Available: DOI: https://doi.org/10.54117/gjpas.v1i1.9 *Review article*

Gadau Journal of Pure and Allied Sciences
Gadau J Pure Alli Sci, 1(1): 27-39 (2022)
ISSN: 2955-1722
DOI: https://doi.org/10.54117/gjpas.v1i1.9

An overview of the general characteristics, pathogenicity, transmission, and diagnosis of human papilloma virus (HPV)

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Abstract

Mucous membrane infections are brought on by the double-standard DNA virus known as human papillomavirus (HPV). Cervix cell alterations are caused by a sexually transmitted illness. E6 and E7 oncogenes play a critical role in HPV infection. Finding these genes to identify HPV strains, particularly the HPV16 strain, will have a significant impact due to its exceptional sensitivity, the dielectric electrochemical biosensor stands out among other pathogen detection methods. Recent evidence suggests that E6 and E7 are also important in inhibiting the innate immune response of the host cell to HPV. Viral replication is mediated by the E1 and E2 proteins in conjunction with other biological stimuli. E2 has also been linked to viral and cellular transcriptional regulation. Despite decades of research, the function of other viral proteins still remains unclear. Therefore, analysis of Human Papilloma Virus (HPV) characteristics, pathogenicity, transmission and diagnosis were reviewed. We concluded that we now have the tools and methodologies necessary to answer this critical question about viral tropism. The discovery of efficient treatments to cure or prevent HPV-induced illness is just as vital as studying the underlying mechanisms of HPV pathogenesis. The identification of drugs specifically to treat HPV infection has not been highly successful due to the complexities of the HPV life cycle and the limited number of enzymatic activities identified for HPV proteins. The development of drug treatments for existing HPV disease is an important undertaking that deserves further attention. In this regard, the development of therapeutic vaccines and self-protection are promising area of investigation and needs to be further supported.

Article History
Received: 25/02/2022
Accepted: 28/06/2022
Published: 30/06/2022

Keywords
Human Papilloma Virus;
Pathogenesis;
Characteristics;
Pathogenicity;
Transmission;
Diagnosis

How to cite this paper: Magaji Y.G., Kolo I., Baba J., and Suleiman K.O. (2022). An overview of the general characteristics, pathogenicity, transmission, and diagnosis of human papilloma virus (HPV). *Gadau J Pure Alli Sci, 1(1): 27-39.* https://doi.org/10.54117/gjpas.v1i1.9.

1.0 Introduction

One of the most common health issues for females globally is HPV infection, which has a global prevalence of 11.7 percent and is linked to the development of cervical cancer. A total of more than 100 HPV genotypes have been identified and described; 40 of them infect the mucosal epithelium, with an annual incidence of 6.2 million cases. In Sub-Saharan Africa, the prevalence rate is 24 percent (Li *et al.*, 2020). Cervical cancer cases in Nigeria have been estimated at 25 cases per 100,000 people, with prevalence rates of HPV in women at 23.3% (Yahaya *et al.*, 2019), 24.0% (Jing *et al.*, 2019), and 44.9% (Maria *et al.*, 2017). During sexual intercourse, a virus belonging to the papillomaviridae family infects the cervical epithelium (Yahaya *et al.*, 2019).

It is now well accepted that HPV infection is the primary cause of cervical cancer and a significant number of other anogenital neoplasms in many parts of the world (anal, vaginal, vulvar, and penile cancer). Several additional malignancies, such as head and neck (oral cavity, pharynx, larynx) cancer and non-melanoma skin cancer, have been linked to HPV, and it is also considered to have a role in the genesis of

several other tumors, such as prostate cancer (Komatsu *et al.*, 2018). The virus spreads mostly through sex and is difficult to detect in the early stages (Palma *et al.*, 2018). Aside from sexual transmission, HPV infection can also spread through other means. Nonsexual transmission of genital HPV is conceivable, such as through a mother's milk or changes in cervical tissue during pregnancy. Warts may become larger or proliferate during pregnancy due to hormonal changes, which may lead to bleeding (Maria *et al.*, 2017). Mucosal human papilloma virus (HPV) epidemiology has been widely investigated in recent years, particularly cervicovaginal HPV infection in young women, and additional epidemiologic data are also available for older women, men, and children. The HPV virus is responsible for the majority of all sexually transmitted illnesses. Sexual contact is the primary mode of transmission, however vertical and horizontal transmissions are also possible.

1.1 General Characteristics

1.2 Classification of Human Papilloma Virus

The phylogenetic classification of HPV genotypes has so far yielded more than 100 taxa and species (De Villiers *et al.*, 2004) (Table 1). Among all HPV genotypes, the L1 protein has a high degree of conservation and is thus employed for taxonomical purposes. More than 60 percent nucleotide sequence similarity in the L1 protein of different genera of Papillomaviridae (Alpha, Beta, etc.) is shared by the species within the same genus. When the L1 gene nucleotide sequence changes by more than 10% from the genotype with which it has the most DNA sequence similarity, a novel HPV isolate is identified as a new genotype (Table 1).

A papillomavirus's tissue tropism (mucosal or cutaneous) and carcinogenic potential can also be used to categorize it (De Villiers *et al.*, 2004). Although these genotypes may be found in both mucosal and cutaneous tissue, they are grouped together based on the tissues in which they occur (Table 1). Based on their relationship with cervical cancer and other anogenital malignancies, 13–25 HPV genotypes have been classified as probable or confirmed high-oncogenic risk (HR-HPV) (review in Wei *et al.*, 2021; IARC, 2011).

According to the World Health Organization's International Agency for Research on Cancer (IARC) classification, HPV-16, 18, 31, 33, 35 and 39 are HR-HPVs (IARC, 2011). Other HPV genotypes classified as possibly carcinogenic included in this categorization were: 26, 30, 34, 53, 66-67, 68, 69, 70-73-82-85-97. Condylomata acuminate (genital warts), a substantial percentage of low-grade squamous intraepithelial lesions (LSIL) in the cervical cavity, oral papillomas, and conjunctival papillomas can all be caused by infection with low-oncogenic risk HPVs (LR-HPVs), such as HPV-6 and 11. Recurrent respiratory papillomatosis may also be caused by HPV-6 or 11 in rare cases, which in newborns and young children can be highly morbid and generally perinatally transferred, although in adults it is usually sexually transmitted and less severe than in children (Armstrong *et al.*, 2000).

2.0 Pathogenicity of Human Papilloma Virus

During HPV's life cycle, mature virion particles are produced only in cells that have undergone keratinocyte differentiation, such as the supra-basal cells. Papillomaviruses are assumed to enter the epithelium through micro-wounds in the basal layer of the epithelium. Heparin sulfate facilitates the first attachment of virions to cells, but the receptor for viral penetration into cells is yet unclear (Figure 1 - 2). (John *et al.*, 2016). A constant supply of cells is maintained in the suprabasal areas by stem cells and transit-amplifying cells found in the basal layer (Andrew *et al.*, 2018). As these cells are infected with HPV, a viral gene expression cascade results in the generation of around 20 to 100 copies of extrachromosomal viral DNA per cell. For undifferentiated basal cells, the average copy number stays the same during the infection. In the early stages of viral replication, E1 and E2 are two of the first viral proteins to be expressed. To facilitate viral replication, this group of proteins binds to sequences near the viral origin of replication and recruits cellular polymerases and auxiliary proteins (John *et al.*, 2016). The E1 protein is also a helicase, allowing the viral DNA strands to be separated before the replication complex arrives (Graham, 2017). To recruit E1 to the origin and also to regulate viral transcription from the early promoter, E2 serves as a site-specific DNA-binding protein (Graham, 2017). Nearby transcription factors that activate the early promoter, and binding sites for E2 can be found (Klymenko *et al.*, 2016). High quantities of E2 repress transcription by inhibiting the binding of cellular transcription factors, whereas low levels of E2 activate the early promoter by binding to its recognition sequences (Klymenko *et al.*, 2016). It is important to note that the capacity of E2 to activate and repress expression helps in the regulation of viral copy numbers in undifferentiated cells.

Table.1.: Classifications of HPVs according to their tissue tropism and oncogenic potential

Tissue tropism	Genotypes of HPV
Mucosal	High-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 Low-risk (or probably carcinogenic): 6, 11, 13, 26, 30, 32, 34, 42, 44, 53, 54, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 89, 90
Cutaneous	1, 2, 4, 5, 8, 9, 12 ,1 4, 15 ,17, 19, 20, 21, 22, 23, 25, 27, 36, 37, 38, 41, 47, 48, 49, 50, 57, 60, 63, 65, 75, 76, 80, 88, 92, 93, 95, 96
Both (mixte)	3, 7, 10, 28, 29, 40, 43, 78, 91, 94

Adapted from De Villiers *et al.*, 2004. Carcinogenic potential classification based on IARC monograph, Vol 100B, 2009.

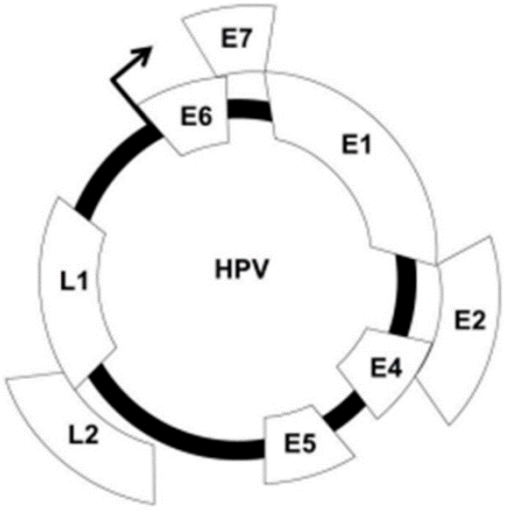

Figure 1: Map of the HPV genome. Early (E) and Late (L) genes are indicated. The arrow indicates the early promoter (Moody and Laimins, 2010).

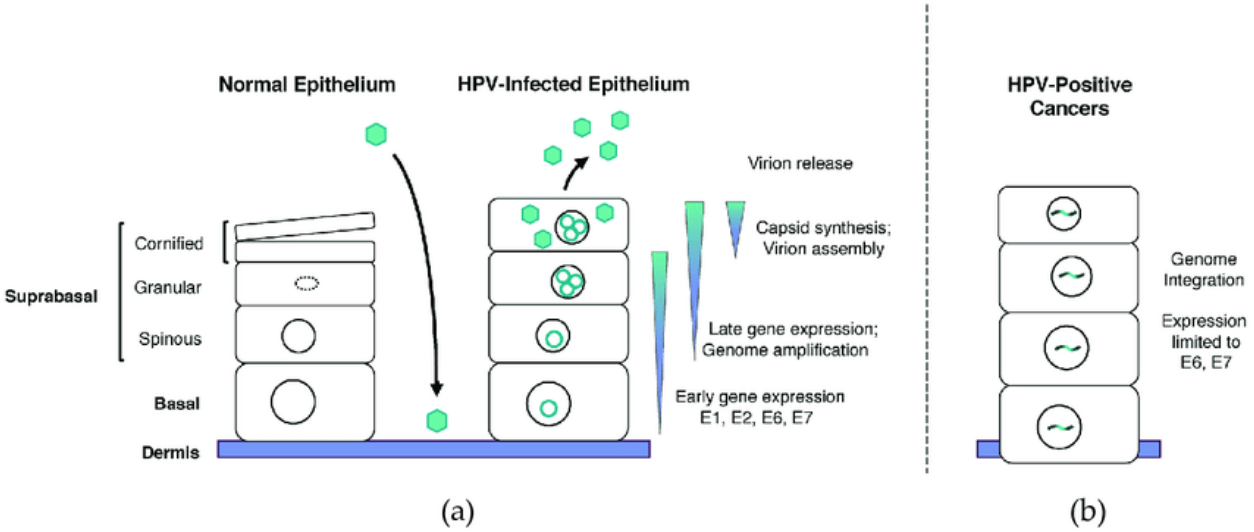

Figure 2. Replication cycle of a papillomavirus (Moody and Laimins, 2010)

High-risk HPV types' E6 and E7 proteins are viral oncoproteins, although the homologous proteins from low-risk HPV types have no such activities. Because of its ability to form an E6AP trimeric complex with the tumor-suppressor protein, E6AP, high-risk E6 causes p53 to be rapidly degraded (Andrew *et al.*, 2018). Rb family tumor suppressors and other proteins involved in cell cycle control are all targets of E7 (Klymenko *et al.*, 2016). When infected basal cells divide, viral genomes are divided into daughter cells, one of which migrates toward the stratum granulosum and undergoes differentiation (Figure 1 - 2) (Graham, 2017). When cells depart the basal layer in normal uninfected epithelia, they quit the cell cycle, which frequently leads to nuclei being lost in suprabasal cells. It is because of the function of the E7 protein that infection cells continue in the cell cycle when they exit the basal layer (Klymenko *et al.*, 2016). When cells have undergone significant differentiation, they rejoin the S phase and begin to express viral replication factors. When E7 is present, infected epithelia retain their nuclei across all of their layers. E6 and E7 have also been found to be essential for the preservation of HPV extrachromosomal forms in undifferentiated basal cells (Graham, 2017). Exact mechanisms are unknown, however, checkpoint that inhibit long-term storage extrachromosomal DNAs are likely to be removed. Both the E4 and E5 proteins have been postulated to be involved in the control of late viral activities; however, their specific roles have not yet been established (Graham, 2017). After a few days, the icosahedral capsids of the L1 and L2 proteins are formed. A mature virus is discharged from the highest layers of the epithelium during virion assembly (Klymenko *et al.*, 2016).

HPV's productive life cycle cannot be activated by the differentiation program of the host cell, for reasons that remain unclear. For the viral replication proteins E1 and E2 to be highly expressed as well as late genes, it is most probable that the late viral promoter will be activated, resulting in high levels of transcript expression. Amplification of viral DNA occurs as a result of increased expression of the late promoter, which, in contrast to the early promoter, is not inhibited by the E2 protein (John *et al.*, 2016). When there are more templates available, replication proteins can replicate more efficiently. Cellular or other viral factors may be increased during differentiation and contribute to the activation of late activities, although the discovery of these proteins is still in its early stages. While the high-risk HPV genomes are present as episomes in the early stages of infection, they have commonly discovered integrated into host sequences when lesions or carcinomas advance. Integrating into the E2 ORF, the E2 repressive function is lost, and this leads in greater amounts of E6, E7 mRNA production (Klymenko *et al.*, 2016). In the sections that follow, we go into great depth regarding the current level of knowledge about how viral factors work during the productive life cycle and development to malignancy.

3.0 Transmission of Human Papilloma Virus

Sexual contact is the most common means of transmission. Sexual activity is strongly linked to HPV infections of the mucosa (Schwarzer, 2018). One of the most significant risk factors for both the prevalence of infection and the acquisition of illness in adulthood is the number of lifetimes and recent partners (So *et al.*, 2020). It has been shown that genital warts are transmitted through sexual contact, that there is concordance in genital infection with genotype-specific HPV infection, that virgin women are extremely unlikely to contract genital HPV infection, and that there is an increased risk of contracting HPV after having sex with new or recently sex partners (Di *et al.*, 2017). HPV detection in the anal canal is also connected with anal intercourse in males who have sex with men and to a lower degree for women (Schwarzer, 2018).

Other sexual behaviors, such as oral sex, digital-vaginal intercourse, and the use of protective sex devices, can potentially transmit the virus (Alemany *et al.*, 2015). The oral sex of a woman with cervical cancer may explain why her husband is more likely to get upper aerodigestive tract cancer (Cuschieri *et al.*, 2016). Evidence suggests that sexually transmitted diseases can be passed from one woman to another in the case of genital HPV infection among sex partners (Maria *et al.*, 2017). More research has been done on the transmission of HPV, and it is clear that this virus is very contagious (Jing *et al.*, 2019). For example, Motevaseli *et al.* (2016) calculated that HPV-16's per-partner male-to-female transmission rate was as high as 60%.

3.1 Vertical Transmission

Non-sexual transmission is thought to be significantly less prevalent, but it is not completely excluded. In 1956, a case of juvenile laryngeal papillomatosis was described in which HPV was transmitted from mother to kid (Vriend, 2015). (JLP). Multiple studies have confirmed the transfer of HPV from mother to kid in various mucosa (genital, oral) but the method of transmission is still a mystery (Brotman *et al.*, 2014). If the mother's membranes rupture early during delivery, the virus can be transmitted directly to the baby by the mother's semen or ascending infection from the mother's genital canal during vaginal birth or caesarean surgery (Schwarzer, 2018). Since HPV DNA has been identified by PCR in the amniotic fluid of pregnant women who are infected with the virus, the virus might likely be transmitted across the placenta.

Syrjänen *et al.*, 2000, detected HPV 16 DNA in cord blood cells (Yahaya *et al.*, 2019).

3.2 Horizontal Transmission and other Route

Outside of sexual contact, the fingers and mouth, fomites, and skin contact are also possible entry points for an illness to spread. Self-inoculation, for example, can spread the disease from the anogenital area to the hands (Hernandez *et al.*, 2008). Aside from sexual contact, this non-sexual method of transmission is thought to be significantly less prevalent, especially in adulthood. However, among youngsters (excluding those who have been sexually abused), the horizontal route is clearly more essential than the sexual route. For example, 19–35 per cent of healthy children aged 6–11 years have oral HPV DNA in their buccal canals (Borgdorf *et al.*, 2016). Since HPV infection does not result in viremia, it cannot be transmitted through blood or breast milk. (Di *et al.*, 2017).

4.0 Diagnosis of Human Papilloma Virus

To accurately identify HPV, molecular biology methods must be used in the majority of instances. The Nucleic probe technique is the test of choice for identifying HPV in clinical specimens because of the genome's double-stranded DNA size of around 8000 base pairs (bp) and its well-known physical structure and gene organization (Chao *et al.*, 2020).

It is possible to determine the presence of HPV based on morphological, serological, and clinical results (Ilhan *et al.*, 2019). However, molecular-biology techniques are required for HPV diagnosis in order to accurately identify and type the virus (Chao *et al.*, 2020). Nucleic acid hybridization, signal amplification, and nucleic acid amplification are now accessible.

4.1 Nucleic acid-hybridization assays

Radio-labelled nucleic acid hybridization tests were initially employed to identify HPV infection in cervical samples in Southern blotting, in situ hybridization, and dot-blot hybridization procedures. Although these methods produced high-quality data, they had several of drawbacks, including limited sensitivity, the requirement for vast quantities of pure DNA, and lengthy processes (Wei *et al.*, 2021).

4.2 Signal-amplification assays

The FDA (Food and Drug Administration) has approved only the Digene® HPV test employing hc2 technology and the Cervista® HPV HR assay for diagnostic testing in the United States (Moody and Laimonis, 2010).

For the non-radioactive amplification of signals, the Hybrid Capture® 2 system (hc2, Digene Corp., USA) uses tagged RNA probes in solution to hybridize target HPV-DNA (Li *et al.*, 2020). As a result, this test can identify 13 of the most common forms of HPV (-16 and 18, respectively) or five of the most common forms of HPV (-6 and 11, respectively) (Kim *et al.*, 2015).

This test differentiates between the HR and LR groups, although it was not designed to genotype single HPV genotypes with this method (Fernandez-Duarte *et al.*, 2018). This is a remarkable result, as the probability of a precancerous lesion with persistent infection is between 10% and 15% for HPV types -16/18, and less than 3% for all other HR types put together. As a result, HPV genotyping is critical in the search for a single carcinogenic strain of the virus (Kim *et al.*, 2015; Fernandez-Duarte *et al.*, 2018). a better understanding of infection persistence and risk stratification (Fernandez-Duarte *et al.*, 2018).

Among the 14 HR-HPV types detected by the Cervista HPV (Hologic, Inc., Marlborough, MA) are the following: 16, 18, 31; 33; 35; 39; 45; 51; 52; 56; 58; 59; 66; and 68. (Lu *et al.*, 2021). For the identification of particular nucleic acids, this assay also employs a signal-amplification approach.

For CIN III, the Cervista® test had a sensitivity of 100 per cent, whereas the hc2 assay had a sensitivity of 98 per cent (Kim *et al.*, 2015; Motevaseli *et al*, 2016). A decreased false-positive rate and good sensitivity and specificity for genotyping HPV -16/18 were also demonstrated by this test (Kim *et al.*, 2015; Motevaseli *et al*, 2016).

4.3 Nucleic acid-amplification methods

i. Microarray analysis

It employs probe amplification, PCR (Polymerase chain reaction) product hybridization, and a DNA chip scanner to display hybridized signals (Jing *et al.*, 2019). Automated approaches based on microarrays can analyze several DNA samples simultaneously. Gene expression profiling and mutation analysis are two of the most common uses of DNA microarrays nowadays (Komatsu *et al.*, 2018).

Several studies have shown that DNA microarray analysis and PCR may be used to identify and genotype HPV. The HPV DNA chip outperformed gel electrophoresis in terms of sensitivity and specificity, and it even outperformed direct DNA sequencing in several circumstances (Komatsu *et al.*, 2018; Jing *et al.*, 2019).

PapilloCheck®

Detects and genotypes 24 kinds of HPV in a single response (HPV -6, -11, -16, -18, -31, -33, -35, -39, -40, -42, -43, -44, -45, -51, -52, -53, -55, -56, -58, -59, -66, -68, -70, -73, and -82). HPV E1 gene fragment 350 bp in length is amplified using a multiplex polymerase chain reaction (PCR) using fluorescent primers and 28 probes, each in five replicate locations, on a DNA chip. Human ADAT1 co-amplification is employed as an internal control. A microarray chip is

used for the hybridization, and the CheckScannerTM and Check-ReportTM software are used to scan and analyze it automatically at 532 and 635 nm (Arbyn *et al.*, 2018)

It's safe to claim that the PapilloCheck® assay (Greiner Bio-One GmbH, Frickenhausen, Germany) is a trustworthy screening test due to its ability to identify HR/LR-HPV and to detect multiple infections (Mitra *et al.*, 2016). This assay, on the other hand, does not amplify HPV-35 and -53, is still expensive and necessitates specialized equipment (Brotman *et al.*, 2014; Mitra *et al.*, 2016).

ii. Polymerase chain reaction (PCR)

There are several advantages to using PCR-based procedures. Thermal DNA polymerase may identify and expand oligonucleotides flanking the target area in the traditional PCR. After 30 rounds of amplification, the PCR may produce one billion copies of a single double-stranded DNA molecule (Borgdorf *et al.*, 2016).

Many different HPV genotypes can be amplified in a single reaction by using consensus primers such as PGMY09/PGMY1 and GP5+/GP6+. For example, the primers focus on the L1 capsid gene of HPV (Ilhan *et al.*, 2019). After amplification, HPV genotypes can be identified using methods such as RFLP, linear probe assays, direct sequencing, or genotype-specific primers. The lengthy L1 control region and the E6/E7 promoter region have been amplified by a type-specific PCR, which some researchers have exploited (Wei *et al.*, 2021).

As with any new technology, there is an inherent risk of false-negative findings when multiple infections are present in low-copy samples, due to a lack of competition for reagents. HPV genotypes may not be detected using the PCR approach because of this issue. Another drawback is that it is fairly unusual to suffer from repeated infections (Ilhan *et al.*, 2019). Samples having DNA from more than one HPV genotype might result in a significantly more powerful amplification for one sequence that would make it more difficult to identify all HPV genotypes in the same sample. Sequencing or type-specific PCR, both of which are labor-intensive, may be necessary in some cases (Wei *et al.*, 2021).

iii. Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP)

By using PCR-RFLP, HPV genotyping may be done, which makes it more convenient and less expensive than sequencing (Brotman *et al.*, 2014). The procedure is easy and reliable, and it doesn't necessitate expensive equipment. It's perfect for situations when there aren't many resources available (Brotman *et al.*, 2014; Borgdorf *et al.*, 2016). It is feasible to identify single or numerous infections using PCR-RFLP since it has strong discriminating power when determining if the virus is in HR or LR. Restrictive enzymes are used to digest the amplified DNA, resulting in DNA fragments of varying lengths, in this method. BamHI, Dd6eI, HaeIII, HinfI, PstI, and RsaI are the most often seen restriction enzymes (Borgdorf *et al.*, 2016). A single restriction enzyme (HpyCH4V) can be employ for the detection of HPV genotypes 21 HR and 31 LR (Schwarzer, 2018).

iv. Real-time PCR

For the identification and genotyping of HPV genotypes in tissue specimens (Chao *et al.*, 2020) and cellular samples, this test is an excellent diagnostic tool. With the use of different fluorochromes that emit fluorescence as the PCR reaction proceeds, it is possible to perform multiple PCR reactions and amplify different nucleic-acid targets; (iii) nucleic acids can be detected even in very low concentrations, using a 7-log dynamic range to extrapolate the viral load/concentration over a standard curve; and finally, (iv) it is extremely reproducible (Mitra *et al.*, 2016).

v. Abbott real-time PCR

The Abbott Real time HPV assay uses PCR to generate amplified product from the DNA genome of HPV in clinical specimens. A DNA sequence that is unrelated to the HPV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated DNA sequence is simultaneously amplified by PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HPV target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott m2000rt is inversely proportional to the log of the HPV DNA concentration present in the original sample.

Individual genotyping for HPV-16/18 and pooled detection of 12 HPV genotypes: -31, 33, 35, 39, 45, 51, 52 and 56 are used in the Abbott Real Time HR HPV test (Mitra *et al.*, 2016).

vi. COBAS® 4800 HPV test

Real-time PCR technique is used to identify 14 HR-HPV in this test, which is automated. There is only one PCR tube used for the amplification and detection of HPV-16, 18 and 12 HR pools (-31, -33, -35, -39, -45, -52, -56, -58, -59, -66 and -68) and one tube for extracting and amplifying beta--globin as the quality control (Chao *et al.*, 2020).

COBAS® 4800 (Roche Molecular Systems) and Real-time PCR (Roche Molecular Systems) were shown to be very consistent in research assessing the

reproducibility of a series of sequential procedures in both intra and interlaboratory (Motevaseli *et al.*, 2016). Because it is designed for primary specimens, the test is simple to perform, and results may be provided in as little as 4 hours after receiving the sample. COBAS® 4800 is clinically proven and reliable in the detection of HR-HPV, according to the worldwide criteria for screening. It has been clinically proven to be useful for ASC-US triage using this particular test (Komatsu *et al.*, 2018).

vii. HPV genome sequencing

It was more than 30 years ago that the dideoxy chain-termination approach (Sanger technique) was originally published for genome sequencing (Li *et al.*, 2020). The use of fluorescence-labeled nucleotides into Sanger sequencing has contributed to the extension and development of high-quality, comprehensive sequencing (Kim *et al.*, 2015; Li *et al.*, 2020). In spite of this, it hasn't been clinically proven. Like dideoxy sequencing, pyrosequencing is suitable for PCR-amplified DNA and RNA from any source (blood, saliva, cell line, plasma, serum, tissue, formalin-fixed paraffin-embedded samples, and whole genome-amplified DNA). Many advantages over dideoxy sequencing may be gained by using this approach for short- to medium-sequence applications, such as in the field of genetics and genomics. To begin with, the readout sequence is obtained rather than a fluorescent signal that has to be translated into a sequence. As a second benefit, it is more efficient and less expensive, thanks to the sequence-by-synthesis approach in which a DNA sequence is read in real time and synthesized by the addition of inexpensive, unlabeled nucleotides. Finally, the technology is also quantitatively unique (Fernandez-Duarte *et al.*, 2018).

viii. CLART® human papillomavirus 2

To detect the HPV L1 area, the CLART® Human Papillomavirus 2 (Genomica, Madrid, Spain) approach makes use of biotinylated primers. A control for adequate DNA extraction and PCR efficiency is provided by co-amplifying an 892 bp portion of the FTR gene with a 1.202 bp fragment of a modified plasmid. By using a low-density microarray that contains DNA probes specific for 35 HPV, amplicons may be identified (-6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -42, -43, -44, -45, -51, -52, -53, -54, -56, -58, -59, -61, -62, -66, -68, -70, -71, -72, -73, -81, -82, -83, -84, -85 and -89). Accurate, precise, and repeatable semi-quantitative data may be achieved using an automated reader with very similar results (Lu *et al.*, 2021).

ix. INNO-LiPA

Real-time PCR genotypes all 14 HPV strains (Vriend *et al.*, 2015). Using SPF10 biotinylated primers and genotyping, INNO-LiPA (LiPA HBV GT; Innogenetics N.V., Ghent, Belgium) is based on the co-amplification of the HPV L1 gene and the 270 bp of the human HLA-DP1 gene (Di *et al.*, 2017; Borgogna *et al.*, 2020). It was shown to be the least effective approach for genotyping HPV-42/-59, which included HPV-35, -39, -52, -56, and -66, as well as HPV-42 (Di *et al.*, 2017).

Only a few techniques of nucleic-acid amplification, such as Real-time PCR, are suited for archival clinical specimens because they target a very tiny fraction of the HPV genome, although they can consistently identify HPV in cervical-swab specimens (less than 160 bp). Consequently, the observed discrepancies in internal control amplification efficiency between Real-time and INNO-LiPA may be ascribed most fairly to the differences in target amplicon length: 136 bp vs. 270 bp (Brotman *et al.*, 2014). Swabs, brushes, tampons, and lavage are all examples of sample types that may be tested with this kit (Di *et al.*, 2017; Borgogna *et al.*, 2020).

x. The Linear array®

HPV genotyping with the Linear Array® assay is performed using reverse line blot hybridization (PCR), and the results are sent to Roche Molecular Diagnostics, located in Pleasanton, California, in the United States. It is possible to differentiate between 36 HPV genotypes (-16, 18, 31, 33;-35; 39;-45; 51; 52; 56;58;59;68;73;82; -82), as well as 15 HR (-16, 18, 31, 33; 35; 39; 45; 51; 52; 56;58;59;68;73;82) and three probable HR (-26, 53; and-66). It is also possible to differentiate between 10 LR (-6;-11;-40;-42; -40; 61; 70; 72;81;-81;-CP6108) and nine genotypes for which the risk is still undetermined (55; 62; 64; 65; 64; 65; 65) (Jing *et al.*, 2019).

The polymorphic L1 region of the HPV genome is amplified using biotinylated PGMY09/11 primers and a 450 kb fragment. For accurate DNA extraction and high PCR efficiency, the 268-bp region of the human -globin gene is co-amplified. The Auto-LIPATM device (Innogenetics, Ghent, Belgium) can process up to 30 strips concurrently in a uniform manner for hybridization and detection of the amplified result. The Linear Array® reference guide is used to interpret the color-coded signals on the strip. Cross-hybridization probes can detect HPV-52 with ambiguous findings when -33, -35, or -58 are also present in the sample. Probes for HPV-33 and 35, but not for HPV-52, are included on the test strip to confirm the presence of HPV-33 and 35. (Motevaseli *et al.*, 2016).

xi. Clinical arrays® HPV

HPV may be detected and genotyped using this kit (Genomica SAU, Madrid, Spain). Absorption columns are used in the DNA extraction process. It uses

biotinylated PCR primers to identify a 451-nucleotide sequence in the HPV genome's L1 region. The PCR process and DNA integrity are both checked using a human cystic-fibrosis transmembrane conductance regulator (CFTR) gene and control plasmids (Palma *et al.,* 2018). As a result, it is possible to identify the 35 genotypes that have been found to be related with either HR (genotypes 16, 18, 26, 31, 33 and 35), or with the Long Rare (LR) HPV (genotypes 51 to 85). (-6, -11, -40, -42, -43, -44, -54, -61, -62, -71, -72, -81, -83, -84 and -89). Detection of uncomplicated infections or co-infections is achievable (Jing *et al.,* 2019).

xii. Microplate colorimetric hybridization assay (MCHA)

It is based on the amplification by PCR of the 150-bp fragment within the L1 region by consensus primers GP5+/6+ followed by colorimetric hybridization to six type-specific probes (ImmobilizerTM Amino Surface, Nunc, Roskilde, Denmark) on microwell plates (Immobilizer Amino Surface, Nunc, Roskilde, Denmark) (Arbyn *et al.,* 2018).

HPV-31, -33, -45, and -45 were all correctly identified by the MCHA, while -39 was incorrectly identified by PapilloCheck®. Probes for HPV-35, -52, -56, and -58 should be incorporated in MCHA to increase detection of additional genotypes (Arbyn *et al.,* 2018).

xiii. HPV-mRNA detection

Cell transformation by HR-HPV is primarily facilitated by E6 and E7, which influence the activity of cellular proteins that control the cell cycle (Arbyn *et al.,* 201). Precancerous lesions can be detected using HPV and the presence of E6/E7 can serve as a particular marker (Borgogna *et al.,* 2020). As a result, searching for transcripts of E6/E7 may improve the specificity and sensitivity of tests for the diagnosis of cervical lesions that have a larger likelihood of developing than a simple detection of HPV-DNA (Motevaseli *et al.,* 2016; Arbyn *et al.,* 2018; Borgogna *et al.,* 2020).

PreTect® Proofer and APTIMA® HPV Assay are the two most often used commercial tests to detect mRNA for E6/E7 oncogenes (Cuschieri *et al.,* 2016). E6/E7 transcripts are amplified by transcription-mediated amplification, which is pre-empted by target capture.

NorChip AS, Klokkarstua, Norway's Pretect® HPV-Proofer test identifies E6/E7 mRNA from five HR-HPVs (-16, -18, -31,-33, and -45). High sensitivity has been demonstrated in clinical investigations. For the identification of underlying high-grade squamous intraepithelial lesions (HSIL), this technique is based on Real-time multiplex PCR and is more specific than HPV-PCR (Alemany *et al.,* 2015).

It is possible to identify 14 HR-HPV E6/E7 mRNAs with the APTIMA® HPV assay, which is manufactured by Gen-Probe in San Diego. The Proofer test only identifies five HR HPV E6/E7s. The APTIMA® HPV assay is more sensitive than the Proofer test since it detects 14 HR HPV E6/E7 mRNAs (Alemany *et al.,* 2015). Among its many advantages over other HPV tests, this one detects HPV E6/E7 mRNA, which may be a better indicator of advanced disease than the more commonly used hc2; it has a lower limit of detection than other tests; it does not cross-react with the LR-HPV types examined in the current study; and it can be processed by an automated system (Alemany *et al.,* 2015; Alemany *et al.,* 2016).

Telomerase reverse transcriptase (TERT) expression is also activated by oncoprotein E6, resulting in cell immortalization (Cuschieri *et al.,* 2016). There are two types of catalytic subunits in the telomerase complex: a structural RNA subunit (hTR) that acts as an example during telomere extension, and a reverse transcriptase subunit (hTERT) (Cuschieri *et al.,* 2016). Quantitative Real-Time PCR can identify high amounts of telomerase in tumor cells, which are almost always the consequence of dysregulated hTERT expression (Cuschieri *et al.,* 2016; Arbyn *et al.,* 2018). hTERT expression often increased during cervical carcinogenesis, suggesting that it might serve as a useful marker for the progression of cervical lesions (Cuschieri *et al.,* 2016; Arbyn *et al.,* 2018).

4.4 HPV viral load quantification and integration

i. HPV-DNA viral load

The link between the HPV viral load and cervical lesions with malignant potential is not yet established. Predicting whether or not a condition will advance or worsen is an important aspect of many research on HPV burden (Cuschieri *et al.,* 2016; Motevaseli *et al.,* 2016; Arbyn *et al.,* 2018; Borgogna *et al.,* 2020). Although few researchers, found changes in load between CIN I and CIN II, they did not find any differences between the two types of the disease when comparing the two types of CIN (Borgogna *et al.,* 2020). As a result, both the histology and the cytology of the load are in sync. Further clinical studies can be conducted if the viral load decreases in response to treatment, as described by Maria *et al.,* 2017. Another study found that viral load evaluation had no additional benefit over cytology, and that testing for high viral load levels may not be clinically beneficial, especially in the case of HPV-16 (Alemany *et al.,* 2015; Alemany *et al.,* 2016).

By using Real-time PCR methods, the HPV viral load may be measured. The hc2 method may also be utilized for the semi-quantification of clinical samples using these technologies (Borgogna *et al.,* 2020).

ii. HPV-DNA integration

Extra-chromosomal or episomal HPV-DNA is commonly found in the earliest stages of cervical

precursor lesions. Viral integration is common in HSIL and CC, and both episomal and integrated HPV-DNA can be found in the same lesions (Maria *et al.,* 2017). Integration of HPV-DNA into the host cell often breaks at the E1 and/or E2 open reading frames (ORFs), while the E6/E7 ORFs and lengthy regulatory region are intact (Arbyn *et al.,* 2018). Oncogenic proteins E6 and E7 are over-expressed when the E2 gene function is lost. E6 and E7 expression is elevated when HPV-DNA has a large copy number and HPV-DNA integration is facilitated (Cuschieri *et al.,* 2016). The initiation of morphological alterations precedes the viral integration, which happens far earlier. The integration of molecular processes with morphological characteristics does not necessarily correlate with a high-grade lesion. The expression of viral E1 and/or E2 after viral integration is not always guaranteed, however (Maria *et al.,* 2016).

PCR, fluorescence in situ hybridization, and Real-Time PCR are the most often utilized techniques for HPV integration detection. With the latter, it is possible to calculate the ratio of E2 to E6/E7 HPV gene levels. HPV integration results in a 1:1 ratio of E2 and E6/E7 genes in the viral genome (Ilhan *et al.,* 2019).

Primers specific to the E2 type are often used in HPV -16 and -18 PCR integration while primers specific to the E1 type are only utilized for HPV 16. In contrast to other methods that can discriminate between extra-chromosomal and episomal forms, the PCR approach can only detect the fully integrated form (Mitra *et al.,* 2016). If HPV integration isn't detected by PCR, a complementary test, such as fluorescence in-situ hybridization, can be employed to check for the presence of HPV in the cervical cytology specimens (Schwarzer, 2018).

4.5 Immunological Techniques for Detection of Human Papilloma Virus Infection

Antigens and antibodies may be detected in a wide range of biological samples using the ELISA (enzyme-linked immunosorbent assay), also known as the enzyme immunoassay (EIA). A labelled antibody is used to detect an antigen that has been immobilized in this method. Direct adsorption onto the test plate or indirect immobilization via a capture antibody already attached to the solid phase can both be used to achieve immobilization. If the primary antibody is labelled, then the secondary antibody can be used to accomplish the detection. When the antibody is tagged, it combines with a colorless substrate and creates a colorant. The Sandwich ELISA technology is the most extensively utilized for identifying antigens in ELISA tests (Jing *et al.,* 2019). The antigen is sandwiched between two antibodies, one of which has previously been bound to the solid phase and the other of which is enzyme-conjugated, in this setup. Visual observation or spectrophotometer measurement were both possible when the enzyme interacted with the substrate and produced a color response. The enzyme activity is proportional to the quantity of the test antigen. In situations when the antigen concentration is low or contaminated protein is present, sandwich ELISA provides a higher level of sensitivity than other Elisa methods (Jing *et al.,* 2019). For this test, no purification of the antigen is required, making it very specific and straightforward. Monoclonal antibodies, on the other hand, can only be utilized as matched pairs. Without interfering with each other's ability to attach to the antigen, they detect a variety of epitopes (Komatsu *et al.,* 2018). "Indirect" and "competitive" techniques are the most often utilized ELISA systems for antibody detection. When using the indirect technique, the antigen is directly adsorbed onto a solid phase, and the primary and conjugate antibodies are added sequentially. The amount of antigen present is indicated by the intensity of the color development between substrate-conjugate enzymes. Using an indirect ELISA, more enzymes may be identified since there are more antibody binding sites. Commercially available enzyme-linked secondary antibodies provide a wide range of options. The approach is adaptable and responsive. Multiple primary antibodies can be detected using the same enzyme-linked secondary antibody from a single species. If the secondary antibody has cross-reactivity with the primary antibody, an additional incubation phase may be necessary to avoid a non-specific signal. Both antigen and antibody detection and quantification may be accomplished using the "Competitive"technology. After incubating the antigen in the presence of the unlabeled antibody, the complex is transferred to an antigen-coated plate. Antibody-antigen-substrate complexes produce color in inverse proportion to the quantity of antigen present in the sample. Competition between the main antibody's antigen and the secondary antibody's antigen occurs in ELISA. The fewer antibodies are retained in the plate and the lower the signal, the more antigens there are in the sample. The test is precise, quick, accurate, and repeatable, even with impure samples (Li *et al.,* 2020), and it is conceivable to employ impure samples (Motevaseli *et al.,* 2016). Because monoclonal antibodies are more difficult to get and more expensive, their usage is not recommended. The sandwich assay has a low sensitivity and specificity than this method (Maria *et al.,* 2017).

HPV antigens may be detected using the ELISA technique (Alemany *et al.,* 2015). HPV16L1 capsid protein antibody and HPV16E6 and HPV16E7 oncoprotein antibodies are the topics of many studies. In terms of total viral proteins, L1 accounts for between 80% and 90% of the total (Palma *et al.,* 2018). The L1 antigen is the target of the majority of

antibody-mediated immune responses (Rocha *et al.*, 2004). Patients with high-risk HPV-associated LSIL and cervical cancer had elevated levels of anti-16L1 antibodies. While women with low-risk HPV-associated LSIL had a poor antibody response, those with no evidence of HPV infection had a high antibody response (Palma *et al.*, 2018). In addition, the L1 capsid protein epitopes can be used to separate high-risk HPV types from low-risk HPV kinds. Oncogenes E6 and E7 are linked to cancer because they alter the cell cycle to promote viral genome replication and subsequent late gene expression, which is essential for cancer progression. E6 and E7 are expressed in the majority of HPV-positive cancer cells (Komatsu *et al.*, 2018). Patients with cervical cancer had a greater level of HPV16E6 and HPV16E7 seropositivity compared to healthy individuals or those with early signs of cervical disease (de Villiers, 2014). To monitor affected women at the very end of the illness, E6 and E7 oncoproteins might be employed. Biomarker identification for early cervical cancer stages was the subject of several researches, which allowed for an early diagnosis of women at risk of infection. The identification of viral HPV16E2 and HPV16E5 proteins was investigated as a result. Cellular differentiation status influences the expression of the HPVE4 protein, which plays a key role in the viral life cycle (Schwarzer, 2018). This is the first time that HPVE2 has ever been expressed. E6 and E7 oncoprotein expression is repressed by high E2 levels. Because of the disruption of E2 activities caused by HPV genome integration, suppression of E6/E7 is not possible (Di *et al.*, 2017). There are competing views on the expression of HPV16E2 and HPV16E4. Patients with cervical cancer have increased responses to the E2 antigen, according to certain studies. Anti-E2 antibodies have been found in healthy women and those with CIN lesions (Mitra *et al.*, 2016). Pre-malignant and cancerous lesions, as well as healthy persons, all showed greater levels of E4 seropositivity (Vriend *et al.*, 2015), whereas other research reported that cancer patients had higher levels of E4 antibodies than healthy controls (Vriend *et al.*, 2015; Di *et al.*, 2016). The E4 protein was also shown to be expressed in individuals with HPV-associated high-risk and low-risk HPV, but not in those with cervical cancer (Chao *et al.*, 2020). Weakly carcinogenic HPV16E5 (Lu *et al.*, 2021) protein enhances E7's transforming activity, but nothing else is known about HPV16E5's function in the body. The HPV-16 E5 protein was identified to be expressed in the bottom third of the epithelium in LSIL by Chang *et al.* (IARC, 2011).

Because of its speed, portability, and high precision, ELISA tests are widely used. High sensitivity and specificity may be achieved with this approach, making it a good match for other radio-immunoassays (Brotman *et al.*, 2014). The Benefit of this method is that it may be done in a small laboratory with the help of automation and multiplexing (So *et al.*, 2020). There is no need for radioisotopes or a piece of expensive radiation equipment with ELISA tests, which also require a minimal volume of blood. It can accommodate a big number of animals. An enzyme/substrate reaction that requires a quick readout may provide false positive findings if the blocking solution is inefficient. It's a time-consuming procedure that requires chilled chemicals and a complicated probe design, although several kits are available. When compared to PCR, ELISA approaches are less sensitive (81.8%, 53.0%, 72.2% vs. 100%) but more specific (100%, 99.0%, 92.20% vs. 78.2%) for invasive cervical cancer (Wei *et al.*, 2021). PCR is the gold standard in cervical cancer detection.

The IgG Antibody to Human Papillomavirus Antibody IgG (PV-IgG) in human blood, plasma, or other bodily fluids may be qualitatively determined using ELISA Kit, a sandwich enzyme-linked immunosorbent test (ELISA) (Borgdorf *et al.*, 2016). Microtitre plates are used as the solid substrate for an unlabeled probe, which captures the sample's target for testing. Following the detection of this duplex, a second probe is used to look for a different part of the sequence. To reduce non-specific reactions and boost specificity, sandwiching the target between two probes might be difficult because of the larger number of processing and washing steps required (Yahaya *et al.*, 2019).

5.0 Prevention

HPV is a group of more than 150 viruses, around 40 of which are generally transmitted from person to person during intimate, or sexual, contact. HPV infections spread through sexual contact are fairly frequent and can affect both men and women. According to the Centers for Disease Control and Prevention, more than 79 million Americans are already infected with HPV, with another 14 million becoming infected each year. HPV is usually harmless and will go away on its own. However, because some HPV types can cause genital warts and others might trigger alterations in cells that can lead to cancer, it's critical to employ the following HPV prevention strategies to protect yourself from HPV transmission (Alex *et al.*, 2022).

5.1 Abstinence from Sexual Activity.

Abstinence from any sexual intercourse, including oral, anal, and vaginal sex, is the only approach to completely avoid HPV transmission. If you're not in a committed relationship, if you're in a relationship but don't feel ready to have sex, or for any other reason, you may choose to forego sex (Alex *et al.*, 2022).

5.2 Limit the Number of Sexual Partners.

Limiting the number of sexual partners is another HPV prevention approach. The more sexual partners you

have, the more HPV you may be exposed to. However, simply one sexual partner who has been exposed to HPV is enough to cause HPV infection (Alex *et al.*, 2022).

5.3 Get an HPV Vaccine.

Gardasil, an HPV vaccination, protects against the kinds of HPV that cause the majority of cervical cancers, as well as HPV-related anal, vaginal, vulvar, penile, and oropharyngeal cancers, which include malignancies of the soft palate, base of the tongue, and tonsils. Gardasil also guards against the majority of genital warts (Alex *et al.*, 2022).

The CDC recommends that all boys and girls get vaccinated at the age of 11 or 12, before they are likely to be exposed to sexually transmitted HPV strains. The vaccine, on the other hand, is safe for girls, boys, women, and men aged 9 to 45 (Centres for Disease Control, 2020).

5.4 Use of Condoms During Sex.

Condoms can help reduce the risk of HPV transmission if you are sexually active. Every sexual action should begin with the use of a condom and end with the use of a condom. Condoms do not completely protect you from HPV infection, however they do aid in HPV prevention (Centres for Disease Control, 2020).

5.5 Keep a Fit and Healthy Lifestyle.

While no one diet has been proven to prevent HPV infection or HPV-related malignancies, there is evidence that eating healthy, plant-based diet rich in naturally occurring vitamins and minerals boosts the immune system and may protect against the development of at least some cancers (Centres for Disease Control, 2020).

5.6 Regular Pap Smear:

A Pap smear, also known as a Pap test, is a procedure that includes collecting cells from the vagina and cervix, which is the lower, thin end of the uterus that sits at the top of the vagina. It's frequently combined with a pelvic examination. Pap smears are used to detect aberrant cell changes that could develop to cancer, as well as to screen for precancers. HPV is a common cause of precancers (Rebecca *et al.*, 2022).

The American Cancer Society (ACS) recommends that all sexually active women aged 21 to 29 get a Pap test every three years without an HPV test. If a woman's Pap test results are abnormal, HPV testing may be recommended (Rebecca *et al.*, 2022).

6.0 Conclusion

The field of HPV pathogenesis is expanding quickly. Many of the high-risk E6 and E7 proteins' biological targets have been discovered. Though each of these interactions must have a physiological significance, it is crucial that they do so. Low-risk E6 and E7 proteins, in contrast to those with a high prognosis, remain a mystery, even though that they must play a crucial part in the viral life cycle, and therefore further research is needed in this area. E6 and E7 protein structural studies by themselves or in conjunction with their substrates are equally relevant.

An understanding of their methods of action might be aided by this knowledge, which could then be used to improve medication development. Analysis of the crystal structures reveals a great deal of information about the actions of viral replication proteins E1 and E2. Researchers have gained insight into basic principles of replication regulation by studying the molecular interactions between these proteins and other cellular replication proteins.

This protein's involvement in plasmid segregation and chromatin modification during viral pathogenesis merits further research.

Also, under investigation is the role of the E4 and E5 proteins in modifying the late stages of the viral life cycle. It has been challenging to research HPV's differentiation-dependent life cycle because of a paucity of genetic systems and ways to differentiate epithelial cells in tissue cultures. Such assessments may now be easily carried out because of the development of new techniques. An examination of viral protein activities must be conducted in the context of whole viral genomes rather than using over-expression tests on cell lines from other species. The processes and variables that mediate viral entrance will also be a prominent focus of research.

To have a better understanding of viral tropism, new tools and methodologies are now accessible. An equally important area for investigation is the analysis of the innate and cellular immune responses to HPV infection. The lack of a good mouse model for HPV infection has hampered these studies, but these questions remain highly important and need to be addressed. Equally important as the investigation into the basic mechanisms of HPV pathogenesis is the development of effective therapeutics to treat or prevent HPV-induced disease. A significant advance in this area has been made through the use of virus-like particle-based vaccines. Initial studies suggest that this vaccine will be effective in blocking initial infection, and it is anticipated that it will prevent the development of HPV-induced malignancies. While this seems likely, cervical cancers arise from single transformed cells, and it will be important to demonstrate the effectiveness of the vaccine in this process. At the same time, it is unlikely that the vaccine will benefit individuals already infected with HPV.

The identification of drugs specifically to treat HPV infection has not been highly successful due to the

complexities of the HPV life cycle and the limited number of enzymatic activities identified for HPV proteins. The development of drug treatments for existing HPV disease is an important undertaking that deserves further attention. In this regard, the development of therapeutic vaccines is a promising area of investigation and needs to be further supported. In summary, much has been accomplished in expanding our knowledge of this important human pathogen but, more needs to be done.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

All authors have read and consented to the submission of the manuscript.

Availability of data and material

Not Applicable.

Competing interests

All authors declare no competing interests.

Funding

There was no funding for the current report.

References

Alemany L, Cubilla A, Halec G, Kasamatsu E, Quirós B and Masferrer E, (2016). Role of human papillomavirus in penile carcinomas worldwide. Eur Urol. 69, 953–61.

Alemany L, Saunier M, Alvarado-Cabrero I, Quirós B, Salmerón J and Shin HR, (2015). Human papillomavirus DNA prevalence and type distribution in anal carcinomas worldwide. *Int J Cancer*. 136:98.

Alex Vorsters, Xavier F. Bosch, Mario Poljak, Dur-e-Nayab Waheed, Margaret Stanley and Suzanne M. Garland (2022). HPV prevention and control – The way forward. IPVS Prev Med. 156: 106960.

Andrew Stevenson, Kim Kavanagh, Jiafeng Pan, Lynne Stevenson, Heather Griffin, John Doorbar, Evelyn Scott, Miriam Deeny, Kate Cuschieri and Sheila V Graham (2018) Risk stratification of cervical disease using detection of human papillomavirus (HPV) E4 protein and cellular MCM protein in clinical liquid-based cytology samples *Journal of Clinical Virology* Volume 108,Pages 19-25

Arbyn, M., Snijders, P.J., Meijer, C.J., Berkhof, J., Cuschieri, K and Kocjan B. J., (2018) Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect21:817–26.

Borgdorf H, Gautam R, Armstrong SD, Xia D, Ndayisaba GF and van Teijlingen NH, (2016). Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier. Mucosal Immunol, 9:621–33.

Borgogna, J. C., Shardell, M.D., Santori, E.K., Nelson, T.M., Rath JM., and Glover ED, (2020). The vaginal metabolome and microbiota of cervical HPVpositive and HPV-negative women: a cross-sectional analysis. *BJOG*, 127(2), 182–92.

Brotman RM, Shardell MD, Gajer P, Tracy JK, Zenilman JM and Ravel J, (2014). Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *J Infect Dis*, 210(11), 1723–33.

Centers for Disease Control and Prevention "CDC" (2020): FDA licensure of bivalent human papillomavirus vaccine (HPV2, Cervarix) for use in females and updated HPV vaccination recommendations from the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep; 59:626-9.

Chao X, Sun T, Wang S, Tan X, Fan Q and Shi H, (2020). Research of the potential biomarkers in vaginal microbiome for persistent high-risk human papillomavirus infection. Ann Transl Med.;8(4):100.

Cuschieri K, Bhatia R, Cruickshank M, Hillemanns P and Arbyn M. (2016). HPV testing in the context of post-treatment follow up (test of cure). *J Clin Virol*, 76 Suppl. 1:S56–61.

de Villiers, E.-M. (2004) Relationship between steroid hormone contraceptives and HPV, cervical intraepithelial neoplasia and cervical carcinoma. *Int. J. Cancer*, 103, 705–708

Di Paola M, Sani C, Clemente AM, Iossa A, Perissi E and Castronovo G, (2017). Characterization of cervico-vaginal microbiota in women developing persistent high-risk Human Papillomavirus infection. Sci Rep., 7(1):10200.

Documento de Consenso. Guía de cribado del cáncer de cuello de útero en Espana, (2014). Prog Obstet Ginecol.;57 Suppl. 1:1–20153

Fernandez-Duarte KP, Olaya-Galán NN, Salas-Cárdenas SP, Lopez-Rozo J and Gutierrez-Fernandez MF. (2018). Bifdobacterium adolescentis (DSM 20083) and Lactobacillus casei (Lafti L26-DSL): Probiotics Able to Block the In Vitro Adherence of Rotavirus in MA104 Cells. *Probiotics Antimicrob Proteins*, 10(1), 56–63.

IARC (2011). Human Papillomaviruses. IARC Library Cataloguing in Publication Data

Ilhan ZE, Laniewski P, Thomas N, Roe DJ, Chase DM and Herbst-Kralovetz MM. (2019). Deciphering the complex interplay between microbiota, HPV, infammation and cancer through cervicovaginal metabolic profling. *EBioMedicine*, 44:675–90.

Jing, L. S, Gopinath. C.B. Thangavel, L. Yeng Chend, Wong Ruen and Yuane Mei Yangf (2019). Target DNA detection of human papilloma virus-16 E7 gene by capture-target-reporter sandwich on interdigitated electrode sensor. *International Journal of Biological Macromolecules*, 141, 564-569.

John Doorbar, Wim Quint, Lawrence Banks, Ignacio G Bravo, Mark Stoler, Tom R Broker, Margaret A and Stanley (2016) The biology and life-cycle of human papillomaviruses *J.vaccine*. PMID: 23199966

Kim MJ, Lee DK, Park JE, Park IH, Seo JG and Ha NJ. (2015). Antiviral activity of Bifdobacterium adolescentis SPM1605 against Coxsackievirus B3. Biotechnol Biotechnol Equip.28(4):681–8.

Klymenko T., Hernandez-Lopez H., MacDonald A. I., Bodily J.M. and Graham S.V. (2016) Human Papillomavirus E2 Regulates SRSF3 (SRp20) To Promote Capsid Protein Expression in Infected Differentiated Keratinocytes *Journal of Virology* Vol. 90, No. 10

Komatsu A, Igimi S and Kawana K. (2018). Optimization of human papillomavirus (HPV) type 16 E7-expressing lactobacillus-based vaccine for induction of mucosal E7-specifc IFNγ-producing cells. Vaccine.36(24):3423–6.

Li Y, Yu T, Yan H, Li D, Yu T and Yuan T, (2020). Vaginal microbiota and HPV infection: novel mechanistic insights and therapeutic strategies. Infect Drug Resist.13:1213–20.

Lu W, Fang Z, Liu X, Li L, Zhang P, and Zhao J, (2021). The potential role of probiotics in protection against infuenza virus infection in mice. Foods.;10(4):1–16.

Maria, L. M. L., Sonia Pérez-Castrob, Manuel Rodríguez-Iglesiasc, Maria Teresa and Pérez-Graciad (2017). Microbiological diagnosis of human papilloma virus infection. Enferm Infecc Microbiol Clin. 2017;35(9):593–602

Mitra A, Macintyre DA, Marchesi JR, Lee YS, Bennett PR, and Kyrgiou M. (2016). The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome.;4:58.

Moody, C. A. and Laimonis A. L. (2010). Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer, 10(8), 550-60.

Motevaseli E, Azam R, Akrami SM, Mazlomy M, Safari M and Modarressi MH, (2016). The efect of Lactobacillus crispatus and Lactobacillus rhamnosus culture supernatants on expression of autophagy genes and HPV E6 and E7 oncogenes in the HeLa cell line. Cell J.;17(4):601–7.

Palma E, Recine N, Domenici L, Giorgini M, Pierangeli A and Panici PB. (2018). Longterm Lactobacillus rhamnosus BMX 54 application to restore a balanced vaginal ecosystem: a promising solution against HPV-infection. BMC Infect Dis.18(1):13–9.

Rebecca L Siegel, Kimberly D Miller, Hannah E Fuchs and Ahmedin Jemal (2022). Cancer statistics *CA Cancer J Clin*: 72(1):7-33. doi: 10.3322/caac.21708

Schwarzer M. (2018). Gut microbiota: puppeteer of the host juvenile growth. Curr Opin Clin Nutr Metab Care, 21(3):179–83.

Sheila V. Graham (2017) The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review *J. Clinical science* S20160786.

So KA, Yang EJ, Kim NR, Hong SR, Lee JH, and Hwang CS, (2020). Changes of vaginal microbiota during cervical carcinogenesis in women with human papillomavirus infection. PLoS ONE., 15;(9): e0238705.

Terese E. Azua, P. Y. Orka'a and R. I. Ega (2017) Prevalence of Human Papilloma Virus (HPV) Infection among Pregnant Women attending Antenatal Care at General Hospital, Minna – Nigeria *Journal of Microbiology and Biotechnology Research*216949492

Vriend HJ, Bogaards JA, van Bergen JE, Brink AA, van den Broek IV and Hoebe CJ, (2015). Incidence and persistence of carcinogenic genital human papillomavirus infections in young women with or without Chlamydia trachomatis co-infection. Cancer Med, 4(10),1589–98.

Wei ZT, Chen HL, Wang CF, Yang GL, Han SM and Zhang SL. (2021). Depiction of vaginal microbiota in women with high-risk human papillomavirus infection. Front Public Health, 8:587298.

World Health Organization. Globocan (2012). Estimated cancer incidence, mortality and prevalence worldwide in 2012. Available from: *http://globocan.iarc.fr*

Yahaya G.M., I. D., Aisha R.S. and Abdullahi B. I (2019) Human Papilloma Virus Infection and Associated Risk Factors Among Pregnant Women Attending Outpient Clinic In General Hospital Lapai, Niger State Nigeria *International Journal of Scientific and Research Publications (IJSRP)*202797073.