



## Original article

## Genetic diversity and phylogenetic analysis of HPV 16 &amp; 18 variants isolated from cervical specimens of women in Saudi Arabia

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## ABSTRACT

Human papillomavirus (HPV) are well known to be associated with the development of cervical cancer. HPV16 and HPV 18 are known as high-risk types and reported to be predominantly associated with cervical cancer. The prevalence and genetic diversity of HPV have been well documented globally but, in the Kingdom of Saudi Arabia, data on HPV genetic diversity are lacking. In this study, we have analyzed the genetic diversity of both HPV16 and HPV18 based on their L1 gene sequence because L1 gene is a major capsid protein gene and has been utilized to develop a prophylactic vaccine. In January 2011–2012, a total of forty samples from cervical specimens of women in Saudi Arabia were collected. The association of HPV16, HPV18 was detected by polymerase chain reaction, sequenced and submitted to GenBank. The sequences identity matrix and the phylogenetic relationship were analyzed with selected HPVs. The highest sequence identity (99.5%) for HPV16 and (99.3%) for HPV was observed with selected HPVs. The phylogenetic analysis results showed that HPVs from Saudi Arabia formed a closed cluster with African, Asian, East Asian as well as American HPVs distributed into multiple lineages from various geographical locations. The results provided the valuable information about genetic diversity, but there is an urgent need to generate full genome sequence information which will provide a clearer picture of the genetic diversity and evolution of HPVs in Saudi Arabia. In conclusion, the generated data will be highly beneficial for developing molecular diagnostic tools, analyzing and correlating the epidemiological data to determine the risk of cervical cancer and finally to develop a vaccine for Saudi Arabian population.

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## 1. Introduction

Cervical cancer is a major cause of death among women globally (Ervik et al., 2016). Human papillomavirus (HPV) is a circular dsDNA virus belongs to the family *Papillomaviridae*. The viral genome size range from ~7.9–8.0 kb in length containing six early genes (E1, E2, E4, E5, E6 and E7) and 2 late genes designated as L1, L2 (Doorbar et al., 2012; Van Doorslaer et al., 2013; El-Aliani

et al., 2017; Van der Weele et al., 2018). Currently, approximately 200 HPVs genotypes have been identified and divided based on more than 10% nucleotide diversity of L1 gene (Van Doorslaer et al., 2013; Ervik et al., 2016; Basto et al., 2017; Islam et al., 2018). Based on criteria defined by the International Agency for Research on Cancer, the development of cervical cancer is predominantly reported to be caused by fifteen high-risk HPVs (hrHPV) (IARC, 2012). HPV16 and HPV18 are well reported as “high-risk” due to their high carcinogenic potentials (Crosbie et al., 2013; Van der Weele et al., 2018). HPV16 and HPV18 are reported to be associated with more than seventy percent of low-grade CIN, but the viral persistence induces the cellular changes leads to high grade CIN and invasive cervical cancer (Mazumder et al., 2011; IARC, 2012; Van Doorslaer et al., 2013; Steenbergen et al., 2014; Chen et al., 2015; Dutta et al., 2015; Wilting and Steenbergen, 2016; Basto et al., 2017; Van der Weele et al., 2018; Liu et al., 2018). Additionally, HPV16, is known to be associated with many other types of cancers like; head and neck squamous cell cancer,

Abbreviations: HPV, Human papillomavirus; CIN, Cervical Intraepithelial Neoplasia.

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prostate cancer as well as breast cancer (Michaud et al., 201; Islam et al., 2017; Islam et al., 2018). Globally it is well known that all hrHPV types had differential risks for CIN and cervical cancer (Bae and Kim, 2016; Michaud et al., 2014; Munoz et al., 2003; Shukla et al., 2009; Van der Weele et al., 2018). The taxonomy of HPVs is fully based on more than 10% sequence variation of L1 gene (Bernard, 2013). The clustering of HPVs genotypes is based on multiple properties like pathogenicity, different organs tumor, ethnicity and geographical location. Currently, based on sequence diversity, HPV16, have been divided into four phylogenetic lineages A, B, C and D and their sub-lineages A1(European), A2(European), A3(E), A4(Asian), B1(African-1a), B2(African-1b), D1(North American 1), D2 (Asian-American 2) and D3(Asian-American1) (Burk et al., 2013).

In 1984, the HPV18 was identified for the first time (Boshart et al., 1984) and reported as the second highest carcinogenic genotype after HPV16. Globally, HPV 18 are known to be associated with invasive cervical cancers in approximately 55–65% of cases. The genetic variants of HPV18 formed closed clustering with lineages A, B and C and that also includes the earlier designated as As/Ai, E and Af lineage (Chen et al., 2009; Guan et al., 2012; Chen et al., 2013, 2015; Xu et al., 2018). HPV18 shares close cluster trait which represents the higher propensity to develop adenocarcinoma as compared to other HPV genotypes (Xu et al., 2018). The HPV encodes the major capsid protein known as L1 gene which could be a potential target for the development of a prophylactic vaccine (Erickson et al., 2014). A prophylactic vaccine known as AS04-adjuvanted HPV16/18 vaccine designated as Cervarix® has been developed by GlaxoSmithKline Biologicals, Belgium, consisting of virus-like particles derivatives of HPV16 and 18 L1 proteins (McKeage and Romanowski, 2011; Zhu et al., 2017). Based on previous studies, the genetic variation in L1 gene significantly affects the viral assembly, protein structure/confirmation results into altered biological functions and host immunogenic recognition with clinical significance (El-Aliani et al., 2017). The information about genetic diversity of HPV16 and HPV18 genotypes have been published in many reports worldwide (Shen et al., 2013; Assoumou et al., 2015; King et al., 2016; Nicolas-Parraga et al., 2016; Doorslaer et al., 2017; Islam et al., 2018; Van der Weele et al., 2018; Liu et al., 2018; Xu et al., 2018; Aziz et al., 2018; Tao et al., 2018; Ramas et al., 2018; Yoon et al., 2018). The current status of HPVs prevalence in Saudi Arabia has been published in many reports, but data on genetic diversity and evolutionary history in Saudi Arabia are lacking (Bondagji et al., 2013; Turki et al., 2013; Alsbeih et al., 2013; Alsbeih, 2014; Al-Ahdal et al., 2014; Al Obaid et al., 2014; Omran and AlSheeha, 2015; Alhamlan et al., 2015; Hussain et al., 2016; Ahmed et al., 2017a). Thus, it is very important to analyze the genetic variability of HPVs with geographical and racial characteristics in the Kingdom. In our previous study, more than forty-three percent HPV prevalence were observed in tested specimens collected from women in Saudi Arabia. The prevalence of genotypes was found as HPV16 (30%) HPV 18 (8.0%) and HPV 45 (5.0%) (Turki et al., 2013). The purpose of this work was to identify the genetic variability of L1 genes amplified from tested positive samples and to elucidate the genetic relationship between HPV16 and HPV18 variants as well as cervical cancer risk in the Saudi population.

## 2. Materials and methods

### 2.1. Ethics statement

The approval was taken from King Abdulaziz University Hospital Ethical Committee and consent was received from all the participating individuals during the study.

### 2.2. Samples collection

The sampling was conducted between January 2011 and January 2012 at King Abdulaziz University Hospital. Total forty samples were collected from women with different age group. Examination performed by gynecological practitioners at King Abdulaziz University Hospital. The HPV16 and 18 positive samples from our previous studies were randomly included in this study. The age of the patients enrolled in this study varied from 36 to 80 years.

### 2.3. DNA isolation, HPV screening and genotyping

DNA extraction was performed using Qiamp DNA mini kit (Qiagen, Valencia, CA) following the manufacturer's instructions. After extraction, all specimens were subjected to PCR amplification of the  $\beta$ -globin gene to serve as an internal control as described previously (Shadrina et al., 2007) and stored subsequently at  $-20^{\circ}\text{C}$  until tested. The presence of HPV was previously detected by polymerase chain reaction (PCR) using Biofilm Chip hybridization assay. The hybridization and detection were performed using auto genomics INFINITI Analyzer (Vista, CA) according to the manufacturer's instructions (Turki et al., 2013).

### 2.4. PCR amplification and sequencing

The PCR amplification was performed by using purified genomic DNA. For amplification of E6-L1 gene (~3.3 kb) from HPV16 was amplified by using primers 5-TAATATAACTGACCAAGCTCCTT CATTAAATTC-3 and 5-TACATAAAACCATCCATTACA TCCCGTACC-3 (Shang et al., 2011). The PCR mixture contains  $1 \times$  PCR buffer, 2.5 mM  $\text{MgCl}_2$ , 0.2 mM dNTPs mix, 1 mM both primers (F/R), 25 ng purified DNA, and 1.5 U *Taq* DNA polymerase (MBI fermentas) in 50  $\mu\text{l}$  final volume. The initial denaturation was given at  $94^{\circ}\text{C}$  ( $3 \text{ min}^{-1}$  cycle) and 35-cycle of denaturation at  $94^{\circ}\text{C}$  (60 sec), annealing at  $60^{\circ}\text{C}$  (35 sec), and extension at  $68^{\circ}\text{C}$  (2 min), with final extension (10 min) at  $68^{\circ}\text{C}$ . The amplicon was visualized on 1% Agarose gel under UV transilluminator.

For amplification of HPV18 E6-L1 genes, type-specific primers were used (Xu et al., 2018). The PCR mixture contains  $1 \times$  PCR buffer, 2 mM  $\text{MgCl}_2$ , 0.2 mM dNTPs mix, 1 mM of both primers (F/R) and 1 U *Taq* DNA polymerase (MBI fermentas) and 25 ng purified DNA in a final volume of 50  $\mu\text{l}$  under following PCR conditions: initial denaturation at  $95^{\circ}\text{C}$ , for 5 min (1 cycle) followed by 35 cycles of  $94^{\circ}\text{C}$  for 30 s,  $55/60^{\circ}\text{C}$  for 30 s,  $72^{\circ}\text{C}$  for 30 s, and a final extension at  $72^{\circ}\text{C}$  for 10 min. The PCR product was visualized on 1% Agarose gel under UV transilluminator. All the amplicons were eluted and gel purified and finally sequenced. The sequences were assembled and analyzed by NCBI-BLAST search. The top matching sequences from various locations were selected for further analysis. Based on the best sequence homology and L1 gene properties as major capsid protein gene which have been extensively used in vaccine development, we selected the L1 sequences for further analysis.

### 2.5. Sequence analysis and phylogenetic tree construction

The L1 gene sequences for both HPV16 and HPV18 were used for genetic diversity analysis in this study. For HPV16 and HPV18 genetic analysis, lineage specific reference sequences were retrieved from GenBank database. The full-length sequences were assembled and BLAST for sequence similarity and percent sequence identity matrix of selected HPV 16 and HPV18 was analyzed by using, BioEdit (version 5.0.9) software programme (Hall, 1999). The multiple sequence alignment was performed by using CLUSTALW program. A phylogenetic tree using maximum

likelihood was generated with selected HPV16 and HPV18, using MEGA7.0 program. The reliability was assessed by the calculation of bootstrap with 1000 replicates (Kumar et al., 2016).

### 3. Results

#### 3.1. Detection and screening of HPV

In this work, total forty samples were collected from women with different age group patients. The results obtained by Biofilm Chip hybridization assay showed that HPV16, HPV18 and HPV 45 were the most common genotypes in Saudi women population. Among them, the prevalence of HPV16 was the highest (30%), HPV18 (8%) followed by HPV 45 (5%) among 35–76-year-old age group in the tested samples collected (Turki et al., 2013).

#### 3.2. PCR amplification and sequence analysis

The E6–L1 gene from both HPV16 and HPV18 were amplified from the positive samples using specific primers and further sequenced in both directions. Based on the sequence homology of L1 gene, total eight from HPV16 and seven from HPV18 were isolated, analyzed and finally submitted to GenBank with accession numbers (MH057735, MH057736, MH057737, MH057738, MH057739, MH057740, MH057741, MH057742, MH057743, MH057744, MH057745, MH057746, MH057747, MH057748, MH057749). The total sequences (nt) of L1 gene isolated from HPV16 ranged from 1596 to 1606 nt while HPV18 ranged from 1607 to 1707 nt. The nucleotide sequence comparison of L1 gene with selected HPV16 from multiple locations revealed that the HPV16 from Saudi Arabia shared a variable similarity ranged from 99.5 to 93.7% with other selected HPV16 from distinct locations distributed in different lineage (Table 1). They were tentatively designated as HPJ16 and HPJ18. The highest identity (99.5%) was observed with two HPV16 (HQ644241, AY686579) reported from the USA and Asian American type 2. Additionally, the comparative nucleotide sequence analysis of L1 gene of HPV18 generated from our study with selected HPV18 also showed variable sequence similarity ranged from 99.3 to 88.0% with various locations laying in different lineage (Table 2). The highest identity (99.3%) was found with HPV 18 identified from Brazil (KU298886).

#### 3.3. Phylogenetic analysis of L1 gene of HPV16 and HPV 18

##### 3.3.1. HPV-16

The phylogenetic tree analysis based on L1 gene sequences of HPJ16 with selected HPV16 showed that the HPJ16 from Jeddah, Saudi Arabia were distributed in six lineages like (i) A1, (ii) A2, (iii) A4, (iv) (B1), (v) C, (vi) D2 (Fig. 1). All the eight isolates were clustered with various lineage. The lineage A had both HPJ16–Jeddah-6, HPJ16–Jeddah-7. Interestingly, HPJ16–Jeddah-6 formed a closed cluster with some isolates from China, Brazil, and UK. The HPJ16–Jeddah-7 clustered mostly with an HPV16 from USA and Brazil. The Linage A2 had only two isolates HPJ16–Jeddah-4 and HPJ16–Jeddah-8 and they formed a closed cluster with an isolates from Thailand, European and Germany. The HPJ16–Jeddah-5 distributed in Linage A4 and clustered with Asian and East Asian only. The lineage D2 had only HPJ16–Jeddah-1 and formed closed cluster with Asian American type 2 and American HPV isolates while HPJ16–Jeddah-3 clustered with only African type one isolates and formed in lineage B1 and HPJ16–Jeddah-2 formed closed cluster with African type two isolates and fall under lineage C. The results obtained in this study showed the wide range of genetic diversity among HPV16 detected from Saudi Arabian population and geographically different and distributed in multiple lineages and

**Table 1**

Sequence identity matrix of HPV16–Jeddah-1 (MH057735) isolate with selected HPV-16.

Serial No	Accession Number	Locations/isolate	% Identity
1	MH057736	Jeddah-2–Saudi Arabia	97.3
2	MH057737	Jeddah-3–Saudi Arabia	97.7
3	MH057738	Jeddah-4–Saudi Arabia	98.6
4	MH057739	Jeddah-5–Saudi Arabia	98.6
5	MH057740	Jeddah-6–Saudi Arabia	98.5
6	MH057741	Jeddah-7–Saudi Arabia	93.4
7	MH057742	Jeddah-8–Saudi Arabia	98.7
8	AF125673	USA	99.1
9	AY686584	USA	99.3
10	NC_001526	USA	94.3
11	HQ644241	Asian American type-2	99.5
12	AY686579	USA	99.3
13	FJ006723	China	99.1
14	KU298885	Brazil	99.5
15	KU298880	Brazil	93.7
16	AF472508	African-Type1	98.3
17	AF536180	African-Type1	98.1
18	HQ644296	African-Type1	98.4
19	AF472509	African-Type2	98.4
20	AB818690	African-Type	98.3
21	K02718	UK	98.7
22	AF536179	European-German	99.0
23	AF534061	East Asian- Asian	99.2
24	HQ644261	Asian	99.2
25	FJ610152	Thailand	99.1
26	LC193821	Japan	99.2
27	AB818689	Japan	98.5
28	HQ644285	Asian-American	98.4
29	HQ644276	Asian-American	98.4
30	HQ644289	Asian-American	98.4
31	HQ644257	Asian-American	98.5
32	HQ644270	Asian-American-2	98.3
33	AF402678	Asian-American variants	98.4
34	HQ644241	Asian-American variants-2	98.4

formed the closed cluster with Asian, American as well as African HPV types.

##### 3.3.2. HPV18

The phylogenetic tree analysis was performed by using L1 gene of all HPV18 sequences detected from Saudi population with selected isolates. The results showed that all seven HPJ18 from Jeddah were distributed in 4 lineages like (i) A1, (ii) A3, (iii) A4, (iv) (A5) (Fig. 2). The lineage A1 contains three HPV types ie., HPJ18–Jeddah-3, HPJ18–Jeddah-4, HPJ18–Jeddah-5 and formed closed cluster with an isolates from China and USA while the Linage A3 had only HPJ18–Jeddah-1 and HPJ18–Jeddah-2 forming closed cluster with multiple isolates from Brazil, Netherland, China and USA and lineage A4 has only one type HPJ18–Jeddah-7 which formed closed cluster with an isolate from the USA. Interestingly, HPJ18–Jeddah-6 come into Linage A5 and formed a closed cluster with Asian isolates like Thailand and China. The wide range of genetic variability was observed among HPJ18–Jeddah isolates identified in the samples tested from Saudi population and they were observed to be genetically and geographically different as they were distributed in different lineages.

### 4. Discussion

HPV infection is the most serious and important etiological aspect and valuable prognosticator in cervical cancer globally. Based on the current research and published information, more than seventy percent cervical cancer cases are caused by hrHPVs. According to WHO, currently, total 561,200 cases are accredited to HPV infection. Based on the oncogenic potential, approximately 200 HPVs have been identified and classified as high risk-HPV and

**Table 2**  
Sequence identity matrix of HPV18-Jeddah-1 (MH057743) isolate with selected HPV-18.

Serial No	Accession Number	Locations/isolate	% Identity
1	MH057744	Jeddah-Saudi Arabia-2	88.2
2	MH057745	Jeddah-Saudi Arabia-3	87.5
3	MH057746	Jeddah-Saudi Arabia-4	88.0
4	MH057747	Jeddah-Saudi Arabia-5	81.8
5	MH057748	Jeddah-Saudi Arabia-6	87.9
6	MH057749	Jeddah-Saudi Arabia-7	88.0
7	KU298886	Brazil	99.3
8	KU707824	Netherland	88.5
9	KC456642	China	88.0
10	KU721791	China	88.4
11	KU721790	China	88.3
12	KY457805	China	88.4
13	KY457821	China	88.2
14	KY457826	China	88.2
15	KY457829	China	88.4
16	KY457831	China	88.5
17	KY457833	China	88.3
18	KY457835	China	88.2
19	KY457840	China	88.1
20	EF202143	USA	88.0
21	EF202144	USA	88.3
22	EF202147	USA	88.4
23	EF202150	USA	88.3
24	EF202155	USA	87.7
25	KC470224	USA	87.6
26	KC470229	USA	87.4
27	NC_001357	USA	88.1
28	KC470211	USA	88.3
29	KC470213	USA	88.4
30	KC470214	USA	87.6
31	KC470223	USA	87.5
32	KC470226	USA	87.7
33	KC470227	USA	87.6
34	GQ180787	Thailand	88.2

low risk-HPV. Cervical cancer caused by HPVs is known as the 4th most common cause of death worldwide (Ervik et al., 2016; Durzynska et al., 2017). HPV16 and HPV 18 are well known to be the most significant risk factors for cervical cancer globally (Dutta et al., 2012; Crosbie et al., 2013; WHO, 2014; Steenbergen et al., 2014; Alhamlan et al., 2015; Chen et al., 2015; Omran and AlSheeha, 2015; Chen et al., 2016; Xu et al., 2016; Krashias et al., 2017; Liu et al., 2018; Aziz et al., 2018; Tao et al., 2018; Ramas et al., 2018; Yoon et al., 2018; Leo et al., 2018; Bahrami et al., 2018). The HPV screening and their genetic diversity in clinical settings could be an important factor for molecular diagnostic and vaccination strategies (Li et al., 2011). Globally, the prevalence and genetic diversity of HPVs, especially HPV16 and HPV18 have been reported by various research groups and published in many reports (Assoumou et al., 2015; Wilting and Steenbergen, 2016; El-Aliani et al., 2017; Xi et al., 2017; Basto et al., 2017; Doorslaer et al., 2017; Islam et al., 2018; Xu et al., 2018; Van der Wee et al., 2018). Recently, HPV infection in females with normal cervical cytology and their genotyping with phylogenetic tree was performed by PCR in total 1011 patients in Pakistan and found that 4.74% HPV prevalence with HPV6 (in 25% of infected individuals), followed by HPV55 (22.9%), HPV11 (20.8%), and high-risk types HPV45 (12.5%), HPV33 (8.33%), HPV18 (6.25%), and HPV16 (4.16%). The sequence identity matrix was observed to be 80–99% similar with same genotypes and formed closed clusters with HPV reported from China, India, Mexico, Iran, Slovenia, and Germany and it was concluded that the HPV prevalence with high risk types was very low and L1 gene sequences were highly similar (Aziz et al., 2018). Additionally, in a similar study performed in 4305 gynecological patients from South west China during March 2014 and March 2015 and total Seventeen high-risk (HR) genotypes (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58,

-59, -66, -68, -73, and -82) and seven low-risk (LR) genotypes (HPV-6, -11, -42, -43, -61, -81, and -83) were detected and a total of 3,785 (87.9%) HPV positive women were found to have HR HPV infection, while 859 (20.0%) were found to have LR HPV infection (Tao et al., 2018). A study was performed to analyze the HPV 16 E6, E7 genes and LCR in women from Uruguay, and high variation was observed in LCR sequence distributed in 18 different variants while E6 and E7 sequences exhibited novel non-synonymous substitutions which mainly belonged to the European lineage and 6 new variants from European and African clusters were identified (Ramas et al., 2018). Recently, Preclinical and genome-wide association studies have reported the associations of genetic variations in several susceptibility loci for the development of cervical cancer (Leo et al., 2018; Bahrami et al., 2018).

In Saudi Arabia, the cervical cancer ranks as 11th most frequent cancer and 8th most common cancer ranges from 15 to 44-year-old women (ICO/IARC, 2017). Based on information provided by WHO, more than 9.29 million women from 15-year-old or older are the risk of developing cervical cancer in the Kingdom of Saudi Arabia. Limited data is available on the HPV prevalence in Saudi Arabian population. Recently, 241 women are diagnosed and 84 died from cervical cancer in Saudi Arabia (ICO/IARC, 2017). About 2.3% of women are assessed for harbor cervical cancer caused by HPV16/18 and 72.4% of invasive cervical cancers are credited to HPV 16 or 18 in Saudi Arabian population (CSR, 2007; WHO, 2014; Alsbeih, 2014; Al-Ahdal et al., 2014; Alhamlan et al., 2015; ICO/IARC, 2017). The prevalence and detection of HPVs in cervical cancer specimens using various technological tools have been performed and published in many reports from Saudi Arabia and Yemen (Bondagji et al., 2013; Turki et al., 2013; Alsbeih et al., 2013; Alsbeih, 2014; Al-Ahdal et al., 2014; Al Obaid et al., 2014; Omran and AlSheeha, 2015; Alhamlan et al., 2015; Ahmed et al., 2017b) but the analysis of genetic diversity based on L1 gene was done for the first time in this study.

In this study, we have identified the HPV16, HPV18 and, HPV45 in cervical cancer specimens by using polymerase chain reaction (PCR) using Biofilm Chip hybridization assay in the time of January 2011 to January 2012 from Jeddah, Saudi Arabia. Additionally, we have analyzed the genetic diversity of L1 gene isolated from HPV16 and HPV18 and identified the phylogenetic relationships by comparing with selected HPVs collected from geographically different lineage. The sequence analysis results based on L1 gene of HPJ16 showed the highest identity (99.5%) with Asian American type 2 (HQ644241; AY686579) from lineage D2. Similarly, higher similarity (99.0–88%) was also observed in multiple HPV16 reported from Asian, East Asian, European-German and African type 1&2 which were geographically different and distributed in various lineage (A1, A2, A4, B1, C, D1). Our data is supported by previously published information as the frequency and sequence diversity of lineage A1 have been reported in cervical cancer in Spanish (87.5%) Tunisian (64%), Indian (66.6%) and Greek (84.6%) patient' population (Hrubec et al., 2011; Ntova et al., 2012; Perez et al., 2014; Islam et al., 2018). A variable prevalence cervical cancer with B (0–1.3%), C (1–2.7%) D (6.2–8.4%), D3 (19%) lineages has also been reported from Asian (China, Pakistan, and Nepal) and Spanish patients population (Nicolas-Parraga et al., 2016; Perez et al., 2014; Islam et al., 2018).

Interestingly, in phylogenetic relationship analysis, the HPV16 identified from Saudi Arabia formed a closed cluster with HPV16 from different locations, distributed in different lineages. HPJ16-Jeddah-1 laid in Lineage D2 and formed a closed cluster with Asian and American type 2 and HPJ16-Jeddah 2 and 3 clustered with African type 2 in Lineage B1 and C. while HPJ16-Jeddah-4,5 and 8 laid into Lineage A2 and A4. Although, similar variants were observed in HPV16, there were also unique variants like HPJ16-Jeddah-6 and 7 laid in a single lineage A1, while HPJ16-Jeddah-4 and 8 laid

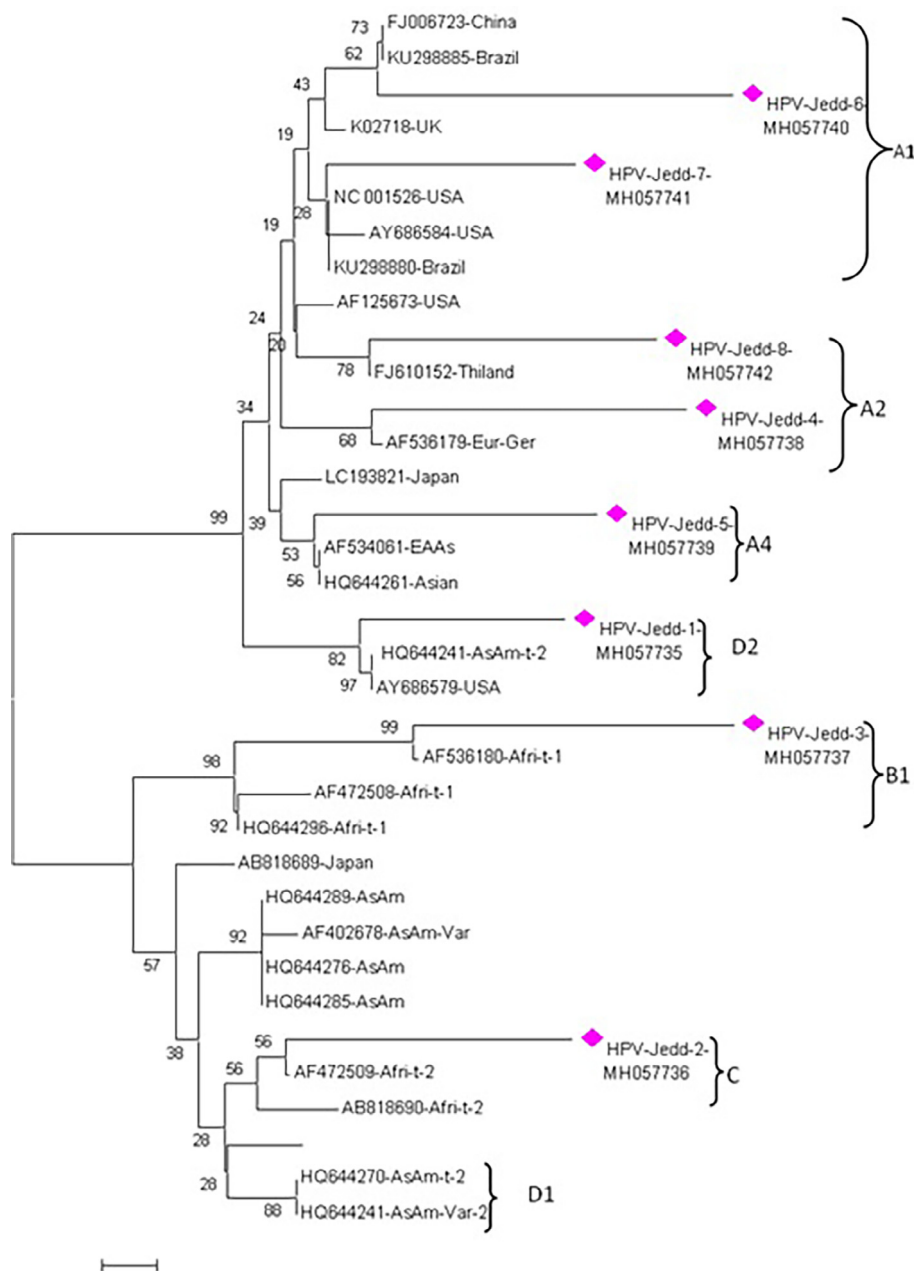


Fig. 1. Phylogenetic analysis of L1 gene with selected HPV16: Phylogenetic tree was constructed by Maximum Likelihood method of MEGA v7.0 software.

into lineage A2. The higher diversity itself indicates that they are different in both sequences as well as geographical locations. The differential unique variations in L1 gene of cervical cancer indicate the age and tissue-specific genetic variation of HPV16 in Saudi women population and this variation might be playing a significant role in the pathogenesis of HPV as similar variation was present in 22% tonsillar squamous cell carcinoma of Swedish patients but absent in cervical cancer of the same population (Du et al., 2012). Similar variation has also been reported in both breast and cervical cancer cases in Indian population (Islam et al., 2018).

Similarly, HPV18 is well known to be predominantly associated with the development of cervical adenocarcinoma (Clifford and Franceschi, 2008; Xu et al., 2018). The genetic diversity of HPV18 in Saudi Arabia is scarce but reports have been published from other locations (Xu et al., 2018). In our study, based on L1 gene sequences of HPJ18; the highest identity (99.3%) was observed

with HPV18 identified from Brazil. Additionally, variable sequence similarity ranged from 99.0 to 88% was observed with HPV18 reported from multiple locations, distributed in different lineage with various geographical locations. In phylogenetic relationship analysis, HPJ18 formed a closed cluster with many geographically different HPV18. The most important findings were observed that, HPJ18-Jeddah-3,4, and 5 formed closed cluster with American and Asian HPV18 and laid into Linage A1 while HPJ18-Jeddah-1&2 laid into lineage A3 and formed closed cluster with HPV18 reported from USA, Brazil, Netherland, and China and HPJ18-Jeddah-6 and 7 laid in Linage A4 and A5 (Chen et al., 2015; Chen et al., 2016). The distribution and prevalence of HPV variants in Asia and South East Asia were found to be geographically different (Chen et al., 2013; Bondagji et al., 2013; Turki et al., 2013; Alsbeih, 2014; Al-Ahdal et al., 2014; Al Obaid et al., 2014; Omer and AlSheeha, 2015; Alhamlan et al., 2015; Ahmed et al., 2017b; Xu et al., 2018,

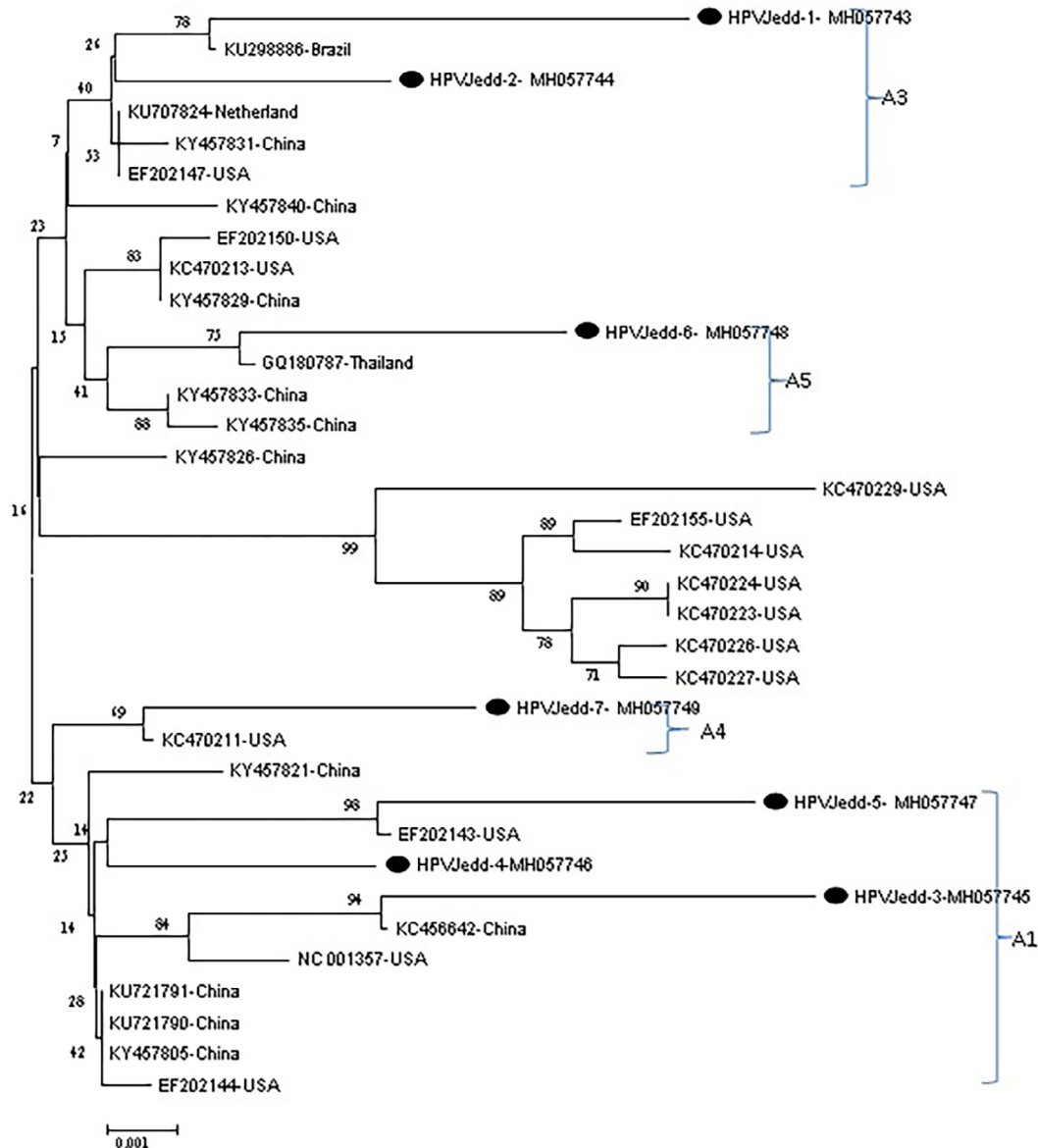


Fig. 2. Phylogenetic analysis of L1 gene with selected HPV18: Phylogenetic tree was constructed by Maximum Likelihood method of MEGA v7.0 software.

Islam et al., 2018). Additionally, we have detected the HPV 45 genotype from tested samples which shows that the prevalence of this genotype may be more in the near future which requires further detailed study with more samples collected from multiple locations in the Kingdom. Based on previous studies it was observed that the development of high-grade CIN is higher in non-European variants (Arroyo et al., 2012). The apparent genetic diversity and geographical differences disclose the intrinsic complication of HPV variants and development of cervical cancer. This provides the alarming situation to generate and pooled the data across the countries/different geographical regions.

## 5. Conclusion

We have reported the genetic diversity of L1 gene isolated from HPV16 and HPV18 in cervical cancer specimens from Saudi women. This study provided very essential information and a practical approach for genetic diversity and phylogenetic analysis which can be very useful for correlating the epidemiological studies as well as natural history and evolution of HPV in Kingdom. The

whole genome sequences from more samples collected from various locations will strengthen the detailed representation of HPV diversity and evolution in Kingdom of Saudi Arabia. The data generated about the genetic diversity of HPVs in Saudi Arabia will be helpful for designing and development of effective disease awareness and management strategies, an effective molecular diagnostic tool, probes and a next-generation prophylactic vaccine and vaccination strategies for Saudi Arabian population.

## Conflict of interest

All authors have no potential conflict of interest including any financial, personal, or other relationships.

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