REVIEW ARTICLE
Iran J Allergy Asthma Immunol
October 2024; 23(5):467-475.
DOI: 10.18502/ijaai.v23i5.16755

Research Progress of Immune Mechanisms Related to Persistent HPV Infection in CIN after Cervical Conization

Jingyi Yang and Fengxia Xue

Department of Gynecology and Obstetrics, Tianjin Medical University General Hospital, Tianjin, China

Received: 10 March 2024; Received in revised form: 20 May 2024; Accepted: 9 June 2024

ABSTRACT

Persistent human papillomavirus (HPV) infection is associated with the grading of cervical intraepithelial neoplasia (CIN), high-risk HPV infection, multiple HPV infections, high HPV load, HPV infection of surgical margin, and age in CIN after conization. The immune mechanism is complex and is primarily related to vaginal microecology disorders, immune escape, immune response impairment, and the release of regulatory cytokines. Currently, the treatment methods for postoperative persistent HPV infection include surgical treatment, antiviral treatment, vaccination, and other approaches.

Keywords: Cervical intraepithelial neoplasia; Cervical conization; Immune mechanisms; Persistent human papillomavirus infection; Research progress

INTRODUCTION

Cervical Intraepithelial Neoplasia (CIN) is an atypical hyperplasia of cells in the cervical epithelium and is regarded as a precursor lesion of cervical cancer. CIN is classified into mild, moderate, and severe categories, with severe CIN (i.e., CIN3) considered the direct precursor of cervical cancer. Cervical smears (Pap smears) are a major diagnostic tool for CIN, detecting changes in cervical cells to assess the presence and severity of CIN. With the increasing attention to women's health, the screening and treatment of CIN have garnered more focus.[1] Persistent human papillomavirus (HPV) infection is widely recognized as a clinical risk factor for cervical lesions. Approximately 95% of cervical cancer patients test positive for HPV infection, with the development of CIN closely related to HPV infection, especially high-risk types such as HPV 16 and 18. Additionally, genetic factors and long-term chronic cervical inflammation may also contribute to the development of CIN. Analyzing HPV infection provides a valuable reference for clinical screening, diagnosis, and treatment of cervical diseases.[2,3] Research indicates that CIN development is associated with HPV infection, and its treatment and prognosis are closely related to HPV infections.[4] Recent research findings largely support established guidelines for managing CIN and HPV infections, aligning with recommendations from the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC). Studies on the effectiveness of HPV vaccination reinforce the importance of routine immunization, while research on HPV testing as a primary screening method

Corresponding Author: Fengxia Xue, MD;
Department of Gynecology and Obstetrics, Tianjin Medical University General Hospital, Tianjin, China. Tel: (+86 015) 9221 5459, E-mail: xuefengxia1962a@163.com

echoes WHO's endorsement of this approach. Additionally, insights into HPV genotyping and biomarkers for risk assessment complement guidelines advocating personalized screening and follow-up strategies. However, debates persist regarding the optimal age for vaccination and screening intervals, challenging existing recommendations. Controversies also arise regarding the management of low-grade CIN lesions, highlighting the need for ongoing guideline refinement to ensure optimal patient care and public health impact. In current medical practice, the treatment of CIN mainly relies on surgical methods, with cervical conization being the most commonly used treatment. This procedure involves removing abnormal cells from the cervix, effectively controlling the development of CIN and greatly reducing the risk of progression to cervical cancer. Cervical conization not only serves as a treatment but also provides pathological samples to help doctors more accurately evaluate the condition and formulate subsequent treatment plans. Therefore, it is regarded as the mainstream procedure for CIN treatment.[5] However, the rate of persistent HPV infection after cervical conization is high, affecting the surgery's effectiveness and significantly increasing the risk of CIN and cervical cancer recurrence. Research shows that 10% to 25% of patients experience recurrence and progression within 2 years after surgery.[6] Imbalances in the vaginal microenvironment may lead to excessive growth of harmful bacteria and inhibition of beneficial microbes, affecting the local immune response. Additionally, HPV has an immune escape mechanism that can interfere with the host's immune system, increasing the persistence of infection. To deepen the understanding of the intricate relationship between HPV and the immune system, it is essential to explore the specific immune escape mechanisms employed by the virus and the components of the immune system involved in the dynamics of HPV infection. HPV is known to employ several strategies to evade the host's immune surveillance, such as downregulating the expression of molecules crucial for immune activation and manipulating the local immune environment to favor viral persistence and lesion development. By elaborating on the cellular and molecular pathways implicated in the immune response to HPV, this discussion aims to provide a comprehensive overview of the immune challenges and considerations that must be addressed in the management and treatment of CIN.

MATERIALS AND METHODS

Literature Search Strategy

This review systematically searched the PubMed, Scopus, and Web of Science databases to collect literature published from January 2000 to December 2023 on the immune mechanisms of persistent HPV infection following cervical conization. The search terms used included "persistent human papillomavirus infection," "cervical intraepithelial neoplasia," "cervical conization," and "immune mechanism." The search was limited to articles published in English. Additionally, manual searches were conducted on the reference lists of the articles to ensure coverage of all relevant studies.

Inclusion and Exclusion Criteria

Inclusion criteria were: (1) studies focused on the immune mechanisms following cervical conization for HPV infection; (2) studies providing data on immune escape, impaired immune response, or the release of regulatory cytokines; (3) types of studies included observational studies, clinical trials, reviews, and meta-analyses. Exclusion criteria were: (1) non-English literature; (2) conference abstracts or articles not peer-reviewed; (3) studies with duplicated publication or overlapping data.

Data Extraction and Analysis

Two researchers independently screened the literature and extracted data, resolving any discrepancies through discussion or third-party review. Extracted information included authors, publication year, study design, sample size, main findings, and immune factors affecting persistent HPV infection. The review employed a narrative analysis approach to summarize the immune mechanisms of persistent HPV infection and discussed current research limitations and future research directions.

Influencing Factors of HPV Persistent Infection After CIN Conization

It has been reported[7] that there is still a risk of recurrence after CIN conization that cannot be ignored, with persistent HPV infection being one of the important reasons leading to recurrence. Analyzing the influencing factors of persistent HPV infection after CIN conization is crucial for controlling infection, reducing recurrence, and ensuring a better prognosis for patients.

Cervical Intraepithelial Neoplasia Classification

CIN refers to the replacement of the cervical epithelium by pleomorphic cells of varying degrees. Pathological changes are divided into CIN 1, CIN 2, and CIN 3. Low-grade squamous intraepithelial lesions (LSIL) usually refer to CIN 1, while CIN 2 and CIN 3 are collectively referred to as high-grade squamous intraepithelial lesions (HSIL). Studies have shown that HSIL can increase the risk of persistent high-risk HPV infection after cervical conization in patients with CIN by 1.793 times. Strengthening clinical intervention for patients with high CIN grade can reduce the risk of persistent HPV infection after CIN conization.[8,9]

High-risk HPV Infection

Different HPV types show varying infection durations and carcinogenic potential, allowing them to be classified into high-risk and low-risk types. It is generally believed that high-risk persistent HPV infection has a more significant impact on HPV-negative conversion and the efficacy of surgery. High-risk subtypes such as HPV 16, 18, 52, and 58 are particularly valuable for diagnosing recurrent lesions.[10] The German S3 guidelines directly recommend high-risk HPV surveillance in the early postoperative period.[11] The ASCCP recommends high-risk HPV reexamination at 1 and 2 years after surgery and HPV testing or combined screening every 3 years for those with histological HSIL (CIN 2, CIN 3), with follow-up for at least 25 years.[12] Koh et al showed that the 2-year CIN recurrence rate was as high as 71.3% in patients with persistent HPV16/18 positivity after surgery, which was significantly higher than in patients who were HPV negative after surgery.[13]

Multiple HPV Infections

The integration of multiple types of HPV into the host DNA will make the virus more difficult to be cleared by the immune system.[14] Ouh et al,[15] reported that the persistent infection rate of HPV in CIN patients with multiple HPV infections before surgery was significantly higher than in CIN patients with a single HPV infection before surgery. However, some researchers believe that[16] there is no obvious correlation between single and multiple HPV infections. Currently, the relationship between single and multiple HPV infections and the postoperative HPV clearance rate in CIN patients is not clear. It is possible that the more types of HPV infections present before surgery, the longer the body's clearance of HPV infection, the longer the body's clearance of HPV infection takes, leading to more severe damage to the body's immunity and a higher persistent infection rate of HPV. However, this hypothesis needs further confirmation.

High HPV Viral Load

HPV viral load directly reflects the number of infected cells and the quantity of viruses within those cells. The higher the viral load, the more active HPV replication is, increasing the probability of HPV DNA integration into host cells. This makes it easier to enter the deeper skin layers that cannot be reached by cervical conization, reducing the conization clearance rate and leading to persistent HPV infection.[17] However, current research on HPV viral load is inconsistent. Some studies suggest that a high HPV viral load significantly influences the postoperative recurrence of CIN and HPV infection, while others argue that there is no correlation between preoperative and postoperative HPV viral load and the clearance rate.[9,17-19] The discrepancies may be more related to individual differences in immune regulation and other complex factors.[20,21]

Positive Surgical Margin Infection

CIN conization can eliminate HPV by excising cervical lesions, but cases with positive conization margins are often reported. Reoperation or conservative observation can be performed based on the actual situation and regular follow-up is necessary. Data showed that in 60% to 80% of patients with positive margins after cervical CIN conization, the lesion can regress.[22] However, for patients with a positive medial margin, the risk of residual lesions is significantly higher than that of patients with a positive outer cervical margin, and conization should be repeated to obtain a clear margin. Regardless of which margin has a positive HPV infection, there is a risk that the lesion tissue has not been completely removed, leading to persistent infection after CIN conization. Therefore, scholars suggest that patients with positive HPV infection at the surgical margin after CIN conization should undergo elective secondary surgery or regular follow-up according to fertility requirements and pathological characteristics to reduce the risk of persistent HPV infection and promote HPV-negative conversion.[23]

Age

Ninety percent of patients with HPV infection can clear the virus naturally within 2 years, but some patients

still develop cervical cancer and precancerous lesions. This natural negative conversion rate is related to the age of the infected person, with infection symptoms becoming more severe as age increases.[24] A survey based on gynecological clinics[25] showed that the perimenopausal period and after is the peak period of women's HPV exposure, and patients infected with HPV during this period find it difficult to clear the virus on their own, causing persistent HPV infection. This property is related to the decrease of estrogen secretion and the decrease of vaginal resistance with age.[26]

In conclusion, to control the risk of HPV infection after CIN conization, it is necessary to improve the preoperative and postoperative examinations, closely monitor changes in HPV infection risk factors, and actively implement intervention measures.

Immune Mechanisms of Persistent HPV Infection after CIN Conization

At present, the understanding of the interaction between autoimmunity and HPV infection, as well as the immune mechanism of persistent HPV infection after surgery, remains to be improved.

Local Immune Environment of the Genital Tract in HPV Infection

The vaginal microecosystem is a complex system composed of microbial flora, endocrine regulation, and the local immune system. It serves as a woman's local immune defense barrier, playing a role in immune regulation to remove HPV and protect vaginal epithelial cells and local vaginal tissue. When the vaginal microecological balance is disrupted by endogenous or exogenous factors, it leads to impaired immune function and an increased risk of viral infection.[27-29] In recent years, the study found that vaginal microecology and the actual status of the cervical local immune system influence the HPV infection process. By simultaneously treating CIN patients with vaginal microbial agents during surgical treatment, the subsequent efficiency of HPV virus removal can be accelerated.[30,31] Specific immune responses produced by cervical epithelial cells cooperate with conventional immune cells to protect the female reproductive tract from bacterial infiltration. Cervicovaginal mucus, a mixture of cervical mucus and vaginal microbiota, contains many proteins associated with immune defense. The immunoglobulin A (IgA), IgM, and IgG in the mucus strengthen the cervicovaginal mucus barrier. The formation of physical and immune barriers through the cervical epithelium and mucus is one of the ways to maintain the immune function of the female lower reproductive tract.[32,33] When HPV infection occurs, the secretion of various cytokines such as IL-10 and Transformational growth factor-β (TGF-β) in the genital tract increases to resist infection.[34]

Studies have shown that there are four types of antigen-presenting cells in the lower genital tract: intraepithelial Langerhans cells, $CD14^-$ dendritic cells in the lamina propria, $CD14^+$ dendritic cells and macrophages, and $CD8^+$ lymphocyte subsets in the lower genital tract. The cervical transformation zone is the primary effector site for cell-mediated immunity in the female lower genital tract. However, when the cervix is infected by foreign pathogens, epithelial cells mediate an immune inflammatory response by releasing a large number of pro-inflammatory factors and chemokines, such as IL-8 and IL-10.[35,36] Studies have shown that Human defensin-5 (HD-5) is an effective antagonist in skin and mucosa to resist HPV infection, which has an effect by interfering with HPV entry into cells.[37] Hu Rui's study[38] showed that the levels of interferon-γ (INF-γ) and $CD8^+$ T-cell percentage in the vagina decreased and the levels of IL-2 and $CD4^+$ T-cell percentage in peripheral blood increased in patients with HSIL after surgery. It was believed that the changes in these immune factors were related to the prognosis of HPV after surgery. The levels of IL-4, IL-6, and IL-10 in vaginal and peripheral blood were not associated with HPV-negative conversion.

Cellular and Humoral Immunity

Cellular immunity and humoral immunity are important components of the human immune defense system and are involved in the process of HPV infection. When HPV infects the damaged skin or epithelium and reaches the basal layer, it activates the immune response of the body, producing corresponding antibodies to clear HPV and reduce the viral load.[39] T cells are the most important group in cellular immune function, while immunoglobulins (Ig) play key roles in humoral immune function. When both cellular and humoral immunity are inhibited, the risk of persistent HPV infection increases. Zhang Feifei et al,[40] showed that the levels of peripheral blood T lymphocyte subsets and immunoglobulins (Ig) in CIN patients with persistent HPV infection after surgery were significantly lower than in those with HPV-negative status after surgery. This indicates that the immune function of patients with persistent HPV

infection post-surgery is impaired, possibly due to the consumption of antibodies and antigens by preoperative HPV infection. Patients' immunity may be insufficient to clear HPV for various reasons, such as HPV at this time, through the integration to the host genes, the *E6*, *E7* protein synthesis, HPV persistent infection induced by immune escape mechanism, and to further reduce the immune function.[41]

Immune Escape Mechanisms of HPV

The immune escape mechanism of HPV is complex, involving protein transcription, antigen presentation, cytokines, adhesion molecules, and a series of signaling pathway regulations. After HPV infection, the virus can synthesize particulate molecules in epithelial cells, preventing cytolytic enzymes from dissolving them, reducing the body's immune response, and leading to persistent infection.[42] In addition, HPV viral genes can integrate into host genes, leading to the synthesis of *L1*, *E6*, and *E7* proteins, which make it easier for HPV to evade immune system surveillance, consequently, low levels of HPV to coexist with cellular immunity, which is one of the immune mechanisms underlying HPV persistent infection.[43,44] As mediators of the immune system, cytokines such as interleukin (IL)-2 and IL-10 are secreted after HPV infection, which participates in the regulation of the body's immune function and play a role in both cellular and humoral immunity. The levels of related cytokines can also reflect the systemic and local immune status. Persistent HPV infection after CIN conization can affect changes in host gene expression, and IL-2 is involved in continuous immune regulation and the inflammatory response throughout the process, promoting HPV clearance.[45] Previous studies have shown[46] that IL-2 levels in patients with high-risk HPV infection are lower than that in patients without high-risk HPV infection. It is suggested that high-risk HPV infection may inhibit the immune function of the body and lead to the decrease of IL-2 level, which is not conducive to the clearance of HPV. A decreased cellular immune response is associated with persistent HPV infection. After a high-risk HPV infection, the body can clear the virus through physical barriers and immune response, but HPV *E6* and *E7* proteins can regulate the host's immune response, causing an imbalance in Th1/Th2 responses. This helps the virus escape the initial immune recognition and interferes with adaptive immunity, thereby achieving the immune escape and causing HPV infection.[47] Melamed et al,[48] found that IL-10 can help clear HPV through cellular immunity. However, IL-10 can also lead to immune escape by producing an immunosuppressive state. Research on HPV-infected vaginal local IL-10 levels shows that continuous HPV infection impairs the Th1 immune response, shifting the Th1/Th2 balance towards Th2. This shift leads to the formation of an immunosuppressive state and the inhibition of cytokines such as IL-4 and IL-10, weakening the body's ability to clear HPV.[49] Relevant literature shows that IL-10 can promote the expression of HPV oncogenes *E6* and *E7*, and *E6* and *E7*-related proteins can regulate the transcription of the gene encoding IL-10. This vicious cycle keeps the cervical microenvironment in a state of immunosuppression, significantly reducing the efficiency of HPV clearance, and leading to persistent HPV infection after CIN conization.[50] Other studies have shown that HPV can inhibit the *TP53* tumor suppressor gene, weakening its normal physiological function, and inducing the progression of CIN. In addition, HPV affects the proliferation and differentiation of normal cervical cells and postoperative recovery of patients by stimulating related genes to produce oncoproteins.[51]

Patients with Immune System Diseases and HPV Infection

Systemic lupus erythematosus (SLE) is an autoimmune disease. Patients with SLE have a higher susceptibility to HPV and are more prone to HPV infections, particularly certain subtypes. The risk of HSIL is also significantly increased in these patients. The European League Against Rheumatic Diseases recommends that SLE patients reduce the risk of HPV infection by vaccination.[28]

Treatment of Persistent HPV Infection After CIN Conization

At present, there is no specific drug for the treatment of HPV infection after CIN conization. In most cases, close follow-up observation is recommended to confirm the disease progression.

Surgery

For patients with a pathological diagnosis of LSIL or less severe lesions, if there is no diagnostic error upon re-examination, a follow-up observation period of 6 to 12 months can be performed, and diagnostic conization can be considered appropriate. For patients over 50 years old

with a positive surgical margin infection or postoperative follow-up pathological diagnosis of residual HSIL or recurrence, resectable surgery such as cervical conization is recommended. If section pathology indicates HSIL lesions but secondary cervical resection can not be implemented in patients with residual disease, total hysterectomy can be adopted as a treatment.[52]

Antiviral Treatment

The main active components of the anti-HPV protein biological dressing are JB protein and carbomer. JB protein can destroy the conformation of HPV proteins, causing their inactivation and preventing the virus from infecting host cells. Carbomers can adsorb inactivated HPV side by side in vitro and also release JB protein to the target area for adsorption. The anti-HPV biological protein dressing can physically block HPV from contacting cervical basal cells, achieving the purpose of eliminating HPV, promoting cervical wound repair and healing, and improving the cervix's ability to resist virus invasion. However, due to its high price, it cannot become a priority treatment for most patients.[53]

Recombinant human interferon α-2b has antiviral and immunomodulatory effects. By directly acting on cervical lesions, it binds to cell surface receptors and induces cells to produce antiviral proteins. At the same time, it inhibits HPV replication, transcription, and subsequent protein synthesis at the site, regulates immune function, promotes the release of lymphocytes and macrophages, and reduces the risk of postoperative wound infection. Shi et al,[54] used high- and low-dose interferon combined with red light to treat HPV infection, and found that high-dose interferon had significant therapeutic effects and high safety. Nikakhtar et al's double-blind randomized study found that the active ingredients of myrtle suppository have antiviral properties, induced resistance, and promote the effect of apoptosis, which can accelerate the virus removal.[55]

Vaccine

The HPV vaccine activates the immune system to produce antibodies to neutralize and eliminate invading HPV.[56] Therapeutic vaccines mainly exert their efficacy by enhancing the effect of cytotoxic T cells. *E6* and *E7* genes in HPV DNA can encode oncogenic proteins, so *E6* and *E7* are often used as antigens of therapeutic vaccines. To expand the therapeutic scope of vaccines, some researchers have included conserved proteins of E1 and E2 in the antigen category of vaccines.[57]

The use of immune adjuvants can enhance the immune response of therapeutic vaccines. Talebi et al used HMGB1 and Hp91 proteins as immune adjuvants to stimulate the body's immunity.[58] De Rosa et al used echinacea extract as an immune modulator to treat genital condyloma acuminatum and found that the recurrence rate of HPV infection in patients was significantly reduced.[59]

Summary and Prospect

The article underscores the significance of identifying and managing risk factors associated with persistent HPV infection following cervical conization, aiming to enhance clinical control and minimize recurrence rates. It highlights HPV's impact on immune function and its mechanisms for immune evasion, leading to persistent infection. Understanding these postoperative immune mechanisms is pivotal for targeted treatment approaches. Immunotherapy emerges as a promising avenue for intervention, offering the potential to clear HPV in affected individuals. Although this review already encompasses a range of studies on the immune mechanisms of persistent HPV infection following cervical conization, a comprehensive examination of the contradictions and variations found in the current literature would further enhance the depth and value of this text. For instance, some studies suggest that persistent infection with high-risk HPV types is associated with general suppression of the host immune system, while others have found that specific regulatory cytokines such as IL-10 and TGF-β may significantly influence the persistence of infection due to their varied expression across individuals. These discrepancies highlight that a single treatment strategy may not suffice for all patient groups, underscoring the need for customized treatment approaches. By discussing these conflicting findings in detail, this review could not only provide more precise guidance for clinical practice but also expose the current gaps in understanding and potential directions for future research. Adding a discussion of these research differences would help the article more comprehensively explain the immune mechanisms of persistent HPV infection and provide a theoretical basis for resolving these disputes. This approach not only enhances the breadth of the literature review but also improves the practicality and scientific rigor of the research, making it a valuable reference for future studies in the field. To effectively translate these

findings into clinical practice, specific recommendations are imperative. Firstly, implementing stringent screening protocols post-conization, integrating HPV testing alongside Pap smears, can aid in early detection. Secondly, adopting personalized treatment strategies based on individual immune responses and HPV genotypes can optimize outcomes. Thirdly, the integration of immunotherapy warrants consideration, given its potential to target HPV-related immune evasion mechanisms. Multidisciplinary collaboration among specialists, along with long-term follow-up plans, is crucial for comprehensive patient care and monitoring. Moreover, educating both healthcare providers and patients about HPV risks and treatment options is essential for informed decision-making and adherence to recommended protocols. Incorporating these recommendations into clinical practice can effectively manage persistent HPV infection post-cervical conization, ultimately improving patient outcomes.

STATEMENT OF ETHICS

Not applicable.

FUNDING

This study did not receive any funding in any form.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

1. Sand FL, Frederiksen K, Kjaer SK. Risk of recurrent disease following conization of cervical intraepithelial neoplasia grade 3 according to post-conization HPV status and surgical margins. Gynecol Oncol. 2022;165(3):472-7.
2. Haring CT, Bhambhani C, Brummel C, Jewell B, Bellile E, Heft Neal ME, et al. Human papilloma virus circulating tumor DNA assay predicts treatment response in recurrent/metastatic head and neck squamous cell carcinoma. Oncotarget. 2021;12(13):1214-29.
3. Penberthy L, Rivera DR, Ward K; The Contribution of Cancer Surveillance Toward Real World Evidence in Oncology. Semin Radiat Oncol. 2019;29(4):318-22.
4. Salvadó A, Miralpeix E, Solé-Sedeno JM, Kanjou N, Lloveras B, Duran X, Mancebo G. Predictor factors for conservative management of cervical intraepithelial neoplasia grade 2: Cytology and HPV genotyping. Gynecol Oncol. 2021;162(3):569-74.
5. Ansari A, Urooj U. Study of causes behind abnormal uterine bleeding according to PALM- COEIN classification at a tertiary care hospital. J Pak Med Assoc. 2020;70(1):154-57.
6. Garland SM, Paavonen J, Jaisamrarn U, Naud P, Salmerón J, Chow SN, et al. Prior human papillomavirus-16/18 AS04-adjuvanted vaccination prevents recurrent high grade cervical intraepithelial neoplasia after definitive surgical therapy: Post-hoc analysis from a randomized controlled trial. Int J Cancer. 2016;139(12):2812-26.
7. Kim G, Taye J, Yu K, Park S, Kim J, Kim S, et al. HPV *E6*/*E7*, hTERT, and Ki67 mRNA RT-qPCR Assay for Detecting High-Grade Cervical Lesion with Microscope Slides. Anal Cell Pathol. 2019;2019:9365654.
8. Ni P, Wu M, Guan H, Yuan Y, Zhang L, Zhang F, et al. Etiology distribution of abnormal uterine bleeding according to FIGO classification system: A combined study of ultrasound and histopathology. J Obstet Gynaecol Res. 2022;48(7):1913-20
9. Mutakha GS, Mwaliko E, Kirwa P. Clinical bleeding patterns and management techniques of abnormal uterine bleeding at a teaching and referral hospital in Western Kenya. Plos One. 2020;15(12):e0243166.
10. Dueholm M, Hjorth IM; Structured imaging technique in the gynecologic office for the diagnosis of abnormal uterine bleeding. Best Pract Res Clin Obstet Gynaecol. 2017;40(9):23-43.
11. Kudoh A, Sato S, Itamochi H. Human papillomavirus type-specific persistence and reappearance after successful conization in patients with cervical intraepithelial neoplasia. Int J Clin Oncol. 2016;21(3):580-7.
12. Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, et al. 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors. J Low Genit Tract Dis. 2020;24(2):102-31.
13. Koh WJ, Abu-Rustum NR, Bean S, Bradley K, Campos SM, Cho KR, et al. Uterine Neoplasms, Version 1.2018, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2018;16(2):170-99.
14. Kops NL, Caierão J, Bessel M, Horvath JDC, Domingues CM, Benzaken AS, et al. Behavioral factors associated with

multiple-type HPV genital infections: data from a cross-sectional study in young women in Brazil. Reprod Health. 2021;18(1):201.
15. Ouh YT, Cho HW, Kim SM, Min KJ, Lee SH, Song JY, et al. Risk factors for type-specific persistence of high-risk human papillomavirus and residual/recurrent cervical intraepithelial neoplasia after surgical treatment. Obstet Gynecol Sci. 2020;63(5):631-42.
16. Toz E, Sanci M, Ozcan A, Beyan E, Inan AH. Comparison of classic terminology with the FIGO PALM-COEIN system for classification of the underlying causes of abnormal uterine bleeding. Int J Gynecol Obstet. 2016;133(3):325-8.
17. Munro MG. Practical aspects of the two FIGO systems for management of abnormal uterine bleeding in the reproductive years. Best Pract Res Clin Obstet Gynaecol. 2017;40(8):3-22.
18. Kawai S, Fujii T, Kukimoto I, Yamada H, Yamamoto N, Kuroda M, et al. Identification of miRNAs in cervical mucus as a novel diagnostic marker for cervical neoplasia. Sci Rep. 2018;8(1):7070.
19. Lili E, Chatzistamatiou K, Kalpaktsidou-Vakiani A, Moysiadis T, Agorastos T. Low recurrence rate of high-grade cervical intraepithelial neoplasia after successful excision and routine colposcopy during follow-up. Medicine. 2018;97(4):e9719.
20. de Sanjose S, Brotons M, Pavon MA. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. 2018;47(3):2-13.
21. Liu Y, Xu C, Pan J, Sun C, Zhou H, Meng Y. Significance of the viral load of high-risk HPV in the diagnosis and prediction of cervical lesions: a retrospective study. Bmc Womens Health. 2021;21(1):353.
22. Simoes RB, Campaner AB. Post-cervical conization outcomes in patients with high-grade intraepithelial lesions. APMIS. 2013;121(12):1153-61.
23. Abdulaziz AMA, You X, Liu L, Sun Y, Zhang J, Sun S, et al. Management of high-grade squamous intraepithelial lesion patients with positive margin after LEEP conization: A retrospective study. Medicine. 2021;100(20):e26030.
24. Vänskä S, Luostarinen T, Lagheden C, Eklund C, Kleppe SN, Andrae B, et al. Differing Age-Specific Cervical Cancer Incidence Between Different Types of Human Papillomavirus: Implications for Predicting the Impact of Elimination Programs. Am J Epidemiol. 2021;190(4):506-14.
25. Liao G, Jiang X, She B, Tang H, Wang Z, Zhou H, et al. Multi-Infection Patterns and Co-infection Preference of 27 Human Papillomavirus Types Among 137,943 Gynecological Outpatients Across China. Front Oncol. 2020;1049.
26. R SJ. The Immune Microenvironment in Human Papilloma Virus-Induced Cervical Lesions-Evidence for Estrogen as an Immunomodulator. Front Cell Infect Microbiol. 2021;11:649815.
27. Wang J, Li Z, Ma X, Du L, Jia Z, Cui X, et al. Translocation of vaginal microbiota is involved in impairment and protection of uterine health. Nat Commun. 2021;12(1):4191.
28. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007;7(1):11-22.
29. Qingqing B, Jie Z, Songben Q, Juan C, Lei Z, Mu X. Cervicovaginal microbiota dysbiosis correlates with HPV persistent infection. Microb Pathogenesis. 2021;152:104617.
30. Zhai Q, Zhang W, Zhang Z, Fu Y, Li Y, Wang X, Li L, et al. Characteristics of the Cervicovaginal Microenvironment in Childbearing-Age Women with Different Degrees of Cervical Lesions and HR-HPV Positivity. Pol J Microbiol. 2021;70(4):489-500.
31. Li Y, Yu T, Yan H, Li D, Yu T, Yuan T, et al. Vaginal Microbiota and HPV Infection: Novel Mechanistic Insights and Therapeutic Strategies. Infect Drug Resist. 2020;13(9):1213-20.
32. Barrios DTJ, Opata MM, Mowa CN. Immunity in the Cervix: Interphase between Immune and Cervical Epithelial Cells. J Immunol Res. 2019;2019(7693183).
33. Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS. Cervicovaginal Microbiota and Reproductive Health: The Virtue of Simplicity. Cell Host Microbe. 2018;23(2):159-68.
34. Du GH, Wang JK, Richards JR, Wang JJ. Genetic polymorphisms in tumor necrosis factor alpha and interleukin-10 are associated with an increased risk of cervical cancer. Int Immunopharmacol. 2019;66(8):154-61.
35. Monin L, Whettlock EM, Male V. Immune responses in the human female reproductive tract. Immunology. 2020;160(2):106-15.
36. Osiagwu DD, Azenabor AE, Osijirin AA, Awopetu PI, Oyegbami FR. Evaluation of interleukin 8 and interleukin 10 cytokines in liquid based cervical cytology samples. Pan Afr Med J. 2019;32:148.
37. Gulati NM, Miyagi M, Wiens ME, Smith JG, Stewart PL. alpha-Defensin HD5 Stabilizes Human Papillomavirus 16 Capsid/Core Interactions. Pathog Immun. 2019;4(2):196-234.
38. Tuerxun G, Abudurexiti G, Abulizi G. Prevalence,

persistence, clearance and risk factors for HPV infection in rural Uyghur women in China. Bmc Womens Health. 2023;23(1):433.
39. Boilesen DR, Nielsen KN, Holst PJ; Novel Antigenic Targets of HPV Therapeutic Vaccines. Vaccines-Basel. 2021;9(11):1262.
40. Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020;40(5):602-8.
41. McBride AA. Human papillomaviruses: diversity, infection and host interactions. Nat Rev Microbiol. 2022;20(2):95-108.
42. Dharmaraj N, Piotrowski SL, Huang C. Anti-tumor immunity induced by ectopic expression of viral antigens is transient and limited by immune escape. Oncoimmunology. 2019;8(4):e1568809.
43. Torres-Rojas FI, Alarcon-Romero L, Leyva-Vazquez MA, et al; Methylation of the *L1* gene and integration of human papillomavirus 16 and 18 in cervical carcinoma and premalignant lesions. Oncol Lett. 2018;15(2):2278-86.
44. Huang SS, Hao DZ, Zhang Y, Liu HM, Shan WS. Progress in studies of the mechanisms and clinical diagnosis of cervical carcinoma associated with genomic integration of high-risk human papillomavirus DNA. Yi Chuan. 2017;39(9):775-83.
45. Barros MJ, de Oliveira T, de Melo C, Venuti A, de Freitas AC. Viral Modulation of TLRs and Cytokines and the Related Immunotherapies for HPV-Associated Cancers. J Immunol Res. 2018;2018:2912671.
46. Paradkar PH, Joshi JV, Mertia PN, Agashe SV, Vaidya RA. Role of cytokines in genesis, progression and prognosis of cervical cancer. Asian Pac J Cancer Prev. 2014;15(9):3851-64.
47. Westrich JA, Warren CJ, Pyeon D. Evasion of host immune defenses by human papillomavirus. Virus Res. 2017;231(9):21-33. .
48. Melamed A, Margul DJ, Chen L, Keating NL, Del Carmen MG, Yang J, et al. Survival after Minimally Invasive Radical Hysterectomy for Early-Stage Cervical Cancer. New Engl J Med. 2018;379(20):1905-14.
49. So KA, Lee IH, Kim TJ, Lee KH. Risk factors of persistent HPV infection after treatment for high-grade squamous intraepithelial lesion. Arch Gynecol Obstet. 2019;299(1):223-7.
50. Berti F, Pereira A, Cebinelli G, Trugilo KP, Brajao DOK. The role of interleukin 10 in human papilloma virus infection and progression to cervical carcinoma. Cytokine Growth Factor Rev. 2017;34:1-13.
51. Niyibizi J, Mayrand MH, Audibert F, Monnier P, Brassard P, Laporte L, et al. Risk factors for placental human papillomavirus infection. Sex Transm Infect. 2022;98(8):575-81.
52. Meng D, Song L, Qi Z, Wang J, Liu H, Lyu YJ, Jia HX, Ding L, Hao M, Tian ZQ, Wang JT. [Prognosis of high-risk HPV infection and its influences by vaginal micro-environmental factors]. Zhonghua Liu Xing Bing Xue Za Zhi. 2021 Jun 10;42(6):1103-1107.
53. Shi N, Lu Q, Zhang J. Analysis of risk factors for persistent infection of asymptomatic women with high-risk human papilloma virus. Hum Vacc Immunother. 2017;13(6):1-7.
54. Shi HJ, Song H, Zhao QY, Tao CX, Liu M, Zhu QQ. Efficacy and safety of combined high-dose interferon and red light therapy for the treatment of human papillomavirus and associated vaginitis and cervicitis: A prospective and randomized clinical study. Medicine. 2018;97(37):e12398.
55. Nikakhtar Z, Hasanzadeh M, Hamedi SS, et al. The efficacy of vaginal suppository based on myrtle in patients with cervicovaginal human papillomavirus infection: A randomized, double-blind, placebo trial. Phytother Res. 2018;32(10):2002-8.
56. Jee B, Yadav R, Pankaj S, Shahi SK; Immunology of HPV-mediated cervical cancer: current understanding. Int Rev Immunol. 2021;40(5):359-78.
57. Ragonnaud E, Andersson AC, Mariya S, et al. Therapeutic Vaccine Against Primate Papillomavirus Infections of the Cervix. J Immunother. 2017;40(2):51-61.
58. Talebi S, Bolhassani A, Sadat SM, Vahabpour R, Agi E, Shahbazi S. Hp91 immunoadjuvant: An HMGB1-derived peptide for development of therapeutic HPV vaccines. Biomed Pharmacother. 2017;85(8):148-54.
59. De Rosa N, Giampaolino P, Lavitola G, et al. Effect of Immunomodulatory Supplements Based on Echinacea Angustifolia and Echinacea Purpurea on the Posttreatment Relapse Incidence of Genital Condylomatosis: A Prospective Randomized Study. Biomed Res Int. 2019;2019:3548396.