Human Immuno Deficiency Virus Infection and Invasive Cervical Cancer in South Africa, What has changed?

STUDENT:

Lusandolwethu Nwabisa Shimange

SUPERVISORS:

Dr T. Smith, Dr T Mosehle

DEPARTMENT:

Obstetrics and Gynaecology Charlotte Maxeke Johannesburg Hospital

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DEDICATION

To my father Dr Oscar Shimange who is the

gynaecologist I want to be. My mother Mrs Zuki

Shimange ‘ndi nje nje nje ngumama’

My husband Mr Meshack Matsose my biggest fan, thank

you for all your support.

My sons Kemoboagong and Milani Matsose, one day it will all make sense.

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ABSTRACT

Introduction

Cervical carcinoma is the second most common malignancy worldwide after carcinoma of the breast and most common in the developing world[1,2].

In Africa, the population of women who are 15 years and older is estimated to be 267.9 million with approximately 78 897 diagnosed with invasive cervical carcinoma annually and a 78% mortality[1].

Aim

The aim of the study was to ascertain whether HIV sero-positive women in South Africa present with a more advanced disease of invasive cervical carcinoma than their HIV sero-negative counter parts as well as to assess the degree of immuno-suppression and its effect on the stage of the disease at initial presentation.

Is there a difference between the studies done then and what is presented now?

Methods

This was a descriptive retrospective record review. A total of 1300 cases of cervical carcinoma were seen at Charlotte Maxeke Johannesburg Academic Hospital, Combined Oncology Clinic from 2009 to 2010. Variables analyzed were patient age, HIV status, ARV standing, CD_4 count, parity, race, papsmear result, cell type FIGO staging. This was done

using the SPSS (Statistical Package for Social Sciences)

version 13.

RESULTS

The mean age of the patients analyzed was 50.74 $^{+}_{-}$ 13.08. There were 436 (37.1%) HIV seropositive patients, with a mean CD_4 count of 357.59 $^{+}_{-}$ 361.15. The mean age of presentation for HIV sero-positive patients was 55.4 $^{+}_{-}$ 11.8 and for sero-negative patients 42.1$^{+}_{-}$ 9.5 (p=0.000). A majority of the patients presented with stage IIIB disease. The HIV status had no bearing on the stage of the disease at presentation (p=0.363), nor the degree of immunosuppression (p=0.999). Due to the HIV pandemic, sero-positive patients presented with invasive cervical carcinoma 10 years earlier than their sero-negative counterparts. Black patients were mostly affected when compared to other races with a (p= 0.004). Antiretroviral seemed to make no difference on clinical staging at presentation (p=0.152)

Conclusion

HIV sero-positive patients presented with invasive cervical carcinoma 10 years earlier than their sero-negative counterparts. The degree of immunosuppression and HIV sero-positivity has no bearing on severity of the disease.

CONTENTS

LIST OF ABBREVIATIONS

AIDS: Acquired Immune Deficiency Syndrome

ASC-H: Atypical Squamous Cells- Cannot

Exclude HGSIL

ASCUS- Atypical Squamous Cells of Unknown

Significance

CDC: Centre of Disease Control

CIN: Cervical Intraepithelial Neoplasia

DNA: Deoxyribose Nucleic Acid

FIGO: International Federation of Gynaecologist and

Obstetricians

HGSIL: High Grade Squamous Intraepithelial

Lesion

HIV: Human Immune Deficiency Virus

HPV: Human Papillomavirus

HSV: Herpes Simplex Virus

STI: Sexually Transmitted Infection

VAIN: Vaginal Intraepithelial Neoplasia

VIN: Vulval Intraepithelial Neoplasia

WIHS: Women Interagency HIV Study

WHO: World Health Organization

Definitions

Cervical cancer is a malignancy that arises primarily from the tissues of the uterine cervix. It is imperative that a histological diagnosis is made, although staging is ascertained clinically. Cervical cancer is staged according to the FIGO (Federation Internationale de Gynecologie) 2009 (Table 1)

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CHAPTER 1: BACKGROUND AND LITERATURE REVIEW:

Background

Previous studies have reported that HIV-sero-positive patients present with invasive cervical cancer 10-15 years earlier than HIV-negative women.[1,2]

The reasons given for these findings are that HIV-sero-positive women are at a higher risk of having persistent human papilloma virus (HPV) infection with subtypes that are associated with high grade squamous intraepithelial lesion (HGSIL,) and subsequently, invasive cervix cancer if not detected early[3].

Pre-invasive cervical lesions and invasive cancer occur at a much younger age in HIV –sero-positive women[4,5] This has been attributed to the alteration of the HPV by the presence of HIV-1 infection resulting in a more active and faster promotion of cancer growth[6,7] . As reported by Van Bogart (2010), there is a shorter pre-invasive stage in HIV Sero-positive women compared with their sero-negative counterparts [8,9].Lomalisa, Smith and Guidozzi (1999) also suggested that the difference between HIV sero-positivity and sero-negativity regarding age at the time of disease presentations, is that HIV sero-positive patients have a shorter latency period from cervical intra-epithelial neoplasia (CIN) to invasive cervical carcinoma and therefore will present at a younger age.

According to Moodley (2001), human immunodeficiency virus may influence the pathogenesis of HPV-associated

cervical pathology by molecular interaction between HIV and HPV genes, as a result of up-regulation of the E6 and E7 HPV oncogenes by HIV[2]. With the global HIV pandemic, it has been estimated that 67% of the people infected are in sub-Saharan Africa, South Africa having the highest infection rate, with 5.7 million people living with the virus and, 60% being women[10].

HIV has been classified as a carcinogen due to the incidence of malignancies observed in its presentation [11]. Although the relationship between HIV sero-positivity and invasive cervical carcinoma is slightly blurred, several studies have reported that women who are HIV sero-positive have an increased risk of lower genital tract neoplasia such as CIN, vaginal intraepithelial neoplasia (VAIN), and vulval intraepithelial neoplasia (VIN)[12,13,14,15]. Currently the prevalence of HIV in South African patients with cervical cancer varies between 13.1% and 21.8%.

Significance of the study

Following the introduction of HAART in South Africa, we found it imperative to investigate the effect, if any, on the patient presentation of invasive cervical carcinoma i.e are patients presenting with a less severe form of the disease. A study in Italy has shown that antiretroviral therapy has no preventative effect on invasive cervical carcinoma but has a protective effect on premalignant cervical lesion[16]. Antiretroviral therapy causes an increase in CD4 count however, Lomalisa et al (1999) reported that women who are HIV-seropositive with a CD4 count of less than 200cell/mm^3 are more likely to have advanced stage of invasive cervical carcinoma at initial presentation than their HIV-Seronegative counterparts.

LITERATURE REVIEW

Invasive Cervical Carcinoma

Cervical carcinoma is a malignancy that arises primarily from the tissues of the uterine cervix. It is the second most common malignancy worldwide after carcinoma of the breast and most common in developing countries. In Africa where the population of women who are 15 years and older is approximately 267.9 million, it is estimated that 79 000 cases of cervical carcinoma are diagnosed annually resulting in a 78% mortality [1] .These proportions are found to be significantly higher in developing countries than those found in the developed world.(WHO.INT(HPV CENTRE) The South African Cancer Registry from 1998-1999 reported 6061 and 5203 new cervical carcinoma cases respectively, with 80% of the affected population being black women[17] .

Reports have shown that cervical carcinoma arises from persistent infection of the cervix with oncogenic subtypes of HPV [2], supported by clinical and epidemiological evidence. Persistence of HPV on the cervix results in dysplasia on the squamocolumnar junction of the cervix[19].

This is observed following microscopy slide from a pap smear. This method of analysis and diagnosis was introduced by Papanicolaou and Babes in the 1920's [18].The degree of dysplasia graded histologically from grade 1 to 3 where 3 is considered severe. Cervical intraepithelial neoplasia (CIN) is graded 1-3 on histology and also squamous intraepithelial neoplasia (SIL), low grade or high grade on cytology[19].

Human papilloma virus and Cervical Cancer

Human Papillomavirus (HPV) is a non-enveloped double stranded DNA virus that belongs to the family papillomaviridae[25]. Approximately 120 subtypes have been identified to date and more than 40 of these infect the epithelial and mucosal surfaces of the anogenital tract[20]. HPV has a carcinogenic component, which can be classified into low or high-risk types. High risk HPV are known as the oncogenic group that comprises of subtypes 16/18/31/33/35/39/45/51/56/58/56, with 16 and 18 being closely linked to carcinoma of the cervix (table 2).
HPV-induced cervical carcinogenesis is a multistep process, which involves the survival of the dormant episomal genetic particle inside epithelial cells[25].

When the host' environment becomes favourable for the HPV viral DNA, it is induced into integration within the host's DNA, leading to the formation of abnormal, dysplastic cells.

Although high risk HPVs are precursors of cervical carcinomas, other contributing factors such as high risk sexual behaviour (which may lead to sexually transmitted infections (STIs)[21] , multiparity[22,23,24],use of oral contraception of more than 5 years[25] , prolonged immunosuppression , smoking [26] and persistence of the virus[25,26].

High-risk behaviour has already been stated as a contributing factor to invasive cervical carcinoma, herpes simplex virus (HSV) is a recognized STI and a marker of previous unsafe sexual practice[30]. It has a sero-positivity of up to 60% in sexually transmitted disease clinics [27]. A co-infection of HPV and HSV, results in increased risk of invasive cervical carcinoma[28]. Oral contraception was introduced in the 1960's, at the same time, use of condoms decreased substantially to 25% of people involved in sexual activities[29]. This practise of increased oral contraceptive use and decreased barrier contraceptive use was also noted in 80% of unmarried women as a result increased STI, mycoplasma, Chlamydia and other STI's have been linked to the use of hormonal contraception[30,31].

TABLE 1: Clinical staging for invasive cervical carcinoma:

FIGO classification 2009

FIGO	SURGICAL PATHALOGICAL FINDINGS
I	Carcinoma strictly confined to the cervix
IA1	Confined to the cervix, diagnosed only by microscopy with invasion of <3mm in depth and lateral spread <7mm
IA2	Confined to the cervix, diagnosed with microscopy with invasion of >3mm and < 5mm with lateral spread <7mm
IB1	Clinically visible lesion or greater than A2, <4cm in greatest Dimension
IB2	Clinically visible lesion, >4cm in greatest dimension.
II	Tumour invades beyond the cervix and uterus, but not to pelvic sidewall or lower third of the vagina
IIA1	Involvement of the upper two-thirds of the vagina, without parametrial invasion, <4 cm in greatest dimension
IIA2	>4cm in greatest dimension
IIB	With parametrial involvement
III	Tumour extends to the pelvic sidewall and/or involves lower third of the vagina and/or causes hydronephrosis or non functioning Kidney
IIIA	Tumor involves lower third of the vagina, no extension to pelvic Wall
IIIB	Tumor extends to pelvic wall and/or causes hydronephrosis or non functional kidney
IV	Tumour beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema, as such, doesn't permit a case to be allotted to stage IV.
IVA	Tumour invades mucosa of bladder or rectum
IVB	Tumour extends beyond true pelvis

Table 2. Human Papilloma Virus Types Associated with Cervical Cancer

HIGH RISK	HPV Types
Carcinogenic	16,18,31,33,35,45,51,52,56,58,59
Probably carcinogenic	68
Possibly carcinogenic	26,53,66,67,70,73,82
Tested for in commercially available detection systems	16,18,31,33,35,45,51,52,56,59,66,68
LOW RISK	6,11,40,42,43,44,54,61,72,81,89

Human Papilloma Virus infection and Human Immunodeficiency Virus

The Women's Interagency HIV Study (WIHS) was conducted by Barkah et al (1994) at six sites around the United States[32]. It was a cohort study of 2056 HIV sero-positive and 569 HIV sero-negative women. Its aim was to look at the natural history and pathogenesis of HIV infection and complications in a large geographically and ethically diverse population[32]. Palefsky et al (1994) then went on further to assess cervicovaginal HPV infection in the participants of the WIHS. In this study cervico vaginal lavage specimens were collected. 29 different HPV subtypes were revealed together with HPV infection, medical and sexual history, substance abuse, HIV status, CD4 count and viral load were assessed as contributing factors to HPV infection[33]. They found that the presence of HPV among HIV positive women was a reflection of persistence or reactivation of pre-existing HPV rather than acute HPV infection[36]. This was further confirmed by Matorras et al (1990) who found that persistence and recurrence of oncogenic HPV was more likely on HIV sero-positive women[37]. Furthermore HIV sero-positive women had a 19-fold risk of developing genital condylomata with more biological aggressive behaviour. The recurrence and persistence rates of 41.7% compared to 12% in HIV sero-negative women[34].

In a separate study by Ellerbrock (2000) who looked at the incidence of

cervical squamous intraepithelial lesions in HIV sero-positive women which has been mentioned is due to HPV infecting the basal keratinocytes of the cervix[35,36]. Although the association between HPV and HIV is more likely than not, it has been postulated by Moodley,(2001) that HIV may influence the pathogenesis of HPV associated cervical pathology by molecular interaction between HIV and HPV genes, results in up regulation of the E_6 and E_7 HPV oncogenes by HIV[5].

HIV infection and cervical cancer

In 2013, South Africa had an estimated population of 52,98 million, with 51% being female. Approximately 5.2 million people were reported to be HIV infected furthermore: 17% of women in the reproductive age group were revealed to be HIV positive[37].

The degree of immunosuppression measured by the CD_4 count, it was found in two separate studies by Lomalisa, Smith and Guidozzi(1999), Gemignani, Maiman and Fructer(1990) that the degree of immunosupression is adversely related to the severity of invasive cervical carcinoma[1]. Women with invasive cervical carcinoma have lower CD_4 counts.[20] The mean CD_4 counts in both these studies, one undertaken in South Africa and the latter in the United States were 316 cells/mm^3 , 312 and 360 cells/mm^3 respectively.[21,22]

In 1993, cervical cancer was added as an AIDS defining illness[38].

The prevalence of invasive cervical carcinoma was revealed to be higher in HIV sero-positive women than their HIV sero-negative counterparts[1]. A possible explanation for this is that the sero-positive individuals were more effectively screened[41]. In a Kenyan study it was found that the HIV sero-positive patients with invasive cervical carcinoma are 10 years younger at presentation than their HIV sero-negative counterparts. It has been suggested by the author that HIV

infection possibly shortens the progression of premalignant lesions, namely high risk HPV to invasive cervical carcinoma. Another possible explanation for the previously-mentioned hypothesis is that, early coitarche, multiple frequent sexual partners puts one at risk for HIV and HPV[42].

Objectives

The aim of the study was:

- To determine and document the characteristics of women with invasive cervical carcinoma at the radiation oncology unit
- To determine if antiretroviral treatment affects the stage of the disease at initial presentation
- To determine if there were differences between HIV sero- positive women with invasive cervical carcinoma to those who are HIV sero-negative with regards to demographics including risk factors
- To determine whether the degree of immunosuppression (measured by CD4 T-lymphocyte count) has an effect on extent of invasive cervical carcinoma at initial presentation.

CHAPTER 2: METHODOLOGY

Materials and Methods

This was a descriptive retrospective record review. A total of 1300 cases, of cervical cancer that were seen at Charlotte Maxeke Johannesburg Academic Hospital, Combined Oncology Clinic from 2009 to 2010 were analyzed. This was done using the SPSS (Statistical Package for Social Sciences) version 13.

Women with a histological diagnosis of cervical cancer that were seen at Charlotte Maxeke Johannesburg Academic Hospital, combined Oncology Clinic were included. All with any other genital cancer such endometrial, vulval, vaginal carcinomas were excluded.

STUDY POPULATION

The study cohort were patients referred from surrounding hospitals in Southern Gauteng including patients from the North West province of South Africa.

There were patients that were referred from Botswana and Swaziland however, they were excluded from the study as they were primarily not seen from hospitals that refer directly to Charlotte Maxeke. These patients were already started on radiotherapy treatment in their home countries.

Other patients that were excluded were those with other cancers besides cervical cancer. E.g. vulva cancer and endometrial cancer.

STUDY VARIABLES

The variables that were attained from the files included:

- Age of the patients
- HIV status
- CD4 count of HIV infected patients.
- HIV infected patients on antiretrovirals or not
- The parity
- Papsmear results
- Histological diagnosis
- FIGO staging of cancer at presentation

DATA ANALYSIS

Information that were collected included: age of the patient, histological diagnosis of the cancer, FIGO staging of the cancer at presentation, HIV status of the patient, CD_4 count of the HIV infected patients, antiretroviral therapy. Patients were assigned a study number, so patients' name or hospital numbers were not used. All data was entered into a data collection sheet.

ETHICAL CONSIDERATION

Ethical clearance has been granted by The University of Witwatersrand's Health Science Research Ethics Committee (Medical), Clearance certificate number: M131141 (appendix 1).Permission was given the by the Head of the Radiation Oncology Department to access patient files. Institutional permission was obtained from the CEO of Charlotte Maxeke Johannesburg Academic Hospital (appendix 2).

CHAPTER 3: RESULTS

1. Introduction

This chapter deals with the results of statistical analyses carried out in SPSS (Statistical Package for Social Sciences) version 13. Firstly, frequency distributions of variables are described in graph formats either pie chart or bar graphs, where the first number is the frequency and the second is the percentage. Secondly, potential correlations (associations) between variables are examined and results are presented in Table formats. The chi Square test of independence is carried out to determine whether the associations between variables are statistically significant.

We test the null hypothesis

H_0: Variable A and Variable B are independent (that, knowing the level of variable A does not help to predict the level of variable B) against the alternative hypothesis

H_a: Variable A and Variable B are not independent (knowing the level of variable A can help you to predict the level of variable B).

All the statistical tests are carried out with 5% significance level. H0 is rejected if p-value (the probability of getting the observed result by chance alone) is less than 0.05 in favour of the alternative hypothesis).

One of the conditions for the use of the chi square test is that the expected frequency count (row-total multiplied by column-

total divided by the grand-total) for each cell, in the contingency table, must be at least 5. So, some of the p-values for the association will not be reported if the condition above is violated.

A total of 1300 files were reviewed. 1174 were patients from South Gauteng, South Africa, 125 were from Botswana and 1 Swaziland. 126 files were excluded from the research, as they did not meet the criteria for inclusion as the research focuses on patients on patients from South Gauteng.

2. Descriptive statistics

Table 3: Mean of age and CD_4 Count

	n	Min	Max	Mean ± Standard Deviation	Median
Age		19	90	50.74 ± 13.08	50
CD_4 Count	316	7	1435	357.59 ± 361.15	303

HIV status of patients expressed in numbers and percentages

1082 patients had HIV results documented in their files. A total of 660 women were HIV sero-negative, which is more than half the total number at 56.2%. 436 women HIV sero-positive at 31.1% and the remainder of the patients (78: 6.7%) their HIV status was unknown.

When analyzing the HIV prevalence according to race, it was revealed that 52% black patients were HIV sero-negative and 37% were HIV sero-positive. Whereas among white seronegative (3%): Seropositive (1%) followed by Indian and coloured patients where the prevalence was lower. The same trend is demonstrated where more patients are HIV sero-negative. This is significant as it has a p-value of 0.04.

Majority of the HIV sero-negative patients were between the ages of 50-59. The HIV sero-positive group was between the ages of 40-49. These mentioned groups also had the most people in the research.

HIV seropositive patients and their antiretroviral status

Regarding the antiretroviral status, 436 patients were HIV Seropositive. 138 (31.7%) were on antiretroviral treatment, with 196 (45%) not on treatment. Of the 436 patients 102 (23.3%) their antiretroviral status was unknown

Parity in numbers and percentages

When looking at the results regarding parity, which is the number of pregnancies after twenty-four completed weeks or above that have been delivered irrespective of outcome.

A total of 464 patients had their parity documented in their files. Of the total number 277 patients were HIV sero-negative and 187 were sero-positive. The group with the parity of between 3-5 had the most patients with 128 being sero-negative and 68 being sero-positive. The group with low parity (0-2) had 164 patients, 69 were sero-negative and 95 sero-positive. This was followed by the grandmultiparity group (parity of more than five) with a total of 98. Seventy-eight patients were sero-negative and twenty patients were sero-positive. The nulliparous group (no births after 24 weeks) had a total of 6 patients, two being sero-negative, and four being sero-positive.

Race of patients studied in numbers and percentages

Black patients constituted the majority (1044: 88.9%) of the total number of patients reviewed. They were followed by white patients (66: 5.6%), coloured (37 : 3.2%) then Indian patients (14 : 1.2%) the remainder of the race category was unknown.

Clinical Staging of cervical cancer in numbers and percentages

Most of the patients that were researched presented with stage IIIB disease as described by FIGO (international Federation of Obstetricians and Gynecology) accounting for a total of 493: 42.0% patients out of a total of 1058 with 278 sero-negative patients and 190 sero-positive patients.

Stage IIB disease had 333: 28.4% patients, with 208 sero-negative and 115 sero-positive patients. A similar trend is observed with the other stages were there a more HIV sero-negative patients than sero-positive patients.

Papsmears of patients studied in numbers and percentages

Only 128 patients were documented to have papsmear results, so statistical analysis was not done.

Cell types expressed as numbers and percentages

The dominant histological cell type was squamous cell carcinoma, which comprises (973 : 82.9%) of the total number, with adenocarcinoma (84 : 7.1%) being the second most common in this research. This is followed by adenosquamous (21 : 1.8%) and the unknown number was (75 : 6.4%).

Age groups of patients in numbers and percentages

Majority of the patients were between the ages of 40-49, with the youngest patient being less than 20 years at 19 years of age and the oldest being more than 80 years of age at 90 years. The mean age was 50 years.

CD_4 Counts of HIV sero-positive patients in numbers and percentages

Regarding the CD_4 count, majority of the patients had CD_4 counts ranging between 200-500 (156 : 35.8%), followed by an unknown group (120 : 27.6%). 97 : 22.2% patients had CD_4 counts that were less than 200. The remainder of the patients (63 : 14.4%), their CD_4 counts were more than five hundred.

3. Association/Relationships

Table 4: Patients age groups and HIV status

Characteristics		HIV status		Total
		Negative	Positive	
Age (years) Chi-square = 270.40; P-value= 0.000	<20	0	2	2
	20 – 29	3	21	24
	30 – 39	36	138	174
	40 – 49	156	161	317
	50 – 59	219	77	296
	60 – 69	142	29	171
	70 – 79	69	3	72
	>80	25	0	25
	Total	650	431	1081
	Mean ± STD	55.4 ± 11.8	42.1± 9.5	
	Median; Range	54;67	41;56	

Table 4 indicates the relationship between age and HIV status of patients. In groups age 30-39 and 40-49, the majority are HIV positive while in groups age 50-59, 60-69, 70-79 and >80 the majority are negative. The younger the patient, the more likely that they would be HIV positive and the opposite is true. This relationship is statistically significant at 5% significant level, as the p-value (0.000) is less than 0.05.

Table 5: Tumour Characteristics and HIV status

Characteristics		HIV status		Total
		Negative	Positive	
Cell Type	Squamous	545	375	920
	Adenocarcinoma	57	19	76
Chi-Square=	Adenosquamous	14	6	20
8.88; P-value	Other	9	9	18
= 0.031	Total	625	409	1034
	IA_1	2	3	5
	IA_2	4	0	4
	IB_1	27	21	48
Clinical	IB_2	18	22	40
Staging	IIA_1	10	9	19
	IIA_2	11	8	19
Chi-Square = 10.93; P-value	IIB	208	115	323
	IIIA	15	11	26
= 0.363	IIIB	278	190	468
	IVA	39	29	68
	IVB	23	15	38
	Total	635	423	1058

Cell type of patients are significantly associated with the HIV status (p-value =0.031< 0.05) while the Clinical Staging is not.

Table 6 Patients parity, Race, Pap smear, and HIV status

Characteristics		HIV status		**Total**
		Negative (n)	Positive (n)	
Parity Chi-square= 41.59; P-value = 0.000	0	2	4	**6**
	0 – 2	69	95	**164**
	3 – 5	128	68	**196**
	> 5	78	20	**98**
	Total	**277**	**187**	**464**
	Mean ± STD	3.5 ± 38.7	2.5 ± 22.2	
	Median; Range	4; 5	4;5	
Race Chi-Square = 13.40; P-value = 0.004	Black	572	408	**980**
	White	38	14	**52**
	Coloured	28	8	**36**
	Indian	12	2	**14**
	Total	**650**	**432**	**1082**
Papsmear	ASC-H	0	0	0
	ASC-US	0	0	0
	LGSIL	1	0	1
	HGSIL	72	48	120
	Total	**73**	**48**	**121**

Table 6 : The women with low parity (0 to 2), are more likely to be HIV positive, those with high parity (3 or more) are more likely to be HIV negative. Black women are likely to be HIV positive than women of other races. These two relationships are statistically significant at 5% (P-values < 0.05).

Table 7: Relationship between Age, Parity and CD4 count of patients.

Patients Variables		Patients CD 4 count			Total
		< 200	200 - 500	>500	
Age Chi-square = 23.95; P-value = 0.021	20 -29	3	0	0	3
	30 – 39	20	12	9	41
	40 – 49	22	46	15	83
	50 – 59	17	41	17	75
	60 – 69	20	19	8	47
	70 – 79	5	13	4	22
	≥ 80	2	2	3	7
	Total	89	133	56	278
	Mean ± STD	49.4 ± 13.6	51.8 ± 11.6	51.7 ± 13.9	
	Median ;Range	48; 64	51; 57	50; 57	
Parity Chi-Square = 4.02; P-value = 0.674	0	1	0	0	1
	1 – 2	11	15	9	35
	3 – 5	13	27	14	54
	> 5	5	13	7	25
	Total	30	55	30	115
	Mean ± STD	2.9 ± 10.3	3.4 ± 16.8	3.3 ± 11.7	
	Median; Range	4; 5	4; 5	4; 5	

Table 7 shows the relationships between patients'CD4 count and their age groups and parity. Most of the Patients younger than 39 years had CD4 count less than 200, and most of those between 40 to 59 years old had CD4 count between 200 and 500. Conclusion can be drawn that on the association of age and CD4 count. However a caution must be made regarding the reporting of the Chi square test of independence as many cells have expected count less than 5.

The parity of patients was not associated with the CD4 count as the majority of patients, in each category of parity, have CD4 count ranging between 200 and 500.

Similarly, observation is made concerning the Chi square test of independence.

Table 8: Relationship between Race, Cell type and RVD and

CD4 count of patients.

Patient variables		Patients CD4 count			Total
		<200	200 – 500	>500	
Race Chi-Square = 2.64; P-value = 0.853	Black	79	119	48	246
	White	3	7	5	15
	Indian	5	5	3	13
	Coloured	2	2	1	5
	Total	89	133	57	279
Cell type Chi-Square=10.80; P-value=0.095	Squamous	75	112	49	236
	Adenocarcinoma	6	6	4	16
	Adenosquamous	1	3	0	4
	Other	0	8	0	8
	Total	82	129	53	264
Antiretroviral Treatment Chi-Square= 5.49; P- value= 0.064	Yes	35	63	28	126
	No	42	46	14	102
	Total	77	109	42	228

Table 8 shows the relationships between CD4 count of patients and their race, cell type and whether they are in ARV treatment. Race is not associated with CD4 count as the majority, Blacks and Whites, have CD4 count between 200 and 500. But with many cells with expected count less than 5, Chi square test should not be reported.

Also, neither cell type nor ARV is associated with CD4 count as the majorities are between 200 and 500 in each of the two variables. For ARV, the relationship is not statistically significant as the p-value is not less than 0.05 (0.064 > 0.05).

Table 9: Relationship between Papsmear, Clinical staging,

and CD4 count of patients

	Patients variables	Patients CD4 count			Total
		<200	200- 500	>500	
Papsmear Chi-Square = 1.35; P- value = 0.853	ASC-US	0	1	0	1
	LGSIL	0	1	0	1
	HGSIL	7	17	5	29
	Total	7	19	5	31
Clinical staging Chi-square= 4.18; P-value = 0.999	IA_1	0	0	0	0
	IB_1	7	7	6	20
	IB_2	3	6	2	11
	IIA_1	4	4	1	9
	IIA_2	2	1	1	4
	IIB	21	34	14	69
	IIIA	3	5	2	10
	IIIB	37	57	25	119
	IVA	4	7	2	13
	IVB	3	6	2	11
	Total	84	127	55	266

Table 9 Shows relationships between Papsmear, Clinical staging and CD4 count are presented in Table 10. Neither of them is associated with CD4 count as the majority of patients are observed in the middle group of CD4 count (200-500). We cannot rely on the chi square test of independence as many cells have expected count less than 5.

Table 10 Relationship between Age group of patients and their clinical staging

		Age								Total
		<20	20-29	30-39	40-49	50-59	60-69	70-79	>=80	
Clinical staging Chi Square = 106.83; P-value = 0.003	1A1	0	0	4	0	0	1	0	1	6
	1A2	0	0	2	1	0	0	0	0	3
	1B1	0	1	11	13	13	13	1	1	53
	1B2	0	3	14	14	6	3	1	1	42
	IIA1	0	2	3	5	6	5	1	1	23
	IIB	1	5	51	104	101	37	24	3	326
	IIIA	0	0	4	1	11	9	4	1	30
	IIIB	1	10	68	131	130	86	43	17	486
	IVA	0	0	11	21	18	17	4	2	73
	IVB	0	1	4	15	12	8	2	4	46
Total		2	22	174	315	301	180	81	32	1107

Table 10 shows us that the clinical stages IIB and IIIB encompass the majority of patients in all the age groups. For patients younger than 70 years old, there are also a good number of them in clinical stages 1B1 and 1B2. There is therefore an association between age and clinical staging. Because of many empty cells and cells with expected counts less than 5 in the Table, the chi square test of independence cannot be interpreted.

Table 11 Relationship between patient on ARV and Clinical staging of patient on ARV.

		No	Yes	Total
Clinical Staging Chi square = 13.25; P-value = 0.152	$1A_1$	0	1	1
	$1B_1$	11	10	21
	$1B_2$	9	2	11
	IIA_1	4	5	9
	$11A_2$	3	3	6
	IIB	48	43	91
	IIIA	2	6	8
	IIIB	79	71	150
	IVA	6	12	18
	IVB	8	2	10
		170	155	
Total				

Table 11 presents the relationship between clinical staging and ARV. Of patients who are on ARV, the majority is in clinical stages IIB and IIIB. The same applies to the patients not on ARV. So, being on ARV or not does not affect the clinical stages of patients.

CHAPTER 4: DISCUSSION, LIMITATIONS, AND CONCLUSION

DISCUSSION

All the variables noted in the results chapter were documented in patient files, so every result was influenced by what the clinician has recorded.

The aim of this research was to follow up what Lomalisa, Smith, and Guidozzi had previously researched, to determine whether HIV sero-positive women with invasive cervical carcinoma presented with a more advanced disease than that of their sero-negative counterparts and also if the degree of immunosuppression as evidenced by the CD_4 count, had a bearing on the extent of the disease at presentation. Regarding this particular research we also wanted to ascertain whether anything has changed since with regards to the above.

We looked at whether antiretroviral treatment since its inception has had any effect on the stage of the disease and the characteristics of the women with invasive cervical carcinoma at the Charlotte Maxeke Johannesburg Hospital regarding parity, age and HIV status. The patients that were researched were mostly black patients. The average age of the patients was 50 years. Most of the patients presented with squamous cell carcinoma stage IIIB followed by stage IIB. Our youngest patient was 19 years and oldest 90 years of age.

Lomalisa , Smith, and Guidozzi found that patients with a greater immunosuppression, CD_4 count <200/mm^3 are more likely to have advanced disease. Maiman also found that patients with invasive cervical cancer had lower CD_4 counts HIV sero-positive patients are more likely to present earlier than their sero-negative counterparts, 44 years and 53 years of age respectively.

Our study did not support the above findings. On the contrary, we found that the degree of immunosuppression did not adversely affect the clinical staging. Lomalisa and Maiman yielded similar results regarding the mean CD_4 counts as they were around the same value, although our degree of immunosuppression had no bearing on disease state, the mean CD_4 count was also similar at $357.59^{\pm}$ 361.15.

Like Lomalisa, Smith and Guidozzi who found that HIV sero-positive patients present an average of 10 years earlier with invasive cervical carcinoma than their sero-negative counterparts. Our research also demonstrated that HIV sero-positive patients present about 10 years earlier than their sero-negative counterparts i.e. 42.1 $^{\pm}$ 9.5 VERSUS 55.4 $^{\pm}$11.8 years respectively (p=< 0.005). We were also able to show that HIV Sero-positivity in its own entity has no bearing on the clinical staging of invasive cervical carcinoma (p = 0.363). Lomalisa, Smith, and Guidozzi had a similar finding.

Studies by Dorruci, Suligoi,and Serraino et al done in Italy

have shown that antiretroviral therapy has no preventative effect on invasive cervical carcinoma but has a protective effect on premalignant cervical lesion. We found after looking at antiretroviral therapy that there is no effect on clinical staging of disease on presentation.

LIMITATIONS

- This was a retrospective review of patient records, which meant data collected was dependent on the clinicians note taking. As noted in the results there were a number of unknown variables.
- They may have been errors in staging of the patients, as it is user dependent and staging of cervical cancer is a clinical one.
- There was no mention of other associated risk factors such as smoking, number of sexual partners, coitarche and use of barrier contraception.
- Not all the files reviewed had documented staging, age, parity, histological cell type, or HIV status recorded.
- Not all the files reviewed had documented staging, age, parity, histological cell type, or HIV status recorded.
- Not all HIV reactive patients had CD4 counts recorded.
- Regarding HIV reactive patients, it was not always documented whether patients are on antiretrovirals or not. This may have affected the overall immune competency of the patient.
- There may have been bias regarding the race profiling of the patient, as it was not stated in the files.

CONCLUSION

Cervix carcinoma has always been known as a disease that ensues in the fifth decade. Now with the HIV pandemic we are seeing cervical carcinoma at least ten years earlier than previously found. As we have demonstrated that the severity of disease state at presentation is independent of HIV status and also independent of degree of immuno-suppression. Antiretrovirals have not made a difference either.

With the government's latest policy on rolling out the HPV vaccine, it would be interesting to see whether we can go back to cervical carcinoma being a disease of the fifth decade or perhaps a non-entity.

As a follow up study one could look whether in terms of long term survival antiretrovirals have made a difference.

For now outside of HIV, cervical carcinoma continues to be a burden in South Africa and the rest of our developing nations.

REFERENCES

1. Lomalisa P, Smith T, Guidozzi F. Human Immunodeficiency Virus Infection, and Invasive cervical Cancer in South Africa. Gynecol Onco 2000;77:460-3.
2. Moodley M, Moodley J, Kleinschmidt I. Invasive cervical cancer and human immunodeficiency virus (HIV) infection: a South African perspective. Int J Gynecol Cancer 2001; 11: 194-197.
3. Sun XW,Kuhn L,Ellerbrock TV et al. Human papilloma virus infection in women with the human immunodefiency virus. N Engl J Med 1997;337:1343-9.
4. Chirenje ZN. HIV and cancer of the cervix. Best Pract Res Clin Obstet Gynaecol. 2005;19:269-276
5. Moodley S, Mould S. Invasive cervical cancer and human immunodeficiency virus (HIV) infection in KwaZulu-Natal, South Africa. J Obstet Gynaecol. 2005;25:706-710
6. Schuman P, Ohmit SE, Klein RS, et al. HIV Epidemiology Research Study(HERS) Group. Longitudinal Study of cervical squamous intraepithelial lesion in human immunodeficiency virus(HIV) sero-positive and at risk HIV-sero-positive women. J infect Dis.2003;188:128-136
7. Aoki Y,Tosato G, Neoplastic conditions in the context of HIV Infection.Curr HIV Res. 2004;2:343-349

8. Van Bogaert LJJ. Age at diagnosis of preinvasive and invasive cervical neoplasia in South Africa.Int J Gynaecol Cancer 2011;21:363-366
9. Nel CPG, Schoeman LC, Van Wyngaardt M, et al. The prevalence of HIV amongst women with cancer of the cervix. SA Fam Pract. 2006;48:17
10. Castro, K.G., Ward, J.W., Slutsker, L., Buehler, J et al Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults 1993.http://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm[4].
11. Conley, L.J. Ellerbrock, T.V. Bush, T.J. HIV-1 infection and risk of vulvovaginal and perianal condylomata acuminata and intraepithelial neoplasia: a prospective cohort study. Lancet 2000;359:108-136.
12. Bradbeer C: is infection with HIV a risk factor for CIN? Lancet 1987 2:1277-1278,
13. Byrne M, Taylor-Robinson D,Munday PE, Harris JRW: The Common Occurrence of Human Papilloma Virus Infection and Intraepithelial Neoplasia in women infected by HIV. Aids 1988,3:379-382.
14. Spurrett B, Jones SD, Stewart G: Cervical Dysplasia and HIV infection. Lancet 1988 ,1:237-238.

15. Spinillo A, Tenti P,Zappatore R et al: Langerhans cell count and Cervical Intraepithelial Neoplasia in women with Human Immunodeficiency Virus Infection. Gynecol Oncol 1993, 48;210-213.
16. Dorruci M,Suligoi B,Serraino D,Tirelli U. Italian HIV-Seroconversion Study.J Acquir Immune Def Syndr 2001,26(4),377-380.
17. Mqoqi,N.Kellet,P.Sitat,F ET AL.Incidence of histologically diagnosed cancer in South Africa 1998-1999 National Cancer Registry of South Africa. Johannesburg: National Health Laboratory Service, December 2004
18. Denny L, Best Practice and Research Clinical Obstetrics and Gynaecology 2012,189 – 196.
19. Denny L. Cervical Cancer in South Africa:An overview of current status and prevention strategies. CME, 2010;February; 28: 23
20. Gemignani M, Maiman M, Fruchter et al, CD4 lymphocytes in women with invasive and preinvasive cervical neoplasia, Gynecol Oncol 1995 Dec;59(3):364-9
21. Maiman M, Fruchter RG, Guy L et al. HIV infection and invasive cervical carcinoma. Cancer 1993,71:402-406.
22. Maiman M, Fruchter RG,Clark M et al, Cervical Cancer as an AIDS defining illness.Obstet Gynecol 1997,89:76-80.
23. Bai J, Wong FW, Bauman A, Mohsin M, Parity and Pregnancy Outcomes, Am J Obstet Gynecol 2002 Feb;186(2) 274-8

24. The interpretation and clinical application of the word 'parity': a survey, BJOG 2007;114:1295-1297
25. Steen M.Duarte-Franco E.Human Papilloma Virus infection Epidemiology and Pathophysiology. Gynecol Oncol 107 (2007) S2-S5
26. Smith J, Herrero R,Basetti C Et Al.Herpes Simplex Virus-2 As A Human Papillomavirus Cofactor In The Aetiology Of Invasive Cervical Cancer.J Natl Cancer Inst2002;949210;1604-13)
27. Munoz N,Franceschi S, Basetti Et Al. Role Of Parity And Human Papillomavirus In Cervical Cancer: The IARC Multicentre Case-Control Study. Lancet 2002; 359(9312): 1093-10
28. Plaxe S, Saltstein S. Estimation Of Pre-Clinical Phase Of Cervical Adenocarcinoma Suggests That There Is ample Opportunity For Screening. Gynecol Oncol1999; 75:55-61.
29. International Collaboration Of Epidemiological Studies Of Cervical Cancer. Carcinoma Of The Cervix And Tobacco Smoking; Collaborative Reanalysis Of Individual Data On 13 541 Women With Carcinoma Of The Cervix And 23 017 Women without Carcinoma Of The Cervix From 23 Epidemiological Studies. Int J Cancer 2006; 118:1481-95.
30. Janier M, Lassau F, Bloch J Et Al. Sero Prevalence Of Herpes Simplex Virus Type 2 Antibodies In An STD Clinic In Paris. Int J Std Aids 1999; 10:522-

31. Franchesci S. The IARC Commitment To Cancer Prevention; The Example Of Papillomavirus And Cervical Cancer Res 2005;166:277-97.
32. Xi L, Carter J, Galloway D, Et Al. Acquisition And Natural History Of Human Papillomavirus Type 16 Variant Infection among A Cohort Of Female University Students. Cancer Epidemiol Biomarkers Prev 2002; 11:343-51).
33. Detzer M, Wendt S, Solomon L et al. Barriers To Condom Use Among women attending Planned parenthood clinics. Women Health 195;23:91
34. Creatsas G. Sexually Transmitted Diseases And Oral Contraception Use During Adolescence. Ann N Y Acads Sci1997; 816:404-10
35. Barkah SE.Melnic SL.Preston-Martin S et al.The Women's Interagency HIV Study. WIHS collaborative Study Group,Epidemiology 1998:9;117-125
36. Palefsky JM, Minkoff H, Kalish LA et al.Cervicovaginal HPV Infection in HIV-1 positive and high risk HIV negative women.J Natl Cancer Inst 199;3:226-236
37. Matorras R, Human Immunodeficiency Virus induced immunosuppression: a risk factor for human Papilloma Virus Infection. Am J Obstet Gynaecol 1991; 164:42-4
38. Ellebrock TV. Incidence of cervical squamous intraepithelial lesions in HIV infected women. JAMA 200; 283:1031-1037.

39. Shravya G.Maria BS,Jason.Cervical Neoplasia.Clinical Obst and Gyn;54:4:528-536
40. STATSSA: Midyear Population Estimates 2013
41. CDC 1993 Revised Classification System for HIV infection and expanded surveillance case definition for Aids among adolescents and adults: MMWR 193; 41:1-19
42. Franceseni S,Dal Maso L, Arniani S et al.Risk for cancers other that Kaposi sarcoma and non-hodgkins lymphoma in persons with Aids in Italy.Br J Cancer 1998;78:966-70
43. Goedert J.Cote J.Virgo P et al.Spectrum of AIDS associated malignant Disorders.Lancet 1998;351:18339.
44. Kevin M.Chin AB .Jasjeet S. et al Invasive Cervical Cancer and HIV. Obstet and Gyn 1998;92:1 july
45. Gichangi PB,Bwayo J.Estambale B et al Cervical Cancer and HIV Infection in Kenya, AIDS, 2003;17:1963-1968
46. Lax S, Histopathology of Cervical precursor Lesions and Cancer. Acta Dermatoven APA, 2011;3;vol 20

APPENDIX 1

R14/49 Dr Lusandalwethu Nwabisa Shimange

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131141

NAME: (Principal Investigator)	Dr Lusandalwethu Nwabisa Shimange
DEPARTMENT:	Obstetrics and Gynaecology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Human Immunodeficiency Virus Infection and Cervical Cancer in South Africa. What has Changed?
DATE CONSIDERED:	29/11/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Trudy Smith
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/02/2014

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature _____ Date 24/February/2014

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 2

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Office of the Chief Executive Officer
Tell: 011 488 3792
Fax: 011 488 3753
Email: lindiwe.mngomezulu@gauteng.gov.za
7 November 2013

Dr. L.N. Shimange
O & G Department
CMJAH

Dear Dr. Shimange

RE: Request to conduct research on "Cervical Cancer and HIV, what has changed?"

Please note that permission to conduct the above mentioned study is provisionally approved. Your study can only commence once ethics approval is obtained. Please forward a copy of your ethics clearance certificate as soon as the study is approved by the ethics committee for the CEO's office to give you the final approval to conduct the study.

Ms. G. M. Bogoshi
Chief Executive Officer

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