Ref: Ro J Infect Dis. 2024;27(3)
DOI: 10.37897/RJID.2024.3.2

Genotyping and phylogenetic analysis of human Papillomaviruses from women with genital warts

Ghufran M. Al Azzawi, Anmar A. AlTaie

Department of Biology, College of Science, University of Mosul, Mosul, Iraq

ABSTRACT

Background and objectives. The pathophysiology of Human Papillomavirus (HPV) infections is dependent on the genotype of virus, the host immune system and the local environmental conditions. HPV infections are linked to a variety of benign and malignant disorders. Anogenital warts is a Sexually Transmitted Infection (STI) primarily caused by Low-Risk HPV (LR-HPV) genotypes 6 and 11. The aims were to Investigation of the prevalence and genotypic distribution of HPV in women with genital warts in Mosul/Iraq, HPV-DNA sequencing and Phylogenetic analysis including minimum spanning trees of amplified HPV L1 gene comparison the present sequences with similar genotypes in GenBank – National Center Biotechnology Information (NCBI) using mega-7-software.

Materials and methods. A total of 150 samples were collected from women with genital wart infections. Polymerase Chain Reaction (PCR) was performed using GP+5/GP+6 and MY09/MY11 consensus primers.

Results. About 84.6% prevalence of HPV genotypes. The highest frequency (32% and 28.6%) were observed in the 19-28 and 29-38 age groups.

Conclusion. Women in Mosul, Iraq, who have genital warts are more likely to have different genotypes of HPV. HPV 11 type was found to be the most common, followed HPV6 and HPV10 as LR HPV, while HPV 16 and HPV45 were the most common as HR HPV. The analysis of the phylogenetic tree revealed a genetic relationship between our isolates and global isolates ranging from (10-100%) bootstrap ratio this indicating phylogenetic relationships (divergent and convergent evolution) this was meant the diversity in origin of the various genotypes common in Mosul, Iraq.

Keywords: HPV, genital warts, DNA-sequencing, genotypes, phylogenetic tree

Abbreviations

BLAST – Basic Local Alignment Search Tool
CC – Cervical Cancer
HPV – Human Papilloma Virus
HR-HPV – High-Risk HPV
IARC – International Agency for Research on Cancer
LR-HPV – Low-Risk HPV
MEGA – Molecular Evolutionary Genetics Analysis
NCBI – National Center Biotechnology Information
PCR – Polymerase Chain Reaction
STI – Sexually Transmitted Infection

INTRODUCTION

The pathophysiology of HPV infections is dependent on the genotype of virus, the host immune system and the local environmental conditions, HPV infections are linked to a variety of benign and malignant disorders, anogenital warts is a STI primarily caused by LR-HPV genotypes 6 and 11, however, co infections with HR-HPV genotypes can also be observed [1]. HPV has been found to play a significant role in the development of Cervical Cancer CC [2]. CC is the fourth most prevalent disease in women globally and is caused by a prolonged infection with HR-HPV specifically with HPV 16 and HPV 18 [3]. According to the most recent global burden of cancer study, roughly 600,000 incident cases and 340,000 deaths annually are caused by CC, which also poses a significant risk to global health [4]. To

Corresponding author:
Anmar A. AlTaie
E-mail: anmaraltaee1978@uomosul.edu.iq

Article History:
Received: 27 May 2024
Accepted: 30 July 2024

date about 450 HPV genotypes following their identification and sequencing have been classified as either HR-HPV or LR-HPV genotypes [5]. The International Agency for Research on Cancer (IARC) classified HR-HPV to be an oncogenic HPV genotypes. The most common HR-HPV genotypes responsible for causing cancer are HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59, which are often linked with penile cancer. The second most common genotypes are HPV 68, 26, 53, 66, 67, 70, 73, 82, 30, 34, 69, 85 and 97 which were considered to be potentially carcinogenic [6]. LR-HPV genotypes included HPV genotypes 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81 [7]. LR-HPV leads to minor benign hyperproliferative lesions, while HR-HPV mostly leads to malignant lesions [8]. The most common HR-HPV and LR-HPV linked to genital lesions, according to various studies from neighboring countries, are HPV16 and HPV6, respectively [9,10]. In most patients HPV infection is asymptomatic and can be controlled by the immune system [11]. Modern screening techniques have been developed as a result of the realization that HPV is the primary cause of malignant cancers in the cervical region. CC can be prevented from progressing if precancerous symptoms are identified early and treated [12]. For accurate identification and genotyping of HPV molecular tests are necessary [13]. Additional testing in cytological investigations are molecular diagnostic tests that identify HPV DNA and differentiate between HR-HPV and LR-HPV genotypes these tests are very sensitive and specific [14]. PCR methods or gene amplification originally needed a small portion of the genome sequence an exponential amplification step is performed on a characteristic of the HPV genome sequence in vitro, allowing its duplication for identification purposes [15]. To identify the genotype and alignment with reference strains, another method is DNA sequencing of pure DNA amplified by traditional PCR using consensus primers GP+5/GP+6 and MY09/MY11, which yield 150 and 450 bp amplicons, respectively [16].

MATERIALS AND METHODS

Sample collection

A total of one hundred fifty specimens (PBS embedded tissue, cervical swab, and cervical brush) were taken from women with genital warts who were referred to Al-Zahrawy private Hospital and Mosul General Hospital in Mosul/Iraq from August/2023 to April/2024. Their ages range between 19 and 58 years old.

DNA Extraction

Using a QIAamp DNA mini kit, DNA was extracted from the genital warts (tissues), cervical swabs and cervical brushes. following the manufacturer's instructions (QIAGEN, Germany). All the extracted DNA was stored at -20 °C until analyzed by PCR.

Identification and sequencing of HPV DNA

The PCR was performed using consensus GP+5/GP+6 and MY09/MY11 oligonucleotide primers to amplify the 150 bp and 450 bp, respectively (Table 1). The steps involved in amplification typically include a step of denaturation with 40 cycles at 94 °C for 5 min and a step of annealing at 48 °C (for GP+5/GP+6 and 55 °C for MY09/MY11) for 0.45 sec. Lastly, there is an extension step lasting 0.45 seconds at 72 °C.

4 µl of PCR product was analyzed by electrophoresis on 2% w/v agarose gel containing 1µl of Red Safe Nucleic Acid (diamond nucleic acid dye, Promega). HPV genotyping was performed by sequencing GP+5/GP+6 and MY09/MY11 PCR in Macrogen (Macrogen Co., Seoul, Korea). The software BioEdit (version 7.2.5.0) was used to perform multiple alignments. After that, the Fasta format files were uploaded to the GenBank database, where the BLAST was used to do the best sequence homology search, which is available at the NCBI online website. The phylogenetic tree was constructed by bootstrap (100X) analysis using the MEGA-11 software [19].

RESULTS

PCR was used to amplify the HPV L1 gene using specific primers MY09/MY11 450 bp and GP+5/+6 150 bp. from 150 samples from women with genital warts. our study on molecular detection of HPV PCR, which was performed in Mosul, Iraq, Only 127 cases (84.6%) were isolated and identified, the types of samples collected were variable among patients. In the current study, we used three types of samples (PBS-embedded tissues, endo cervical swabs, and

TABLE 1. Primers used in this study

Primers		Sequence	Size (bp)	Reference
MY09	F	5-CGTCCMARRGGAWACTGATC-3	450	[17]
MY11	R	5-GCMCAGGGWCATAAYAATGG-3		
GP+5	F	5 TTTGTTACTGTGGTAGATACTAC-3	150	[18]
GP+6	R	5 GAAAAATAAACTGTAAATCATATTC-3		

cervical brushes) to ensure the presence of viruses. Patients' ages ranged from 19 to 58 years old. and participants were classified into four age groups the variable distribution of HPV positives across all age categories, with the highest frequency (32% and 28.6%) occurring at the 19-28 and 29-38 years age (Table 2).

TABLE 2. The relation between age group/years and HPV frequency

Age Groups	PCR Results				Total	
	Positive		Negative			
	N.	%	N.	%	N.	%
19-28	48	32	3	2	51	34
29-38	43	28.6	4	2.7	47	31.3
39-48	21	14	6	4	27	18
49-58	15	10	10	6.7	25	16.7
Total	127	84.6	23	15.4	150	100

The DNA sequencing of the positive PCR products was followed by analysis. The results for all samples demonstrated the evolutionary relationships between the strains under study and the closest related species of the HPV genus found in the Genbank data. The Nucleotide Basic Local Alignment Search Tool (BLAST) program [20] was used to search for homology to the input sequences against entire sequences that are available on the sequence NCBI GenBank database. according to the present sequence alignment results, all isolates are HPV-positive, with a similarity ratio between the query and subject sequences of 93-100%, as indicated in Table 3, Based on the Capsid Protein (L1) Gene, Representative Genotyping Analysis of HPV Sample Isolates was conducted.

The genetic variation in the nucleotide sequence, which was relatively few is due to the occurrence of some point mutation (insertion, deletion, and base substitution) as Figure 1.

TABLE 3. Genotyping Analysis of HPV Sample Isolates based on Capsid Protein (L1) Gene and make homology against entire sequences, which are available on the sequence, NCBI GenBank database

No.	Geno-types	Ident per%	Accession Number	Country	Year
5	HPV6	98.79	ON053201.1	Iran	2022
6		99.24	KC706454.1	Saudi Arabia	2013
1	HPV 10	98.32	OP971088.1	South Africa	2022
14	HPV11	96.23	KM501531.1	Thailand	2014
15		99.03	KR674044.1	Kenya	2015
16		98.98	GU344758.1	Iran	2009
22	HPV16	94.29	MG849876.1	USA	2018
23	HPV45	100	OP971081.1	South Africa	2022
24		100	OP712095.1	France	2022

A phylogenetic tree (Figure 2) was built using sixteen typical HPV L1 gene sequences from the current study and thirty-one sequences of other HPV genotypes that were obtained from the NCBI nucleotide databank based on percentage similarity. Using the MEGA-11 program and bootstrap (100X) analysis, the phylogenetic tree was created [19].

DISCUSSION

The anogenital HPV burden varies across different geographic groups and even within the same country's diverse areas. Also, genital warts were considered HPV-related geographic regions and/or population subgroups [21,22]. The highest frequency (32% and 28.6%) occurring at the 19-28 and 29-38 years age. So, there was a tendency for women in the third and fourth decades to have greater viral

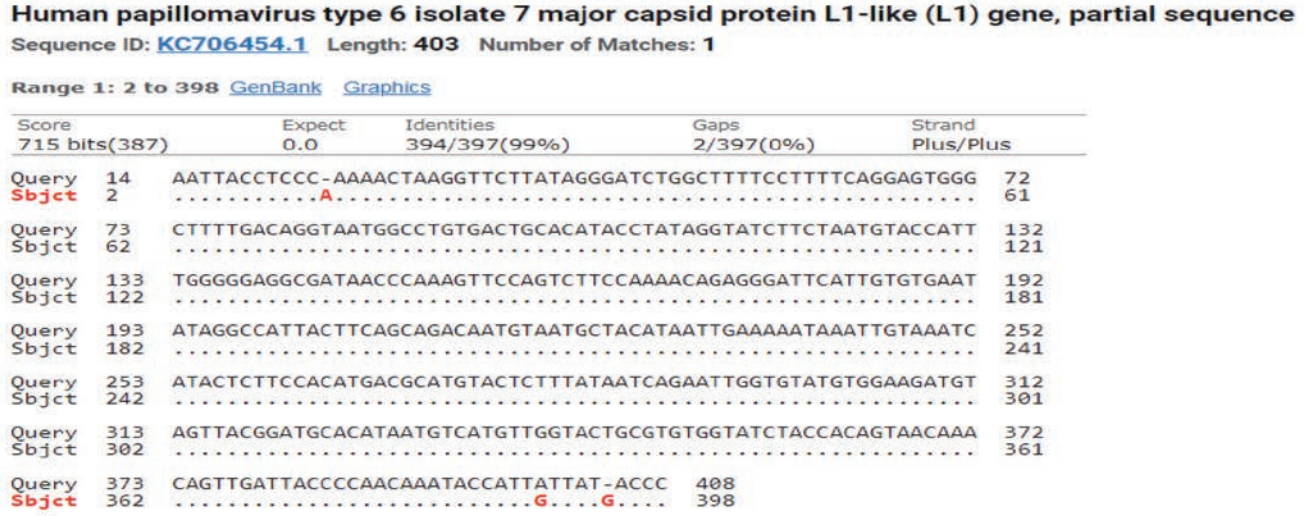

FIGURE 1. The isolate of HPV 6 (HPV No. 6) using MY09/11 primer was performed by gene sequence alignment with reference strains in NCBI. The point mutation (deletion and base substitution) occurred at positions 13, 389, and 394 to the reference sequence (ACCN: KC706454.1) isolated in Saudi Arabia

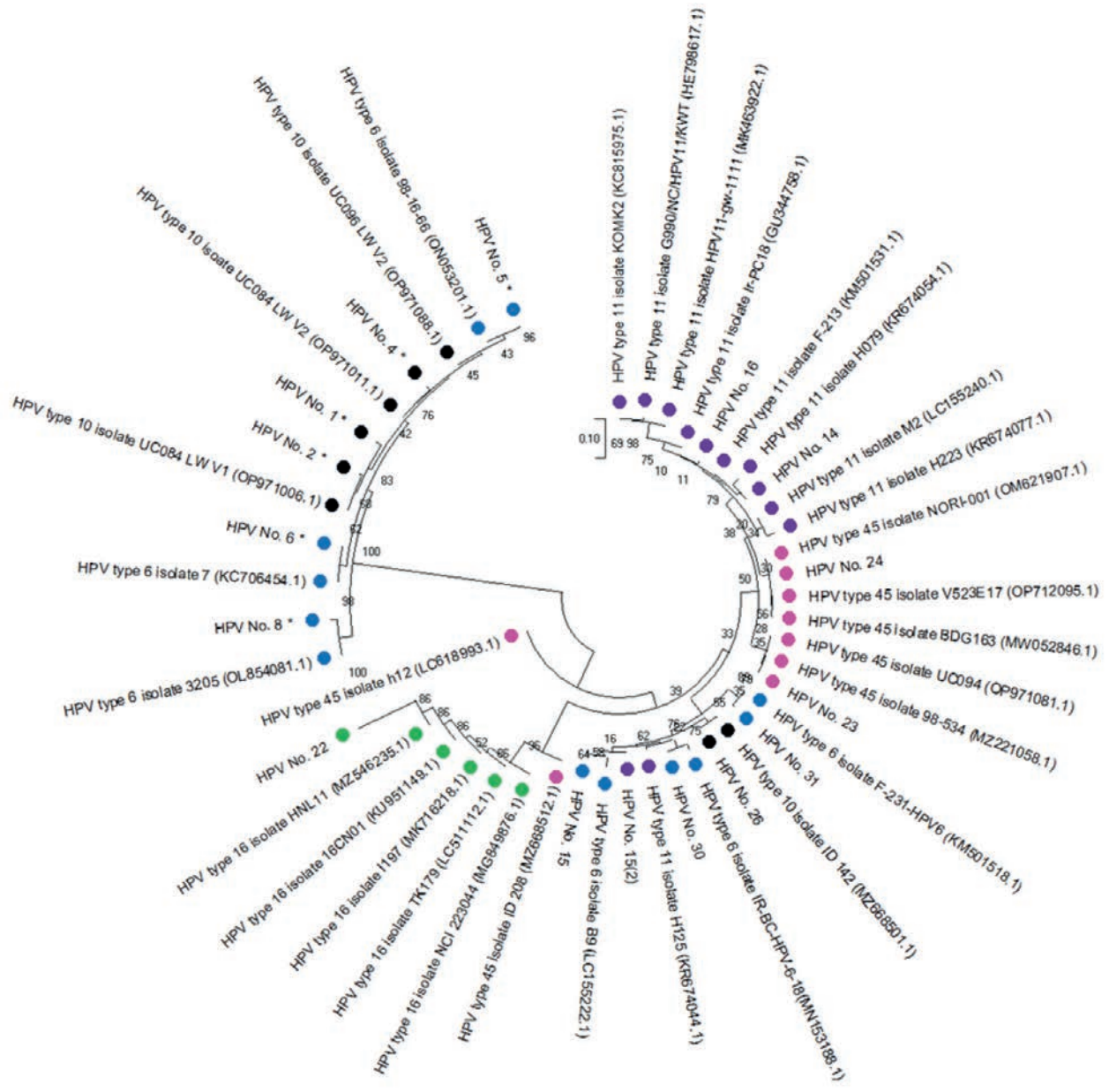

FIGURE 2. Neighbor-joining phylogenetic trees showing the relationship between our results indicate as (HPV No.) based on MY09/11 sequences with an asterisk and GP+5/+6 using MEGA-11 software with a scale length of 0.1 and the reference HPV types obtained from the NCBI Genbank database each color indicate different genotypes

infections because during this period sexual activity begins and marriage becomes more popular [23,24]. HPV infections are transient, which explains the progressive decline in HPV infection rates among middle-aged and older people and the potential that certain tested and immunized women may eventually have a stronger immune system, which would, in most circumstances, eliminate the virus cases [25,26]. Among the most widely used primers in traditional PCR experiments is the MY09/11 consensus primer, which targets the 450 bp conserved sequence of the HPV L1 region. Its drawbacks, particularly its low sensitivity, have been demonstrated by earlier research [27-29]. This was enhanced by missing a positive case [30]. Because the GP-PCR method amplifies tiny DNA fragments, it is possible to explain why the detection of HPV was higher for GP+5/GP+6 oligonucleotides than for MY09/MY11 oligonucleotides [31]. Viruses evolve genetically through a variety of mechanisms including Evolutionary fitness, Recombination, and mutations. Successful reproduction is referred to as evolutionary fitness. Viruses can go through natural selection-based evolution to maximize their fitness since they are self-replicating creatures with a genotype/phenotype relationship. The prevalence (frequency) of an HPV type in the host population can be used to determine its fitness. Prevalence is a function of both incidence (rate of successful transmission to new hosts) and persistence (length of productive infection). HPV genomes have low mutation rates because they replicate using host DNA polymerases [32]. The analysis

of the phylogenetic tree (Figure 2) revealed a genetic relationship between our isolates and global isolates ranging from (10-100%) bootstrap ratio this indicating phylogenetic relationships (divergent and convergent evolution) with types related to the same genotype (intra typic) and with types related to the different genotype (extra typic). The sequences from Iran, South Africa, Kenya, France, Pakistan, China, and Indonesia have coevolved with various HPV types were grouped to many clusters with our isolates of five HPV genotypes 6,10,11,16 and 45 (each genotype has a distinct color). The genotype clustering suggests a taxonomical separation of HPV types, and the intra-typic variations also demonstrate a very low level of genomic diversity. The phylogenetic tree showed that HPV 6 (HPV No 6) isolated in this study was close match to reference strain (ACC. OL854081.1) globally isolate of China 2021 while HPV11 (HPV No.16) showed the lowest similarity with global isolates of Iran 2009 (ACC. GU344758.1), China 2019 (ACC. MK463922.1) and Kuwait 2012 (ACC. HE798617.1). These findings suggest that nucleotide heterogeneity. The potential for viral isolates from other nations that were spread by the entry of infected individuals into the country might be the reason for the discrepancy in replicate ratios between our local isolates. This, however, is in line with research by [33-35] which highlights the significance of nucleotide polymorphism and mutational changes in viral genes rather than the fact that geographic distance does not entirely influence the distribution of HPV types. The variation analysis corroborates a study by Bernard et al. 2010 [36], which found that differences in the genetic makeup of the women from whom HPV was isolated could account for disparities in HPV strains. Similarly, the study by Xi et al 2003 [37], which found that differences in the genetic makeup of the host contribute to disparities in HPV strains.

CONCLUSION

According to the current study, women in Mosul, Iraq, who have genital warts are more likely to have different strains of HPV. HR HPV varieties are very low. LR HPV type 11 was shown to be the most common HPV genotype, followed by HPV types 6 and 10. The partial L1 region's sequencing revealed that it was extremely conserved across all sequences that have been published. The prevalence of different HPV types in this study emphasizes the need for more research on the factors that influence HPV infection and its associated risk factors, including previously unrecognized factors like race and ethnicity, income and socioeconomic status, marital status, sexual partners, education level, and use of contraceptives. The information is important for the creation of novel HPV screening tests as well as for evaluating the impact of upcoming vaccinations on infections with varying degrees of severity. The report also emphasizes the necessity of routine HPV screening to prevent the problem from getting out of control and leading to cervical cancer. Additionally, prompt and accurate HR-HPV diagnosis may help in the early detection and prevention of cervical cancer in the area.

Conflict of interest: none declared
Financial support: none declared

REFERENCES

1. Stuqui B, Provazzi PJS, Lima MLD, Cabral ÁS, Leonel ECR, Candido NM, et al. Condyloma acuminata: An evaluation of the immune response at cellular and molecular levels. *PloS one.* 2023;18(4):e0284296. http://doi.org/10.1371/journal.pone.0284296.
2. Kamal M, Lameiras S, Deloger M, Morel A, Vacher S, Lecerf C, et al Human papillomavirus (HPV) integration signature in Cervical Cancer: identification of MACROD2 gene as HPV hot spot integration site. *Br J Cancer.* 2021;124(4):777-85. http://doi.org/10.1038/s41416-020-01153-4
3. Chen Q, Qu W, Zhao Y, Shu L, Wang Y, Chen X. The prevalence of HPV among 164,137 women in China exhibited some unique epidemiological characteristics. *Infect Agent Cancer.* 2023;18(1):72. http://doi.org/10.1186/s13027-023-00553-4
4. Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-Secretan B, et al. Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. *Lancet Glob Health.* 2023;11(2):e197-e206. http://doi.org/10.1016/S2214-109X(22)00501-0
5. McBride AA. Human papillomaviruses: diversity, infection and host interactions. *Nat Rev Microbiol.* 2022;20(2):95-108. http://doi.org/10.1038/s41579-021-00617-5
6. Zheng L, Zheng L, Chen S, Liu W, Qi J, Li K. Human papillomavirus prevalence and genotype distribution in Liaocheng men between 2016 and 2022. *J Med Virol.* 2024;96(1):e29360. http://doi.org/10.1002/jmv.29360
7. Ehteram H, Sadat Mousavian M, Mazoochi T, Khamehchian T, Karimian M. Association of Some High-Risk Mucosal Types of Human Papillomavirus with Cutaneous Squamous Cell Carcinoma in an Iranian Population. *Iran J Pathol.* 2019;14(4):313-6. http://doi.org/10.30699/ijp.2019.101544.2011
8. Malik S, Sah R, Muhammad K, Waheed Y. Tracking HPV Infection, Associated Cancer Development, and Recent Treatment Efforts-A Comprehensive Review. *Vaccines (Basel).* 2023;11(1):102. http://doi.org/10.3390/vaccines11010102
9. Moossavi M, Fereidouni M, Zardast M, Khazaei Z, Ghanbarzadeh N. Genotype distribution of human papilloma virus among women with genital warts biopsies in southern Khorasan, eastern Iran. *Meta Gene.* 2020;25(2):100720. http://doi.org/10.1016/j.mgene.2020.100720
10. Huang Z, Yao S, Zou L, Xie W, Xie D, Li W, et al. Evaluation of HPV infection and presence of licensed HPV vaccine genotypes among genital warts in Foshan, China. *Front Microbiol.* 2024;15:1376141. http://doi.org/10.3389/fmicb.2024.1376141
11. Georgescu SR, Mitran CI, Mitran MI, Tocut SM, Popa MI, Tampa M. New Insights in the Pathogenesis of HPV Infection and the Associated Carcinogenic Processes: The Role of Chronic Inflammation and Oxidative Stress. *J Immunol Res.* 2018;2018:5315816. http://doi.org/10.1155/2018/5315816
12. Venkatas J, Singh M. Cervical cancer: a meta-analysis, therapy and future of nanomedicine. *Ecancermedicalscience.* 2020;14:1111. Published 2020 Sep 24. http://doi.org/10.3332/ecancer.2020.1111

13. Ramesh PS, Krishnamurthy S, Shrestha S, Devegowda D. Where do we Stand on the Molecular Diagnostics for the Detection of Human Papillomavirus? A Comprehensive Review. *JCDR.* 2020;14(7):BE01-BE067. http://doi.org/10.7860/JCDR/2020/44371.13825
14. Ghosh S, Seth S, Paul J, Rahman R, Chattopadhyay S, Bhadra D. Evaluation of Pap smear, high risk HPV DNA testing in detection of cervical neoplasia with colposcopy guided or conventional biopsy as gold standard. *Int J Healthcare Biomed Res.* 2014;2(2):192-7.
15. Melón S, Alvarez-Argüelles M, de Oña M. Molecular diagnosis of Human Papillomavirus Infections. Human Papillomavirus and Related Diseases – From Bench to Bedside A Diagnostic and Preventive Perspective. *InTech.* 2013:1-26. http://doi.org/10.5772/55706
16. Tadlaoui KA, Hassou N, Bennani B, Ennaji MM. Emergence of oncogenic high-risk human papillomavirus types and cervical cancer. In Emerging and Reemerging Viral Pathogens Academic Press. 2020; p. 539-570. http://doi.org/10.1016/B978-0-12-819400-3.00024-7
17. Bauer HM, Ting Y, Greer CE, Chambers JC, Tashiro CJ, Chimera J, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA.* 1991;265(4):472-7. http://doi.org/10.1001/jama.1991.03460210055023
18. Jacobs MV, Snijders PJ, Van Den Brule AJ, Helmerhorst TJ, Meijer CJ Walboomers JM. A general primer GP5+/GP6 (+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *J Clin Microbiol.* 1997;35(3):791-5. http://doi.org/10.1128/jcm.35.3.791-795.1997
19. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol.* 2021;38(7):3022-7. http://doi.org/10.1093/molbev/msab120
20. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990;215(3):403-10. http://doi.org/10.1016/S0022-2836(05)80360-2
21. Diakite I, Martins B, Owusu-Edusei K, Palmer C, Patterson-Lomba O, Gomez-Lievano A, et al. Structured Literature Review to Identify Human Papillomavirus's Natural History Parameters for Dynamic Population Models of Vaccine Impacts. *Infect Dis Ther.* 2024;13(5):965-90. http://doi.org/10.1007/s40121-024-00952-z
22. Khan I, Harshithkumar R, More A, Mukherjee A. Human Papilloma Virus: An Unraveled Enigma of Universal Burden of Malignancies. *Pathogens.* 2023;12(4):564. http://doi.org/10.3390/pathogens12040564
23. Muderris T, Afsar I, Yıldız A, Akpınar Varer C. HPV genotype distribution among women with normal and abnormal cervical cytology in Turkey. *Rev Esp Quimioter.* 2019;32(6):516-524.
24. Oztürk S, Kaleli I, Kaleli B, Bir F. Servikal orneklerde insan papillomavirus DNA varliğinin hibrid yakalama yöntemiyle araştirilmasi [Investigation of human papillomavirus DNA in cervical specimens by hybrid capture assay]. *Mikrobiyol Bul.* 2004;38(3):223-32.
25. Ferrall L, Lin KY, Roden RBS, Hung CF, Wu TC. Cervical Cancer Immunotherapy: Facts and Hopes. *Clin Cancer Res.* 2021;27(18):4953-4973. http://doi.org/10.1158/1078-0432.CCR-20-2833
26. Garbuglia AR, Lapa D, Sias C, Capobianchi MR, Del Porto P. The Use of Both Therapeutic and Prophylactic Vaccines in the Therapy of Papillomavirus Disease. *Front Immunol.* 2020;11:188. Published 2020 Feb 18. http://doi.org/10.3389/fimmu.2020.00188
27. Sahiner F, Gümral R, Sener K, Yiğit N, Dede M, Yapar M. et al. Servikal Sürüntü Örneklerinde İki Farklı Yöntemle HPV-DNA Varlığının Araştırılması: MY09/11 Konsensus PCR ve Tipe Özgül Gerçek Zamanlı PCR [Investigation of HPV-DNA in cervical smear samples by two different methods: MY09/11 consensus PCR and type-specific real-time PCR]. *Mikrobiyoloji bulteni.* 2012;46(4):624-36.
28. Depuydt CE, Boulet GA, Horvath CA, Benoy IH, Vereecken AJ, Bogers JJ. Comparison of MY09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types. *J Cell Mol Med.* 2007;11(4):881-91. http://doi.org/10.1111/j.1582-4934.2007.00073.x
29. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlée F, Hildesheim A,et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol.* 2000;38(1):357-361. http://doi.org/10.1128/JCM.38.1.357-361.2000
30. Othman AA, Goreal AA, Pity IS. Genotyping and Phylogenetic Analysis of Human Papillomaviruses in Formalin Fixed Paraffin Embedded Sections from Cervical Lesions in Duhok-Iraq. *Asian Pac J Cancer Prev.* 2023;24(4):1313-1319. Published 2023 Apr 1. http://doi.org/10.31557/APJCP.2023.24.4.1313
31. Remmerbach TW, Brinckmann UG, Hemprich A, Chekol M, Kühndel K, Liebert UG. PCR detection of human papillomavirus of the mucosa: comparison between MY09/11 and GP5+/6+ primer sets. *J Clin Virol.* 2004;30(4):302-308. http://doi.org/10.1016/j.jcv.2003.12.011
32. Nelson CW, Mirabello L. Human papillomavirus genomics: Understanding carcinogenicity. *Tumour Virus Res.* 2023;15:200258. http://doi.org/10.1016/j.tvr.2023.200258
33. Cui F, Zhang Z, Xu J, Ding X, Mu X, Wan Q, et al. Genetic variability and lineage phylogeny of human papillomavirus type 45 based on E6 and E7 genes in Southwest China. *Virus Res.* 2018;255:85-9. http://doi.org/10.1016/j.virusres.2018.07.003.
34. Ramas V, Mirazo S, Bonilla S, Ruchansky D, Arbiza J. Analysis of human papillomavirus 16 E6, E7 genes and Long Control Region in cervical samples from Uruguayan women. *Gene.* 2018;654:103-9. http://doi.org/10.1016/j.gene.2018.02.023
35. Conde-Ferraez L, Pacheco-Arjona R, Novelo Canul C, Gomez-Carballo J, Ramirez-Prado JH, Ayora-Talavera G, et al. Genetic Variability in E6 and E7 Oncogenes from Human Papillomavirus Type 58 in Mexican Women. *Intervirology.* 2017;60(6):235-246. http://doi.org/10.1159/000489306
36. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology.* 2010;401(1):70-79. http://doi.org/10.1016/j.virol.2010.02.002
37. Xi LF, Touré P, Critchlow CW, Hawes SE, Dembele B, Sow PS, et al. Prevalence of specific types of human papillomavirus and cervical squamous intraepithelial lesions in consecutive, previously unscreened, West-African women over 35 years of age. *Int J Cancer.* 2003;103(6):803-9. http://doi.org/10.1002/ijc.10876