

Research Article

Molecular diagnosis of human papilloma virus (HPV) genotypes in women with genital warts in Mosul / Iraq

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Abstract

Human Papillomavirus (HPV) belongs to the Papillomaviridae family and can contribute to Cervical Cancer (CC). The present study aimed to investigate the genotypic distribution and prevalence of HPV using Phosphate Buffered Saline (PBS) embedded tissue, a cervical swab and a cervical brush in Viral transport medium (VTM) from women with different genital warts with no other disease in Mosul City/ Iraq. A total of 150 samples collected from women with genital warts infections were analyzed. A sequencing analysis of the amplified HPV L1 gene was made and compared with that of the National Center for Biotechnology Information (NCBI). The existence of HPV nucleic acid DNA was examined in all samples by Polymerase Chain Reaction (PCR) using MY09/MY11 and GP+5/GP+6 primers, and then HPV genotypes were identified in MacroGen (MacroGen Co., Seoul, Korea) BioEdit (version 7.2.5.0). From 150 (cervical swab, brush, and tissue) patients with genital warts, the distribution of HPV genotype frequency in females with vaginal warts was 127 patients (84.6%), 10 patients (8%) with High-Risk (HR-HPV) serotypes (4%) of each HPV (16 and 45), while the rest 117 patients (92%) with Low-Risk (LR-HPV) included 60.5% HPV11, 20.5% HPV6 and 11.% HPV10. For the first time in Mosul, Iraq, the study used PBS-embedded tissue from patients with various anogenital lesions to examine the prevalence and genotypic distribution of HPV.

Keywords: Genital warts, Genotype, Human Papillomavirus (HPV), Phosphate Buffered Saline (PBS), Polymerase Chain Reaction (PCR)

INTRODUCTION

Human Papillomavirus (HPV), which belongs to the *Papillomaviridae* family, is a tiny, icosahedral, and non-enveloped virus with 8,000 bp of circular double-stranded DNA that combines with histones to form structures resembling chromatin (Hareža *et al.*, 2022). It mostly affects the keratinized and mucosal epithelium, having a cytopathic effect on the vaginal mucosal epithelium (Alacam and Bakir, 2021). HPV was categorized into five genera and was represented by the Greek letters nu, mu, gamma, beta, and gamma, which indicate the phylogenetic and pathogenic characteristics of the nucleotide sequences in their genome (Malik *et al.*, 2023). Based on their capacity to cause cancer, alpha HPV strains are further divided into two major groups: HR-HPV and LR-HPV (Medda *et al.*, 2021). HPV is a commonly occurring sexually transmitted virus that can also spread non sexually through mother-to-child and horizontal (fomites, fingers, mouth,

and skin contact) channels (Petca *et al.*, 2020). Until this day, about 450 different HPV genotypes have been identified and sequenced (Mcbride, 2022). HPV primarily infects squamous epithelial cells to spread to other organs (Wu *et al.*, 2020). Genital warts, which are mostly benign lesions with a clinical appearance, are the result of infections with LR-HPV types like HPV 6 and 11. 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 (Chen *et al.*, 2023). The HPV genome is composed of three unique regions: the Long Regulatory or Non-Coding Region (LCR or NCR, respectively), late (L), which encodes structural proteins, and early (E), which encodes the virus's nonstructural proteins. The host epithelial differentiation program is closely connected to the three stages of transcription that arise from the viral genome: early, intermediate, and late (Graham, 2017). The majority of HPV infections are asymptomatic or subclinical. LR-HPV genotypes 6 and 11 are commonly

associated with genital warts, which are benign epithelial proliferations that often form in places that are vulnerable to abrasion during sexual activity. About 120 genotypes known as HPVs can infect the skin and mucosa; they can lead to psychological stress, which is exacerbated by the necessity for uncomfortable and time-consuming therapy (Fawzy *et al.*, 2023). For accurate identification and genotyping of HPV, molecular tests are necessary (Ramesh *et al.*, 2020). 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 for its duplication for identification purposes (Melón *et al.*, 2013). PCR targets the L1 gene portion of the viral capsid by amplifying only a highly conserved area of viral DNA. The variety of HPV genotypes has led to the development of many genome amplification methods employing various primer pairs. This technique is sensitive, affordable, and rapid. This technique's approach uses two consecutive PCR processes on the same target, each using a distinct combination of primers. This increases the sensitivity of the diagnostic. The primary consensus primers for identifying HPV are GP+5/GP+6 and MY09/MY11, which produce 150 and 450 bp amplicons, respectively (Tadlaoui *et al.*, 2020). The aim of study was to investigate the prevalence and genotypic distribution of HPV using Phosphate buffered saline- embedded tissue (PBS-embedded tissue) from patients with different anogenital lesions in Mosul / Iraq .

MATERIALS AND METHODS

Sample collection

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 Hospital and Mosul General Hospital in Mosul/ Iraq from August /2023 to April /2024. And their ages ranged between (19- 58) years old. The specimens were transported in plain tubes containing PBS ; cervical swabs and cervical brush were transported in plain tubes containing Viral Transport Medium (VTM) to the postgraduate laboratory in the Biology department/ College of Science /University of Mosul.

DNA extraction

Using a QIAamp DNA mini kit, DNA was extracted from

the genital warts (tissue), cervical swab and cervical brush. Following the manufacturer's instructions (QIAGEN, Germany) in the central laboratory for the College of Science / University of Mosul. All the extracted DNA was stored at -20°C until analyzed by Polymerase Chain Reaction (PCR) .

HPV DNA detection and sequencing

The extracted DNA was tested to check for the presence of HPV by PCR amplification of conserved regions of the L1 genes. The PCR was performed using consensus GP+5/GP+6 and MY09/MY11 oligonucleotide primers to amplify the 150bp and 450bp, 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.

Four 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 Basic Local Alignment Search Tool (BLAST) was used to do the best sequence homology search.

Ethical approval

The study was carried out following the moral guidelines found in the Helsinki Declaration. It was conducted with the verbal and analytical consent of the patients before the sample was collected. The local ethics committee reviewed and accepted the research protocol, the consent form, and subject details based on document number 40297 (8/10/2023) to obtain this approval.

RESULTS AND DISCUSSION

Among 150 samples of women with genital warts, only 127 cases (84.6%) were isolated and identified. The types of samples collected were variable among patients. The present study used three types of samples (PBS-embedded tissues, endocervical swabs, and cervical brush) to ensure the presence of viruses. These

Table 1. Showing primers in the present study

Primers	Sequence	Size	Reference
MY09	F 5-CGTCCMARRGGAWACTGATC-3	450bp	(Bauer et al., 1992)
MY11	R 5-GCMCAGGGWCATAAYAATGG-3		
GP+5	F 5-TTTGTTACTGTGGTAGATACTAC-3	150bp	(Jacobs et al., 1997)
GP+6	R 5-GAAAAATAAACTGTAAATCATATTC-3		

Table 2. Showing the relation between age group and sample types

Age groups	Samples types					
	Tissue		Swab		Brush	
	N	%	N	%	N	%
19-28	27	21.3	5	3.9	4	3.2
29-38	24	18.9	11	8.7	4	3.2
39-48	14	11	10	7.9	6	4.7
49-58	11	8.7	6	4.7	5	3.9
Total	76	59.9	32	25.1	19	14.8

N= Number of samples

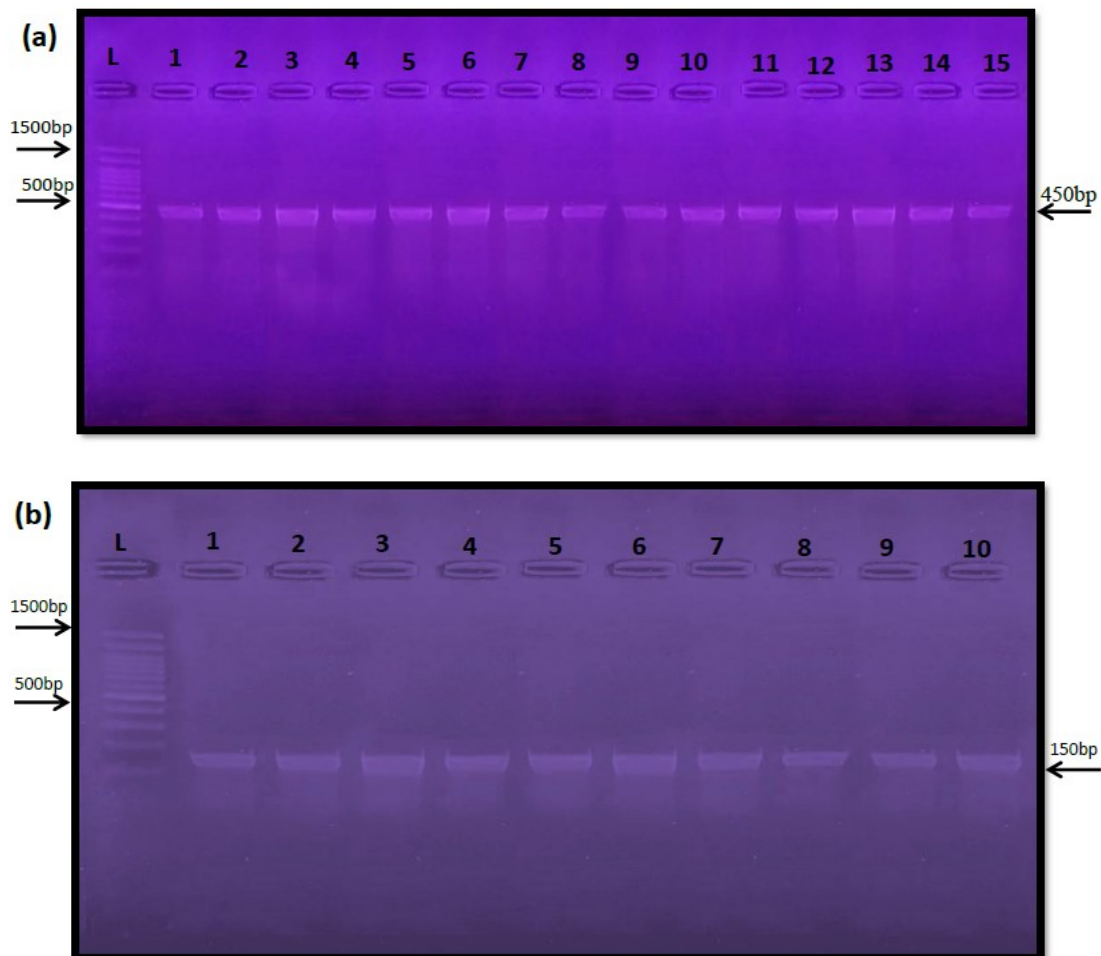


Fig. 1. Showing detection of Human Papillomavirus (HPV) for both gene (MY 450 bp) and (GP 150 bp) , (a) Line (L) marker (100bp); Lines (1-15) represent positive results of Human Papillomavirus for gene MY. (b) Line (L) marker (100bp); Lines (1-10) represent positive results of Human Papillomavirus for gene GP (Electrophoresis conditions: 5v / cm²)

differences were because, in some cases, it was not possible to take a swab or brush from virgin women, and in other cases, it is not possible to take a tissue because the warts can not be removed (Table 2). PCR was used to amplify the HPV L1 gene using specific primers MY09/MY11 450 bp and GP+5/+6 150 bp. Fig. 1 a and b shows the bands of primers and confirms their size, about 78.8% had positive results for GP+5/+6 and 21.2% for MY09/11 (Table 4). Among this, 10

patients (8%) were with high-risk (HR-HPV) serotypes (4%) of each HPV (16 and 45), while the rest 117 patients (92%) were with low-risk (LR-HPV) included 60.5% HPV11, 20.5% HPV6 and 11% HPV10 (Table 4). The fourth most frequent cancer in the world to affect women is cervical cancer, with an expected 604,100 new cases and 341,831 deaths in 2020 (IARC, 2020). Cervical cancer affects women and people with cervixes, even though it is preventable and treatable. At the

advanced stages of the disease, it results in severe suffering and has a significant negative influence on quality of life. The World Health Organization (WHO) published a global strategy to eradicate cervical cancer as a public health issue in 2020 (WHO, 2020). Of this rate, 4,000 deaths are associated with HPV disease (Moossavi *et al.*, 2020). The anogenital HPV burden varies across different geographic groups and even within the same country's diverse areas and also genital warts were considered HPV-related geographic regions and/or population subgroups (Diakite *et al.*, 2024; Khan *et al.*, 2023). The present study on molecular detection of HPV PCR in Mosul, Iraq, showed (84.6%) HPV positive cases. Far higher rates have been reported in Brazil and Iran, and similar frequency rates have been observed in surrounding regions such as China, Iran, Turkey, Jordan, and China. Compared to numerous descriptions of frequencies in various other geographic states, the current rate in Mosul/Iraq appears to be high (Table 3).

The elevated frequency rate may be related to the reduced level of HPV immune protection by the abbreviated national immunization program (Muderris *et al.*, 2019; Oztürk *et al.*, 2004). Some countries' lower HPV rates may be attributed to socioeconomic situation variations, education level, age, and awareness (Sand *et al.*, 2023). 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 earlier (Sahiner *et al.*, 2012; Depuydt *et al.*, 2007; Gravitt *et al.*, 2000). This was enhanced by missing a positive case (Othman *et al.*, 2022). 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 (Remmerbach *et al.*, 2004). For this, the frequency for GP+5/GP+6 was 78.8% higher than the MY09/MY11 frequency, which was 21.2% (Table 4). Regarding the ages, the present study showed the variable distribution of HPV positives across all age categories, with the highest frequency (71.7%) occurring at 19–28 and 29–38 years of age (Table 4). So, there was a tendency for women in the third and fourth decades to have greater viral DNA frequencies. Because this is the time, sexual activity begins, and marriage becomes more popular due to observing religious and cultural customs, as well as sinful illicit sex, in this specific region (Muderris *et al.*, 2019; Oztürk *et al.*, 2004). This was confirmed in the present study.

As shown in Table 5, 92% were LR-HPV and 8% were HR-HPV genotypes. The high present 75.5% of the LR-HPV group was dominated by the third and fourth decades, while the mix between HR-HPV (8%) and LR-HPV 45.6% came from the fourth and fifth decades. The last age group shows a very low presence of 3.9% and only with LR-HPV.

Table 3. Frequency of human papillomavirus (HPV) risk types reported in various geographical countries

Research year	Town/ Country	Number of cases	HPV (%)	HR HPV (%)	Common HR-HPV genotypes	LR HPV (%)	Common LR-HPV genotypes
Present study, 2024	Mosul / Iraq	150	84.6	8	HPV16,45	92	HPV6,10,11
Othman <i>et al.</i> , 2022	Duhok/ Iraq	125	72.0	17.8	HPV33,33,35,5, 1,58, 59,73	73.3	HPV42,11,6
Ağar, and Aker, 2023	Istanbul / Turkey	108	83.7	34.3	HPV16, 18	72.2	HPV6,11
Bitarafan <i>et al.</i> , 2021	Iran	12076	38.7	17.4	HPV16,52,18,3, 9,31, 51,45,68	32.0	HPV6,11,62,
Deluca <i>et al.</i> , 2021	Argentina	957	56.3	42.2	HPV16,52,56,5, 1,58, 31	30.8	HPV42,6,40, 81,11
De Oliveira <i>et al.</i> , 2013)	Brazil	172	98.8	99.4	HPV35,33,31,1, 8,16	0.6	HPV 11
Alizadeh <i>et al.</i> , 2021	Guilan/ Iran	157	77.7	16.6	HPV68,66,52,5, 1,45, 16,18	83.4	HPV40,11,6
Krashias <i>et al.</i> , 2017	Cyprus	596	72.8	59.4	HPV68,31,58,6, 6,18,56	34.8	HPV6,54,42, 61
Ghobadi <i>et al.</i> , 2023	Iran	50	20	10	HPV16,18,35,6, 6, ,68,33,34,35		
Moossavi <i>et al.</i> , 2020	Iran	2135	92	12.6	HPV 16,	87.4	HPV 6,
Huang <i>et al.</i> , 2024	China	1,625	80.3	5.97	HPV16,18,35,6, 6,67,68,33,34, 35,,45,58	49.48	HPV6,11,54, 42, 61,41,23,41,4 4

Table 4 . Relation between age group and human papillomavirus (HPV) and the frequency of HPV primers

Age Groups	PCR Results				Total	
	MY09/ MY11		GP+5 /GP+6		N	%
	N	%	N	%		
19-28	9	7.1	39	30.7	48	37.8
29-38	8	6.3	35	27.6	43	33.9
39-48	6	4.7	15	11.8	21	16.5
49-58	4	3.1	11	8.7	15	11.8
Total	27	21.2	100	78.8	127	100

Table 5. Relationship between age groups human pappilo virus (HPV) Genotypes risk group

Age groups	HPV Genotypes									
	LR- HPV					HR – HPV				
	HPV6		HPV10		HPV11		HPV 45		HPV16	
N.	%	N.	%	N.	%	N.	%	N.	%	
19-28	10	7.9	9	7.1	35	27.5	Zero	Zero	Zero	Zero
29-38	5	3.9	5	3.9	32	25.2	2	1.6	3	2.4
39-48	11	8.7	Zero	Zero	5	3.9	3	2.4	2	1.6
49-58	Zero	Zero	Zero	Zero	5	3.9	Zero	Zero	Zero	Zero
Total	26	20.5	14	11	77	60.5	5	4	5	4

N= Number of samples

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 (Ferrall *et al.*, 2021; Garbuglia *et al.*, 2020). However, there are conflicting findings of viral peaks among older Saudi Arabian women. (Bondagji *et al.*, 2013), and younger Greek women (Stamataki *et al.*, 2010). The present results agree with what has been seen in Duhok, Iraq (Othman *et al.*, 2022). It is also important to note that the HR-HPV viruses were most prevalent in the fourth decade. This result is consistent with research among women in Kazakhstan (Babi *et al.*, 2021). However, a Chinese study found that younger groups had higher rates of high-risk viral dominance, but rates were lower in middle-aged groups (Wang *et al.*, 2015).

Accession Number of HPV

Depending on the sequence homology of the L1 gene, HPV was isolated, analyzed, and finally submitted to Gen Bank, and the accession number obtained was HPV 11 PP577025.

Conclusion

The present research reported that women with genital warts were more likely to have LR HPV and HR HPV infections, underscoring the substantial prevalence of these infections in the age range 19-58 . This is the first study conducted in Mosul/Iraq . Due to the high rate of

infection dissemination, which is the primary cause of cervical cancer, adequate measures for preventive programs, such as education, screening, immunization and treatment, should be considered. Additionally, this study highlights the need to broaden HPV vaccination programs to encompass men as a tactic to lower the prevalence of genital warts. This strategy addresses the transmission dynamics of HPV infections in addition to preventing HPV-related disorders in women, which helps to lower the prevalence of HPV in the general population.

Conflict of interest

The authors declare that they have no conflict of interest.

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