Genotyping of Human Papilloma Virus Causing Skin Wart among Iraqi Patients

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Abstract

The papillomaviruses are a large group of tiny, non-enveloped DNA viruses that in several different anatomical locations can cause squamous epithelial tumor's (warts and papilloma's). There is a close association between HPV and cervical uterine cancer . The genome is composed of 7200–8000 base pairs and is divided into three parts, early region (E) comprising of E1, E2, E4–E8 and reflecting 50 percent of the L1 and L2 genome, Genomic DNA from wart tissue samples were extracted by using gSYAN DNA mini kit extraction kit . The Real Time PCR primers for detection and genotyping of DNA Human papillomavirus type 1 and type3 and type6 based on L1 gene that designed in this study using NCBI-Genbank Sequence database and primer 3 plus . The genetic study included analysis of a common DNA to HPV that was found in 20 % of cases and DNA fragment specific to HPV type 1, 3 and 6 based on real time PCR. Type 1 HPV was not approved to present in any sample in the study. Type 3 HPV was identified in a single case. HPV 6 was identified in 8 cases, Therefore, type 6 HPV is the most common genotype, followed by type 3.

Keyword : *HPV, Wart skin disease , L1 gene, RT-PCR .*

Introduction

Skin warts are considered the main manifestation of the cutaneous Human papilloma viruses (HPV) types [(1)] . Human papillomaviruses are small, double-stranded DNA viruses that infect the epithelium. More than 120 HPV types have been identified; they are differentiated by the genetic sequence of the outer capsid protein L1. Most HPV types infect the cutaneous epithelium and can cause common skin warts [(2)] .

Characterization and analysis of human papillomaviruses of skin warts are ver clinically important . Common warts represent 70% of skin warts and occur primarily in children, whereas plantar and flat warts occur in a slightly older population [(3)] .

Mucosal HPV are small double-stranded DNA viruses that infect mainly anogenital epithelium [(4-5)] .

The etiological role of the human papillomavirus (HPV) in cancers of the uterine cervix has resulted in the development of molecular technologies for viral detection [(6)] .

The aim of this study was to develop a SYBR Green-based real-time for genotyping of Human Papilloma Virus causing skin Wart among Iraqi patients.

Materials and Methods

Genomic DNA from wart tissue samples were extracted by using gSYAN DNA mini kit extraction kit (Tissue protocol) Geneaid. USA, and done according to company instructions with modification as following procedures : 200mg frozen wart tissue samples was transferred to sterile 1.5ml microcentrifuge tube. Then homogenised by liquid nitrogen 200µl GST (Tissue lysis buffer) and 20 µl proteinase K (10mg/ml) were added and homogenized by sterile micropestle and mixed by vortex. Then incubated at 60C for 1 hour. After that, 200µl of binding buffer GSB was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 70C for 15 minutes, and inverted every 3 minutes through incubation periods. Then, 200µl absolute ethanol were added to lysate and immediately mixed by shaking vigorously. After that, DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture

(including any precipitate) to column. Then centrifuged at 10000rpm for 5 minutes. And the 2 ml collection tube containing the flow-through were discarded and placed the column in a new 2 ml collection tube. 400µl W1 buffer were added to the DNA filter column, then centrifuge at 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube. 600µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of pre-heated elution buffer were added to the center of the column matrix. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA (table 1) .

Table 1. : The kits used in this study with their companies and countries of origin

No.	Kit	Company	Country
1	gSYAN DNA Extraction Kit	Geneaid	USA
	GST buffer		
	GSB buffer		
	W1 buffer		
	Wash buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
2	RealMOD™ Green SF 2X qPCR Mix (SYBER)	INtRON	Korea
	SYBER Green DNA dye HS-Taq DNA Polymerase, PCR buffer, MgCl2 and dNTP's		

The Real Time PCR primers for detection and genotyping of DNA Human papillomavirus type 1 and type3 and type6 based on L1 gene that designed in this study using NCBI-Genbank Sequence database and primer 3 plus. (5) All these primers was provided from Macrogen company, Korea as in table (2) .

Table 2. : HPV PCR primers that are used in the study

Primer	**Sequence (5'-3')**		**Product Size**
HPV DNA primer	F	TGAAAATCCCGCCTTTGAGC	118bp
	R	TGTGCCTTCAGGTGTTTCAC	
HPV-1 Primers	F	TTCTGGGAAGTCGATCTCACAG	226bp
	R	TTTGCGCTTTGTGGTGGAAC	
HPV-3 Primers	F	CCGTGGGTCATCCTTATTTTGC	117bp
	R	ACTTATTTGGGTCGGGCAAC	
HPV-6 Primers	F	TTCGATCCCACAACACAACG	90bp
	R	ATGTCCACTTACACCCACACC	

In this study ,collection of 50 samples (skin wart) . Liquid nitrogen was used as a preparatory step for genetic material extraction . The Real Time PCR technique was performed for detection and genotyping human papillomavirus based L1 gene from common wart patient samples

Results and Discussion

The genetic study included analysis of a common DNA to HPV that was found in 20 % of cases and DNA fragment specific to HPV type 1, 3 and 6 based on real time PCR as shown in figures (1) ; (2) ; (3) ; (4) . We fail to identify any case with type 1 HPV. Type 3 HPV was identified in a single case. HPV 6 was identified in 8 cases . In 11 patients who were positive for the common HPV DNA, there was no positivity to HPV 1, 3 or 6 specific DNA fragment, (as shown in table. 3) .

Table.3: The frequency distribution of patients according to HPV genotype

HPV type	Negative	Positive	Total
Negative	20	0	20
Unidentified	0	11	11
HPV 3	0	1	1
HPV 6	0	8	8
Total	20	20	40

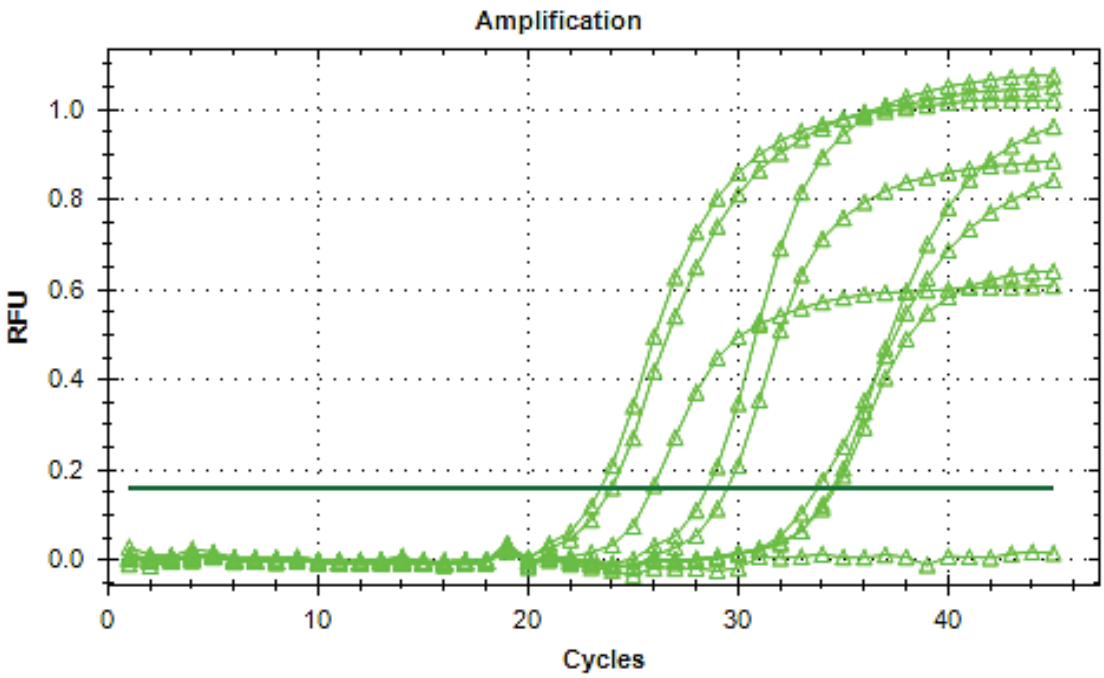

Figure.1: Real time PCR amplification plot based SYBER Green DNA dye for detection Human Papilloma virus DNA from extracted DNA of wart patient samples

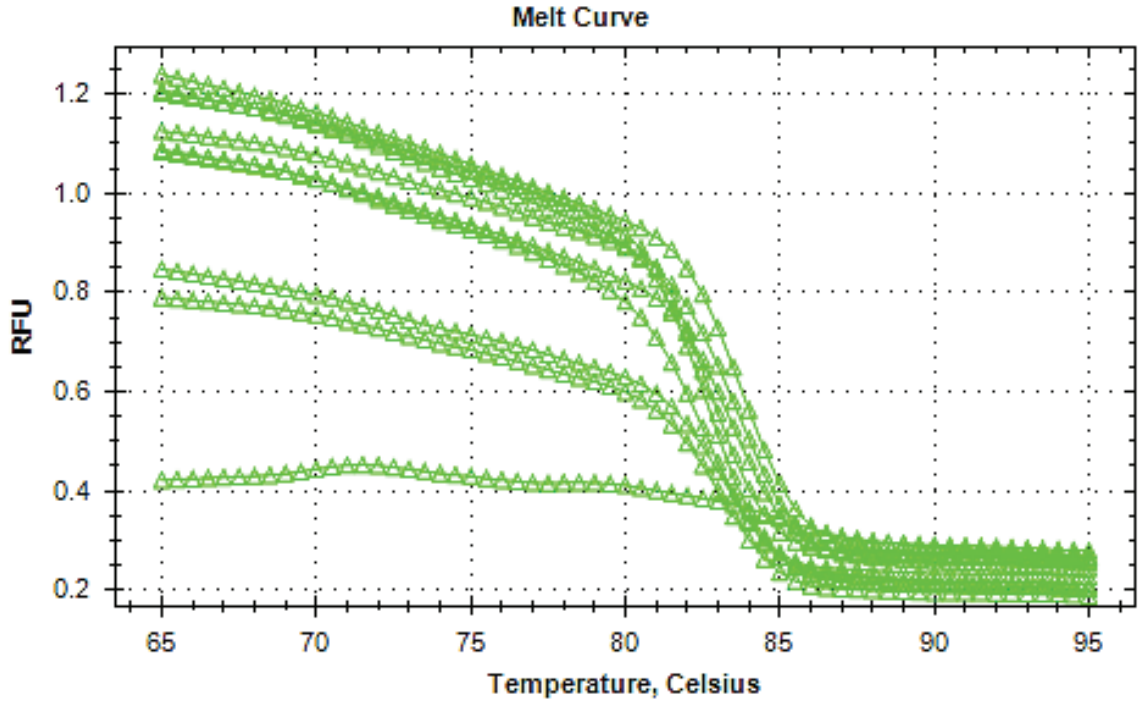

Figure.2 : Real time PCR Melt curve based SYBER Green DNA dye for qPCR specificity in Human Papilloma virus DNA .

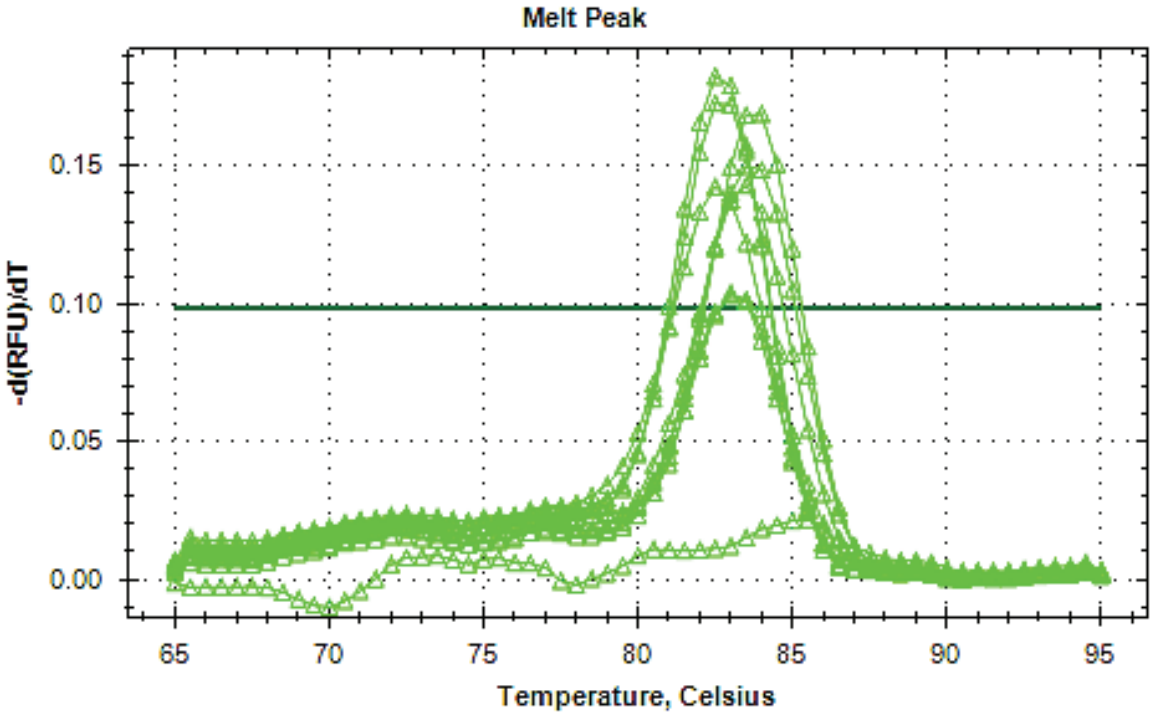

Figure.3: Real time PCR peak curve based SYBER Green DNA dye for qPCR specificity in Human Papilloma virus DNA that showed positive primer amplification at 80-85°C.

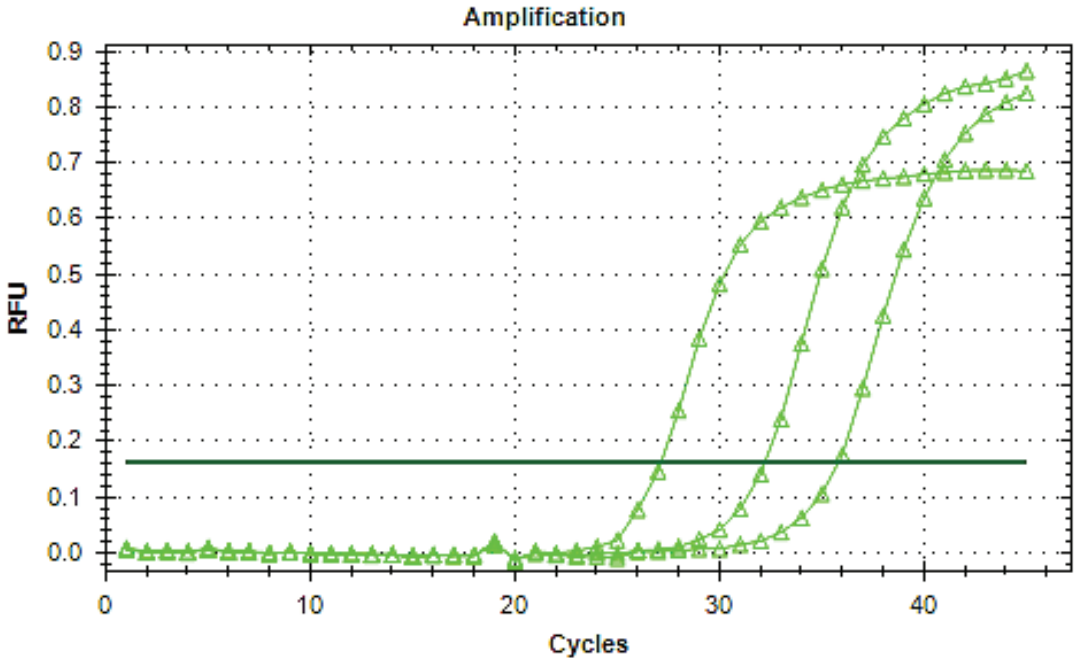

Figure.4 : Real time PCR amplification plot based SYBER Green DNA dye for detection Human Papilloma virus type 3 based L1 gene from extracted DNA of wart patient samples.

HPV genotyping has been used in epidemiological studies and in pre- and post-vaccination trials to determine the overall vaccine effectiveness and to unmask niche replacements by non-vaccine HPV types [7] .

In contrast to conventional PCR, a real-time approach enables accurate quantification of the PCR target. A standard curve that shows a highly significant correlation between the log of the PCR target and the Cp value could be obtained for our SPF10 real-time PCR. Standard curves for HPV 16 and 31 showed a dynamic range from 10 copies to 106 copies in a linear fashion. Other have used the SPF10 primers in a qPCR format to determine the viral load, but the compatibility of the real-time PCR amplicon with the LiPA assay was not evaluated groups [8] .

The high amplification efficiency of the real-time PCR results in higher amplicon concentration than with conventional PCR, leading to cross-reactivity when the LiPA assay is performed under default conditions.

Conclusions

The age average of HPV patients between 10-19 years accounted for 12 (30 %) is considered as a high group frequency and the Frequency distribution of patients with wart according to gender included 26 (65.0 %) males and 14 (35.0 %) females . The genetic study included analysis of a common DNA to HPV that was found in 20 % of cases and DNA fragment specific to HPV type 1, 3 and 6 based on real time PCR . Type 6 HPV is the most common genotype, followed by type 3.

Ethical Clearance: Taken from University of Al-Qadisiyah ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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