Jurnal Kesehatan As-Shiha
Avalilable Online https://ejurnal.umri.ac.id/index.php/JKU/index

Detection of Gene E6 HPV 18 With Real Time PCR (Polymerase Chain *Reaction)* Using Isolated Samples of Uterine Cancer Patients in Riau Province

Dewi Gulyla Hari[1]*, Marlina[2], Andani Eka Putra[3]
[1]Departement Of Pharmacy, University of Muhammadiyah Riau
[2]Faculty of Pharmacy, Andalas University
[3]Faculty of Medicine Andalas University

ARTICLE INFORMATION

Received: 1 December 2021

Revised: Month, 15 December 2021

Available online: 31 December 2021

KEYWORDS/KATA KUNCI

Gene E6, HPV 18, cervical cancer, RT PCR

CORRESPONDENCE

E-mail:

lyla@umri.ac.id

A B S T R A C T

One indication that there has been malignancy in cervical lesions due to exposure to *high-risk* hpv types such as HPV 16 and 18 is the detection of oncogene DNA in the cervical tissue of cancer patients. The oncogenes are the E6 and E7 genes. Previous research data showed evidence that the prognosis of HPV 18 is more massive than that of other *high-risk* HPV types such as HPV 16. Thus, the effort to detect the E6 gene from biopsy samples of patients *suspected of* cervical cancer is the right and effective step in order to establish an accurate diagnosis of cervical cancer itself, especially caused by HPV 18.

INTRODUCTION

Until now HPV 16 and 18 are still considered as the main etiology of cervical cancer *(IARC 2012a).* Although most genital HPV infections are not realized and can resolve on their own, persistent infections can gradually turn into malignancies characterized by lesions in the cervix (Villa *et* al., 2000; Arroyo *et al*, 2012). In 2015, WHO released data stating that in developing countries (including Indonesia), cervical cancer is included in the top 5 cancers mostly served by women (estimated to be > 85%). Especially for cancer cases in Indonesia, quoting on Riskesdas data conducted in 2013, after breast cancer then the second position is occupied by cervical cancer.

The obstacle in knowing exactly how much the prevalence of cancer in Indonesia (including cervical cancer) is because until now there has never been a study or survey in the field coordinated systematically, on a national scale, comprehensive and done massively. The information obtained so far is usually only sourced from government referral hospitals, the majority of which are on the island of Java. For example, the data for the top three most HPV types obtained from RSCM Jakarta are HPV 16, 18 and 52 (44%, 39% and 14%). Research on HPV types conducted in Jakarta, Tasikmalaya and

Bali, obtained the top three HPV types, namely 52, 16 and 18 (23%, 18% and 16%) (Schellkans et *al*, 2004; Boer *et al, 2005;* Vet *et al* 2008). The data on HPV in other areas in Indonesia outside Java such as Sumatra, Kalimantan, Sulawesi, Papua and other areas can be said to be very minimal or almost non-existent.

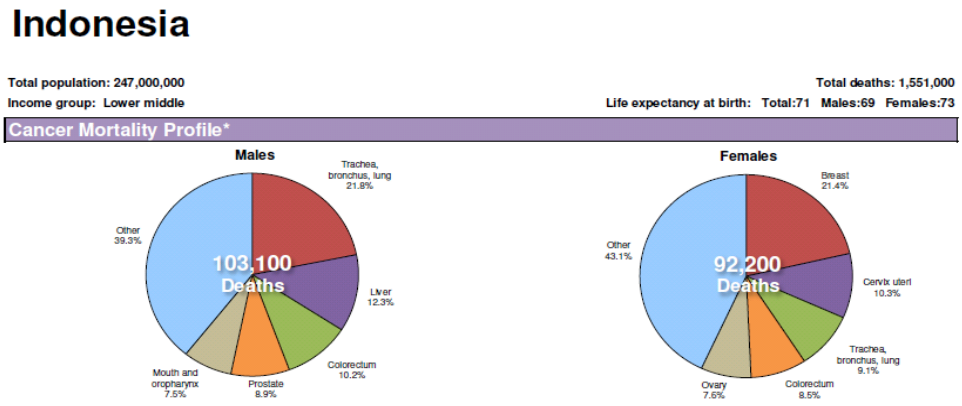


Figure 1. Number of cancer deaths in Indonesia

(Excerpted from WHO – *Cancer Country Profilles,* 2014)

Morphology of HPV

Papillomavirus is a member of the family *Papovaviridae* which usually infects humans and animals. If this virus is present in humans, it is known as Human *Papillomavirus* or commonly abbreviated as HPV. HPV is relatively small, un webbed, with a diameter of 52-55 nm. It has an icosahedral capsid composed of 72 capsomeres and contains 2 capsid proteins namely L1 and L2. Each capsomer is pentamer-shaped from the major L1 capsid protein. Each capsid virion contains some copies (about 12 per virion) of the protein capsid minor L2. When viewed with an electron microscope, the virus resembles a golf ball *(IARC Monographs Volume 90).*

The HPV genome consists of double-strand molecules, with circular DNA containing approximately 7,900-8,000 bp incorporated with *histones.* All *Open Reading Frames (ORFs),*i.e. proteins that code sequencing are located on a single strand. This genomic function is divided into 3 regions, namely: (i) *Upstream Regulatory Region (URR),* (ii) Early *region (ER)* and (iii) *Late region.* In ER there are 6 viral proteins that play a role in replication and malignancy, namely E1, E2, E4, E5, E6 and E7 while in LR there are L1 and L2 proteins that function in encoding to produce viral capsids (de Viller *et al,* 2004).

Malignant E6 begins with the interaction between E6 and p53 which is a gene suppressor tumor. p53 has an important role in protecting the genome by causing apoptosis in damaged cells or by inducing *cell-cycle arrest* until the damaged cell's DNA is repaired. The E6 gene has a target of p53 degradation through *the ubiquitin pathway* mechanism, preventing apoptosis of damaged cells and allowing cancer cells to replicate (Lechner *et al.*,1994; Boulet *et al*,2007; Kao *et al*,2012; Nedecky *et al*, 2013).

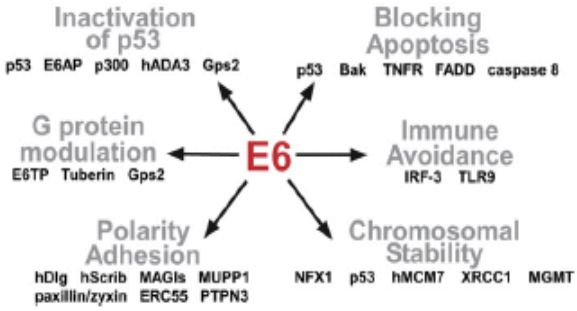


Figure 2. Mechanism of work E6 (quoted from Howie et al, 2009)

METHOD

Materials and Tools

HPV 18 DNA that has been identified genotype, Consensus primers GP5+/GP6+ and MY09/11, primary forward and reverse specific HPV 18, primary specific gene E6 HPV 18 design forward are: 5'-AGA AAC ACA CCA CAA TAC TAT GGC G-3' and reverse design: 5'-GTC GGG CTG GTA AAT GTT GAT-3', SYBR® safe DNA stain gel (Invitrogen ®), Mastermix PCR (Promega ®) with details of its composition are Nuclease Free Water 1.25 mL and Mastermix PCR which is contains taq DNA polymerase at pH 8.5 inside reaction buffer, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl2, DNA ladder 100 bp (Vivantis®), TopVision Agarose (Thermoscientific®), buffer 10x Tris Boric acid EDTA or TBE (Thermoscientific ®).

The method used in this study is descriptive analysis. The samples came from 15 HPV 18 DNA isolates, then identified the E6 gene. Amplification using PCR (Brian J. Morris, 2005; Piana et al,2009).

Procedure

1). Amplification is carried out on Sample Isolate using PCR methods For this amplification process it takes as much as 2.5 µL primary forward and 2.5 µL primary reverse, mastermix PCR (brand Promega®) as much as 12,5 µL, 5 µL templet DNA and Nuclease Free Water to sufficient the volume until the total obtained as much as 25 µL. Initial denaturation for 5 minutes to obtain profil amplification PCR is done at a temperature of 94oC, denaturation for 30 seconds at a temperature of 95oC, annealing for 30 seconds also at a temperature of 55oC, elongation for 1 minute at a temperature of 72oC and final extension for 10 minutes at a temperature of 72oC. The entire stage of the process is designed for 35 cycles.

2). The amplification results of sample are visualized by the method of eletrophoresis PCR amplification analysis is performed by electrophoresis method using 1.5% agarose gel that has been given SYBRdye®. The next step is visualization of safe DNA gel using a UV Transluminator.

Research Ethics

Samples were obtained from several regions in Riau province. This sample is a collection from the UNAND Padang HPV Research Center and has received approval from the UNAND ethics team. The sample used in this study is a biological sample that does not require a review of ethics.

RESULT AND DISCUSSIONS

Total isolated samples detected have HPV DNA with CONCENSUS PRIMERS GP5 +/6+ and MY09/11 as many as 45 pieces, of which 15 samples are HPV type 18. Running PCR conducted using a specific primary E6 gene against all 15 HPV sample isolates 18, found that the E6 gene was detected in 11 sample isolates that were ampted by the presence of tape at the position of 700 bp. There are four of 18 HPV sample isolates that do not show bands with primary specific E6 genes (likely caused by damage to the target gene DNA).

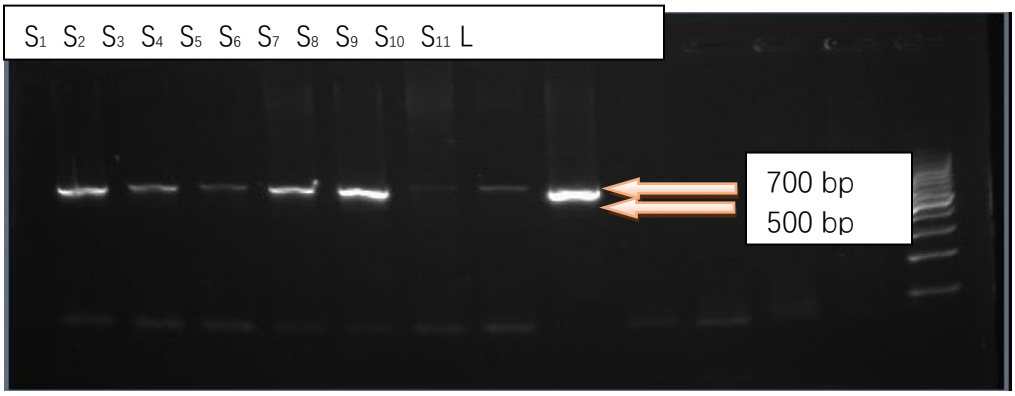
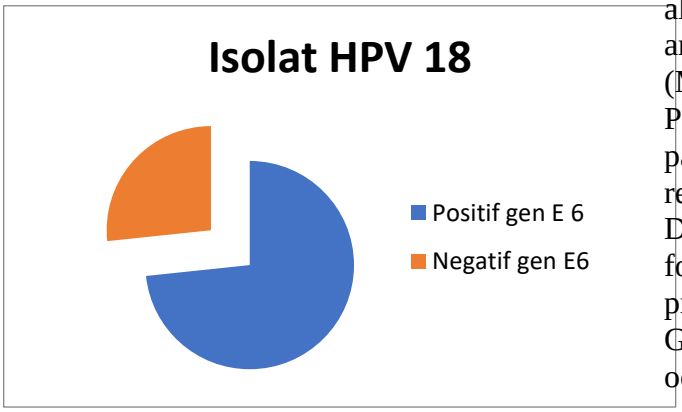


Figure 3. Electrophoresa results of detection of the HPV E6 gene 18

Description :
L : ladder
S_{1-11} : detected sample of E6 gene with specific primer

Table 1. Diagram showing the percentage of samples detected by the E6 gene and those not with a specific primer



The PCR method or also known as reaksi polimerase chains reaction is a method based on the synthesis of enzymatick in vitro to amplify nucleotides. With metoda PCR this can be doubled the number of target DNA sequences up to10^6-10^7 times the original number. Every times cycle of the PCR will produces 2^n target DNA. The main principle of the PCR mechanism of work is to condition that amplification occurs only in the target DNA sequence by selecting a specific primer. Specific primary selection is also very helpful in minimizing amplification on non-target DNA sequences (Morris, *2005;* Piana *et al, 2009).* With PCR it is also possible to synthesizea particular region exponentially against a region of DNA using 2 special designs of DNA fragments (primers). This primer will form two terminal ends for the amplification process of the target nucleic acid molecule. Generally the amplification reactiary that occurs in PCR is very specific.

PCR amplification is actually a cyclical process in which a DNA sample is initially denatured to separate double helix DNA into single strands. This is usually achieved by heating a DNA sample in a watery environment, usually at a temperature of 94°C for 30 seconds to 5 minutes. Hibridization of the primary oligonucleotides specific to each strand is

achieved by lowering the temperature of the reaction mixture to *annealing* temperature (Tm) which is usually set between 40°C and 65°C (depending on the design of the oligonucleotide sequence used as the primer). Once primary hybridization is complete, the temperature is raised to about 72°C, (the optimal temperature of DNA polymerase for replicating the DNA strand) and this entire cycle is then repeated about 20 to 50 cycles. Once each replication cycle is complete, each newly synthesized double-stranded DNA molecule (known as an amplimer or amplicon) will contains terminal sequences that are complementary to the primary sequence used. This process allows each amplimer to be a *template* for replication at a later spin resulting in double amplification (exponential amplification) of the number of target molecules during each cycle (Verkuil et al., 2008). PCR is still the *"gold standard"* for diagnosing HPV, where the target DNA is selectively amplified (Molijn et *al*, 2005). But there is still the possibility of contaminants from outside or even amplifying nucleate material from other samples.

References

Andrea Piana, Giovanni Sotgiu, Paolo Castiglia, Stefania Pischedda, Marco Dettori, Clementina Cocuzza, Elena Muresu, Alessandro Maida, Molecular methods for the detection of human papillomavirus infection : new insights into their role in diagnostics and epidemiological surveilance, Italian Journal of publich health, volume 6, number 2, 2009.

Arroyo SL, Basaras M, Arrese E, Herna´ez S, Andı´a D, et al. (2012) Human Papillomavirus (HPV) genotype 18 variants in patients with clinical manifestations of HPV related infections in Bilbao, Spain. Virol J 9: 258. doi:10.1186/1743-422X-9-258.

Boulet, G., Horvath, C., Broeck, D. V., Sahebali, S., & Bogers, J. (2007). Human papillomavirus: E6 and E7 oncogenes. *The international journal of biochemistry & cell biology*, *39*(11), 2006-2011.

Branislav Ruttkay-Nedecky, Anna Maria Jimenez Jimenez, Lukas Nejdi, Dagmar Chudova, Jaromir Gumulec, Michal Masarik, Vojtech Adam and Rene Kizek, Relevance of infection with human papillomavirus : The role of the p53 tumor suppressor protein and E6/E7 zinc finger proteins (Review), International Journal of oncology, Vol 43, 2013

Brian J. Morris, Cervical human papillomavirus by PCR : advantages of targetting the E6/E7 region, Clin Chem Lab Med 2005.

De Boer, M. A., Peters, L. A., Aziz, M. F., Siregar, B., Cornain, S., Vrede, M. A.,& Fleuren, G. J. (2005). Human papillomavirus type 18 variants: histopathology and E6/E7 polymorphisms in three countries. *International journal of cancer*, *114*(3), 422-425.

Ethel-Michele de Villiers, Claude Fauquet, Thomas R. Broker, Hans Ulrich Bernard and Harold zur Hausen, Classification of papillomaviruses, Virology 324 (2004) 17-27

Heather L. Howie, Rachel A. Katzenellenbogen, Denise A. Galloway, Papillomavirus E6 Proteins, Virology 384, 2009

IARC Monographs Volume 90

JNI Vet, MA de Boer, BE, BEWM van deb Akker, B Siregar, Lisnawati, S Budiningsih, D Tyasmorowati, Moestikaningsih, S Cornain, AAW Poters and GJ Fleuren, Prevalence of

human papillomavirus in Indonesia : a population-based study in three regions, British Journal of Cancer (2008) 99, 214-218.

Maaike C. Schellekens, Anneke Dijkman, Mohammad Farid Aziz, Budiningsih Siregar, Santoso Cornain, Sandra Kolkman-Uljee, Lex A. W. Peters and Gert Jan Fleuren, Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia, Gynecologic Oncology 93 (2004) 49-53.

Mark S. Lechnert and Laimonis A. Laimins, Inhibition of p53 DNA Binding by Human Papillomavirus, International Journal of Virology, July 1994.

Basic Health Research 2013, Results of Health Research and Development, Ministry of Health of the Republic of Indonesia, 2013.

Villa LL, Sichero L, Rahal P, Caballero O, Ferenczy A, et al. (2000) Molecular variants of human papillomavirus types 16 and 18 preferentially associated with cervical neoplasia. J Gen Virol 81: 2959–2968.

WHO, Evidence based recommendations on Human Papilloma Virus (HPV), Vaccines Schedules , Background paper for SAGE discussions, March 11, 2014

WHO/ICO, 2015

Wynn H. Kao, Sylviel, Beaudenon, Andrea L. Talis, Jon M. Huibregtse and Peter M. Howley, Human Papillomavirus Type 16 E6 Induces Self-Ubiquitination of the E6AP Ubiquitin-Protein Ligase, Journal of Virology, July 2000