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Short Communication

Genetic variations of the *L2* gene in human papillomavirus (HPV) type 16 from cervical cancer patients in Sumatra region, Indonesia

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Abstract

The L2 protein, a minor capsid component of human papillomavirus (HPV), plays a critical role in the HPV life cycle by packaging the viral genome with the L1 protein and facilitating DNA transport to the nucleus. Identifying genetic variations in the *L2* gene is essential for improving vaccine development, diagnostic accuracy, and understanding viral evolution, potentially contributing to more effective HPV vaccines. The aim of this study was to investigate the genetic variation of the *L2* gene in cervical cancer specimens collected from patients in Riau Province, Indonesia. A single-center, cross-sectional study was conducted at Arifin Achmad General Hospital, Riau Province, involving cervical cancer patients with confirmed HPV16 infection between January 2018 and August 2020. Demographic, clinical, and risk factor data were collected through structured interviews and direct assessments. Cervical biopsy specimens were collected, and viral DNA was extracted for *L2* gene amplification using polymerase chain reaction (PCR). Sequencing was conducted on PCR products, followed by single-nucleotide polymorphism (SNP) identification through alignment with the HPV16 reference genome. The amplification and sequencing of the HPV16 *L2* gene from 22 cervical cancer specimens revealed 36 SNPs, including 31 nonsynonymous and five synonymous mutations. High-frequency mutations were observed at nucleotide positions 4,074 and 4,177, each detected in 95.45% of the samples. Notable insertions were found at positions 3,668–3,669 and 4,275–4,276, indicating substantial sequence variation. Phylogenetic analysis grouped the sequences into three clusters, with most belonging to sub-lineage A2 (European), while others aligned with A4 (Asian) and East Asian lineages. The observed genetic diversity in the HPV16 *L2* gene may reflect regional viral evolution and has potential implications for future vaccine development.

Keywords: Cervical cancer, human papillomavirus, HPV16, *L2* gene, L2 protein

Introduction

Cervical cancer is the second most prevalent cancer among women in Indonesia, with the integration of human papillomavirus (HPV) into the host's DNA being a pivotal factor in its

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pathogenesis [1]. Persistent HPV infection can lead to a cascade of events, starting with lesions in the cervical epithelial cells, progressing to precancerous lesions, and eventually culminating in invasive cervical cancer (ICC) [2-4]. HPV types 16 and 18 are responsible for approximately 87% of ICC cases, with an estimated 4.0% of the general population carrying these high-risk types [5]. These types fall under the high-risk (hrHPV) category, which also includes other oncogenic types such as 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The varying presence of hrHPV types influences clinical outcomes due to differences in transmission, persistence, lesion progression, and immune response [5].

The HPV genome consists of eight genes: six early (*E*) genes encoding non-structural, oncogenic proteins and two late (*L*) genes, which encode structural proteins. The L2 protein, a minor capsid component [6-8], plays a critical role in viral genome packaging and facilitates the transport of HPV DNA into the host cell nucleus [9]. L2-based vaccines are promising candidates for broader protection, targeting a wider range of HPV types, including those not covered by existing vaccines, as well as low-risk mucosal HPVs and some beta papillomavirus, a subgroup of HPVs primarily associated with cutaneous infections [10]. Investigating genetic variations in the *L2* gene is crucial for enhancing vaccine development, improving diagnostic accuracy, understanding viral evolution, and optimizing treatment strategies. Additionally, L2-based vaccines could be particularly beneficial for immunocompromised individuals, who are at an elevated risk of persistent HPV infections, and for low-resource settings where access to current vaccines and cervical cancer screening programs is limited. The aim of this study was to examine the genetic variation of the *L2* gene in cervical cancer patients.

Methods

Study design, setting and sampling

A single-center, cross-sectional study was conducted at Arifin Achmad General Hospital in Riau Province, Indonesia, a referral center for cervical cancer patients from various regions within the province. The study began with the screening of HPV16 infections among cervical cancer patients, during which demographic and clinical data were collected. Subsequently, the *L2* gene was sequenced from patients with confirmed HPV16 infections to assess genetic variation. A total sampling approach was employed, including all cervical cancer patients who underwent biopsy and met the predetermined inclusion criteria between January 2018 and August 2020.

Patients and criteria

Specimens were collected from eligible patients who met the predetermined criteria, provided informed consent, and agreed to participate in the study. The inclusion criteria were established based on clinical suspicion of cervical cancer, determined through patient-reported complaints, clinical symptoms, evaluations, Pap smear results, and the feasibility of obtaining cervical tissue via punch biopsy. Patients whose diagnostic results did not indicate cervical cancer, as well as those who tested negative for the *L2* HPV16 gene, were excluded. All biopsies obtained from eligible patients who met the inclusion criteria were included in the study. Specimens with partial or unreadable HPV16 DNA sequences were excluded.

Data collection

Demographic and clinical data of cervical cancer patients who underwent cervical tissue biopsies at Arifin Achmad General Hospital, Riau Province, were collected. Structured interviews were conducted to obtain demographic data and risk factors, including age, number of children, educational background, duration of marriage, number of sexual partners, smoking history, and history of intrauterine device (IUD) use. Clinical profiles, including histological tumor types (squamous cell carcinoma and adenosquamous carcinoma) and clinical staging, were also collected. Tumor types were classified according to World Health Organization (WHO) criteria [11], while staging followed the International Federation of Gynecology and Obstetrics (FIGO) classification (1B, IIA, IIB, IIIA, IIIB, IVA, IVB) [12]. In addition, clinical symptoms, such as vaginal bleeding, postcoital hemorrhage, postmenopausal bleeding, leukorrhea, pelvic discomfort, lumbar pain, fatigue, leg pain, decreased appetite, limb edema, and urinary or stool incontinence, were recorded through both interviews and physical examinations.

Human papillomavirus (HPV) detection

Viral DNA was extracted from cervical cancer tissue biopsies using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). HPV16 was specifically identified by amplifying the *L2* gene at nucleotide positions 3,668 to 4,277 (GenBank Accession No: NC_001526). Primers for this amplification were designed using Primer3 (https://primer3plus.com), with the forward and reverse primer sequences of 5′-ACCACTTCACATGCAGCCTC-3′ and 5′-GGGACAGGAGGCAAGTAGAC-3′, respectively. Polymerase chain reaction (PCR) was carried out in a total reaction volume of 50 µL, consisting of 5 µL of extracted DNA, 50 mM Tris-HCl (pH 8.4), 10 mM KCl, 2.0 mM $MgCl_2$, 1% β-mercaptoethanol, 0.05% bovine serum albumin, 0.2 mM deoxynucleoside triphosphates (dNTPs), 20 pmol of each primer, and 2.5 U of *Taq* polymerase (Boehringer Mannheim, Mannheim, Germany). The amplification protocol consisted of an initial denaturation at 94°C for five minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 60 seconds, and extension at 72°C for 60 seconds, with a final extension at 72°C for ten minutes [13]. PCR products of approximately 450 base pairs were visualized using 1% agarose gel electrophoresis.

Gene sequencing and variation analysis

The PCR products were sequenced to determine the nucleotide sequences of the HPV16 *L2* gene. Sequence assembly and alignment were performed using Molecular Evolutionary Genetics Analysis (MEGA) version 11 to ensure accuracy and consistency [14]. The assembled sequences were aligned with the HPV16 reference genome (GenBank Accession No. NC_001526) to identify single-nucleotide polymorphisms (SNPs). SNP detection was carried out by comparing nucleotide positions with the reference genome, allowing the identification of variations indicative of genetic diversity [15].

Phylogenetic tree analysis

Phylogenetic analysis was conducted on 22 HPV16 *L2* gene sequences by comparing them to the reference genome (NC_001526) and 20 representative HPV16 genomes from various sub-lineages to assess the genetic relatedness of *L2* sub-lineages. Initial sequence alignment was performed using ClustalX software [16], and the phylogenetic tree was subsequently visualized using MEGA version 11. The alignment process ensured accurate base pairing for reliable tree construction. Phylogenetic inference was carried out in MEGA using the maximum likelihood method. Bootstrap support values were calculated to evaluate the reliability of the tree nodes. Evolutionary distances were estimated using the Kimura–Nei model, which accounts for multiple nucleotide substitutions and provides a robust measure of evolutionary divergence.

Results

Characteristics of the patients

During the study period, 110 cervical tissue specimens were analyzed, of which 35 tested positive for HPV16 and 22 had the presence of the *L2* HPV16 gene (**Figure 1**). These 22 patients were then included in the final analysis, and their characteristics are summarized in **Table 1**. The average age of patients was within the reproductive age range. The majority of participants were multiparous and had an educational level of elementary or junior high school (63.64%), with a marriage duration of 15–20 years. Regarding cervical cancer risk factors, only a small proportion of participants had a history of multiple sexual partners or smoking. Additionally, the use of IUD contraception was limited to a small number of participants (**Table 1**).

Clinical manifestations were recorded using direct interviews, in which patients provided details of the clinical symptoms they experienced before seeking medical attention. The most frequently reported symptoms were fluor albus, vaginal bleeding, and pelvic pain (**Table 1**). Additionally, the most prevalent histopathological type was squamous cell carcinoma accounting for 72.73% of cases, while the most common clinical stage was stage IIA (27.27%) (**Table 1**).

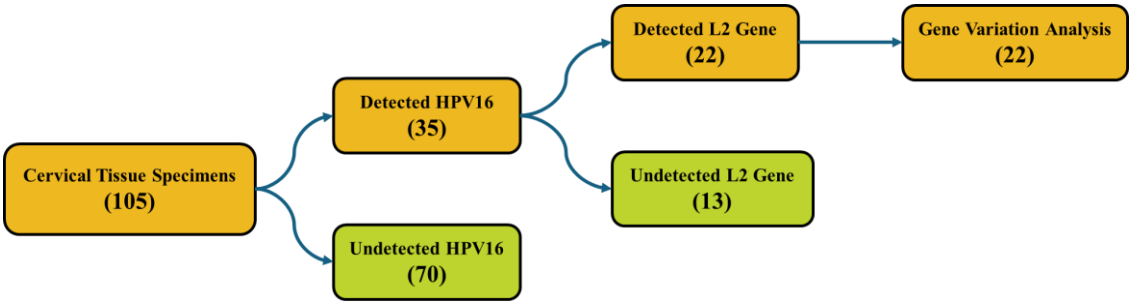

Figure 1. Number of investigated cervical tissue specimens from cervical cancer patients at Arifin Achmad General Hospital, Riau Province, between January 2018 and August 2020.

Table 1. Characteristics of patients with confirmed human papillomavirus (HPV16) infection from whom HPV16 L2 gene sequences were successfully generated (n=22)

Characteristics	Frequency (percentage)
Age (years), mean±SD	47.90±11.20
Number of childbirths, mean±SD	1.70±2.16
Education level	
Elementary school - junior high school	14 (63.64%)
Senior high school or higher	8 (36.36%)
Length of marriage	
<15 years	6 (27.27%)
15–20 years	14 (63.64%)
>20 years	2 (9.90%)
Lifetime sexual partners (>1 person)	1 (4.54%)
History of smoking	5 (22.7%)
Intrauterine device (IUD)	3 (13.63%)
Clinical symptoms	
Vaginal bleeding	12 (54.54%)
Post coitus hemorrhage	8 (36.36%)
Post menopause hemorrhage	7 (31.82%)
Fluor albus	15 (68.18%)
Pelvic pain	11 (50.00%)
Back pain	5 (22.73%)
Fatigue	12 (54.55%)
Leg pain	4 (18.18%)
Decreased appetite	3 (13.64%)
Limb edema	2 (9.09%)
Urine/stool leakage	1 (4.55%)
Histopathological type	
Squamous cell carcinoma	16 (72.73%)
Adenosquamous carcinoma	4 (18.18%)
Neither	2 (9.09%)
Cancer staging	
IB	1 (4.55%)
IIA	6 (27.27%)
IIB	6 (27.27%)
IIIA	3 (13.64%)
IIIB	3 (13.64%)
IVA	1 (4.55%)
IVB	2 (9.09%)

HPV16 *L2* gene sequence variations

The amplification of the HPV16 *L2* gene yielded sequences of 609 base pairs. These sequences were aligned with the reference sequence of the HPV16 *L2* gene (GenBank Accession No: NC_001526) to identify SNP variations. A total of 36 SNPs were detected across the 22 samples, including 31 synonymous (did not alter the amino acid sequence) and five nonsynonymous variations (altered the amino acid sequence) (**Table 2**). A detailed list of mutation points in the human HPV 16 *L2* gene sequences of the included patient is presented in **Table 2**.

Mutations were present in all samples, with nucleotide positions 4,074 and 4,177 exhibiting the highest frequency of mutations, presented in 21 samples (95.45%). The analysis revealed notable insertion events at positions 3,668–3,669 and 4,275–4,276, which were detected in 17 (77.27%) and 14 (63.64%) samples, respectively (**Table 2**). A point mutation was also observed at

positions 3,708–3,709, involving the insertion of adenine in three samples (8.33%). At this site, a silent mutation at the amino acid level was identified. Among all samples positive for HPV16, L2HPV16_03 and L2HPV16_44 exhibited the highest number of mutations. Based on clinical data, L2HPV16_03 was classified as adenosquamous carcinoma at stage IIB, while L2HPV16_44 was diagnosed as squamous cell carcinoma at stage IIA. At position 4,244, a G>T mutation was detected in all but one sample (L1HPV16_25), in which a G>A substitution was present. The mutation at this position was considered low-frequency, occurring in 11% of samples. At position 4,271, both a G deletion and a nucleotide substitution from G to A were identified; however, these mutations were only detected in three samples (8.33%). Several mutations were found exclusively in individual samples, including those at positions 3,864; 3,957; 4,210; 4,245; 4,249; 4,250; 4,251; 4,252; 4,272; and 4,273 (**Table 2**). In addition to silent mutations, the majority of nucleotide changes observed in this study were predicted to alter the amino acid sequence of the *L2* gene. These findings highlight the significant genetic variability in the *HPV16 L2* gene, particularly in the regions of frequent SNPs and insertions, which may have implications for the virus's evolution and potential vaccine development.

Phylogenetic tree analysis

The phylogenetic tree was constructed on 22 HPV16 *L2* gene sequences from this study, the reference sequence (NC_001526), 20 intra-typic variants of *HPV16*, and the outgroup sequence (hepatitis B virus, AB981583). The phylogenetic tree showed the genetic relationships and clustering patterns among the HPV16 *L2* gene sequences, with both the reference and variant sequences presented in **Figure 2**.

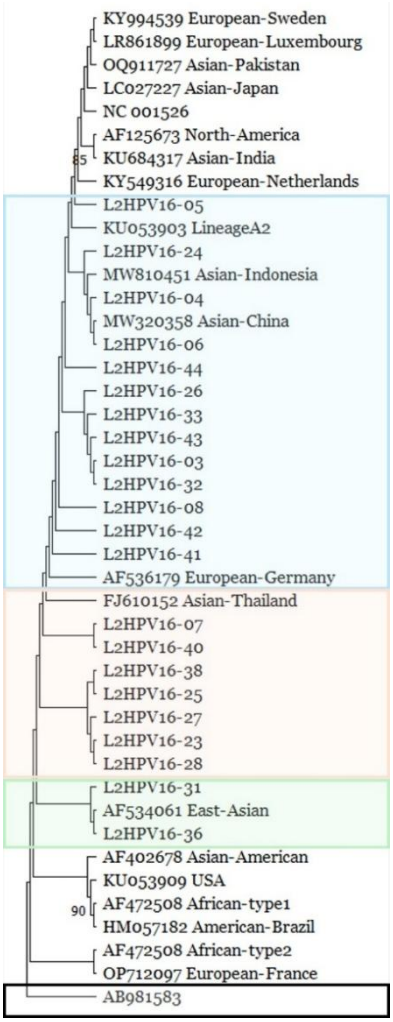

Figure 2. Phylogenetic tree based on the *L2* gene sequences of HPV16, illustrating the genetic relationships among the strains. The samples from this study (L2HPV16_03, _04, _05, _06, _08, _24, _26, _32, _33, _41, _42, _43, and _44), highlighted in the blue box, were closely related to sub-lineage A2 (European). Samples highlighted in the orange and green boxes (L2HPV16_07, _23, _25, _27, _28, _31, _36, _38, and _40) were closely related to sub-lineage A4 (Asian).

Table 2. Mutation points of human papillomavirus (HPV) 16 *L2* gene sequence from the study

Samples	Point of mutation																					
	3668–3669	3708–3709	3861	3864	3957	3983	4074	4177	4181	4210	4244	4245	4246_4247	4249	4250	4251	4252	4271	4272	4273	4275_4276	4276–4277
NC_001526 (*ref.*)	-	-	C	T	A	G	G	T	G	G	G	G	-	G	C	A	T	G	C	A	-	-
L2HPV16_23	Ins C	-	-	-	-	-	A[a]	C[a]	-	A	T[a]	-	-	-	-	-	-	-	-	-	Ins T[a]	-
L2HPV16_24	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L2HPV16_25	Ins C	Ins A[a]	-	-	-	-	A[b]	C[b]	-	-	A[b]	-	-	-	-	-	-	-	-	-	-	-
L2HPV16_26	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	-	-	-	-	-	-	-	-	Ins T[a]	-
L2HPV16_27	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	T[a]	-	-	-	-	-	-	-	-	-	Ins T[a]	-
L2HPV16_28	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	T[a]	-	-	-	A	-	-	-	-	-	-	-
L2HPV16_31	Ins C	-	T	-	-	-	A[a]	C[a]	A[a]	-	-	-	-	-	-	-	-	-	-	-	-	-
L2HPV16_32	Ins C	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L2HPV16_33	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	-	-	-	-	-	-	-	-	Ins T[a]	-
L2HPV16_36	Ins C	-	T	-	-	-	A[a]	C[a]	A[b]	-	-	-	-	-	-	-	-	-	-	-	Ins T[b]	-
L2HPV16_38	Ins C	Ins A[b]	-	-	-	-	A[b]	C[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L2HPV16_40	Ins C	Ins A[b]	-	-	-	C[a]	A[b]	C[b]	-	-	-	-	-	-	-	-	-	Del G[a]	-	-	Ins T[c]	-
L2HPV16_41	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L2HPV16_42	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	-	-	-	-	-	-	-	-	Ins T[b]	-
L2HPV16_43	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	-	-	-	-	-	Del G[b]	-	-	Ins T[b]	-
L2HPV16_44	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	-	-	-	-	-	A[c]	A	T	Ins T[b]	-
L2HPV16_03	-	-	-	-	C	-	A[c]	C[c]	-	-	-	C	Ins A[a]	C	-	G	G	-	-	-	Ins T[b]	-
L2HPV16_04	-	-	-	-	-	-	A[c]	C[c]	-	-	-	-	-	-	-	-	-	-	-	-	Ins T[d]	-
L2HPV16_05	-	-	-	-	-	-	A[c]	C[c]	-	-	-	-	-	-	-	-	-	-	-	-	Ins T[d]	-
L2HPV16_06	-	-	-	-	-	-	A[c]	C[c]	-	-	-	-	-	-	-	-	-	-	-	-	Ins T[d]	-
L2HPV16_07	-	-	-	-	-	C[b]	A[c]	C[c]	-	-	-	-	-	-	-	-	-	-	-	-	Ins T[d]	-
L2HPV16_08	Ins C	-	-	-	-	-	A[a]	C[a]	-	-	-	-	Ins T[b]	-	-	-	-	-	-	-	-	Ins A
Type of mutation	Silent	[a] Silent [b] 12T>*fs	163 F>Y fs	164 T>R fs	195E>D	[a]204 S>P fs [b]204 S>T	[a]234 Q>S fs [b]234 Q>K fs [c] Silent	[a]269 S>S fs [b]269 S>F fs [c]269 S>P	[a]270 S>S fs [b]270 S>*fs	280 D>K fs	[a]291 R>Y fs [b]291 R>S fs	291R >S	[a]292 P>H [b] Silent	293 A >T fs	293 A>R	294A>Gfs	295 L>G fs	[a]300 G>H fs [b]300 G>C fs [c]300 G>*fs	300 G>I fs	301 I>I fs	[a]301I>I fs [b]301I>L fs [c]301I>F fs [d]302R>* fs	Silent

Different superscript letters in a column represent different types of mutations within the column, and the type of mutation is presented under the main table
Types of mutation: Asterisk (*): stop codon; Del: deletion; fs: frameshift; Ins: insertion; (>): substitution; Silent: silent mutation
Nucleic acids abbreviation: A: adenine; C: cytosine; G: guanine; T: thymine
Amino acids abbreviation: A: alanine; C: cysteine; D: aspartic acid; E: glutamic acid; F: phenylalanine; G: glycine; H: histidine; I: isoleucine; K: lysine; L: leucine; P: proline; Q: glutamine; R: arginine; S: serine; T: threonine; Y: tyrosine

Phylogenetic analysis revealed a close relationship between the HPV16 strains in this cohort and the A2 (European) and A4 (Asian) sub-lineages. Based on the phylogenetic tree, the sequences from Riau, Indonesia, were grouped into three distinct clusters. The majority of sequences were assigned to sub-lineage A2 (European), showing high similarity to sequences from China (blue box). A previously reported Indonesian sequence available in the database also demonstrated a close relationship with Chinese sequences. Based on shared mutation sites, L2HPV16_04 and L2HPV16_06—both clustering with the Chinese sequences—were found to possess identical mutations in both number and position. This observation suggested a potential common ancestry or the influence of similar evolutionary pressures acting on these sequences. In this study, the second most prevalent group of sequences clustered closely with those from Thailand (orange box), representing the A4 (Asian) sub-lineage. Additionally, two sequences (L2HPV16_31 and L2HPV16_36) were closely related to sequences from East Asia (green box). Both of these sequences shared mutations at positions 3,861; 4,074; 4,177; and 4181.

Discussion

Our sequencing analysis confirmed the successful amplification of the *L2* gene from HPV16 in all samples. A comparison with the reference sequence (NC_001526) identified 36 SNPs across 22 specimens. The L2 capsid protein plays a pivotal role in viral pathogenicity, contributing to viral particle assembly, facilitating viral entry, ensuring efficient DNA transport into the nucleus of infected cells, and interacting with the host's immune response [17-19]. Although studies on the HPV16 *L2* gene in clinical samples remain limited, previous studies have highlighted the gene's polymorphism in various geographic locations [20], including China [21], India [22] and Pakistan [23]. For instance, a study conducted in Pakistan identified several non-synonymous mutations in the *L2* gene, underscoring its variability [23]. However, the N-terminal domain of the L2 protein exhibits a high degree of conservation across various strains [3,24].

This research revealed an amino acid substitution, S269P, in five *L2* gene sequences from our samples. This discovery corresponds with a review article that examined the mutation profiles of *L1* and *L2* genes of HPV16 across different geographical locations, indicating that the S269P mutation is one of the most prevalent variants in the L2 protein. This behavior has primarily been reported in isolates from Asian [20]. The significant occurrence of this mutation in Asian populations indicates it may signify a region-specific evolutionary adaptation of HPV16, perhaps affecting viral persistence, immunological evasion, or vaccine effectiveness. Additional research is required to investigate the functional ramifications of this mutation and its significance in HPV-related pathophysiology.

The HPV16 *L2* gene sequences analyzed in this study revealed significant genetic diversity within HPV16, suggesting multiple geographic and evolutionary origins. Phylogenetic analysis further demonstrates that these sequences cluster into various sub-lineages, with notable associations to both Asian and European variants, underscoring the global genetic variability of HPV16. This finding aligns with a study conducted in Padang, Indonesia, which also reported that most of their HPV16 *L2* isolates clustered with European lineages [25]. These results suggest that HPV16 variants circulating in Indonesia may have historical links to European and Asian populations, possibly due to human migration and population interactions over time.

The European sublineage of HPV16 is the most prevalent globally, accounting for the majority of HPV16 infections, as evidenced by the examination of cancer and non-cancer control samples. Chronic HPV16 infection is the primary etiological factor in virus-induced carcinogenesis. Prior research contrasting the "European" variety of HPV16 with the "non-European" variation revealed that the non-European variant exhibited a heightened risk of viral persistence and cervical cancer progression [26]. The European sublineage of HPV16 is the most prevalent globally, accounting for the majority of HPV16 infections, as evidenced by the examination of cancer and non-cancer control samples. Chronic HPV16 infection is the primary etiological factor in virus-induced carcinogenesis. Prior research contrasting the "European" variety of HPV16 with the "non-European" variation revealed that the non-European variant exhibited a heightened risk of viral persistence and cervical cancer progression [27].

Supporting this, whole-genome sequencing of HPV16 from Thai women revealed a significant non-synonymous mutation in the *L2* gene, specifically an alteration from L2-269S to

L2-269D in cases of CIN III. Phylogenetic analysis indicated that Thai HPV16 strains are more closely related to European strains, underscoring the genetic diversity within the region [28]. In Nepal, the HPV16 genome sequences exhibited high similarities, particularly with two major indels in the non-coding region between *E5* and *L2* genes. The *L2* gene displayed 52 nucleotide variants, and phylogenetic analysis positioned the Nepalese HPV16 across the A, C, and D lineages, highlighting a broad spectrum of genetic diversity [29]. The distribution of HPV16 variants, including those with specific mutations in the *L2* gene, exhibits significant geographical variation. This variation can impact the risk of viral persistence and the subsequent development of cervical cancer [30,31]. Gaining insights into the genetic diversity and phylogenetic relationships of the *L2* gene is essential for creating vaccines and therapeutic strategies tailored to specific regions. In designing prophylactic vaccines, it is important to account for the variability in the *L2* gene to ensure comprehensive protection against a wide range of HPV16 variants [20,32].

The main limitation of this study was the small sample size, comprising only 22 *L2* gene sequences of HPV16, which may not adequately represent the genetic diversity of HPV16 circulating in Riau Province. Future studies should include a larger cohort to provide a more comprehensive understanding of *L2* gene variability. Although this study focused on genetic variation within the *L2* gene, functional analyses are required to evaluate the effects of these mutations on protein structure, viral infectivity, and immune response. Additional in vitro and in vivo experiments will be essential to determine the biological relevance of these alterations. The integration of whole-genome sequencing with functional characterization in future research will further advance our understanding of *L2* gene variants in HPV pathogenesis and inform vaccine development strategies.

Conclusion

This study identified 36 SNPs in HPV16, with 31 being nonsynonymous and five synonymous. Phylogenetic analysis revealed a close genetic relationship of HPV16 isolates to sub-lineages A2 (Asian) and A4 (Asian-American). Notably, variations in the *L2* gene were observed in HPV16 isolates from Riau Province, indicating potential genetic diversity in this region. These findings suggest that the *L2* gene variations may have implications for developing an L2-based HPV vaccine, particularly in addressing regional viral strain variations. Further studies are warranted to assess the clinical relevance of these variations in vaccine efficacy.

Ethics approval

This study was approved by the Health Research Ethics Committee of Arifin Achmad General Hospital and the Faculty of Medicine, Universitas Riau, Pekanbaru, Riau, Indonesia (089/UN.19.5.1.1.8/UEPKK/2018).

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Competing interests

The authors have no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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