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Research Article

Molecular Detection of HPV E7 Gene Using TaqMan Probe-Based RT-PCR: A Case-Control Study in Hilla City, Iraq

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Abstract

Background: Human papillomavirus (HPV) is a key driver of cervical cancer, with high-risk genotypes, mainly HPV 16 and 18, responsible for approximately 70% of cases. Persistent infections and immunological evasion by HPV increase the risk of malignant transformation. ***Objective***: To evaluate the effect of TaqMan probes in real-time PCR pivotal role in HPV DNA detection, targeting a highly conserved regions like the E7 gene. ***Methods***: A case-control study was conducted on 71 women in Hilla City, Iraq, to detect HPV DNA. The study used cervical swabs and blood samples, with real-time PCR targeting the E7 gene. Statistical analysis, including chi-square tests, evaluated associations between demographic, clinical, and behavioral variables. ***Results***: Out of 71 samples, 22 tested positive for HPV, primarily using cervical swabs (95.5%). Amplification of the E7 gene showed high efficiency (100.9%) and specificity. Significant associations were found between HPV infection and factors such as smoking, rural residence, and family history of cancers. No co-infections were observed. ***Conclusions***: This study underscores the effectiveness of TaqMan probe-based RT-PCR in HPV detection and highlights critical demographic and behavioral risk factors. The findings advocate for expanding HPV vaccination and diagnostic access, particularly in underserved regions.

Keywords: Cervical cancer, E7 oncoprotein, Human papillomavirus (HPV), Real-time PCR, TaqMan probe.

الكشف الجزيئي عن جين فيروس الورم الحليمي البشري E7 باستخدام RT-PCR القائم على مسبار TaqMan: دراسة حالة وشواهد في مدينة الحلة، العراق

الخلاصة

الخلفية: فيروس الورم الحليمي البشري (HPV) هو المسبب الرئيسي لسرطان عنق الرحم، مع الأنماط الجينية عالية الخطورة، وخاصة فيروس الورم الحليمي البشري 16 و 18، المسؤولة عن حوالي 70٪ من الحالات. تزيد العدوى المستمرة والتهرب المناعي من فيروس الورم الحليمي البشري من خطر التحول الخبيث. **الهدف**: تقييم تأثير مجسات TaqMan في تفاعل البوليميراز المتسلسل في الوقت الفعلي للكشف عن الحمض النووي لفيروس الورم الحليمي البشري، والتي تستهدف المناطق المحفوظة للغاية مثل جين E7. **الطرائق**: أجريت دراسة حالة وشواهد على 71 امرأة في مدينة الحلة بالعراق للكشف عن الحمض النووي لفيروس الورم الحليمي البشري. استخدمت الدراسة مسحات عنق الرحم وعينات الدم ، مع تفاعل البوليميراز المتسلسل في الوقت الفعلي الذي يستهدف جين E7. قام التحليل الإحصائي، بما في ذلك اختبارات مربع كاي ، بتقييم الارتباطات بين المتغيرات الديموغرافية والسريرية والسلوكية. **النتائج**: من بين 71 عينة، تم اختبار 22 عينة إيجابية لفيروس الورم الحليمي البشري، باستخدام مسحات عنق الرحم بشكل أساسي (95.5٪). أظهر تضخيم الجين E7 كفاءة عالية (100.9٪) وخصوصية. تم العثور على ارتباطات كبيرة بين عدوى فيروس الورم الحليمي البشري وعوامل مثل التدخين والإقامة الريفية والتاريخ العائلي للسرطانات. لم يلاحظ أي عدوى مشتركة. **الاستنتاجات**: تؤكد هذه الدراسة على فعالية RT-PCR القائم على مسبار TaqMan في الكشف عن فيروس الورم الحليمي البشري وتسلط الضوء على عوامل الخطر الديموغرافية والسلوكية الحرجة. تدعو النتائج إلى توسيع نطاق التطعيم ضد فيروس الورم الحليمي البشري والوصول إلى التشخيص ، لا سيما في المناطق المحرومة.

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INTRODUCTION

Human papillomavirus (HPV), a pervasive viral agent, is implicated in a spectrum of lesions encompassing cutaneous, mucosal, and neoplastic growths, with a principal role in the etiology of cervical cancer [1]. Among the approximately 210 identified genotypes, HPV strains are classified into low and high-risk categories based on their oncogenic potential, with high-risk genotypes 16 and 18 alone responsible for approximately 70% of cervical cancer cases [2]. These malignancies disproportionately affect women in regions where screening programs are deficient [3]. While HPV infections are often asymptomatic and resolve spontaneously, immunosuppression or cofactors such as early sexual debut, multiple sexual partners, smoking, and extended oral contraceptive use can

enhance the likelihood of persistent infection and subsequent malignant transformation [4]. Cervical intraepithelial neoplasia (CIN), a precursor to cervical cancer, is graded into CIN 1, 2, or 3 based on dysplasia severity, with advanced CIN lesions posing a significant risk of progression to invasive cervical carcinoma [5]. The oncogenic activity of HPV is mediated by its viral oncoproteins E6 and E7, which disrupt key cellular regulatory pathways, as E6 facilitates the degradation of the tumor suppressor protein p53. At the same time, E7 inactivates the retinoblastoma protein (pRb), driving unchecked cell cycle progression and proliferation [6]. The integration of the HPV genome into the host's cellular DNA, driven mainly by these oncoproteins, is a critical event in tumorigenesis [7]. Epidemiological data indicate that over 80% of sexually active women will encounter HPV during their lifetime, with the majority clearing the infection within two years [8]. Nevertheless, a subset of infections persists, advancing to malignancy over a prolonged latency period. HPV employs sophisticated immune evasion strategies, notably impairing the host's Th1 immune response, particularly in patients with high-grade CIN and cervical carcinoma, making the elicitation of a robust immunological response against HPV antigens imperative for thwarting malignant transformation [9]. TaqMan probes are widely used in real-time PCR assays for the detection of HPV DNA due to their high specificity and sensitivity, as these probes consist of a short, sequence-specific oligonucleotide labeled with a fluorescent reporter dye at the 5' end and a quencher dye at the 3' end [10]. When the probe is intact, the quencher suppresses the fluorescence of the reporter dye. During the amplification process, the Taq polymerase enzyme, which possesses 5'–3' exonuclease activity, cleaves the probe bound to its complementary target sequence, separating the reporter dye from the quencher and generating a detectable fluorescence signal. For HPV DNA detection, TaqMan probes are designed to target conserved regions of the HPV genome, such as the E6, E7, or L1 genes, which are highly conserved across various HPV genotypes, with the E7 gene being particularly popular due to its role in viral oncogenesis, making it an ideal target for diagnostic assays [11].

METHODS

Study design and setting

This case-control study was conducted at private clinics in Hilla City, Babylon Governorate, Iraq, between February 2024 and November 2024, involving 71 women suspected of harboring human papillomavirus (HPV) infections. Among the participants, 22 women were confirmed positive for HPV through real-time polymerase chain reaction (RT-PCR). They constituted the positive control group, whereas the remaining 49, despite being clinically suspected of HPV infection, tested negative and were designated as the negative control group. Patient selection was rigorously guided by clinical evaluations conducted by physicians, who assessed participants based on a spectrum of cervical abnormalities. These ranged from mild symptoms, such as abnormal vaginal bleeding and foul-smelling discharge, to moderate symptoms, including persistent pelvic pain, and severe findings, such as cervical ulceration or the presence of a palpable cervical mass. Additionally, some patients were asymptomatic, with cervical abnormalities identified incidentally during routine medical examinations. A structured questionnaire was meticulously employed to obtain comprehensive demographic and clinical data directly from the participants, ensuring the robustness of the study's methodology (Table 1).

Specimen collection

A total of 71 meticulously selected specimens were collected to ensure a comprehensive and representative foundation for this study, comprising 61 cervical swabs and 10 blood samples. The cervical swabs (n=61) were expertly obtained from women across diverse age groups using precise sterile techniques, with each swab carefully preserved in 3 mL of Viral Transport Medium (VTM) and stored at -80°C to maintain the pristine integrity of viral nucleic acids. Simultaneously, 10 blood specimens were thoughtfully collected in EDTA tubes, paired with the corresponding cervical swabs, and stored in deep freeze conditions to ensure optimal preservation. This strategic and methodical approach culminated in a total of 71 specimens.

Primers

The PCR detection of HPV based on the E7 gene was designed according to the design in this study using the NCBI-Genbank database (GenBank: FJ158594.1) and Primer3 plus (Table 1).

Table 1: Primer sequences for PCR detection of HPV E7 gene designed using NCBI-GenBank database (GenBank: FJ158594.1) and primer 3 plus

Primers		Sequence 5'-3'	Product size
HPV E7 gene	F	AGAGGAAACAACCCAACGCT	281bp
	R	CAGCTAGGGCACACAATGGT	

Primers and probes

Human papillomavirus qPCR and PCR primers for the E7 gene were designed in this study using the NCBI-GenBank database sequence (Table 2) and the Primer 3 plus Primer Design online program. Moreover, these primers provided by Macrogen/ South Korea.

Table 2: Primer and probe sequences for qPCR and PCR detection of HPV E7 gene designed using NCBI-GenBank database and primer 3 plus

qPCR Primers and probe		Sequence (5'-3')	Amplicon	Genbank code
Common type HPV E7 primer	F	GAGGAGGAGCGAGCACCTTA	143bp	MH777342.2
	R	GAAGGGCAAAGCAAACTCAA		
Common HPV E7 probe		FAM-TGAAACAGGTGTAAGGCTGTG-BHQ1		
qPCR Primers and probe		Sequence (5'-3')	Amplicon	Genbank code
Type16 HPV E7 primer	F	ACAAGCAGAACCGGACAGAG	94bp	KM058634.1
	R	TACGTGTGTGCTTTGTACGC		
Type 16 HPV E7 probe		FAM-TGCAAGTGTGACTCTACGCTTCGG-BHQ1		
Type18 HPV E7 primer	F	ACATTTACCAGCCCGACGAG	146bp	OP712008.1
	R	AGGACAGGGTGTTCAGAAACAG		
Type 18 HPV E7 probe		FAM-GCTCAGCAGACGACCTTCGAGC-BHQ1		
PCR Primers		Sequence (5'-3')	Amplicon	Genbank code
Common HPV E7 primer	F	TGGTAATAAGCCCACTATTCAAGA	258bp	MH777342.2
	R	CTTGAGCACGAAGGGCAAAG		

Ethical approval

All participants in this study provided informed consent, with verbal agreements obtained prior to the collection of specimens. The study protocol was reviewed and approved by the Committee on Publication Ethics at the College of Medicine, Babylon University, Iraq, certificate number: BMS/0231/016.

Statistical analysis

Descriptive statistics were employed to comprehensively summarize the demographic, behavioral, and clinical characteristics of the study participants. Associations between categorical variables were evaluated using chi-square (χ^2) tests to determine statistical significance. For variables with insufficient variability, statistical analysis was deemed inapplicable. All analyses were conducted using IBM SPSS Statistics, version 26 (IBM Corp., Armonk, NY, USA), adhering to rigorous methodological standards. A *p*-value less than 0.05 was considered indicative of statistical significance.

RESULTS

Figure 1 shows the real-time PCR amplification plots of the *E7* protein gene used for direct detection of HPV. The results showed 22 positive real-time PCR amplification samples. It also demonstrates results of real-time PCR targeting the *E7* protein gene for direct detection of HPV. All 22 samples exhibit positive amplification, indicated by the characteristic sigmoidal curves crossing the threshold, which confirms the presence of the HPV *E7* gene in these samples. The amplification efficiency and consistent curve profiles suggest reliable and robust detection across all samples, validating the assay's specificity and sensitivity in identifying HPV DNA. This analysis underscores the effectiveness of the *E7* gene as a molecular target for diagnosing HPV infections. Figure 2 illustrates the standard curve generated during real-time PCR amplification targeting the E7 oncoprotein gene for HPV detection.

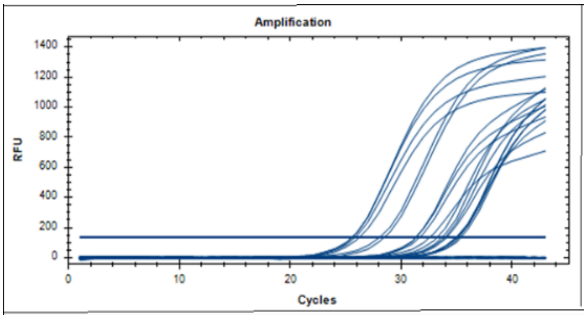

Figure 1: Real-time PCR amplification plots of the *E7* protein gene used for direct detection of HPV. The results show 22 positive real-time PCR amplification samples.

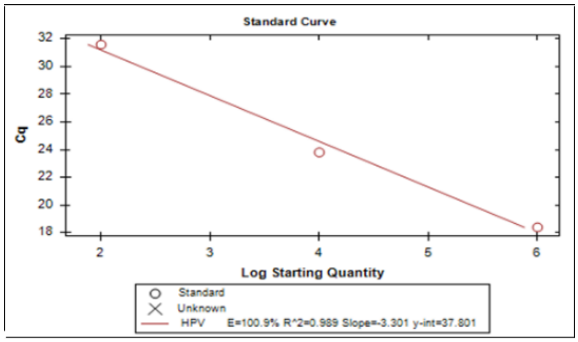

Figure 2: Real-time PCR amplification positive samples standard curve plots of E7 protein gene that show 100 qPCR efficiency used for detection of HPV.

The y-axis represents the cycle quantification (Cq) values, while the x-axis shows the log of the starting quantity of template DNA. The assay achieved an efficiency of 100.9%, indicating highly accurate and reliable amplification. The R^2 value of 0.989 reflects a strong linear correlation between the Cq values and the log of the starting quantity, ensuring the robustness of the assay. The slope of -3.301 closely approximates the theoretical ideal of -3.322 for optimal amplification efficiency, and the y-intercept is 37.801. Open circles represent the standards used for serial dilution, and crosses indicate unknown samples. This plot confirms the assay's precision and sensitivity in detecting HPV DNA, validating its reliability for clinical applications targeting the E7 gene. The detection of HPV in this study was conducted using a highly specific and sensitive method targeting the E7 oncoproteins of HPV types 16 and 18. This was achieved through TaqMan

probe-based real-time polymerase chain reaction (RT-PCR), ensuring accurate identification of these high-risk HPV types. Table 3 elucidates the relationship between HPV infection and various demographic, behavioral, and clinical factors among 22 confirmed cases. Smoking was reported by only 4.5% of participants, demonstrating a highly significant association with HPV infection ($p< 0.001$). Vaccination against HPV was absent in all participants, precluding statistical evaluation of its association. Oral contraceptive use was observed in 40.9% of cases; however, this variable did not exhibit a statistically significant relationship with HPV infection ($p= 0.394$).

Table 3: Association of demographic, behavioral, and clinical variables with HPV infection among positive cases (n=22)

Variable	n(%)	*p*-value
Smoking		
Yes	1(4.5)	<0.001
No	21(95.5)	
Vaccination		
Yes	0(0.0)	NA
No	22(100)	
Oral contraceptives		
Yes	9(40.9)	0.394
No	13(59.1)	
Infection site		
Cervix	22(100)	NA
Specimen type		
Cervical swab	21(95.5)	<0.001
Blood sample	1(4.5)	
Family history of cancers		
Yes	1(4.5)	<0.001
No	21(95.5)	
Residence		
Urban	3(13.6)	0.001
Rural	19(86.4)	
Co-infection (Chlamydia trachomatis)		
Yes	0(0.0)	NA
No	22(100)	
Immune status (HIV previous exposure)		
Yes	0(0.0)	NA
No	22(100)	

The cervix was the sole site of infection, with cervical swabs being the primary diagnostic specimen type (95.5%), and a single blood sample (4.5%) contributed to specimen analysis. The strong association between specimen type and infection ($p< 0.001$) underscores the relevance of cervical swabs in HPV detection. A family history of cancers was reported by 4.5% of participants and was significantly linked to HPV infection ($p< 0.001$). Additionally, 86.4% of cases were from rural areas, with residents exhibiting a significant association with infection ($p= 0.001$), highlighting the potential impact of geographic and socio-environmental factors. Co-infections with *Chlamydia trachomatis* and HIV exposure were absent among participants, rendering these variables statistically inapplicable. This comprehensive analysis emphasizes significant associations between HPV infection and factors such as smoking, family history of cancers, and rural residence. At the same time, other variables, like oral contraceptive use, showed no significant correlation. Figure 3 illustrates the distribution of HPV test results and sample types among a total of 71 specimens analyzed in the study.

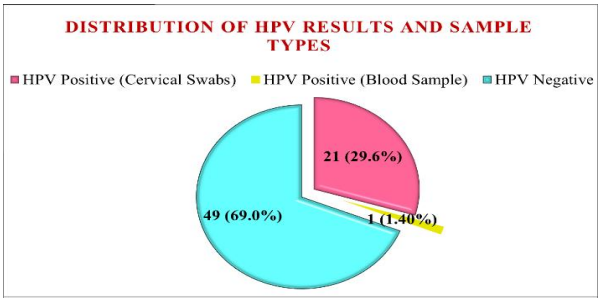

Figure 3: HPV Test Results and Sample Type Distribution in the Study Population.

Of these, 49 specimens (69.0%) tested negative for HPV, while 22 specimens (31.0%) were positive for HPV. Among the HPV-positive samples, 21 (29.6% of the total specimens) were cervical swabs, representing the predominant type of sample yielding positive results. In contrast, only 1 specimen (1.4% of the total) was a blood sample that tested positive for HPV. The figure underscores the predominance of cervical swabs as the primary sample type in HPV detection, aligning with the established preference for cervical specimens in studies of HPV-associated infections. Additionally, the high proportion of negative results highlights the critical role of molecular diagnostics in distinguishing infected from non-infected cases within the study population.

DISCUSSION

Human papillomavirus (HPV) infections, particularly those caused by high-risk genotypes such as HPV 16 and 18, represent a leading global driver of cervical cancer. The urgent need for precise and timely identification of these genotypes' success is the E7 oncoprotein, a pivotal player in HPV-mediated oncogenesis. By targeting the retinoblastoma protein (pRb), E7 disrupts tumor suppressor pathways, driving unregulated cell cycle progression and proliferation [12]. This dual role as a driver of carcinogenesis and a molecular diagnostic target underscores the significance of E7 in HPV diagnostics [13]. In this investigation, among the 71 specimens analyzed in this study, as shown in Figure 3, 22 tested positive for HPV (31.0%), with 95.5% originating from cervical swabs and only a single blood sample (4.5%) yielding a positive result. The presence of HPV DNA in blood is a rare and intriguing finding, warranting deeper exploration into its clinical implications. While HPV predominantly infects epithelial tissues, detection of viral DNA in blood suggests potential mechanisms such as transient viremia during acute infection, viral dissemination in advanced disease, or latent infections within hematopoietic cells [14]. The singular blood positivity observed in this study opens avenues for further research. It may reflect

transient viremia, where viral particles circulate temporarily during infection [15]. Alternatively, it could signify systemic dissemination, although this is typically rare and more likely in immunocompromised individuals or cases with advanced pathology. The absence of co-infections (e.g., HIV) in the study population suggests that this blood detection is not linked to broader immune suppression. However, technical considerations, including potential contamination, must also be ruled out to validate this result. Regardless, the presence of HPV DNA in blood highlights the potential utility of blood-based diagnostics, particularly for monitoring latent or disseminated infections, though its clinical significance remains unclear. The study's findings, encapsulated in Table 1, highlight critical associations between HPV infection and several demographic and behavioral variables. Smoking emerged as a statistically significant risk factor ($p< 0.001$) despite being reported by only one participant (4.5%). Smoking's role in HPV-related pathogenesis may stem from its immunosuppressive effects and the carcinogenic compounds it introduces, which can amplify the oncogenic activity of HPV. This aligns with prior studies identifying smoking as a facilitator of persistent HPV infections and cervical dysplasia [16,17], underscoring the need for integrated smoking cessation programs within HPV management frameworks. An equally critical finding was the absence of HPV vaccination among participants, reflecting a glaring gap in preventive strategies [18]. Despite the demonstrated efficacy of vaccines targeting HPV 16 and 18 in reducing cervical cancer incidence, this study's cohort lacked immunization coverage, highlighting the need for robust public health initiatives to increase vaccine access, particularly in rural and underserved areas. Table 1 further reveals that while 40.9% of participants reported oral contraceptive use, no statistically significant association with HPV infection was observed ($p= 0.394$). This finding resonates with existing literature that presents mixed conclusions about the relationship between hormonal contraception and HPV pathogenesis [19], warranting further research to clarify this complex interaction. The exclusive identification of the cervix as the site of infection, with cervical swabs as the predominant diagnostic specimen type (95.5%), underscores their critical role in HPV detection. The strong statistical association ($p< 0.001$) between specimen type and diagnostic success, as detailed in Table 1, reinforces established guidelines favoring cervical swabs for screening. However, the singular positive result from a blood sample invites questions about its diagnostic role in systemic or non-epithelial HPV infections. Geographic and socio-environmental factors also play a pivotal role in HPV epidemiology. The significant association between rural residence and HPV infection ($p= 0.001$) in this study reflects disparities in healthcare access. It emphasizes the need for targeted interventions such as mobile screening units and community vaccination campaigns, and this is confirmed in another study [20]. Additionally, the observed link between HPV infection and a family history of cancers ($p< 0.001$) underscores the potential interplay of genetic predisposition and viral oncogenesis, advocating for the integration of familial risk assessments into screening protocols [21]. These findings align with and extend the growing body of evidence supporting the TaqMan probe-based RT-PCR assay's utility in HPV detection. This assay's quantitative accuracy, combined with its exceptional specificity and sensitivity, solidifies its status as a cornerstone in HPV diagnostics [22]. While cervical swabs remain the primary specimen type, the exploratory potential of blood-based testing warrants further investigation, particularly for advanced or systemic HPV infections. To combat HPV-associated diseases effectively, future efforts must prioritize expanding vaccination and screening access in underserved populations, alongside advancing research into alternative specimen types and diagnostic methodologies. By leveraging tools like TaqMan probe-based RT-PCR within comprehensive prevention and management frameworks, the global burden of HPV-related cancers can be substantially reduced. To be overstated, early detection forms the cornerstone of effective screening, diagnosis, and intervention. In this context, the TaqMan probe-based real-time polymerase chain reaction (RT-PCR) emerges as a transformative molecular diagnostic tool, offering unparalleled specificity and sensitivity. This advanced method, as demonstrated by the amplification efficiency of 100.9% ($R^2 = 0.989$) in Figure 2, highlights its superior capability for real-time quantification while minimizing false positives, setting it apart from conventional PCR and hybridization assays.

Conclusion

This study highlights the utility of TaqMan probe-based real-time PCR in detecting HPV DNA by targeting the E7 gene, a pivotal marker in HPV-mediated oncogenesis. The high efficiency (100.9%) and specificity observed underscore the assay's robustness, especially in identifying high-risk HPV genotypes such as 16 and 18. The predominance of cervical swabs in HPV detection emphasizes their reliability as diagnostic specimens. However, the rare detection of HPV DNA in blood samples suggests the need for further exploration of systemic HPV dissemination. The significant associations between HPV infection and factors like smoking, rural residence, and family cancer history underline the complex interplay of demographic and behavioral factors.

Future prospects

Future research should improve molecular diagnostic methods like TaqMan probe-based RT-PCR to increase

sensitivity and usefulness in various clinical contexts. Broader specimen types (blood samples) reveal new information about HPV pathogenesis, especially systemic dissemination and latent infections. Research on the link between blood-detected HPV DNA and disease development could be useful diagnostic and prognostic markers. Additionally, the inclusion of HPV genotyping into routine screening can improve risk classification and tailored patient care. Lastly, new therapies targeting the E7 oncoprotein may lead to better HPV-related cancer treatments and prevention.

Conflict of interests

No conflict of interest was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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