



## Review

# Association of High-risk Human Papillomavirus Titer and Pathogenic Co-infections with Cervical Tissue Cytopathology

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### Abstract

Human papillomaviruses (HPV) are one of the first viral organisms acknowledged to causing carcinogenesis. Among gynecologic cancers, Pap smear represents a gold standard diagnostic procedure for precancerous cervical lesions. It is efficiently interpreted through a standardized reporting system; The Bethesda System, which aids in distinguishing squamous categories from other entities. Co-infections with other sexually transmitted infections (STIs) could exacerbate cervical lesion severity caused by initial HPV infection as co-infections can lead to distinct reprogramming of host cells and genome integrity. The intricate pathways and effect of the unique cellular microenvironment that HPV and co-infecting STIs create that cause local inflammation and eventually cervical lesion progression will be reviewed in this manuscript. Besides, it is also crucial to consider HPV viral load and distinguish its correlation with cervical lesion severity. Varying amounts of viral titer and its impact on cervical lesions could indicate a mutagenic transformation of the human host cells and HPV. Thus, this review aims to discuss the correlation of co-infections and viral titer on cervical lesion severity and its progression to cancer. Based on these factors, clear clinical reasoning with more effective treatment plans and specific diagnostics can be achieved.

**Keywords:** Cervical cancer, Cervical lesions, Co-infection, Human papillomavirus, Viral titer

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Viral infections can be the root cause of human cancers which constitute about 15–20%, including viruses which contribute to its multiple-stage progression of malignant cancers.<sup>[1]</sup> In the past 20 years with countless research conducted, it is evident that several viruses contribute to the development of human cancers. Viruses that cause carcinogenesis among humans consist of; Kaposi sarcoma-associated herpesvirus (or known as human herpesvirus 8, hepatitis B virus, human T-lymphotropic virus 1, Epstein-Barr virus (EBV), Merkel cell polyomavirus (MCPyV), hepatitis C virus, and finally human papillomaviruses (HPVs).<sup>[2]</sup>

KSHC and EBV represent as oncogenic viruses with large DNA genomes that lead to the development of lymphoid malignancies and solid tumors,<sup>[3]</sup> whereas MCPyV and HPV and have shorter DNA genomes.<sup>[4]</sup>

HPV represents a double-stranded DNA virus originated from the Papovaviridae family. Within this family, it has been discovered that over 200 HPV types exist, that of which colonizes the genital tract while molecular diagnostic systems have streamlined them into more than 40 distinct HPV types, particularly correlated with high grade squamous intraepithelial lesion (HSIL).<sup>[5]</sup> HPV infections

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can be classified into two groups depending on their carcinogenic severity: High-risk (HR) and low-risk (LR). LR-HPV types consist of 6, 7, 11, 42, 43, 70, and 90.<sup>[6]</sup> Meanwhile, HR-HPV types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, and 59. HPV represents one of the oncogenic viruses that contribute to the development of cancer and is sexually transmitted. HR-HPVs are identified in 99.7% of cervical cancer specimens.<sup>[7,8]</sup> As of now, HPV-16 and HPV-18 are among the most virulent HR genotypes that represent 70% of all invasive cervical cancer (ICC) cases worldwide.<sup>[9]</sup> Regardless of the HPV strain, most HPV infections can be eliminated by the host's immunity in 1–2 years' time frame proceeding infection, with a median of 6 months. That being said, only a sum of <1% of HPV patients further develop into HPV-mediated cancers.<sup>[10]</sup>

HPV is evidently the central etiologic agent for cervical carcinogenesis.<sup>[11]</sup> All cervical carcinoma specimens examined had viral DNA present with a consistent link between HPV infection and development of cancer having been recognized.<sup>[11]</sup> With over 200 strains of HPV identified to date, categorized either as; cutaneous or mucosotropic HPV types, these groups comprise of the *Alpha*, *Beta*, *Gamma*, *Mu*, and *Nu* genera with clinical implications of inducing various skin lesions from warts to carcinomas.<sup>[12,13]</sup> The genus *Gamma* includes most of the recognized HPVs with 99 strains, continued by the *Alpha* genera with 65 strains and *Beta* with 54 strains. The *Mu* and *Nu* genera on the other hand each consist of 3 and 1 type of strains.<sup>[14]</sup> A subclass of 12 mucosal HR-HPV types grouped in the genus *Alpha-papillomavirus* (alpha HPV) contributes to being HR due to their vigorous carcinogenicity.<sup>[15,16]</sup> This genus constitutes etiological agents of many types oncogenesis which also constitutes cancer of the anus, vagina, vulva, cervix, penis, and a subtype of head and neck cancers.<sup>[17]</sup> Besides having a subgroup of HPV strains that are carcinogenic, the *Alpha* genus also has a subset of LR-HPV strains that facilitates benign lesions or genital warts as well as cutaneous HPV types which leads to the development of common and plantar warts.<sup>[18]</sup> Moreover, the genera *Beta*, *Gamma*, *Mu*, and *Nu* also consist of HPV subgroups that invade cutaneous epithelia with the *Beta* genus having more than 54 HPV strains, *Gamma* with 98 strains while *Mu* and *Nu* with only a few.<sup>[17,18]</sup> Among these clusters of HPV strains, there is only a cluster of the *Alpha* genus that are heavily implicated with the development of cervical carcinoma.<sup>[19,20]</sup>

Despite extensive amount of research on HPV's infection and virion life cycle, oncogenic properties, prevention, screening method improvements, and vaccination efforts, infection persists especially in the developing countries. Cervical cancer is globally, the fourth most frequently diagnosed cancer and fourth in place as a highly common

type of cancer in the female population with an estimate of 604,000 new cervical cancer cases diagnosed and 342,000 deaths recorded worldwide in the year 2020 alone.<sup>[21]</sup> In Malaysia, 19.7/100,000 females are diagnosed with cervical cancer based on the age-standardized rate according to a recent systematic literature review.<sup>[22]</sup> Annually, according to the estimates in 2018, 1,682 new cervical cancer cases are diagnosed, ranked as the third leading cause of female cancers and ranked as the fourth leading cause of female cancer deaths with 944 deaths yearly in Malaysia.<sup>[23]</sup> This could be due to a number of factors such as low socioeconomic status, lack of awareness primarily in communities of rural areas, inconsistent and inadequate diagnostic screenings, as well as poor implementation of vaccination rollouts.<sup>[24,25]</sup> Therefore, it is important to continue the chain of discussion and focus the attention of specialists towards identifying better algorithms when it comes to earlier and more specific detection of HPV and cervical cancer precursors. Thus, this review aims to highlight an association between HR-HPV viral titer and potential co-infections along with the clinical cytopathological impact on cervical tissue.

### HR-HPV Infection and Cervical Lesions

HPV targets epithelial cells and is contingent on the differentiation pathway of epithelial cells to regulate its lifecycle.<sup>[26]</sup> There is a significant amount of HPV infections that are transitory and undetectable for up to 2 years.<sup>[27,28]</sup> After 12–24 months of infection, 90% of people exposed to the virus are naturally cleared by the immune system or becomes dormant. However, an ongoing infection with a carcinogenic strain of HPV is denoted as the main risk factor of cervical invasive carcinoma that could range from atypical squamous cells of undetermined significance (ASC-US) to HSIL, based on The Bethesda System (TBS), and ultimately its progression to cervical cancer.<sup>[28-32]</sup>

In a study conducted by Skinner et al.,<sup>[28]</sup> (2016) the results concluded that an ongoing infection with a cancer causing HPV strain contributed as the principal cause in the detection of a cervical intraepithelial neoplasia (CIN) lesion. The HPV strains of HPV-16 and HPV-33 represented the largest risk factor, continued by HPV-18, HPV-31, and HPV-45. Detection of a developing lesion had 30–40 times of higher tendency for HPV-33 and 20 times more for HPV-16. This study also concluded that only 1 out of 10 HPV infections failed to be cleared in a span of 48 months while the middle range span of infections was within 12 months, with HPV-31 having the most prolonged detection window, continued by HPV-16, HPV-45, and HPV-18. The conclusions in this study was congruent with the sample size of a younger age group.<sup>[28]</sup>

TBS is a two-tiered system which varies between low-

grade squamous intraepithelial lesion (LSIL) and HSIL.[33] This grading system corresponds to the HPV carcinogenic potential, which is also suitable in categorizing vaginal cytology and anal Pap.[33,34] Meanwhile, CIN lesions' basis of nomenclature are classified by its grade. CIN1 represents a LSIL which is disclosed as a condition where the patients are infected instead of disease progression. LSIL constitutes of morphological conversion in the nether side of the spectrum of squamous intraepithelial lesion (SIL). Approximately 1.7% of total Pap smears are categorized as LSIL, which consist of >80% cases that are positive for HR-HPV.[35] LSIL lesions are usually distinguished among women a decade younger compared to patients diagnosed with invasive cancer.[36] As LSIL occurrences are due to short-term HPV infections that are eventually eliminated by the host, HPV infections usually persists for <24 months, while ongoing infection can denote higher risks of progression into neoplastic transformation.[37] HPV staging is crucial in prognosticating the immediate risk of HSIL. Therefore, in cases of HSIL determination, CIN2 and CIN3 are both included in the HSIL category which represents morphologic transformations correlated to the more critical end of the SIL spectrum. HSIL grading consists of 0.3% of all Paps where, 95% are HR-HPV positive.[35] Ultimately, HSIL has a higher tendency of development to cancer and are inferior to regression, while prolonged development to invasive cancer is approximated at 30% of the cases which takes 30 years to reach this stage.[35] Situations where HSIL cells possess precursor of LSIL on the background should be reported as HSIL. Identifying potential higher risk in cases with other squamous or glandular abnormalities is the ideal method of classification when it comes to diagnosis. Meanwhile, if an insignificant amount of irresolute HSIL cells are observed in the surroundings of LSIL case, then this brings to categorizing the case as ASC-H or LSIL, although high-grade dysplasia cannot be ruled out (LSIL-H).[38,39] HPV positive LSIL cases are correlated with a higher immediate risk of CIN3 than HPV negative cases. Therefore, to assess the risk of CIN3 on a Pap test graded as LSIL which ranges from 1% to 4.3%, heavily relies on the HPV status. The risk of CIN3 is evaluated as a determinant for clinical intervention.[35,37,40] CIN3 is labeled as genuine precancer, distinguished by developing to invasive cervical carcinoma at a factor of 0.2–4% within a year.[41,42] If untreated, CIN3 has the potential to become invasive over a 30-year period.[41,43] Adenocarcinoma, however, is separated from squamous cell carcinoma (SCC) as it originates from the glandular epithelium of the endocervical canal, therefore labeled as a precursor of adenocarcinoma *in situ*.<sup>[44]</sup> In spite of the quantitative and qualitative factors, a fraction of morphological characteristics may be categorized as ASCs that can be further divided into two

subcategories, including ASC-US or ASCs-HSIL cannot be excluded (ASC-H). These subcategories strictly depend on the underlying lesion, whether it is LSIL or HSIL.<sup>[45]</sup>

### HPV-Induced Cervical Cancer Pathogenesis

Studies have proven that HPV infection by itself, anticipates cervical cancer progression by decades. Continuous and consistent HR-HPV infection is the sole contributor in the development and exacerbation of precancerous lesions of the cervix to higher grades of precancerous disease or to cancer itself.<sup>[46,47]</sup> This makes HPV's life cycle an intrinsic and well-choreographed pathogenic pathway which begins with HPV infecting epithelial cells and relying on the differentiation pathway of epithelial cells to complete its life cycle. Initial infection normally begins in the basal epithelium through micro-fissures on the epithelial surface. The HPV viral particle exploits these micro-abrasions by accessing the dividing basal cells. Adherence of the viral particle to heparan sulfate proteoglycans and alpha-6-intergrin on the basement membrane leads to unspecified secondary receptor transference onto keratinocytes. These mechanisms induce cellular and conformational changes to favor viral entry.<sup>[26,48,49]</sup>

The pathogenesis of HPV that transcends into cervical cancer involves an intricate mechanism of uncontrolled cellular division involving the integration of the HPV gene assisted by other epigenetic factors and cellular change. The HPV genome is made up of circular double stranded DNA, with an estimated size of 8 kb and are separated into three sections: early, late, and long control region. The early region in the HPV genome functions as open reading frames which encodes non-structural proteins; E1, E2, E4, E5, E6, and E7 while the late genes include; L1 and L2.<sup>[50]</sup> Meanwhile, E4, E5, E6, and E7 in HR-HPV genome represent the accessory genes which enforces alterations in the host basal epithelium to promote favorable conditions for viral replication and persistence, hence evading host immunosurveillance.<sup>[51,52]</sup> These early gene products are responsible in regulating the viral life cycle while manipulating cell processes to transcribe, translate, and replicate viral proteins (E1 and E2), modulate early viral gene by-products, stimulate cytoskeleton reformation (E4), and deregulate cell cycle (E6 and E7). On the other hand, the two late genes L1 and L2 encode for structural viral capsid proteins necessary for virion synthesis, transmission, and consistent infection of HPV to the neighboring basal cells.<sup>[53]</sup> As a whole, these genes cooperate to substantiate continuous HPV infection while also accommodating HPV's viral reproduction.<sup>[52]</sup>

As cervical cancer originates from the cervical lining, the squamocolumnar junction is a crucial cytologic and colposcopic landmark due to its high susceptibility to HPV

infection. Besides, it is the location where 90% of lower genital tract neoplasia occurs.<sup>[54]</sup> CIN I and CIN II (mild dysplasia) lesions have relatively lower expression of E6 and E7 as these genomes multiply episomally, meanwhile CIN III and invasive cancer lesions often consist of high expression of E6 and E7. Integration of these viral DNAs into host cell genome is the cellular action that initiates neoplastic development.<sup>[55]</sup> One mechanism that induces integration of viral DNA into the host cell starts with the DNA damage response (DDR) that is essential for cell repair and is a precursor for cell division. As viral oncoproteins downregulate the process of DDR and diminish damage repair, the break points in the host DNA provides an easy entrance for viral integration. Besides, infection of HPV also triggers inflammatory responses and oxidative stress, for instance, the interferon response. Interferon stimulates the loss of episomal HPV and inhibits the HPV's E2 protein which is responsible for controlling transcription, replication, and viral genome division along the process of viral infection progression.<sup>[56]</sup> Hence, this ensues the election of cells with HPV integrated genome that has higher E6 and E7 expression levels which encode for HPV transforming oncoproteins that moderates the progression of cervical cancer.<sup>[57,58]</sup> Consequently, as the Toll-like receptor 9's function is decreased with an impaired interferon response, this leads to impairment of immune defense and persistent HPV infection. This results in the exponential expression of E6/E7 that encourages genetic vulnerability and chromosomal reformation, which further amplifies HPV viral integration.<sup>[59]</sup>

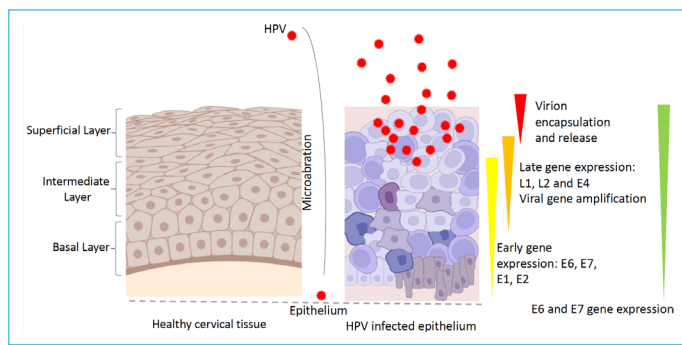
When HPV infection occurs, DNA in the basal cells of the cervix undergo mutation that allows the incorporation of viral DNA into the host DNA's synthesis machinery. This allows the virus to conceal itself thus providing the opportunity to escape from the host's immune processes and accommodating cell division along with evading cellular apoptotic cascades.<sup>[60]</sup> HPV-16 is among the viral strain with the highest carcinogenic potential as nearly half of the entirety of cervical cancers are analogous with this strain that leads to benign and precancerous lesions of the cervix, resulting in development of cervical carcinoma.<sup>[31,61,62]</sup> A secure multicopy plasmid initiates when the first viral proteins, E1 and E2 are expressed. Though it is assumed that early expression requires E1 and E2; however, it has been suggested that E1 may not be needed once viral copy number overcomes a viral division of 50–100 copies.<sup>[63]</sup> Every cycle produces a replicate of one viral genome during the S phase, (the latent phase of the viral division) that ensures persistent infection of basal cells up to years or decades. After which, a switch from stable replication to dormant viral DNA replication occurs to allow the continuation of genome production for packaging into virions. Two of the

proteins, E1 and E2 are heavily implicated in this process. E1 is the sole viral encoded enzyme that operates as helicase that is dependent on ATP.<sup>[64]</sup> E1 interlocks to the AT-rich regions which acts as the origin of replication bonded with weak affinity, which brings E2 as a stabilizer to maintain the binding at the origin of replication that results in a strong binding of the E1/E2 complex. This will lead to successful viral DNA synthesis via initiation and elongation.<sup>[65]</sup> As HPVs do not possess the ability to encode replication enzymes, hijacking the host's DNA synthesis to achieve replication is crucial. Since differentiated cells are incapable of advocating DNA synthesis due to their exit from the basal epithelium as a defense mechanism through withdrawing from continuing cell cycle, this is where the activation of HPV's viral E6 and E7 is expressed which reactivates cellular DNA replication machinery to allow vegetative viral DNA synthesis.<sup>[66]</sup>

E6 and E7 are capable of modifying the host cell's natural environment which upregulates viral genome replication among terminally differentiated cells in their vegetative state, which usually does not permit DNA replication. HR-HPV E6 and E7 pilots cell division in the basal and parabasal layers which results to increasing the infection radius. This is achieved when HR-HPV E6 proteins inactivate p53 tumor suppressor by proteasomal degradation through the associated E3 ubiquitin ligase, UBE3A. This inhibits the anti-proliferative and apoptotic capabilities of p53 in response to DNA damage and cellular stress due to aberrant S-phase entry. Not only that, but telomerase activity is also upregulated via HR-HPV E6 expression. This maintains the integrity of telomeres regardless recurrent cell proliferation. Moreover, HR-HPV E6 targets cellular PDZ domains that facilitate cell junctions and signaling pathways.<sup>[61]</sup> On the other hand, E7 predominantly targets the retinoblastoma tumor suppressor, pRB and "pocket proteins" p107 and p130,<sup>[67]</sup> resulting in their degradation via ubiquitin/proteasome system. This leads to the upregulation of E2F-modulated gene expression mechanism that regulates DNA synthesis and cellular division.<sup>[68]</sup> Figure 1 summarizes the infection mechanism of HPV in cervical mucosal epithelium and the disease progression.

### HPV-Induced Cytomorphological Changes in Cervical Tissue

Healthy cervical tissue possesses a smooth and shiny mucosal surface with a small rim of vaginal cuff when observed from a hysterectomy specimen. The cervix can be divided into zones of the endocervix, endocervical canal, and the ectocervix. The endocervix is referred as the region that meets the corpus of the uterus; meanwhile, the ectocervix is the most outer part of the cervix that protrudes into



**Figure 1.** Route of HPV infection via micro-abrasions in cervical mucosal epithelium. There is a highly controlled expression of several early viral gene products; E1, E2, E6, and E7, where E1/E2 are responsible in regulating the viral life cycle whilst manipulating cell machinery to replicate, transcribe and translate viral proteins during early infection. E6/E7 oncogenes on the other hand, target their respective substrates p53/pRb which promotes continual cell proliferation throughout infection and disease progression. This allows the virus to amplify its genome along with late gene expression of the L1, L2, and E4 genes. These series of expressions aid in completing its productive life cycle and ultimately produce new virions. If the immune system of the host does not resolve the viral infection and it persists for a long period of time, this may result in HPV-induced malignancy, in which the virions DNA are encapsulated, and the progeny released rapidly, thus reinstating infection while the E6 and E7 proteins are highly overexpressed.

the vagina.<sup>[69]</sup> On a cellular level, the endocervix comprises of columnar epithelium equipped with goblet cells that secrete mucus, scattered in between. When approaching the outer region of the cervix, the epithelial cellular morphology transitions into squamous cell epithelium.<sup>[70]</sup> The borderline between these two transition zones of epithelium is termed the “transition zone,” which is denoted to be clinically significant in the development of cervical cancer.<sup>[69]</sup> The location of the squamocolumnar junction changes with age, as it is located in the ectocervix during puberty, then migrates to the endocervix during adulthood due to squamous metaplasia, the replacement of friable glandular surface lining with resilient stratified squamous. Immature squamous metaplasia and ongoing chronic inflammation usually leads to increased susceptibility of infection with HPV.<sup>[71]</sup>

### Normal Cervical Tissue Morphology

The ectocervix of a normal cervical specimen consists of stratified nonkeratinizing squamous epithelium. The basal cell represents the deepest layer with compact nuclear chromatin, perpendicular oriented oval nuclei to the basement membrane with scant cytoplasm. The next layer, placed above the basal layer is the parabasal cells that has a higher ratio of cytoplasmic volume compared to basal

cells and consist of multiple layers of cells. Meanwhile, the intermediate cells are rich in cytoplasmic volume which, at the accumulation of glycogen, can appear pink or clear. Finally, the superficial cells are smaller in size with circular nuclei, pink, or clear cytoplasmic matter. In respect to the basement membrane, this cellular layer are arranged in a parallel order.<sup>[72]</sup>

The endocervix, on the other hand, has a single layer of mucus producing columnar cells with oval, dense, uniform, apical mucin, and basally oriented nuclei. When preparing H&E staining, the PAS-Alcian blue stains apical mucin with a rich blue or purple as a result from the reaction between the acidity of the mucin. This is where ciliated cells can be found with inconspicuous underlying reserve cell layer with clefts, infoldings, and glands of multiple shapes.<sup>[72]</sup>

The border between these two zones, as mentioned previously, is the transformation zone where metaplastic cells can be found, produced by endocervical cells that differentiate toward the squamous boarder. This differentiation occurs between the transitional sites of the glandular and squamous epithelia and is similar to parabasal cells with relatively scant cytoplasm and dense nuclei. It is normal for the endocervical epithelium to overlap with the metaplastic cells as well as the presence of nonspecific inflammatory infiltrate consisting of, plasma cells, lymphocytes, and neutrophils which are not commonly correlated with infection. Finally, the cervical stroma of the cervix is mostly composed of fibrous tissue with smooth muscle fibers and is richly supplied with blood vessels.<sup>[72]</sup>

### LSIL

When it comes to LSIL, it is characterized by the morphologic transformations on the lower end of the SIL spectrum with one of the major and distinctive cytomorphologic features: The presence of koilocytes. Koilocytes are focally angulated with clear, large, perinuclear intracytoplasmic space with irregular accentuated outlines. A majority of LSIL cells possess significant volume of cytoplasm, similar to superficial and intermediate squamous cells that are identifiable with darker and larger stained nuclei. They can be clumped together or exist as single cells. Among other distinguishable features, the enlarged nuclei of 3 times the size of intermediate cell nuclei (ICN) are also noted. These differentiated cells consist of chromatin that appear coarse but may be cloudy and dark with raisinoid characteristics that is evident in HPV-related lesions. Nuclear enlargement, irregularities, hyperchromasia as well as binucleation and multinucleation are also distinguishable at this stage.<sup>[45]</sup> Signs of increased keratinization are observed with dense orangeophilia or also known as atypical parakeratosis can be observed. However, LSIL cells with immature metaplastic

cytoplasm may be undeciphered between ASC-H or HSIL as this could be correlated with eosinophilic dysplasia.<sup>[36,73]</sup>

## HSIL

HSIL fits the criterion of changes analogous with the higher end of the SIL spectrum which is also categorized with both CIN 2 and CIN 3. HSIL cytomorphologic changes strongly that correlate to squamous or glandular abnormalities. In liquid-based cytology (LBC), HSIL is identified based on the scattered single dysplastic cells. Under low magnification, multiple three-dimensional hyperchromatic crowded groups (HCGs) or two-dimensional sheets of cells can be observed.<sup>[45]</sup> In general, the appearance of CIN 2 cells with checkerboard pattern and CIN 3 cells with syncytial aggregates can be seen. Under high magnification; however, significant nuclear atypia can be easily identified. Cells in HSIL specimens are observed to have a smaller radius than LSIL specimens, with high nuclear/cytoplasmic (N/C) ratio. That being said, the nuclei tend to display dysplastic characteristics such as coarse but delicate chromatin and irregular nuclear membrane without distinguishable nuclear quality and hyperchromasia,<sup>[45]</sup> Tulip flow-like or longitudinal nuclear grooves can be seen. HSIL specimens tend to have presence of apoptotic and mitotic bodies. SCC including dysplastic tadpole-shaped cells or fiber cells and necrotic debris are immediately classified as SCC or HSIL with attributes that show suspicion for invasive SCC based on the degree of cervical lesions. Moreover, another key feature of HSIL includes cervical glands that exhibit clumps of atypical HCG with nucleoli present in the cells along the circumference of HSIL cells with nuclear absence.<sup>[45,71]</sup> In spite of specific cytomorphological classifications, unsure patterns of HSIL such as keratosis (keratinizing high-grade lesions) and background atrophy resulting to the interpretation of ASC-H can occur, especially in LBC. Such cytology with limited intact HSIL cells for an affirmative categorization are thus categorized as ASC-H.<sup>[45]</sup>

## ASC-US

This classification is often administered with cytomorphological characteristics that manifest excessive nuclear atypia than reactive changes. Their cytomorphological features can differ based on the increase of nuclear size such as irregular membranes and hyperchromasia. Some of the distinguished characteristics of ASC-US classification comprise atypical repair and atypical parakeratosis.<sup>[36]</sup> Other typical cytomorphological features include enlarged nuclei with 2–3 times ICN and a slight increase in N/C ratio. Meanwhile, differential diagnosis of ASC-US heavily relied on coinfections such as herpes simplex virus (HSV), *Candida* and *Trichomonas vaginalis* where cells would manifest tight

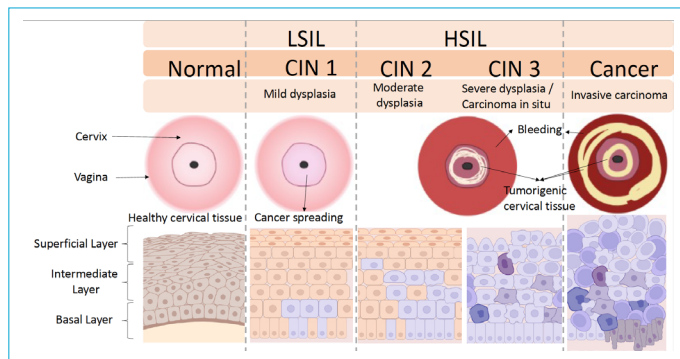
perinuclear halos, reactive inflammatory changes, as well as repair-like features showing cohesive groups of undefined “school of fish pattern” with polarized cells and pointed ends that also denotes a low N/C ratio.<sup>[74]</sup> Finally, parakeratosis describes feature of miniature cells clustered with densely orangeophilic cytoplasm that can be observed in whorls of keratin (squamous pearls) without the presence of nuclear atypia.<sup>[45,74]</sup>

## ASC-H

ASC-H is dedicated for specimens with cytomorphologic alterations that correlate to high-grade dysplasia due to qualitative or quantitative shortcomings. In conjunction to ASC-US, the cellular features range from reactive LSIL to HSIL plausibility. HPV status is dependent on the prompt risk of HSIL in cases with ASC-H categorization.<sup>[38]</sup> HPV cases in ASC-H possess higher risks of precancerous lesions which could lead to HSIL as compared to HPV-positive ASC-US and LSIL classifications, but still possess lower risk than HSIL. That being said, the instant risk of HSIL in HR-HPV positive ASC-H cases represent HSIL on the more critical end of the SIL spectrum compared to other SIL classifications.<sup>[75]</sup> The cytomorphological spectrum of ASC-H comprises atrophic smears with single atypical squamous metaplastic cells (ASMs). Among these ASMs, parabasal cells can imitate single-cell pattern, or termed as litigation cells of HSIL. The absence of nuclear dysplastic components of atrophic cells as compared to HSIL, which typically consist of cells with irregular nuclear membrane, hyperchromatic nuclei, and higher N/C ratio. Another class along the spectrum for ASC-H characterization is the presence of small atypical parakeratotic cells. HCG of small parakeratotic cells with clumps of hyperchromatic small cyanophilic atypical parakeratotic cells that can have a range of undefined to defined cell membranes. The N/C ratio are higher among these cells with blurred or smudged-like chromatin.<sup>[45,74]</sup> Figure 2 illustrates the epithelial cell features and characteristics as well as the histological characteristics of normal, precancerous lesions, and cancer stages of the cervix.

## HPV and Cervical Cancer Screening

Cervical cancer screening such as the Papanicolaou test (“Pap smear”) that examines the cervix by utilizing acetic acid which promptly aids in detecting abnormal cells and early cancer cells that can potentially develop into cancer.<sup>[76]</sup> Regular screening provides the chance of early and proactive treatment that results to desirable outcomes and helps in reducing deaths from cervical cancer.<sup>[77]</sup> Cytology-based screening or more commonly known as the Pap smear was the sole method of screening until the dawn of HPV testing’s. To date, cervical cancer screening comprises



**Figure 2.** Precancerous lesions of the cervix are referred to as cervical intraepithelial neoplasia (CIN). Based on histological observations, the changes that indicate intraepithelial neoplasia include enlarged nuclei, increased nuclear-cytoplasmic ratio, increased hyperchromasia, increased nuclear polymorphism, and increased anisokaryosis. As the severity of CIN increases, the number and abnormal configurations of mitotic figures also increase. The lesions are defined by the amount of the squamous epithelium that is dysplastic. Based on The Bethesda System, LSIL, or CIN1, displays dysplastic changes in approximately one third of the thickness of the epithelium. CIN2 involves one half to two thirds of the thickness, and CIN3 can show full-thickness involvement. Finally, carcinoma in situ is diagnosed when dysplasia is seen throughout the epithelium and resembles cervical cancer but has not invaded into the basement membrane. High-grade lesions (HSIL), such as CIN-2 and CIN-3, are true precursors of invasive cancer.

three methods,<sup>[1]</sup> HPV testing such as antigen tests via immunohistochemistry that detects the presence of HR-HPV types of cervical cells,<sup>[2]</sup> Pap testing and<sup>[3]</sup> HPV/Pap co-testing that utilizes the same cell sample for detection of both HR-HPV and abnormal cervical cell cytology.<sup>[78]</sup> Another method of managing HPV patients is through repeated testing in 4–6 months that makes the possibility of distinguishing between persistent and transient HPV infections as approximately 40% of women who are HR-HPV positive would have their infection cleared during this duration.<sup>[79]</sup> Indeed, the previous studies have shown that a substantial proportion of women who are tested positive for HR-HPV have normal cytology.<sup>[80,81]</sup> Other options of increasing specificity of triage, biological markers such as immunohistochemical staining of cervical smears for tumor markers such as p16INK4a methylation of host or HPV genes, and the quantification of HPV viral load could be useful in diagnostic testing.<sup>[82]</sup>

### HR-HPV Viral Titer and Cytology

It is now evident that HR-HPV is highly correlated with the development of cervical cancer. When it comes to the severity of viral load, this depends on the totality of infected cells and aggregate of viruses per infected cell that heavily relies on the degree of HPV infection on the cervical sur-

face, as well as the level of viral production in the area.<sup>[83]</sup> Varying amounts of viral titer and its impact on cervical lesions could denote a composite interaction between the human host and HPV, thus being an additional potential predictive marker for diagnostics.<sup>[83]</sup>

Chang et al. (2014) conducted a study to investigate the implication of viral load in HR-HPV against CIN and cancer, while also detecting potential biomarkers for cervical disease through collecting pathological reports among 343 women who were examined with both tests of HPV liquid-based cervical cytology and DNA load. Among this sample size of women, 143 consented to cervical tissue biopsy. The cytology and histopathological observations of all cases were examined microscopically by their respective pathologists. It was found that the correlation between the viral titer and cervical lesions were affirmative ( $p < 0.005$ ). Based on the cytological screening, the rate of HPV infection increased to 9% among women with negative or reactive results while among women with ASC-US a 25% increase was observed. Meanwhile, women diagnosed with LSIL and HSIL have HPV infection rates increased to 70% and 79%, respectively. In addition to that, the mean HPV load was also found to be increased respective to the severity of CIN grade.<sup>[84]</sup>

In another study, Wu et al. (2020) discovered an association between STAT1 and HPV16 viral load in cervical lesions extracted from 141 cervical tissue samples. To ensure that these specimens were infected with HPV-16, its L1 gene, which is a papillomavirus major capsid protein with the ability to spontaneously assemble virus-like particles, was primarily detected. The entirety in the normal group consisted of tissues that were HPV-16 negative. Using the  $2^{-\Delta\Delta CT}$  method following PCR detection for gene expression of HPV 16 viral load performed for 49 in the HSIL group, 30 in the cancer group and 34 cases in the LSIL group gave results that's were apparent that the viral loads gradually increased from LSIL to HSIL to cervical SCC with the mean values of  $26.98 \pm 12.34$ ,  $51.30 \pm 21.377$ , and  $169 \pm 79.47$ , respectively. This data concluded that the copy number of HPV-16 demonstrated a positive correlation with the continuous development of cervical lesions, along with the highest total copy number recorded in cervical cancer samples.<sup>[85]</sup>

In addition, Sun et al. (2001) collected cervical swabs from 73 women referred for colposcopy and these samples were processed to investigate an association between HR-HPV DNA viral titer with lesion size and cervical histologic severity. The results deduced a distinct increasing trend of HR HPV viral load and histologic severity of cervical lesions ( $p = 0.001$ ). Not only that, the study also discovered higher

viral titer in conjunction to larger radius of cervical lesions ( $p=0.003$ ). This study proved that women with higher viral titer had a significantly higher risk of having a larger lesion size, hence tends to be capable of harboring more HPV viral copies. Apart from this, higher HR-HPV viral load was also shown to be correlated with high-grade CIN and was more likely to persist in the host than a lower viral load of HR-HPV.<sup>[86]</sup>

Similarly, a large-scale study conducted by Long et al. 2018 to distinguish the most carcinogenic HPV type in South Western China and if the viral titer has a correlation with severity of cervical premalignant lesions. Between 2013 and 2017, 7747 patients were screened for various HPV genotypes; HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Among the above patients, 1728 patients were diagnosed with HR-HPV subtypes. These cervical samples were quantified through RT-PCR and a receiver operating characteristic curve analysis was used in predicting severe intraepithelial lesions of each case (CIN I, CIN II, CIN III, and ICC). Findings obtained demonstrated that the severity of cervical lesions had a positive correlation to the viral load ( $p<0.001$ ). When analyzing the specific HPV genotypes, HPV16, HPV58, and HPV33's increased viral loads were directly proportional to the severity of lesions, meaning these three HPV genotypes were the main cause to cervical cancer exacerbation through persistent infection. This is proven by significant correlation coefficients of 0.209 ( $p=0.026$ ), 0.189 ( $p<0.001$ ), and 0.121 ( $p=0.016$ ) for HPV33, HPV58, and HPV16, respectively. Their study further explained that most HPV infections does not lead to cancer due to the requirement of persistent infection for the progression of low-grade lesion to a high-grade lesion or cancer, which explains that the clearance of HR-HPV among low viral titer patients was easier compared to those with high viral titer of HPV-infected patients. Moreover, this study also concluded that HPV-16 was the most pronounced in carcinogenicity and its viral load found in cervical smears is correlated to an increased risk of future ICC. Meanwhile, HPV-18 or HPV-31 did not carry the risk of progressing to ICC. These observations showed an increasing level of carcinogenicity between HPV genotypes, from HPV-33 to HPV-58 and then HPV-16 that is the most carcinogenic.<sup>[87]</sup>

The positive association between HPV viral titer and severity of histological grades in the progression of cervical cancer was also shown in a cross-sectional study performed by Wu et al. (2017). Consisting of 2513 women with varying grades of cervical lesions, histological diagnosis was conducted by local pathologists and an automated RT-PCR test was used to measure the viral titer of HPV-16, HPV-18, and other HPV genotypes. From the analysis, they have deduced that the viral titer of HPV-16 increases with cervical

lesion grade and is predominant in cervical cancer ( $p<0.05$ ) compared to other HPV genotypes. Besides, the HPV viral load differed significantly among the HR-HPV genotypes, where women with SCC who were HPV-16 positive had the highest amount of viral titer, about 5 times higher than those infected with HPV-18 while 10 times greater than the other HR-HPV genotypes-infected group. Moreover, in this study, HPV-18 viral titer showed low amount in precancer cases but increases sharply in the cancerous stage. Meanwhile, other HR-HPV genotypes only manifested a large viral load in LSIL and HSIL but decreased sharply in SCC and ADC. This variation of viral titer among HPV genotypes is most likely due to the HPV type-specific difference in replication or its capability to integrate into the host DNA. As the mechanism of the viral lifecycle involves a complex and multistep processes, small changes at the molecular level can bring a huge magnitude of change in the observed viral load. In addition, this investigation inferred that the viral titer among patients with singular infections of HPV-16 and HPV-18 was higher than those patients with multiple and with prior infections in HSIL positive results. When it comes to the age factor, there was a higher viral load observed among younger women that may be due to new exposure of HPV; therefore, the ongoing immune response to HPV has yet to be cascaded.<sup>[88]</sup>

Although viral titer represents the viral copies in singular cells and the total number of infected cells has been proposed as a useful marker in non-transient HPV infection<sup>[89]</sup> and cervical lesion progression, this correlation can be controversial as the correlation of viral load and cervical lesion progression may also be affected by age, presence of different viral type and area of the cervical lesion.<sup>[90]</sup> This is shown by Lu et al. (2021) who investigated the correlation of viral titer and the severity of cytopathological impact on cervical lesions, where he had deduced a positive correlation between viral load and cervical lesion severity. This significance became faint when other factors such as age, location of lesions, and presence of multiple HPV types were added to the variable. Among the 273 women in their study, the median age was 39 years, where a concurrent increasing pattern between age and viral titer was observed. Furthermore, both ecto- and endocervical sampling showed a significantly higher viral load in multiple HPVs type infection compared to the singular HPV type infection group.<sup>[90]</sup>

### **Pathogenic Co-Infections with Cervical Lesion Severity**

Co-infection is defined as concurrent infection of a singular cell by multiple virus types or two or more pathogens from different genera or kingdom (bacterial or fungal), which



can arise incrementally beginning with an initial microbe infection, followed by another infection. Since presence of multiple pathogen species may result in cross-interaction within the host, co-infection plays an important role in human health. This is because such an interaction within the host may lead to either a positive or a negative effect on each of the co-infecting pathogen, which may potentially stem into syndemic. Syndemics refers to the aggregation of two or more diseases or afflictions in a population where there is a synergistic relationship between them, which enhances and exacerbates the negative health effects of any or all of the diseases.<sup>[91]</sup> Several key pathogens responsible for sexually transmitted infections (STIs) that are commonly involved in the exacerbation of cervical carcinogenesis include *Chlamydia trachomatis* (*Chlamydia*), *Neisseria gonorrhoeae* (*Gonorrhea*), *Mycoplasma genitalium*, HSV 1 and 2, *Treponema pallidum*, *T. vaginalis* and *Candida*.<sup>[92,93]</sup> These pathogens commonly elicit inflammatory processes and micro-abrasion on the cervical epithelium, thus compromising cellular integrity and may subsequently promote the persistence of HPV infection.<sup>[94,95]</sup> Besides, they themselves might compromise the host immune system, hence increasing risk of HPV infection as well as exacerbating an existing infection.<sup>[94,95]</sup>

### Biological Interaction Between HPV and Co-Infectious Pathogens

When it comes to the biological interaction between HPV and other pathogens, there are two schools of thought. The first speculation is that HPV or vaginal infections favors susceptibility to more infections or second, the co-infection of HPV and a separate pathogen simultaneously affect the development of CIN.<sup>[95,96]</sup>

There were previous studies that showed a noteworthy correlation between HPV and *Candida* infections. *Candida* species represents as a common fungal infection of the genital tract. Most cases are asymptomatic and although, no fixed mechanism has been deduced, certain pathogenic and virulent strains of *Candida* are known to cause breakdown of proteins and amplify antigenic responses that ushers mucosal abrasion and endogenous invasion.<sup>[97]</sup> The state of epithelial cell maturity is also a factor which determines the magnitude of cellular damage. A person infected with vulvovaginal candidiasis grants access for infection with HPV, which allows its entry and proliferation. In endogenous fungal infection, tissue debris and accumulation of free-radicals exponentiates the virulence of the pathogen and increases susceptibility of the host.<sup>[98]</sup> This further solidifies the correlation between cervical lesion progression and *Candida* spp. due to its inflammatory effects.

*C. trachomatis* infection primarily occurs in columnar epithelial cells of the endocervix due to its magnified prevalence of cases with cervical ectropion. This represents the region of squamous metaplasia of cervix that is mostly infected by chlamydia due to a majority of cases that account for the increased prevalence of SCC in association with the infection.<sup>[99]</sup> When co-infection with *C. trachomatis* occurs, facilitation of HPV entry to the basal layer is facilitated by micro-abrasions. This subsequently causes accumulation of HPV virions and derangement of host immunity where immune response is shifted from T-helper cell I (active immune system primarily in HPV control) to T-helper cell II, along with plasma cell infiltration.<sup>[100]</sup> This co-infection results in a synergistic interaction between those two infectious pathogens that accelerated the development of neoplasia.<sup>[99]</sup> With persistent Chlamydial infection, cytotoxic substances such as nitric oxide and anti-apoptotic mechanisms are activated. This results in further proliferation of infected cells and initiating carcinogenesis that is magnified alongside HPV co-infection.<sup>[101]</sup> Consequently, as co-infection persists, the cells are eventually reprogrammed from their usual cell proliferative capacity to an accelerated proliferation of daughter cells with modified genetic material with higher tendency for neoplastic development due to DNA damage.<sup>[102]</sup>

Apart from that, *T. vaginalis* is often associated with high grade cervical intraepithelial lesions due to significant alteration and damage of the epithelial cells. These conditions further contribute to the HPV virions entry into the epithelial cells and subsequent proliferation of the pathogen.<sup>[103]</sup> The activation of cell mediated immunity against this pathogen involves a vast recruitment of leukocytes as seen on cytology smears. Besides, the pathogen often feed on nutritional elements such as iron and fatty acids via the disintegration of red blood cells of the host. This is a result of trypsin-like substances which are cytotoxic that aids in cell detachment. Examples of these substances are; N-nitrosamines and CDF which also elevate epithelial atypia and dysplasia.<sup>[104]</sup> Infection with *Trichomonas* increases vaginal pH, which grants a suitable environment for proliferation of the pathogen. *T. vaginalis* primarily feeds on serous exudate and tissue debris, resulting in tissue damage that is substantial and atypical. This pathogen possesses a high prevalence of interaction with both local and systemic which denotes of great clinical implication.<sup>[105]</sup> This being said, Yang et al. (2018) deduced that patients infected with trichomoniasis had 6.5 times probability to have HPV-16 compared to patients who are *T. vaginalis* negative.<sup>[106]</sup> Concordantly, *T. vaginalis* infection with HPV could arise simultaneously instead of *T. vaginalis* being a direct exacerbate factor of HPV. Evidence based on epidemiology has

proven a more probable risk of cervical cancer for women with a history of *T. vaginalis* infection. To a certain extent, *T. vaginalis* can facilitate HPV infection and cervical lesions development.<sup>[107-109]</sup>

Furthermore, Wohlmeister et al. (2016) had investigated the relationship between the severity of cytological modifications in cervical epithelium and the conduct of co-infections between different STIs. The results depicted a significant association between the co-infection of HR-HPV (HPV-16 and HPV-18) and *C. trachomatis* which led to cervical carcinogenesis. STI co-infection has been proven to result with inflammation which contributes to tumor development, hence exacerbating epithelial lesions. With this in mind, the exacerbation of lesions provides an optimum environment for virus lodging and persistence. Based on the 169 endocervical and ectocervical specimens gathered, those tested positive for inflammatory or reactive benign cellular changes (RI) consisted of 16.2% that were also detected for one or more STI etiological agents through molecular testing. It is therefore inferred that *C. trachomatis* infection has an impact on HPV infection and persistence, as the co-infection increases the rate of transformation and progression of precursor lesions. As most infections are asymptomatic, patients could go untreated and thus, chronic inflammation is favored. Not only that, *C. trachomatis* has the tendency to damage epithelial tissue and inflammatory pelvic disease.<sup>[110]</sup> *C. trachomatis* has an affinity of infecting immature endocervical cells as this facilitates metaplastic epithelial transformation. This represents as a solid risk factor in explaining its exaggerated lesion progression with HR-HPV infection as HPV also preferable infects metaplastic epithelia.<sup>[111]</sup> Moreover, in this study, co-infection between HPV and other pathogens among 53.6% of patients was observed to have abnormal vaginal discharge or pruritus.<sup>[112]</sup>

Likewise, Hanisch et al. (2014) examined the significance of HPV-16 viral titer of the cervix and the severity of cervical cancer in association with immunosuppression by HIV infection. HPV-16 viral titers were investigated in this cross sectional study, by quantifying the viral titer derived from cervical swab samples from 498 HPV-16 positive Senegalese women of which 126 HIV-1 and/or HIV-2 seropositive and 368 were HIV-seronegative. Their analyses demonstrated a positive association between these variables as they have found that despite an absence of cervical disease, HIV-positive women had a higher mean HPV-16 viral load than HIV-negative women. In contrast to women with normal cytology, the probability of CIN I (ORa:1.21, 95% CI 0.93–1.57), CIN II–CIN III (ORa:2.38, 95% CI 1.72–3.29), and cancer was found to increase along with an increase of HPV-16 viral titer.<sup>[113]</sup> This observation might be attributed

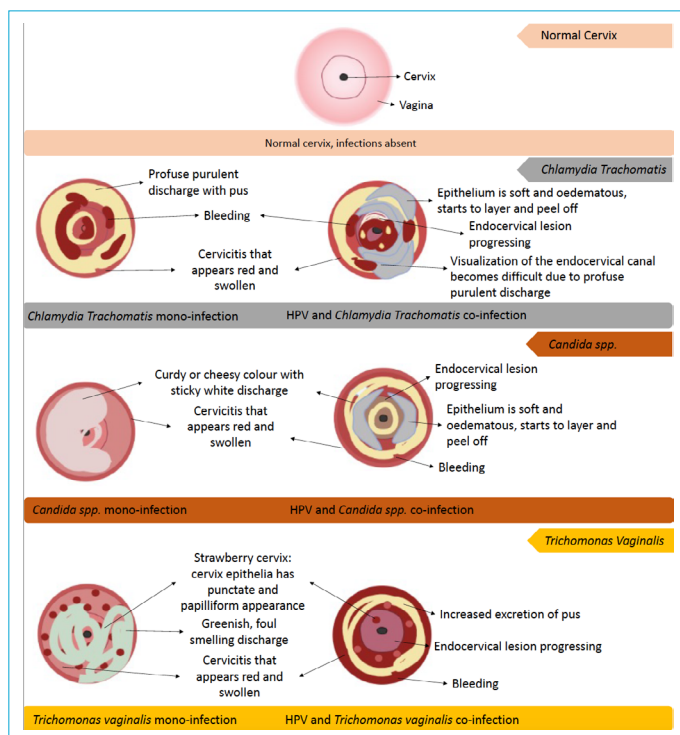
to HIV's Tat protein that effects directly on the proliferative ability of HPV.<sup>[114]</sup> Furthermore, HIV is proven to manipulate the expression of cytokines such as interleukin-10 that induces HPV transcription.<sup>[115,116]</sup>

In a broader study conducted by Kiseki et al. (2017), those with ASC-US cytology with the presence of a vaginal infection with HR-HPV had a positive impact the progression of CIN. After analyzing the data for 56 ASC-US patients who have conducted HR-HPV testing and cytological screening, positive rate of infection for HPV was 73.2%. Among these patients, seventeen of the study's volunteers were confirmed to have an infection with one or more vaginal pathogens which were *Gardnerella vaginalis*, *C. trachomatis*, *Candida* spp., coagulase negative *Staphylococcus*, and Group B *Streptococcus*. The rate of  $\geq$ CIN II was significantly higher among patients with an STI infection and HPV as to the study volunteers without an STI ( $p=0.0498$ ). One possible explanation is that certain STI agents established as virulent and pathogenic types are known to elevate antigenic response and cause protein degradation and enhance antigenic response that accelerates endogenous invasion and mucosal injury.<sup>[117]</sup> For instance, cells infected with *C. trachomatis* can aid entry of HPV to the basal layer. Once HPV viral particles are accumulated, host immunity is deranged. This shifts the immune defense from T-helper cells type 1 which aids in the clearance of HPV lesions to T-helper cell type II.<sup>[100]</sup> Therefore, the infectious nature as a result by a singular pathogen can act as a pathway for co-infection of other pathogenic species.<sup>[93]</sup> However, there have been contradictions with some literature regarding the correlation of HR-HPV and STI. For example, Kim et al. (2016) did not detect a positive correlation between STIs, HR-HPV infection and abnormal cervical lesions. The sample group of this study consisted of 1000 women who had conducted cervical cytology screening with LBC technique. Among them, 800 patients showed atypical cervical cytology that was contrasted with 200 other patients from the control group. They were congruently examined using the multiplex RT-PCR for detection of seven STI pathogen types. The results stated that there was no apparent distinction in HR-HPV positive group and co-infection with STIs based on abnormal cervical cytology. The group with HR-HPV negative results had a significantly greater total STI positive rate among cervical cytology grade equal to or higher than ASC-US than the control group (OR, 1.87; 95% CI, 1.20–2.90;  $p=0.0053$ ). On the other hand, no differences were found in the overall STIs positive rates among the groups with or without grades equal to or higher than ASC-H. However, there was a significant association between total STIs and HR-HPV ( $p=0.0057$ ) whereby, STI positive cases had 1.47 times higher positive rate of co-infection with HR-HPV than STI negative cases, despite

the causative pathogen. Another interesting inference of this study suggested that the cytological changes graded as ASC-US among women without HR-HPV is potentially a false positive due to the inflammation and cytological manipulation of cervical epithelial cells due to the impact of STI pathogens.<sup>[92]</sup> The previous studies have shown that certain STI pathogens were capable of modifying the morphology of cervical epithelial cells as this was one of the most common cause in misdiagnosis of ASC-US.<sup>[118,119]</sup> Therefore, patients negatively diagnosed with HR-HPV with ASC-US cervical Pap smear results should consider the probability of cytological changes due to STI pathogenic co-infection. Clinical evaluation for both STI and HPV infections should be considered as it could aid in diagnosis and prescription of more specific treatment.<sup>[120]</sup> Figure 3 illustrates in brief the co-infection pathogenic agents associated HPV infection, and possible mechanisms that could exacerbate cervical lesion progression.

### Epigenetic Reprogramming of Cervical Tissue During HPV Infection

Epigenetics represent the post-translational modification processes that influence gene expression without altering



**Figure 3.** Co-infection with other STI pathogens such as *Chlamydia trachomatis*, *Candida spp.* and *Trichomonas vaginalis* can increase the susceptibility to HPV infection as well as contributing to viral persistence. This in turn causes an exacerbation on cervical lesion progression with distinctive characteristics which are indicative of the specific type of co-infecting STI pathogen.

the original DNA sequence. In the virions and infected epithelial cells, HPV genomes are organized in nucleosomes packed as chromatin.<sup>[121]</sup> The epigenetic processes in cervical host cells that could accommodate HPV genomes generally involves post-translational modifications of histones, including methylation, acetylation, phosphorylation, and DNA methylation.<sup>[122]</sup>

Atypical DNA methylation of cervical epithelial cellular genome is apparent in various HPV-associated cancers.<sup>[123]</sup> As majority of cervical cancers are HPV dependent, the captivation of HPV-driven carcinogenesis involves the atypical DNA methylation in a variety of tumor suppressor genes (TSGs).<sup>[124]</sup> For instance, in a study conducted by He et al., (2019) it was observed that the occurrence of hypermethylation of the cyclin A1 gene (CCNA1), which represents an A-type cyclin distinguished by typical regularity in excessive protein synthesis along the cell division cycle,<sup>[125]</sup> was undetected (0%) in the control groups whereas 93% of hypermethylation was observed in HSIL patients.<sup>[126]</sup> Not only that, methylated sites were observed to have an increase in the promoter of hTERT gene that is responsible for the regulation of telomerase activity and this is mostly expressed in cancer cells and immortal stem cells.<sup>[127]</sup> Repressed sequences inhibited by methylation increases the hTERT production in HPV-dependent cervical cancer cells.<sup>[128]</sup> High risk HPV-16 promotes methylation by silencing E-cadherin, a TSG involved in Langerhans cell movement through stratified epithelium, assuring immune response to HPV persistent infection. Based on two independent studies, both HPV-16's E6 and E7 can cause inhibition of E-cadherin upon hindering DNA-methyltransferase activity.<sup>[129,130]</sup> In another study, interferon-k (IFN-k) was found to be suppressed by E6 of HPV-16, hence resulting in the methylation of CpG islands regulating the IFN-k expression. On further analysis, it was revealed that components of IFN-k, p53 protein and IFN-k and signaling pathways are factors that contribute to maintaining the antiviral microenvironment.<sup>[131]</sup>

### Conclusion

Cervical cancer is analogous with substantial mortality, while HR-HPV strains are the foremost causative pathogens of cervical cancer that can be prevented through vaccination, early and consistent screenings with proactive treatment plans. Despite HPV being the major risk factor for cervical precancerous lesions that ultimately progresses to cancer, it has also been reported that the potential role of STIs and cervical lesions progression are contributing factors to disease exacerbation. Interaction between other pathogens such as *T. vaginalis*, *Neisseria gonorrhoeae*, and *C. trachomatis* and HPV contributes to the epithelial

and epigenetic impairment as a consequence of HPV. The culmination of co-infections causes chronic inflammation which facilitates carcinogenesis by altering molecular and cellular processes. The spread, increase of reactive oxygen species and upregulation of inflammatory cells recruitment during inflammatory processes can cause the inhibition and impairment of DNA repair. Therefore, these changes are proven to transform the cervical epithelium's environment to be more vulnerable to mutations by stimulating the upregulation of oncogenes or inhibiting tumor suppressors and hence, accelerating HPV infection which determines the outcome cervical lesion severity. Increasing amount of research has proven that cervical lesions based on cytology results among patients who are co-infected with HPV and STIs are more severe than patient groups who are solely HPV positive. Without a doubt, the impact of cervical microbiome and its functions on cervical pathophysiology differ between individuals. Therefore, performing both HR-HPV testing and cervical cytology screenings for patients who are potentially STI positive may be helpful in formulating efficient diagnosis and innovative therapeutic approaches.

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