Profile of human papillomavirus genotypes in breast and oesophageal cancer patients in Pretoria, South Africa

N Maroga,[1] MSc; **T Mokoena**,[1] FRCS, DPhil (ORCID); **N Ledibane**,[2] MB ChB, MPH; **A Musekiwa**,[2] PhD;
M Bida,[3] MSc, MMed (Path); **M Kgomo**,[4] FCP (SA), PhD; **R Lebelo**,[5] PhD

[1] *Department of Surgery, School of Medicine, Faculty of Health Sciences, University of Pretoria and Steve Biko Academic Hospital, Pretoria, South Africa*
[2] *School of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria, South Africa*
[3] *Department of Anatomical Pathology, School of Medicine, Faculty of Health Sciences, University of Pretoria and National Health Laboratory Service, Pretoria, South Africa*
[4] *Gastroenterology Unit, Department of Internal Medicine, School of Medicine, Faculty of Health Sciences, University of Pretoria and Steve Biko Academic Hospital, Pretoria, South Africa*
[5] *Department of Virology, National Health Laboratory Service, Sefako Makgatho Health Sciences University, Pretoria, South Africa*

Corresponding author: T Mokoena (taole.mokoena@up.ac.za)

Background. The association between human papillomavirus (HPV) and cervical cancer is well established, and cervical cancer can be prevented through HPV vaccination. Little has been reported on the association between HPV and breast carcinoma (BC) or oesophageal squamous cell carcinoma (OSCC) in Africa. It is possible that use of appropriate HPV vaccines against genotypes responsible for these cancers may also prevent their development.
Objectives. To investigate HPV genotype prevalence in BC and OSCC patients in Pretoria, South Africa (SA).
Methods. A retrospective cross-sectional study of BC and OSCC patients managed at Steve Biko Academic Hospital from 2015 to 2019 was undertaken. Patient medical records were analysed, and DNA was extracted from their archived pathology material and amplified by polymerase chain reaction before hybridisation for HPV genotypes.
Results. There were 101 patients with BC and 50 with OSCC. The prevalence of HPV infection in BC patients was 77.2%, with 35.6% high-risk (HR) genotypes, and that in OSCC patients 90.0%, with 56.0% HR genotypes. The most prevalent HPV genotypes (>20% each) were HPV 16, 70 and 51 for BC and HPV 51, 70, 16 and 82 for OSCC, with 31.7% and 60.0% of patients, respectively, having co-infection with ≥2 genotypes.
Conclusion. The high prevalence of infection with multiple HPV genotypes in BC and OSCC patients, with HPV 16, 51, 70, 35 and 82 the most common genotypes in these cancers, warrants expansion of the current SA bivalent HPV 16/18 vaccine for girls to include boys, and inclusion of HPV 51, 70, 35 and 82, in order to prevent BC and OSCC as well as cervical cancer.

According to the World Health Organization, global estimates of the burden of cancer reached 18.1 million new cases and 9.1 million deaths in 2018.[1] A meta-analysis of the global burden of disease for the period 1990 - 2017 indicated that the burden of cancer quantified as disability-adjusted life-years had risen from sixth place in 1990 to second in 2017.[2]

Breast carcinoma (BC) is the most prevalent cancer among women worldwide.[1] The association of human papillomavirus (HPV) with cervical and other genital squamous cell carcinomas is well established.[3,4] A number of studies from around the world have identified the presence of HPV infection in patients with BC,[5-7] although a few failed to demonstrate this association in some regions.[8-10]

Oesophageal squamous cell carcinoma (OSCC) is a significant public health problem in many countries, including South Africa (SA). Persistent HPV infection has been implicated in the causation of OSCC.[11,12] HPV infection has been demonstrated in patients with OSCC in many geographical areas, especially those with a high OSCC prevalence.[13-18]

HPV oncogene products can inhibit tumour suppressor genes such as p53[19,20] and retinoblastoma protein (pRb),[21,22] inhibit antigen presentation and effective local immune response,[19,23] and induce carcinogenic host cell somatic mutations and genomic instability.[24]

These changes have also been observed in BC.[25]

There are no scientific reports on the association between HPV infection and BC in sub-Saharan Africa or SA, and there is a paucity of reports on the association of HPV with OSCC in the region.[26,27] A cross-sectional study was therefore undertaken to investigate the presence and genotypes of HPV infections in patients with BC or OSCC, the leading causes of cancer deaths among women and men, respectively, in southern Africa.[28] HPV infection affects >60% of young women in SA.[29]

It is hoped that the demonstration of HPV infection in patients with these cancers, especially BC, in SA would motivate for expansion of HPV vaccination, which is currently limited to girls for the prevention of cervical cancer,[30] to include prevention of BC and OSCC and therefore to be extended to boys as well.

Methods

Study population and data collection

A retrospective cross-sectional analysis of patients with confirmed BC or OSCC treated at Steve Biko Academic Hospital, Pretoria, SA, from 2015 to 2019 was conducted. Medical records were accessed, and patient demographic details were recorded. The patients' archived formalin-fixed paraffin-embedded (FFPE) pathology blocks were retrieved from the Department of Anatomical Pathology,

National Health Laboratory Service (NHLS), for DNA extraction and amplification and HPV genotyping. Patients who had undergone prior neoadjuvant chemotherapy or radiotherapy were excluded, as were patients whose medical records were incomplete and those whose archived pathology samples were inadequate.

Tissue preparation
All selected pathology blocks were histologically re-examined to confirm the pathological diagnosis. FFPE tissue blocks were first placed on ice for 1 hour to harden the wax to facilitate sectioning. Three 20 μm sections were trimmed from the blocks before the test sections were cut. Ten sections of 8 μm each were cut from the selected blocks using a Leica 2245 microtome (Leica Biosystems, Germany) and placed in sterile 2 ml Eppendorf tubes (Merck KGa, Germany) using disposable toothpicks. The microtome and blades were cleaned with xylene followed by 70% ethanol after cutting each block, and a fresh toothpick was used for each sample. The tubes were labelled and stored at room temperature until DNA extraction.

DNA extraction
DNA extraction was performed according to the QIAamp DNA Mini and Blood Mini Handbook (Qiagen, Germany).[31] This involved cell lysis using physicochemical methods. Lipids were removed by the addition of detergent (Qiagen, USA), followed by the removal of proteins with Proteinase K solution (Qiagen, USA). RNA was removed with RNase (Qiagen, USA), and the remaining DNA was quantified.

Polymerase chain reaction
HPV genotyping was performed using the Roche Linear Array HPV genotyping test (Roche Diagnostics, Germany), according to the manufacturer's recommendation. This method uses biotinylated primers to define a sequence of nucleotides within the polymorphic L1 region of the HPV genome that is approximately 450 base pairs long (supplementary file, available online at https://www.samedical.org/file/2040).

Ethical considerations
Study patients' information was kept confidential, and their identity was protected by assigning each with a unique study number.

The study was approved by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria (ref. no. 343/2020). The study was conducted according to the Helsinki Declaration ethical principles for research on human subjects.

The hospital and NHLS management, as custodians of patient records and materials, gave permission to access patients' medical records and their archived pathological material, respectively, and to publish the research findings.

Statistical analysis
Both descriptive and inferential statistics were used. To determine the prevalence of HPV infection in patients with histologically confirmed BC or OSCC, the number of cases with HPV infection was divided by the total number tested and expressed as a percentage. Descriptive statistics were used to describe demographic characteristics and HPV genotypes. Numerical characteristics such as age in years were summarised using means, standard deviations (SDs) and ranges. Categorical characteristics such as genotypes were tabulated, showing counts and percentages for each category. Bar charts were used to visualise categorical data. For inferential statistics, Pearson's χ^2 test was used to compare high-risk (HR) HPV infection between BC and OSCC patients. All statistical analyses were performed using Stata version 15 (StataCorp, USA) software, and a p-value <0.05 was considered statistically significant.

Results
One hundred and fifty-one patients (101 BC and 50 OSCC) had complete medical records and pathological samples for analysis. All 101 BC patients were female, with a mean (SD) age of 56.2 (15.7) years (range 19 - 96). Seventy-eight BC patients (77.2%) tested positive for HPV infection (Table 1). The highest HPV infection rate was in the middle age range (50 - 65 years).

There were 26 male and 16 female OSCC patients (sex was not stated in 8 patient records and could not be deduced from the patients' names), with a mean (SD) age of 51.9 (25.7) years (range 18 - 88). Forty-five OSCC patients (90.0%) tested positive for HPV infection (Table 1). The highest HPV infection rate was also in the middle age range (50 - 65 years).

HPV genotypes in patients with BC
Fig. 1 shows the number of BC patients with HR-HPV or low-risk (LR) HPV genotypes. It was noted that the most common HPV genotypes were HPV 16 and HPV 70 at ~37% each, followed by HPV 51 at 23%, with HPV 35 and HPV 82 trailing at 16% each.

HPV genotypes in patients with OSCC
Fig. 2 shows the number of OSCC patients with HR-HPV or LR-HPV genotypes. The most common genotypes were HPV 51 (62.0%), HPV 70 (48.0%), HPV 16 (44.0%) and HPV 82 (34.0%).

Overall, 35 genotypes were identified, of which 21 were HR and 14 LR.

Comparison of HR-HPV infection between BC and OSCC patients
A comparison between BC and OSCC patients in respect of selected HPV genotypes is shown in Table 1. It was noted that OSCC patients had a higher prevalence of HPV infection overall (90.0% v. 77.2%; p=0.057) and had significantly more HR-HPV genotypes (56.0% v. 35.6%; p<0.017). The profile of HR-HPV genotypes was similar but not identical in the two cancer types. While HPV genotypes 16, 70 and 51 were the most prevalent in both, OSCC patients had a higher proportion of HPV 51 than BC patients (62.0% v. 21.8%; p<0.001). Interestingly, HPV 82 was significantly more prevalent in OSCC patients than in BC patients (34.0% v. 15.8%; p=0.011). In contrast, BC patients had a higher proportion of HPV 35 (15.8% v. 8.0%; p=0.181). It was noted that the prevalence of HPV 18 was relatively low in both cancer types (5.0% in BC and 2.0% in OSCC).

Multiple HR-HPV co-infection in BC and OSCC patients
A number of patients in both cancer groups were co-infected with different HR-HPV genotypes (Tables 2 and 3). It was noted that 60.0% of OSCC patients had ≥2 HR-HPV genotypes (Table 2), compared with 31.7% of BC patients (Table 3). However, these differences were not statistically significant. Interestingly, 19 OSCC patients (38.0%) had co-infection with 3 or 4 HR-HPVs, namely 16, 51, 70 or 82, compared with only 14 BC patients (13.9%).

A qualitative comparison of the degree of HPV co-infection between BC and OSCC patients is shown in Table 4. The median number of HPV co-infections in OSCC was 3 genotypes, compared with 2 in BC.

Table 1. Comparison of selected HPV genotypes in BC and OSCC patients

HPV genotype	BC (*n*=101*), *n* (%)	OSCC (*n*=50*), *n* (%)	*p*-value
HPV negative	23 (22.8)	5 (10.0)	0.057
HPV, HR	36 (35.6)	28 (56.0)	0.017
HPV, LR	5 (5.0)	2 (4.0)	0.794
HPV, both HR and LR	37 (36.6)	15 (30.0)	0.419
HPV 16	37 (36.6)	22 (44.0)	0.383
HPV 70	36 (35.6)	24 (48.0)	0.144
HPV 51	22 (21.8)	31 (62.0)	<0.001
HPV 35	16 (15.8)	4 (8.0)	0.181
HPV 82	16 (15.8)	17 (34.0)	0.011
HPV 18	5 (5.0)	1 (2.0)	0.382

HPV = human papillomavirus; BC = breast cancer; OSCC = oesophageal squamous cell carcinoma; HR = high risk; LR = low risk.
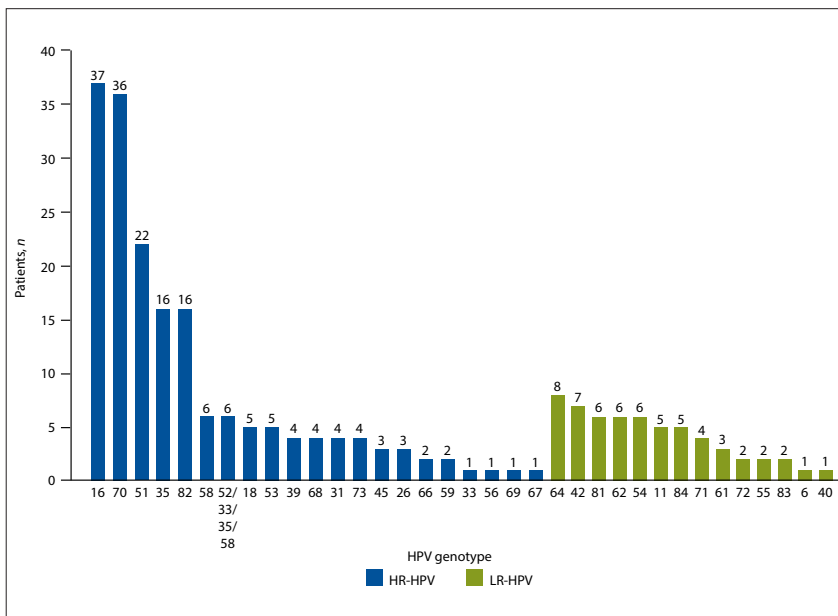*Some patients were co-infected with ≥2 HR-HPV genotypes.

Fig. 1. HR-HPV and LR-HPV genotypes in 101 breast carcinoma patients. (HR-HPV = high-risk human papillomavirus; LR-HPV = low-risk human papillomavirus.)
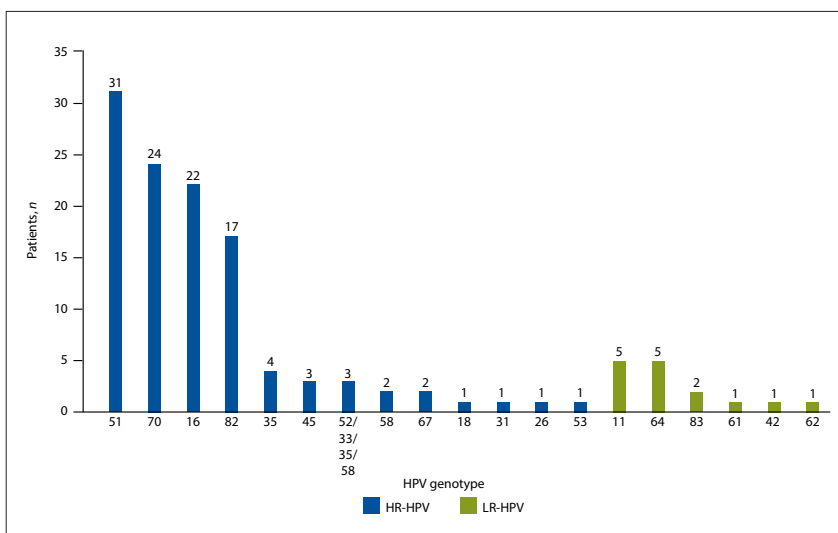

Fig. 2. HR-HPV and LR-HPV genotypes in 50 oesophageal carcinoma patients. (HR-HPV = high-risk human papillomavirus; LR-HPV = low-risk human papillomavirus.)

Fig. 3 shows a composite summary of the key findings of the study.

Discussion

The study identified 35 HPV genotypes in BC and OSCC patients, and the majority (62.5%) were HR-HPV genotypes. The prevalence of HR-HPV in BC was 64.0%, compared with 64% in India,[32] 61% in Syria,[33] 48.6% in Iran,[34] 42% in Venezuela[35] and 42% in the UK,[36] with relatively high prevalence rates, and 25% in Morocco,[37] 21% in Japan,[38] 17.5% in Korea,[39] 17.3% in China[22] and 5.8% in Australia,[40] with relatively low rates. The most common HPV genotypes were 16, 70, 51, 35 and 82, where 16 and 70 accounted for the majority (72.2%). The 36.6% HPV 16 prevalence rate is similar to 35% reported in Mexico[41] and 33% in Argentina,[42] but contrasts with 19.3% in Japan[38] and 69% in India,[32] which were much lower and higher, respectively. The low 5% HPV 18 prevalence contrasts with a high prevalence in Australian BC patients (55%).[40] Interestingly, the high HPV 51 prevalence in our BC patients (21.8%) was also noted in Venezuela (30.8%)[35] and Korea (63.6%).[39] HPV 70, which was as prevalent as HPV 16 (35.6% and 36.6%, respectively) in this study, has not been prominent in BC studies performed elsewhere. It was noted that HPV 70 was also very prominent in OSCC patients in the present study, as was HPV 16, which probably reflects a regional population HPV infection pattern.

Many BC patients (31.7%) were infected by ≥2 HR-HPV genotypes, with 13.9% having 3 or 4 co-infections. A high prevalence of multiple HR-HPV genotype infections has been reported in SA women with cervical cancer, but the predominant HPV genotype profile of HPV 16, 56, 18 and 39 was different from the profile found in BC in the present study.[43] This contrasts

with concordant HPV infection profiles in Australian BC patients with prior cervical cancer.[44]

There was a very high prevalence of HR-HPV (70.0%) in OSCC patients. This is in contrast to the generally much lower HPV infection rates reported in many other geographical areas, especially in the West.[12,13] This high HPV prevalence rate may partly account for the endemically high rate of OSCC in SA. High HPV prevalence has also been reported in other high OSCC prevalence countries such as China[15] and Iran.[16]

The 60.0% rate of co-infection with multiple HR-HPV genotypes in OSSC patients in this study was much higher than that in BC women (31.7%), but their HPV genotype profiles were qualitatively similar although quantitatively different. This finding may reflect the HPV genotype infection pattern in the SA population, since a similar HPV profile was reported in asymptomatic young women in Cape Town and Johannesburg.[29] In the present study, HPV 51 and 70 accounted for the majority of genotypes in OSSC (62.0% and 48.0%, respectively), while HPV 16 and 70 were most common in BC (36.6% and 35.6%, respectively). It was further noted that the prevalence of HPV 82 was proportionately double in OSSC compared with BC (34.0% v. 15.8%, respectively). Interestingly, a case control study

Table 2. Co-infection with HR-HPV genotypes in 50 patients with OSCC

HR-HPV genotypes	Patients, n (%)
51, 70	3 (6.0)
70, 82	2 (4.0)
16, 51	5 (10.0)
16, 18	1 (2.0)
51, 70, 82	8 (16.0)
16, 51, 70	4 (8.0)
16, 51, 70, 82	7 (14.0)
Total co-infections	30 (60.0)

HR-HPV = high-risk human papillomavirus; OSCC = oesophageal squamous cell carcinoma.

Table 3. Co-infection with HR-HPV genotypes in 101 patients with BC

HR-HPV genotypes	Patients, n (%)
16, 51	2 (1.98)
51, 70	5 (4.95)
70, 82	1 (0.99)
16, 18	4 (3.96)
16, 70	6 (5.94)
16, 51, 70	2 (1.98)
16, 70, 82	3 (2.97)
51, 70, 82	3 (2.97)
16, 51, 70, 82	6 (5.94)
Total co-infections	32 (31.68)

HR-HPV = high-risk human papillomavirus; BC = breast cancer.

from Johannesburg analysed serum anti-HPV-16 antibody levels in a cohort of patients with different cancers including OSCC and BC.[45] The BC patients' results were pooled together with those of other cancers that were not thought to be associated with HPV infection as a putative 'negative' case comparison. The study found a significantly high rate of anti-HPV-16 seropositivity in OSCC patients, but not in the putative 'negative' case control patients, which included BC patients. The BC patients constituted 50% of the 'negative control' cancers. It would have been salutary to have analysed the BC patients' results separately to see whether the findings would still indicate a non-significant anti-HPV-16 seropositivity.

The profile of HR-HPV in OSSC differs significantly from that previously reported in SA, where there was predominance of HPV 11 (an LR-HPV genotype), HPV 16 and HPV 39.[26,27] It also contrasts with that reported for Chinese and US patients, who predominantly had HPV 16, 57 and 26,[46] and with Japanese[47] and Iranian[16] patients, who predominantly had HPV 16 and 18.

Co-infection with multiple HPV genotypes in BC and OSCC has been reported previously in other studies from various countries and regions.[26,27,35,38] Such co-infections have been shown to be associated with more advanced or high-grade squamous intraepithelial neoplasia in the uterine cervix.[48] Multiple HR-HPV infections probably act synergistically to induce malignant transformation of target tissue cells, since their mechanism or intensity of action on the cell cycle may differ; for example, HPV 16 E6 inhibits p53 tumour suppressor gene protein[19,20] and HPV 18 E7 targets pRb[21,22] in the cell cycle checkpoints. The transforming effect of HPV is enhanced or facilitated by the growth effect of oestrogen on its target breast and reproductive tissues.[20] It has been reported that patients who develop cervical and breast cancers associated with HPV are younger than those with cancers that are not, and their cancers are more aggressive, probably as a result of synergy between HPV and oestrogen.[44]

Although HPV infection has most frequently been associated with squamous cell carcinoma of the uterine cervix and the oesophagus, it has also been demonstrated in adenocarcinoma of the uterine cervix[49] and of the oesophagus, including the premalignant Barrett's dysplasia of the oesophagus.[12,17,50] It is therefore not surprising that HPV infection is also associated with adenocarcinoma of the breast.

Despite the fact that oncogenesis is a multistep process that involves a variety of risk factors, such as oestrogen for BC[20] and lifestyle factors, such as diet and smoking, in addition to oncogenic viral infections working sequentially in tandem or synergistically contemporaneously, HPV-induced cancers are preventable by mass HPV vaccination.[51] A national HPV vaccination campaign was introduced in SA in 2014 to provide free vaccination to all girls aged ≥9 years with a bivalent HPV 16/18 vaccine aimed at cervical cancer prevention.[30] The finding of multiple HPV co-infections in patients with breast and oesophageal cancers in the present study highlights the need to broaden the scope of HPV vaccine to include both girls and boys. A tailor-made polyvalent vaccine targeted at this population should therefore be designed to include HPV 70, 51, 82 and 35 genotypes in addition to HPV 16 and 18 in a nanovalent

Table 4. Comparison of degree of co-infection with ≥2 HPV genotypes in BC and OSCC patients

Number of HIV genotypes	BC (n=101), n (%)	OSCC (n=50), n (%)	p-value
2	18 (17.8)	11 (22.0)	0.376
3	8 (7.9)	12 (24.0)	0.006
4	6 (5.9)	7 (14.0)	0.097
Total	32 (31.7)	30 (60.0)	<0.001

HPV = human papillomavirus; BC = breast cancer; OSCC = oesophageal squamous cell carcinoma.

HPV vaccine,[52] or at the very least a pentavalent HPV vaccine incorporating HPV 16, 51, 70, 82 and 35, for it to be completely effective in the prevention of breast or oesophageal cancer in our region. The paucity of HPV 18 in both BC (5.0%) and OSCC (2.0%) in the present study is noteworthy and diminishes its relevance and importance in the design of vaccines specifically aimed at these cancer types in the region.

The limitation of the current study is that it examined HPV genotype profiles at a single academic institution, and its findings may therefore need to be confirmed in other parts of the country. However, while previous HPV genotyping in SA women with cervical squamous cell carcinoma showed a quantitatively and qualitatively different HPV genotype profile,[43] a recent study of HPV prevalence among sexually active but asymptomatic young women in Cape Town and Johannesburg found similar HPV genotype profiles to the

ones demonstrated in the current study, indicating their widespread distribution in the country.[29] These authors also advocate for a vaccine with expanded HPV genotypes. The research on HPV genotype infection profiles in cancer patients, especially BC, needs to be confirmed in other southern African regions to demonstrate the generalisability of our findings.

Conclusion
The present study has demonstrated similar and novel HPV genotype infection profiles in SA BC and OSCC patients. In addition to HPV 16, described for cancer of the uterine cervix, the study identified HPV 51, 70, 82 and 35 as other prevalent HR-HPV genotypes in this patient population. This finding calls for the expansion of the current bivalent HPV 16/18 vaccination for girls to include boys, and the addition of HPV 51, 70, 35 and 82 genotypes as well in a vaccine for SA.
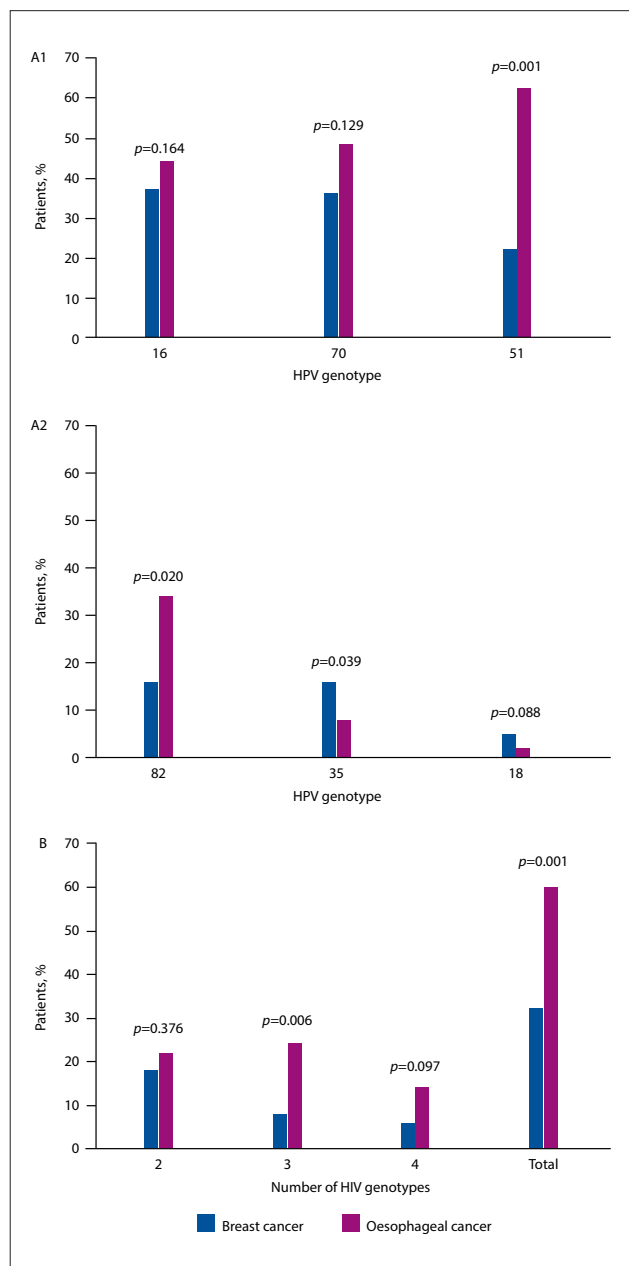
Fig. 3. Composite summary of the study findings, showing percentages of carcinogenic HPV genotypes (A1 and A2) and percentages of co-infection with ≥2 HPV genotypes (B). (HPV = human papillomavirus.)

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