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ORIGINAL RESEARCH

The demographics of women with multizonal anogenital HPV disease: a retrospective cohort study in Cape Town, South Africa

TS Adams,[1,2] M Mhembere,[2] NH Mbatani,[1,2] LJ Rogers[1,2]

[1] Department of Obstetrics and Gynaecology, Groote Schuur Hospital, University of Cape Town, South Africa
[2] South African Medical Research Council, University of Cape Town Gynaecological Cancer Research Centre, South Africa
Corresponding author, email: tracey.adams@uct.ac.za

Objectives: Multizonal human papillomavirus (HPV)-associated disease refers to HPV involvement at different anatomical sites of the lower genital tract. This study aimed to document the prevalence of multizonal HPV-associated disease (multizonal intraepithelial neoplasia [MUZIN]) in women attending the colposcopy clinic at Groote Schuur Hospital in 2018, and describe the demographics of this population.

Methods: We conducted a retrospective review of all new women at the colposcopy clinic in 2018. Evidence of HPV disease was based on clinical findings and pathology reports. HPV deoxyribonucleic acid (DNA) testing was not routinely done at our centre during the study period. We specifically aimed to calculate the prevalence of multizonal HPV-associated disease and identify risk factors.

Results: In 2018, 653 women attended the clinic, of whom 104 (16%) had evidence of MUZIN. Of the entire cohort, 320 (49%) were human immunodeficiency virus (HIV)-positive. This contrasts with the 78/104 women (75%) with multizonal disease who were HIV-positive ($p < 0.001$). Tuberculosis was also a significant association among MUZIN patients and all patients ($p = 0.004$). In the MUZIN cohort, most women were on antiretroviral treatment, and 89% were virologically suppressed. In South Africa, CD4 counts are taken at diagnosis, and 75.6% of women had initiated treatment when their CD4 counts were < 500.

Conclusion: This study suggests that MUZIN may be related to an inadequate or non-functional immune response, although prospective studies are required to confirm this hypothesis.

Keywords: human papillomavirus, multizonal, lower genital tract, squamous intraepithelial lesion, squamous carcinoma, condylomata acuminata

Introduction

Skin-to-skin acquisition of human papillomavirus (HPV) is the most common sexually transmitted infection. Most of these infections are cleared by a competent immune system, but infection persistence can ultimately result in precancerous lesions of the lower genital tract and, eventually, malignancy. As cervical cancer is the second most common cancer in women globally, its prevention has been the focus over the years. In 2020, there were 604 000 new cervical cancer cases worldwide and 342 000 resulting deaths. Cervical cancer is the leading cause of cancer deaths in 42 countries, including sub-Saharan Africa and Southeast Asia.[1] South Africa is no different, where it is the leading gynaecological cancer, with 10 702 new cases and 5 870 deaths annually.[2]

The "field effect" of HPV is a term that is not often used in the literature. In 1953, Slaughter et al.[3] described this term. They postulated that a single stem cell undergoes a genetic transformation, resulting in daughter cells acquiring similar genetic alterations and that these changes were bound to progress to premalignant and malignant lesions at some stage.[3] In contemporary times, field effect or "multizonal HPV-associated disease" in the setting of lower genital tract diseases refers to the involvement of different sites (cervix, vagina, vulva, perianal area, and anus) with the HPV-associated disease. These lesions can occur simultaneously (synchronous) or at different times (metachronous) in the same individual.

Managing women with multizonal disease is challenging as they are often young. Their management demands intense follow-up and time off work, and the interventions (medical or surgical) can have debilitating side effects with an impact on body image if mutilating surgery is required.[4,5] This article aims to describe the demographics of the population attending a colposcopy clinic at Groote Schuur Hospital, Cape Town, South Africa, in 2018, focusing on women with multizonal HPV-associated disease of the lower genital tract (multizonal intraepithelial neoplasia [MUZIN]).

Methods

Study design

A retrospective descriptive study of the women attending the colposcopy clinic at Groote Schuur Hospital, Cape Town, South Africa, was performed.

Study population

In South Africa, health services are divided into the government-funded public sector and the private sector, which relies on payment from medical aid insurance schemes and patients who privately pay for services. Groote Schuur Hospital is a state-

funded, tertiary-level care hospital that services parts of the Western Cape province in South Africa. Women with abnormal cervical cytology or vulval lesions are referred to this clinic. As a state hospital in South Africa, a limited-resource country with a poor follow-up rate, women are often managed with a "see and treat" approach if abnormal cytology (high-grade squamous intraepithelial lesion [HSIL]) is confirmed on colposcopy.

The population included all new women seen at this clinic in 2018. Evidence of HPV disease was based on clinical findings (colposcopy or visual evidence of HPV disease on the vulva, perianal area, or vagina) and biopsy pathology reports. HPV DNA testing was not routinely done at our centre during the study period. Women are often referred with one complaint, but the clinicians examine and document all HPV-associated lower genital tract diseases. Specifically, the database can document cases where extensive HPV disease is found. The database also collected demographic data, including HIV status.

Data collection

Data were extracted from an existing colposcopy database at Groote Schuur Hospital, which has Human Research Ethics Board approval from the University of Cape Town (reference: 344/2011). This database documents demographic information, sites of HPV-associated disease, and the treatment offered. In South Africa, antiretrovirals were previously only initiated when the CD4 counts were < 200. In 2015, this changed to CD4 counts < 500, and the database collected CD4 levels according to this parameter. Currently, all women diagnosed as HIV-positive are offered antiretroviral treatment.

Ethical considerations

The colposcopy database has existing ethical approval, as mentioned above. This review was performed per the Declaration of Helsinki.[6]

Objectives

This study aimed to document the prevalence of multizonal HPV-associated disease in women attending the colposcopy clinic in 2018 and describe the demographics of this population. It is our impression that human immunodeficiency virus (HIV) infection is the most important associated factor/aetiology of multizonal HPV disease in our population. Moreover, to treat these women more effectively, with less treatment-related morbidity, we needed to understand and document this population's demographics.

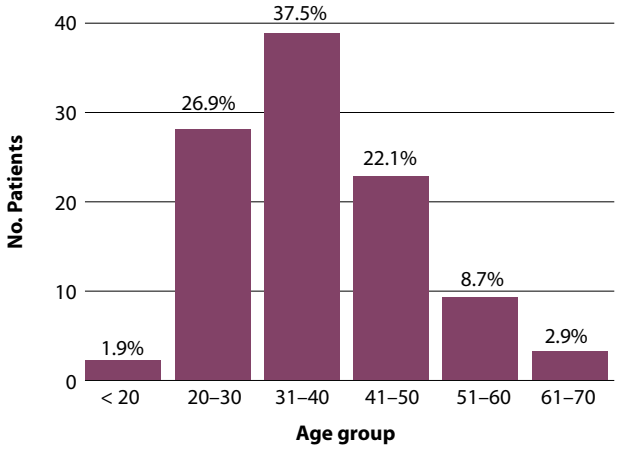

Figure 1: Age distribution of women with multizonal HPV-associated intraepithelial neoplasia
HPV – human papillomavirus, MUZIN – multizonal intraepithelial neoplasia

Statistical analysis

In preparation for analysis, each data variable was checked for input errors and cleaned. To explore these fields, basic descriptive statistics (minimum, maximum, and mean) were calculated for each variable. The categorical fields were also standardised. The overall statistical analysis was executed using R statistical software (R version 4.0, 2020), and the ggplot R package was employed to produce graphics. Different plots, such as pie and bar charts, were computed to provide data visualisation. The chi-square test assessed the relationship between the MUZIN data and the overall 2018 data. In all analyses, $p < 0.05$ was taken as statistically significant.

Results

Prevalence

In 2018, 653 new women attended the colposcopy clinic at Groote Schuur Hospital, Cape Town. Of these, 104 women had evidence of multizonal HPV-associated lower genital tract disease. The prevalence of MUZIN was 16%. Most women with

Table I: Reason for referral to the colposcopy clinic

Reason for colposcopy referral	Overall cohort *n* = 653	MUZIN cohort *n* = 104
High-grade cytology		
HSIL Atypical squamous cells, HSIL cannot be excluded	359 (55%) 41 (6.3%)	41 (39.4%) -
Cancer cytology	5 (0.8%)	-
Low-grade squamous intraepithelial lesion cytology	27 (4.1%)	5 (4.8%)
Atypical squamous cells of undetermined significance cytology	9 (1.4%)	-
Atypical glandular cells cytology	7 (1.1%)	-
Negative for intraepithelial lesion or malignancy cytology	27 (4.1%)	6 (5.8%)
Other cytology	10 (1.5%)	1 (1%)
Vulval lesion referral with no cytology	168 (25.7%)	51 (49%)

HSIL – high-grade squamous intraepithelial lesion, MUZIN – multizonal intraepithelial neoplasia

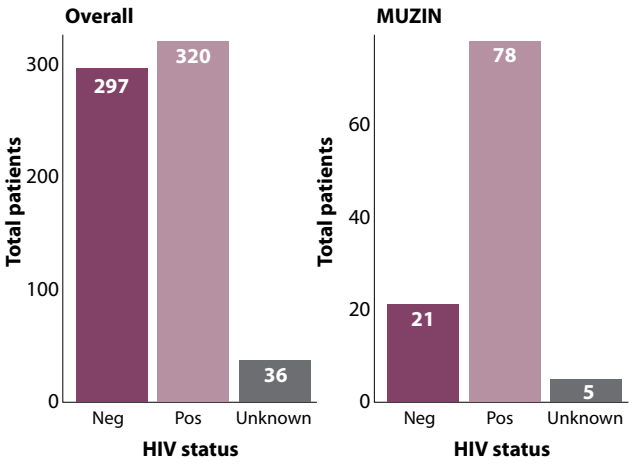

Figure 2: HIV status of the entire cohort versus women with multizonal HPV-associated disease

HIV – human immunodeficiency virus, HPV – human papillomavirus, MUZIN – multizonal intraepithelial neoplasia

Table II: CD4 counts in the HIV-positive group

CD (cells/mm³)	HIV-positive overall cohort (*n* = 320)	MUZIN WLWH (*n* = 78)
> 500	51 (16%)	7 (9%)
< 500	105 (33%)	59 (75.6%)
Unknown	164 (51%)	12 (15.4%)

HIV – human immunodeficiency virus, MUZIN – multizonal intraepithelial neoplasia, WLWH – women living with HIV

Table III: Comparison of diabetes and tuberculosis in the overall cohort and MUZIN cohort

Medical disorder	Overall cohort (*n* = 653)	MUZIN (*n* = 104)
Diabetes	42 (6.4%)	6 (5.8%)
Tuberculosis	89 (13.6%)	26 (25%)

MUZIN – multizonal intraepithelial neoplasia

MUZIN (86.5%) were aged between 20 and 50 years, with the biggest group (37.5%) aged between 31 and 40 years. The mean age was 37.5, and the age range was 18–69 years (Figure 1).

Of the entire cohort attending the colposcopy clinic in 2018, 320 (49%) were HIV-positive. This contrasts with the 78/104 women (75%) with multizonal disease who were HIV-positive ($p < 0.001$, 2.2e-16) (Figure 2). The CD4 count was unknown for most women (51%) in the overall cohort, with only 51 women living with HIV (WLWH, 16%) in this group having a CD4 count > 500. Similarly, only seven women (9%) with MUZIN had a CD4 count > 500(Table II).

Of the 104 women with MUZIN, only six had HPV DNA testing. These women were tested in the private sector and were later referred to our state hospital. Three of them were positive for HPV DNA 16, two were positive for HPV DNA 18, and one was also positive for HPV DNA 45. The last woman tested positive for other high-risk HPV.

The focus was diabetes and tuberculosis, which could lower immunity. Tuberculosis was found in 89 women (13.6%) of the overall cohort, compared to 26 women (25%) in the MUZIN group (Table III). Tuberculosis has a significant association among women with MUZIN and all patients ($p = 0.004$).

Table IV: CD4 counts of WLWH with MUZIN

CD4 count	*n* = 78	%
Unknown	12	15.4
< 20	4	5.1
20–50	2	2.6
51–100	6	7.7
101–250	26	33.3
251–500	21	26.9
501–1 000	5	6.4
> 1 000	2	2.6

MUZIN – multizonal intraepithelial neoplasia, WLWH – women living with HIV

Table V: Viral load of WLWH with MUZIN

VL (copies/ml)	*n* = 78	%
Unknown	5	6
Not done	7	9
Lower than detectable	24	31
< 50	12	15
50–1 000	12	16
> 1 000	18	23

MUZIN – multizonal intraepithelial neoplasia, VL – viral load, WLWH – women living with HIV

In the entire cohort, 169 women (26%) were smokers, compared to the 22 women (21%) with multizonal disease who smoked.

As HIV positivity was statistically significant when comparing the overall cohort and the women with multizonal HPV-associated disease, we specifically looked at WLWH with MUZIN. We looked up the CD4 counts and the viral loads (VL) of women who were HIV-positive in the MUZIN cohort. There were 68 women (87.2%) who were on or had been on first-line antiretroviral therapy, whereas 10 women (12.8%) had never been on antiretroviral therapy. Second-line antiretroviral therapy was utilised by 16 women (20.5%), and none of them required third-line therapy.

We had no access to the CD4 counts in 12/78 WLWH. The remaining 66 CD4 counts are presented in Table IV. The majority (75.6%) had CD4 counts < 500, with only seven women (9%) having CD4 counts > 500. There were 18 women (23%) who were not virologically suppressed (VL > 1 000) (Table V).

Discussion

There is limited data on multizonal HPV-associated disease of the lower genital tract in the literature. Most studies focus on HPV prevalence and outcomes by specifically addressing one anatomical part, such as the cervix, vagina, vulva, anal, or perianal area.[7-11] The terminology is controversial and not well-defined.[12,13] Multizonal HPV disease refers to having more than one area of premalignant or malignant HPV-associated disease in two or more anatomical locations. Currently, there is no standardised terminology. Previously, we published articles in which we referred to it as the field effect of HPV disease or multifocal HPV-associated disease (multifocal intraepithelial neoplasia [MUFIN]), as very little was written about it previously.[4,5] We have shifted to multizonal disease to align with evolving understanding.

In our review, we found that multizonal disease of the lower genital tract was prevalent in 16% of women who were referred to our colposcopy and vulval clinic at Groote Schuur Hospital in Cape Town, South Africa, in 2018. These women were referred with abnormal cervical cytological smears or vulval premalignant disease. This contrasts with the prevalence of 4.4% found by Menguellet et al.[13] when they reviewed "multicentric" lesions in their colposcopy clinic. One of the reasons for our higher prevalence may be attributed to the women included with perianal disease and genital warts, categories excluded by Menguellet et al.[13] In addition, we had a larger cohort of 653 women who attended our colposcopy and vulval clinic in 2018. Menguellet et al.[13]'s study revealed a cohort of 998 women attending colposcopy over six years.

The prevalences found in the studies mentioned refer to premalignant disease. However, there may be a higher risk in cancer patients. At our institution, a retrospective study of a cohort of vulval cancer patients between 2002 and 2012 found a 38.6% prevalence of multizonal HPV-related disease.[14] A more recent study by Albuquerque et al.[15] found a 22% (n = 56) prevalence of multizonal HPV-associated disease in 253 women over five years. The authors included premalignant and malignant disease in women with a history of anogenital neoplasia in their sample size, possibly explaining the higher prevalence found in their study.[15] They also found that women with previous HPV-associated malignancies had a higher incidence of multizonal disease.

Our 2018 review of the colposcopy and vulval clinic attendees revealed that in the entire cohort, 61.28% were referred with high-grade cervical cytology, and only 25.7% were referred with vulval pathology. This contrasts with the 104 women with multizonal disease, where 49% were referred with vulval disease (genital warts or HSIL of the vulva), and 39.4% had high-grade cervical cytological findings. The vulval examination included the vulva and perianal area.

HIV positivity was a statistically significant risk factor for multizonal HPV-associated disease: 49% in the entire cohort versus 75% in the women with multizonal disease ($p < 0.05$). This contrasts with the study by Alberquerque et al.,[15] where 13% of women with multizonal HPV anogenital disease were HIV-positive. This study did not find a significant difference in HIV status between women with or without multizonal disease. However, they did find a statistically significant difference in women with MUZIN who were more commonly taking immunomodulators or immunosuppressive drugs.[15]

South Africa is a country known to rank first in the world in the annual number of new HIV infections.[16] An analysis published in 2015 found a 1 000 new infections per year in South Africans aged 15–49 years.[17] In addition, with the use of antiretrovirals, many more people live longer with HIV. The high prevalence of HIV in our colposcopy clinic reflects this. However, it must be remembered that WLWH are screened more frequently with cervical cytology in South Africa than HIV-negative women. There have been many challenges in establishing a systematic cervical cancer screening policy in South Africa, and there is diversity within the state and private sectors.

Opportunistic cervical cancer screening exists, but the policy adopted in 2000, which aimed to reduce cervical cancer in South Africa by 67% by offering women three cervical cytology smears in their lifetime (at 30, 40, and 50 years), has not been achieved. In 2011, WLWH with a CD4 count < 500 were offered a Pap smear at diagnosis and every three years, if normal. More recently, in 2016, a Universal Test and Treat (UTT) strategy was adopted, where all women were offered HIV testing and, if positive, were offered antiretroviral treatment and a Pap smear at diagnosis and three-yearly if normal.[18]

In this study, we could not comment on HPV DNA genotyping as it was not part of South African national guidelines in 2018 and was not performed routinely. HIV-positive women normally have a CD4 count taken at diagnosis, and thereafter, VL is used to assess treatment effect and adherence. Therefore, we do not have the CD4 counts of the entire cohort, and CD4 counts are not routinely repeated once women are on treatment.

Diabetes was not significant in our study. However, tuberculosis in our MUZIN cohort was significant (p = 0.004). Tuberculosis could be a marker for poor cell-mediated immunity; it is more common in the HIV-positive population, or it could be that tuberculosis further suppresses immunity.

Smoking was not significantly different in our study compared to the entire cohort and the women with multizonal disease (26% versus 21%, respectively). This again contrasts with Albuquerque et al.[15]'s study, where 51% of women with multizonal disease were smokers compared with 33% of women without MUZIN (p = 0.021). Our smoking prevalence is in keeping with the people with HIV in the Western Cape, South Africa, and national levels. Mutemwa et al.[19] found an overall smoking prevalence of 22% in HIV-positive adult attendees at clinics in the Western Cape: 26% prevalence in men and 21% in women.

As HIV was a significant factor for multizonal HPV-associated disease, we specifically looked at the 78 women who were HIV-positive in this group. Most women in this group were on antiretrovirals, with only 10 (12.8%) never accessing this treatment. Despite this, only seven (9%) had CD4 counts > 500. In addition, most women were virologically suppressed, with 12 women (15%) having VLs < 50, and 24 (31%%) having a lower than detectable VL. Only 18 women (23%) were not virologically suppressed.

Previously, antiretrovirals were only initiated in women with a CD4 count < 500 in South Africa. This policy has evolved over the years, with all having access to treatment now.[18] It is known that CD4 count is an important predictor of high-risk HPV infections and the incidence of precancerous neoplasia.[20,21] It has also been found that WLWH on antiretroviral treatment are at a higher risk of developing cervical disease if the antiretrovirals are initiated at a lower CD4 count.[21] We believe that the findings in our study support this, as most of these women had CD4 counts < 500 despite being virologically suppressed. As mentioned earlier, in South Africa, CD4 counts are taken before antiretroviral initiation and not repeated routinely. Despite being virologically suppressed, their immunity may be functionally impaired.

Study limitations and strengths

The retrospective nature of the study is recognised as a limitation. In addition, we have limited HPV genotyping results and no immunological samples to support our theories.

The strength of our study is that there are few studies on multizonal disease, and this is one of the largest cohorts collected over a year in the literature. Suggestions for future research:

- HPV genotyping in women with multizonal disease.
- Immunological comparisons (blood and tissue analysis) between WLWH with and without MUZIN.
- Assessment of quality of life in women who require treatment for multizonal HPV-associated disease.

Conclusion

There is limited data on multizonal HPV-associated disease. This study attempts to identify the demographics that render women susceptible to persistent HPV infection and the manifestation thereof in various genital tract organs, either simultaneously or at intervals. HIV and previous or current tuberculosis infection were significant factors in our MUZIN cohort. Monitoring the VL as a measure of viral suppression and treatment adherence may not be sufficient in managing these women. CD4 counts may be more useful in assessing the ability to mount an adequate and functional immune response.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

The database used for this research was approved by the University of Cape Town's Human Research Ethics Board (reference: FWA00001637; IRB00001938).

ORCID

TS Adams https://orcid.org/0000-0002-3686-3540
M Mhembere https://orcid.org/0000-0001-9952-1858
NH Mbatani https://orcid.org/0000-0001-8826-5182
LJ Rogers https://orcid.org/0009-0003-1759-5485

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Addendum: abbreviations and acronyms

CD4 – CD4 counts are white blood cells called T lymphocytes that fight infection/play an important role in the immune system's function

DNA – deoxyribonucleic acid

HIV – human immunodeficiency virus

HPV – human papillomavirus

HSIL – high-grade squamous intraepithelial lesion

MUFIN – multifocal intraepithelial neoplasia (MUZIN previously referred to as MUFIN in prior articles by authors)

MUZIN – multizonal intraepithelial neoplasia

See and treat approach – immediate treatment of a HSIL Pap result with a cervical excisional procedure/large loop excision of the transformation zone/loop electrical excision procedure if colposcopy confirms a high-grade lesion

SAMRC – South African Medical Research Council

UTT – Universal Test and Treat strategy (in the context of HIV testing in South Africa)

VL – viral load