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Human Papillomavirus: Detection Method and Infection

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Abstract. The Human Papilloma Virus (HPV) is a small non-enveloped DNA virus, around 8000 bp in size and only humans become its host by infecting skin epithelial tissue, human oral mucosa and anogenital epithelium. Human Papillomavirus is often found in patients and is ranked as the second most malignant disease in women, belonging to the Alphapapillomavirus genus. HPV infection can be identified through the structure of the HPV virus itself and the particles contained therein which initiate the carcinogenic process of its host. The research methods used in this study are literature studies. The literature study method is a series of activities related to the method of collecting library data, reading, recording and managing research materials. The method often used to detect HPV is the polymerase chain reaction (PCR). The viral cell reproduction process is aided by activated proteins E1, E2, E4, E5, E6, and E7, which also delay apoptosis and alter the host cell cycle in various ways to cause cell alterations that result in cancer cells.

Keyword: Human Papilloma Virus (HPV), Detection Method, Infection.

Abstrak. *Human Papilloma Virus (HPV) merupakan virus DNA non-envelop yang kecil ukurannya berkisar 8000 bp dan hanya manusia yang menjadi inangnya dengan menginfeksi jaringan epitel kulit, mukosa mulut manusia maupun epitel anogenital. Human Papillomavirus banyak dijumpai pada pasien dan menduduki peringkat kedua penyakit paling ganas pada wanita, masuk dalam genus Alphapapillomavirus. Infeksi HPV dapat diketahui melalui struktur virus HPV itu sendiri dan partikel yang terkandung didalamnya yang menginisiasi proses karsiogenik inangnya. Metode penelitian yang digunakan dalam penelitian ini adalah studi literatur. Metode studi literatur merupakan rangkaian kegiatan yang berkaitan dengan metode pengumpulan data pustaka, membaca, mencatat dan mengelola bahan penelitian. Metode yang sering digunakan untuk mendeteksi HPV yaitu polymerase chain reaction (PCR). Aktivasi protein E1, E2, E4, E5, E6, E7 membantu proses replikasi sel virus, mencegah apoptosis, dan mempengaruhi siklus sel inang sehingga terbentuk mutasi sel yang menyebabkan sel kanker.*

Kata Kunci: *Human Papilloma Virus (HPV), Metode Deteksi, Infeksi.*

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1. Introduction

HPV is a double-stranded DNA virus that has 8000 bp, is not enveloped, and belongs to the Papillomaviridae family. The HPV genome consists of 8 open reading frames, namely 6 early genes and 2 late genes. Of the 16 genera belonging to the Papilomaviridae family, 5 of them infect humans, namely Alphapapillomavirus, Kapapillomavirus, Gammapapillomavirus, Mupapillomaviurs and Nupapillomavirus. The human papillomavirus that is most commonly found in patients is in the genus Alphapapillomavirus [1].

HPV can cause warts to form on various infected parts of the body. Based on previous studies, there are 60 types of HPV that cause warts that infect other parts of the body, such as the feet and hands, but among them 40 types trigger the appearance of genital warts. HPV is very dependent on the life cycle of its horpes because it is unable to synthesize its own enzymes, usually the nature of enzymes in general. Exploited The HPV genome is covered by an icosahedral capsid (T=7) with a diameter of 55nm, encoding only 8 genes (8 ORFs), namely 6 genes in the Early gene (ER) and 2 genes in the Late gene (LR). Early Region (ER), contains genes that code for non-structural proteins. The early region (ER) encodes proteins for viral forms or replication processes, viral transcription and regulation of viral genes as well as oncogenes. Human Papillomavirus has 6 genes in the Early Region (ER) and 2 genes in the Late Region (LR), and in LR it is formed in L1 and L2 proteins. The major capsid protein L1 is a 55kD protein that has the ability to form itself into a virus-like particle (VLP) [2].

There are 6 L1 proteins that function as intermediaries for the reaction between the virus and Tissue specific heparin sulfate proteoglycan (HSPG) in the extra cellular matrix during the initial HPV infection. [3]. This is consistent with the viral life cycle where L1 will encapsulate the viral genome in the nucleus during the replication phase, forming viral progeny that can initiate infection. The major capsid protein L1 is a target protein for the humoral immune response and is useful as a marker for active HPV infection with HPV. L1 can transform itself into VLP which is immunogenic and stimulates antibody reactions [4]. The viral genome also encodes proteins that stimulate cell cycle entry and cell proliferation, as well as proteins that mediate the viral genome for replication, virus assembly, and may also be effective in virus release and transmission [5].

2. Research Purpose

This study aims to determine the molecular technique detecting HPV and the infection in human.

3. Methods

The research methods used in this study are: literature studies. The literature study method is a series of activities related to the method of collecting library data, reading and recording, and managing research materials.

4. Result and Discussion

A. Molecular HPV Detection Methods

HPV does not grow on conventional culture media and serological diagnostic methods have limited accuracy. The diagnosis of HPV infection is made by histopathology of lesions or detection of viral DNA in infected cells. There are a few molecular techniques that are employed to detect HPV DNA:

Nucleic acid-hybridization assays

The Southern Blot is a technique for detecting DNA amplification in a particular DNA sample. This method combines the agarose gel electrophoresis process to measure DNA according to the size of the electrophoresis DNA fragment transferred to the membrane. The membrane used in the southern blot process is the nitroselulosa membrane. In the following steps, the membrane will be hybridized using a specific test. In the beginning of HPV testing, Southern Blot was used to analyze the HPV genome. Southern Blot has a high sensitivity for detecting HPV in specific clinical specimens or identifying the HPV type [6]. Weaknesses of the nucleic acid hybridization method are that it takes a long time, requires a relatively large amount of pure DNA, and cannot use degraded DNA [7].

Signal-amplification assay

The Hybrid Capture II system (HC-II) is a non-radioactive signal-amplification method based on the hybridization of the target HPV-DNA to labeled RNA probes in solution. This test detects 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) or 5 LR-types (6, 11, 42, 43, and 44) [7]. The HC-II technique is used to detect the possibility of someone being infected with HPV before the virus makes changes to the cervix that can lead to cervical cancer. HC-II has high accuracy in detecting HPV infection because it can detect the presence of very small amounts of HPV DNA. In general, HC-II is a DNA-RNA-based technique that can detect accurately and quickly with a sensitivity of 98% and a specificity of 98% [6].

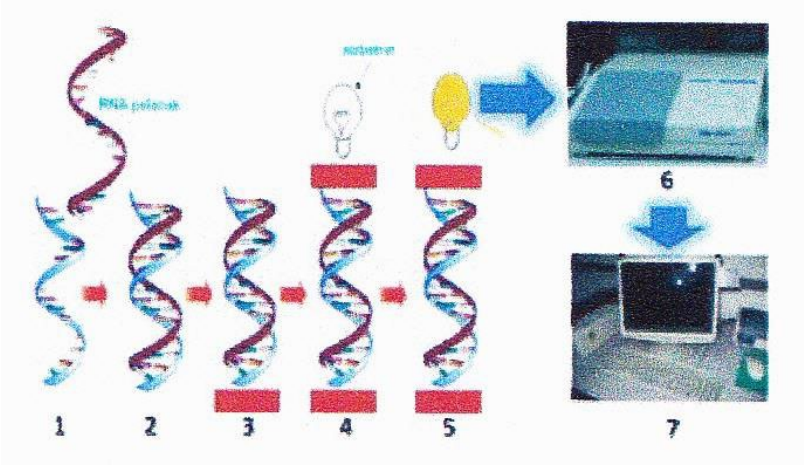


Figure 1. DNA that has been denatured (1), Hybridization of viral DNA with an RNA probe (2), Specific antibodies bind to hybrid DNA-RNA (3), The antibody binds to hybrid DNA-RNA, which will react with alkaline phosphatase (4), This reaction produces a chemiluminescent signal, followed by signal amplification in the form of emission (5).

Nucleic acid-amplification methods

The PCR-based techniques are highly sensitive, specific, and widely used. In a conventional PCR, the thermostable DNA polymerase recognizes and extends a pair of oligonucleotide primers that flank the region of interest. In the final process, the PCR can generate one billion copies from a single double-stranded DNA molecule after 30 cycles of amplification [7]. Detection of the presence of HPV by using PCR has been successfully carried out in several previous studies, especially by building primer pairs that are specifically capable of detecting genes in HPV. In general, primers that have been designed have the target of detecting high-risk HPV viruses and/or detecting more than one type of HPV in a single PCR process. One example of a primer that includes a broad primer is GP5(+) / GP6(+) [8]. After amplification, the genotype of the HPV can be determined separately, using techniques such as restriction fragment length polymorphism (RFLP) [7].

The method of restriction fragment length polymorphism (RFLP) is frequently employed to find changes at the DNA level. Based on the potential for variations in the length of the target DNA fragment created after being cut with a restriction enzyme, RFLP detection is conducted. The following are the phases of the PCR-RFLP approach for identifying HPV that causes cervical cancer: 1. Collecting cell samples, specifically epithelial cells in the cervix. 2. DNA isolation. This DNA isolation aims to collect DNA in cell samples. HPV DNA detection HPV DNA strands are detected using a bagian from the L1 (conserved) region with a panjang of 450 base pairs. The area in question was chosen because it has been affected by the HPV infection mechanism, which has resulted in increased humoral resistance to various types of HPV virus. DNA HPV detection is carried out by amplifying certain HPV sequences using primer MY09/114, which is typically

used in this process. 4. Sequencing results of the PCR products were then carried out for sequencing analysis and processing of the sequenced data using several software programs and bioinformatic analysis. 5. The PCR results are then electrophoresed on polyacrylamide gels with specific enzymes. DNA that is cut into pieces to form DNA fragments can be visualized using a UV transilluminator to see the type of HPV present in the cervical epithelial cells, so that it can be seen whether there is a potential for cervical cancer [2].

B. Genome group and Gene Expression

The gene promoter site is only activated after infection with a host cell. After infecting the host cell, the papillomavirus DNA performs transcription (DNA becomes RNA). After the transcription process, the primary mRNA produced contains all the ORFs. ORF is the region that encodes a protein or polypeptide. This mRNA can code for more than one protein or polypeptide because it has six ORFs (E1, E2, E4, E5, E6, and E7), and all of them are capable of coding for proteins or polypeptides. The type of RNA that is characterized is known as polycistronic RNA [9]. This polycistronic RNA consists of two introns (non-coding sequence of DNA) and three exons (coding sequence of DNA). After the production of this RNA, it undergoes post-transcriptional modification in alteration mechanism more specifically splicing and produces multiple mRNAs. Splicing is a mechanism where the introns are removed [10].

The promoter site of late region (L) activates when DNA replication (DNA to DNA) takes place. After transcription, late region also produces polycistronic messenger RNA. This mRNA also consists of three exons and two introns. In transcription process, this mRNA codes L1 and L2 protein which is essential for the formation of capsid of this virus. Post- transcriptional modification in late region is also splicing, and in this case, it is regulated by some splicing factors of host and cis-elements of RNA[10]

The E1 protein helps the viral genome get ready for replication. The E2 protein controls transcription and preserves the structure of the viral genome. While the E5 protein alters the activity of the receptor, the E4 protein promotes viral multiplication and suppresses the cytoskeleton for the method of releasing viruses from differentiated cells. The key carcinogenic elements of the HPV genome are the E6 and E7 proteins. The p53 tumor suppressor protein is bound by the E6 product, which induces the cell to enter the S phase of the cell cycle, inhibits apoptosis, and enhances the capacity of the cell to undergo transformation [11].

The E5 protein can interact with pathways that induce the epidermal growth factor receptor (EGFR). Overexpression of EGFR will regulate gene transcription and modulate cell proliferation, apoptosis, angiogenesis, tumor invasion, and metastases through the Ras-Raf-MAP kinase and PI3K-Art pathways. Expression of E5 can also reduce the expression of MHC/HLA class 1, which facilitates the mechanism of evasion of the virus against the immune response. UV-

induced apoptosis can also be inhibited by E5 proteins by activating the MAP kinase and PI3K-A pathways. Finally, the E5 protein can inhibit hydrogen peroxide-induced apoptosis [12].

The E6 protein's major target is p53. Due to DNA damage, P53 functions as a transcription factor that can halt the cell cycle or start apoptosis. P53 levels will rise in response to DNA damage to start apoptosis or cell cycle arrest. According to Figure 2, the E6 protein interferes with this process by interacting with p53 and the E6-associated protein ligase (E6AP), which results in the ubiquitination and destruction of p53 [11].

P53 tumor suppressor gene plays a role in controlling the cell cycle and the cessation of growth due to DNA damage. Mutationof the p53 tumor suppressor geneis the most common aberration in human malignancies. There is a correlation between the expression of HPV E6 protein and p53 protein in cervical adenocarcinoma. The increase in HPV E6 protein expression is usually in line with the increase in p53 protein expression [13].

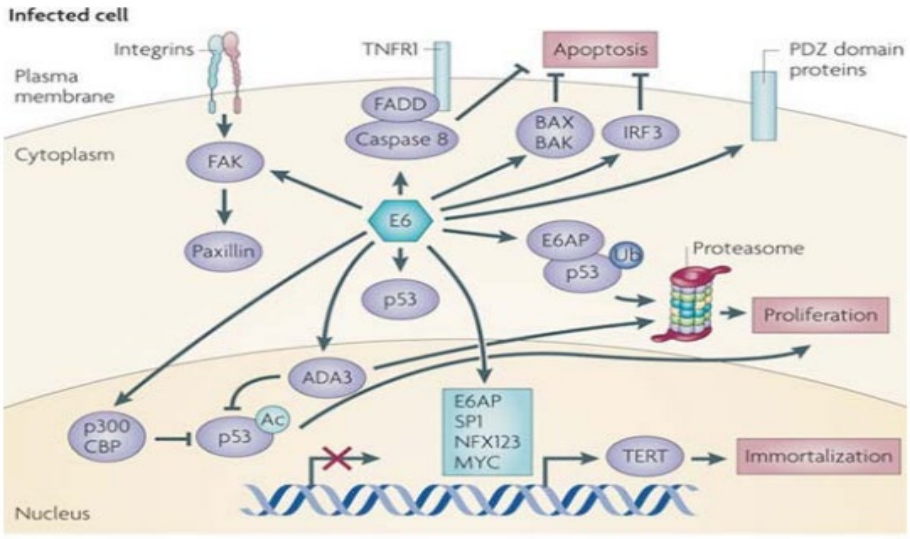
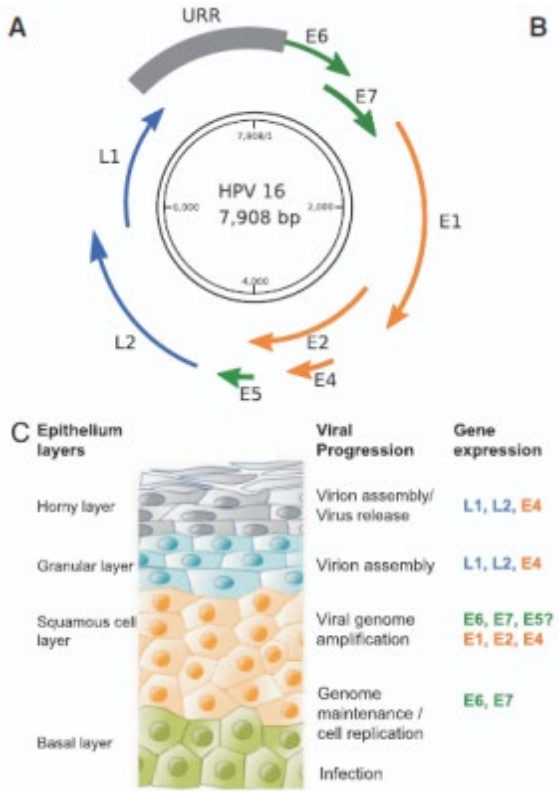


Figure 2 The E6 protein inhibits P53 to stop growth and carry out apoptosis which leads to genome instability and accumulation of cellular mutations. The E6-E6AP-P53 complex will culminate in the degradation of p53, and the E6 interaction also inhibits apoptotic signaling. The interaction of E6 with SP1, MYC, NFX123, and E6AP activates TERT and telomerase, preventing telomere shortening. E6 modifies the IFN response by interacting with IRF3 and inhibiting p53 activity. FAK: focal adhesion kinase; ub; ubiquitin.

E7 protein is a multifunctional oncoprotein, this protein can inhibit differentiation and activate the cell cycle. The main target of the E7 protein is the retinoblastoma protein (pRb). This protein binds to the transcription factor E2F which regulates the transition from the G1 phase to the S phase. Activated E2F will make the cell cycle enter the S phase. The E7 protein has a function that is analogous to pRb phosphorylation so that the viral protein will bind to pRb and release

E2F. This leads to the activation of E2F-responsive genes that encode both the cell cycle and viral replication. The Papilloma virus double-stranded DNA genome is depicted in the following schematic (figure 2a and 2b). The following describes the functions of the various types of genes in the Papilomavirus (figure 2c) [12].

HPV infection is one of the main causes of cervical cancer. HPV infection generally resolves spontaneously. The condition of immunocompression is closely associated with persistent HPV infection which can progress to precancerous lesions and malignancy with the role of proteins E6 and E7 [14].



B

Locus	Function
URR	-Harbours transcription factor-binding sites -Controls gene expression
E6	-Promotes cellular immortalization by degradation of p53 -Modifies cell adhesion and differentiation by degradation of TAp63 and p73
E7	-Promotes pRb degradation, permitting cell progression to S-phase of cell cycle -Induces chromosomal instability
E5	-Induces cell proliferation -Contributes to evasion of apoptosis -Downregulates MHC expression
E1	-ATP-dependent DNA helicase activity -Role in viral DNA replication
E2	-Coactivator of viral DNA replication -Transcrition repressor of HPV E6 and E7 -Regulates cell cycle and apoptosis -Interacts with chromatin for segregation of viral genome
E4	Binds cytoskeletal proteins and disrupts cytoskeletal structure of the G2 arrested cell
L2	-Minor capsid protein -Recruits L1 protein for virus assembly
L1	-Major capsid protein -Can autoassemble into VLPs

Figure 3. (A) A schematic depicting the double-stranded DNA genome of the Papilloma virus, for example HPV 16, illustrating the positions of the E, L, and URR genes (B) A summary of the functions of the various types of genes in papillomavirus Green: oncogenesis; orange: viral replication gene; blue: viral capsid gene. (C) A schematic depiction of the life cycle of HOV 16.

In HPV16, an intron of E6 ORF does not remove and endure intact after splicing. This intron codes E6 oncoprotein in translation and also expresses E7 oncoprotein. The viral genome s E2 ORF and enters into host genome, and thus, repression of E2 on both E6 and E7 is prevented. When this viral genome enters into host genome, the expressions obtained by E6 and E7 are increased, which help cells to proliferate and lead to malignancy [10].

C. Types of Infections Caused by HPV

Cancer in vagina, anus, vulva, penis, and most important in cervix is cased due to HPV infections. The HPV16, HPV18, HPV31, and HPV45 are known to be highly risky for all the types of previously said cancers. Some infected patient's immune system does not respond after this infection and this will enhance the growth of cancers. Additional effects such as cigarette smoking enhance the chance of getting cancer associated with HPV [15]. According to Chakraborty *et al*. types of cancers associated with HPV infections are as follows cervical cancer, genital cancer, oropharyngeal cancer, lung cancer and cancer on head and neck.

Cervical cancer

HPV infection is the foremost cause of cervical cancer, which is the leading cancer in India and second utmost common cancer worldwide. Approximately, all the types of cervical cancers are caused due to the infection of HPV. In 70% of cervical cancers, the HPV18 and HPV16 infections are found to be responsible. About 41–54% cervical cancers are found to be occurring for the HPV16, the one strain which is mostly malignant In general, in cervical cancer, the rate of transformation of normal cells into cancerous cells is relatively slower. The persons infected with HPV more than a decade or a much longer duration are more prone to having cervical cancers [10].

4. Conclusion

The Human Papilloma Virus (HPV) is an 8000 bp-sized, non-enveloped DNA virus. Molecular approaches such as target amplification techniques, signal amplification techniques, and nucleic acid hybridization procedures can all be used to detect HPV (PCR). The viral cell reproduction process is aided by activated proteins E1, E2, E4, E5, E6, and E7, which also delay apoptosis and alter the host cell cycle in various ways to cause cell alterations that result in cancer cells. The types of cancers associated with HPV infections are cervical cancer, genital cancer, oropharyngeal cancer, lung cancer and cancer on head and neck.

Reference

[1] Savira, M. "Biologi Molekuler Human Papilloma Virus," *JIK*, jilid 11, no 1, hlmn 1-6, 2017.

[2] Erni, E. and Wathon, S. "Pengembangan Sistem Deteksi Hpv (Human Papilloma Virus) Berbasis Marka Molekuler Pcr-Rflp," *BioTrends*, vol. 9, no.2, 2018.

[3] Kines RC, Thompson CD, Lowy DR, Schiller JT, Day PM, "The Initial Steps Leading To Papillomavirus Infection Occur On The Basement Membrane Prior To Cell Surface Binding," *Proceedings of the national academy of the united states of America*, vol 106, no 48, hlmn 20458- 20463, 2009.

[4] Egawa N, Egawa K, Griffin H, Doorbar J, "Human papillomaviruses; epithelial tropisms, and the development of neoplasia." *Viruses,* vol 7, no 7, hlmn 3863-90, 2015.

[5] Wang, J. W. and Roden, R.B, "L2, the minor capsid protein of papillomavirus," *Virology,* vol 445, halman 175-86, 2013.

[6] Lipinwati, "Diagnosis Molekuler Human Papilloma Virus (HPV) Penyebab Kanker Serviks," *Jambi Medical Jurnal*, vol 2, no 1, hlmn 78-86, 2014.

[7] Abreu, A.L.P., Souza, R.P., Gimenes, F. and Consolaro, M.E.L, "A Review of Methods For Detect Human Papillomavirus Infection," *Virology Journal*, vol 9, 2012.

[8] Wahjudi, M., Setiawan, E., and Tofnastri, E.N, "Penggunaan Gen E6 Sebagai Target Deteksi Human Papillomavirus Tipe 11 dengan Metode Polymerase Chain Reaction," *Jurnal Farmasi Klinik Indonesia*, vol. 9, no. 3, halman 205-218, 2020.

[9] Schiller, J.T., Day, P.M., Kines, R.C, "Current understanding of the mechanism of HPV infection," *Gynecol Oncol*, vol 118, 2010.

[10] Chakraborty, S., Das, R., Mishra, V., Sharma, N., and Khurana, N, "Human Papillomavirus And Its Nature Of Infection: An Overview," *Asian Journal of Pharmaceutical and Clinical Research*, vol 11, no 6, halaman 12-16, 2018.

[11] Efthimios D, Aikaterini G, Nikolaos A, Vasileios K, Anastasia V, Konstantinos D, "HPV infection: Immunological aspects and their utility in future therapy, *Infectious diseases in obstetrics and gynecology*, halaman 1-9, 2013.

[12] Zhao Q, Yorgo M, Katrina H, Victoria T, Yuan M, Yang W, *et al*, "Disassembly and reassembly of Human Papillomavirus virus-like particles produces more virion-like antibody reactivity," *Virology Journal*, vol 9, no 52, 2012.

[13] Alia, R., Mastutik, G., & Hoesin, F, "Ekspresi protein HPV16 E6/18 E6 dan protein P53 pada adenokarsinoma serviks," *Patologi*, halaman 26-30, 2016.

[14] Gultom, D. A, "Patogenesis Human Papillomavirus (HPV) Dalam Onkogenesis Kanker Serviks dan Pengembangan Vaksin Pencegahannya," *Kemenkes RI*, halaman 134-147, 2021.

[15] Alam S., Conway M.J., Chen H.S., Meyers C, "The cigarette smoke carcinogen benzo[a]pyrene enhances human papillomavirus synthesis," *Journal of Virology*, vol 82, no 2, halaman 1053-1058, 2008.