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RESEARCH ARTICLE

Genetic diversity in the *E6* and *E7* gene of human papillomavirus type 16 among cervical cancer patients in Riau Province, Indonesia

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Abstract

Cervical cancer mostly occurs due to persistent high-risk human papillomavirus (HPV), with type 16 being the most frequent. In Indonesia, cervical cancer ranks second in mortality, with a fatality rate of 57%. The *E6* and *E7* genes of HPV-16 play crucial roles in the virus's oncogenic transformation, leading to cervical cancer. This study was conducted to determine the prevalence and genetic diversity of the *E6* and *E7* genes of HPV-16 in Riau Province, Indonesia. There were 37 HPV-positive samples analyzed using the MY09/11 primers. This was followed by the amplification of the *E6* and *E7* genes. Nineteen samples were detected as HPV-16, thirteen of which were coinfected with HPV-18. Seven *E6* and *E7* sequences were aligned compared with the reference sequence NC_001526.4. The most common nucleotide changes in the *E6* gene, 7318A>G, was detected in 30.7% of samples, leading to an amino acid change from 65N>S. Three nucleotide changes were identified in the *E7* gene of sample 89: two synonymous (7831T>C, 7837T>G) and one non-synonymous (7989A>G), resulting in an amino acid change of 29N>S. The most frequent *E7* nucleotide change, 7708G>A, was found in 80% of samples. Phylogenetic analysis revealed that HPV-16 isolates from Riau have close kinship with the European lineage, with 57.1% (*E6*) and 85.7% (*E7*). In conclusion, the incidence of cervical cancer in Riau Province caused by HPV-16 is 52.8% and 7318A>G and 7708G>A are the most common genetic diversity. Furthermore, the majority of HPV-16 isolates in Riau Province show close kinship with the European lineage.

1. INTRODUCTION

Cervical cancer is in the top four most prevalent malignancy suffered by women worldwide. According to statistical data from the Global Cancer Observatory (GLOBOCAN) of the World Health Organization (WHO), the global incidence of cervical cancer in 2022 reached 662,301 new cases. The Asian continent exhibited the highest incidence and fatality rates. In Indonesia, 2022 data indicate that cervical cancer ranks as the second most prevalent malignancy among women, with

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1,964 cases and a mortality rate of 56%. Cervical cancer remains a major health issue, characterized by high prevalence and substantial mortality both globally and in Indonesia (1,2). Over 95% of cervical cancer is associated with chronic infection by high-risk Human papillomavirus (HPV) (3). HPV type 16 and 18 are genotypes with the highest oncogenic potential, accounting approximately 70% of cervical cancer cases. HPV-16 is responsible for over 65% of worldwide cervical cancer incidences (4-6). Global preventive measures for cervical cancer have been established through the HPV vaccine program and have shown significant effectiveness. Countries with high vaccination coverage show a significant decrease in the incidence of HOV-related disease, including cervical lesions and genital warts. HPV vaccines targeting high-risk genotypes, such as HPV-16 and HPV-18, demonstrate sustained effectiveness for up to 9.5 years and have a well-established safety profile. However, worldwide vaccination coverage is inconsistent, with striking differences found between high-income and low-income countries (7). Therefore, it is important to understand the genetic diversity of HPV-16 in areas with low vaccination coverage and high cervical cancer incidence, such as Indonesia, as this can provide insights into the evolution of the HPV virus and how it evades the immune system against the vaccine.

The HPV genome is a circular double-stranded DNA, approximately 8 kb in length, containing an upstream regulatory region (URR) and two open reading frames (ORFs). The ORF consists of early (E) and late (L) regions. The early ORF is expressed during the early stage of viral cycle and encodes six early genes include *E6* and *E7*. Simultaneously, the late ORF is expressed at the end of the viral cycle and encodes L1 and L2 genes. The main driver of cell development to malignancy is the oncogenic activity of the *E6* and *E7* genes, which disrupt tumor suppressor mechanisms. The *E6* gene inhibits the tumor suppressor protein p53 and the *E7* gene inhibits the retinoblastoma protein (pRb) (4,8,9). Additionally, *E6* and *E7* interact with several host cell proteins to inhibit cellular differentiation, promote viral DNA replication, and escape host immune responses (4,10).

Genetic diversity in HPV-16, particularly in the *E6* and *E7* genes, can significantly affect oncogenic potential and the transformation of cervical lesions to invasive cancer. These genes play a crucial role in cervical carcinogenesis by inactivating primary tumor suppressor pathways. Meanwhile, the *E7* protein inactivates the pRb leading to dysregulation of the cell cycle and increased risk of genomic instability (11,12). The *E6* gene is an oncogene that is 450 bp long and encodes protein with 151 amino acids involved in cellular transformation. Single-nucleotide polymorphisms (SNPs) have been widely reported throughout the *E6* gene, indicating exposure to diversifying selection. Research shows that mutations in *E6* may alter its antigenic properties and immunogenicity, thereby enhancing transformation ability of the virus. The *E7* gene, an oncogene measuring 297 bp, encodes a protein of 98 amino acids. A big scale epidemiological studies have shown that genetic variation in *E7* gene in cervical cancer patients is lower than in the control group, suggesting that conservation of the *E7* sequence is vital in cervical cell carcinogenesis (11).

Currently, the latest research focuses on developing a therapeutic HPV vaccine for cervical cancer that targets the *E6* and *E7* genes. The best approach is the DNA therapeutic vaccine, which has demonstrated favorable outcomes in clinical trials, including safety profiles, strong immunogenicity, and potential for lesion regression (13). The presence of mutations in the *E6* and *E7* genes may influence the binding affinity of the vaccine or alter immune recognition sites, potentially reducing therapeutic efficacy. Consequently, identifying genetic diversity in these genes is crucial, as it can inform the development of targeted therapies aimed at local HPV variants.

Geographical diversity in the mutation of the *E6* and *E7* genes indicates the regional evolution of the virus. Nonetheless, research on HPV-16, specifically the *E6* and *E7* genes in Indonesia remains limited. The majority of HPV research in Indonesia emphasizes prevalence over genetic diversity, creating a critical gap in understanding the local molecular epidemiology and its implication for cervical cancer. Investigating the genetic diversity of HPV-16 in Indonesia is crucial, given that distinctive sociocultural factors, including early marriage and a low HPV vaccination rate. Furthermore, environmental factors might affect the dynamics of HPV transmission and facilitate the formation of region-specific variants. This study was designed to calculate the incidence of cervical cancer caused by HPV-16 and to analyze the genetic diversity of the *E6* and *E7* genes among cervical cancer patients in Riau Province, Indonesia. Riau is an ideal study area due to its diverse demographic composition, which includes indigenous and migrant populations with different cultural behaviors that may influence HPV transmission. Additionally, its strategic location along the Strait of Malacca promotes cross-border interactions, which can add to the genetic diversity of the virus. Limited coverage of cervical cancer screening emphasizes the need for region specific genetic epidemiology studies to give information for public health interventions.

The findings of this study are expected to considerably contribute to cervical cancer control efforts in Indonesia by offering crucial insights into the genetic diversity of HPV-16, notably in the *E6* and *E7* genes. These data may support the development of region-specific therapeutic vaccination strategies for local HPV variants. Ultimately, such efforts may help reduce the national burden of cervical cancer in Indonesia.

2. MATERIALS AND METHODS

2.1. Study Design and Sample Collection

This descriptive study analyzed the E6 and E7 genes of HPV-16 detected in cervical cancer patients between September 2023 and May 2024. Biological samples consisted of total DNA extracted from thirty-six cervical tissue

specimens obtained via the punch biopsy at the Gynaecological Oncology section of Arifin Achmad Hospital, Riau Province. Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine, University of Riau (B/106/UN19.5.1.1.1.8/UEPKK/2023).

2.2. HPV Detection

Sample preparation began with the collection of 10–20 mg of cervical biopsy tissue, followed by the addition of viral transport medium (VTM), and homogenization using a micro-pestle. DNA was isolated using the TORAX Nucleic Acid Extraction system by processing the samples with 15 µL of proteinase K and 200 µL of the homogenized sample, which were loaded into columns 1 and 7 of the TORAX plate. Upon completion of the extraction run, the purified DNA was retrieved from columns 6 and 12 of the TORAX plate.

The isolated DNA was amplified using universal HPV primers, specifically MY09 (5'CGT CCM ARR GGA WAC TGA TC'3) and MY11 (5'GCM CAG GGW CAT AAY AAT GG'3). A positive result was indicated by the presence of an amplification band with a product size of 450 bp (14). The polymerase chain reaction (PCR) was conducted using a final reaction volume of 10 µL consisting of 5 µL GoTaq® Green Master Mix (Promega Inc., Madison, WI, USA), 0.6 µL nuclease-free water (NFW), 0.2 µL primer MY09, 0.2 µL primer MY11 and 4 µL of sample DNA. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 1 minutes, and extension at 72°C for 1 minutes with a final extension at 72°C for 10 minutes.

2.3. *E6* and *E7* Gene Amplification and Sequencing

PCR products that tested positive using the MY09/MY11 primers were further amplified for the *E6* and *E7* genes using specific primers, as shown in Table 1. The optimized master mix for target gene amplification had a total reaction volume 15 µL. It consists of 7.5 µL GoTaq® Green Master Mix (Promega Inc., Madison, WI, USA), 4.9 µL nuclease-free water (NFW), 0.3 µL forward primer (5µM), 0.3 µL reverse primer (5µM) and 4 µL of sample DNA. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 12 cycles of amplification at 95°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds, with the annealing temperature reduced by 2°C every two cycles. This was followed by 30 additional cycles of amplification at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 15 minutes and a hold at 4°C indefinitely. Visualization of PCR results was performed using a 2% agarose gel run in agarose gel electrophoresis. The PCR results that yielded positive results were subsequently subjected to sequencing (1st Base Oligo Sequencing) to determine the nitrogen base sequence of the targeted genes.

2.4. Sequence Analysis

The forward and reverse sequencing data were processed using BioEdit software (version 7.2.5) to obtain consensus sequences. In this study, BioEdit was applied only in the pre-analysis stage, including visual inspection for chromatograms, manual trimming of low-quality bases, and generating consensus sequences. The entire technique is crucial for reducing sequencing errors and enhancing the accuracy of subsequent analysis.

The consensus sequence of each sample was the aligned with the reference genes *E6* and *E7* of HPV-16 retrieved from GenBank NCBI (National Center for Biotechnology Information) with accession number NC_001526.4 using he Nucleotide Basic Local Alignment Search Tool (BLASTn). This alignment was performed to identify potential nucleotide and amino acid mutation in each isolate. To ensure the reliability of the BLASTn results, the default E-value threshold was applied. In addition, the percentage identity and query coverage values exceeding 90% were used as supplementary criteria to validate the significance of the identified mutations.

2.5. Phylogenetic Analysis

Phylogenetic analysis was performed using MEGA11 software, employing the maximum likelihood method with the Kimura-2 model, and 1,000 bootstrap replicates. This approach is widely recognized for its accuracy in reconstructing evolutionary relationships (16), while the Kimura-2 model provides an appropriate substitution framework by accounting for different rates of transitions and transversions, which is relevant to HPV genetic variation (17). To construction of distinct phylogenetic lineages, sixteen reference HPV-16 sequences were retrieved from the GenBank sequence database, representing the major phylogenetic lineages: European (EUR), African-1 (AFR1), African-2 (AFR2), Asian-American (AA), and East Asian (As).

Table 1. Specific primers sequences

Gene Target	Primer (15)	Product Size (bp)	Tm (°C)	Gene Location
E6-HPV16 (F)	ACCGGTTAGTATAAAAGCAGACA	700	57.59	56–755
E6-HPV16 (R)	AGCGTAGAGTCACACTTGCA		59.33	
E7-HPV16 (F)	TGTCAAAAGCCACTRTGTCC	553	59.73	419–972
E7-HPV16 (R)	GTCATYTGATAYRGCATCCCCTGT		59.83	

3. RESULTS AND DISCUSSION

3.1. Distribution of Human Papillomavirus Genotypes

Out of 36 HPV-positive samples, 6 (16.7%) were infected with HPV type 16, 16 (44.4%) with HPV type 18, 13 (36.1%) with mixed HPV type 16 and 18 infections, and 1 (2.8%) with an undetermined HPV genotype. The distribution of HPV genotypes is illustrated in Figure 1. Statistical analyses were conducted to assess the significant difference in genotype distribution. Both the Chi-square and Fisher's Exact test revealed a significant difference in HPV genotype distribution among the 36 samples ($p = 2.6 \times 10^{-6}$ and $p = 1.3 \times 10^{-5}$, respectively), indicating that the observed distribution does not follow a uniform pattern.

In this study, the frequency of HPV-16 infection study participants is lower than HPV-18 infection. This finding is inconsistent with previous study by Wu et al. (18), who observed 52.38% single HPV-16 infections, 14.98% single HPV-18 infections, and 1.13% HPV-16 and 18 coinfections among 4,933 women with high-risk HPV infections in Asia. In contrast, several studies reported a higher HPV-16 infection frequency of HPV-16 in Indonesia. For example, Savira et al. (19) reported 48.8% of HPV-16 infection cases, 10.5% of HPV-18 infection cases, and 12.8% of mixed HPV-16 & 18 infections among 86 HPV-associated cervical cancer cases. The differences in HPV genotype distribution in various studies may be influenced by several factors, such as demographic characteristics, including age and sexual behavior. In young women under 30, the prevalence of HPV-16 is higher compared to other high-risk HPV (20). Additionally, differences in sample size and geographic scope may contribute to the observed discrepancies. For instance, the study by Wu et al. (18) included a large cohort of 4,933 women from multiple locations in Hubei Province, China and encompassed not only cervical cancer patients but also individuals diagnosed with cervical intraepithelial neoplasia (CIN).

According to Chet et al., (21) cervical cancer patients may have one or more HPV genotypes with 18.82% of patients have multiple HPV infections; however, these multiple infections were not associated with increased severity of cervical lesions. Similarly, Ao et al., (22) using multivariate analysis adjusted for several confounding factors, reported that single HPV-16 infection was associated with a higher risk of CIN3+ compared with co-infection involving low-risk HPVs and/or other high-risk HPVs, excluding HPV-18. The biological mechanisms underlying these contrasting associations among HPV genotypes remains unclear. One proposed explanation is intergenotypic competition, whereby co-infection with multiple HPV types may inhibit progression to CIN3+ by interfering with critical stages of HPV infection, including receptor binding, utilization of host cellular organelles, viral DNA replication, and integration into the host genome. In some cases, variation in HPV genotypes infecting the host may reduce the pathogenicity of subsequent infections through immune-mediated mechanisms. An immune response elicited by one HPV genotype may facilitate partial control or clearance of other HPV types, thereby lowering overall oncogenic risk. Furthermore, prior infection with low-risk HPV genotypes may prime the host immune system, potentially diminishing the pathogenicity of subsequent HPV-16 or HPV-18 infections (18,22). Another proposed mechanism involves immunological cross-protection, whereby co-infection with multiple HPV genotypes induces a more robust immune response compared with single HPV infection, further contributing to reduced disease progression (22).

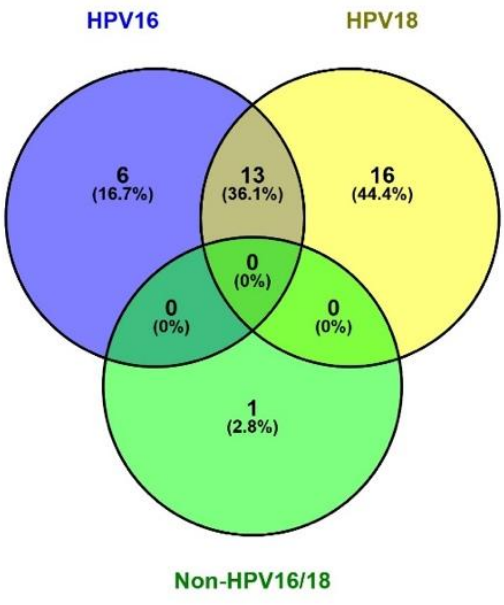

Figure 1. Distribution of HPV genotypes. Diagram was prepared on *Venny 2.1*

3.2. HPV-16 *E6* and *E7* Gene Sequence Variation

Sequences of 700 base pairs and 553 base pairs were obtained following amplification of the HPV-16 *E6* and *E7* genes, respectively. These sequences were analyzed using the deposited HPV-16 *E6* reference sequence (NC_001526.4) from the GenBank database to examine single-nucleotide polymorphism (SNP) variation among the sequences. As shown in Figure 2, a total of 22 mutations were identified across the HPV-16 *E6* and *E7* genes. In the *E6* gene, 13 SNPs were detected among the seven analyzed samples, comprising six synonymous SNPs (7151 T>C substitution, 7187 G>T substitution, 7328 T>A substation, 7331 A>G substitution, 7445 A>G substitution, 7484 A>G substitution) and seven nonsynonymous SNPs (7174 G>T substitution 7185 C>G substitution, 7318 A>G substitution). A recurrent point mutation involving an adenine-to-guanine substitution at position 7318 (7318A>G) was identified in samples 65, 79, 83, and 87, making it the most frequently observed variation, with four occurrences (30.7%), as shown in Figure 2 and Table 2. This nucleotide substitution resulted in an amino acid sequence, specifically a missense mutation which asparagine was replaced by serine at position 65 (65N>S).

Total of nine SNPs were detected among the seven analyzed samples, comprising eight synonymous SNPs (7708 G>A substitution, 7831 T>C substitution, 7837 T>G substitution) and one nonsynonymous SNPs. A non-homologous point mutation involving an adenine-to-guanine substitution at nucleotide position 7689 (7689A>G) was identified, resulting in a missense mutation in which asparagine was replaced by serine at amino acid position 29 (29N>S). In addition, one isolate, designated HPV-16 *E7* 89, found with three nucleotide mutations, including two synonymous mutations and one nonsynonymous substitution, as illustrated in Figure 2 and summarized in Table 3.

In this study, the most frequently identified point mutation in the *E6* gene was 7318A>G (30.7%), which results in a missense mutation at amino acid position 65 (65N>S). This finding is consistent with previous research by De Boer et al., (23) who reported that 40.9% of HPV-16 infection in cervical cancer cases showed the 7318A>G mutation, leading to the same 65N>S missense mutation. That study also identified the 7318A>G mutation in 12% of samples from Suriname, a finding attributed to the historical migration of Javanese populations to the region. It is even mentioned that the Javanese ethnic group in Suriname has been reported to have a higher incidence of cervical cancer compared with other ethnic groups (23). In contrast, a study conducted by Mahendra et al. (24) in Bali between 2019 and 2020 did not identify the 7318A>G mutation or any similar mutations observed in the present study. Several studies have reported that the 7318A>G point mutation occurs at a relatively low frequency (1-4%) among individuals with precancerous lesions and cervical cancer (25).

Table 2. Mutation points of HPV16 *E6* gene sequences

Isolate	Point of Mutation									
	7151	**7174**	**7185**	**7187**	**7318**	**7328**	**7331**	**7377**	**7445**	**7484**
NC_001526.4(ref)	T	G	C	G	A	T	A	C	A	A
HPV16 65	-	-	-	-	G	-	-	-	-	-
HPV16 79	-	-	-	-	G	-	-	-	-	G
HPV16 83	-	-	-	-	G	-	-	-	-	-
HPV16 87	-	-	-	-	G	-	-	-	-	-
HPV16 89	C	T	G	T	-	A	G	T	G	
HPV16 94	-	-	-	-	-	-	-	-	-	-
HPV16 104	-	-	-	-	-	-	-	-	-	-
Amino Acid Mutation		17R>I	21Q>D		65N>S			85H>Y		
		Missense	Missense		Missense			Missense		

Table 3. Mutation points of HPV16 *E7* gene sequences

Isolate	Point of Mutation			
	7689	**7708**	**7831**	**7837**
NC_001526.4(ref)	A	G	T	T
HPV16 65	-	A	-	-
HPV16 79	-	A	-	-
HPV16 83	-	A	-	-
HPV16 87	-	A	-	-
HPV16 89	G	-	C	G
HPV16 94	-	A	-	-
HPV16 104	-	A	-	-
Amino Acid Mutation	29 N>S	E>E	I>I	T>T
	Missense	Silent	Silent	Silent

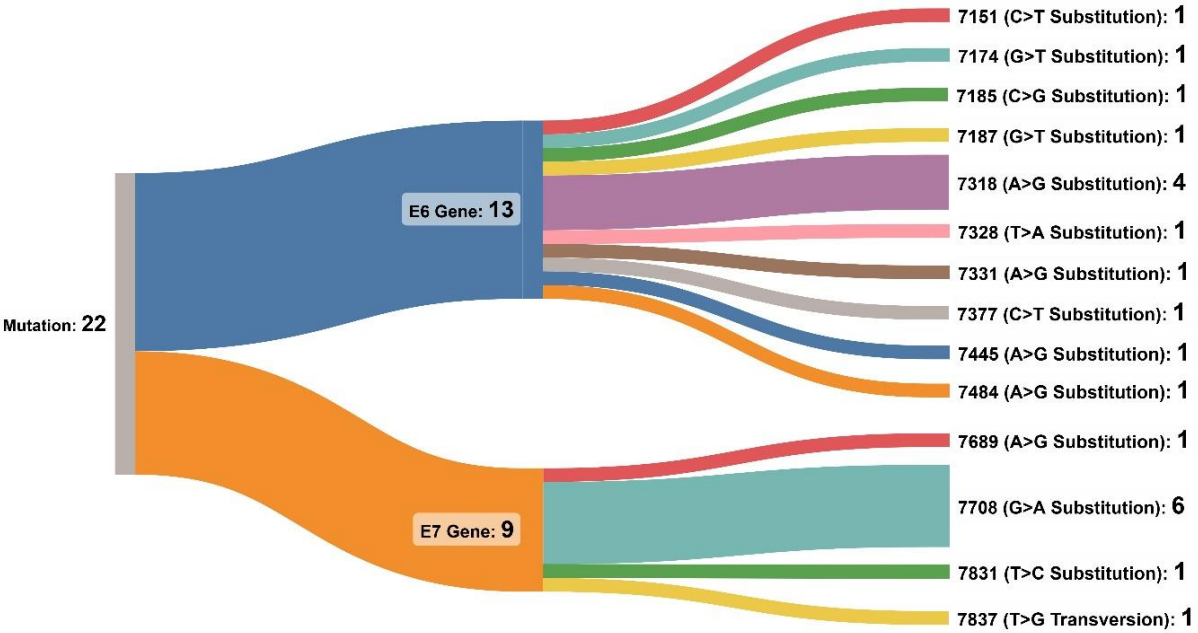

Figure 2. Mutation points of HPV 16 isolates. Diagram was prepared by *sankeymatics.com*

The 7318A>G mutation leads to an amino acid substitution from asparagine (N) to serine (S) at position 65 (65N>S). Both asparagine and serine play important roles in protein structure and function. Asparagine involved in hydrogen bonding and can influence substrate specificity and enzyme activity, whereas serine, owing to its hydroxyl group, contributes to protein polarity and serves as a primary site for phosphorylation (26,27). The *E6* protein initiates the oncogenesis process by attaching to the tumor suppressor protein p53, which is the primary mechanism in carcinogenesis of cervical cells caused by HPV (28). Although our study did not explicitly assess the influence of the 65N>S mutation on *E6*-p53 interactions, previous studies have indicated that amino acid substation in *E6* can modify this interaction and potentially affect the oncogenic properties of the gene (29). Consequently, the 65N>S mutation also changes the binding affinity of *E6*-p53, thereby influencing the development and severity of cervical cancer lesions.

Mutation in HPV may also confer a selective advantage that enables the virus to endure longer within the host without eliciting a robust immune response or inducing rapid tissue damage (30). This state may enhance virus transmission by extending the infection duration and sustaining a virus reservoir within the population. The virus that endures in extended mild infections will be able to cause higher transmissions rate by avoiding rapid immune clearance or host death, thus securing an evolutionary advantage (31). Further functional studies are warranted to determine whether the 65N>S mutation affects viral fitness, pathogenicity, or transmission dynamics.

The results of this study regarding the *E7* gene are consistent with reports from Chinese studies by Li et al. (12) and Dai et al. (32), which identified nucleotide variants in the *E7* gene, including the amino acid substitution N29S, with reported frequencies of 60.1% and 64.4%, respectively. The amino acid N>S change results from a missense mutation that arises in the nitrogen base sequence A>G (12,32). This N29S substitutions has been reported to be closely associated with the development of cervical cancer, as it may interfere with retinoblastoma (Rb) function, thereby facilitating persistent HPV infections and cervical cancer development (32).

The study by Lou et al. (33) investigated the impact of *E7* gene variations on carcinogenesis and identified that 7 of the 13 *E7* variants were identified in non-cervical cancer samples, including the N29S variant. In non-cancer samples exhibiting the N29S substitutions, the steady-state nuclear level of *E7* protein was reduced compared with those of the wild-type *E7* protein. However, transforming activity does not always correlate directly with *E7* protein abundance. The substitution of asparagine with serine at position 29 may enhance the affinity of *E7* for Rb, leading to increased Rb degradation and promoting cellular transformation (33).

In the present study, the synonymous SNP G7708A (7708 G>A) was frequently detected in HPV-16 *E7* gene isolates, aligning with a study by Alsanea et al. (15) in Saudi Arabia. In that study, seven mutation sites were identified in the HPV-16 *E7* gene, including a silent mutation at G5968A (5968 G>A), which was associated with a lower viral load compared with other mutations (15).

3.3. Phylogenetic Tree Construction

Phylogenetic trees were constructed using the HPV-16 *E6* and *E7* gene sequences obtained in this study (n = 7), the HPV-16 reference sequence (NC_001526.4), 16 intra-typic HPV-16 variants sequences representing major sub-lineage and AB981583 (Hepatitis B-virus) as an outgroup. Evolutionary distances were calculated using the Kimura-Nei model, as shown in Figure 3.

Figure 3 illustrates the phylogenetic relationships of the HPV-16 *E6* and *E7* genes isolates., demonstrating their genetic relatedness to global HPV-16 variants available in the NCBI database. As shown in Figure 3a, most *E6* gene isolates clustered within the European lineage. Specifically, isolates 94 and 104 showed close relatedness to accession number K02718, while isolates 83 and 87 clustered with accession number HQ644236, which also belongs to the European lineage. In contrast, isolates 79 and 65 clustered with accession number AF534061 from the East Asian lineage, whereas isolate 89 was closely related to accession number KP67755 from the African lineage. Figure 3b presents the phylogenetic analysis of the *E7* gene. The majority of *E7* gene isolates, including isolates 94, 104, 83, 87, 79, and 65, clustered with accession number HQ644236, which belongs to the European lineage. One *E7* gene isolate clustered with accession number AF536180, representing the African lineage.

The results of the phylogenetic analysis indicate that the majority of HPV-16 isolates from Riau Province are closely related to the European lineage, with 57.1% (4/7) based on the *E6* gene and 85.7% (6/7) based on the *E7* gene. In contrast, 28.6% (2/7) of *E6* isolates clustered with the East Asian lineage, while 14.3% (1/7) clustered with the African lineage. For the *E7* gene, 14.3% (1/7) of isolates were associated with the African lineage.

These findings align with previous reports demonstrating that the European lineage of HPV-16 is the most prevalent variant worldwide (12,34). This predominance may be attributed to lineage-specific viral characteristics, as well as host-related factors, such as ethnicity and differences in carcinogenic potential among HPV-16 lineages (35). A study conducted in Pakistan by Ahmed et al. (36) also reported a predominance of the European sub lineage A1 in HPV-16 *E6* and *E7* gene isolates, further supporting the widespread distribution this lineage. Identification of HPV genetic variations is essential for mapping regional HPV distribution, optimizing PCR-based diagnostic assays, guiding vaccine development, especially therapeutic vaccines, and informing personalized treatment strategies for cervical cancer associated with high-risk HPV (hrHPV) infection (36). While currently available HPV vaccines are prophylactic and based on the L1 capsid protein. In contrast, the *E7* gene, which encoding a consistently expressed oncoprotein crucial for HPV-mediated carcinogenesis, is being actively explored as a target for therapeutic vaccines aimed at inducing robust cellular immune responses against infected or transformed cells (37).

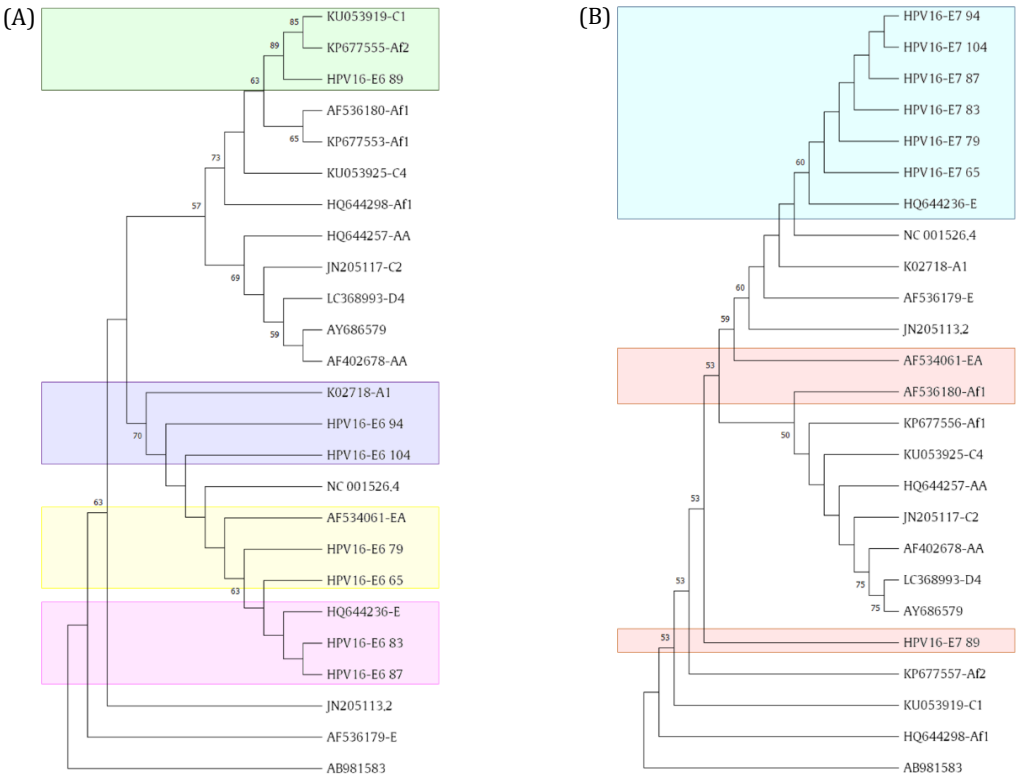

Figure 3. Phylogenetic tree on HPV-16 isolates based on *E6* (A) and *E7* (B) gene. Bootstrap values (displayed at branch points) represent the confidence levels for the grouping. The color-coded boxes highlight clusters of closely related isolates

Historical human migration and demographic dynamics likely contribute to the predominance of the European HPV-16 lineage in Riau Province. HPV-16 variants are broadly classified into three major lineages based on geographic origin: European, Asian-American, and African. Phylogenetic analyses suggest that genetic divergence among these lineages reflect periods of genetic isolation during early human migration out of Africa. The global distribution of HPV-16 variants is therefore thought to mirror human migratory routes and interpopulation contact throughout history (38).

Riau province is located in a strategic position along the Straits of Malacca, which is one of the most important maritime trade routes in the world, and is positioned at crossroads of international economic and cultural intersections. During the colonial period, interactions between European traders and local population may have led to the introduction and dominance of European lineage HPV variants in the Riau region, presumably accounting for the prevalence of the European lineage in Riau (39).

The carcinogenic characteristics of non-European HPV-16 lineages, especially African and East Asians variants, are affected by functional differences in the *E6* oncoprotein, which leads to the degradations of the tumor suppressor protein p53. Studies have shown that African lineages, such as African-2 (Af2), exhibit a higher ability to degrade p53 compared to the European lineages. The degradation level in the African lineage was 81-86%, whereas the European lineage exhibited a degradation level of 76%. This enhanced degradation ability leads to higher oncogenic risk. Genetic polymorphism in the *E6* gene, particularly in regions associated with binding to the ubiquitin ligase *E6AP* and interacting with the p53 core domain, explain for these functional differences. The *in-silico* study revealed that mutation in the amino and carboxyl terminal regions of *E6* can modify its structural features, thereby influencing its binding affinity to p53 and its carcinogenic potential. East Asian lineages possess distinct genetic characteristics that may influence *E6*-p53 interactions, although further studies are needed to measure this phenomenon more precisely. Overall, these findings provide basic molecular information on the differences in potential oncogenic risks associated with distinct lineages and emphasize the necessity for lineage-specific approaches in cervical cancer prevention and treatment (40).

Evolutionary pressure can influence the prevalence of HPV-16 lineage in diverse population through genetic drift, natural selection, and host adaptation. Variations in pathogenicity and immunogenicity may occur in HPV-16 variants, potentially affected by environmental factors and host genetic backgrounds (41). The rapid viral adaptation to the environment changes may result from positive selection, characterized by the elevation in the prevalence of abnormal alleles (12). A study conducted by Sun et al., (41) in the Anyang region of Central China showed that the European and Asian lineages of HPV-16 have significant genetic variability. Their findings indicate that the virus gains advantage due to positive selection occurring at several codons in the *E6* gene, thereby enhancing the viral survival capability (41).

The prevalence of the European HPV-16 lineages in Riau Province will influence HPV vaccination strategies in Indonesia, including preventive vaccine and the development of a therapeutic vaccine. Although in this study the prevalence of HPV-16 was lower compared to HPV-18, the coverage of vaccination against all high-risk HPV remains important, considering the regional genetic diversity.

This study has several limitations, the primary is the small sample size, which means that the results of this research cannot be fully generalized to a larger population. Moreover, the application of conventional sequencing techniques also reduces the ability to identify genetic variants with low frequency or minor variants. Therefore, subsequent research should involve a larger and more diverse sample size to elucidate the genetic variants of HPV-16 more specifically. The use of next generation sequencing (NGS) is also recommended because this method can detect rare mutations and provide a more comprehensive overview of mutation patterns. Furthermore, future research is required to evaluate the biological effects of mutations to elucidate the clinical relevance of *E6* and *E7* mutations. The studies include protein structure modelling to predict conformational changes, as well as in vitro assessments to determine oncogenic potential, the viral ability to escape the immune system, and the interaction between the virus and the host cell. This approach will enhance the understanding of the role of these mutation in cervical carcinogenesis and facilitate the development of more targeted prevention and therapy strategies.

4. CONCLUSIONS

This study found that HPV-16 was detected in nineteen patients with cervical cancer. Among these, thirteen (36,1%) patients exhibit co-infection with HPV-18. Analysis of the *E6* and *E7* gene sequences show that the most prevalent genetic variations were the 7318A>G and 7708G>A mutations in HPV-16. The phylogenetic analysis results indicate that the majority of HPV-16 isolates are closely related to the European lineage. Although the precise effects of the mutation on viral properties remain unclear, it si believed to influence the structure and function of the viral protein. This will affect the interactions between virus and host cells, viral immune evasion, or viral ability to survive in the host cell. These findings suggest that the mutation may provide an evolutionary advantage to the virus, necessitation further research to understand how this mutation impact on virulence and transmission patterns of the virus.

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