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Prevalence and genotypes of HPV in pre-invasive ocular surface squamous neoplasia in Pretoria

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Background: Pre-invasive ocular surface squamous neoplasia (OSSN) are tumours of the ocular surface that include squamous neoplasms arising from the conjunctiva and the cornea, characterised by a multifactorial aetiopathogenesis. The role of human papillomavirus (HPV) in the aetiopathogenesis of OSSN is still controversial.

Aim: This study aimed to determine the prevalence and genotypes of HPV in pre-invasive OSSN samples.

Setting: The study setting was a tertiary laboratory in Northern Pretoria, South Africa, from 01 January 2012 to 31 December 2016.

Methods: This was a retrospective cross-sectional study that utilised 58 formalin-fixed paraffin-embedded (FFPE) specimens of previously diagnosed pre-invasive OSSN cases. Immunohistochemistry (IHC) and molecular pathology techniques were used to detect HPV and data analysis was conducted using STATA statistical software.

Results: The study sample had a higher proportion of females (60.3%; 35/58) compared to males (39.7%; 23/58) with an overall mean age of 41.8 ± 10.82 years. Human papillomavirus was detected in 20.7% (12/58) using IHC, 0% (0/58) by chromogenic in situ hybridisation, 19.0% (11/58) by linear array (LA) and 17.2% (10/58) by real-time polymerase chain reaction (RT-PCR). The HPV genotype commonly detected by LA and RT-PCR was HPV 16 at 12.1% and 8.6%, respectively.

Conclusion: A minimal proportion of OSSN cases tested positive for HPV, predominantly exhibiting oncogenic high-risk HPV 16 positivity. The role of HPV in the oncogenesis of OSSN remains controversial.

Contribution: This study contributes valued insights about HPV involvement in OSSN oncogenesis and HPV detection techniques in FFPE samples.

Keywords: ocular tumours; koilocytes; HPV; OSSN; CIN.

Introduction

Ocular surface squamous neoplasia (OSSN) is a term that encompasses a spectrum of epithelial squamous malignancy of the ocular surface, histologically ranging from mild dysplasia conjunctival intraepithelial neoplasia I (CIN I), to moderate (CIN II), to severe dysplasia (CIN III and carcinoma in situ (CIS) and invasive squamous cell carcinoma (SCC). In CIN and CIS of the ocular surface, the dysplasia has not yet invaded the substantia propria of the conjunctiva or the Bowman layer of the cornea.[1] The predisposing factors encompass elevated P53 expression, ultraviolet light exposure, a compromised immune system, and infections with human immunodeficiency virus (HIV) and human papillomavirus (HPV).[1]

Ocular surface squamous neoplasia lesions are typically described as low-grade malignancies that manifest in the sixth decade of life, predominately affecting males residing in regions with elevated ambient solar exposure.[2] The majority of OSSN patients are asymptomatic and mainly present with irritation, redness and pain. Early lesions mainly occur in the limbus, vary in size, and rarely invade the eye or orbit unless left untreated.[3]

Non-communicable diseases are on the rise globally, with rising mortality and morbidity rates attributed to cancer in both developing and developed countries. The global mortality rate attributed to cancer is increasing, with HPV and other oncogenic viruses attributing to about 25% of the cancers in developing nations.[4,5,6] This significant cancer burden in these developing

countries is attributed to the higher rates of HIV and HPV epidemic.[5,6] Over 50% of OSSN cases diagnosed in patients under 50 years of age are associated with HIV, frequently presenting with aggressive variants characterised by higher recurrence rates and having poor prognosis.[2,7,8,9,10] In some developed countries, numerous patients have limited or no access to highly active antiretroviral therapy (HAART), hence heightening individual susceptibility to certain opportunistic infections.[9]

The African continent exhibits the highest incidence of HPV infection globally, with an age-adjusted prevalence of 25.6% among women aged 15–74 years, followed by South America (14.3%), Asia (87%) and Europe (5.2%).[11] The most elevated prevalence of HPV in ocular lesions recently documented in South Africa was 66.7%, as reported by Odendaal et al. in the Western Cape.[11] Other studies have also reported on the correlation between HPV and malignancies in the ocular region, particularly the conjunctiva, with HPV 16, 11, 6 and 18 as the most commonly reported genotypes.[10,11,12,13,14,15,16,17] The conjunctival epithelium is occasionally vulnerable to HPV infection because of its exposure to the external environment.[13] One proposed mechanism of transmission to the ocular surface is inoculation by contaminated fingers.[18]

Integration of HPV DNA into the host genome is essential for oncogenesis. The early proteins E6 and E7 encoded by the high-risk (HR) HPV, disrupt the functions of the tumour suppressor gene P53 and the retinoblastoma gene, which are crucial for regulating cell-cycle checkpoints by promoting cell-mediated arrest and apoptosis.[1,4,5,6,11] Since 2014, two vaccines have been introduced in South Africa, Gardasil® and Cervarix® protecting against HPV types (16 and 18) and (6, 11, 16, 18, 31, 33, 45, 52 and 58), respectively.[19] Identification of prevailing HPV genotypes is critical for effective vaccination programmes.

The oncogenic role of HPV is still controversial as some studies have found higher percentages of HPV 16 in benign compared to malignant ocular lesions, suggesting a hit-and-run mechanism observed with these oncogenic viruses.[20,21] The hit-and-run theory suggests that the virus is responsible for the tumour cell initiation but it is not responsible for tumour cell maintenance and progression, hence the detection on pre-invasive and not on malignant lesions. This study evaluated the presence and genotypes of HPV in pre-invasive lesions of the ocular surface using molecular techniques.

Research methods and design

Study design and data collection

This was a retrospective cross-sectional study that analysed formalin-fixed paraffin-embedded (FFPE) tissue samples of pre-invasive OSSN conducted at the tertiary laboratory in Tshwane, National Health Laboratory Service (NHLS), Anatomical Pathology Department, from January 2012 to December 2016. The study used 58 pre-invasive OSSN FFPE tissue specimens, with their corresponding clinical data (histopathological diagnosis, age, gender and HIV status), obtained from the NHLS central data warehouse (CDW). Demographic and clinical data that were absent were retrieved from the laboratory information system (TrakCare) and captured in Microsoft Excel. The haematoxylin and eosin (H&E) slides and tissue blocks of the cases were retrieved by researcher and reviewed by two experienced pathologists to confirm the initial diagnosis. All pre-invasive or dysplastic OSSN samples diagnosed during the study period were included in this study after microscopic confirmation of the diagnosis.

Microtomy and sample preparation

Haematoxylin and eosin staining, immunohistochemistry (IHC) and chromogenic *in situ* hybridisation (CISH) slides were sectioned using the same protocol. Four micrometres' sections were cut from the archived FFPE tissue samples as per in-house Dr George Mukhari (DGM)-NHLS standard operating procedures (SOP) using the Leica 2245 rotary microtome (Leica Biosystems, Germany), then the sections were picked using charged slides (Cell Path services, South Africa) from the water bath (Leica Biosystems, Germany). For molecular tests, five sections of 5 μm were cut with a microtome blade from the FFPE tissue block and transferred into a 2 mL Cryo's® polypropylene vial (Merck KGa, Germany). To prevent cross-contamination between the samples, for molecular testing, the sections for each case were picked with a new applicator stick and the microtome and blade were cleaned with Xylene (Merck KGa, Germany), DNA AWAY™ Surface Decontaminant (Thermofisher, SA) and 70% ethanol (Merck KGa, Germany), after sectioning of each study sample. The slides and the tubes were stored at room temperature after sectioning until processing.

Haematoxylin and eosin staining

The H&E slides were oven-dried at 60 degrees Celsius for a minimum of 30 min prior to staining. The slides were stained using the automated Shandon Varistain H&E staining system (Thermo Fisher Scientific, United Kingdom) and subsequently mounted on the automated Leica Tissue-Tek cover slipper machine (Leica Biosystems, Germany) in accordance to the DGM-NHLS (SOP). The H&E-stained slides were reviewed to evaluate the morphological features of the lesions. The OSSN lesions were reclassified according to the American Joint Committee on Cancer guidelines (AJCC; 2010).

Human papillomavirus immunohistochemistry

For IHC testing, the slides were incubated overnight at a 60°C oven prior to staining. In addition, two internal controls were added with each run. A known cervical intraepithelial neoplasia HPV-positive tissue was used as an internal control. Briefly, sections were deparaffinised and rehydrated using the 30 ml Envision Flex target retrieval solution (K8004) 50× concentrate Tris/EDTA at pH 9 (Dako, Denmark) diluted in 1.5 L distilled water. Following antigen retrieval for 20 min at 95°C and cooled to 65°C using the Dako 48 PT link (Dako, Denmark). Then slides were incubated with Monoclonal

Mouse-Anti-Human HPV clone (K1H8) for 60 min diluted at 1:20. Staining was performed using the Dako Autostainer (Dako, Denmark) as per the manufacturer's standard operating procedure. The primary antibody used was a Dako Monoclonal Mouse Anti-Human HPV which stains 11 HPV genotypes, eight HR-HPV genotypes (16, 18, 31, 33, 51, 52, 56, and 58) and three LR-HPV (6, 11, and 42) genotypes. The Dako HPV stain signal is observed by a brown staining pattern on the cellular nucleus.

Human papillomavirus chromogenic in situ hybridisation

The slides were preheated in the oven at 60°C overnight and subsequently treated with enzymatic solution (code S3007, Dako, Denmark) at room temperature for 3 min, followed by the quenching of endogenous peroxidase activity using 0.3% hydrogen peroxide (H_2O_2). Slides were hybridised overnight (16 h) with the HPV biotinylated deoxyribonucleic acid (DNA) probe (Y1443) (Dako, Denmark), which catalyses signal amplification system for the 13 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and subsequently washed in 1x TBST (code S3306) (Dako, Denmark) at room temperature. Manual counterstaining with haematoxylin and followed by mounting with Entellan (Merck KGa, Germany). The HPV-CISH stain is a nuclear stain, with positive signals manifesting within the nucleus as either episomal (uniform nuclear staining) or integrative (distinct punctate staining). Each run incorporated established HPV-positive and negative internal controls.

DNA extraction

The deparaffinisation and rehydration of the sample were performed using xylene and the decreasing grades of ethanol (100%, 90%, 85% and 70%) before the actual DNA extraction could commence. Total DNA was extracted from the FFPE samples using a Nucleospin® DNA FFPE XS (Life Technologies, South Africa) extraction kit as per the manufacturer's guidelines. The quality and concentration of DNA were assessed with the Nanodrop technology, which measures at 260 nm and 260/280, respectively, using the Quanti-IT Pico Green DNA quantification kit (Thermo Fisher Scientific, United Kingdom). The extracted DNA was stored in a -20°C freezer until tested.

Conventional nested polymerase chain reaction

Human papillomavirus DNA was analysed by conventional nested-PCR assay. The human β-Globin primers; Forward PCO4 and Reverse GH20 were used for the detection of internal control. This method employed consensus primers forward-MY09/reverse-MY11 (450 bp) and forward GP5+/Reverse-GP6+ (140 bp), which target the conserved HPV L1 gene. The primers and the PCR method were published by Hildesheim et al.[22] Amplification was performed in a 96 gold plate well Applied Biosystems 2720 thermal cycler with the following programme: 1 denaturation cycle at 95°C for 4 min, followed by 40 three-step cycles; denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 5 min and finally elongation at 72°C for 5 min. The 268 bp generated by GH20 and PCO4, the 450 bp generated by MY11/MYO9 and the 140 bp PCR products generated by GP5+/GP6+ were detected using gel electrophoresis (2% agarose gel) and visualised with ethidium bromide on Vacutec Syngene G-BOX; both the 450 bp and 150 bp products were visualised. The PCR products were used as a template to perform Linear Array (LA) HPV genotyping test.

Linear array genotyping test kit

The HPV genotyping was performed on all study samples using the HPV LA genotyping kit (Roche Diagnostics, Germany). The HPV LA genotyping test kit detects about 37 different HPV genotypes, 15 HR-genotypes (HPV 16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59,67, 68, 73 and 82), three probable HR-genotypes (HPV 53, 66, 26), and 18 LR genotypes (HPV 6, 11, 40, 42, 54, 55, 61, 64, 62, 69, 70, 71, 72, 81, 83, 83, 89 and 39). The positive reaction is indicated by a blue line next to the HPV type that is positive.

Real-time quantitative type-specific polymerase chain reaction

TaqMan-based real-time quantitative type-specific PCR is a high-throughput approach for DNA detection and genotyping of 16 HPV genotypes. Human papillomavirus genotypes were detected using TaqMan-based real-time quantitative type-specific PCR (qPCR) on the LightCycler® 480 II system (Roche Diagnostics, Basel, Switzerland) with additional HPV 11 primers and probe. TaqMan-based quantitative qPCR enables the detection of an 80 bp fragment of 16 HPV genotypes that target 14 HR-HPV genotypes (HPV16 E7, 18 E7, 31 E6, 33 LI, 33 E6, 35 E6, 39 E7, 45 E7, 51 E7, 53 E6, 56 E7, 58 LI, 58 E6, 66 E6, 67 LI, 68 E7) and 2 LR-HPV genotypes (HPV6 E6 and 11 E6).

Statistical analysis

Descriptive statistics was used to analyse this study data. Descriptive statistics was used to analyse demographic data and clinical data; age was presented as minimum, maximum and mean; and other clinical and pathological data (HIV status, HPV pathology and solar elastosis) were presented in frequencies and percentages. To determine the prevalence of HPV in OSSN samples, descriptive statistics were used, and the data were presented in percentages. Categorical data and HPV genotypes were tabulated and presented as percentages.

Ethical considerations

Ethical clearance to conduct this study was obtained from the Sefako Makgatho University Research Ethics Committee (No. SMUREC/P/260/2016:PG).

Results

Clinico-pathological characteristics

Table 1 illustrates the demographic and clinical data with HPV detection. The study observed a higher number of

females (60.3%; *n* = 35/58) compared to males (39.7%, *n* = 23/58); the male-to-female ratio was 1: 1.5. The minimum age was 26 years, mean age of 41.8 ± 10.82 years. The mean age for males was 43.3 ± 8.31 with a range between 31 years and 63 years and the mean age for females was 40.9 ± 12.10 with a range between 26 years and 70 years. Clinical data on the HIV status of the samples was incomplete, HIV was positive on (36.2%; 21/58), negative on 3/58 (5.2%) and a higher percentage of samples had an unknown HIV status (58.6%, 34/58). Solar elastosis was observed in 24/58 (41.4%) of the cases. The HPV Pathology or Cytopathy (defined microscopically by the presence of koilocytes) was present in 15/58 (25.9%) of cases. Fifty-seven (98.3%) of the tumours were primary ocular tumours and only 1 (1.7%) was recurrent. Bilateral tumours were observed in only 1 (1.7%) and 57 (98.3%) were unilateral. With regard to tumour location, 23 (39.7%) were located in the right eye compared to 17 (29.3%) that were located in the left eye (Table 2). Most of the cases were CIN III (56.9%, 33/58) and 20.7% (12/58) were CIS.

The human papillomavirus prevalence in pre-invasive ocular squamous neoplasia samples

There was not much difference in HPV positivity between all three techniques although the IHC technique had a slightly higher HPV prevalence. The prevalence of HPV genotypes targeted by LA and real-time polymerase chain reaction (RT-PCR) showed minor variations. The positivity rate of LA and RT-PCR were 19% and 17%, respectively. Table 2 illustrates the HPV positivity with the different techniques; all the samples were negative for the CISH test and IHC detected the highest percentage. The RT-PCR technique did not detect any multiple infections; the multiple infections were only detected by LA, although HPV 11 and HPV 16 were the common detected genotypes in both the techniques (Figure 1).

Table 2 illustrates the HPV findings in relation to the histopathological subtypes. All techniques detected a higher prevalence of HPV on CIN III and CIS (the higher grades of the pre-cancerous OSSN lesions), 100%, 91% and 83% by RT-PCR, LA and IHC, respectively. The LA kit detected 5 mixed infections (HPV 11&16) as the common finding (45%) from the 11 positive cases. The commonly detected genotype by RT-PCR was HPV 16 (*n* = 5; 50%). More HR HPV genotypes were detected by RT-PCR (*n* = 7; 70%), compared to LA (*n* = 3; 27%) (Figure 1).

Comparison of human papillomavirus genotypes as detected by linear array and real-time polymerase chain reaction

The HPV genotypes as detected by LA and RT-PCR showed some minor differences.

Figure 1 summarises HPV genotypes as detected by LA and RT-PCR. The LA kit detected 5 mixed infections (HPV 11&16) as the common finding (45%) from the 11 positive cases.

Discussion

This study aimed to investigate the HPV prevalence and genotypes in pre-invasive lesions of OSSN. At the time of writing this article, there was no study in literature that used the three different techniques used in this study, and most studies had a smaller sample size compared to this study. The overall HPV prevalence was 21% when using IHC; however, reports in the literature have shown a very wide difference in detection rates ranging from 0% to 100% from

TABLE 1: Demographic, clinico-pathological characteristics and human papillomavirus findings in ocular surface squamous neoplasia cases (*N* = 58).

Variables	Sample *N* = 58		HPV Tests							
	n	%	IHC POS (*N* = 12)		CISH POS (*N* = 0)		LA POS (*N* = 11)		RT-PCR-POS (*N* = 10)	
			n	%	*n*	%	*n*	%	*n*	%
Gender										
Male	23	39.7	4	33.3	0	0	4	36.4	5	50.0
Female	35	60.3	8	66.7	0	0	7	63.6	5	50.0
HIV status										
Positive	21	36.2	4	33.3	0	0	3	27.3	3	30.0
Negative	3	5.2	1	8.3	0	0	1	9.1	0	0
Unknown	34	58.6	7	58.3	0	0	7	63.6	7	70.0
Histopathology										
CIN I	5	8.6	1	8.3	0	0	0		0	0
CIN II	8	13.8	1	8.3	0	0	1	9.1	0	0
CIN III	33	56.9	5	41.7	0	0	10	90.9	9	90.0
CIS	12	20.7	5	41.7	0	0	0	0	1	10.0
Solar Elastosis										
Present	24	41.4	7	58.3	0	0	3	27.3	4	40.0
Absent	34	58.6	5	41.7	0	0	8	72.7	6	60.0
HPV Cytopathy										
Positive	15	25.9	3	25.0	0	0	1	9.1	3	30.0
Negative	43	74.1	9	75.0	0	0	10	90.9	7	70.0

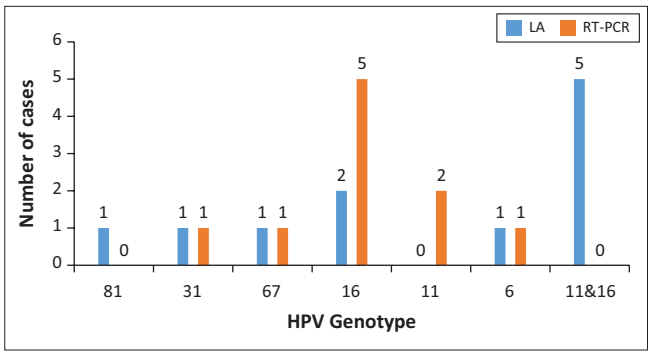
n, number of case; HIV, Human immunodeficiency virus; CIN, conjunctival intraepithelial neoplasia; CIS, Carcinoma in Situ; IHC, Immunohistochemistry; CISH, Chromogenic in situ hybridisation; HPV, Human Papillomavirus; LA, Linear array; POS, positive; RT-PCR, real-time polymerase chain reaction.

TABLE 2: Summary of human papillomavirus DNA positivity with histological grades (N = 58).

OSSN histological grade	IHC	CISH	LA-PCR	HPV		RT-PCR	HPV	
				Genotype	*n*		Genotype	*n*
CIN I	1	0	0	N/A	N/A	0	N/A	N/A
CIN II	1	0	1	6	1	0	N/A	N/A
CIN III	5	0	10	81	1	9	11	2
	-	-	-	31	1	-	16	4
	-	-	-	67	1	-	67	1
	-	-	-	16	2	-	31	1
	-	-	-	11&16	5	-	6	1
CIS	5	0	0	N/A	N/A	1	-	-
Negative cases	46	58	47	N/A	N/A	48	N/A	N/A
Total	**58**	**58**	**58**	**N/A**	**N/A**	**58**	**N/A**	**N/A**

Note: P16INK4a Positive (N = 13); IHC positive (N = 12); HPV Linear Array Positive (N = 11); Real-time PCR positive (N = 10).

DNA, deoxyribonucleic acid; OSSN, ocular surface squamous neoplasia; CIN, conjunctival intraepithelial neoplasia; CIS, Carcinoma *in situ*; IHC, Immunohistochemistry; CISH, Chromogenic *in situ* hybridisation; HPV, Human Papillomavirus; LA-PCR, linear array polymerase chain reaction; N/A, not applicable; RT-PCR, real-time polymerase chain reaction.



HPV, human papillomavirus; LA, linear array; RT-PCR, real-time polymerase chain reaction.

FIGURE 1: Summarises human papillomavirus genotypes detected by linear array and real-time polymerase chain reaction.

different geographical regions using IHC, PCR and other molecular techniques.[20,21,23,24,25]

The different findings might be attributed to the different HPV detection methods used, the different sample preservation methods affecting the quality of DNA, and different sample types and regions. The study's findings suggest that HPV might be a co-factor in the oncogenesis of OSSN. These findings agree with several studies that observed the possible role of HPV as a co-factor in the pathogenesis of the lesion. However, more data are needed to elucidate the association of HPV infection with OSSN and pathways linked to its oncogenesis, and this will assist with prevention, management and overall prognosis.[21]

The study has indicated different prevalence rates of 20.7%, 0%, 19.0% and 17.2% using IHC, CISH, LA and RT PCR, respectively. The varying results from this study might be attributed to the HPV genotypes detectable by each technique; the IHC technique was the most sensitive technique. The detected HPV types were HR-HPV; 16, 31, and 67 and the LR-HPV; 6, 11, and 81 (Figure 1). There were fewer variations between genotypes detected by LA and RT-PCR; the differences might be attributed to the genotypes that are not detectable by the other technique and few HPV-positive samples in this study. Although LR HPV genotypes are detected, these might be bystanders as earlier studies have associated them with benign and not malignant lesions.

Multiple HPV infections were only detected by LA (HPV 11&16), and RT-PCR only detected HPV 16 for those samples (Figure 1). Contrary to this study's findings, the Cape Town study reported a mot of multiple, triple, quadruple and five HPV infections in some of the OSSN lesions.[11] The CISH method in histopathology is a useful technique for demonstrating the presence of the HPV infection *in situ* in the presence of the morphological architecture of the tissue. The negative results of the HPV-CISH technique might be attributed to a low copy number of HPV in infected cells. Furthermore, the OSSN samples are minute tissue specimens in nature, and therefore the small session might have influenced the HPV viral load on the samples. Similar findings were reported by Peterson et al. in the US, where RNA ISH detected HPV in 8% of the samples but DNA ISH did not detect HPV in all pre-invasive OSSN samples.[12]

This study detected HPV 16 in 50% of positive samples using RT-PCR, and other studies have also reported HPV type 16 to be the most prevalent in Uganda[26] in India[27] and in Germany.[20] Similar to the current findings, Odendaal et al., in Western Cape, SA reported a 66.7% HPV prevalence on OSSN lesions with a 52.4% prevalence of HPV 16.[11] A Mexican study reported a 40.9% HPV prevalence in OSSN with a 32% HPV 16 prevalence.[28]

Galor et al. reported a 78% HPV positivity in OSSN lesions; HPV 16 was the commonly detected genotype at 48% prevalence in Miami. The difference in prevalence might be attributed to the different techniques utilised in different studies because the techniques have different sensitivity and specificity.[29] The most common HPV genotypes detected in this study were HPV 16, 11 and 6; similar to those reported by Carrilho et al. Galor et al. and Chauhan et al.[21,27,29] Similar findings were also reported by Odendaal et al. in Cape Town, where HPV 11, 16 and 18 were the commonly detected genotypes.[11] A different finding was reported by Tornesello et al. in Uganda where only HPV 18, 38 and 20 were detected.[26]

In this study, a higher prevalence of HPV infection was detected in CIN III as has been observed in other studies.[23,24,29,30] The study's findings suggest a relationship between the higher CIN grades and HPV infection; however, more studies are warranted to establish the relationship. The higher rate of

high-grade dysplasia samples (> 70%) in this study and the relationship between HPV and CIN III emphasise the need for early detection before the bridging of the epithelial.

The presence of koilocytes in tissue or fluids (for cytopathology) is commonly used for confirmation of HPV infection. In this study, koilocytes were present in only 26% of all the OSSN samples, while less than 30% of the samples with koilocytes on H&E were positive for HPV by other techniques. These varying findings with the different techniques create a need to develop a sensitive, standardised and reliable methods for detecting HPV in FFPE samples, especially samples with small amount of tissue such as OSSN. Because koilocytes are an indication of HPV infection and other samples with koilocytes were HPV negative, it could mean that the test used for testing HPV need to be further investigated in these tissue samples, with clinical data correlation. Furthermore, HPV pathology was commonly observed in high-grade OSSN lesions; 80% of the samples with HPV pathology were CIN III and 13% were CIS.

There are limited data on the correlation of koilocytes with HPV infection in OSSN tissue samples. Miyahara et al. reported a poor correlation between koilocytes and HPV infection in oropharynx SCC; some of the HPV DNA-positive samples did not have koilocytes, while other samples with koilocytes were negative for HPV DNA analysis.[31] Khangura et al. reported a correlation between HPV 16 and 18 with koilocytes and no correlation with other HPV genotypes in oral SCC tissue samples.[32] A case report by Knutsson et al. in Italy reported koilocytes in HPV 16 positive grade I pre-invasive OSSN case.[33] Thus, the correlation of koilocytes with HPV infection in OSSN cases needs further investigation.

Chauhan et al. and Carreira et al. attributed the OSSN aetiology to multiple factors. They indicated UV light as one of the major causes of OSSN, and that UV light interferes with the DNA repair mechanisms.[19,23] Representation of UV exposure is indicated by solar elastosis on OSSN tissue samples, which was present in 41% of this study samples. Of these samples, 58% were positive for HPV. Solar elastosis was also prevalent in high-grade OSSN lesions, 54% and 12% in CIN III and CIS, respectively. However, the presence of solar elastosis does not confirm UV light as the primary causative agent of OSSN, and therefore, the relationship between HPV in OSSN and UV light remains inconclusive.

Of the HIV-positive patients in this study, only 30% had HPV; therefore, the association between HIV and HPV infections with OSSN remains inconclusive. However, a prospective study with a powered sample size might help in elucidating this association. In this study, when the samples were analysed further based on the HIV status, it was observed that most HIV-positive samples were diagnosed with high-grade OSSN variants; 57% were CIN III and 14% were CIS. A study by Masanganise et al. reported more aggressive variants of OSSN in younger HIV OSSN patients with a likelihood of high recurrence.[7] While other studies have associated HIV positivity with the development of OSSN.[10,21]

The main limitation of this study was incomplete information of patients because of the retrospective nature of the study and a lack of studies on OSSN. The HPV infection that applied the same laboratory techniques used in this study and the small sample size. Furthermore, HPV quantification was not performed because of financial constraints; this limits the researchers' ability to conclude the relationship between CISH sensitivity and the concentration of HPV as described in the literature.

The low prevalence of HPV indicated by this study may suggest that HPV is not the principal causative agent but the co-factor in the aetiopathogenesis of OSSN. The scarcity of studies on oncogenic HPV genotypes on OSSN was a limitation as no exact comparison could be made with other studies. The commonly detected genotypes in this study were HPV 16, 6 and 11; they are included in the available approved vaccines (Cervarix and Gardasil) where Cervarix is administered through the national immunisation programme given to young girls in South Africa.

Conclusion

The prevalence of HPV in OSSN lesions was low (21%), and the commonly detected HPV genotypes consisted of both the HR- (HPV 16) and LR-HPV (HPV 6 and 11) genotypes. The HPV-CISH technique was negative in all samples. This study's findings suggest that HPV might be a co-factor in OSSN oncogenesis in this population, but not the necessary causative agent. The results also suggest that individuals might be protected from HPV infection with these genotypes found in the current HPV vaccines being rolled out in South Africa.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

L.N.M. was involved in data collection, data analysis, writing-original draft preparation and editing of the manuscript. C.M.N. performed conceptualisation, data collection, methodology, data analysis and editing of the manuscript. M.C.K. was responsible for data analysis and reviewing of the manuscript. R.L.L. was methodology, data analysis and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author, L.N.M., upon reasonable request.

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