INVASIVE CANCER OF THE VULVA AT THE CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

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A research report submitted to the University of the Witwatersrand Faculty of Health Sciences, Johannesburg in partial fulfillment for the Degree of Master of Medicine in Obstetrics and Gynecology

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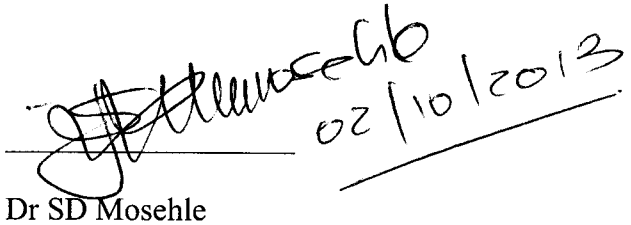
DECLARATION

I, Setheme Daniel Mosehle, declare that this research is my own work. It is being submitted to the University of the Witwatersrand as partial fulfillment for the degree of MMed in Obstetrics and Gynecology (Wits). It has not been submitted before for any other degree or examination at this or any other university.



02/10/2013

Dr SD Mosehle

DEDICATION

To my wife Mathlodi and son Aobakoe

PUBLICATIONS AND PRESENTATIONS

This research was presented at the annual Ethicon Registrars Symposium, Midrand, Gauteng on the 17 September 2010.

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I would like to thank my supervisors Dr T Smith, Prof F Guidozzi and Dr K Frank for their contributions and guidance towards my study.

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ABSTRACT

Introduction.

Invasive vulval cancer is most common in the elderly patients, affecting women above 60 years of age. Historically this disease is known to follow two different etiological and pathological pathways i.e. VIN- related and non-VIN related. In sub-Saharan Africa HIV infection rates are increasing in younger women under the age of 35 years. Over the years there has been a trend of invasive vulval cancer presenting in younger women especially those infected with HIV. This study was undertaken, of women admitted to Charlotte Maxeke Johannesburg Academic Hospital with a diagnosis of vulva cancer to determine whether there had been a trend for the patients to present at an earlier age, whether HIV infected women with invasive cancer of the vulva presented with advanced stage disease and whether the degree of immune suppression affects the stage of the disease at presentation.

This was a retrospective record review of 222 cases of vulval cancer that attended the Charlotte Maxeke Johannesburg Academic Hospital Combined Oncology Clinic from 1999 to 2009. Data was analysed using the Stata 11 statistical package.

The mean age of the patients was 53 years with the majority between 50 and 59 years. Fifty-seven patients (25%) were HIV positive, 61.4% HIV negative and 12.7% with unknown HIV status. Approximately ninety-one percent of the women had Squamous cell carcinoma (SCC) of the vulva, 80.5% were Black and 50%presented with stage 3 disease Of the patients with SCC of the vulva (n=201), the mean age at presentation for HIV positive and HIV negative patients was 40 years (95% CI 36.7 – 43.0) and 56.0 years (95% CI 49.7 – 53.8) respectively (p value = 0.000). HIV positive women

with SCC of the vulva were on average 16 years younger than HIV negative patients, whilst patients between the ages 20 to 29 showed 100% HIV seropositivity. What does this mean? The peak age for HIV positive patient and HIV negative patients was 40 to 49 years and 50 to 59 years, respectively. There was no statistical significance between the two groups for the stage of the disease at presentation. The level of the CD4 count did not affect the stage at presentation. The histological background within which the SCC of the vulva had developed could not be adequately evaluated since the majority of the histological did not have this information.

Conclusion

The mean age of the patients in this study that presented with carcinoma of the vulva was 53 years which is younger than that reported in the literature. HIV positive patients with SCC of the vulva were on average 16 years younger than the HIV negative patients and patients below the age of 30 years presenting with SCC of the vulva are most likely to be HIV seropositive. There was no statistical difference in the stage and age of presentation or the HIV status. The CD4 count does not affect the stage of the disease at presentation.

INTRODUCTION AND LITERATURE REVIEW.

Background

In 2000, Lamolisa et al published a study looking at the South African perspective of the relationship between human immunodeficiency virus (HIV) infection and invasive cancer of the cervix. The authors retrospectively analyzed files of 836 patients attendinga combined oncology clinic at Charlotte Maxeke Hospital in Gauteng Province, between January 1997 and June 1998. The prevalence of HIV in their study was 7.2%. They found that HIV positive patients presented 10 years earlier than HIV negative women and CD4 count levels did not play influence the stage of the disease at presentation. [1]

Five years later, another group of investigators, Moodley et al, performed a similarstudy in KwaZulu Natal. They studied 271 patients retrospectively for a period of 1 year. In this study the HIV prevalence was 21.8%. They reached the same conclusions as Lomalisa et al, in that HIV infected patients were 13 years younger than HIV negative patients at the time of presentation, with the same disease stage as HIV negative women. The CD4 count levels did not affect the stage of the disease at presentation.[2]

With the above-mentioned in the background, this study was undertaken to determine whether there has been a change in the age at presentation of women with squamous cell carcinoma of the vulva and whether HIV infection was associated with it.. The characteristics of squamous cell carcinoma of the vulva in HIV-infected versus non-infected women will also be described.

Despite a thorough literature review, I was not able to find any South African studies that correlated the relationship between HIV infection, invasive vulval carcinoma and the stage of the disease. It was felt that this study would contribute to scientific knowledge and that it would be of epidemiological value if we could demonstrate how associated HIV infection affects the characteristics of invasive vulval cancer. If an association is shown, it will have major implications on health planning, resources and management of at-risk patients.

The study will also concentrate on the aspects of the complex relationship between HPV, HIV and cancer of the vulva and briefly look at how HPV is associated with other malignancies of the anogenital tract.

The National Health Laboratory Service (NHLS) publishes vulval and vaginal cancer statistics in the National Cancer Registry. In 2000, cancer of the vulva and vagina combined, constituted 1.2% of all histologically diagnosed cancers affecting women in South Africa.[3] The report of the NHLS published in 2002, also confirmed that histologically diagnosed cancers showed a peak in the ages between 40 and 59 years. [4]

According to an unpublished report from the National Health Laboratory Service's National Cancer Registry [5], the number of vulval cancers (excluding vaginal) showed a steady increase between the years 1997 and 2001 as indicated below:

- 1997 : 23/100 000
- 1998 : 46/100 000
- 1999 : 201/100 000
- 2000 : 166/100 000

- 2001 : 199/100 000

It is estimated that about 67% of people who are HIV positive live in sub-Saharan Africa. South Africa has the highest HIV prevalence with an estimated 5.7 million people living with the disease and 60% being women. Between the years 1997 to 2002 there was a sharp increase in HIV seroprevalence in South Africa [31]. The latest figures show that females between the ages of 25 and 34 have the highest incidence of HIV infection with an increasing incidence over the past 3 years. [6]

At Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) it is customary to offer HIV testing to antenatal patients and over the last decade or so, the prevalence has been about 27% among pregnant women seen. However, about 20% of pregnant women decline testing. A study was conducted in 2004 among women who initially declined HIV testing, and it was found that 44% were HIV positive. [7] The sero-prevalence among the gynecologic patients is unknown as routine HIV testing is not offered, although all patients with gynecologic cancer are offered HIV testing. The HIV sero-prevalence among these patients has risen over the last decade and at present it is estimated to be about 10%.

Vulval Cancer

Cancer of the vulva is defined when the primary site of the growth is the vulva and secondaries from other genital or extra-genital sites have been excluded. Even if the tumor extends into the vagina, it should still be considered as carcinoma of the vulva. There must be histological confirmation of the cancer. The cancer is staged according to the International Federation of Gynecology and Obstetrics (FIGO) staging system.

[8]:

Stage 0: Carcinoma in situ or Vulval Intraepithelial Neoplasm III (VIN).

Stage 1: Tumor confined to the vulva or vulva and perineum, 2cm or less in greatest dimension.

1A: Tumor confined to vulva or vulva and perineum, 2 cm or less in greatest dimension and with stromal invasion no greater than 1.0 mm.

1B: Tumor confined to vulva or vulva and perineum, 2 cm or less in greatest dimension and with stromal invasion greater than 1.0 mm

Stage 2: Tumor confined to the vulva or vulval perineum, more than 2cm in greatest dimension.

Stage 3: Tumor invades any of the following: lower urethra, vagina, anus and/or unilateral regional nodes metastasis.

Stage 4

4A: Tumor invades any of the following: bladder mucosa, rectal mucosa, upper urethral mucosa, or is fixed to bone and/or bilateral regional node metastases.

4B: Any distant metastasis including pelvic lymph nodes.

Histological types of vulval cancer [9] include/comprise:

- Squamous cell carcinoma
- Melanoma
- Bartholin's gland carcinoma
- Adenocarcinoma
- Basal cell carcinoma
- Verrucous carcinoma
- Sarcomas

  Leimyosarcoma

Epitheloid sarcoma

Rhabdomyosarcoma

Lymphoma

Endodermal sinus tumor

Merkel's cell carcinoma

Dermatofibrosarcoma protuberans

Malignant Schwannoma

The classification of VIN has evolved over the years. In 1986, VIN was classified as VIN 1, VIN 2 and VIN 3. VIN 1 as a mild form and VIN 3 as a severe form in a continuum. It was later realized that VIN 1 is not a precancerous lesion, was an uncommon histological finding and that it was not reproducible in the laboratory. The majority of VIN lesions are VIN 2 and VIN 3 and there appears to be good histological agreement on them. VIN 2 and VIN 3 were subsequently combined into a single entity to form high grade VIN. Presently the high grade VIN category includes 2 types of lesions, which are different in morphology, biology and on clinical presentation, namely VIN usual type and VIN differentiated type. [10]

The International Society of the Study of Vulval Disease modified the classification of VIN in 2004 and classifies it as follows [10]:

- VIN, usual type

  VIN, warty type

  VIN, basaloid type

  VIN, mixed (warty/basaloid) type
- VIN, differentiated type

In my study I used the 1986 classification of VIN 1, 2 and 3 because that is what the histopathologist followed and used in their histopathological report.

Cancer of the vulva is regarded as a rare malignancy although it is the 4th most common gynecological cancer in high income countries, after cancers of the endometrium, ovary and cervix and occurs in approximately 1 to 2 per 100 000 women per year in various population groups in the USA. [11]

It has been frequently stated in the literature that the prevalence of vulval cancer is between 3-5% of genital cancers. [11, 12, 13] However these studies span several years and the same figures are quoted for high and low income countries. Locally there is a paucity of data on the epidemiology of cancer of the vulva.

In their annual report published in 2006, FIGO addressed treatment of vulval cancer patients from 1999 to 2001, and showed that the peak incidence (30.6%) for vulval cancer was in patients between 70 and 79 years of age . The incidence then decreases sharply in patients above the age of 80 years to about 13.7% and women below the age of 50 years comprise 18.6% of this report. The authors claim the age factor to be explained partially by the delay in patients seeking medical attention for the disease. [12]

It has been hypothesized that 2 types of vulval cancer exist. [14] The first type involves human papillomavirus (HPV) infection, which leads to vulval epithelial neoplasm (VIN) and predisposes the patient to vulval cancer. HPV is the most common sexually transmitted infection. [14] It occurs in relatively young patients and is related to sexual factors and immune status. The high risk infectious subtypes, HPV

16 and 18, are responsible for non-keratinizing carcinomas, VIN, warty and/or basaloid VIN and can simultaneously affect the cervix, vagina and/or anus. [14]

The second type of vulval cancer involves vulval non-neoplastic epithelial disorders (VNED), which usually occurs in older women, leading to cellular atypia and cancer. It is related to chronic vulval inflammation, lichen sclerosis and hyperplasia and is not associated with high-risk HPV infections. [14]

It is believed that this second type of vulval squamous cell carcinoma is caused by chronic tissue damage brought about by itching and scratching in certain women with a susceptible immunophenotype. The "itch-scratch-linchen sclerosis hypothesis" is referred to as the "Kobner phenomenon" an occurs in susceptible women who scratch because of genital irritants such as urine, vaginal secretions and smegma, or lichen sclerosis. Lichen sclerosis leads to a vicious cycle of itching and scratching which leads to lichen simplex chronicus, squamous cell hyperplasia and ultimately cancer. [15]

Crum in 1992 published a review of epidemiology and pathogenesis of vulval cancer and demonstrated the incidence to be about 1.8 per 100 000 which is half that of cervical cancer, and 30% of vulval cancer developed in women older than 70 years. This disease rarely occurred in younger women. Above the age of 75 years vulval cancer is as common as cervical cancer and the incidence was 20 per 100 000. The review states chronic inflammatory disorders, VIN, smoking and a history of genital warts as risk factors for development of invasive vulva cancer. [16]

A retrospective study by Messing et al of 78 women who had been treated for vulva cancer reported that the average presenting age for their patients decreased from 69 to 55 years over a period of 15 years. But they did not comment on why there was such a decrease in age at presentation. They reported that condylomata accuminata was more common in younger patients, whilst patients above the age of 45 years were more likely to present with advanced stage disease i.e. stage III or IV. [17]

HPV infection and genital cancers Several infectious agents are considered to be causes of cancers in humans. In a paper published by Parkin in 2002, it was estimated that the total infection- related cancers in 2002 were 1.9 million cases, which was about 17.8% of global cancer burden. In that study, human papillomavirus was responsible for about 5.2% of world cancer burden. HPV infection is responsible for most cancers of the cervix occurring worldwide (n=492,800). The analysis showed that HPV infection plays a significant role in the etiology of vulval, vaginal, penile, anal, oral cavity and oropharyngeal cancers. According to the same study, the distribution is different between the developed and developing world with the attributable fraction being higher in developing world (7.7%) than in developed world (2.2%). [18]

Human papillomavirus belongs to the family papillomaviridae and is a non-enveloped double-stranded DNA virus. About 120 different subtypes have been isolated. Forty of these subtypes infect the mucosal and epithelial lining of the anogenital tract. HPV subtypes are classified according to high and low risk groups. Low risk types, HPV 6 and 11 cause benign lesions in the anogenital areas and cause about 90% of external genital warts. [19]

While the literature has shown that almost 100% of cervical cancers are caused by HPV, this does not seem to be the case in vulval, vaginal and anal cancers. In a meta-analysis conducted by Vuyst et al, only 40% of vulval cancers, 69,9% of vaginal cancers and 84,3% of anal cancers were found to be HPV PCR positive. Overall HPV 16 was found more frequently (>75%) than HPV 18 in these lesions. Vulval cancers were commonly HPV positive if they where warty basaloid in type and occurred in younger patients who were 60 years or less. VIN 1, anal intraepithelial neoplasia (AIN) 1 and vaginal intraepithelial neoplasia (VAIN) 1 is usually caused by low risk HPV types such as HPV 6 and 11. AIN 2 and 3, VIN 2 and 3 and VAIN 2 and 3 are more likely to be HPV high risk type positive. [20]

HIV infected women and men are more likely to be infected with high risk oncogenic HPV types. Therefore they will be more likely to have cervical intraepithelial neoplasia or anal intraepithelial neoplasia which may lead to both invasive cervical cancer and anal cancer respectively and this risk remains high even with availability of highly active anteretrovial therapy (HAART). [21]

Epidemiological studies and HPV DNA studies supported a causal role for high risk HPV 16 in the pathogenesis of vulval cancers. Type 16, 18 and 33 are the commonest HPV type found in vulval carcinoma. Fourteen other high risk types of HPV have been associated with VIN grades 1-3 including 6/11/16/18/31/33/35/39/45/51/52/56/58/59. [22]

Persistent infection and increased viral loads of high risk HPV leads to incorporation and integration of high risk HPV DNA into the infected basal cell genome. This leads to upregulation of E6 and E7 oncoproteins which leads to inactivation of P53 and

Retinoblastoma tumor suppressor genes. This induces chromosome duplication errors and activation of telomerase, which further leads to infected cell genomic instability. Genomic instability together with host immune fitness, viral genetic factors, environmental and dietary mutagenic factors, tobacco and co-infection with other STI leads to development of HPV associated squamous cell carcinoma. [23]

VIN lesions can either progress to invasive vulval cancer or undergo spontaneous regression if not treated. A review by van de Nieuwenhof (2008) reported various rates of progression from VIN to invasive vulval cancer. They reported rates of between 9% and 15.8% of untreated patients progressing to invasive vulval cancer. [14]

Human papilloma virus and Human immunodeficiency virus

In my review of the literature, I make several references to cervical carcinoma. This is because the causative organism, HPV is common to both malignancies (cancer of the vulva and cancer of the cervix) and while more is known about cancer of the cervix, one may be able to extrapolate these findings to vulva carcinoma.

Cervical cancer is the second most common cancer in women in the developing world. The causal role and association of persistent HPV infection in the development of cervical pre-malignant lesions and carcinoma is well established. [20]

In a study published in the Lancet in 2002, Conely et al, showed in a series of 385 women that HIV positive women were more likely to have HPV DNA in a cervicovaginal lavage sample and that the incidence of vulvovaginal lesions was 16

times higher in HIV positive women than in their HIV negative counterparts. This study showed a large number of cases with high-grade vulval intraepithelial neoplasia and one case of invasive vulval cancer. [24]

What is undisputed is the fact that malignancies associated with HPV infection occur more frequently in HIV infected women. The incidence of perianal lesions was 16 times higher in HIV positive than in HIV negative women. Their study reports a hazard ratio of 5.8 [95% CI 3.0-10.2] in invasive vulvovaginal carcinoma associated with HPV in 50 000 HIV positive women in the USA between 1995 and 1998. It is likely that HIV-related immunosuppression may also contribute to the development of cancer by failing to clear HPV as would a healthy individual. [24]

Jamieson et al (2006) performed a multicenter prospective study to evaluate and compare the incidence of vulval, vaginal and perianal intraepithelial neoplasia in HIV infected women with a well-matched high-risk group of HIV negative controls. One hundred and ninety two HIV positive women and 88 HIV negative women were followed over a period of 6 years. During the study 8.5% of HIV infected women and 1.1% of HIV negative women developed vulval, vaginal, or anal intraepithelial neoplasia. The incidence of vulval, vagina, and anal intraepithelial neoplasia was found to be 1.96 per 100 person years for the HIV positive women and 0.26 per 100 person years in HIV negative women. The above-mentioned paper recommended that women undergoing colposcopy for cervical lesions should also have a colposcopic examination of the entire perineum including the vulva and perianal regions. [25]

Chiasson and colleagues (1997) compared the prevalence of HPV associated vulvovaginal lesions in 397 HIV positive and 375 HIV negative women and

demonstrated that the prevalence of VIN and condyloma accuminata was 7 times higher in HIV positive women as compared to HIV negative women. It was shown in the study that 5.6% of HIV positive and 0.8% of HIV negative patients had vulval condyloma accuminata (OR 7.3, $p<0.001$). [26]

Conley et al have published similar results in a prospective study of 925 patients. The purpose of the study was to look at HIV-1 infection as a risk factor for developing vulvovaginal and perianal condylomata accuminata and intraepithelial neoplasia. The study subjects had twice yearly gynecological examinations including colposcopy and cervicovaginal lavage for HPV DNA testing. The authors concluded that HIV infection, HPV infection, lower CD4 count levels and frequent intravenous drug abuse are the risk factors for development of vulvovaginal or perianal condylomata accuminata and intraepithelial neoplasia. [24]

However, it needs to be borne in mind that this was an older study published in 2002, and there was already a trend towards a discrepancy in pathology between HIV+ and HIV- women. It was also demonstrated that HIV infected women with CD4 counts below 200 were more likely to have high grade VIN than were women with counts of at least 200, nine of 79 (11%) compared to 13 of 248 (5%) (OR 2.3, 95% CI 0.6-6.1). The authors then concluded that HIV infection confers an additional risk for the development of HPV- related lesions of the vulva . [26]

HIV infection and the risk of cancer

The local vulval immune response is important for the progression or regression of HPV- related VIN. Host immune response comprises first-line innate immune

response and second-line adaptive immune response. Pertaining to the innate immune response, the dendritic cells are important as they are the first- line cells that recognize the antigen, process it and present it to the T cells of the adaptive immune response. The function of the dendric cells is disturbed in women who smoke, are on corticosteroids, have autoimmune diseases and who are infected with HIV. The dendritic cells express CD4 receptors on their surfaces and are therefore a target for destruction by the human immunodeficiency virus. This leads to an ineffective local innate immune response. The ineffective innate immune response then leads to an ineffective and inaccurate adaptive immune response. [27]

Taube et al analysed Langerhans cell density and high-grade vulva intraepithelial neoplasia in HIV infected women. Langerhans cells are antigen-presenting cells that ingest antigen, process antigen and present it to T helper cells. In their study the mean Langerhans cell count was investigated in 48 HIV positive and 40 HIV negative women with high grade VIN using S100 immunohistochemical staining. HIV positive patients had significantly lower Langerhans cell count (5.82) compared to HIV negative patients who had Langerhans cell count of 9.86. According to the authors this demonstrated that local immunity in HIV positive patients is significantly impaired, leading to progression of HPV-related vulval lesions. [28]

There are proven links between HIV infection, HPV infection, cervical cancer and both cervical and vulval intraepithelial neoplasia. It is expected that the high prevalence of HIV may cause a similar increase in vulval cancer, Kaposi's sarcoma, non-Hodgkins lymphoma, cancer of the cervix, Hodgkins lymphoma, cancers of other anogenital organs, and squamous cell carcinoma of the skin. [29]

HIV has been classified as a carcinogen and is related to a number of cancers. It plays a critical role in development of several cancers. Women infected with HIV are at high risk of cancers mainly through the mechanism of immunosuppression, which leads to increased replication of oncogenic viruses. In 1996, the International Agency for Research on Cancer (IARC) Working Group determined that the infection with HIV-1 is associated with an increase in the risk of developing Kaposi's sarcoma, non-Hodgkin's lymphoma, squamous conjunctival cancers and leiomyosarcomas. Significant risks associated with HIV infection was found only for Kaposi's sarcoma (OR=61.8) and non-Hodgkin lymphoma (OR=4.8), but there was some uncertainty about the ability of HIV infection to increase the risks of other cancers. [29]

Crulich et al performed a meta-analysis of the incidence of cancer in people infected with HIV and immunosuppressed transplant recipients. In this meta-analysis, 7 studies of HIV infected people and 5 of transplant patients were analyzed. The incidence of infection-related cancers was found to be significantly increased in both groups of patients. These infection-related cancers included all three types of AIDS defining cancers (Kaposi's Sarcoma, cervical cancer and non-Hodgkins Lymphoma), HPV related cancers, Hodgkins lymphoma, liver cancer and stomach cancer. The authors concluded that immune deficiency is responsible for this increased risk rather than other risk factors. The investigators further noted that with HIV/AIDS becoming a chronic disease, infection-related cancers will become an important complication of long-term HIV infection. [30]

A new assessment and a review published by the IARC as Part B of Volume 100 done in 2009, now shows a newly identified association between HIV and invasive cancer

of the cervix. According to the authors the risk is associated with a suppressed immune system and remains high even with the use of anti-retrovirals. The same report suggests that a link between HIV and vulval cancer exists although the evidence is limited and insufficient. [22]

In 1997 Sitas et al performed a case control study of 913 black patients to look at association between HIV and cancer in a black population in South Africa. The study found significant association of Kaposi's sarcoma OR=61.8, 95%CI 19.5-194.2) and non-Hodgkins lymphoma (OR=4.8, 95% CI 1.5-14.8) to HIV infection. No association was demonstrated between HIV infection and cancers of the liver and cervix. [32]

A South African study by Sitas et al in 2000 examined the relationship between HIV infection and number of cancers that are known to be common in Africa. In total, 4,883 patients were recruited and were tested for HIV. The prevalence of HIV was found to be 8.3% for males and 9.1% in females. A significantly increased risk of vulval cancer in HIV infected women was shown. (OR=4.8, 95% CI 1.9-12.2). It also showed a significant HIV-associated risk for Kaposi's sarcoma, non-Hodgkins lymphoma, vulval cancer and cervical cancer. [33]

Subsequently in 2008, Stein et al published an ongoing study similar to the one by Sitas et al in 2000. The authors reached the same conclusion as the aforementioned study. Although Stein et al did not report on vulval cancer separately, it reported a significant increase in the cancer of anogenital organs other than the cervix (OR=2.2, 95% CI 1.4-3.3). It also showed an association between HIV and cancers such as

Kaposi's sarcoma, Hodgkin's lymphoma, cervical cancer and squamous cell carcinoma. [34]

Case reports have documented the presence of vulval cancer in younger patients infected with HIV. These case reports are becoming more frequent in the literature. The first report by Wright in 1996 published 2 cases of women aged 32 and 59 years old, both infected by HIV. Both patients presented with advanced stage disease (Stage IV) and one died from the disease and the other one experienced recurrence after treatment. [35] The other case report was of a 12-year-old patient with vertically acquired HIV infection who developed invasive vulval cancer. [36] Elit also reported on the case of a young HIV-infected patient with invasive cancer of the vulva. [37] Sekowsky(2008) reported 5 cases of stage IV vulval cancer occurring in patients younger than 50 years old all of whom where HIV infected. According to his report, 70% of patients first consulted traditional healers and only presented for hospital care at an advanced stage when symptoms were unbearable (Sekowsky 2008). Beliefs in traditional medicine and delaying contact with medical professionals may play a significant role in my patients and account for their late presentation. Late presentation can also be due to delay in diagnosis by the treating physician although it has not been demonstrated in my setting. [38]

A review by Consolati in 2003 noted that there is conflicting data about severity of the immune depression, expressed by CD4 count, as a risk factor for persistence, recurrence or progression to vulval cancer. Overall though, the current information about the effect of HAART is conflicting and scanty. [39]

Stage of the disease at presentation.

A study by Stroup and colleagues, demonstrated that 90% of their patients presented at an early stage (stage I and II) and that only older patients were more likely to present with advanced disease. The study states that the majority of these older patients also had 2 or more co-morbid diseases. Their study states that clinical and behavioral barriers lead to delayed diagnosis in the older patients. [40]

In a review of 47 case files by Anderson et (1995) which was performed to review their institution's 18 year experience with vulval cancer treatment, they found that 60% of their patients presented with Stage I and II disease, 25% in Stage III and 14% in Stage IV disease. [11]

Rosen and colleagues (1997) reviewed records of 328 patients with histologically confirmed vulval cancer treated between 1948 and 1994. The stage of the disease in their patients were as follows: Stage I: 35%; Stage II: 37%; Stage III: 15%; and Stage IV: 37%. [41]

A study by Canavan and colleagues (2002) stated that both patients and physician contribute to the delayed diagnosis of vulval cancer. Patients took up to 16 months before reporting vulval lesions or chronic pruritis. The physician contributed to this delay in diagnosis by offering medical treatment for up to 12 months before performing a biopsy or referring patients for further management. Canavan advocated teaching female patients self-examination of the vulva as a preventative method for serious vulval pathology. [9]

STUDY OBJECTIVES

a) To document the characteristics of women who presented with invasive cancer of the vulva at Charlotte Maxeke Johannesburg Academic Hospital.
b) To investigate whether there is a relationship between invasive vulval cancer and human immunodeficiency virus (HIV) infection in South African women.
c) To determine whether HIV-infected women with invasive vulval cancer present at an earlier age with more severe disease than HIV- negative women.
d) To ascertain whether the degree of immunosuppression affects the extent of disease at initial presentation

METHODOLOGY

Study setting

This study was conducted at the Charlotte Maxeke Johannesburg Academic Hospital, formerly Johannesburg General Hospital. This is a tertiary/quaternary referral hospital offering radiotherapy and other oncology services to the Southern Gauteng region through a combined Oncology Clinic.

Study Population

A retrospective study of the case files of all women who were seen in Gynecologic Oncology unit and subsequently treated at the CMJAH for vulval cancer was undertaken. These files were analysed for age of onset of disease (if it was documented), histology, FIGO staging and history of VIN-background, HIV status and CD4 T-lymphocyte count. The patients seen in the Gynecologic Oncology unit are derived either from the gynecologic outpatient department of CMJAH or are

referred from surrounding hospitals to the unit for further management because of vulva cancer. The admission registry was used to review all case files of patients vulval cancer from 1999 to 2009. A list of these cases was made categorizing them into years. This list was then used to obtain patients files from the records department. All the files where searched for the clinical presentation, demographics of the patient, histology report, HIV staus and CD4 count. Files wrongly assigned as vulval cancer in the registry were excluded e.g. vaginal cancer, urethral cancer, cervical cancer and VIN. The information was then entered into a data sheet using Microsoft Excel.

Study design:

This was a cross-sectional retrospective audit, which spanned 11 years from 1999 to 2009.

Statistical analysis:

Data management and analysis was performed using the Stata11 statistical software package, employing descriptive and analytic methods. Descriptive methods involved expressions of frequencies and percentages. Continuous data was presented as means with standard deviations, and medians with ranges. Comparison of categorical variables was done using the Chi-square test. Statistical significance was accepted at $P<0.05$.

Ethics

The University of Witwatersrand's Health Science Research Ethics Committee (HREC) granted ethics approval (M090909).

There was no external funding for the study and hospital permission was sought from the Head of Radiation Oncology Department.

RESULTS

Two hundred and twenty files were reviewed. The majority of the patients were Black (80.3%) followed by Caucasian and mixed race. The mean age of the patients in the study was 52 years; the youngest being 14 years and the oldest 88 years of age. Overall the majority of women (26.4%) were between 40 to 49 years old.

The majority of women (61.4%) were HIV negative. Fifty-seven (25.9%) of the patients were HIV positive while the results of 12.7% (28) were unknown. Squamous cell carcinoma was the dominant histological type occurring in 91.4% (201) of patients, while the other histological subtypes comprised 8.6% (19).

The majority of the patients had locally advanced disease as defined by the International Federation of Gynecology and Obstetrics with 50% having stage III at presentation. (See Table 1 for more detailed data). Thirteen patients (5.9%) where unstaged and there was no clinical description in the files of the tumour size or spread. Table 1 show the characteristics of the women who presented with vulva malignancies, and includes the different histologic subtypes.

Table 1. The available demographics of our patients with cancer of the vulva.

Characteristics		Frequency (n)	Percentage (%)
RACE	Black	177	80.5
	Mixed race	5	2.3
	Caucasian	35	16.0
	Unknown race	3	1.4
HIV STAUS	Negative	135	61.4
	Positive	57	25.9
	Unknown	28	12.7
HISTOLOGY	Adenocarcinoma	2	0.9
	Glassy cell carcinoma	1	0.5
	High grade spindle cell tumour	1	0.5
	Kaposi's sarcoma	6	2.7
	Neuroendocrine tumour	1	0.5
	Alveolar rhabdosarcoma	3	1.4
	Choriocarcinoma	1	0.5
	High grade liposarcoma	1	0.5
	Smooth muscle tumour of uncertain malignant potential	1	0.5
	Squamous cell carcinoma	201	91.4
	High grade leiomyosarcoma	2	0.9
STAGE	I	13	5.9
	II	21	9.6
	III	110	50.0
	IV	63	28.6
	unstaged	13	5.9
AGE GROUPS (years)	<20	1	0.5
	20-29	15	6.8
	30-39	21	9.6
	40-49	58	26.4
	50-59	56	25.5
	60-69	41	18.6
	70-76	17	7.7
	>80	11	5.0

Sub-analysis of patients with squamous cell carcinoma of the vulva

The mean age of the patients with squamous cell carcinoma (SCC) (excluding the other histological subtypes) was 53 years. The majority of patients were within the age group 40 to 49 years (26.9%). The youngest patient was 22 years and the oldest 88 years old. The majority of the patients with SCC were Black (79.6%), followed by Caucasian (16.9%) the mixed race patients (2.0%). The HIV seropositivity was 24.9% and HIV negative patients were 63.2%. The majority of patients (52.5%) did not have a histological background in which the carcinoma had developed recorded in the histology report. Fifty two percent of patients with SCC presented with stage 3 disease (52.7%), 29.9% with stage 4, 20% with stage 2 and 6.7% with stage 1. Most patients had grade 2 differentiation (68.6%). See Table 2.

The average age of the HIV positive patients was 40 years (SD +/- 10.5), while the mean age for the HIV negative patients was 56 years (SD +/- 12.5). The youngest HIV positive patient was 22 years old and the oldest was 72 years old. The youngest HIV negative patient was 32 years and the oldest, 88 years of age.

The majority of HIV infected women (46.0%) were within the age group 40 to 49 years, while most of HIV negative women (28.4%) were between 50 and 59 years of age. A comprehensive breakdown of the age groups and their HIV serostatus is shown in Table 3.

Table 2. Characteristics of patients with SCC of the vulva (Total=201).

Characteristics		Frequencies (n)	Percentage (%)
RACE	Black	160	79.6
	Mixed race	4	2.0
	Caucasian	34	16.9
	Unknown	3	1.5
AGE GROUPS	< 20		
	20-29	12	6.0
	30-39	19	9.5
	40-49	54	26.9
	50-59	51	25.4
	60-69	39	19.4
	70-79	15	7.5
	>80	11	5.5
HIV STATUS	HIV negative	127	63.2
	HIV positive	50	24.9
	Unknown status	24	11.9
STAGE	1	13	6.7
	2	20	20.0
	3	106	52.7
	4	60	29.9
	Ustaged	2	1.00
BACKGROUND	VIN	93	46.3
	VIN/Lichen sclerosis	2	1.00
	Unknown	106	52.7
YEARS	1999	24	11.9
	2000	17	8.5
	2001	11	5.5
	2002	18	9.0
	2003	17	8.5
	2004	27	13.4
	2005	16	8.0
	2006	14	6.97
	2007	23	11.4
	2008	27	13.4
	2009	7	3.5
DIFFERENTIATION	Grade 1	24	12%
	Grade 2	137	68.6%
	Grade 3	15	7.4%
	unknown	24	12%

Table 3. Peak age for both HIV positive and negative for squamous cell carcinoma in relation to whole group

AGE GROUP (years)	HIV STATUS			
	Negative	Positive	Unknown	
20-29	0	24.0	0.0	
30-39	7.1	16.0	8.3	P<0.01
40-49	24.4	46.0	0	
50-59	28.4	12.0	37.5	
60-69	27.6	0.0	16.7	
70-79	7.9	2.0	16.7	
>80	4.7	0.0	20.83	

There is 100% HIV seropositivity in the vulva cancer (SCC) patients between the ages of 20 and 29 years, 42.1%, 42.6% and 11.8 in the age groups 30 to 39, 40 to 49 and 50 to 59 years respectively. A comprehensive breakdown is shown in Figure 1.

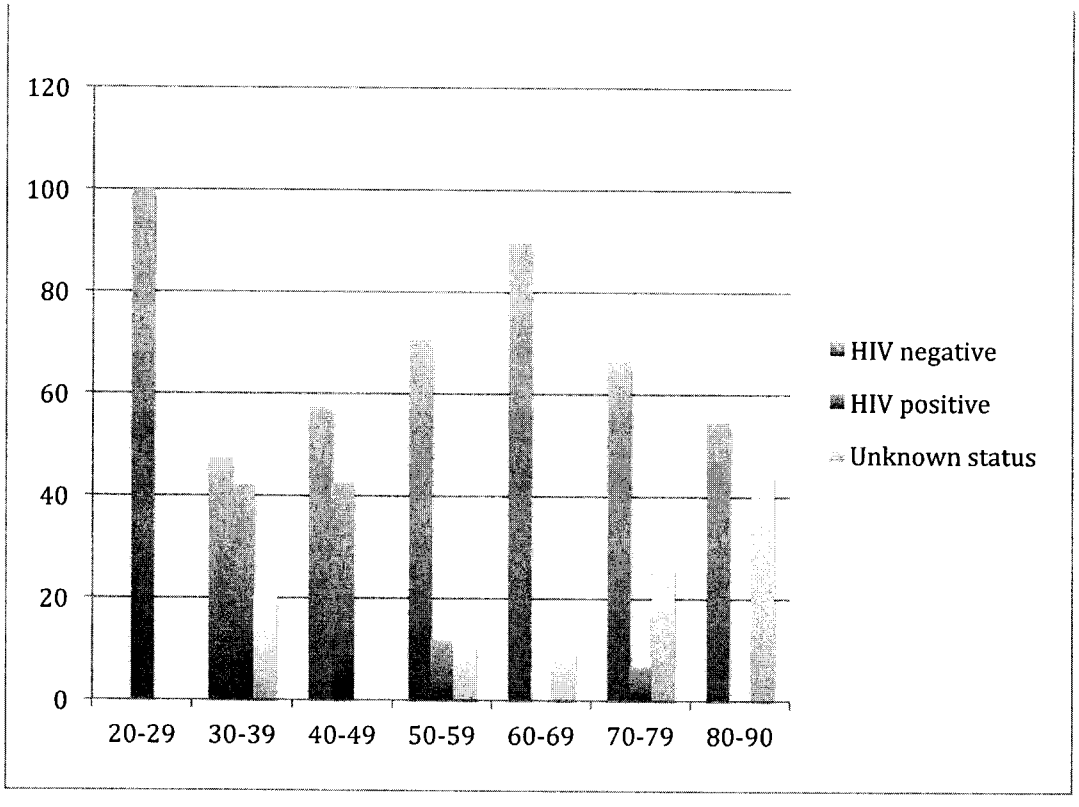

Figure 1 The prevalence of HIV seropositivity by age in patients with SCC of vulva

As demonstrated in Table 4, both young and older women presented with either stage 3 or 4 of the disease. There was however no significant difference (p=0.422) in stage at presentation among the age groups.

Table 4. Correlation between stage and age groups.

AGE GROUP (years)	STAGE				
	I	II	III	IV	unstaged
20-29	16.7	0.0	25.0	50.0	8.3
30-39	10.5	10.5	57.9	21.1	0.0
40-49	3.7	7.4	55.6	31.5	1.8
50-59	3.9	15.7	49.0	31.4	0.0
60-69	7.7	7.7	59	25.6	00
70-79	6.7	20.0	40.0	33.3	0.0
>80	9.1	0.0	72.7	18.2	0.0

Ten percent of the HIV positive patients presented with stage I, 10% with stage II, 40% with stage III and 38% with stage IV disease. In HIV negative patients, 3.9% presented with stage I, 11% with stage II, 56% with stage III and 27.7% with stage IV. There was no significant difference in the stage of presentation when both HIV positive and negative patients were compared (p=0.350). (see Table 5)

Table 5. Correlation of stage of the disease by HIV status.

HIV STATUS	STAGE					
	I	II	III	IV	unstaged	
Negative	3.9	11.0	56.7	27.7	0.7	P=0.350
Positive	10.0	10.0	40.0	38.0	2.0	
unknown	12.5	4.2	58.3	25.0	.0	

The majority (52.7%) of the patients with SCC of the vulva had no histological background stated in their histology report and files. Forty-six percent (93) of the patients had the histological background recorded as only VIN and 1% had both VIN

and lichen sclerosis-related SCC of the vulva. The breakdown of this is demonstrated in Table 6.

Table 6. Correlation between histological background and the age groups

Age group (n)	VIN	VIN and Lichen sclerosus	Unknown
20-29	49.7	0.00	58.3
30-39	42.1	0.00	57.9
40-49	50	0.00	50
50-59	52.9	3.9	43.2
60-69	43.6	0.00	56.4
70-79	46.7	0.00	53.3
80-89	18.2	0.00	81.8
Total (208)	93	2	106

Forty-four out of 50 patients who were HIV positive had CD4 counts recorded in the files. The lowest CD 4 count was 9 and the highest 766. The data regarding frequencies is shown in the Table 7.

Table 7. Frequencies of CD 4 count levels.

CD4 lymphocyte count	Frequency (n)	Percentage (%)
<200	15	34
201-500	18	40.9
>500	11	25

Forty-six percent of patients with CD4 < 200 and 54.6% of patients with CD4 counts of more than 500 presented with stage 3 disease, whilst 47% of patients with CD4 counts between 200-500 presented with stage IV disease. The breakdown is shown in Table 8. There was no significant difference in stage at presentation among the CD4 count levels

Table 8: The stage of disease and CD4 count levels.

CD4 COUNT	STAGE					
	I	II	III	IV	unstaged	
<200	13.3	6.7	46.7	26.7	6.7	P=0.653
200-500	16.7	11.0	27.8	44.5	0.0	
>500	0	9.1	54.6	36.4	0	

The incidence of squamous carcinoma of the vulva cancer did not increase in a linear function or predictably over the period of the study (1999-2009).What is evident is that there were 4 peaks in incidence: 2000, 2002 and 2007 with 2004 having the highest incidence. (See Figure 2)

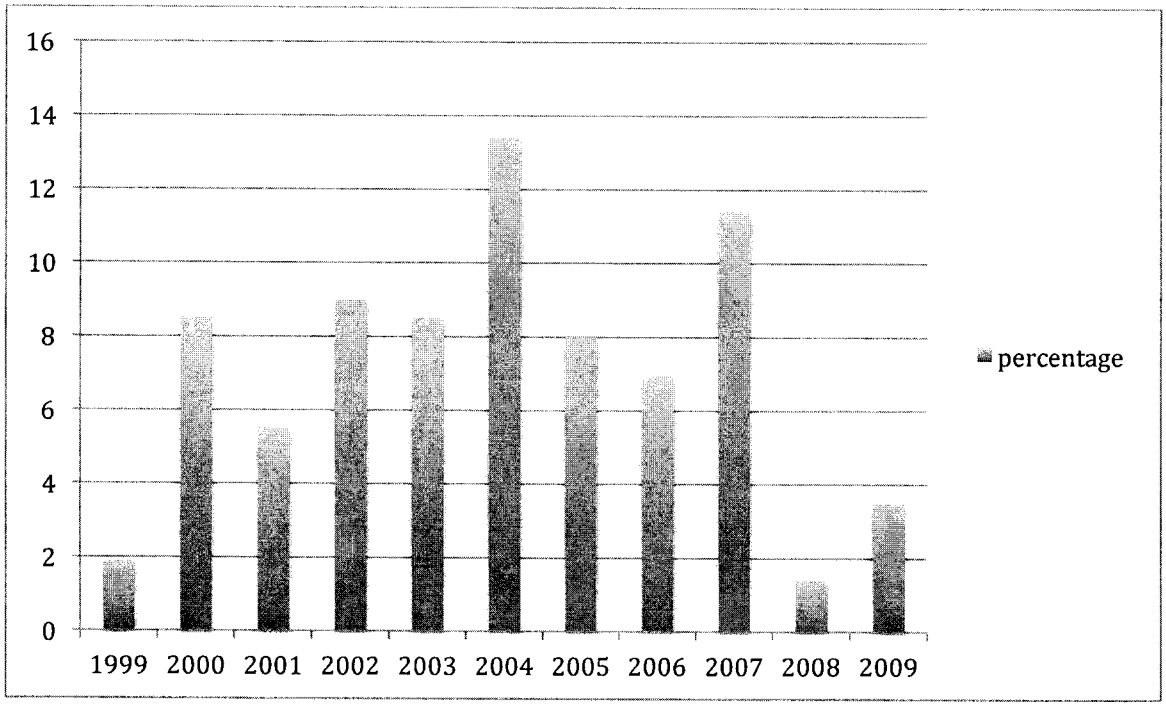

Figure 2. The prevalence of the SCC of the vulva from the year 1999 to 2009.

DISCUSSION

All the patients had histological or clinically confirmed vulval cancer. Although the background in which the cancer had developed in only 46% of the report The objective of the study was to determine whether the prevalence of vulval cancer was increasing in the younger patients at a tertiary care hospital which provides services to most of the women within the referral area; especially in those who are immune-compromised. I also aimed to determine the stage at presentation amongst the women presenting with vulva cancer and specifically determine the likely impact of HIV serostatus.

Squamous cell carcinoma was the most common histological type in my study. This is comparable to published reports. [40, 41, 13] The second most common histological types were Kaposi's sarcoma which occurred in 6 cases (2,7%) and the other histological types are shown in Table 1.

Kaposi's sarcoma of the vulva occurred exclusively in the HIV infected patients. It had been common in African countries but the incidence has increased 20 fold since the HIV/AIDS epidemic began. Kaposi's sarcoma shows a pattern similar to the prevalence of HIV infection.[18] In a study from Soweto, Sitas showed an association between HIV seropositivity and development of generalized Kaposi's sarcoma with an odds ratio of 61.8 [95%(CI 19.7-194.2.)].[32]

The majority of the patients in my study were black, which demonstrated the socio-demographics in my institution and the province as the majority of the patients are black or of African descent. The mean age of my cohort was 53 years of age. The

youngest patient in my cohort was a 14 year-old girl who was HIV negative and presented with stage 3 alveolar rhabdo-myosarcoma of the vulva. The majority of the patients was HIV negative and presented in stage 3 of the disease.

Based on the fact that squamous cell carcinoma formed the majority of my cohort, the majority of the sub-analysis centered specifically around these patients. This resulted in 21 patients with the other histological subtypes not forming part of the studies. This was done as the number of the different histological types varied greatly and data about each case would have made the final analysis very difficult.

In the sub-analyis of the women presenting with vulva cancer, my cohort showed that irrespective of HIV status the patients with SCC of vulva were two decades younger (mean of 53 years with peak in the 5^{th} decade) than those reported in some publications [16, 12] who demonstrated a peak age of 70 years. Unfortunately due to the lack of data from the files of our patients, this inconsistency cannot be explained, as I do not have access to other risk factors etc.

The epidemiological data on vulva cancer in South Africa is outdated. The most recent available data from National Health Laboratory Services cancer registry is from 2002. These findings are mentioned earlier in the literature review. [4]

The 2002 report of the NHLS also confirmed that histologically diagnosed cancers showed a peak in the ages between 40 and 59 years, a finding similar to that found in this study. In 2002 the NHLS reported that vulva and vaginal cancer represented about 1.04% of all cancer in women for that year. [4] Since then the incidence of vulva cancer is reported to be between 3-5% [34]. These studies are also subject to

selection bias since it is a histologically based registry and only samples sent to NHLS laboratory will be analyzed.

My study shows that the patients, both young and old, present with stage 3 and 4 disease. It must be borne in mind that the older patients in the study may have had co-morbid diseases, which rendered them immune-compromised even in the absence of HIV. Studies by Anderson [11] and Rosen et al [41] demonstrated that the majority of their patients presented with early stages of the disease. These two studies are in obvious contrast to mine. Their work was conducted in high-income countries, which may contribute to the difference due to several factors including access to health care.

The infection with HIV did not increase the likelihood of having advanced stage disease. The majority of both HIV negative and HIV positive presenting with stage 3 and 4 disease and there was no significant difference in the presenting stage between patients who were HIV negative or HIV positive.

Why did my patients present with locally advanced stage of the disease? I could not find a reason for this phenomenon and can only reiterate the findings of a local survey which demonstrated that approximately 70% of patients first consult the traditional healers and consult hospital care at an advanced stage when symptoms are unbearable. [38] The culture of consulting traditional healers first and thereby delaying in seeking conventional medical care may play a significant role in our patients and account for their late presentation. Another possibility may be because of the delay in making the diagnosis by the treating physician, [38] although my study was unable to detect this notion, as the data was not available in the retrospective review of records.

The average age of HIV positive and HIV negative patients was 40 years and 56 years respectively and this difference was statistically significant. The study demonstrated that a young patient (<30 years) presenting with vulval cancer, was significantly more likely to be HIV infected than her older counterpart by about a mean of 16 years. Sixty four percent of patients who were less than 40 years with SCC of the vulva were HIV positive. My study therefore hypothesizes that HIV infection is a definite risk factor for developing invasive cancer of the vulva at an early age.

Several case studies have recently reported that the age at presentation of women with squamous cell cancer of the vulva is declining and a girl as young as 12 having been described. . Giaquinto et al reported a case of a 12 year old girl with invasive vulval cancer within a background of HIV infection and a CD4 count of 148. The patient was classified as having had Stage II disease. HPV16 was isolated from the histology. [36] A case report of 2 HIV positive women, 32 and 59 years respectively, with vulval cancer had a background of VIN2/3. The older patient had a CD4 count of 330 and was diagnosed with Stage II disease. The younger patient had a CD4 count of 735 and presented with stage IV disease.[35]All these studies implicate immunosuppression, especially HIV infection and HPV infection (mostly HPV 16), as etiological agents in vulval cancer and HIV as an "enabler".

It might be possible, although not demonstrated, that most of the patients with unknown HIV status might be immune-compromised, therefore contributing to the severity of their disease.

The histological background in my study could not be confidently analyzed and interpreted since the majority of our patients had no information in their files.

However there is evidence from other studies, with view specifically to VIN, that show similar trends as my study, namely the median age for VIN lesion is now in the late 4th decade of life rather than in the 60's as was the opinion 13 years ago. A study by Messing reported that the average presenting age for their patients decreased from 69 to 55 years over a period of 15 years. But their study did not report the reason for such a decrease in age. They reported a trend toward a younger age at presentation and that HPV infection was common in younger patients.[17]

Again the relationship between HIV infection and VIN-related SCC of the vulva could not be clearly demonstrated in my study for the same reasons stated above. Published reports claim that HIV infected patients are expected to have persistent HPV infection with high risk subtypes, such as HPV 16, due to their inability to naturally clear the infection.[13,26]

The way to overcome this shortcoming of my study would be to for the pathologist to review all the slides with missing information on the histological background. This would be a mammoth task that will require extra funding and perhaps even the assistance of a private pathology laboratory. This would nevertheless enable us to see if there is an increase or decrease in either VIN or non-VIN related SCC carcinoma in my institution.

Prospective cohort studies may be able to establish whether HIV infection affects prognostic factors such as lymph node involvement, margins of resection and survival rate. This is a difficult undertaking since vulval cancer is rare and therefore would have to rely on cancer registries to study these factors.

in my study, the CD4 count, a marker of immune-suppression surprisingly did not seem to affect the stage of the disease at presentation. There was no statistical difference in the stage of presentation across all CD4 count levels. I was not able to offer any plausible reason as to why the degree of immune suppression did not relate to the severity of the disease at presentation.

The effect of HAART on vulval cancer was not assessed in my study. A recent review suggests that treatment with antiretroviral has not been shown to lower the natural history of intraepithelial lesions in women, although it does increase life expectancy.[20] Overall, the current information about the effect of HAART is conflicting and scanty with no obvious data pertaining to vulva cancer. [39]

LIMITATIONS

This study has several limitations:

- It is a retrospective review of patient's records and not only may some data have been incorrectly recorded, but a fair amount of important data is missing.
- There is a risk of referral bias since this study only included those women who came to the CMJAH and does not include those patients who were referred but either did not come or may have demised prior to being assessed at our clinic.
- Since it is a cross-sectional study, there will be no data pertaining to follow-up of these patients. Comparison of survival rates between groups of patients was not possible.
- There is no information about whether or not the patient was on HAART. This may have affected the immunecompetence of the patient.

- No mention was made of risk factors e.g. smoking, number of sexual partners, drug use and previous HPV infection.
- Not all patients had documented staging, age, parity, histological type or HIV status recorded in their files.
- Not all HIV positive patients had CD4 counts documented in their records.
- Since my objective was to study vulval cancer which is a rare disease, all files were reviewed whether information was complete or not.

CONCLUSION

The study did not show a direct association between vulval cancer and HIV infection but a significant link was shown between *young* women infected with HIV and vulval cancer. There was a mean age difference of 16 years between HIV positive and HIV negative women with the HIV positive patients being younger. Public health programs that address sexual behavior and HIV/AIDS prevention may be helpful in prevention of HPV-related vulval cancer. Measures such as screening vulvoscopy of younger HIV + women who present with cervical abnormalities may prevent vulva cancer. Available HPV vaccines at the moment are specifically design to prevent the cancer of the cervix and not that of the vulva. The most significant development above all, which so far has failed, will be development of HIV vaccine or cure.

It was also demonstrated that HIV infection is not related to advanced stage at presentation. The presence of HIV infection at and early young young age is a risk factor for developing SCC of the vulva. What has been shown quite conclusively is that the mean age for vulval cancer has been decreasing in the last 3 decades where

the mean age of presentation was in the 6th decade; it is now in the latter 4th and early 5th decades. It is surmised that the reason for this is the significantly greater incidence in the HPV infection of the anogenital tract.

This study has reached the same conclusions as in other published studies that have analysed the impact of HIV status on cervical cancer in South Africa. The studies by Lamolisa and Moodley both concluded that HIV positive patients were younger and that HIV infection and the level of the CD4 count did not affect the stage of the disease at presentation. A prospective study would be helpful to overcome limitations of my study such as missing information and lack of follow up. It can also be valuable to assess response to treatment and survival rates. The role of anti-retroviral and other prognostic factors such as number of lymph nodes and margins of resection can also be assessed more accurately in a prospective study.

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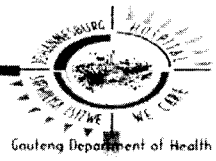
APPENDIX A

DATA SHEET

PATIENTS STUDY NUMBER	
YEAR	
AGE	
RACE	
HIV STATUS	
CD4 COUNT	
HISTOLOGY	
DIFFERENTIATION	
VIN BACKGROUND	
STAGE	
TREATMENT MODALITY	
LYMPH NODES AND MARGINS	

APPENDIX B



Office of the CEO
Charlotte Maxeke Johannesburg
Academic Hospital
Enquiries: M. Motjelele
(011): 488-3792/3
(011) 488-3753
07th March 2010

Dr. Daniel S. Mosehle
Department of Obstetrics & Gynaecology
CMJAH

Dear Dr. Mosehle

RE: "Invasive cancer of the vulva at the Charlotte Maxeke Johannesburg Academic Hospital"

Permission is granted to you for the abovementioned as requested.

1. The Charlotte Maxeke Johannesburg Academic hospital will not in anyway incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the Head of Department and Unit Manager or Sister in Charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Yours sincerely

Dr. T.E. Selebano
Chief Executive Officer

APPENDIX C.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Daniel S Moodley

CLEARANCE CERTIFICATE	M0909O9
PROJECT	Invasive Cancer of the Vulva at the Charlotte Maxeke Johannesburg Academic Hospital (Revised title)
INVESTIGATORS	Dr Daniel S Moodley
DEPARTMENT	Department of Obstetrics & Gynaecology
DATE CONSIDERED	2009/10/02
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 06/12/2009 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor: Dr T Smith

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.