THE ASSOCIATION BETWEEN INFECTION WITH THE HUMAN IMMUNODEFICIENCY VIRUS AND CANCER OF THE VULVA

Dan Kirwana Kibuuka

Student Number: 0110757X

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DECLARATION

I, Dan Kirwana Kibuuka declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Epidemiology and Biostatistics, in the School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or Examination at this or any other university.

SIGNED: ..... AT JOHANNESBURG

DATE: 30 January 2004

DEDICATION

This research report is dedicated to the two most important women in my life, my wife Gloria Nono Kibuuka and my mother Leah Kibuuka, through whose support of all kind this qualification has been made possible.

Also to my Late Father Edmund Kirwana Kibuuka.

ABSTRACT

Background: Human immunodeficiency virus (HIV) infection has been associated with the development of various malignancies. There has been concern that HIV-infected women may be at higher risk of developing cancer of the vulva. In Johannesburg, investigators noted an association between HIV infection and cancer of the vulva, but it was unclear whether the increased risk was real, or due to incomplete adjustment for sexual activity.

Study Objectives: This study aimed to determine whether there is an association between HIV infection and increased risk of cancer of the vulva, and to determine any confounding factors in this association.

Methods: Data from an ongoing case control study run by the Cancer Epidemiology Research Group (CERG) in Johannesburg was processed using STATA and analysed using unconditional unmatched logistic regression.

Results: Cancer of the vulva was associated with HIV, with an OR = 3.72 (95% CI: 1.68 - 7.56). Smoking was also independently associated with cancer of the vulva (OR = 3.83; 95% CI: 1.90 - 7.72), as was number of lifetime sexual partners (OR = 3.68; 95% CI: 1.84 - 7.34). Age at first sexual intercourse was not found to be associated with vulvar cancer.

Conclusion: There was overwhelming evidence for a significant association between HIV and cancer of the vulva that it is not due to confounding. The major independent risk factor was smoking and number of lifetime sexual partners was a confounder, however the association remained significant even after adjusting for

these factors. Age at first sexual intercourse and level of education were not found to be associated with cancer of the vulva.

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1.0 INTRODUCTION:

1.1 BACKGROUND

The vulva is the external portion of the female genital organs. Vulvar cancer is a malignancy that can occur on any part of the external organs, but most often affects the labia majora or labia minora. Worldwide, cancer of the vulva is a rare disease, accounting for 0.5% of all cancers in women. [1, 2, 3, 4, 5]

Nearly 90% of vulvar cancers are squamous cell carcinomas.[6] Melanoma is the second most common type of vulvar cancer, usually found in the labia minora or clitoris. [6] Other types of vulvar cancer include adenocarcinoma, paget's disease, sarcomas, verrucous carcinoma and basal cell carcinoma.[6]

Human immunodeficiency virus (HIV) infection and the associated acquired immune deficiency syndrome (AIDS), leads to the progressive deterioration of the immune system and the development of opportunistic infections and various malignancies.[7] Infection with HIV-1 has been associated with an increased risk of developing a number of infection related cancers, for example Kaposi's Sarcoma, Hodgkin and non- hodgkin lymphoma, squamous conjuctival cancers and leiomyo sarcoma in children. [8, 9, 10, 11]

As more experience is being accumulated on the nature and complications of AIDS, the gynecological implications of neoplasia in the HIV-positive patient are becoming apparent.[12] Invasive cervical cancer was designated in 1993 as an AIDS case defining illness by the Centres for Disease Control and Prevention. [5, 13] Cervical cancer was associated with HIV infection in South Africa in 2000, however the odds ratio was small (OR= 1.6, 95% CI: of 1.1 – 2.3), [14] as was also found in Uganda in 2001 (OR = 1.6, 95% CI: 0.7 – 3.6). [6]

As cervical and vulvar cancers share certain risk factors, and invasive cervical cancer can occur simultaneously or metachronously with invasive vulvar cancer, the concern arose that HIV-infected women may also be at risk for developing vulvar cancer. [15] This concern was supported by data from tumour registries of renal transplant recipients in the United States, which indicate that in these immuno-suppressed patients, invasive vulvar carcinoma is more common than invasive cervical carcinoma. [15]

A study in Johannesburg, South Africa, found a significantly increased risk of vulval cancer associated with HIV infection. In this study there were 53 cancer of the vulva cases and 10 of these were HIV positive while 43 were HIV negative. The control group included 556 female patients. The investigators noted an odds ratio of 4,8, and 95% confidence interval of 1.9-12.2, but it was unclear whether the excess risk was real or due to incomplete adjustment for sexual activity. [14] These results suggested that further research was needed to clarify the association between HIV infection cancer of the vulva.

1.2 RISK FACTORS FOR VULVAR CANCER

A number of risk factors for vulval cancer have been suggested, including increased age (three quarters of cases are over age 50, and two-thirds are over 70), chronic vulvar inflammation, infection with the human papillomavirus (HPV), HIV infection, lichen sclerosus (which can cause the vulva skin to become very itchy and may slightly increase the possibility of vulvar cancer), melanoma or atypical moles on non-vulvar skin (a family history of melanoma and dysplastic nevi anywhere on the body may increase the risk of vulvar cancer), low socioeconomic status, vulvar intraepithelial neoplasia (VIN), other genital cancers and smoking.[1, 16]

1.3 CONFOUNDING

In a study of the association between exposure to a disease cause (or risk factor) and the occurrence of disease, confounding can occur when another exposure exists in the study population that is associated both with the disease and the exposure being studied.[17] A problem arises if this extraneous factor - itself a determinant or risk factor for the health outcome is unequally distributed between the exposure subgroups.

Confounding (from the latin word Confoundere, to mix together) occurs when the effects of two exposures (risk factors) have not been separated and it is therefore incorrectly concluded that the effect is due to one rather than the other variable.[17, 18, 19] The most common concern over confounding is that it may create the appearance of a cause-effect relationship that in reality does not exist. For a

variable to be a confounder, it must, in its own right, be an independent determinant of the occurrence of disease (i.e. a risk factor) and be associated with the exposure under investigation. Age and social class are often confounders in epidemiological studies. [17, 18, 19]

An example of confounding occurs in the association between coronary heart disease (CHD) and high alcohol consumption. In this case, smoking history is a potential confounder. This is because smoking is an independent risk factor for coronary heart disease, and heavy smokers may also be heavy consumers of alcohol. As a result, there may appear to be an association between alcohol consumption and CHD, which is in fact due to the confounding effect of smoking. However, if one stratified by smoking, it would be found that within each smoking group there was no association between level of alcohol consumption an and CHD; that is, adjusting for the confounding effect of smoking history, there is no association between level of alcohol consumption and CHD.It is therefore important to adjust every analysis for potential confounding factors.

This study examined a number of potential confounders that were suspected to play a role in the relationship between HIV and cancer of the vulva. The main objectives were to determine whether there is a relationship between HIV and cancer of the vulva and whether age at first sexual intercourse is a confounding factor in this relationship.

1.4 EFFECT MODIFICATION

Effect modification occurs when the association between an outcome and an exposure factor is different for each level of confounding. Effect modification can be additive or multiplicative. It is additive when the effect of one risk factor adds to the effect of the other risk factor. Multiplicative effect modification occurs when the effect of one risk factor multiplies the effect of other risk factors. This is controlled by logistic regression.

1.5 TEST OF HOMOGENEITY

To determine whether there is confounding or effect modification, it is important to test whether the odds ratios can be considered to be constant over the strata. The tests used are tests of homogeneity for the odds ratios over the strata. The most commonly used test is the Mantel-Haenszel Chi square test of homogeneity calculated as $\Sigma^{k}_{i=1} \{\ln (R_i) .. \ln (\check{R})\}^2 / \text{var} \{\ln (R_i)\}$

Where R_i are the stratum specific odds ratio (I_i=1,2...k) and $\check{R}$ the combined Mantel-Haenszel odds ratio[20]. It is important to establish whether there is confounding or effect modification; if there is confounding then it is sensible to present a single (combined) odds ratio. However, if there is effect modification then separate odds ratios should be presented for each level of the confounder.

1.6 AIDS IN SOUTH AFRICA

Knowledge of AIDS is almost universal in South Africa. According to the South African Demographic and Health Survey (SADHS), 97% of women in all nine Provinces aged between 15-49 said they had heard of the disease. In Gauteng, 98.9% of all women surveyed said they had heard of the disease, while 95.9% of black women had heard of the disease.[21]

In South Africa, the HIV-1 epidemic was first noted in 1982, and is primarily due to HIV-1.[14] In 1996, in the province of Gauteng, where Johannesburg is situated, 18,8% of women between 15-44 years of age surveyed at antenatal clinics tested positive for HIV-1. [14] Nationally, about 16% of surveyed women attending antenatal clinics were infected with HIV and it was estimated that about 2.4 million adults (male and female) and 157,000 children were infected. [14, 22]

In the antenatal survey conducted in October 2002, 26,5% of pregnant women were HIV positive. Extrapolating from the result of this survey, the Department of Health estimated that 5.3 million South Africans were HIV positive by the end of 2002. This translates to an estimated 2.95 million women between the ages of 15 to 49 years, and 2.3 million men between 15 and 49 years being infected with HIV in 2002. [23]

Based on speculation that HIV is associated with cancer of the vulva, this study aimed to confirm the association, and to clarify whether confounding had affected the results of previous studies. Given the HIV/AIDS statistics quoted above, this is clearly an important public health issue in South Africa.

2.0 MATERIALS AND METHODS

This project entailed analysis of pre-existing data from an ongoing case control study started in 1995 and run by the Cancer Epidemiology Research Group, National Health Laboratory Service, Johannesburg, South Africa.
The data was initially collected to study the spectrum of HIV-1 related cancers in South Africa. [22, 24]

2.1 STUDY DESIGN

The data obtained for this project was generated from a case control study conducted among black cancer patients admitted to Chris Hani-Baragwanath, Hillbrow and New Johannesburg Hospitals in Gauteng. The data was collected using a questionnaire. Questions asked were in the home language of the patients. The data collected included demographic and behavioural characteristics - including place of birth, years of education, reproductive and sexual history, and smoking habits. The questionnaires were anonymous and the women gave written or verbal consent (if illiterate) to participate in the study.

For the purpose of this analysis, cases were selected from the overall database as women diagnosed for the first time with vulvar cancer. The controls were women who had other cancers that were not gynaecological. Gynaecological cancers were excluded as they may have similar risk factors to vulvar cancer.

The choice of controls comprising cancers other than those thought to be associated with the exposure of interest has been used successfully with data from this case control study in other contexts.[22, 24]

2.2 STUDY POPULATION

6,478 incident black (mainly cancer) patients who were women aged 18 years or older, presenting at the medical, surgical, radiation therapy and oncology wards of Chris Hani-Baragwanath, Hillbrow and New Johannesburg hospitals in Gauteng were interviewed by trained nurses. Data used for this analysis was collected from 1996 to 1999.

2.3 STUDY SAMPLE

A total of 1,680 women were used for this analysis. There were 57 cases and 1,621 controls for whom HIV status was available. Given the fact that cancer of the vulva is a rare disease, a sample of 57 cases is considered adequate.

2.4 DATA ANALYSIS

Data was processed using STATA [25]. Data analysis was done using unconditional unmatched logistic regression, because the response was binary. The variables examined in association with vulvar cancer were HIV status, number of lifetime sexual partners, education, smoking and age at first sexual intercourse. As this was a cancer-based study, detailed indicators of sexual activity were not

collected, therefore age of oldest child subtracted from age of the mother was used as a proxy for age at first sexual intercourse.

3.0 RESULTS

The results are presented before and after adjusting for confounders. The results also give details of smoking status as well as number of lifetime sexual partners that women had had, up to the time of the study, as these variables were suspected confounders.

TABLE 1: Sociodemographic description of cases and controls

Variable	Cases	Controls
Total	n= 57	n= 1,621
Age		
Mean	51.4	53.1
Standard deviation	12.57	14.75
HIV Status		
Positive (%)	11 (19%)	98 (6%)
Negative (%)	46 (81%)	1,523 (94%)
Education Level		
≤ Std 5 (%)	45 (79%)	932 (58%)
Std 6-8 (%)	12 (21%)	519 (32%)
≥ Std 9 (%)	0 (0%)	160 (10%)
Smoking Status		
Never Smoked	31 (54%)	1,280 (80%)
Past smoker	17 (16%)	222 (14%)
Current Smoker	9 (30%)	104 (6%)
Marital Status		
Divorced	9 (16%)	144 (9%)
Married	11 (19%)	607 (38%)
Single	24 (42%)	460 (29%)
Widowed	13 (23%)	405 (25%)

Table 1 shows the demographic description of cases and controls. The mean age of the cases was 51.4 with a standard deviation (sd) of 12.57.The mean age of the controls was 53.1 (sd 14.75). Among the 57 cases, 11 (19%) were HIV positive and 46 (81%) were HIV negative. Out of the 1,621 controls 98 (6%) were HIV positive and 1,523 (94%) were HIV negative. 58% of cases had an education level less than or equal to standard 5.Only 10% of cases had standard 9 or a higher level of education.79% of women with cancer of the vulva had an education level less than or equal to standard 5. There were no cases of vulval cancer among those with standard 9 or a higher level of education. 31 (54%) of the cases had never smoked, 17(16%) were past smokers and 9 (30%) were current smokers. 1,280 (80%) of the controls had never smoked, 222 (14%) were past smokers and 104 (6%) were current smokers. Of the 57 cases 9 (16%) were divorced, 11 (19%) were married, 24(42%) were single and 13 (23%) were widowed. Of the 1,621 controls 144 (9%) were divorced, 607 (38%) were married, 460 (29%) were single and 405 (25%) were widowed.

TABLE 2: HIV status of cases and controls

	HIV Negative	HIV Positive	Total
No vulva cancer	1,523	98	1,621
Vulva cancer	46	11	57
Total	1,569	109	1,678

Table 2 shows the overall number of cases and controls that were used in the study. The exposure under investigation is infection with HIV and vulvar cancer is the outcome of interest. A total of 1,678 people were studied; 57 cases of vulvar cancer and 1,621 controls. Among the 57 cases, 46 (81%) were HIV negative and 11 (19%) were HIV positive. Among the 1,621 controls, 98 (6%) were HIV positive and 1,523 (94%) were HIV negative. The controls comprised 13 different types of cancers, illustrated in Table 3, below. Cancer of the breast contributed the largest number of controls 542 (33%), followed by cancer of the throat 226

(14%) and cancer of the oesophagus 134 (8%). Cancer of the larynx contributed the smallest number 7 (0.4%).

TABLE 3: Distribution of the number and proportion of cancers in the control group

Type of Cancer	Frequency	Percentage
Breast	542	33.4
Throat	226	13.9
Colon	60	3.7
Endometrium	76	4.7
Hodgkin	42	2.6
Larynx	7	0.4
Leukemia	122	7.5
Lung	37	2.3
Myeloma	48	3.0
Oesophagus	134	8.3
Ovary	58	3.6
Stomach	27	1.7
Vascular	242	14.9
Total	**1,621**	**100**

3.1 SMOKING AND CANCER OF THE VULVA:

Table 4 shows the smoking status of women with cancer of the vulva. Out of a total of 113 women who were current smokers, 104(92%) did not develop cancer of the vulva and nine (8%) developed cancer of the vulva. Of the 239 women who had ever smoked in the past, 222(93%) did not develop cancer of the vulva and 17(7%) developed cancer of the vulva. Out of 1,311 women who had never smoked, 1,280(98%) never developed cancer of the vulva and 31(2%) developed cancer of the vulva. There is an association between smoking and cancer of the vulva (Chisquare = 21.31 at 2 degrees of freedom, p< 0.0001).

TABLE 4: Description of the smoking status of the cases and controls

Smoking status	Cancer of the vulva				Total
	No cancer		Cancer		
	No.	(%)	No.	(%)	
Never smoked	1,280	(97.64)	31	(2.36)	1,311
Past smokers	222	(92.89)	17	(7.11)	239
Current Smokers	104	(92.04)	9	(7.96)	113
Total	**1,606**	**(96.57)**	**57**	**(3.43)**	**1,663**
Chisquare = 21.31 with 2 degrees of freedom (d.f) p< 0.0001					

3.2 SMOKING AND HIV STATUS:

Table 5 below illustrates that out of 113 women who were current smokers, 106 were HIV negative and seven were HIV positive. Of 239 women who were past smokers (ever smoked), 222(93%) were HIV negative and 16(7%) were HIV positive. A total of 1,311 women had never smoked; of which 1,225(93%) were HIV negative and 86(7%) were HIV positive. There is no association between smoking and HIV status (Chisquare = 0.025 with 2 d.f p = 0.988).

TABLE 5: Smoking and HIV status.

Smoking status	HIV Negative		HIV Positive		Total
	No.	%	No.	%	
Never smoked	1,225	(93.44)	86	(6.56)	1,311
Past smokers	222	(93.31)	16	(6.69)	239
Current smokers	106	(93.81)	7	(6.19)	113
Total	**1,554**	**(93.44)**	**109**	**(6.55)**	**1,663**
Chisquare = 0.025 with 2 d.f, p= 0.988					

3.3 LIFETIME SEXUAL PARTNERS AND CANCER OF THE VULVA:

Table 6 shows that the percentage of cases of cancer of the vulva is highest in those women who had five to nine lifetime sexual partners. Among women who had zero or one sexual partner in their lifetime, 97.2% (311/320) did not develop cancer of the vulva and 2.8% (9/320) had cancer of the vulva. Among women who had two to four lifetime sexual partners, 97.3% (986/1013) did not have cancer of the vulva while 2.7% (27/1013) had cancer of the vulva. Among women who had five to nine lifetime sexual partners, 93.2% (264/283) did not have cancer of the vulva and 6.7% (19/283) developed cancer of the vulva. Among women who had 10 or more lifetime sexual partners each, 96.7% never developed cancer of the vulva and 3.2% (1/31) developed cancer of the vulva. There is weak evidence that the risk of developing cancer of the vulva increases with increasing number of lifetime sexual partners (Chisquare for trend = 3.69; p = 0.055). OR for zero or one lifetime sexual partners = 1.00, for two to four lifetime sexual partners = 0.95, for five to nine lifetime sexual partners = 2.49 and for 10 and more lifetime sexual partners = 1.15.The low OR for greater than and equal to 10 partners may be merely due to low numbers of individuals in this category.

TABLE 6: Number of lifetime sexual partners and cancer of the vulva cases

Lifetime sexual Partners	Cancer of the vulva				Odds Ratios	TOTAL
	No cancer		Cancer			
	No.	(%)	No.	(%)		
0-1	311	(97.19)	9	(2.81)	1.00	320
2-4	986	(93.29)	27	(2.67)	0.95	1013
5-9	264	(93.29)	19	(6.71)	2.49	283
≥10	30	(96.77)	1	(3.23)	1.15	31
Missing	30	(96.77)	1	(3.23)	1.15	31
Total	**1,621**	**(96.60)**	**57**	**(3.40)**		**1,678**
Chi square $_{trend}$= 3.69, 1 d.f p = 0.055						

3.4 LIFETIME SEXUAL PARTNERS AND HIV STATUS:

Table 7 shows that 96% (307/320) of the women who had no or one lifetime sexual partner were HIV negative, while 4.1% (13/320) were HIV positive; 94% (949/1013) of the women who had two to four lifetime sexual partners were HIV negative while 6% (64/1013) were HIV positive; 90% (256/283) of the women who had five to nine lifetime sexual partners were HIV negative and 10% (27/283) were HIV positive; and 90% (28/31) of the women who had 10 or more lifetime sexual partners were HIV negative while 10% (3/31) were HIV positive. The percentage of HIV positive women is highest with 10 or more lifetime sexual partners. There is strong evidence that odds of HIV infection increase with increasing number of lifetime sexual partners (Chisquare for trend = 5.544, 1 d.f, p = 0.019).

TABLE 7: Number of lifetime sexual partners and HIV status

Lifetime sexual partners	HIV status				Odds Ratio	Total
	Negative		Positive			
	No.	(%)	No.	(%)		
0-1	307	(95.94)	13	(4.06)	1.00	320
2-4	949	(93.68)	64	(6.32)	1.59	1,013
5-9	256	(90.46)	27	(9.54)	2.49	283
≥10	28	(90.32)	3	(9.68)	2.53	31
Missing	28	(93.55)	2	(6.45)	1.63	31
Total	**1,569**	**(93.50)**	**109**	**(6.50)**		**1,678**

Chisquare $_{trend}$ = 5.544, 1 d.f p = 0.019,

3.5 EDUCATION LEVEL AND CANCER OF THE VULVA:

Table 8 shows that women with less than standard 5 level of education were more likely to be vulvar cancer cases (45), than those between standard 6 and 8 (12 vulvar cancer cases). Those with standard 9 or higher level of education had zero vulvo cancer cases. Thus, there is evidence that the risk of cancer of the vulva decreases with increasing level of education (chi square = 11.99, 2 d.f p = 0.0005)

TABLE 8: Level of education and cancer of the vulva

Level of education	Cancer of the Vulva				Total
	No		Yes		
	No.	(%)	No.	(%)	
≤ std 5	932	(95.39)	45	(4.61)	977
Std 6 – 8	519	(97.74)	12	(2.26)	531
≥ std 9	160	(100.00)	0	(0.00)	160
Total	**1,611**	**(96.58)**	**57**	**(3.42)**	**1,668**
Chi square = 11.99 2 d.f p = 0.0005					

3.6 AGE AT FIRST SEXUAL INTERCOURSE AND CANCER OF THE VULVA:

Table 9 shows women who had their first sexual intercourse at or below age 19 had 12(3%) cases of cancer of the vulva, those who had their first sexual intercourse between ages 20 and 25 had 27 (4%)cases of cancer of the vulva, and those who had their first sexual intercourse at or over age 26 had 13(4%) cases of cancer of the vulva. From these data, it does not appear that cancer of the vulva is associated with age at first sexual intercourse (Chisquare = 1.35, 2 d.f p =0.51)

TABLE 9: Age at first sexual intercourse and cancer of the vulva

Age first sexual intercourse	Cancer of the vulva				Total
	No cancer		Cancer		
	No.	(%)	No.	%	
≤ 19 yrs	446	(97.38)	12	(2.62)	458
20 –25 yrs	694	(96.26)	27	(3.74)	721
≥ 26 yrs	320	(96.10)	13	(3.90)	333
Total	**1,460**	**(96.56)**	**52**	**(3.44)**	**1,512**
Chisquare = 1.35, 2 d.f p = 0.51					

3.7 CRUDE ODDS RATIO:

Table 10, below, shows the association between HIV and cancer of the vulva before adjusting for any confounders, showing a highly statistically significant association. The proportion of those exposed to HIV among the vulvar cancer cases was 19.3% while the proportion among the controls was 6.05%. The overall odds ratio is 3.72 and the 95% confidence interval (CI) is 1.68 to 7.56. Thus if a woman is HIV positive, she has a 3.72 times higher risk of developing cancer of the vulva than one who is HIV negative, without adjusting for confounders.

TABLE 10: Association between HIV and cancer of the vulva: crude odds ratio

	HIV Positive	HIV Negative	Total	Proportion exposed
Vulvo cancer	11	46	57	0.19
No vulvo cancer	98	1523	1621	0.06
Total	109	1569	1678	0.07
Odds ratio 3.72 (95% CI 1.68 – 7.56)				
Chi ^{2} = 15.92 Pr> Chi ^{2} = 0.0001				

3.8 ADJUSTING FOR CONFOUNDERS AND INDEPENDENT RISK FACTORS:

A logistic regression model was fitted to investigate the relationship between infection with HIV and cancer of the vulva, after adjusting for potential confounders or other risk factors. The other risk factors were: education, smoking status, number of lifetime sexual partners and age at first intercourse. Stepwise regression methods (backward elimination) were used to determine which confounders were actually required in the model- these were found to be smoking status and number of lifetime sexual partners. The results from the model fitting are shown in Table 11 on the next page.

TABLE 11: Association between HIV and cancer of the vulva after adjusting for independent risk factors.

Parameter	Odds ratio	Significance level	95% Conf. Interval
Smoking status			
Never smoked	1	baseline	-
Past smoker	3.17	<0.001	1.71; 5.86
Current smokers	3.23	0.004	1.47; 7.08
Number of lifetime sexual partners			
0 -1	1	baseline	-
> 1	2.04	0.014	1.15; 3.60
HIV status – negative	1	baseline	-
– positive	3.48	0.001	1.71; 7.06

Likelihood Ratio chi ^{2}= 34.40 P< 0.0001 Pseudo R^2 = 0.069

Number of observations = 1663

Adjusting for the other risk factors, there is a highly significant association between HIV and cancer of the vulva- those who are HIV positive are at a greater risk of developing vulvar cancer (OR = 3.48; 95% CI = 1.71 to 7.06) independently, non smokers are at lower risk for vulva cancer (OR = 1.0) than current smokers (OR =3.23; 95% CI = 1.47 to 7.08) and past smokers (OR = 3.17; 95% CI = 1.71 to 5.86). The higher the number of lifetime sexual partners, the greater the risk of vulvar cancer (OR =2.04; 95% CI = 1.15 to 3.60).

It was decided to simplify the model by combining the current and past smokers into an "ever smoked" category (Table 12). As table 11 showed that there was no difference between past and current smokers.

COMBINING SMOKERS AND PAST SMOKERS:

TABLE 12: Association between HIV and cancer of the vulva after combining smokers and past smokers.

Parameter	Odds ratio	Significance level	95% Conf. Interval
Smoking Status			
Never smoked	1	baseline	-
Ever smoked	3.27	<0.001	1.90; 5.61
Number of lifetime sexual partners			
0 - 1	1	baseline	-
> 1	1.77	0.022	1.09; 2.88
HIV status – negative	1	baseline	
– positive	3.55	<0.001	1.75; 7.19

Likelihood Ratio chi ^{2}= 33.41 P<0.0001 Pseudo R^2 = 0.067

The important confounder was number of lifetime sexual partners and smoking was found to be an independent risk factor. The OR of developing cancer of the

vulva among smokers is 3.27 (95% CI 1.90 5,60), which means that smokers have 3.27 times increased risk of developing cancer of the vulva compared to non-smokers.

The OR for number of lifetime sexual partners is 1.77 (95% CI 1.8 to 2.9) which means that having one or more lifetime sexual partners increases the risk of developing cancer of the vulva by 1.77 times.

Adjusting for smoking and number of lifetime sexual partners, HIV status is still a significant risk factor for cancer of the vulva with OR = 3.55 (95% CI: 1.75 to 7.19). Given two women who are of the same smoking status and have the same number of lifetime sexual partners, the one who is HIV positive has an approximately 3.5 times higher risk of developing cancer of the vulva compared to the one who is HIV negative.

3.9 MANTEL – HAENSZEL:

STRATIFYING FOR SMOKING:

TABLE 13: Association between HIV and cancer of the vulva stratified by smoking status.

Smoking status	OR*	95% Conf. Interval	M-H Weight (exact)
Never	2.85	(0.83 - 7.80)	1.61
Ever**	5.45	(1.57 - 16.51)	0.97
Crude	3.72	(1.68 - 7.56)	
M-H Combined	3.83	(1.90 - 7.72)	

* OR for association of HIV and cancer of the vulva

** Current and past smokers

Test of homogeneity (M-H) Chi ^{2}= 0.80 Pr>Chi ^{2} = 0.37

Test that combined OR =1, Mantel – Haenszel Chi ^{2} = 15.85, Pr>Chi ^{2} = 0.0001

Table 13 illustrates that the effect of HIV on cancer of the vulva can be treated as homogenous over the two smoking strata, and adjusting for smoking, the odds ratio for being a case among HIV positive women compared to HIV negative women is 3.83.

STATIFYING FOR NUMBERS OF LIFETIME SEXUAL PARTNERS:

TABLE 14: Association between HIV and cancer of the vulva stratified for number of lifetime sexual partners.

Lifetime sexual partners	OR	95% Conf. Interval		M-H Weight (exact)
0-1	3.11	0.65	26.59	0.3
2-4	1.20	0.13	5.00	1.5
5-9	7.12	2.10	22.08	0.8
≥10	14	0.14	111.07	0.06
Crude	3.72	1.68	7.56	
M-H Combined	3.58	1.75	7.13	

Test of homogeneity (M-H) Chi ^{2}= 4.71 Pr Chi ^{2} = 0.19

Test that combined OR =1 Mantel – Haenszel Chi ^{2} = 13.51 Pr> Chi ^{2} = 0.0002

Table 14 above shows that with no or one lifetime sexual partners OR= 3.11 (95% CI 0.065 to 26.583), two to four lifetime sexual partner OR= 1.20 (95% CI 0.13 to 4.97), five to nine lifetime sexual partners OR= 7.12 (95% CI 2.1 to 22.1) and 10 or more lifetime sexual partners OR= 14.00 (95% CI 0.14 to 111.1). The p value of 0.0002 is highly significant. In table 7 and 14 the numbers of lifetime sexual partners in the categories of five to nine and 10 and more were very small. Therefore table 15 gives results when categories two to four, five to nine and 10 and more are combined.

ADJUSTING FOR NUMBER OF LIFETIME SEXUAL PARTNERS USING TWO CATEGORIES:

Table 15 illustrates the outcome for a stratified analysis of the association between HIV and being a case, stratified by number of lifetime sexual partners.

TABLE 15: Association between HIV and cancer of the vulva after adjusting for number of lifetime sexual partners using two categories.

Number of Lifetime sexual partners	OR*	95% Conf. Interval		M-H Weight (exact)
0-1	3.11	0.065	26.59	0.3
>1	3.75	1.61	8.00	2.41
Crude	3.72	1.68	7.57	
M-H combined	3.68	1.84	7.34	

*OR for association of HIV and cancer of the vulva

Test of homogeneity (M-H) chi ^{2} = 0.03 pr> chi ^{2} = 0.87

Test that combined OR = 1 Mantel – Haenszel chi ^{2} = 15.49 Pr> chi ^{2} = 0.0001

The results suggest that the effect of HIV on cancer of the vulva can be treated as homogenous over the two lifetime sexual partners strata (P= 0.87) and adjusting for the effect of lifetime sexual partners, the odds ratio for being a case among HIV positive women compared to HIV negative women is 3.78.

4.0 DISCUSSION

Worldwide, approximately 40% of the estimated 23 million persons infected with HIV are women.[26] Since the first cases of HIV in the world were reported in the early 1980's, the epidemic has spread rapidly throughout the world. Sub-Saharan Africa, and Southern Africa in particular, are experiencing some of the world's worst national epidemics. South Africa's epidemic has been one of the last to develop in Africa, possibly because of this country's most Southerly position on the continent.[27, 28]

In South Africa, it is estimated that in 2001, 2.65 million women between the ages of 15 to 49 were infected with HIV. Clearly HIV is a significant health problem.[29] From data analysis shown in this report, there is strong evidence that cancer of the vulva is associated with HIV (OR = 3.72; 95% CI: 1.68 - 7.57). Thus, after adjusting for smoking status and number of lifetime sexual partners, women who were HIV positive were 3.7 times more likely to have cancer of the vulva than those who were HIV negative. A similar study in Johannesburg, reported an odds ratio for developing cancer of the vulva among those infected with HIV of OR= 4.8. This study did not adjust completely for sexual activity; therefore they did not confirm whether the association was real.[22] This research report confirms the association between cancer of the vulva and HIV, after adjusting for all other variables. There is a slight reduction in the odds ratio after adjusting for other risk factors.

Cancer risk is increased with most types of immune deficiency, including congenital disorders and iatrogenic treatments to prevent allograft rejection. With HIV/ AIDS the increased cancer risk is extraordinarily high.[15, 30, 31, 32, 3] HIV/ AIDS has been demonstrated to be associated with Kaposi's sarcoma and non-Hodgkin's lymphomas, and there are numerous reports of squamous cell neoplasia developing in HIV-infected persons.[9, 10] Since immuno-suppressed women are known to be at high risk for lower genital tract neoplasia, immunodeficient HIV- infected women may also be at such risk.[33, 34, 35, 36]

Since the aim of this study was to find out if there was a true relationship between cancer of the vulva and HIV, logistic regression analysis was used to adjust for potential confounders, which included number of lifetime sexual partners, level of education, age at first sexual intercourse and smoking. It was found that smoking and number of lifetime sexual partners were independently associated with cancer of the vulva. Adjusting for these factors, there was overwhelming evidence of an association between HIV infection and cancer of the vulva.

Because age at first sexual intercourse was not available, age of the oldest child subtracted from age of the mother was used as a proxy. The fact that no association was found between age at first sexual intercourse (P= 0.30) and cancer of the vulva may be a result of this being a poor proxy. Level of education was also not significantly associated with cancer of the vulva in the model

(P= 0.12). However, level of education was associated with smoking. Those with lower education smoked more than those with higher education. This relationship between smoking and education may explain why educational level was significantly associated with cancer of the vulva in the bivariate analysis, but not in the regression.

The controls that were selected for this research report were representative of the population because this was a hospital based study and all the three hospitals studied were hospitals that have a wide catchment area.

Previous epidemiological studies have shown that risk factors for cancer of the vulva include multiple sexual partners, a prior infection with genital warts, current cigarette smoking, and a prior abnormal cervical papanicolaou smear.[16, 37] It is probable that, as in congenitally and iatrogenically immunodeficient populations, the immunodeficiency resulting from HIV infection allows an oncogenic virus to flourish, and inhibits the body's natural immunologic defense mechanisms that suppress the development of neoplasia.[38]

Extraneous factors may complicate the estimation of the measure of association between exposure and disease. By using stratified analysis and adjusting confounders, this research report attempts to adjust for, or remove the effect of, an extraneous factor (confounder) which is related to both the exposure and the disease. Rather than one (crude) measure of association, measures of association were calculated for each of the strata of the confounder and the independent risk

factor, and an adjusted (common) measure computed using Mantel Haeszel methods.[17, 18, 19] In order to confirm that smoking was not an effect modifier, a test of homogeneity was carried out, and this showed that there was insufficient evidence to regard the odds ratios for the association between HIV and cancer of the vulva of smokers (OR 5.46 95% CI 1.57 16.50) and non smokers (OR 2.85 95% CI 0.83 7.80) for the association between HIV and cancer of the vulva as being significantly different ($p = 0.37$). Therefore the effect of HIV can be thought to be the same for smokers and non smokers. After adjusting for smoking, the combined Mantel-Haenzel estimate of 3.83 (95% CI 1.90 7.72) meant that a woman who is HIV positive is 3.8 times at risk of developing vulva cancer than one who is HIV negative. The result is highly significant ($p \geq 0.0001$).

The research proved that smoking is associated with cancer of the vulva. The association between smoking and cancer of the vulva has also been demonstrated in other studies, (OR 2.01,95% CI: 1.3 – 3.2). [1, 24, 39]

South Africa is a society, in transition particularly for the black population. In addition to urbanisation, recent decades have seen many life-style changes for black Africans including increasing rates of smoking.[40, 41, 42, 43] In South Africa, tobacco use is a major public health concern, as it has severe consequences for smokers, non-smokers and the economy.[44] One in four South African adults smokes[21]. Traditionally, black African women, have had among the lowest rates of smoking in South Africa with estimates of their smoking incidence ranging from

7% to 12%. Tobacco usage by women is disturbing because of the disease it produces.[43]

Since cancer of the vulva may occur simultaneously or metachronously with carcinomas of the cervix, vagina and anus, all organs covered by contingious mucous membranes, these neoplasms may share similar etiological or epidemiological profiles.

Recent research suggests that cigarettes may promote the occurrence of cervical neoplasia by mitigating the effect of host mucosal immunity on human papillomavirus (HPV) by reducing the number of langerhans cells. Langerhans cells are dendritic cells that are derived from the bone marrow and play an important role in immune responses of the squamous epithelium. [45, 46, 47, 48] This project suggests that a similar effect may be occurring with cancer of the vulva.

The confounder in this research report was the number of lifetime sexual partners each woman had had. This means that number of lifetime sexual partners is related to cancer of the vulva and as well as with HIV. In this research report, after adjusting for number of lifetime sexual partners, there was again overwhelming evidence of an association between cancer of the vulva and HIV.

In order to confirm that number of lifetime sexual partners is a confounder, a test of homogeneity was carried out, this revealed that there was insufficient evidence to regard the odds ratios of cancer of the vulva in relation to HIV for zero and one

lifetime sexual partners (OR 3.11 95% CI 0.065 26.59) and for more than one lifetime sexual partners (OR 3.75 95% CI 1.61 8.00) as being significantly different ($p = 0.19$).

Therefore the combined Mantel-Haenszel estimate suggests that there was no effect modification but confounding. After adjusting for number of lifetime sexual partners, the combined Mantel-Haenszel estimate of (3.68 95% CI: 1.84 7.34) meant that a woman who is HIV positive is 3.7 times more at risk of developing cancer of the vulva than one who is HIV negative. The result was highly statistically significant ($p \geq 0.0001$).

These results support the idea that the human papilomavious (hpv) may be responsible for cancer of the vulva, as it is often associated with pre-existing or coexisting condyloma acuminatum.[1, 16] Vulvar carcinomas have a heterogeneous aetiology, whereby a subset of vulvar cancers occur in young women with risk factors related to sexual practices, and these cancers of the vulva are associated with the hpv.[49] Similar to cervical cancer, immunosuppressed individuals and those who have had hpv associated malignancy are at an increased risk of invasive vulvar carcinomas. [49]

5.0 CONCLUSION

This research report has produced strong evidence that the association between HIV and cancer of the vulva is real and not due to confounding.

As HIV infected patients begin to live longer because of the use of antiretroviral therapy and prophylaxis and treatment of infections, malignancies such as cancer of the vulva may become more common. However, if cancer of the vulva is related to immune status, and antiretroviral drugs improve the immune status of patients, the incidence of vuval cancer may not increase due to HIV.

After data analysis using logistic regression, age at first sexual intercourse and education were not found to play any role in the association between HIV and cancer of the vulva. The major independent risk factor was smoking and number of lifetime sexual partners was a confounder. Since smoking is associated with cancer of the vulva, it is important that women should be educated about this additional adverse effect of smoking on their health, hopefully leading to a reduction in cigarette consumption. It should be emphasised that HIV respects no boundaries in terms of age, race, gender, sexual orientation or social status. People who are in high - risk situations have greatest need for information about HIV/AIDS and they should be empowered to decrease their susceptibility.

As a result of the findings of this research report, I recommend that during a gynaecological examination, HIV positive women should have a thorough

inspection of the vulva and women who present with cancer of the vulva should be tested for HIV.

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