

ORIGINAL ARTICLE

Molecular detection of HPV16 infections in cervical cancer samples of women patients from Dhi-Qar, Iraq

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Received: Oct 12, 2023,
Revised: Nov 10, 2023,
Accepted: Nov 20, 2023,

DOI: 10.57238/jbb.2023.7197.1052



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article online

Abstract

Background and Objective The current study aims to detect the frequency of human papillomavirus (HPV16) 16 from cervical samples in Dhi Qar Governorate / Iraq based on molecular analysis.

Method Between 2017 and 2020, this survey was conducted on 93 adult females who had cervical cancer and 60 healthy people as controls. Patients' ages ranged from 32 to 78. DNA was collected for molecular analysis and treated to PCR for minor capsid protein L2 gene amplification and identification.

Results According to PCR data, HPV16 was present in 60 (65%) of the 93 cervical cancer cases, but only 5 (8%) of the healthy control group were HPV16 positive. The current survey found the highest rates of high-risk HPV16 infections in 2019 (78%) and 2020 (69%) compared to the lowest rates in 2017 (47%) of infections. Additionally, age groups have an impact on the rate of HPV16 infection; according to the most recent findings, the infection rate among elderly women declined while it increased among young women. On the other hand, the distribution of HPV16 infections according to the stages of cervical cancer revealed that stage IV (70%) had the greatest infection rates, followed by stage III (68%) and stage II (60%).

Conclusion For accurate HPV16 detection, PCR is a reputable technique. In addition, viral infections have surged recently, especially in young women, and are unmistakably linked to the advancement of cervical cancer. In the Thi-Qar province of Iraq, HPV16 infections are significantly linked to cervical carcinoma among women.

Keywords: HPV16; Cervical cancer; Women; PCR; L2 gene

1 Introduction

One of the most prevalent illnesses in women and a leading source of morbidity and mortality is cervical cancer. Only breast cancer occurs more frequently in women worldwide, making it the second most common type of cancer in this group [1,2].

The progression of the disease is influenced by a variety of risk factors. Along with a number of other environmental and behavioural factors, including as smoking, age at first sexual contact, sexual behav-

ior, partners, hygiene, and other STDs [3]. There is a significant link between HPVs and cervical cancer, as shown by several epidemiological and experimental investigations. Actually, the primary risk factors for developing cervical cancer are recognised to be HPV [4,5].

The Papillomaviridae family includes human papillomaviruses [6]. These non-enveloped viruses have circular double-stranded DNA genomes of around 8 kb in size, an icosahedral capsid, six early genes (E1, E2, E4, E5, E6, and E7), and two late genes L1, L2

(in that order [7,8]. The nuclei of terminally differentiated cells in the upper layers of the squamous epithelium include expressed L1 and L2, even though the infected basal epithelial cells do not display them [9,10]. In these cells, the expression of L1 and L2 occurs even later than that of E4. When created in various recombinant expression methods, L1 possesses the ability to self-assemble into empty virus-like particles (VLPs), which are the fundamental units of the licensed HPV vaccines. VLPs are not produced by L2. Despite not producing VLPs, L2 can be integrated when co-expressed with L1. Based on genetic variations, HPV comes in 120 different kinds. HPV is separated into high-risk and low-risk viruses based on its connection with precancerous or non-cancerous lesions [9]. High-risk HPV (types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56) is linked to cervical cancer lesions, while low-risk HPV (types 6, 11, 42, 43, and 44) is linked to benign lesions. The most prevalent high-risk HPV strains are HPV-16 and HPV-18. Women without cervical abnormalities are infected with HPV at a rate of 11–12% worldwide, with sub-Saharan Africa having the greatest prevalence (24%) followed by Eastern Europe (21%) and Latin America (16%) [11,12].

The more dangerous HPV type is HPV 16. It is the most common and accounts for more than half of all occurrences of cervical cancer worldwide. Only sporadic information on this cancer is available from Iran, and there have been few research done to identify the epidemiology and risk factors relevant to HPV cases in Middle Eastern nations. Furthermore, there is no organised programme for excision, immunization, or screening to prevent cervical cancer. As a result, without well-organized statistics, it is undoubtedly challenging to implement effective preventive actions against the disease. Although some tests, like the Pap test, are very appropriate, the outcomes might come back falsely negative [13].

The use of molecular biology techniques, such as PCR, real-time PCR, and nucleic acid hybridization assays, are crucial for the accurate identification of HPV16 since the virus cannot be produced in tissue culture [14,15]. The current work focuses on identifying HPV16 using PCR analysis from women with cervical cancer, based on L2 gene amplification. This information may be crucial for the rational design of diagnostic, therapeutic, and vaccine approaches.

2 Materials and methods

2.1 Study design

This case-control study was carried out between 2017 and 2020 by Yanka Kupala State University of Grodno and the histopathology department of Al-Hussein Teaching Hospital in the Iraqi province of Dhi-Qar.

The consent document was obtained by hospital management with the cooperation of the patients. Al-Hussein Teaching Hospital's ethical guidelines were also followed by the current study, and each participating women gave her verbal informed consent.

2.2 Sample collection

93 samples were taken from the cervical cancer tissue blocks that Al-Hussein Learning had preserved between 2017 and 2020 after samples were obtained from them in the histopathology Laboratory. The year the sample was taken, were the name and age of the patient, the cancer's developmental stage, and the histological diagnosis all recorded in the samples' registration.

2.3 DNA extraction

According to manufacturer guidelines, genomic DNA was extracted from paraffin-embedded block tissue samples using the G-spin™ Total DNA Extraction Kit (Fixed tissues technique). s method). Thermo (USA) Nanodrop spectrophotometer was used to measure the absorbance at (260/280 nm) in order to concentrate and purify the viral DNA.

2.4 PCR reaction

In this investigation, the L2 gene primers for the minor capsid protein for HPV16 (F: 5'-CCGGCTACTGAAGT GGTGTT-3' and R: 5'-TACCAGCACGTTTCAGCCAAT-3') were developed using the NCBI-Genbank data base sequence. (MH777342.2). The Maxime PCR PreMix Kit was used to create the PCR master mix, after which the steps specified by the manufacturer were followed. After adding the HPV16 DNA, each PCR tube was placed inside an Exispin vortex centrifuge and spun for three minutes at 3000 rpm. And hence, the PCR thermocycler was employed. (BioRad USA, T100 Thermal cycler). Using a conventional PCR thermocycler system, the following conditions are carried out: first denaturation for 1 cycle at 95°C for 5 minutes; first annealing for 35 cycles at 95°C for 58 seconds; extension for 35 cycles at 72°C for 1 minute; and final extension for 1 cycle at 72°C for 5 minutes. Seeing the PCR findings on a 1% agarose gel stained with ethidium allowed for the confirmation of the presence of a 511 bp band.

2.5 Statistical analysis

Only data with a probability value (P value) less than 0.05 were considered statistically significant. The Statistical Package for Social Sciences version 20

(SPSS20) program and Microsoft Excel 2010 were utilized for the statistical analysis.

3 Results

From tissue samples with paraffin-embedded cervical carcinoma, HPV16 DNA was isolated. As seen in the following Table 1 and Figure 1, readings from the Viral DNA concentration and purity ranged from (1.7 to 3.4) with a mean SD of 2.01 ± 0.11 and (4.0 to 39.0 ng/ μ l) with a mean SD of $24.417.2$ ng/ μ l, according to equipment used in nano-drop spectrophotometers.

Table 1: The Concentration and Purity of viral DNA.

Viral DNA	Concentration (ng/ μ l)	Purity (260/280)
Range	4.0 - 39.0	1.7 - 3.4
Mean $\pm$ SD	24.4 ± 17.2	2.01 ± 0.11
SE	1.76	0.011

SD= Standard Deviation; SE= Standard Error

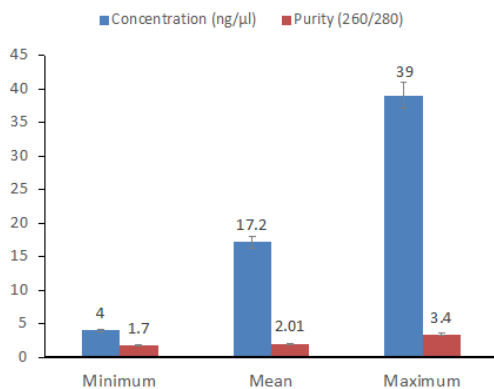


Figure 1: Viral DNA concentration and purity at the lowest, mean, and highest.

The HPV16 L2 gene was genetically screened by Only 5 (8%) of the healthy control group tested positive for HPV16 (Figure 2), compared to 60 (65%) of cases of cervical cancer that underwent conventional PCR (Figure 3). Thus, the frequency of HPV16 in patients and controls differed significantly ($p < 0.05$), as Table 2 illustrates.

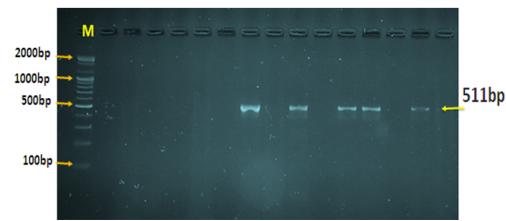


Figure 2: Using DNA recovered from paraffin-embedded block tissue samples, PCR product analysis of the L2 gene in the human papillomavirus is displayed on an agarose gel electrophoresis picture. The PCR result (511 bp) for the positive HPV L2 gene samples showed the M: marker (2000-100 bp).

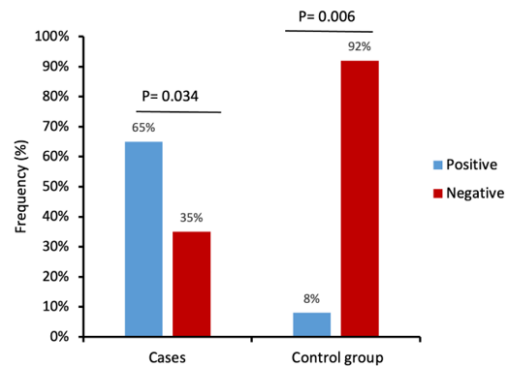


Figure 3: Frequency of PCR diagnostic HPV16 in studied groups.

Table 2: Compared prevalence of HPV16 infection in cases and control.

PCR detection of HPV16	Cases	Healthy control	P value
	N (%)	N (%)	
Positive	60 (65)	5 (8)	0.0031*
Negative	33 (35)	55 (92)	0.0111*
Total	93 (100)	60 (100)	

*Significant correlation ($p < 0.05$)

According to the current survey (Figure 4), 2019 and 2020 had the highest rates of hrHPV16 infections (78%) and 69%, respectively, whereas 2017 had the lowest rates (47%) ($p = 0.045$). According to the current findings, infections were more common in young women in the age categories of 32 to 42 and 43 to 52 years, while infections in older women in the age groups of 53 to 62 and 63 to 78 years were less common ($p = 0.034$). Age groups also have an impact on the rate of high-risk HPV16 infection. On the other hand, HPV16 infections were most common in stage IV cervical cancer (70%), followed by stage III (68%), stage II (60%) and stage 0 (60%) while stage I only had

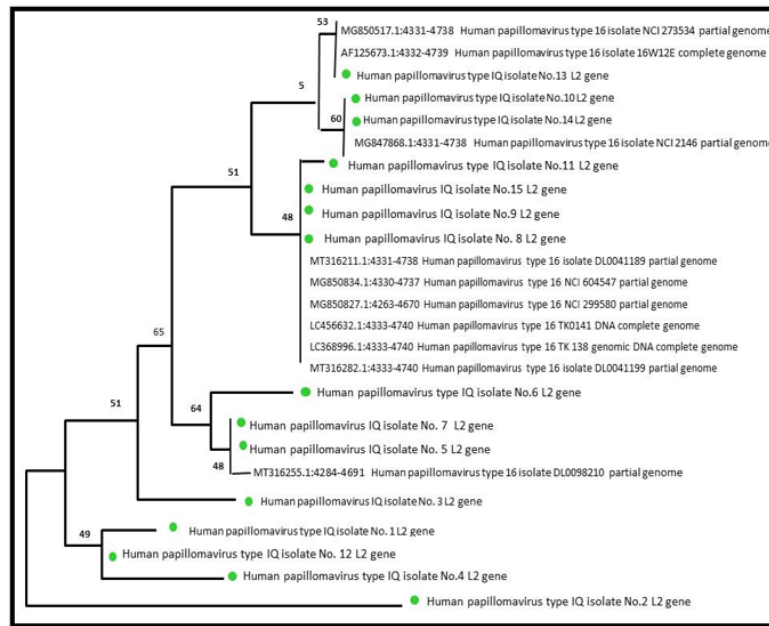


Figure 5: Human papillomavirus genetic analysis using phylogenetic tree analysis based on the partial sequence of the capsid protein (L2) gene. The BLAST alignment tool was used to analyse the nucleotide sequences of all samples, and a phylogenetic tree was built using the Neighbor-Joining technique (in) (MEGA 6.0 version).

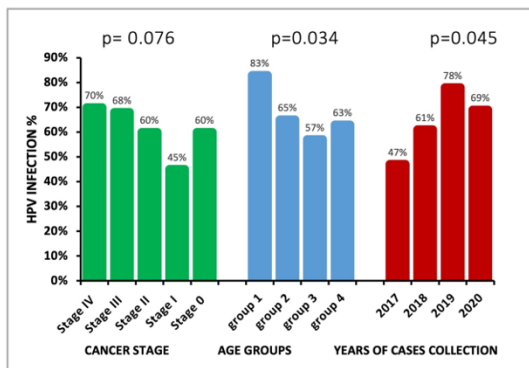


Figure 4: Distribution hrHPV16 infections based on the cervical cancer stages, age groups and years of sample collection.

The evolutionary relationships between the examined strains and the closest related strains of the human papillomavirus genus contained in the gene bank data were revealed by sequencing analysis of the capsid protein (L2) gene for 15 samples (Figure 5). The sequences similarity of our isolates was ranged from 99 to 100% compared to other isolates that recorded in the NCBI-Genbank. The Neighbor-Joining method in the MEGA 6.0 version was used to create nine clusters (49, 48, 51, 64, 65, 51, 5, 69 and 53) in the current phylogenetic tree. Genetically, HPV IQ-№1, IQ-№2, IQ-№3, IQ-№4, IQ-№5, IQ-№7 and IQ-№12 were shown to be connected to the NCBI-BLAST human papillomavirus type 16 DL0098210 isolate partial genome (MT316255.1) that isolated from cervical cancer in

Guatemala/ US in the same line IQ-№6 appeared linked to DL0041189 (MT316282.1) complete genome that also studied in Guatemala/ USA. Moreover, isolates IQ-№10 and IQ-№14 seen linked to human papillomavirus type 16 isolate NCI_2146 (MG847868.1) partial genome from USA study so isolate IQ-№13 observed similar to human papillomavirus type 16 isolate 16W12E complete genome (AF125673.1) also isolated from USA.

4 Discussion

Cervical cancer is often associated with persistent infection by high-risk HPVs such as HPV16 [16]. This is supported by the current study, which found that 60 (65%) of cases of cervical cancer were infected with HPV16, while only 5 (8%) of the healthy control group tested positive for HPV16 based on PCR amplification of the L2 gene [18]. In order to determine the frequency of HPV infection in different patient groups, the most common types of viruses and their geographic distribution, and the risk factors linked to the development of cervical cancer, numerous studies have been conducted [17,18]. To investigate if there is a universal link between HPV infection and cervical cancer, as well as the geographic variation in the distribution of HPV types, Bosch and colleagues carried out a comprehensive epidemiological study. In 32 hospitals across 22 countries, more than 1,000 anatomically verified samples from patients with advanced cervical cancer were

assessed using polymerase chain reaction-based testing, which can distinguish between over 25 different strains of HPV. Their results showed that 93% of the tumors contained HPV DNA and that there was no discernible variation in the prevalence of HPV positive across the participating countries. The majority of samples had positive HPV 16 test results, which was found to be the most prevalent virus type globally [19,20]. Many investigations have revealed that HPV 16. Numerous studies have revealed that HPV 16 is the most prevalent or frequent form of virus. In addition, the results of this investigation revealed a greater HPV frequency than those of Mahmoodi et al., who found that 43.33% of patients with cervical dysplasia or cancer contained HPV DNA in their bodies [20]. However, our results are in close agreement with those of a prior Iranian study that examined 50 patients with cervical cancer and discovered that HPV 16 was present in 66% of cases, HPV 18 in 14%, and HPV 16 and 18 in 14% of cases [21].

In a research on cervical biopsy samples from patients from many various countries, polymerase chain reaction (PCR) detected HPV DNA in 99.7% of cases of cervical cancer, demonstrating that HPV is, in fact, the main cause of this form of cancer. On the other hand, HPV detection rates in individuals without cervical epithelial anomalies vary from 5% to 20% [22,23]. Recall that both recent and past studies have shown that HPV infection is more common in young, sexually active women between the ages of 18 and 37, and that the frequency of infection significantly decreases beyond 45 years of age. However, women over 35 are more likely to have cervical cancer, suggesting a lower frequency and a slower rate of malignant progression [24]. According to a study by Mahmoodi et al., the majority of cervical cancer and HPV positive samples belonged to people aged 48 to 71, particularly people aged 48 to 55 [20]. Munoz and associates conducted a study to increase understanding of high risk HPV kinds.

Data from 11 case-control studies, comprising 1928 control women and 1918 women with histologically proven squamous-cell cervical cancer, were merged from nine different countries. In 1928, there were 259 control women (13.4%) and 1739 instances of cervical cancer (90.7%) with HPV DNA. The most common HPV types in patients were types 16, 18, 45, 31, 33, 52, 58, and 35, in decreasing order of frequency [25]. 52 paraffin-embedded blocks of cervical tissue with cervical cancer and 52 paraffin-embedded blocks of cervical tissue with normal histology were examined in a different study carried out by Sadeghi et al. in 2008. According to the findings, 30.7% of the cervical cancer samples had HPV-positive, while there was not a single positive sample in the healthy specimens [26].

Maleknejad et al. tried to detect HPV infections in 64 paraffin-embedded tissues from patients with cervical cancer. HPV DNA was found in 59.4% of samples; 34.4% of these samples contained HPV 16, and the remaining 25% had HPV 18 [27,28]. Han et al. performed a study on young women (under 35) in Korea. The results revealed that HPV 16 was the most prevalent virus, with HPV 18 and 16 being found in 84.5% of samples [29].

Yang et al. used a survey to investigate the association between viral factors and cervical cancer in Taiwan. Researchers found that 18 (66.7%) of the 27 cervical cancer biopsy samples tested positive for HPV DNA after comparing them to 29 normal cervical scrapings. Out of all of those, HPV types 16 and 18 had the strongest correlations with cervical cancer [28]. For a different investigation, Schellekens and colleagues looked at 74 cervical cancer samples in Indonesia. There was HPV DNA from 12 distinct HPV types in 96% of the samples. HPV types 16 (44%) and 18 (39%), which both showed a notable prevalence of HPV type 16, were the two most common types. Nonetheless, some data suggested that HPV type 18 was the predominant virus in this area, with HPV type 16 coming in second [30,31]. The results of the current investigation confirmed the potential value of the PCR as a test for identifying HPV DNA, and allowed for the identification of distinct forms of HPV that are circulating in a population with the use of the subsequent RFLP analysis. Multiple Therefore, it would appear that HPV 16 is one of the primary viral types that Iraq's immunization program against the illness should address [32].

The capacity of different HPV strains to cause disease can vary, even when they have similar evolutionary histories. Previous investigations of the intratypic evolution of HPV variations depended on viral genome fragments, which were obtained using restriction enzyme polymorphisms and, more recently, viral fragment sequence determination. These fragments were typically restricted to the E6, E7, and LCR regions. The bulk of earlier intratypic evolutionary research used the E6, E7, and LCR regions. Researchers can now thoroughly examine HPV variant lineages and sublineages at the complete genome level thanks to the development of next-generation sequencing [33–36].

Variant lineages and sublineages have been determined by sequence alignments of entire viral genomes in conjunction with phylogenetic analyses; the experimentally characterized differences between these lineages range from 10.0 to 10.0% and 0.5 to 1.0%, respectively. Phylogenetic analysis and genetic variation of HPV L2 are crucial for generating new vaccines and for enhancing our comprehension of the virus's toxicity and infectivity.

5 Conclusions

Through the use of PCR, it was found that HPV16 infections, particularly in young women, were strongly associated with cervical cancer in women in the Iraqi province of Thi-Qar. These results demonstrate the need for more study to fully comprehend the biology of the different HPV kinds and the risk factors that lead to the development of illness. The results of this study could help shape future initiatives for this dangerous illness's management and screening.

Conflict of Interest: No conflicts of interest exist between the authors and the publication of this work.

Ethical consideration: The ethical committee approved the study at Yanka Kupala State University of Grodno, Grodno, Belarus.

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How to cite this article

Jalil A. T.; Molecular detection of HPV16 infections in cervical cancer samples of women patients from Dhi-Qar, Iraq. *Journal of Biomedicine and Biochemistry*. 2023;2(4):9-16. doi: 10.57238/jbb.2023.7197.1052