International Journal of Molecular and Clinical Microbiology 9(1) (2019) 1074-1081

International Journal of
Molecular and Clinical Microbiology

Prevalence of anal HPV in women with cervical neoplastic and paraneoplastic lesions from Bogotá, Colombia

HA Vargas, Ph.D[1*]; JP Sánchez, MSc[1]; DA Obando, BSc[1]; LV Barbosa, BSc[1] ; SL Gomez, MSc[1]; P Arce, MSc[1]; E Teuzabá, MD-Pathologist[2]; S Salamanca, MD-Gynecologist[3]; CD Golijow[4]

1. Public Health Laboratory, District Department of Health; Bogota, Colombia.
2. Pathology Laboratory Patolab Rx Ltda.Bogota, Colombia.
3. Ginesalud IPS, Bogota-Colombia.
4. Institute of Veterinary Genetics "Ing. Fernando Dulout "(IGEVET, CONICET-UNLP, Argentina).

ARTICLE INFO

Article history:
Received 12 Feburary 2019
Accepted 29 April 2019
Available online 1 June 2019

Keywords:
Anal Cancer,
Human Papillomavirus,
Prevalence HPV,
HPV Epidemiology,
Female.

ABSTRACT

The incidence of anal squamous cell carcinoma has increased in the last few decades. Anal cancer has been associated with persistent infection with high-risk human papillomavirus (HR-HPV) types. However, only a few data reflect the status of anal HPV infection in women with cervical neoplastic and paraneoplastic lesions. The objective of the present study was to investigate the distribution of HPV genotypes and abnormal anal cytology in sexually active women with cervical disease from Bogota, Colombia. We therefore performed anal cytology for type-specific HPV identification in 134 anal samples of sexually active women from Bogota, Colombia, presenting cervical disease; using a commercial molecular technique (Linear Array®; Roche, Molecular Systems, USA). Results of anal cytology were normal in 93.3% of samples, while the remaining 5.2% was classified as atypical squamous cells of undetermined significance (ASC-US), 0.75% high-grade anal intraepithelial neoplasia (LGAIN-II/III,) and 0.75% low-grade AIN ((HGAIN-I). The analysis for viral infection in the 134 anal samples showed HPV in 61.5% of the studied population. In general, higher HPV infection values were seen in more compromised anal lesions. The most prevalent viral genotypes were HPV-16 (27.7%), -6 (25.30%), -58 (15.70%), -18 (9.60%) and -53 (9.60%). Multiple infections were more common than single ones, and HR-HPV genotypes were present in 69.80% of positive samples. Updating data concerning the distribution of genotypes that infect the anal mucosa may contribute to the implementation of strategies focused on reducing the rate of anal cancer morbidity and mortality.

1. Introduction

Anal cancer is a relatively rare disease, certainly much less common than other digestive carcinomas, such as those of the colon or rectum. The incidence of this carcinoma has been increasing in recent years in the western world (Chiao et al., 2005; Sunesen et al., 2010; van der Zee et al., 2013). In the general population, the rates of anal cancer range from

*Corresponding author: Dr. Hernan A Vargas
Tel: 3649662
E-mail address: havargas@saludcapital.gov.co

0.7 per 100,000/year in the United Kingdom to 1.7 per 100,000/year in the United States (Palefsky et al., 2014; van der Zee et al., 2013). However, such prevalence can reach values above 35/100,000 in high-risk populations such as men who have sex with men and immunosuppressed HIV-positive and transplanted people (Darragh & Winkler, 2011).

Like cervical cancer, persistent infection with high-risk human papillomavirus (HR-HPV) genotypes has been strongly associated with the development of anal cancer and its precursor lesions (Chiao et al., 2005; Moscicki et al., 2015; Poggio, 2011). More than 90% of anal cancers are linked to anal infection with a variety of oncogenic types of HPV (Darragh et al., 2012; Darragh & Winkler, 2011) being HPV 16 the most frequently associated with the development of anal lesions, followed by HPV 18 (Darragh & Winkler, 2011).

Human papillomavirus is one of the most common sexually transmitted viruses (Faridi et al., 2011). It penetrates the epithelium through the microtraumas suffered during anal intercourse. Once HPV reaches the basal and parabasal epithelium, it enters into the cells and, if conditions become favorable, it initiates replication by altering the cell cycle program. This process contributes to cell immortalization and the subsequent initiation of anal carcinogenesis (Pineda & Welton, 2009).

The average age at anal cancer diagnosis is between 60 and 70 years (Darragh & Winkler, 2011; Disease, 2015). Similar to cervical cancer, the development of anal disease takes between one and three decades (Moscicki et al., 2015). Although anal cytology and high-resolution anoscopy have been used for early diagnosis of anal premalignant lesions in high-risk populations, at present there are no screening protocols for anal carcinoma diagnosis in the general population (Darragh & Winkler, 2011).

Anal cytology was first introduced in 2005 by the College of American Pathologists in an interlaboratory program of non-gynecologic cytology (Darragh et al., 2012). This test is a simple and cost-effective analysis to screen for at-risk populations in order to decrease the incidence of anal cancer (Original, 2006). Near 50% of anal cancers are diagnosed at late stages, reducing the opportunity for identifying people at risk of developing anal neoplasia (Moscicki et al., 2015). Thus far, the impact of anal squamous intraepithelial lesion (ASIL) screening and treatment has not been documented (Darragh & Winkler, 2011). Considering that this carcinoma can be potentially controlled by screening tests (Moscicki et al., 2015) assessment of the best approach for the prevention, diagnosis and treatment of anal neoplasia would help decrease disease prevalence.

Today, vaccination has substantially reduced the burden of infectious diseases. In this sense, complete prevention of persistent infection by certain virus genotypes has been demonstrated for HPV vaccine (Andre et al., 2008). Vaccination protocols against HPV infection were firstly introduced in immunization programs as a prevention strategy for cervical cancer and its precursor lesions. Guidelines for the use of prophylactic HPV vaccine were firstly introduced by the American Cancer Society (Saslow et al., 2007). Recent evidence has shown that quadrivalent HPV vaccination significantly reduced the risk of recurrent high-grade SIL (HSIL) and anal cancer in young men, heterosexual men and men who have sex with men (Deshmukh et al., 2015; Shum et al., 2015) providing an interesting opportunity for the development of prevention programs.

The spectrum of ASIL includes a range of stages (from mild to severe squamous dysplasia and anal cancer) (Pineda & Welton, 2009) and clinical manifestations (from anal warts to intraepithelial lesions) (Darragh & Winkler, 2011). However, symptoms are unspecific and could include bleeding, itching, pain and tenesmus. Often, asymptomatic patients are diagnosed during the treatment of another anorectal disease (Pineda & Welton, 2009; Poggio, 2011).

Although most infections are satisfactorily resolved, certain risk factors favor virus persistence in the anal epithelium, contributing to the development of precursor lesions and anal cancer (Sunesen et al., 2010). Risk factors for anal HPV acquisition include anal intercourse, number of sexual partners, presence of anal fissures, immunosuppresion and infection with HPV in the cervix, among others (Moscicki et al., 2015; Poggio, 2011; van der Zee et al., 2013). Tobacco smoking has been reported as a risk factor for the development of anal cancer (Madeleine, Finch, & Lynch, 2013; van der Zee, et al., 2013), and cervical HPV infection has also been considered in several studies, since the

cervix could act as a reservoir for the virus to infect the anus or *vice-versa* (Moscicki et al., 2015). Moreover, it has been reported that women have twice the risk of anal HPV infection than men (Disease, 2015).

Currently, little data reflect the epidemiological profile of anal HPV infection in sexually active women with cervical disease. The objective of the present study was to investigate the distribution of HPV genotypes and abnormal anal cytology in sexually active women with cervical disease from Bogota, Colombia.

2. Materials and Methods

Anal samples from 134 women (age range, 18-83 years) with cervical disease from the city of Bogota, Colombia, undergoing routine anal cytology were randomly collected between May and November 2015. All patients agreed to voluntarily participate in the study through an informed consent. Women with HIV were excluded. This study was approved by the ethics committee of the institution Patolab Rx Ltd, Bogotá, Colombia. This work was supported by The District Department of Health and the research group from the Laboratory of Public Health, Bogotá, Colombia.

2.1. Anal Sample Collection

Samples were collected by inserting a cytobrush 3-4 cm into the anal canal with circular movements, to obtain the greatest number of cells. Samples were preserved in Cobas® PCR Cell Collection Media (Roche, Indianapolis, Indiana, USA) and sent to the Public Health Laboratory of the Bogotá Department of Health, Colombia.

2.2. Cytological Analysis

Samples for anal cytology were collected using a cytobrush and placed in BD SurePath™ liquid-based Pap test (Becton Dickinson, NJ, USA), a thin-layer cell preparation approved by the FDA. Glass slides were stained and mounted. Then, they were examined by two experienced cytopathologists following the Bethesda system criteria for the evaluation of cervical cytological results (Solomon, 2002) and the recommendations proposed by Darragh et *al*. (Darragh et al., 2012).

2.3. HPV Detection and Typing

Nucleic acid extraction was performed using the QIAamp Media MDx Kit (Qiagen, Hilden Geschäftsführer, Germany). Genotyping of HPV was performed using the Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, Indiana, USA) according to the manufacturer´s instructions and the method described by Rouleau and coworkers (Rouleau et al., 2006). This technique allows the detection of 37 HPV genotypes, 14 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and 23 low-risk (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108), with two levels of β-globin gene controls in order to validate the technical procedure.

2.4. Statistical Analysis

The frequency distribution of viral genotypes, anal cytology and graphics were performed using an Excel® database. Data were also processed using the IBM SPSS™ Statistics 21. Possible associations between variables were analyzed using Fisher's exact test and significant associations were defined by a *p* value below 0.05 in all cases.

3. RESULTS

We collected 134 anal samples to detect anal lesions and type-specific HPV infection. The mean age of participants was 38.3 years (range, 18–83 years), and the majority of them (77%) were between 25 and 54 years. Age at first intercourse was mainly distributed between 15-20 years (58%). Around 41% of participants declared to have had a sexually transmitted disease (STD) before recruitment to this study.

The analysis of cell smears showed that 3.59% of samples had inadequate material or missing data and were therefore excluded from the study. Among the suitable samples, 93.28% showed normal cytology, while abnormal samples (6.7%) were classified as atypical squamous cells of undetermined significance (ASC-US, 5.2%), high-grade anal intraepithelial neoplasia (LGAIN-II/AIN-III, 0.75%) and low-grade anal intraepithelial neoplasia (HGAIN-I, 0.75%).

Overall, HPV DNA was identified in 61.5% of the studied anal samples, 69.80% of which

corresponded to HR-HPV infections. Multiple HPV infections were more prevalent than single infections, appearing in 51.81% of the analyzed samples and being more common in women between 31 and 40 years old. Figure 1 shows the distribution of multiple infections in the different age groups. The number of HPV types infecting the same anal sample ranged from two (53.25%) up to eight different viral genotypes (1.30%).

Table I shows HPV prevalence by age. The highest positivity for HPV infection was observed in the 36-40 year age group (24.10%) followed by people aged 31-35 years (18.10%). Figure 2 shows HPV prevalence sorted by genotype in the studied population. Briefly, the most common HPV types and frequencies were HPV-16 (27.70%), -6 (25.30%), -58 (15.70%), -18 (9.60%) and -53 (9.60%). In fact, HPV-6 and -16 were responsible for more than half of the anal viral infections (53.00%). As a highlight, HPV-16 was more frequent in people between the ages of 31 and 40 years (60.80%), whereas HPV-58 showed the highest prevalence in people between 26 and 30 of age (38.50%).

Table 2. Anal HPV prevalence according to age groups

Age Groups (Years)	HPV Positivity	
	n	%
20-25	9	10,8
26-30	14	16,9
31-35	15	18,1
36-40	20	24,1
41-45	8	9,6
46-50	4	4,8
≥ 51	13	15,7
TOTAL	83	100,0

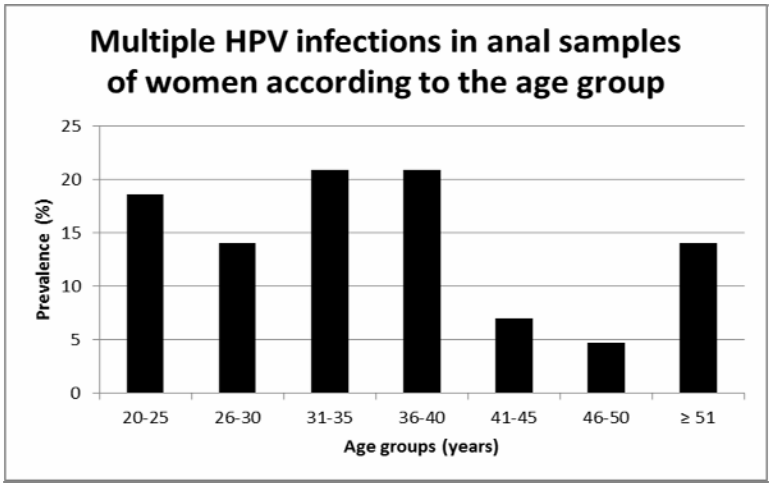

Figure 1. Prevalence of multiple infections in the different age groups

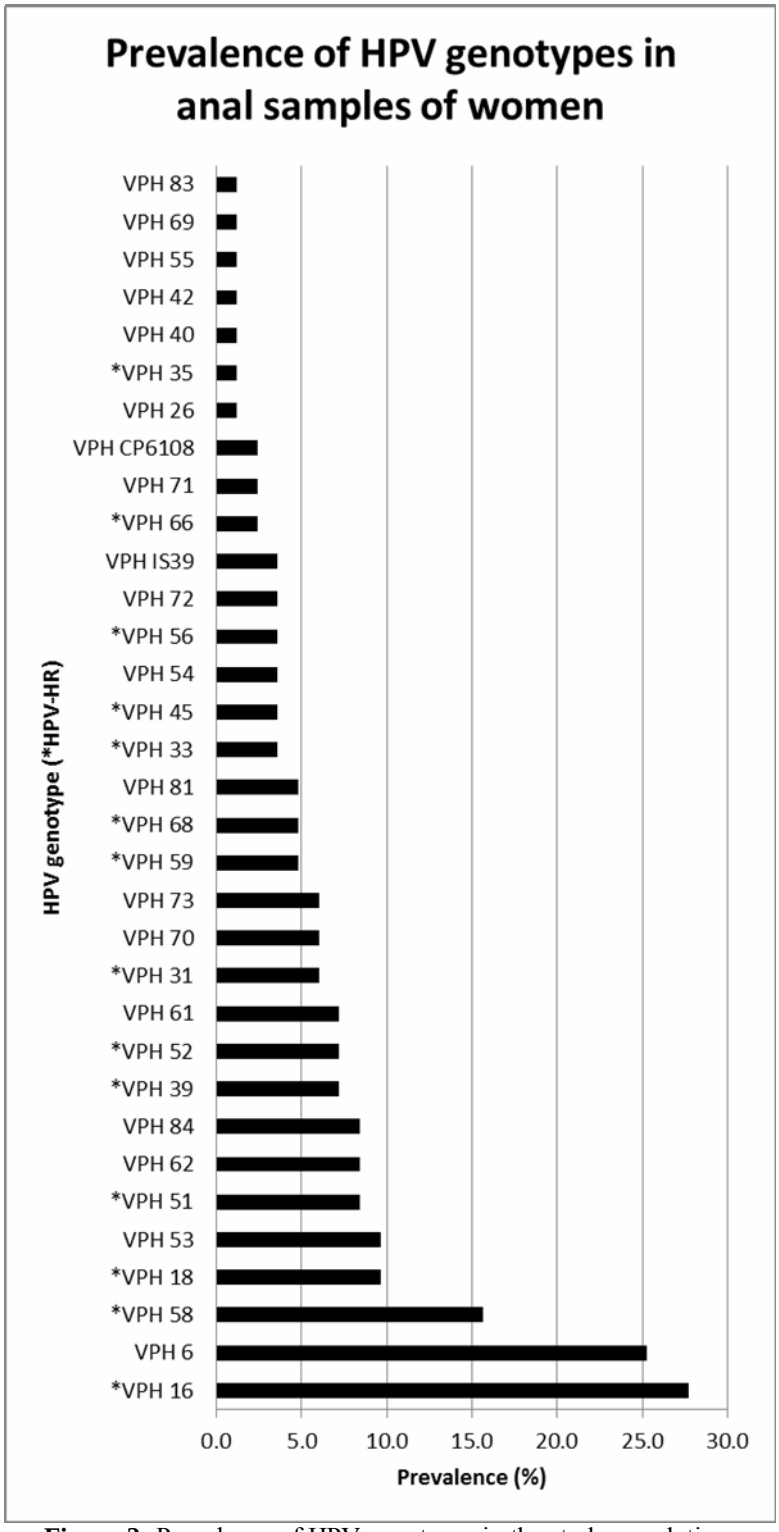

Figure 2: Prevalence of HPV genotypes in the study population.

4. Discussion

This is the first study determining the rate of HPV infection and abnormal cytology in anal samples from women with cervical disease in the city of Bogota, Colombia. Our results showed a low prevalence of abnormal anal cytology (6.7%) but an overall high prevalence of HPV infection (61.94%). Moreover, multiple HPV infections were more frequent than single

ones, and up to eight different viral types were detected in the same anal sample. The infecting viral types HPV-16 and -6 were responsible for more than 50% of HPV infections.

Despite cytology is useful for detecting high-grade anal lesions, the sensitivity of the method is quite low to detect low-grade lesions (Correlation, 2013). In fact, these authors reported 70% sensitivity and 93% specificity in detecting intraepithelial lesions (Correlation, 2013). High-resolution anoscopy and biopsy diagnosis are nowadays considered the Gold Standard for anal cancer detection. However, their efficacy is based on the sampling procedure and the expertise of the pathologist to identify intraepithelial lesions (Darragh & Winkler, 2011). In this work, only 6.7% of the samples analyzed showed abnormal results, a quite low prevalence compared with other studies. For instance, a recent study conducted by Robison et *al.* (Robison et al., 2016) in the United States analyzing high-risk (with cervical lesions) and low-risk (with normal cervical cytology) women found 41.20 and 21.70% of abnormal anal cytology, respectively, being the latter value almost three times higher than that obtained in our work. In the same line, another case-control study performed in Brazil, found that women with cervical lesions have twice the risk of having abnormal anal cytology("Liquid based cytology and HPV DNA testing using intraanal specimens from HIV□negative women with and without genital HPVinduced lesions.pdf," n.d.). Again, the prevalence of abnormal anal results in low-risk women reported in that work was around 18%, almost three times higher than that obtained in our study. Evidently, the prevalence of abnormal anal cytology varies geographically and is tightly associated with the presence of cervical lesions. Unfortunately, such information was not available for women of our study with normal cervical samples.

In this work, the prevalence of anal HPV infection was over 60%, with approximately 75% corresponding to HR-HPV genotypes. In plain numbers, over 43% of the recruited patients showed HR-HPV types in their anal samples. This value is extremely high when compared with that obtained by other authors studying similar patient cohorts. In this sense, a systematic review performed by Stier and collaborators(Stier et al., 2015) showed that HPV prevalence in anal mucosa from HIV-negative women with signs of cervical disease ranged from 23 to 36%, lower than that reported in the present work. Moreover, Hernandez et *al.*(Hernandez et al., 2015) found that age younger than 30 years increased the risk for anal HPV infection, while in the current work the highest HPV prevalence was found in women aged 36-40 years. This situation could be explained by differences in sexual behavior or age at first intercourse.

In the study performed by Schelcht et *al.* (Schlecht et al., 2012) in a group of young women of non-Caucasian and/or Hispanic ethnicity (mean age 18 years), the prevalence of anal HPV reached 42%, a quite high value considering that these women did not present cervical disease. This could be reflecting differences in anal HPV prevalence mostly associated with sexual behavior and/or transient HPV infection mainly seen in young girls.

The presence of multiple HPV types in the anal mucosa constitutes a risk factor for disease development and progression (Poggio, 2011). In our study, the majority of the analyzed patients presented multiple HPV infections (from two up to eight different HPV types in the same anal sample), suggesting that the women belonged to an at-risk population for anal disease development. However, cohort studies performed by Goodman et *al.* (Goodman et al., 2008) and Shvetsov et *al.*(Shvetsov et al., 2009) found that anal HPV infection was quite transient, with a median time for viral clearance of 5 months against 6-18 months reported for cervical HPV infection (Stanley, 2009). Therefore, the presence of multiple HPV-DNA types in the anal mucosa could only be the reflection of multiple transient HPV infections due to sexual activity.

Approximately 90% of anal carcinomas are caused by several HPV genotypes. In this sense, HPV-16 has been the most frequent viral type involved in anal cancer, being present in more than 75% of anal carcinomas (Moscicki et al., 2015; Poggio, 2011). In our study, HPV-16 was the most prevalent HPV type (26.40%). Contrarily, a study performed by Valari and coworkers (Valari, Koliopoulos, Karakitsos, Valasoulis, & Founta, 2011) showed that HPV-6 and HPV-18 were the most common HPV types in the anal mucosa. Our data partially agree with those results, since HPV-6 was second in prevalence. In a the study performed by Castro

and coworkers (Castro et al., 2017) analyzing sexually active women between 22 and 29 years from Costa Rica, the prevalence of anal HPV was 31.60%, similar to the one obtained in the present study for practically the same age group (28.40%). However, they found only 33% HR-HPV types, with an HPV-16 prevalence of only 3%. These results contrast with the 43.28% HR-HPV infection and the 27.70% HPV-16 prevalence reported here, further demonstrating that anal viral dynamics is quite different in different populations.

Considering the high frequency of anal HPV reported in the present work, anal cytology and HPV-16 screening would be an effective strategy to detect low grade ASIL in women with a history of cervical disease, since they are at high risk of vaginal, vulvar and anal cancer(Madeleine et al., 2013) As in the cervix, screening for anal disease could be done through the HPV test, leaving anal cytology for patients positive for the molecular test.

Acknowledgement

The authors are grateful to the professionals from the District Department of Health, and the research group from the Laboratory of Public Health in Bogota. Thanks are also due to A. Di Maggio for manuscript correction and edition.

Refereces

Andre, F.E., Booy, R., Bock, H.L., Clemens, J., Datta, S.K., John, T.J., Ruff, T.A. (2008). Policy and practice Vaccination greatly reduces disease, disability, death and inequity worldwide. 86: 140–146.

Castro, F.A., Quint, W., Gonzalez, P., Katki, H. A., Herrero, R., Doorn, L. Van, … Kreimer, A. R. (2017). Prevalence of and Risk Factors for Anal Human Papillomavirus Infection Among Young Healthy Women in Costa Rica. 206: 1103–1110.

Chiao, E. Y., Krown, S. E., Stier, E. A., & Schrag, D. (2005). Epidemiology And S Ocial S Cience A Population-Based Analysis of Temporal Trends in the Incidence of Squamous Anal Canal Cancer in Relation to the HIV Epidemic, 40(4): 451–455.

Correlation, C. (2013). A Six-Year Experience With Anal Cytology in Women With HPV in the Lower Genital Tract□. 42(5): 396–400.

Darragh, T.M., Colgan, T.J., Cox, J.T., Heller, D.S., Henry, M.R., Ronald, D., Winkler, B. (2012). The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions□: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. Arch Pathol Lab Med. 136: 1266-1297.

Darragh, T.M., & Winkler, B. (2011). Anal Cancer and Cervical Cancer Screening□: Key Differences. 1: 5–19.

Deshmukh, A.A., Chhatwal, J., Chiao, E.Y., Nyitray, A.G., Das, P., & Cantor, S.B. (2015). Long-Term Outcomes of Adding HPV Vaccine to the Anal Intraepithelial Neoplasia Treatment Regimen in HIV-Positive Men Who Have Sex With Men. 61: 1527–1535.

Faridi, R., Zahra, A., Khan, K., & Idrees, M. (2011). Oncogenic potential of human papillomavirus (HPV) and its relation with cervical cancer. Virology Journal, 8: 269-277.

Goodman, M.T., Shvetsov, Y.B., Mcduffie, K., Wilkens, L.R., Zhu, X., Ning, L., Hernandez, B.Y. (2008). Acquisition of Anal Human Papillomavirus (HPV) Infection in Women□: the Hawaii HPV Cohort Study. 197: 957-966.

Hernandez, B.Y., Scanlan, L., Ah, J., Kamemoto, L.E., Thompson, P.J., Zhu, X., Pago, P. (2015). Cervical and anal human papillomavirus infection in adult women in American Samoa. Asia Pacific journal of public health. 25: 19-31.

Madeleine, M. M., Finch, J. L., & Lynch, C. F. (2013). HPV□related cancers after solid organ transplantation in the United States. American Journal of Transplantation, 13: 3202-09.

Moscicki, A., Darragh, T.M., Berry-lawhorn, J. M., Roberts, J.M., Khan, M.J., Boardman, L.A., Palefsky, J.M. (2015). Screening for Anal Cancer in Women. 19(3): 27–42.

Pineda, C.E., & Welton, M.L. (2009). Management of anal squamous intraepithelial lesions. Clinics in colon and rectal surgery. 22: 94-101.

Poggio, J.L. (2011). Premalignant lesions of the anal canal and squamous cell carcinoma of the anal canal. Clinics in colon and rectal surgery. 24: 177-192.

Robison, K., Cronin, B., Bregar, A., Luis, C., Schechter, S., Pisharodi, L., Clark, M. (2016). Anal cytology and human papillomavirus genotyping in women with a history of lower genital tract neoplasia compared with low-risk women. Obstetrics and gynecology. 126: 1294-1300.

Rouleau, D., Petignat, P., Ghattas, G., Kornegay, J. R., Schlag, P., Boyle, S., Macleod, J. (2006). Enhanced Detection and Typing of Human Papillomavirus (HPV) DNA in Anogenital Samples with PGMY Primers and the Linear Array HPV Genotyping Test. 44(6):1998–2006.

Saslow, D., Castle, P.E., Cox, J. T., Davey, D. D., Einstein, M.H., Ferris, D.G., Partridge, E.E. (2007). American Cancer Society Guideline for Human Papillomavirus (HPV) Vaccine Use to Prevent Cervical Cancer and Its Precursors. 57(1): 7–28.

Schlecht, N.F., Burk, R.D., Nucci-sack, A., Shankar, V., Peake, K., Lorde-rollins, E., Diaz, A. (2012). Cervical , Anal and Oral HPV in an Adolescent Inner-City Health Clinic Providing Free Vaccinations. 7(5): 1–10.

Shum, J., Kelsberg, G., & Safranek, S. (2015). Clinical Inquiry: Does qHPV vaccine prevent anal intraepithelial neoplasia and condylomata in men? The Journal of Family Practice. 64(9): 581–583.

Shvetsov, Y.B., Hernandez, B.Y., Mcduffie, K., Wilkens, L.R., Zhu, X., Ning, L., Goodman, M.T. (2009). Duration and clearance of anal human papillomavirus (HPV) infection among women: the Hawaii HPV cohort study. Clinical infectious diseases. 48: 536-546.

Solomon, D. (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. Jama, 287: 2114-2119.

Stanley, M. A. (2009). Immune responses to human papilloma viruses. Indian J Med Res, 130(3), 266–276.

Stier, E.A., Sebring, M.C., Mendez, A.E., Ba, F. S., Trimble, D.D., & Chiao, E.Y. (2015). Infection and anal HPV-related disorders in women□: a systematic review. The American Journal of Obstetrics & Gynecology. 1: 1-32.

Sunesen, G., Nørgaard, M., Thorlacius-ussing, O., & Laurberg, S. (2010). Immunosuppressive disorders and risk of anal squamous cell carcinoma: a nationwide cohort study in Denmark, 1978–2005. International Journal of Cancer. 127: 675-684.

Valari, O., Koliopoulos, G., Karakitsos, P., Valasoulis, G., & Founta, C. (2011). Gynecologic Oncology Human papillomavirus DNA and mRNA positivity of the anal canal in women with lower genital tract HPV lesions□: Predictors and clinical implications. Gynecologic Oncology, 122(3), 505–508.

van der Zee, R.P., Richel, O., De Vries, H.J., & Prins, J.M. (2013). The increasing incidence of anal cancer: can it be explained by trends in risk groups. Neth J Med. 71(8): 401–411.