The Natural History of Low Grade Squamous Intra-epithelial Lesions in Women attending Groote Schuur Hospital Colposcopy Clinic

Title:

The Natural History of Low Grade Squamous Intra-epithelial Lesions (LSIL) in Women attending Groote Schuur Hospital Colposcopy Clinic.

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OBSTETRICS AND GYNAECOLOGY , University of Cape Town

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Author's Declaration

This study represents original work by the author and has not been submitted in any form to another university. Where use was made of the works of others, it has been duly acknowledged in the text.

The research topic is entitled: "The Natural History of Low Grade Squamous Intra-epithelial Lesions (LSIL) in Women attending Groote Schuur Hospital Colposcopy Clinic.**"**

Researcher: Dr K Govender: **Kamendran Govender**

Date: **03/12/2015**

Reason for submission

This dissertation “The Natural History of Low Grade Squamous Intra-epithelial Lesions (LSIL) in Women attending Groote Schuur Hospital Colposcopy Clinic”, is in part fulfilment of the requirement for the degree of MPhil in Gynaecology-Oncology at the University of Cape Town. It is also done in part fulfilment of the Certificate in Gynaecology-Oncology of the College of Medicine of South Africa

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Key Words :

Low grade intra-epithelial lesion (LSIL)

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Progression

Regression

Natural history

Colposcopy

Human immune-deficiency virus (HIV)

Cigarette smoking

Menopause

Cancer Cervix

Contraception

Abstract

Title: The Natural History of Low Grade Squamous Intra-epithelial Lesions (LSIL) in Women attending Groote Schuur Hospital Colposcopy Clinic

Author: Dr K Govender **Supervisor:** Prof L Denny

Aims and Ojectives:

To identify risk factors affecting rates of progression and regression of LSIL

To determine the rates of progression and regression of disease in women with LSIL

To compare the natural history of LSIL in HIV positive and negative women

To determine patient adherence to colposcopy clinic in women with LSIL

Methods: This is a retrospective, descriptive, cohort study of women who were referred to the colposcopy clinic with a cytological diagnosis of LSIL and followed for a minimum period of 2 years. Data was extracted from the colposcopy clinic data. Women who were referred and attended the clinic between 1st January 2009 and 31st December 2013 were included in the analysis. Statistical analysis was performed using Stata version 13.1 (StataCorp LP,4905 Lakeway Drive, College Station, TX 77845, USA). . All p-values <0.05 were deemed statistically significant.

Results: The study population was 154 women with LSIL (N=154). Of these, 27 (17%) women were HIV negative, 106 (69%) were HIV positive and 18 had an unknown HIV status. The overall regression rate from LSIL to normality was 88.5% [95% CI = 83.9 – 92.1%], with 128 of the 154 women having had regression of disease. The overall progression to higher grade lesions included 31 women, giving a progression rate of 17.7% [95% CI = 15.4 -22.8%]. None of these women progressed to invasive cancer.

HIV positive women made up 69% of the study population but there was no significant difference in regression and progression between the HIV positive and negative women. The mean age of the group was 37.8 years with 60% of women screened at this clinic falling between 30-49 years of age. All age groups had similar trends of regression and progression, but those over 60 years of age were 12 times more likely to regress and none of them progressed to HSIL [p=0.002]. Those using an IUCD made up 141 person-months studied, they were shown to have a 6 times greater likelihood to result in regression (p=0.01) compared to women on no contraception.

Conclusion: The high regression and low progression rates of LSIL are in keeping with global data and substantiate the need for surveillance rather than surgical intervention ie LSIL is a risk factor rather than a precursor for cervical cancer. The lack of difference in regression-progression rates despite HIV status means we can follow-up positive and negative women similarly (12 monthly). Older women (60+) are most likely over-called during diagnosis due to genital atrophy and thus follow-up interval can be longer than 12 months. More research is required to assess IUCDs' effect on LSIL regression and to ascertain the possible reasons for patient adherence.

1. INTRODUCTION AND BACKGROUND

1.1 Epidemiology

Cervical cancer is, world-wide, the fourth commonest cancer affecting women, behind breast, lung and colorectal cancer respectively. In 2012, an update of the Globocan datatabase, placed a global estimate of 529 512 cervical cancer cases diagnosed annually. Eighty six percent of these cases (n = 453 032) were diagnosed in developing countries[1]. It was estimated that 274 967 women died from cervical cancer in the same year, of which 88% (n = 214 818) were living in developing countries. The mortality to incidence ratio in developed countries was 36 - 43%. This was far lower when compared to developing countries where estimates of 54 - 80% have been reported[1,2]. This clearly highlights that cancer of the cervix is an important disease that causes significant morbidity and mortality on a global scale, particularly in low to middle income countries (LMICs). The tragedy of these statistics, both internationally but especially in developing countries, is that cervical cancer is a preventable condition. All invasive pathology is preceded by a precursor phase which is detectable through various methods of cervical screening and sampling.

1.2 Histology of the cervix

Most of the cervix is composed of fibromuscular tissue whilst the epithelium is either squamous or columnar in nature. The endocervix is lined by columnar epithelium that secretes mucus. It has has infoldings that resemble glands or clefts, although are not true glandular structures.

The ectocervix is covered by nonkeratinizing, stratified squamous epithelium, and has basal, midzone and superficial layers[3]. After menopause, these layers become atrophic and may resemble dysplastic cells which are characterized by high nuclear:to cytoplasmic ratios and often lead to false positive cytological diagnoses. A similar problem is encountered in women who are hypoestrogenic, including prepubertal girls, who fortunately rarely undergo cytological screening.

The squamo-columnar junction (SCJ) is where squamous and columnar epithelia meet. This junction can migrate depending on oestrogen status. Estrogen is responsible for depositing glycogen into squamous cells. These cells are in a constant state of desquamation and the glycogen is converted to lactic acid by the doderlein bacilli, natural commensals of the vagina. The lactic acid is responsible for the normal vaginal pH being less than 4.5 and it is this acidity that is believed to stimulate the process of metaplasia: a process whereby one mature type of epithelium is converted to another[3]. In the case of the cervix, the exposed columnar epithelium is converted to squamous epithelium, creating the transformation zone. The transformation zone is the area between original SCJ and new SCJ, and is the site of 90% of squamous cell carcinomas and dysplasia (Refer figure 1).

Basal cells are derived from SCJ cells, are cuboidal to low columnar with scant cytoplasm and round/oval nuclei. They become more eosinophilic as they mature.

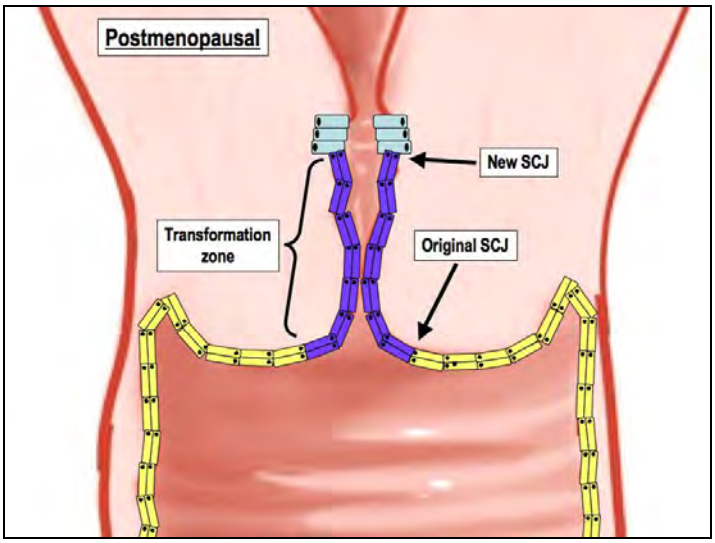

Figure 1: Epithelial lining of the endocervix and ectocervix

(http://micro2tele.com/2013/01/20/histoquarterly-cervix/faces-of-cervix-6/)

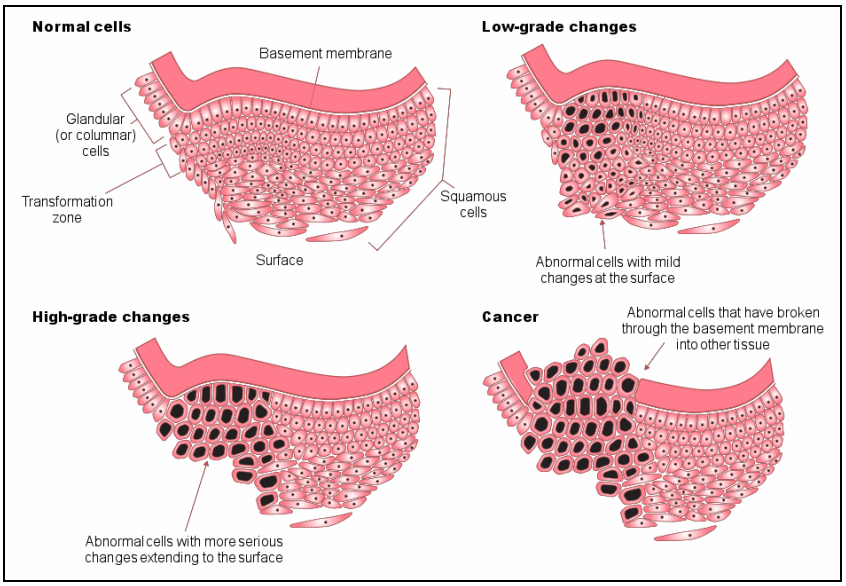

Figure 2: Levels of cellular dysplasia

(https://www.healthed.govt.nz/resource/prevention-cervical-cancer-guide-women-new-zealand)

1.3 Pathophysiology

Human Papilloma Virus (HPV) is a double stranded Deoxyribose Nucleic Acid (DNA) virus with over 100 subtypes. Approximately 40 of these are specific to the ano-genital region, some (like HPV 6 and 11) causing benign but morbid disease, whilst others (like HPV 16 and 18) are responsible for premalignant and malignant conditions. The virus particle, which is sexually transmitted through skin to skin contact, colonises the epithelium of the genital tract and in the cervix, has a preponderance for the squamo-columnar junction.

The natural history of cervical cancer has been studied extensively for the past 30 to 40 years, and persistent infection of the cervix with certain high-risk types of HPV has been well established as a necessary cause of cervical cancer[4]. HPV is a very common sexually transmitted infection, usually acquired soon after initiation of sexual activity. Most HPV infections clear spontaneously within 1 to 2 years, but those that persist, particularly high-risk types of HPV (including HPV 16 and 18), may progress to cervical cancer precursors, and ultimately to invasive cervical cancer. High-risk types of HPV are identified in nearly all cancers of the cervix, and the relative risk of cervical cancer associated with persistent, ongoing infection with high-risk types of HPV is higher than the risk of lung cancer associated with smoking. Munoz and colleagues pooled data from 11 case-control studies involving 1,918 women with histologically confirmed squamous cell carcinoma of the cervix and 1928 control women[5]. The pooled odds ratio for cervical cancer associated with the presence of any HPV infection was 158.2 (95% CI: 113.4 – 220.6). On the basis of the pooled data, 15 HPV types were classified as high-risk (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and are considered carcinogenic. The International Agency for Research on Cancer (IARC) includes the following types as Group One human carcinogens: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. HPV 16 and 18 are responsible for about 70 percent of cases worldwide[6]. There is little geographic variation in the predominant HPV types associated with cervical cancer.

The virus may remain in an episomal state and in the majority of cases, is cleared by cell mediated immunity. However, once the viral DNA incorporates its DNA into that of

the nucleus of the host epithelial cell, the control of the cell cycle is altered, viral genes are expressed and epithelial cells begin proliferating and causing pathologic and morphological changes to the cell. These changes are seen on cytological examination of the cells and after staining with the Papanicoloau stain and graded according to severity as low grade squamous intra-epithelial lesions (LSIL). high grade squamous intra-epithelial lesions (HSIL), invasive cancer and/or glandular abnormalities.

HPV infection can be detected by cytology as well as by molecular testing. In essence HPV infection of the cervix is a proxy for LSIL and should be seen as a risk factor for cervical cancer (as smoking is for lung cancer) but not a true cervical cancer precursor. Fortunately, most patients infected with HPV or with the diagnosis of LSIL will regress spontaneously without any clinical consequences for the patient. . However if infection of the cervix with high-risk types of HPV persists, progression is HSIL or cancer is likely to occur.[7] Once the basement membrane is breeched, causing stromal invasion, progression to invasive cervical cancer occurs. HPV infection of the host cell, integration into host DNA, DNA replication, viral spread and evasion of host immunity are all mediated by proteins coded for by viral DNA. There are 7 early (E) proteins and 2 late (L) ones.

The table below shows the broad functions of the different proteins expressed by HPV DNA.

Table 1: HPV proteins and functions

Protein	Functions
E1	mediates viral DNA replication
E2	Controls viral transcription and DNA replication
E4	regulates the expression of late genes
E5	Enhances the activity of E6 and E7 and assists to immune-response evasion
E6	inhibits apoptosis
E7	Inhibits apoptosis and assists in immune reponse evasion
L1	Major capsid protein and very immunogenic
L2	Minor capsid protein

(Fernandes JV, Araújo JMG, Fernandes TAAM, Biology and natural history of human papillomavirus infection, OAJCT, 2013:5)

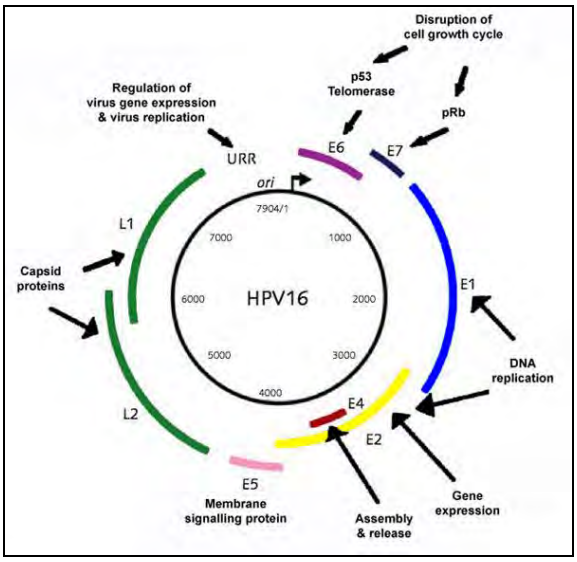

Figure 3: Illustration of HPV protein function

(http://www.microbiologybytes.com/virology/Papillomaviruses.html)

1.4 Genetics and Immunology of Natural History

The genital infection by HPV is transmitted by direct contact with skin or epithelium usually, but not exclusively via vaginal or anal intercourse. In their lifetimes, approximately 50%–80% of sexually active individuals, will acquire a genital HPV infection, with peak incidence being close to sexual debut[7].

Incubation periods are variable ranging from weeks to months to years. At this stage, the cell contains HPV and may lay dormant or have lower levels of infectious virus being produced and released. Higher levels of this occur in the active replication phase, which also has a variable duration but over time most individuals will develop an effective immune response, eliminating cells containing viral DNA and thus the virus itself[8]. Cell-mediated immunity against early proteins, especially E2 and E6, is implicated in

maintaining immunity against the virus. This immune response promotes regression of the intra-epithelial disease[9]. Approximately 10% to 20% of individuals will not effectively clear the virus and will develop persistent infection placing them at higher risk for progression and development of cervical carcinoma[10].

After the infection phase, infectious HPV is exclusively housed in an *episomal state* in the basal cells. This is called the *maintenance phase*, where the virus is maintained in the basal cells of epithelium with minimal viral gene expression present in the proliferating epithelium. This phase is primarily mediated by E6 and E7; and due to low episomal count and minimal genetic expression, immune response is minimal in this phase.

During the *differentiation-dependent* phase, when HPV-infected cells leave the basal layer, they undergo differentiation, and high levels of viral protein synthesis are induced. By restricting viral protein synthesis to highly differentiated cells, there is a delay in the expression of viral antigens to locations, making HPV less susceptible to host immunity[11].

Finally, the *shedding stage* is reached where a putative late promoter activates the capsid genes (L1 and L2), allowing the viral particles to assemble in the host nucleus, just before the complete virions are released from the outer epithelial layers. This occurs during normal physiological desquamation, thus avoiding inflammation (ie host immunity) associated with lysis or necrosis allowing for increased persistence[12].

Persistence and DNA viral integration into the host cell leads to an increased risk of epithelial dysplasia because of the genomic instability. E5, E6, and E7 can induce cellular abnormalities: fusion between cells, aneuploidy, chromosomal instability, and abnormal centrosome reduplication resulting in abnormal numbers of centrosomes. There is also activation and inactivation of p53 and pRB family members respectively allowing for further chromosomal abnormalities[13].

1.5 Screening and Prediction

With the introduction of the Papanicolau smear test (Pap) in the 1950s, cervical cancer rates declined by more than 60% in the United States[14], and in Europe it declined by 84% in Iceland, 50% in Finland, 34% in Sweden, 27% in Denmark and 11% in Norway[15]. Pap smears are cytology based tests looking for dysplastic cells of the transformation zone of the cervix. Depending on specific cellular features, a cytopathologist can grade such cells as normal or atypical. Atypical cells, in turn, may be categorized as HSIL or LSIL as previously described.

Prior to the 1970's, noninvasive intra-epithelial lesions of the cervix were classified using a 4-tiered terminology: mild dysplasia, moderate dysplasia, severe dysplasia, and carcinoma in situ[16]. In the early 1970s, Richart[17] introduced the concept that all precursor lesions were part of a spectrum of a single disease process: cervical intraepithelial neo-plasia (CIN) [refer figure 2]. This 'terminology chaos' led to a large conference held in Bethesda, Maryland, USA in 1988 in order to standardize

terminology. This new system combined clinically similar cytological and histological types into a number of broad categories, the most important being: LSIL (koiliocytic cytologic atypia and CIN 1) and HSIL (CIN 2 and 3)[18]. It is currently the universal standard when describing intra-epithelial lesions. Richart's CIN nomenclature is still used for histological description but as the Bethesda classification has more clinical relevance due to difference in behavior between HSIL and LSIL, our study will be referring to HSIL and LSIL diagnoses for both cytology and histology.

This study focuses on women referred to the Colposcopy clinic at Groote Schuur Hospitalclinic with a cytological diagnosis of LSIL and specifically to determine factors associated with regression or progression of LSIL lesions. This will help to better target, evaluate, and treat patients who are at highest risk of higher grade lesions and invasive cancer. Diagnostic and therapeutic measures triggered by abnormal cytology or colposcopy, which include cold knife conization, large loop excision of the transformation zone (LLETZ) procedures, laser excision of cervical lesions and cautery to the cervix have been shown to be associated with a number of complications which include acute and chronic perineal pain, sexual dysfunction such as dyspareunia, miscarriage and pre-term labour, reduced quality of life, and are an increased financial burden to patient and health facility[19].

If it were possible to predict the risk of a woman with an LSIL Pap smear progressing to an HSIL lesion or invasive cancer, it will improve patient experience by exposing less women to invasive procedures. This will also greatly reduce financial burden to the health care system. Newer and more sensitive screening laboratory tests have been developed over the last few years, like HPV DNA[20], but these tests lack adequate

specificity, are currently expensive and where introduced., positive HPV DNA tests have been seen to increase the number of women who are screened positive rather than decreasing them. This study has therefore been designed to evaluate the value of risk prediction formulae in managing clinical outcomes.

There is a precedent for using *risk prediction formulae* in order to use evidence based models for predicting outcomes, eg as physicians make use of age, BMI, cigarette smoking status, family history etc to evaluate cardio-vascular disease potential in their patients[21]. This helps him or her triage care and improve decision making. All of this is pre-empted by data available in the clinical history.

Several risk factors have been shown to be associated with an increased risk for both the persistence and progression of precancerous lesions of the cervix. These include age, cigarette smoking, number of sexual partners, gravidity, number and regularity of Pap tests, immune suppression (e.g., HIV infection/ long term corticosteroid use among others), and long-term use of oral contraceptives[22]. Can any one of or a combination of these factors be used to predict progression, regression and persistence potential of women with LSIL?

1.6 South African cervical cancer policy for colposcopic referral and risk factors for cervical cancer precursors

In sub-Saharan African countries, human papilloma virus (HPV) screening is not yet available, but formal HPV vaccination campaigns have commenced in in a number of

pilot projects[23]. This has led to the introduction of a South African HPV vaccination program as of 2014 aimed at 9-12 year old school girls.

The South African national cervical cancer screening program offers 3 free Pap smears per lifetime to asymptomatic women, after the age of 30, 10 years apart for those who screen negative. If a low-grade abnormality is found, the smear is repeated after 6-12 months. Referral threshold for colposcopy includes[24]:

- 3 consecutive atypical squamous cells of undetermined significance (ASC –US)
- 1 Pap described as ASC –H (i.e. ASCUS but HSIL not excluded)
- 2 consecutive low-grade squamous intraepithelial lesion (LSIL)
- 1 high-grade squamous intraepithelial lesion (HSIL) or macroscopically suspicious lesion
- Any glandular abnormality in cytology is also a criterion for colposcopy referral.

HIV infected women have been found to have a higher prevalence of HPV infection (up to 68%).[25,26] For women known with HIV infection, the clinical guidelines for the management of HIV and AIDS in adults and adolescents advises 1 Pap smear at diagnosis of HIV and then every 3 years if normal, regardless of antiretroviral therapy (ART) status[27].

In South Africa, referral of HIV-positive women for colposcopy evaluation is encouraged after the first abnormal smear If a high-grade lesion is visualized the policy at the GSH colposcopy clinic is to perform an immediate excisional procedure (LLETZ or large loop excision of the transformation zone) or a punch biopsy is performed is there is doubt and only fi the biopsy confirms a high-grade abnormality, an excision procedure is performed. LLETZ is performed under local anaesthesia in an outpatient setting as long

as the entire lesion is visible, including the upper limit, HSIL is colposcopically confirmed, there is no evidence of a glandular lesion or of microinvasion. Cold knife cone excision (CKC) is performed in cases where there is suspected early invasive carcinoma, adenocarcinoma in situ, large lesions or distorted anatomy not suitable for LLETZ, a discrepancy between cytology and/or histology and colposcopy or where colposcopy and LLETZ are not possible. Many patients are treated solely on the combined assessment of the Pap and the colposcopy – so – called 'see and treat' approach. The LLETZ is performed without prior punch biopsy at the first visit, to avoid patients having to return to the clinic for extra visits. This is the usual approach of Groote Schuur Hospital Colposcopy Clinic. In young women where there is still a wish for future pregnancy, confirmatory biopsies are required before LLETZ or CKC.

1.6.1 HIV

It has been shown that there is an altered course of HPV infection and development of cancer in HIV-infected women as opposed to those who are not HIV-infected. There is an increased persistence of genital HPV infection due to the immune-compromised state[28] and that cervical lesions are more likely to progress to high-grade lesions and do so more rapidly[29]. In addition, it has been reported that there are trends of lower HPV and LSIL rates in those who have initiated anti-retroviral therapy due to lower viral loads and thus greater immunity[30].

However, global opinion regarding regression and progression is not uniform with some studies, like that of Massad et al which shows no evidence of increased progression to

higher grade lesions in women who are HIV positive[31]. This was similar to data published by Heard et al regarding HIV positive women infected with high risk HPV DNA types[32].

In South Africa, the MACH-1(Management of abnormal cytology in HIV-1 positive women) Trial, showed that HIV status alone could not predict the presence of high risk HPV DNA types and in this study, the only significant predictors of abnormal cytology were high viral loads and low CD_4 counts in HIV positive women[33].

1.6.2 Smoking

Cigarette smoking has always been known to be one of the leading preventable causes of death. There is convincing evidence that tobacco smoking causes cardiovascular disease, chronic lung disease, and several epithelial cancers including lung cancer[34,35]. There has been a rise globally in both the incidence and prevalence of cigarette smoking. This is seen particularly among younger women[35]. There is also evidence that it may interfere with regression of cervical precursor lesions, thus resulting in the persistence cervical abnormalities[36].

There are several mechanisms theories implicated in carcinogenesis and halted LSIL regression associated with cigarette smoking. One molecular theory involves direct exposure of cervical epithelial cell DNA to nicotine and cotinine, or aromatic polycyclic hydrocarbons found in tobacco smoke with subsequent dysplasia and carcinogenesis[37]. Cervical mucus of smokers contains cigarette constituents and their metabolites such as benzo-a-pyrene (BaP). BaP causes HPV genome amplification which can increase the probability of viral DNA integration into the host genome[38]. It has also been shown

that long-term nicotine exposure could affect persistent cellular proliferation, inhibition of apoptosis, and stimulation of vascular endothelial growth factor (VEGF), with increased microvessel density[39]. Smoking also results in abnormalities in systemic and peripheral immunity, eg. unbalanced production of pro- and anti-inflammatory cytokines, suppression of T lymphocyte activity, diminished numbers of T lymphocytes, and low levels of immunoglobulins. All of these can result in decreased numbers and activity of Langerhans cells in the cervix of smokers; cells which play an important role in averting carcinogenesis and eliminating dysplastic cells[40].

1.6.3 Contraception

The risk of cervical dysplasia seems to vary with the type of contraceptive method in question. There has been a multitude of research addressing the impact of oral contraception (OC) in HSIL and LSIL, cervical carcinoma *in situ* (CIS), and invasive cervical cancer (ICC) development. The majority found an increased risk of ICC and CIS associated with long-term (>5 years) OC use. A meta-analysis on the association between hormonal contraceptives and cervical cancer concluded that there was a linear dose-response relationship between the two, but that the relationship tends to disappear with time after OC cessation[41]. However, there is no relationship as yet seen between OC usage and LSIL[42-44].

Logically, it can be assumed that although not complete, barrier methods should provide some decrease in HPV infection rate. Consequently, barrier methods have been seen to be associated with an overall reduced risk of LSIL[45].

Levonorgestrel implants and intra-uterine systems have only recently been widely available. Results of studies to date of these contraceptive methods display no association increased risk of cervical SIL, or risks of progression or regression[46].

1.6.4 Menopause

A quarter of all women who are screened within the UK national cervical screening programme are over the age of 50[47]. Most of these women are menopausal and will thus have lower circulating serum oestrogen levels. A hypo-oestrogenic state in the cervical epithelium could result in possible cytological changes. These changes may be difficult for a cytopathologist to distinguish from dysplsia seen in LSIL[48]. A menopausal state also makes colposcopy difficult due to decreased collagen content and atrophy leading to unsatisfactory colposcopy more often than not. In addition, the thinner epithelium results in an aceto-white change that may mimic CIN, compounded by the lack of intracellular glycogen that may result in partial iodine negativity[49]. Also in the background of atrophy, the nucleus to cytoplasm ratio in the basal cells are similar to that of LSIL. The false positive rate in this subset of women is thus high.

1.7 Patient adherence to colposcopy clinic

Patients with abnormal pap smears who fit referral criteria (as previously discussed) are referred to a colposcopy clinic and this forms an integral part of cervical cancer screening programs. However, when patients fail to attend recommended colposcopy appointments, they are defined as non-compliant. This is a common problem on a global scale with rates varying from 0.4% to 47.3%[50].

A failure of recommended follow-up could result in not identifying HSIL timeously and thus potentially allowing progression of premalignant lesions to cancer[51].

From a service point of view, non-adherence results in inefficient use of already scarce health care resources, thus elevating national and global expense[52].

Risk factors for non-adherence may include younger age; lack of knowledge about cervical screening and colposcopy; lower grade abnormalities; lack of child care, time, or transportation; patient anxiety and lower socioeconomic status[53].

2. Material and Methods

2.1 Aim

To identify risk factors affecting rates of progression and regression of disease in women with LSIL

2.2 Objectives

1. To determine the rates of progression and regression of disease in women with LSIL

2. To compare the natural history of LSIL in HIV positive and negative women

3. To determine patient adherence to colposcopy clinic appointments in women with LSIL

2.3 Study Design

2.3.1 Sampling strategy and Study population:

This is a retrospective, descriptive, cohort study of women who were referred to the colposcopy clinic with a cytological diagnosis of LSIL and followed for a minimum period of 2 years. Data was extracted from the colposcopy clinic data base which captures all new and follow up patients referred to the clinic. Women who were referred and attended the clinic between 1st January 2009 (when the database began) and 31st December 2013 were included in the analysis.

2.3.2 Sample Size:

Since this was a descriptive study, the power of the study was not considered in sample size calculation, but rather the precision that the sample size will provide to estimate a given parameter of the population. Since many parameters are being investigated, it was decided the sample size should be decided on logistical and time constraints rather than statistical validity.

2.3.3 Inclusion Criteria

The study included all women with LSIL whose data were entered onto the GSH data base and who attended the GSH Colposcopy Clinic between January 2009 to December 2013.

2.3.4 Exclusion Criteria

Those women who had too much missing data to be able to add to data analysis and those who were incorrectly diagnosed upon referral were excluded from this study.

2.3.5 Variables extracted from database:

1. Age of at each point of contact with the colposcopy clinic
2. Menopause status and years of menopause at each point of contact
3. Contraception type and years of use at each point of contact
4. HIV status and years since diagnosis at each point of contact
6. CD4 counts and trend over time
7. Duration of ARV therapy
8. Cigarette smoking at each point of contact

2.3.6 Data analysis

Data was collected from the Groote Schuur Hospital Colposcopy Clinic Data Base and after all exclusion criteria were met, included subjects were analysed by both actual number and as a product of their visits, expressed as patient-months. Visits and laboratory results were consolidated to 6 monthly and yearly intervals for ease of analysis. At least 64% of our population were compliant for a minimum of 726 days. For this reason, we have chosen the time period of 726 days to analyse and describe regression and progression trends. Histology results were used as the gold standard where available, but not all visits included a tissue biopsy for histology. In these cases, cytology results were used in data analysis. Regression was defined as subsequent histology an cytology showing no evidence of dysplasia or malignancy following and initial LSIL diagnosis. Progression was defined as subsequent histology and cytology showing either HSIL or malignancy following an initial LSIL diagnosis. Descriptions of individual parameters were expressed both in terms of absolute patient number, as well as the number of times the parameter was observed which may been more than once in a given patient (observations).

Statistical analysis was performed using Stata version 13.1 (StataCorp LP,4905 Lakeway Drive, College Station, TX 77845, USA). The distribution of continuous data was assessed graphically and using the Shapiro Wilk test. Normally distributed data were summarised using means and standard deviations and analysed using parametric methods (independent or paired t-tests as appropriate, or analysis of variance for the comparison of 3 or more means), Skewed data were summarised using medians and

interquartile ranges and analysed using non-parametric methods (Mann-Whitney-U-tests or Wilcoxon signed rank tests for paired data as appropriate, or the Kruskal Wallis test for the comparison of 3 or more medians). Binomial 95% confidence intervals were used to report the precision of binary variables. Time-to-event data were explored graphically using Kaplan-Meier plots and log-rank tests, followed by Cox proportional Hazards regression to estimate Hazard Ratios and their respective 95% confidence intervals). All p-values <0.05 were deemed statistically significant.

After univariate analysis of all variables were carried out, those deemed to be of most significance were entered into a multi-variate analysis. These included:

- Age
- HIV status
- CD4 counts
- Contraceptive status

2.4 Ethical Consideration

During this study all principles of the Declaration of Helsinki were upheld. Participation in this study did not affect patient management in any way. This was a retrospective study looking at patient information recorded in a data-base; patient names were replaced by a study number to ensure confidentiality. No patient contact occurred, and there was thus minimal risk to patients. Ethical approval for establishment and maintenance of the colposcopy data base had

been obtained in 2011 (HREC REF: 344/2011). In addition, ethical approval was obtained for this study (HREC REF: 108/2014).

3 RESULTS

Within the study period, 208 women met the criteria for inclusion due to referral with a LSIL Pap. Thirty nine women were excluded, however, due to an incorrect diagnosis entered into the data base upon referral and 15 were excluded due to large amounts of irretrievable data not amenable to analysis. This left a study population of **154 women** with LSIL as the reason for the referral to the colposcopy clinic. Of this group, 27 (17%) women were HIV negative, 106 (69%) were HIV positive and 18 had an unknown HIV status. The study population generated 252 observations during which time there 223 observations of regression from LSIL to normal, making the overall regression rate 88.5% [95% CI = 83.9 – 92.1%]. One hundred and twenty eight of the 154 women had regression of disease to normal. The overall progression to higher grade lesions included 31 women and 48 observations giving a progression rate of 17.7% [95% CI = 15.4 -22.8%]. None of these women progressed to invasive cancer.

3.1 HIV

The following tables and figures contain descriptive statistics of patient population by HIV status, hazard ratios and Kaplan Meier curves for progression/regression.

TABLE 2.1: Description of study population vs HIV status

	HIV NEGATIVE	HIV POSITIVE	HIV UNKNOWN	TOTAL
AGE (in years)				
< 30	4 (2)	23 (15)	2 (1)	29 (19)
30-39	9 (6)	64 (42)	6 (4)	79 (51)
40-49	6 (4)	23 (15)	5 (3)	34 (22)
50-59	4 (2)	1 (1)	4 (2)	9 (5)
60-69	1 (1)	0	1 (1)	2 (1)
70+	1 (1)	0	0	1 (1)
MENOPAUSE				
Pre-menopausal	19 (12)	107 (69)	12 (8)	138 (90)
Post-menopausal	6 (4)	1 (1)	6 (4)	13 (8)
CONTRACEPTION				
None	13 (8)	46 (30)	11 (7)	70 (49)
Combined oral	4 (2)	1 (1)	1 (1)	6 (4)
Injectable	8 (5)	32 (21)	4 (2)	46 (32)
IUCD	1 (1)	1 (1)	0	2 (1)
Tubal ligation	1(1)	16 (10)	2 (1)	19 (13)
SMOKING	3 (2)	17 (11)	1 (1)	21 (14)
PROGRESSION	5 (3)	25 (16)	1 (1)	29 (18)
REGRESSION	22 (14)	89 (58)	17 (11)	128 (88)
TOTAL	27	106	18	154

( ) = %

HIV positive women made up 69% of the women included in this study. However, over a time period of 726 days, there was no significant difference found in both the regression (Table 2.2) and progression (Table 2.3) rates of HIV infected women as compared to those who were uninfected. HIV positive women were further sub-analysed with regard to CD4 counts and found to still have no discernable difference in regression and progression rates compared to their negative counterparts by CD4 count. Commencement of or duration of anti-retroviral therapy also did not alter the trends displayed. Regression and progression curves were not significantly different from those of women whose status was unknown compared to HIV infected and uninfected women.

Table 2.2: Regression rate vs HIV negative women

	Hazard Ratio	**P-value**	**95 % Confidence interval**
HIV positive	0.798	0.357	0.494 - 1.290
HIV unknown	0.908	0.773	0.470 - 1.760

Table 2.3: Progression rate vs HIV negative women

	Hazard Ratio	**P-value**	**95 % Confidence interval**
HIV positive	1.240	0.662	0.473 - 3.245
HIV unknown	0.264	0.225	0.307 – 2.266

Regression decreased with time, primarily in year one. Sixty five out of 89 HIV positive women who regressed (74%) did so in year 1. Similarly, 71% of HIV negative women and 69% of HIV unknown women who regressed, experienced regression in year 1.

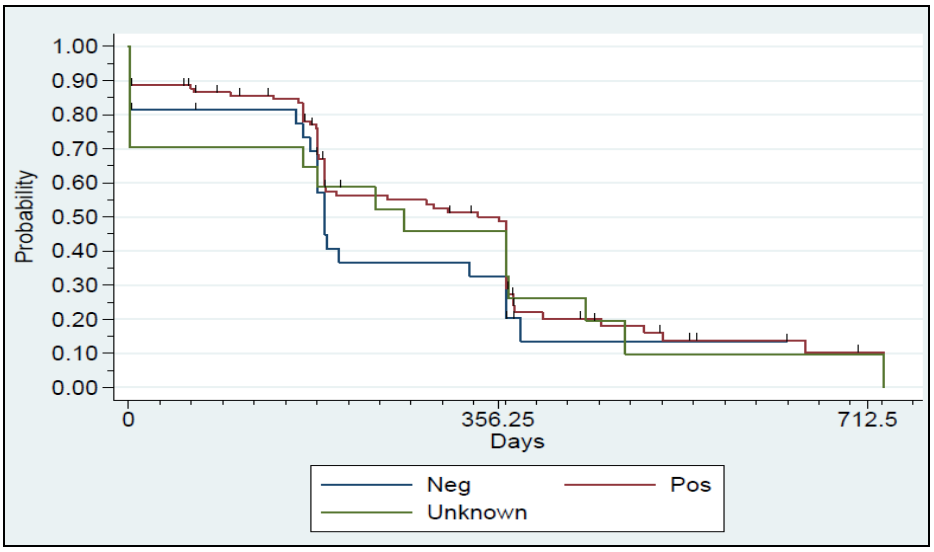

Figure 4.1: Regression by HIV Status

Progression decreased with time in all 3 groups, but patterns differed. HIV positive women had equivalent progression in both year 1 and 2 but HIV negative and unknown women had greater progression in year 2 as compared to year 1. There was 1 progression in the HIV unknown group which occurred after 422 days of surveillance and 3 of the 5 progressions in the HIV negative group (60%) occurred in the second year. Over a 2 year period however, all 3 groups had similar regression and progression.

Both HIV status and CD4 counts were included as variables in a multivariate analysis but showed no difference in results from univariate analysis.

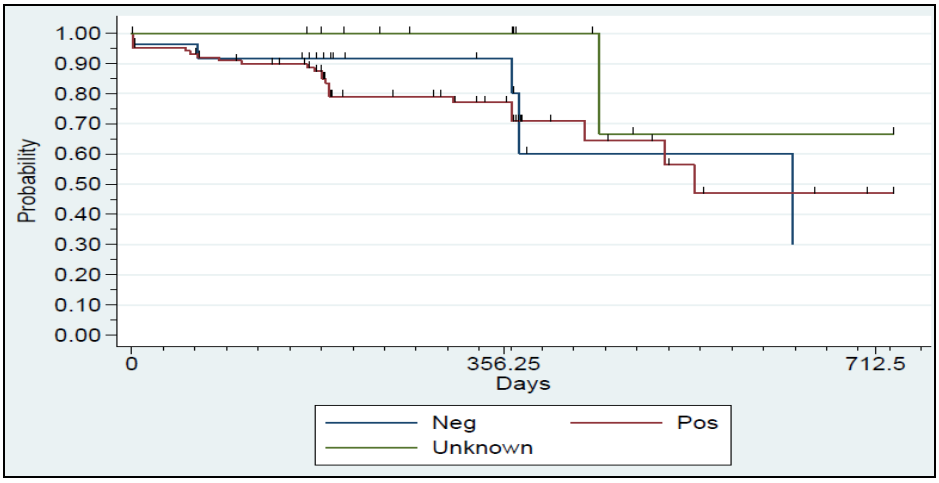

Figure 4.2: Progression by HIV status

3.2 Age

The mean age of the group was 37.8 years with 60% of women falling between 30-49 years of age. All age groups had similar trends of regression, and showed greater regression in year 1 as opposed to year 2. Although regression was shown to decrease with time, the <30 age group displayer much higher regression as compared to other age groups in year 2.

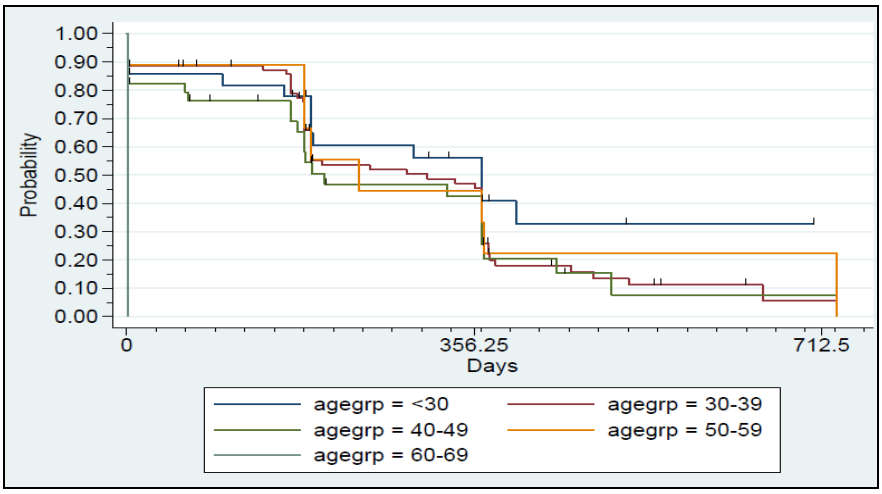

Figure 5.1: Regression by age

Progression was shown to uniformly decrease over the 2 years among all age groups except in those 50 years and over, where none progressed to higher grade lesions.

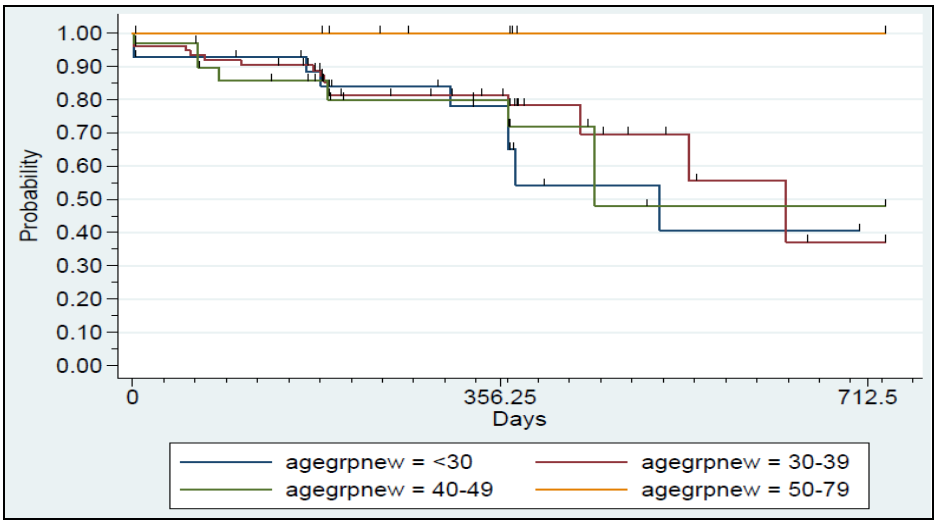

Figure 5.2: Progression by age

Overall, women between the ages of 60 to 69 showed the greatest regression, and were 12 times more likely to regress as compared to their under 30 counterparts [p=0.002] (Table 3) .

Age was included as a variable in a multivariate analysis but showed no difference in results from univariate analysis.

Table 3: Regression of different age groups as compared to women under 30 years old

AGE (years)	Hazard Ratio	P-value	95 % Confidence interval
30-39	1.640	0.090	0.926 – 2.900
40-49	1.861	0.058	0.979 – 3.537
50-59	1.499	0.355	0.635 – 3.540
60-69	11.831	0.002	2.577 – 54.745
70+	2.432	0.392	0.318 – 18.573

3.3 Menopause:

The study population contained 138 pre-menopausal women (89%), 13 post-menopausal women (8%) and 3 with unknown menopausal status. By the second year of follow-up, information regarding the status of these 3 women was consolidated. Regression of both pre-menopausal and post-menopausal women decreased with time and patterns of regression were similar (figure 6.1). There was no significant difference in absolute regression between post-menopausal and pre-menopausal women (9 events [69%] vs 101 events [73%] respectively).

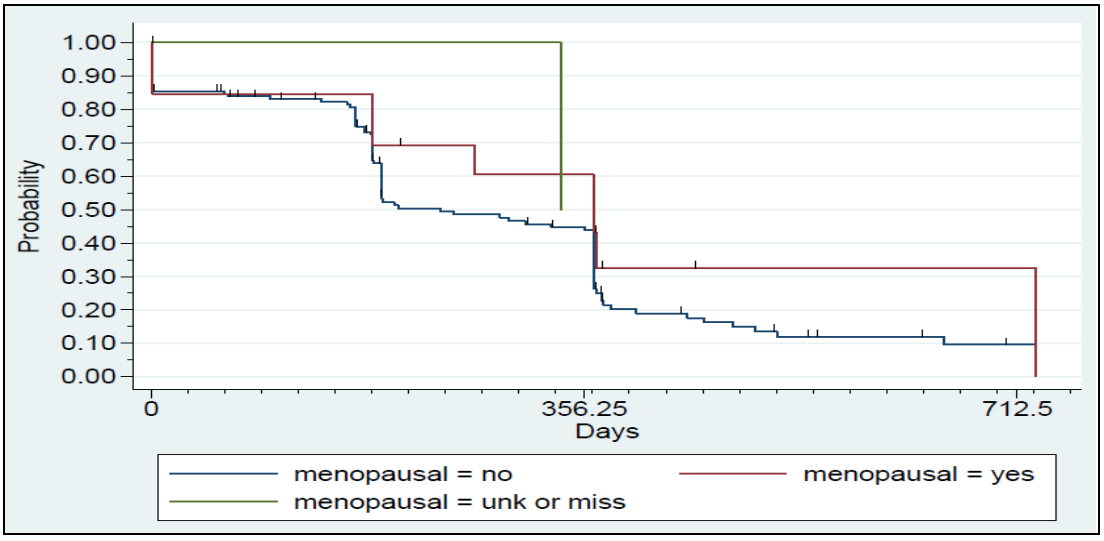

Figure 6.1: Regression by Menopausal status

Progression patterns, however, were not similar: menopausal women had a significantly lower absolute progression (2 events [15%] vs 29 events [23%] p=0.04) but both groups had the same probability of progression after 726 days. It was also noted that there were no events of progression observed in post-menopausal women in the first year of surveillance.

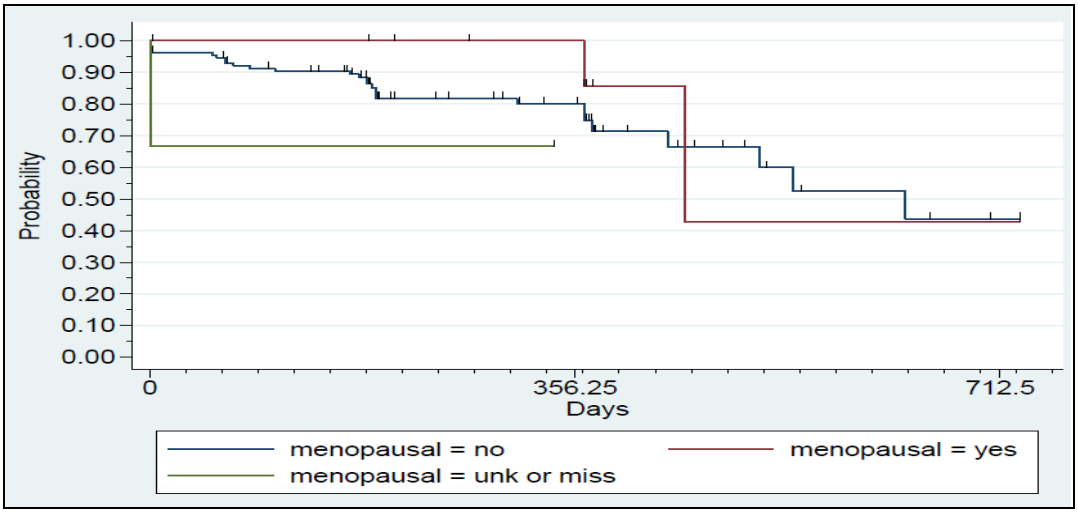

Figure 6.2: Progression by Menopausal status

Seventy of the 143 women (49 %) followed up with known contraceptive status did not consistently use any form of family planning. They made up a total of 58% of the total person-months observed in this study. The largest group using a contraceptive, were those using injectables (32%). A distant third included 19 women who were surgically sterilized. Six women were on oral contraception and only 2 had IUCD's in situ.

Table 4.1: Description of Contraceptive use

Contraceptive	Number of Patients (%)	Person-months
None	70 (48.9)	21934
OCC	6 (4.2)	1737
Injectables	46 (32.1)	11227
IUCD	2 (1.4)	141
Sterilisation	19 (13.3)	3798
Total	143 (100)	38839

Although those using an IUCD made up 141 person-months studied, they were shown to have 6 times greater likelihood to result in regression (p=0.01) compared to women on no contraception. The other forms of family planning displayed no significant difference in absolute regression or patterns of regression with regard to their non-user counterparts.

Table 4.2: Regression vs No Contraceptive use

Contraceptive	Hazard Ratio	P-value	95% Confidence Interval
OCC	0.759	0.596	0.274 – 2.105
Injectables	0.887	0.595	0.570 – 1.380
IUCD	6.499	0.012	1.508 – 28.012
Sterilisation	1.0254	0.080	0.589 – 1.881

As we have already ascertained the overall progression rate of our study population, we can mathematically predict expected progression events of individual variables provided they do not proportionately differ from the overall rate. Table 4.3 highlights this factor and shows clearly that those using the IUCD only, oral contraceptive or those who had a tubal ligation recorded an expected number of events. However those on nil contraception recorded 36% less progression events than expected and those on the injectables, recorded 48% more progressions than expected (p=0.04).

Duration of contraceptive use was sub-analysed revealing no significant difference in absolute or patterns of regression or progression.

Contraceptive type was included as a variable in a multivariate analysis but showed no difference in results from univariate analysis.

Table 4.3: Observed Progression vs Expected progression as per Contraceptive type

Contraceptive	Events expected	Events observed
None	14.16	9
OCC	1.59	2
Injectables	8.93	13
IUCD	0.10	0
Sterilisation	3.21	4

$Chi^2 = 4.20$

P value (chi^2) = 0.03793

3.4 Cigarette Smoking

Only 21 women (14%) were smokers. There were no statistically significant differences seen in patterns of, or variations away from expected events in both regression and progression.

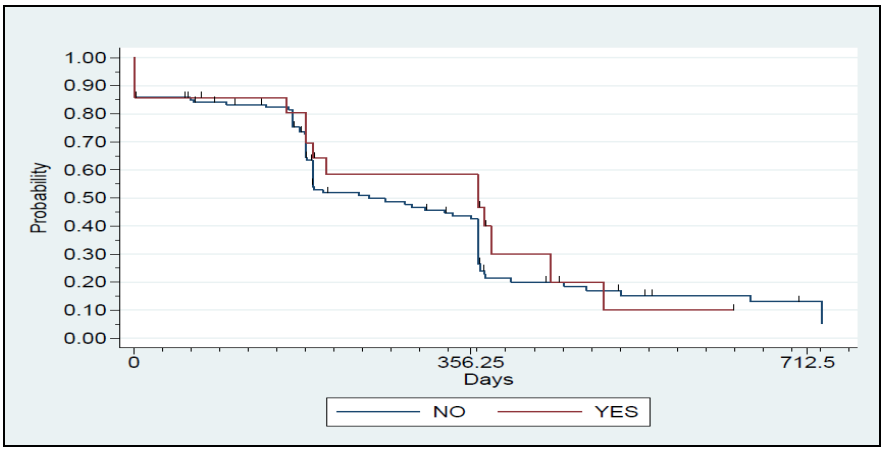

Figure 7.1: Regression by Cigarette smoking status

After 726 days, the probability of progression in smokers was 0.05, whilst that of smokers remained at 0.55, but this was not statistically significant (p=0.6).

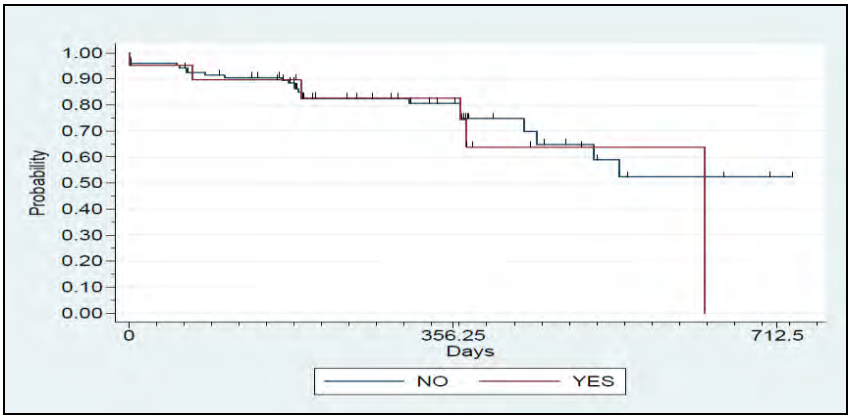

Figure 7.2: Progression by Cigarette smoking status

3.5 Patient Adherence

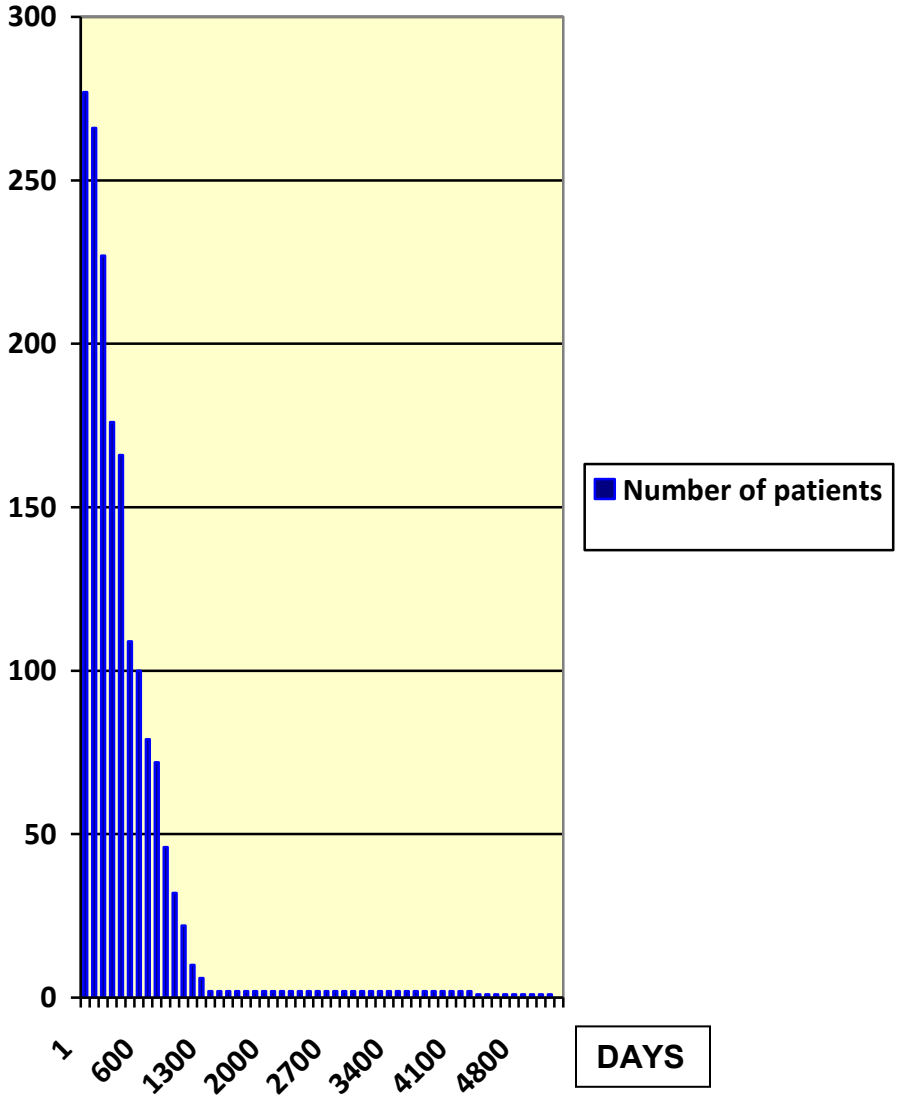

Figure 8: Adherence rates of women attending GSH Colposcopy clinic

There were 277 women with LSIL who had at least one visit to the colposcopy clinic. Of these women, 28 (10%) did not revisit the clinic thereafter. Sixty four percent of the women studied, followed for a minimum of 726 days (+/- 2 years). Hence this was the

timeline used for our study's analysis. Only 39 women followed up for 1000 days (+/- 3 years), 14 women followed up for 1500 days (+/- 4 years), and 2 for 2000 days (+/- 5 years). The intended follow-up for a women who returns to normal pap smear is 3 years and our 3 year adherence rate is 14%.

4. Discussion

Our study has found a high rate of regression (88%) and low rate of progression (17%) of LSIL, with none progressing to invasive carcinoma. This is in keeping with global data: regression rates range between 84-99%[54] and progression rates range between 1.2-26%[55]. These unequivocal rates suggest that intervention (surgical or otherwise) is unnecessary, as almost all will regress, with a small percentage progressing to HSIL which can then be treated. Hence, LSIL may be considered as a risk factor for HSIL rather than a pre-malignant lesion itself. In so doing, the complications of cervical surgery can be avoided in most patients with LSIL, avoiding especially fertility and pregnancy related adverse events. They can also benefit from a longer interval follow-up.

More than two thirds of our eligible patients were found to be HIV positive, far outnumbering their HIV negative counterparts (106 vs 27). The most reasonable explanation for this is that HIV positive women access the health services more frequently and it is policy to offer cytology to all HIV positive women in the Western Cape. Screening of HIV negative women is mostly opportunistic and sporadic and hence many eligible HIV negative or HIV status unknown women are not screened. One of the main reasons for offering screening to HIV positive women is the risk of increased HPV acquisition amongst HIV positive women[28].

Progression and regression of LSIL, in our study, were not affected by HIV status, CD4 count levels, or the use of anti-retroviral therapy. This finding conflicts with international literature which proposes lower rates of regression and higher rates of progression, especially amongst those with very low CD4 counts[28-30]. We propose that LSIL is a

product of HPV DNA integration into the host genome and is thus, more of a genetic disease than an immunity-based one. This, together with the fact that the HPV virus has several mechanisms of evading host immunity, renders HIV status to not be a seemingly major factor in LSIL regression and progression. Large studies by Massad, Denny and Heard do indicate that HIV does not affect the rates in question[31-33].

The mean age of our study population was 37.8 years with almost two thirds of them being between the ages of 30-49 years. This is expected for a colposcopy clinic, as the intended age of screening is 30-50 years of age, with most patients being referred between 30-40 years of age. All women under the age of 60, had no differences in regression and progression but those over the age of 60 had a significantly higher regression and none of these women progressed to higher grade lesions. There are similar results in our pre-menopausal versus post-menopausal groups, but becomes apparent when age stratified. We suspect the reason for this finding to be directly related to oestrogen exposure to cervical tissue. These women are menopausal with decreased circulating oestrogen leading to cervical atrophy. The atrophic cells, under microscope examination, can easily be confused for the atypia of LSIL lesions[48,49]. Thus, in the case of older women, rather than increased regression alone, there may be an element of over-call.

All women attending the colposcopy clinic had several family planning methods available to them but almost half (N=70) were on no form of contraception at all. Those that did use contraception, mostly fell into the group using injectables (N=46). Only 2 women used IUCDs but they were found to increase regression 6 fold, although the clinical significance of this finding cannot be estimated due to such a small number.

Injectables were shown to be associated with a decreased rate of progression, but again, more data is required to confirm that result and as yet there is no literature regarding this contraceptive method and its effect on LSIL. The sub-analysis of the contraception categories in general were limited by the small numbers in each group and lack of statistical significance.

Only 14% of our study population were regular cigarette smokers, making them a subset that could not provide significant results. This low incidence of smoking in women is well recognised Sub-Saharan Africa[56]. Reasons for this include cultural background and paternalistic social structure. There is no peer influence to initiate and maintain cigarette smoking; and a broader social norm of unequal gender liberties where it is acceptable for males to use money on vices and necessary for females to use money for child and household responsibilities. It thus, does not seem that cigarette smoking plays a great role in cervical dyplasia in Sub-Saharan Africa.

Our patients' 1-year adherence rate was 85%, far better than any global data on colposcopy clinic adherence[50]. However, this tends to decrease exponentially. A 2-year adherence rate of 64% is well within global standards, but a 14% 3-year adherence rate is far from satisfactory and needs to be addressed. Specific factors need to be isolated so that they can be acted upon to improve this rate. Factors we suspect may be implicated are those of, transport, finance, lack of education or miseducation, fear of invasive examination, lack of understanding regarding treatment and follow-up of an 'asymptomatic disease'. One must also allow for bias of patient selection, as many were excluded due to previously stated exclusion criteria and their follow-up data may change these results. A detailed structured prospective study will be required for this.

5. Reccommendations

As discussed earlier, high rates of regression and low rates of progression of LSIL found in our study, allow us to recommend that all women with persistent LSIL (3 papsmears 6 - 12 months) apart should be offered follow up colposcopy and only treated once they have developed a HSIL lesion. Follow up can take place at intervals of 12 months until she has normal cytology and remains so for the next **3 - 5 years**. She can then resume routine screening protocol. There is no need for therapeutic intervention but a biopsy can be taken initially to confirm on histology or if unsure of the grade of dysplasia on colposcopy.

As shown by our study, HIV status, CD4 counts, or even the use of anti-retroviral therapy does not appear to impact on LSIL regression or progression, and hence there should be no difference in management protocol of LSIL between this groups.

Twelve monthly intervals of follow-up can even be lengthened to 3-yearly or 5-yearly in women above 60 as they tend to regress much faster and none have been seen to progress. To help the pathologist distinguish between atrophic cells and LSIL, this information must be provided with the specimen and local oestrogen therapy can be used if not contra-indicated prior to repeat cytology.

Family planning must be encouraged in the colposcopy clinic as half of its attendants are not on any, and this is of particular significance for HIV positive women, who need to be encouraged to use dual contraception. . More research is required regarding the roles of IUCD's and injectables on regression and progression respectively, before either can be recommended as a contraceptive of choice in women with persistent LSIL.

Lack of compliance of the patients is worrying with only 64% making the 2-year follow-up. There are several factors, patient and institutional, which may contribute to this outcome but our database was not structured to address this issue. The fact that 15% of our study population did not follow-up for at least 1 year, and 36% for at least 2 years, limits our study in terms of results, conclusions drawn and recommendations. We do not know what the progression and regression were in these non-compliant groups and if they would change our results. A detailed, prospective study looking at factors impacting on adherence is necessary. Once these factors are known, they can be addressed and adherence rates improved. Thereafter, if the results of this study are confirmed, its recommendations should be employed.

6. Limitations

This study, being retrospective in nature, carried with it, its own limitations. There were some statistics that could not be measured. Although variables were assessed to the best of our ability in a dynamic process, the temporal relationship, at times, was difficult to assess. As with all retrospective studies, exposure or outcome assessment cannot be controlled, but instead need to rely on others for accurate record-keeping.

In addition, missing data greatly impeded our analysis, and in some cases there was just too much missing data to successfully analyse and these patients had to be excluded.

A few of our subset populations were small and did not yield significant results. These included patients who smoked and those that used oral contraceptives and IUCD's.

Patient compliance played the largest factor in data analysis, as we only had 60% of the patients by the end of the study as compared to the initial number of women referred.

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