



# Human papilloma virus: A review study of epidemiology, carcinogenesis, diagnostic methods, and treatment of all HPV-related cancers

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## Abstract

**Background:** Human papillomavirus (HPV) infection is considered as the most common viral sexually transmitted infection worldwide. This poses an increasingly interdisciplinary medical challenge. Since there is vast scattered information in databases about HPV and the correlated diseases, we decided to collect useful data so that the experts can get a more comprehensive view of HPV.

**Methods:** In this article, HPV-associated diseases, prevalence, prevention, and new treatments are discussed. The retrieved articles reporting the latest data about the required information for our review were selected through searching in Web of Science, Scopus, Medline (PubMed), EMBASE, Cochrane Library, Ovid, and CINHALL with language limitations of English and German.

**Results:** There are 2 groups of HPVs: (1) low-risk HPV types that can lead to genital warts, and (2) high-risk HPV types that are involved in HPV-associated oncogenesis. About 70% of all sexually active women are infected and most of these infections heal within many weeks or months. In the case of HPV-persistence, a risk of preneoplasia or carcinoma exists. These types of viruses are responsible for the existence of genitoanal, gastrointestinal, urinary tract, and head and neck tumors. There is still no definite successful treatment. The detection of HPV-related condylomata occurs macroscopically in women and men, and the diagnosis of the precursors of cervical carcinoma in women is possible by Pap smear.

**Conclusion:** For extragenital manifestations, there is no structured early detection program. Meanwhile, studies on HPV vaccines confirm that they should be used for the primary prevention of HPV-dependent diseases. However, we need more research to find out the real advantages and disadvantages of vaccines.

**Keywords:** Human Papilloma Virus, Cancer; Epidemiology, Warts, Vaccines, Virology, Diagnostic

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## Introduction

Sexually transmitted infections (STIs) are initially developed by sexual contact and have a high rate of morbidity.

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### ↑What is “already known” in this topic:

There are numerous papers in the field of sexual transmitted diseases (STDs), especially HPV. However, most of them do not have comprehensive data involving the main facts. Some of them have only evaluated the epidemiology or carcinogenesis or screening methods. And some have only assessed one type of cancer correlated with HPV. Thus, there is no distinct information among concepts.

### →What this article adds:

This study presents comprehensive updated data about HPV, the laboratory methods, and all its correlated cancers. These combined data are highly useful for the clinicians and laboratory specialists. This is the main reason that we decided not to divide this article into several papers. The readers do not need to look for scattered information to find their required data. A vast part of the information is available in tables and figures that are easily reachable by the readers.

ty and mortality worldwide, affecting 50% to 70% of sexually active individuals (1, 2). Human papillomaviruses (HPVs) are a large and diverse group of epitheliotropic double-stranded DNA viruses (3). There are up to 225 types of HPVs divided into 5 groups ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\mu$ , and  $\nu$ ) (4). The exact classification of each group is shown in Figure 1. A subgroup of about 15 of the  $\alpha$ -types (high-risk (HR)-HPV types) can lead to invasive carcinomas (5, 6).

Persistent HPV infection is one of the important sexual transmitted diseases (STDs) associated with more than 5% of all cancers in the world (7). In other words, globally more than half of all malignancies related to infection are caused by HPV (8). Approximately 90% of the HPV viruses clear or become dormant in 1 to 2 years after infection. Statistics show that the majority of women who had a positive test for a high-risk HPV serotype developed cervical cancer after 3 to 5 years (9). HPVs mostly cause nonpersistent acute infections. Hence, like other oncoviruses, there is a striking gap between the times of diagnosis of the chronic infection and its early stages (10). Some studies report that infection with at least 1 high-risk HPV will occur during the lifetime of 60% of sexually active individuals (11). They are often eliminated by the immune system within 1 or 2 years after exposure (12). The virus in the remaining cases that persists for a long time affords lesions that can bring malignancies (13). Therefore, early diagnosis of HPV infection and HPV induced lesions is highly important to prevent cancer development (14). In this review, we tried to collect all data about the prevalence, mechanism of action and carcinogenesis, the correlated diseases as well as a brief overview about laboratory studies, screening, diagnosis, and therapies of each disease. This collection has been prepared by the newest data of valid databases and websites, such as Centers for Disease Control and Prevention (CDC) and human papillomavirus (HPV) centers. This information

can help us to be aware of several factors when a patient with HPV comes to us for screening or treatment.

**Methods**

**Search Syntax and Search Strategy**

All original published articles were searched in international databases, including Web of Science, Scopus, Medline (PubMed), EMBASE, Cochrane Library, Ovid, and CINHALL to retrieve all articles reporting the latest data about epidemiology, diagnostic methods, and treatment of diseases related to HPV that were not discussed comprehensively in previous studies. A search of these databases was performed by the researchers with hand searching through the reference lists and gray literature. We searched from January 1985 to March 2020, with language limitation of English and German. All related components were related to the following keywords: “Human Papilloma Virus”, “HPV”, “Epidemiology”, “Carcinogenesis”, “Diagnostic methods”, “Screening methods”, “Treatment”, “Cervical cancer”, “Cervical neoplasm”, “Cervical malignancy”, “Vulvar and vaginal cancer”, “Vulvar and vaginal neoplasm”, “Vulvar and vaginal malignancy”, “Anal cancer”, “Anal neoplasm”, “Anal malignancy”, “Esophageal cancer”, “Esophageal neoplasm”, “Esophageal malignancy”, “Colorectal cancer”, “Colorectal neoplasm”, “Colorectal malignancy”, “Prostate cancer”, “Prostate neoplasm”, “Prostate malignancy”, “Urothelial cancer”, “Urothelial neoplasm”, “Urothelial malignancy”, “Testicular cancer”, “Testicular neoplasm”, “Testicular malignancy”, “Renal cancer”, “Renal neoplasm”, “Renal malignancy”, “Penile cancer”, “Penile neoplasm”, “Penile malignancy”, “Head and neck squamous cell carcinoma”, “Head and neck cancer”, “Head and neck neoplasm”, “Head and neck malignancy”, “cutaneous squamous cell carcinoma”, “Warts”, “HPV Vaccines”, and “HPV DNA Tests”.

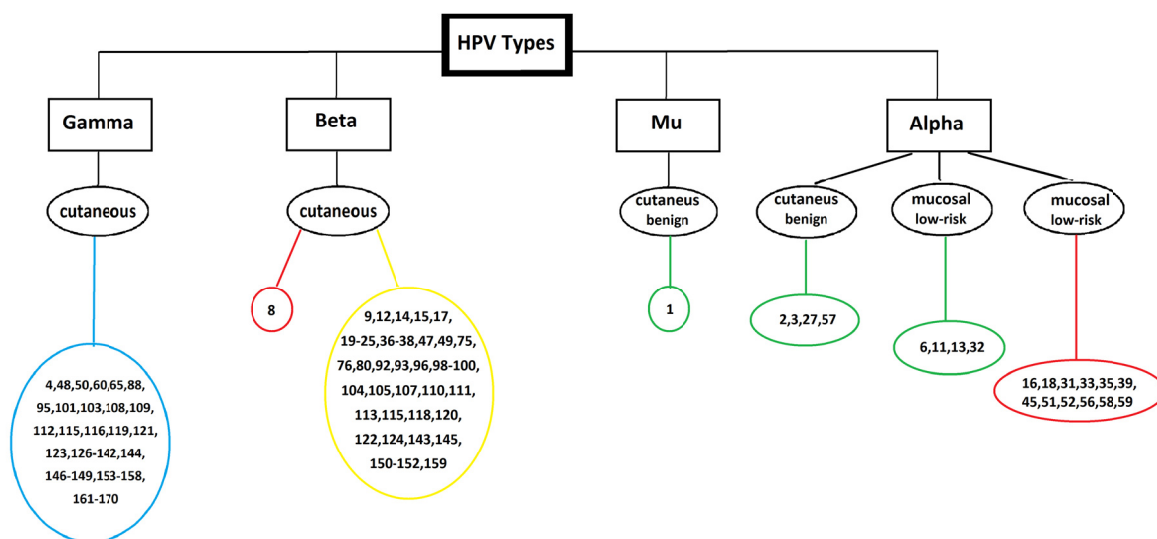


Fig. 1. HPV classification: Red color: High-risk HPVs, Green color: Low-risk HPVs, Yellow colors: probably high-risk HPVs, Blue color: Unknown

### Screening and Selection

The Reference Manager bibliographic software was applied to manage the searched citations. Duplicate entries were checked by considering the title of the published papers, authors, year of publication, and specifications of the sources types. In case of questionable records, the texts were compared. We reviewed the initial search results, and after reviewing each article by title and available abstract, some of the articles were excluded. Evaluating the papers under consideration was based on the inclusion and exclusion criteria by 2 researchers separately (Sh.N. and Maryam S.). About 546 papers were retrieved; 353 of which were selected and evaluated to extract the required information. In case of finding no data in the studies available in databases, we tried to apply valid websites such as HPV center or CDC that have been cited in references.

### Eligibility Criteria

All observational studies that assessed the prevalence of HPV and related cancers, screening and diagnostic methods, and total therapeutic managements were included. We excluded duplicate citations, oral presentation, posters, and articles where their abstract and full-text were not available. The original articles and reviews were included.

### Data Extraction

Three steps of assessment for titles, abstracts, and full-texts were done. The full-text of each selected article was retrieved for detailed evaluation. Data were extracted using a checklist involving publication year, authors, type of cancer, laboratory tests, screening methods, diagnostic methods, and treatment. All steps from search to final data extraction were followed independently by 2 research experts (Sh. N., Marzieh S).

## Results

### Epidemiology and Transmission

About 50% to 80% of sexually active females will be infected with HPV during their lifetime (15). The estimated global HPV prevalence is 11.7%. South Africa (17.4.0%), Eastern Africa (33.6%), Eastern Europe (21.4%), Western Europe (9.0%), Eastern Europe (21.4%), and Caribbean (35.4%) showed the highest HPV prevalences (16, 17). Female sex workers (FSWs) are among the most susceptible groups to acquire HPV infection and develop cervical intraepithelial neoplasia and cervical cancer. In a meta-analysis done by Farhmand et al, it is demonstrated that the pooled HPV prevalence was 42.6%. HPV-16, HPV-52, and HPV-53 were the most common high-risk HPV types found among FSWs (18). Since there are no definite antiviral treatments for HPV, the high prevalence of genital HPV has been a great concern in the world (19). Another research evaluating 645 sexually active innercity young females reported a cervical HPV prevalence of 54% (20). Assessments of 12.7 million cancers occurring globally in 2008 showed that HPV infection is related to almost 100% of cervical cancers, 90% to 93% of anal canal cancers, 12% to 63% of

oropharyngeal cancers, 40% to 64% of vaginal cancers, 40% to 51% of vulvar cancers, and 36% to 40% of penile cancers. Virtually 5% (610 000) of the cases had HPV associated with anogenital or oral cancers. Nonmelanoma skin cancers are the increased risk of cutaneous HPV types. The initial risk factor for occurring the anogenital and oral HPV infection among males and females is sexual behavior (21).

### HPV Infection and Carcinogenesis

HPV infection can be persistent and carcinogenic through integrating the viral DNA into the host genome. Then, it omits the early and late HPV genes known as E2, E4, E5, L1, and L2. Two early viral proteins have oncogenic potentials: E6 and E7 (22, 23). The high-risk HPVs (HR-HPVs) that are also carcinogens have a great ability to remain in human keratinocytes in vitro through a chronic status. These viruses penetrate the cervical epithelial cells and express the oncogenic protein E6 and E7, leading to the inactivation of host regulatory proteins p53 and then retinoblastoma protein (RB). The viral antigen is a very specific type of antigen marking tumor cells. It is presented in viral-mediated carcinogenesis. Thus, the point that should be considered is that the best target to have an antitumor therapeutic goal is this antigen that is expressed just by the infected cells. This is somehow a way to block the autoimmunity cascade. Viral antigens can also act along with tumor-associated antigens in case of the dependence of oncogenes on expressing viral oncoproteins in infected cells. Scientists try to develop anti-cancer gene therapy because of the relationship between HPV infection and cervical cancer (24). When HPV starts to integrate into the host genome, the malignant transformation occurs. Hereby, E6 and E7 are expressed. Therefore, they have been targeted by several types of vaccines. It is proven that vaccines have a satisfactory result against these antigens in HPV-induced cervical dysplasia (25-27). They are applied in the clinical trial phase to treat cervical and head and neck cancers (28). The integration of the genome to the cell is necessary to induce cervical cancer. So persistent HPV infection is not the only or sufficient factor to this point (29). HPV 18 has a great power to integrate into the host genome leading to malignancy (30-32). The fragile sites of chromosome have this integration that causes disruption to the open-reading frame of E2 and less commonly E1, E4 or E5 (29, 30). Tables 1 and 2 present complete data of the statistics as well as present screening methods, diagnostic methods, and available treatments of different types of HPV-related diseases.

### Molecular Diagnosis of HPV

The molecular techniques are the main instrument to detect HPV DNA because of hard cultivation of HPV in culture systems. Although the E1 gene is used, the L1 and L2 late genes encode viral capsid proteins are used for HPV genotypes detection. The diagnostic techniques for detection and genotyping of HPV were classified as shown in Figure 2. All the commercially available tests are listed in Table 3.

**Table 1.** The Statistics of Community, Prevalence, Incidence, Age, Types of Dependent and Mortality of HPV-related Cancers and Warts

Type of disease	Prevalence	Incidence (per year)	In which age it mostly occurs?	Related types	Mortality
Cervical cancer	The 3 <sup>rd</sup> most common cancer in women (156) From 31.6% in eastern Africa to a low prevalence of 6.2% in southeastern Asia (17)	569,847 in 2018 (156)	15 to 44 year (156)	16, 18 (157)	311,365 deaths in 2018 (156)
Vulvar and vaginal cancer	Rare (158, 159) 5.6%, 4.4% (160)	27000 and 13000 new cases in 2008 respectively (156)	Females aged 45 to 49 years old (161)	16, 18 (162)	880 and 400 deaths in 2007 respectively (162)
Anal cancer	Rare (156) 1.5 % of GI cancers (163) and more than 10% in HPV positive patients (164)	27000 new cases (165)	65-69 years old (166)	16,18 (167)	1- and 5-year mortality rates of 40% and 80%, respectively (163)
Esophageal cancer	The 8 <sup>th</sup> most common cancer (168) 11.7%-38.9% (169, 170)	500000 new cases (168)	Mean age 65 years old (171)	16, 57 (172)	406000 deaths each year (168)
Colorectal cancer	The third most common in men and 2d most common in women (173) For prevalence rate Data not found	141,270 new cases in 2016 (166)	65-79 years old (174)	16 (175, 176)	52,286 deaths each year (166)
Prostate cancer	The most common cancer in men (177) 15% (178)	160000 new cases (177)	72-74 years old (179)	16 (180)	11,710 each year in the US between 1988-92 (181)
Urothelial cancer	The 4 <sup>th</sup> most common cancer (182) 3%-50% (183-185)	150,350 new cases (182)	≥65 years old (186)	16, 18 (187)	132,432 worldwide in 2000 (186)
Testicular cancer	1% of all male cancers (188) 0.066% (166)	14.9 per 100,000 (166)	20-39 (166)	16 (189-191)	2% (188)
Renal cancer	The 6 <sup>th</sup> most common cancer in men and the 10 <sup>th</sup> most common cancer in women (160) 0.3% (166)	22.8 per 100,000 men and 11.6 per 100,000 women each year (166)	65-84 years old (166)	Data not found	About 9,000 men and 5,000 women each year (166)
Penile cancer	Rare (156) 6% (192)	22000 new cases (156)	50-70 years old (156)	16, 18, 31, 33 (193)	30% (194)
Head and neck squamous cell carcinoma	Less than 5% of all cancers (195) 6.9% (196)	644,000 new cases each year (195)	≤45 years old (195)	16 (197)	2.2% (195)
Cutaneous squamous cell carcinoma	The 2d most common form of skin cancers (198) 9-14% in men and 4-9% in women (195)	700,000 new cases each year in US (199)	In ages>35 years old (200)	β-HPV such as 5 and 8 (200, 201)	2.1% (198)
Warts	One of the common sexually transmitted Peak prevalence of 53.8% (202)	14,100,000 new cases each year (166)	Peak prevalence 20- to 24 (202)	6, 11, 42, 43, 44 (114)	Significant source of morbidity and mortality worldwide (203). (Data about the exact rate was not found)

## 1. Nucleic Acid Detection-based Methods

### 1.1. Nucleic Acid Amplification-based Methods

These methods are generally based on the polymerase chain reaction that is used for amplifying, detecting, and typing the HPV DNA by the use of degenerate primers MY09/MY11 or PGMY09/11, GP5+/6+ and SPF10 to amplify the viral capsid L1 gene (33-35).

1. The Conventional PCR-based Methods: The conven-

tional PCR based methods are single or double nested-PCR (36, 37) multiplex-PCR (38) and nested-PCR-RFLP assay (34). Then, the PCR product is amplified by targeting a type-specific DNA sequence or treatment with restriction enzyme to determine the specific sequence existing in the sample related to a specific type of HPV (39, 40)



2. The PCR Following by Hybridization-based Methods: The methods-based amplification of target DNA following hybridization include traditional PCR in situ hybridization (PISH), microplate colorimetric hybridization assay (MCHA), the linear array for HPV genotyping, and the reverse line hybridization. The PISH technique is the typical PCR performed on the slide of intact paraffin-embedded tissue, and then hybridized with specific DNA probes (41, 42). The MCHA is a method based on PCR

followed by colorimetric hybridization to type-specific probes on microplates (43). In the reverse line blot assays and the linear array HPV genotyping, after the amplifying step, the sequences are fixed on a membrane strip and detected by type-specific probe (39, 44).

3. PCR-based Fluorescent-based Array: Microarray-based HPV genotyping assays employ the PCR to amplify the viral genome fragment and then hybridize with several HPV-specific oligonucleotide probes attached on

**Table 2.** Pathogenesis, screening methods, diagnostic methods, treatment, and some useful information in brief. Abbreviations: ESCC, esophageal squamous cell cancer; EAC, esophageal adenocarcinoma; GEJAC, gastroesophageal junction adenocarcinoma; BCG: Bacillus Calmette-Guerin; CT scan, computed tomography scan; MRI, magnetic resonance imaging.

Type of Disease	Pathogenesis	Screening & Diagnostic Methods	Treatment	Additional Information
Cervix	Cervical intraepithelial neoplasia exists due to chronic infection that can lead to cervical cancer (204)	Pap smear (149) Colposcopy, cervical biopsy (149), avoid unnecessary excisions (205)	Ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA), synthetic CDCA derivatives like HS-1199 and HS-1200 and the system of the cholic acid-functionalized star-shaped PLGA-b-TPGS (CA-PLGA-b-TPGS), polymeric nanoparticles control delivery of the drug, such as Docetaxel (149)	Progression of precursor lesions for cervical cancer takes more than 10 years (160, 206, 207)
Vulvar and vaginal	Vulvar and vaginal intraepithelial neoplasia (VIN and VaIN) (208, 209)	No screening methods are available (4) Direct visual examination, biopsy and histopathological examination (210)	Surgery, external or internal radiation therapy, and systemic or regional chemotherapy (211)	The current treatment strategies, unfortunately, are not successful. The relapse rate is high (158)
Anal	Strongly links to a complex inflammatory process leading to anal cancers of squamous cell origin (167)	Anal Papanicolaou smears (pap) and Southern blotting (212) High-resolution anoscopy (HRA) (212)	An organized team to plan chemotherapy, radiation therapy and surgery (5)	It has a ratio of female to male being as high as 5:1 (165). HPV was positive in 83%-95% of patients (213). HPV is the reason for 90% of SCCA (214, 215)
Esophageal cancer	Chronic infection leads to ESCC (216)	In high incidence area regular endoscopy (171) Endoscopy and biopsy (217)	Chemotherapy, radiation therapy and surgery (217)	A significant relation between HPV and ESCC but not with EAC and GEJAC. Identification of HPV in this malignancy can be helpful for better response and outcome (216)
Colorectal cancer	Chronic infection leads to mutations in glandular cells of the colorectal mucosa of colon and rectum (218)	Fecal occult blood test and colonoscopy (219) Colonoscopy and biopsy (220)	Chemotherapy, radiation therapy and surgery (220)	Identification of HPV in this malignancy can be helpful for better response and outcome (216)
Prostate cancer	Still unclear (150)	Prostate-specific antigen (PSA) (179) Tissue biopsy (217)	Chemotherapy, radiotherapy and surgery and androgen deprivation therapy (217)	It is important to keep HPV infection in mind when encounters with unusual disease manifestations of the urogenital tract (221)
Urothelial cancer	Still unclear (222)	No recommended screening method (223) Applying CT urography or MRI for upper urinary tract evaluation and cystoscopy for lower urinary tract evaluation (224)	Intravesical chemotherapy or intravesical BCG (224)	Urothelial cancer is an overarching term that describes a number of tumors that arise from the urothelial lining of the bladder, renal pelvis, ureters, and urethra (225)
Testicular cancer	It leads not directly to testicular cancer but can provide a status of higher vulnerability induced by the tumor (226)	No recommended screening method (227) Transscrotal ultrasonography (228)	Orchiectomy, chemotherapy and radiotherapy (229)	No Additional information

Table 2. Ctd

Type of Disease	Pathogenesis	Screening & Diagnostic Methods	Treatment	Additional Information
Renal cancer	Still unclear (230-232)	No recommended screening method (182)	Surgery, chemotherapy, immunotherapy, or targeted therapy (182)	No Additional information
Penile cancer	Penile intraepithelial neoplasia (PIN) (234)	Renal CT scan with and without contrast or MRI with and without gadolinium enhancement (233) Data not found for screening methods No imaging technique can exactly detect micrometastatic lymph nodes. So, invasive inguinal lymph node diagnosis is recommended for all tumor stages from pT1G2 (235)	Surgical methods (circumcision, wide local excision, and glans resurfacing), T-cell immune checkpoint inhibitors, and HPV genome targeting strategies (236, 237)	Penile SCC has four subgroups: warty, basaloid, keratinizing, and verrucous. Only the first two groups (warty and basaloid) are related to HPV (238, 239)
Head and neck squamous cell carcinoma	Still unclear (240-242)	P16 IHC, FISH, HPV genome detection in biopsy specimens (243) Endoscopy (nasopharyngolaryngoscopy, esophagoscopy, and bronchoscopy, as appropriate) and biopsies (195)	EGFR TKI and low-dose radiation, trans-oral robotic surgery, many reduction surgeries and post-operative adjuvant therapies based on pathologic staging (244)	HPV-positive OPSCCs have a better therapeutic response and prognosis (240-242)
Cutaneous squamous cell carcinoma (CSCC)	CSCC is mostly seen in patients with epidermodysplasia verruciformis (EV). EV is an autosomal recessive genodermatosis existed through mutations in EVER 1 and EVER 2 genes (200, 201) Available in the text.	A total-body examination of the skin is the only screening test available (195) Biopsy (195)	Surgical excision, cryotherapy, curettage, electrodesiccation, topical treatments (e.g. imiquimod, 5-FU, ingenol mebutate, diclofenac, and retinoids) and radiation therapy (245)	Although most of CSCC have an excellent prognosis, some of them are susceptible to have poor outcomes (198)
Warts (Condyloma acuminata)	Available in the text.	Regular physical examination, cytology/viral detection (246) Histologic examination of biopsy specimens (118)	Topical and systemic. Trichloroacetic acid (TCA) is the best (124, 125)	Available In the text.

the surface of an insoluble supporter like bead or DNA chip (45-53). The suspension array genotyping assays use bead-based technology, which is based on the use of polystyrene beads dyed with 2 spectrally distinct fluorophores (red and infrared), and each bead set is coupled with a specific oligonucleotide probe for 1 HPV type. The HPV sequences are amplified, denatured, and hybridized with the bead-bound probes. Then, hybridized biotinylated amplicons that are labeled by using phycoerythrin and streptavidin are served as a reporter fluorophore. The bead sets are then read and analyzed on a Luminex analyzer (54-60).

4. The Real-time PCR-based HPV Genotyping-based Methods: The real-time PCR is reliable and sensitive, with high accuracy and validity in HPV-DNA detection and genotyping. Also, the viral load quantification and the capability of multisample qualification with different fluorochromes is the advantage. In this method the fluorescent probes in cooperation with PCR primers allow for quanti-

fication of the viral genome and are presented in a sample as the name "viral load" (61, 62) (63-65). The cobas 4800 HPV test uses multiplex real-time PCR and nucleic acid hybridization with 4 different fluorescent reporter probes that concurrently detects the L1 gene (64, 66).

5. HPV E6/E7 mRNA-based Screening Assays: The detection of viral mRNA is based on transcription-mediated amplification of full-length E6/E7 transcripts accomplished by target capture. Reverse-transcriptase-PCR incorporates a RT step following real-time quantitative PCR. The oncoproteins E6 and E7 are the most relevant transcripts for diagnostic and carcinogenesis follow-up. (67, 68). The main techniques used to detect mRNA for E6/E7 oncogenes are 3 commercial assays shown in Table 3 (45, 68-71).

### 1.2 Signal Amplification

Signal amplification describes methods-based probe molecule, which are hybridized to the target nucleic acid

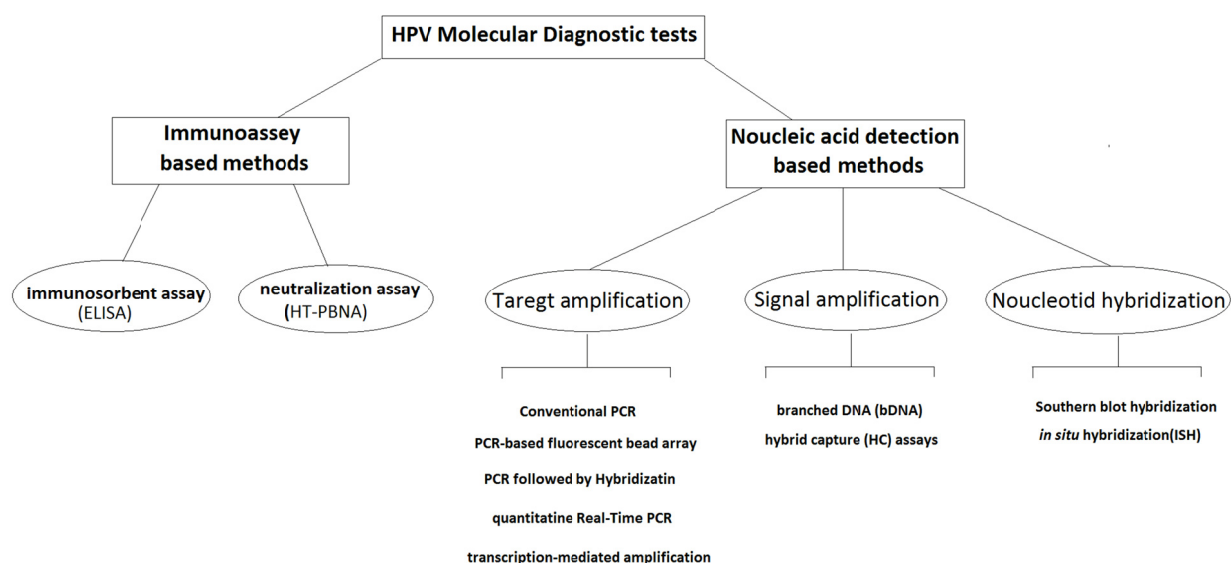


Fig. 2. The schematic classification of diagnostic techniques for detection and genotyping of HPV

sequence and generate the signal related to amplification rate (72). Signal amplification technologies include branched DNA (bDNA) and hybrid capture (HC) assays (73, 74). The hybrid capture method is the most widely-used signal amplification method that briefly samples DNA hybridized with the cocktail of RNA probes. The RNA-DNA hybrids indicate the presence of HPV DNA and subsequently it is revealed by the nonradioactive signal-amplification method to aid detection (73-76). The Digene HCII technology served as the second version of hybrid capture (77). The bDNA assay directly measures nucleic acid molecules and is based on binding the subset of “target probes” bound to specific nucleotide sequences (exist in the sample) as in situ hybridization (bDNA ISH) (72, 74). The Cervista HPV test is a signal amplification method that uses 2 types of isothermal reactions, which is briefly based on the enzymatic cleaves the FRET oligonucleotides between the fluorophore and quencher molecule, resulting in the production of a fluorescence signal (39, 42, 74).

### 1.3 Nucleotid Hybridization-based Methods

The nonamplified HPV techniques include in southern/dot blot hybridization, and in situ hybridization (ISH). This method is generally time-consuming, requires more skill, necessary equipments, and is not as sensitive and reliable as the molecular methods. briefly, in HPV DNA detection by southern blot, the sample extracted DNA is digested by restriction enzymes and then runs in agarose gel electrophoresis to separate the digested DNA based on the size, and then is transferred to a nitrocellulose or nylon membrane and finally hybridized with cloned HPV genomic probes labeled with isotopic ( $P^{32}$ ) or nonisotopic (digoxigenin) techniques. The detection procedure in ISH

occurs right on the fixed nuclei of infected cells (in situ) and hybridization reaction is evaluated microscopically (78, 79) In comparison with PCR, the blot hybridization-based method has higher specificity but is less sensitive (42, 80, 81).

### 2. Immune-biochemical-based Methods

1. HPV serology — ELISA assay: The serological tests to determine the HPV virus are performed based on VLPs (virus-like particles)(82-84), and the sensitivity is about 50%(85). Three forms of Elisa method include (1) Direct assays binding to HPV VLPs on the microplate; (2) indirect assays binding to HPV VLPs to the microplate via anti-VLP antibodies; and (3) Competitive assay by which the antigen is coated on fluorescent beads (Luminex-based assays) exposed to the sample (54, 58, 86).

2. HPV Neutralization Assay: High-throughput pseudo-virion-based neutralization assay (HT-PBNA) with excellent repeatability and run-to-run reproducibility was developed for HPVs (86). HPV neutralization assays rely on neutralization of one of the following items: authentic virions, pseudotyped virions that are capsids carrying a reporter gene on their surface, and pseudovirions (PsVs) that have encapsidated reporter genes to assess anti-VLP conformational antibodies neutralizing activity. The neutralization assays are the gold standard to assess the protective potential of antibodies induced by HPVs vaccines in experimental systems (87-89).

### The Potential Biomarkers for HPV Detection

The biomarkers related to high-risk HPV infection can help to enhance the sensitivity of cervical cytology screening, reduce false-negative diagnoses, monitoring and prognosis of related diseases.

Table 3. The classification of commercially available diagnostic techniques for the detection and genotyping of HPV

The classification of diagnostic techniques for detection and genotyping of HPV			The commercial available
detection based methods nucleotide acid	Nucleic acid amplification	Conventional PCR based methods	❖ PCR, Nested-PCR, multiplex-PCR, PCR-RFLP
		PCR following by hybridization	❖ The LINEAR ARRAY HPV Genotyping Test (Roche), ❖ AMPLICOR HPV test (Roche), ❖ INNO-LiPA HPV Genotyping Extra (Innogenetics) ❖ Microplate colorimetric hybridization assay (MCHA)
		PCR-based fluorescent bead array	❖ Microarray-based HPV genotyping assays ○ The PapilloCheck HPV Screening Test(greiner bio), ○ Clart HPV 2 – papillomavirus clinical arrays( Genomica), ○ HPV GenoArray Test Kit ( HybriBio), ○ GeneTrack HPV DNA Chip( Daejeon), ○ GeneSQUARE HPV Microarray,( Kurabo) ○ INFINITI HPV-HR QUAD (AutoGenomics), ○ PANArray HPV Genotyping Chip( Panagene), ○ GG HPVCHIP ( Goodgene) ❖ Suspension array based HPV genotyping assays ○ The Multiplex HPV Genotyping Kit (Multimetrix, Heidelberg) ○ digene HPV Genotyping LQ Test(Qiagen)
	Signal amplification	Real-time PCR based HPV genotyping	❖ Human papillomavirus Real-Time PCR Kit ( GenoID), ❖ Abbott RealTime High-Risk HPV(Abbott molecular), ❖ QIAScreen HPV PCR(QIAGEN) ❖ TRUPCR® HPV High Risk Kit (TRUPCR Diagnostics) ❖ cobas 4800 HPV test (Roche) ❖
		HPV E6/E7 mRNA-based screening assays	❖ PreTect HPV-Proofer (NorChip), ❖ NucliSENS EasyQ HPV V1 assay (bioMérieux) ❖ APTIMA HPV Assay (Gen-Probe) ❖
	Nucleotid hybridization	Signal amplification technologies include branched DNA (bDNA)	❖ Branched DNA (bDNA), initially developed by Chiron and marketed by Becton Dickinson, and recently by The Quantivirus(®) HPV E6/E7 RNA 3.0 assay
		Hybrid capture (HC) assays	❖ hybrid capture (HC) assays ( Digene Corporation , QIAGEN) ❖ Cervista HPV test ( Invader) ❖
		Southern blot hybridization	❖ Just use in research laboratory
	Immune-biochemical based methods	in situ hybridization (ISH)	❖ No commercial product were found and Just use in research laboratory
		HPV Serology-ELISA assay	❖ Biocompare, Cusabio, Abbexa, Creative-diagnostics,
HPV Neutralization assay		❖ high-throughput pseudovirion-based neutralization assay (HT-PBNA) ❖ No commercial product were found	

The potential biomarker in cervical cancer includes in p16INK4a, Ki-67 that are the target of E6 and E7 oncoprotein (90-93). There are many molecular targets of E6 and E7, such as Wnt/β-catenin/Notch (94, 95), PI3K/AKT/mTOR pathway (96), P53, and PRb(97, 98). The molecular targets of HPV E5 oncoprotein are the cell surface receptors like EGFR (99, 100), p21Waf1/Sd1/Cip(101), p27KIP1(102), COX-2, VEGF, and Cav-1(102). Besides, the host microRNAs have been affected by HPV proteins E5, E6, and E7. Increased expression of miR-16, miR-25, miR-92a, and miR-378, and

decreased expression of miR-22, miR-15a, miR-15b, miR-21 and miR29a miR-16, miR-27a, miR-29a, and miR-100 are attributed to viral oncoprotein E6 or E7 (103-108). The miRNA-944(109) and miRNA-155(110) overexpression as well as downregulation of miRNA-375 (111) can potentially be served as a biomarker for cervical cancer follow-up. Dysregulation of miR-375/AEG-1 Axis by HPV high-risk 16 and 18 E6/E7 promotes cellular proliferation, migration, and invasion in cervical cancer (112).



### Warts

Condyloma acuminata (CA) or wart is one of the most common sexually transmitted diseases in the world (15). The incubation period is 3 weeks to 8 months, and the clinical manifestation takes 2 to 3 months (113). The viruses producing genital warts are usually the low-risk types of HPV: 6, 11, 42, 43, and 44. types 6 and 11 are detected in 90% of cases. A study in 2009 has reported that 31% of patients with CA have a high-risk HPV as coinfection (114). A cohort study in 2019 reported that the high-risk HPV genotypes could also be associated with warts. Particularly, a large number of patients with CIN2-3 and milder lesions with CIN1 were carriers of a virus of a HPV-HR genotype (115). Typically, CA is mostly discrete, skin-colored, brown or whitish, pedunculated, or broad-based with a variation in size from 1 millimeter to several centimeters (116). They are usually found on the external genitalia, perineum, perianal area, and oral cavity (117). Warts are most often diagnosed through their clinical appearance. Laboratory tests for the presence of HPV are not recommended for the diagnosis of CA. Histologic examination of biopsy specimens can be performed to rule out intraepithelial or invasive squamous cell carcinomas SCCs, which can coexist with or appear similar to anogenital warts (118). Their feature in histologic evaluation involves epidermal hyperplasia, parakeratosis, koilocytosis, and papillomatosis (119). Unfortunately, there is not any definite antiviral therapy to treat anogenital warts. Recurrence rate is high (25%-65%), which is may be due to the widespread infection and subclinical lesions (120). Also, most of the treatments are time-consuming and uncomfortable (121). Since there are no specific therapy options, the therapist should consider many points for the treatment such as the number, size, morphology, anatomic location, patient preference and side effects (122). The available therapies are effective for 60% to 90% of nonimmunosuppressed patients (121, 123). About trichloroacetic acid (TCA), it is investigated that 71% to 79% of cases regressed from high grade squamous intraepithelial lesion (HSIL) to low-grade squamous intraepithelial lesion (LSIL) or complete resolution when using TCA (124). The current treatments used for warts are as below:

1. Topical, destructive, surgical: podophyllotoxin and podophyllin, imiquimod, sinecatechins, intralesional immunotherapy with skin test antigens, cidofovir, 5-fluorouracil (5-FU), TCA, cryotherapy, potassium hydroxide, surgical excision, laser therapy, and photodynamic therapy

2. Systemic: interferon, isotretinoin (125).

### The Risk of Infertility

Infertility means the inability of a couple to have a gestational process after 1 year of unprotected sex. Approximately 10% to 20% of couples at the reproductive age are suffering from infertility. Also, 50% of the reasons for infertility among couples relate to male factors, such as sexual dysfunction, congenital dysplasia, endocrine disorders, varicocele, immune factors, and sexually transmitted infections (126-128). It is established that there is a correlation between cervical disease and pregnancy complica-

tions (129). Nonetheless, we do not have any definite evidence recognizing the influence of HPV infection on pregnancy, such as preeclampsia, preterm labor, or fetal growth restriction (130). In men, the story is as in women. The literature supporting a link between HPV and infertility is still controversial (131). Seminal HPV infection is common worldwide, which may be correlated with the risk of male infertility through affecting sperm abnormalities, such as low sperm count and motility. Some studies have reported that HPV16 and 52 in men and HPV 58 in women are the most common types of HPVs leading to infertility (24, 132).

### Vaccination

There is no definite therapy for HPV infection. Sometimes it is eliminated on its own. Some scientists believe that it cannot be cleared completely and may only switch to undetectable levels (133). We have several types of therapeutic vaccines evaluated in preclinical and clinical trials: live vector, protein or peptide, nucleic acid, and cell-based vaccines (134). However, 3 HPV vaccines are currently available in the vaccination program: cervarix (bivalent vaccine for HPV 16, 18), gardasil (quadrivalent vaccine for HPV 6, 11, 16, 18), and nonavalent vaccine (for HPV 6, 11, 16, 18, 31, 33, 45, 52, 58). These vaccines can target between 2 and 7 oncogenic HPV serotypes (135). They promise, in the long-term (30-50 years), to reduce the incidence of disease associated with HPV vaccine types (136). Some studies have proved that HPV vaccination is a secure and efficient method to prevent cancer (137, 138). Steben et al in 2018 evaluated 10 years of clinical experience in Canada. The results indicated that the prevalence of HPV types 16, 18, 6, and 11 was lower in qHPV-vaccinated than unvaccinated individuals (1.5% vs 11%). The risk of anogenital warts incidence had a decrease by up to about 45% in vaccinated population in cohort studies and the incidence of cervical intraepithelial neoplastic had a significant decrease by up to 86%. These researchers concluded that the programs of HPV vaccination constitute an effective and useful public health initiative (139). Unfortunately, there is limited vaccination coverage of large populations in the world. Therefore, a gross unvaccinated population remains at a high risk of HPV-associated disease (14). Additionally, some researches have demonstrated that current vaccines will not be useful for preventing all types of HPV-related cancers (140). Also, the HPV subtype distribution in cervical cancer varies throughout the world (141, 142). Japanese scientists in 2019 reported a significant number of adolescent girls complaining of unusual symptoms after HPV vaccination. The vast majority of them had psychiatric illness in the absence of any pathologic findings in radiological images or laboratory tests. So the recommendation of vaccination was withdrawn by the Japanese Ministry of Public Health (143). At present, the vaccination program is implemented in Argentina, Australia, Austria, Belgium, Bhutan, Brazil, Brunei, Darussalam, Canada, Colombia, Cook Islands, Czech Republic (the), Denmark, Fiji, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Japan, Kiribati, Latvia, Lesotho, Luxembourg, Malaysia, Marshall Islands

(the), Mexico, Micronesia, Netherlands, New Zealand, Norway, Palau, Panama, Paraguay, Portugal, Rwanda, San Marino, Singapore, Slovenia, Spain, Sweden, Switzerland, United Kingdom of Great Britain, the former Yugoslav Republic of Macedonia, Uganda, and Northern Ireland, Trinidad and Tobago, United States of America (the), and Uruguay (144).

### Discussion

HPV transmission risk is increased among multi-partners. It can originate from a genital area and extend to other parts like the anus region. Neonates can be infected by their mothers during pregnancy or the delivery period through vertical and perinatal transmission (145). The transmitted couples are sexually more active with a history of not using condoms regularly. It shows that condoms can be effective, along with the prophylactic vaccines. Despite the 3% of transmitting couples, more than 50% of nontransmitting couples presented that during the previous 4 months they used condoms in 100% of intercourse (146). So condoms can be used to protect against HPV but since they do not cover every possible HPV-infected area of the body, they cannot offer full protection (147). According to a cohort study done by Hernandez et al, genital transmission from women to men occurs more frequently than from men to women. The primary source of transmission to the cervix is the penis. The cervix and urethra are the primary sources of infection to male genitals. Sexual transmission implicates also the scrotum, the anus of women, and the hands of both genders. In heterosexual transmission the anus of women plays an important role as a major source and target. It is found that HPV transmission does not definitely influence the target organs because it depends on the tissue or genotype differences or both (146).

Epidemiologist research finds that HR-HPVs play an important role in cervical cancer and bacterial vaginosis (148, 149). After the integration of the genome, E6 oncogene is expressed. Many cells have changed by the E6 protein, leading to inhibition of apoptosis and increased telomerase function. This is why E6 protein can prolong cellular lifespan. It functions as a transcriptional activator accompanied by this prolonged lifespan and transforms cells (150). HR-HPV E7 allows the cell to increase its transforming activities. Therefore, human keratinocytes are immortalized because they interact with factors that regulate cell growth (151). Based on the our results, the expression of E6 and E7 can be blocked by E2. After disrupting, it enables uninhibited E6 and E7 oncoprotein activity (31). The differences between low-risk and high-risk HPVs in producing warts or cancers is mainly in E6 and E7 function. In low-risk HPVs, E6/E7 expression stimulates cell cycle entry in the upper epithelial layers (differentiating cells), allowing genome amplification in both low and high-risk HPVs. Also, basal cell proliferation may still be stimulated by growth factors, but not E6/E7. There is little or no expression of E6/E7. About high-risk HPVs, E6/E7 expression stimulates additional cell cycle entry and cell proliferation in the basal and parabasal epithelial layers leading to neoplasia. E6/E7 inhibit immune

response to tolerate viral gene expression (152). HPV vaccines provide a promising primary approach to prevent malignancies. Because HPV acquisition generally occurs soon after first sexual activity, vaccine effectiveness will be lower in older age groups because of prior infections. Evidence suggests that although HPV vaccination is safe for adults aged 27 to 45 years, population benefit would be minimal; nevertheless, some adults who are not adequately vaccinated might be at risk for new HPV infection and might benefit from vaccination in this age range. Vaccination is routinely recommended at age 11 or 12 years; it can be given starting at age 9 years. Although catch-up HPV vaccination is recommended for all persons through age 26 years who are not adequately vaccinated, it is not recommended for all adults aged >26 years. Instead, shared clinical decision-making regarding HPV vaccination is recommended for some adults aged 27 to 45 years who are not adequately vaccinated. HPV vaccines are not licensed to be used in adults aged >45 years (153).

### Strengths

This was a comprehensive review study involving 353 valid publications evaluating all important aspects of HPVs and their related cancers. Most of the studies have evaluated only 1 or 2 types of cancers correlated with HPVs. The need for easily accessible comprehensive data in this field seemed to be necessary. Thus, we tried to provide such data for clinicians and laboratory specialists working on HPV infection.

### Limitations

The major limitations of this review were lack of data in some countries and nonvalid data in developing countries. Some papers had poor or unclear information or evaluation. In some cases, it was not possible to find valuable data. Thus, we applied the phrase "not found" for such cases.

### Conclusion

About 50% to 80% of sexually active females will be infected with HPV during their lifetime. The global HPV prevalence has been estimated to be about 11.7%. The major burden of HPV infection is the carcinogenic effect of high-risk HPVs. Since there is no definite treatment for HPV, the high prevalence of genital HPV has been a great concern in the world. At present, vaccination has been introduced as the best prevention and treatment method for HPV infection. If complete effectiveness of vaccination is expected, future vaccines should be multivalent for all described oncogenic HPV types. Nonetheless, these vaccines will be much more expensive than current formulations (14). Although some researchers have reported positive clinical effectiveness of vaccines in reducing malignancy, large population-based clinical studies of these vaccines are necessary to assess the true impact of vaccination (135, 154, 155). We believe that routine use of HPV vaccines needs much more care and assessment because there are many doubts and questions about these vaccines.

**Conflict of Interests**

The authors declare that they have no competing interests.

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