation (P=0.4393).

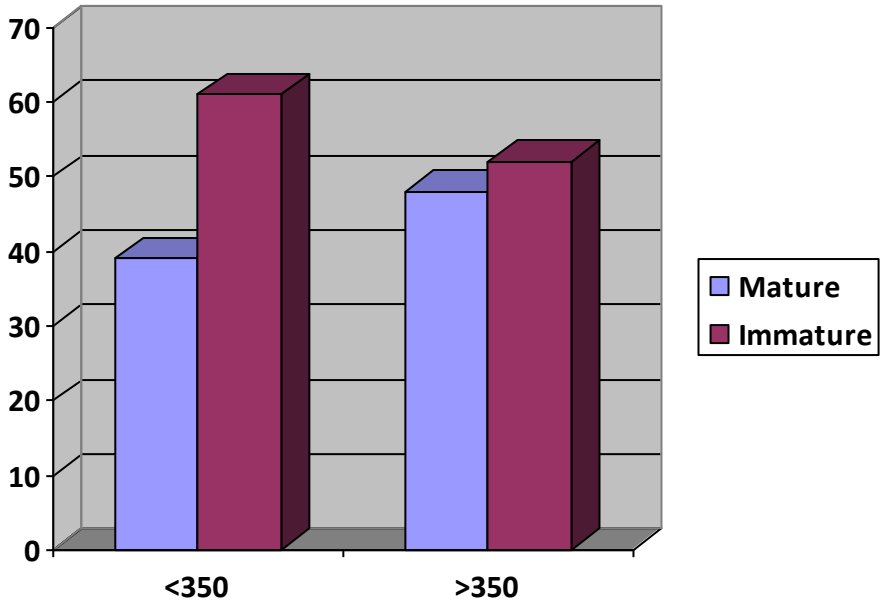


Figure 9: Proportion (%) of each maturation group according to CD4 counts above and below 350 x10^6/L

5.8 Determining if there is a difference in maturation in those started on cART before the postmenopausal period and those started during or after the menopause.

86 patients had a recorded date of menopause and cART initiation. Figure 10 illustrates the following.

- 20 women were started on cART at least six months prior to reported date of menopause, 11 (55%) predominantly mature, and 9 (45%) predominantly immature.
- 18 women were started on cART at the reported date of menopause, 12 (67%) predominantly mature, and 6 (33%) predominantly immature.
- 48 women were started on cART after the reported date of menopause, 22 (46%) predominantly mature, and 49 (54%) predominantly immature.

Chi squared test for trend shows a significant correlation between the stage at which cART was started and maturation (P=0.0127).

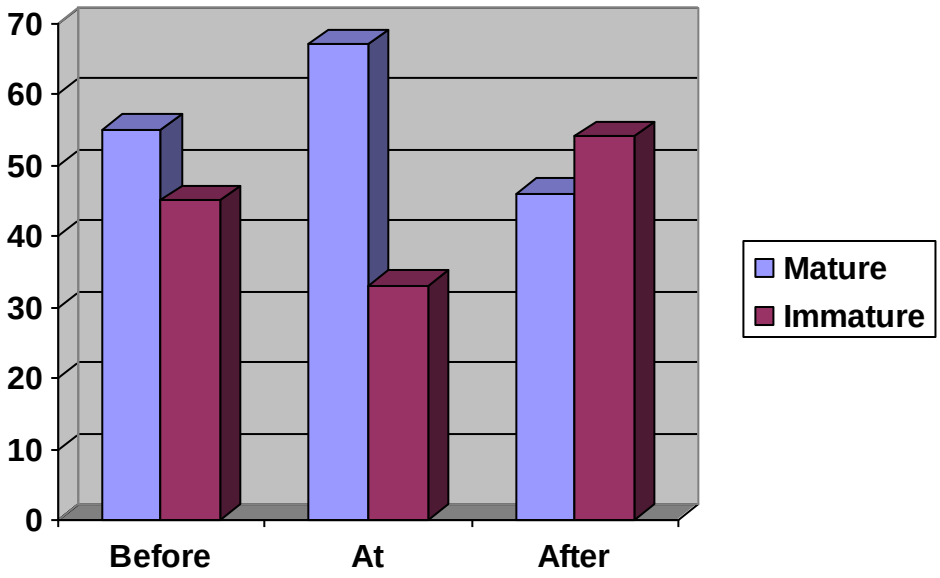


Figure 10: Proportion (%) of cases in each maturation group according to time relative to menopausal date cART started

5.9 Interobserver and intraobserver reliability

5.9.1 Interobserver reliability

129 cases were seen twice (by two different observers independently of each other). 93 cases (72%) were concordant, and 36 (28%) discordant. Of the discordant cases, 16 were major (mature/immature) differences. The remaining discordances were considered minor, eg. mature/obscured by inflammation. Thus there was a major discordance (mature/immature) rate of 12% (interobserver reliability of 88%). This is acceptable (>85%).

5.9.2 Intraobserver reliability

Each observer viewed a set of seven slides, previously seen by them, a second time (5% of the total cases). The results were concordant in 91% and discordant in 9% (91% concordance rate). This is acceptable (>85%).

The above chapter provides the results obtained from the data collected and analysed, with accompanying graphs and figures. This provides the reader with the numerical results obtained from data collection and values obtained from statistical analysis.

CHAPTER 6

6.0 Discussion

In this chapter the results are discussed and interpreted with respect to the aim and objectives of the study.

HIV positive women are expected to live longer with the cART use. The Pap smear is a common screening tool used in the detection of cervical pathology, which occurs with increased frequency in HIV. As a result, Pap smears will be performed more often in this group and thus it is important to be aware of the changes that may be encountered. HIV dyslipidaemia, loss of bone density, anxiety and depression are some of the documented issues facing HIV-infected, postmenopausal women [13,42]. However, the effects of HIV and cART on cervical maturation appears not to have been studied.

6.1 Demographics

The group of HIV positive women not on cART (n = 24) was proportionally much smaller than the group on cART (n = 111). The reason for this is was because of selection bias as a result of using patients from a dedicated HIV clinic. This clinic was chosen due to their use of a dedicated patient record database in an attempt to optimise data retrieval for this study. Ideally, comparisons should be made between groups of equal size. However, using statistical methods (Fisher's exact test), it was possible to compare these groups.

The mean age of menopause in the women included in the study is in keeping with current literature. The mean age of menopause in this study (47.2 years) is slightly less than the average age of 49 years in available literature [5]. HIV positive women are expected to experience menopause at younger ages than HIV negative [11-14]. Thus, the mean age of menopause in this study is in keeping with current literature for this population.

In total, the maturation index was predominantly mature more often than would be expected for the postmenopausal state in day to day practice if one expects that all postmenopausal women should have a predominantly immature cervix. On an individual basis, when one examines a Pap smear from a postmenopausal woman, one would always expect to see a predominance of immature cells, unless there is knowledge of factors which would alter this picture (eg. HRT use). In this study, only 50% of the patients showed a predominance of immature cells. Thus, in day to day practice, a significantly large proportion of Pap smears from postmenopausal HIV positive women are more mature than expected, regardless of cART use. Inflammation can lead to cervical maturation in postmenopausal women. Enhanced vascularity may be important in this regard [43]. There have been some small studies regarding cervical mucosal inflammation in HIV-infected women, with higher rates of CD3(+), CD45(+), CD19(+), CD14(+), Langerin(+), CD24(+), interleukin (IL)-1beta, IL-6 and IL-8 compared to HIV-negative women [44]. Thus it may be possible that increased cervical mucosal inflammation in HIV-infected women is associated with increased cervical maturation. However, this is entirely speculative at this point and further studies would be required to test this hypothesis.

6.2 Determining whether HIV positive women on cART have a significantly different maturation index than expected for the postmenopausal state.

According to this study, postmenopausal HIV positive women on cART are mature more often than expected for the postmenopausal state. In total, the maturation index was predominantly mature more often than would be expected for the postmenopausal state in day to day practice if one expects that all postmenopausal women should have a predominantly immature cervix. On a case by case basis, when one examines a Pap smear from a postmenopausal woman, one would always (100% of cases) expect to have a predominance of immature cells. In this study, only 48% of the patients on cART showed a predominance of immature cells. Thus, in day to day practice, a significantly large proportion of Pap smears from postmenopausal HIV positive women on cART are more mature than expected, regardless of cART use. This value is however not significantly different from that when cART use is not considered.

6.3 Determining if there is a significant difference in maturation between women on cART and those not yet on treatment.

While there appeared to be a nominal difference in proportion of maturation group between the women on cART (52%) and those not on cART (38%), this was not statistically significant. Both groups were significantly more mature than is expected for the

postmenopausal period if one expects all postmenopausal women to have a predominantly immature cervical epithelium, but the apparent nominal difference between the groups is not statistically significant, indicating that the use of cART is at least not the sole reason for this reduced atrophy. As mentioned above, possible cervical inflammation, rather than cART, may be a factor in the cervical maturation seen in HIV positive women, both on and off cART.

6.4 Determining if there is an association between cervical maturation and duration of cART use.

This study has shown no significant association between maturation and duration of cART use. This would seem to support the finding of no significant difference in maturation between those on cART and those not.

6.5 Determining whether there is an association between cervical maturation and the specific cART regimen.

This study has shown no statistically significant difference in maturation according to particular regimen used. However, the number of women on Regimen 2 was very small and these results may not be fully representative.

6.6 Determining whether there is a trend in maturation in relation to number of years postmenopausal.

There is a gradual nominal reduction in maturity with longer duration of menopausal state. However, this trend was not statistically significant in this study. At later stages (>15 years), there is an increase in atrophy. This is in keeping with the physiological decline in oestrogenic stimulation of the cervical epithelium. Although the overall picture in this study is that of an increased likelihood of a mature cervical epithelium into menopause, there is still a slight, although not statistically significant increase in atrophic change with longer duration of menopause.

6.7 Determining if there is a relationship between CD4 count and cervical maturation.

This study has shown no significant association between CD4 count and cervical maturation. Although, according to current literature, women with lower CD4 counts are thought to experience menopause at an earlier age with increased symptoms, [8,11-14] this study has not shown this to translate into a reduced cervical epithelial maturation as one may expect. Again, the reason for this is uncertain.

6.8 Determining if there is a difference in maturation in those started on cART before the postmenopausal period and those started at or after the menopause.

This study has shown a significant association between when cART was started relative to menopause, and cervical maturation. Patients who initiated cART before the menopause showed an increased likelihood of a mature cervical epithelium on Pap smear than those initiated after the menopause, which were more likely to be atrophic.

Thus is seems to be that cART does in fact have an effect on cervical maturation, but only if started before the menopause, and results in a retained mature cervix into the postmenopausal period.

In a postmenopausal woman with a mature epithelium who is not on hormone replacement therapy, expensive investigations looking for the reason for this may be undertaken. Therefore, knowing that HIV positive postmenopausal women, especially those who started cART before menopause, are more likely to have a mature cervical epithelium, will likely reduce the number of additional investigations.

6.9 Interobserver and intraobserver reliability

6.9.1 Interobserver reliability

The interobserver concordance rate with regards to this study is considered acceptable. The lower rate of concordance when minor discordances are included is fair. Thus the rate of agreement between observers with regards to maturation index in this study was acceptable when major discordance is considered.

6.9.2 Intraobserver reliability

The intraobserver concordance rate with regards to this study is considered acceptable. Thus the rate of agreement between maturation index results obtained by the same observer on the same slide at two different points in time in this study is acceptable.

6.10 Limitations

6.10.1 Size of study groups

The size of the group of women not on cART was much smaller than that of the women on cART. The reason for this is thought to be as a result of taking a sample group from a dedicated HIV clinic where cART rollout occurs. It would be ideal to compare groups of similar size and although this was corrected for with statistical methods, may have influenced some of the results in this study and made it less reliable with regards to inferences made in examining this group.

6.10.2 Accuracy and completeness of records

The analysed data relies heavily on accurate and complete record keeping. If records are incomplete or incorrect, the values in this study would be incorrect. When information, such as CD4 count and date of cART initiation, was not available for a particular case, it was not included in that particular test. These cases were not excluded from the study altogether as this cut down the number of the cases not on cART, which was already small.

Some of the information may not be exact. The duration of menopause in some women, may be an estimation. The exact date of last menstrual period is seldom known or recorded. Ideally, exact values should be used and compared.

There was no detail as to the circumstances of menopause, regarding whether this was physiological or as a result of surgical intervention. Women who had undergone hysterectomy without oophorectomy may have been included unknowingly in this study as they would report a last menstrual period in keeping with menopause. Hormonal effect from retained functional ovarian tissue would result in increased cervical maturation as although these women would not menstruate, they would technically not be postmenopausal. However, it is unlikely that there would be enough of such cases to significantly skew this study data.

Cases from the Themba Lethu Clinic, where there is a dedicated database for patient records, were used in an attempt to reduce problems with incomplete and inaccurate records.

6.10.3 Confounding variables

6.10.3.1 Adherence

This study did not take into account patient adherence to cART. Patients are recorded as on cART if that has been prescribed, but does not take into account whether the patient takes the medication regularly and as recommended. This may have an impact on the values in this study, as those patients who do not take cART as prescribed, may not have the same effects as those who do.

6.10.3.2 Other factors affecting menopause

The age of onset and effects of menopause are multifactorial. Several factors may influence the menopause, including BMI, nutrition status, socioeconomic factors and medications, and these would likewise alter the degree of atrophy seen on Pap smear. This study has not taken these factors into account and influence from these variables may explain, at least in part, some of the findings.

6.10.4 Lack of similar research

There is very little available research on the subject of cART use and menopause, or HIV and menopause in general. It would be useful to compare data with existing research on the subject and the challenges experienced in similar studies.

6.10.5 Lack of data from HIV negative women

Ideally, our data should have been compared to a control group of HIV negative postmenopausal women. As addressed in the text, HIV negativity cannot be reliably determined in this retrospective manner with incomplete records. This limitation could be

addressed by performing a prospective study, with a closely monitored HIV negative group where the absence of virus could be proven.

6.11 Recommendations for further studies

More research is needed on this subject, especially given the apparent discrepancy between the results of maturation and cART when stage of initiation is and isn't considered. Ideally, this subject should be studied as a blinded prolonged prospective study, following both HIV positive and proven HIV negative women from before menopause, with detailed, accurate record of age, date of cART initiation, regimen, adherence, additional medication use, date of menopause, menopause symptoms and additional factors such as BMI, diet, socioeconomic status and family history. In addition, clinical examination would be interesting to include, such as colposcopic examination, as well as the incidence and type of squamous intraepithelial lesions.

Any additional study in which HIV positive older people are examined would prove valuable to our current knowledge base on HIV with age.

CHAPTER 7

7.0 Conclusions

There is minimal available literature on the subject on HIV and its treatment in postmenopausal women. This study has demonstrated that on day to day practice, Pap smears from postmenopausal HIV positive women, regardless of cART use and CD4 count, are more likely to appear mature than expected in this age group. In addition, although this study shows no statistically significant difference in maturation with regards to whether cART is used and the regimen given, there is a significant correlation with when cART was initiated in respect to the menopause. This study shows that women started on cART before the menopause retain a mature cervical epithelium, as compared to those initiated after the menopause, who were more likely to be atrophic. Further research is needed to clarify this apparent discrepancy and consider other contributing factors.

This is the first study, to our knowledge, that investigates the effect of HIV and cART on cervical epithelium in postmenopausal women, and the findings thereof will impact on the management of these women, both from a cytologic, as well as a gynaecologic, point of view.

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Appendix 1: Ethics certificate

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131061

NAME: (Principal Investigator)	Dr Gillian Elaine Davies
DEPARTMENT:	Division of Anatomical Pathology National Health Laboratory Services
PROJECT TITLE:	Do Human Immunodeficiency Virus-Positive Postmenopausal Women on Highly Active Antiretroviral Therapy Show Reduced Cervical Atrophy?
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Pamela Michelow
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 15/11/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

03/12/13
M131061Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2: Data capture sheet

Case no.	Adequate (Y/N)*	No. Basal cells	No. Intermediate	No. Superficial	Age	Years menopausal	cART (Y/N) **	**Reg	**Duration	HRT (Y/N)	CD4

*If N, qualify with the following.

1. Obscured by inflammation

2. Extensive dysplasia

3. Inadequate ectocervical component

** If Y, state duration and regimen.