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# Molecular Detection of *Molluscum Contagiosum Virus* Type II (MCV II) and Human Papillomavirus Type1 (HPV1)

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## Abstract

To detect HPV type1 and *Molluscum contagiosum virus* type 2(MCV II) molecularly in patient attending the dermatology clinic in Basrah hospitals. The study was conducted during the period from October 2017 to April 2019 in outpatient clinic of Basrah Teaching hospital, Al-Fayha hospital, and Al-Muanaa hospital in Basrah city/Iraq. A total of 22 warts samples were collected from patients presented with plantar warts and 15 samples from patients with MCV. The samples of plantar warts were identified genetically by using monoplex PCR system and confirmed by DNA sequencing, while the samples of MCV II were identified genetically by using PCR only depending on the size of the PCR products after electrophoresis. out of 22 plantar warts samples, 17 (77.27%) samples were positive according to monoplex PCR method, while 5 (22.72%) samples were negative by this method. All 17 positive samples were sent to sequences and confirmed to HPV type1.And the sample of MCV II 6 (40 %) samples were positive with PCR technique, while 9 (60 %) samples were negative by PCR method. The results of MCV showed that 11 (73%) of the patients are of the age group (1-11 years), of whom 9 (60%) are male, 6 (40%) are female.HPV1 associated with plantar skin warts and can be detected by monoplex PCR and DNA sequences. The Molluscum contagiosum was predominantly in males and the age group 1-10 y was more infected than other age groups.

## Keywords

HPV, plantar warts, and monoplex PCR, MCV II , *Molluscum contagiosum*

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## 1-Introduction

### HPV:

The cutaneous warts are an infectious skin disease commonly found in a dermatology clinic throughout the world. They are caused by human papillomaviruses (HPVs) of the Papillomaviridae family of viruses [1]. HPV infections are primarily transmitted by direct skin- to- skin contact or sexual contact [2]. Four main types of warts are common warts, plantar warts, flat warts and anogenital condylomata [3]. Common warts are often found on feet and hands, but can also be found in other areas [4]. HPV-2, -27, and -57 are usually detected in common warts [5]. Flat warts are developing on the face, dorsal hands, or distal forearms of adolescents. They are caused primarily by HPV-3, -10, -28, -29, -77, -78, -94, and -117 [6]. Plantar warts are benign epithelial tumors which usually develop in the plantar area generally caused by infection by human papilloma virus types 1, 2, 4, 60, or 63, but have also been caused by types 57, 65, 66, and 156 [7,8].

### MCV:

Infectious molluscum is a benign and self-defining viral skin infection that generally affects young children, young adults and individuals with immunodeficiency, but it can occur at any age. It can affect any part of the body surface and is characterized by separate, soft papules or lesions in the form of a dome called "mollusca", usually 2-5 mm in diameter [9]. Infectious molluscum (MC) is a skin infection and mucous membranes caused by DNA viruses called Molluscum virus belonging to the family of Poxviridae subgenus Molluscipoxvirus, the virus belongs to the group of smallpox, and it measures 300 nm at maximum [10], which includes 4 genetically divided but MC viral types cannot Clinically distinguished [11]. However, the first type of virus is responsible for the majority (76% -97%) of MC infection [12]. In contrast, MC 2 virus naturally causes type (60%) of infection in HIV patients [13]. Clinically, MC lesions can be confused with other lesions of viral skin infections such as herpes simplex virus, varicella zoster virus, and human papillomavirus (HPV) infection, especially in HIV patients such as HIV patients [14]. MCV is transmitted directly by skin contact to produce mucous skin lesions and is rarely transmitted by fumit on bath sponges. Bath towels, beauty salons and school swimming pools [15]. In adults, mollusks often spread sexually [16].

## 2-Material and methods

A total of 22 plantar wart and 15 MCV samples collected from patients of both sex attending the dermatology clinic. Each sample were placed in tube containing 300 µl phosphate buffer saline (PBS) and immediately transferred to the laboratory of the virology branch of the College of Science at Al- Basrah university, which were stored at -200C. DNA was extracted by using geneaid viral DNA extraction kit (Viral Nucleic Acid Extraction Kit II). The mixture of PCR was contained 2 µl of HPV1 primers (table 1) [17], 25 µl Master Mix, 10 µl of extracted DNA sample, and the mixture was complete by 11 µl nuclease free water to 50

µl. Nuclease free water was used as negative control. PCR was applied by using thermal cycler and performed by using the following PCR condition; 94°C for 3 min and then 35 cycles of 94°C for 40 secs, 58°C for 40secs, and 72°C for 40 secs. the final step was 72°C for 3 min. The amplification products were analyzed by gel electrophoresis and visualized under UV.

**Table 1- The sequences of forward and reverse specific primers that used in monoplex PCR.**

| Type | Primer sequence | Position | Location | Length (bp) |
|---|---|---|---|---|
| HPV1 | R: 5-CTACCTATCTCTATCCCTCTT-3<br>F: 5-GTCTGGTTACCAGCGCAGAAT-3 | Nt 5437-5457<br>Nt 5730-5750 | L1 ORF | 314 |

**Sequencing of PCR product**

The PCR product samples of HPVs were sent to Macrogen Inc. (Macrogen Korea: 10F, 254 Beotkkot-ro, Geumcheon-qu, Seoul, 08511, Rep. of Korea) and to china (yang ling tianrun aoke biotechnology company). The sequence processing was performed using Chromas version 2.6.2 (http:// technelysium.com.au).

**PCR technique for MCV samples**

Molecular detection of MCV virus has been done by extraction of the viral DNA by using Tissue Genomic Viral Nucleic Acid Extraction Kit II (Geneaid / Taiwan). MCV DNA fragment with the designed primer yielded band corresponding to their molecular size of approximately 575 bp (table 2).

**Table 1- The sequences of forward and reverse specific primers that used in PCR.**

| Primers sequences | | |
|---|---|---|
| F | (5-GGAGGAGTGCCCATCAAGAAT-3) | 575-bp |
| R | (5-GCTTTTCAGTTTTTGTGCGA-3) | |

The PCR technique was applied by adding 5 µl from DNA extracted to the PCR tube containing 5 µl of the master mix and 1 µl of the F and R primers was then added to this PCR tube. In the end, 13 µl of the NFW were added to the tube to get 25 µl as a final size. The mixture then transfers to the thermal cycler machine, the conditions of PCR reaction are listed in (Table 3).

**Table 3- The PCR amplification Condition**

| NO. | Steps | Temperature | Time | Cycles |
|---|---|---|---|---|
| 1 | Initial Denaturation | 94 ºC | 5 mins. | 1 |
| 2 | Denaturation | 94 ºC | 45 sec. | 35 |
| 2 | Annealing | 58 ºC | 45 sec. | |
| 2 | Extension | 72 ºC | 45 sec. | |
| 3 | Final Extension | 72 ºC | 10 mins | 1 |

## 3-Results

### Molecular detection

### Monoplex PCR of HPV

monoplex PCR system was found capable to identify HPV DNA in17(77.27%) samples and the reminder 5(22.73%) samples were negative in monoplex PCR. A 314 DNA band were regarded as positive for monoplex PCR system.

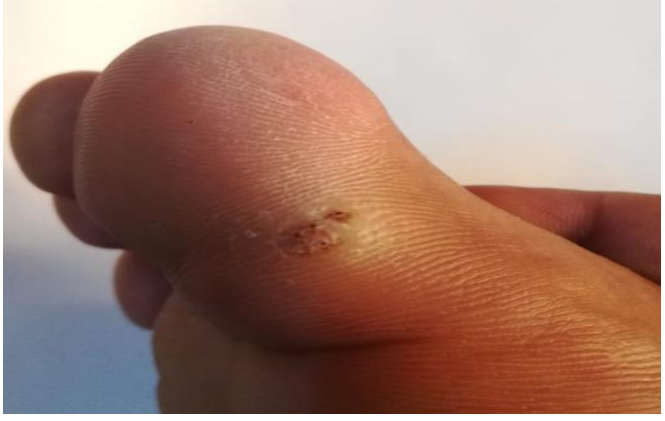


**Fig. 1- plantar wart**

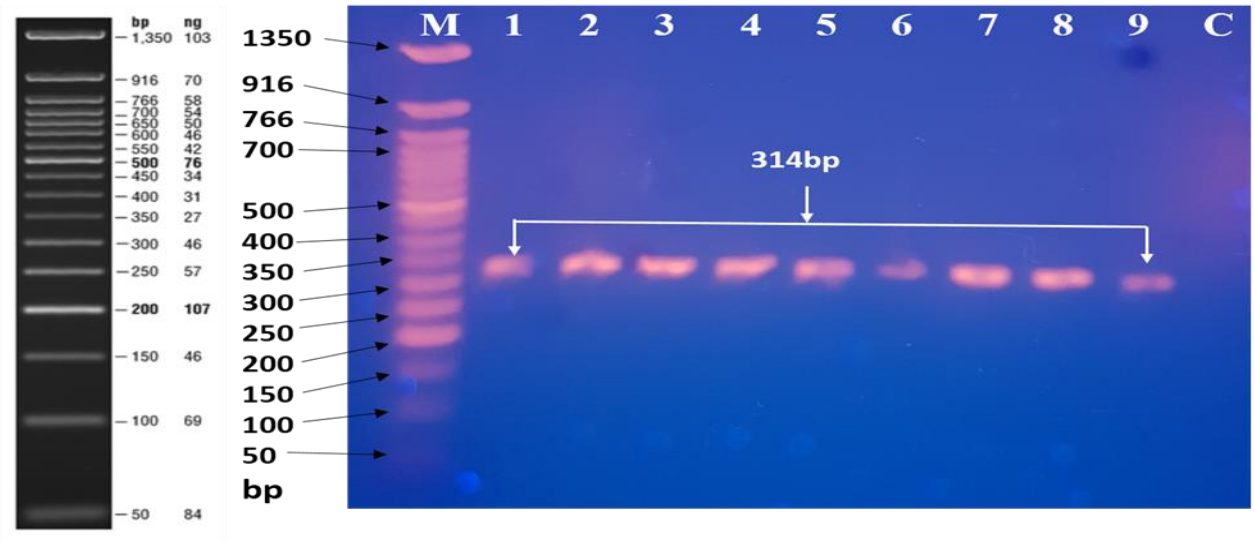



**Fig. 2- Gel electrophoresis of monoplex PCR products of human papillomavirus type 1. Lane M: DNA ladder (50-1350bp).**

## Sequencing of PCR products

A 17 PCR product samples were sent to sequencing. The result showed that HPV genotype 1 that belong to the genus *mu papillomavirus* where detected in all isolate.

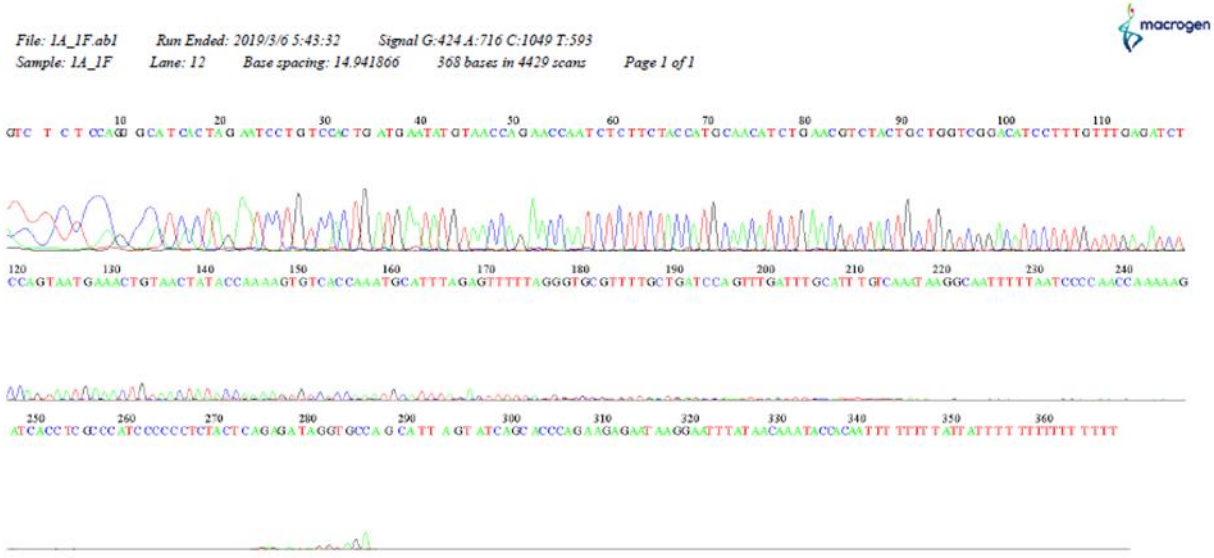
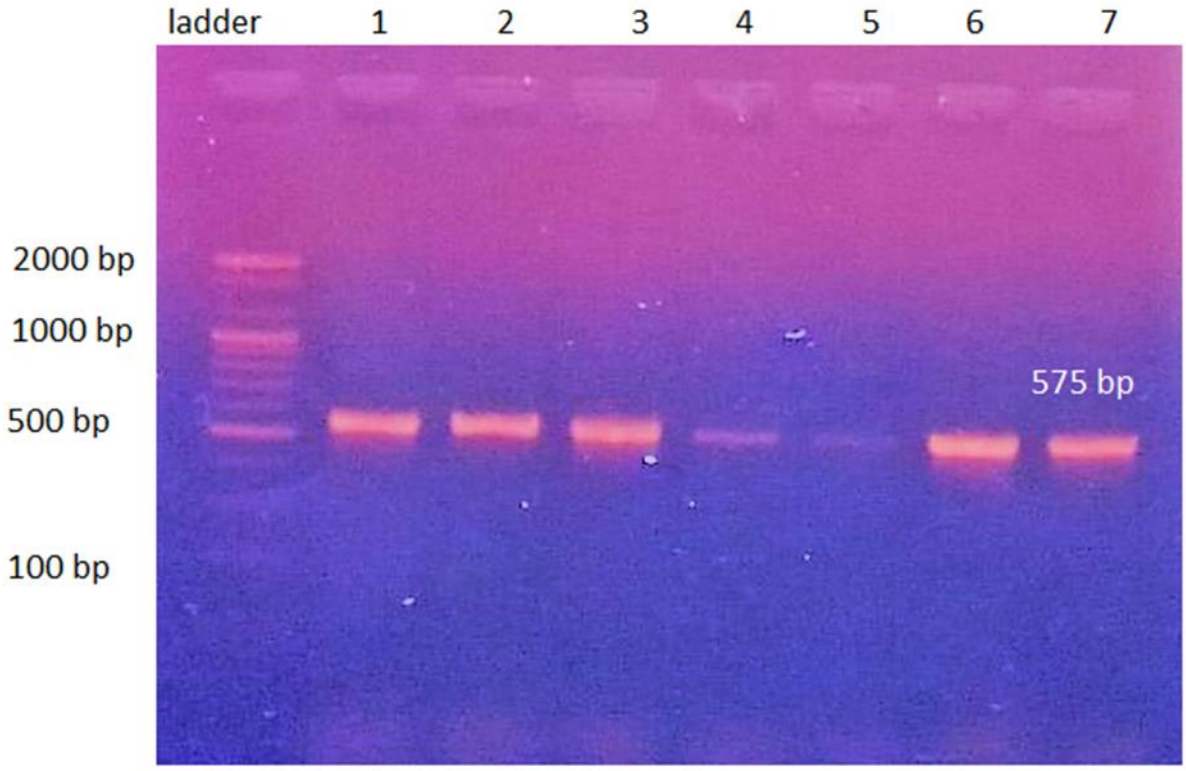



**fig. 3- peak sequences of HPV1 of forward isolate 6**

**PCR result of MCV**

Molecular detection of MCV virus has been done by extraction of the viral DNA by using Tissue Genomic Viral Nucleic Acid Extraction Kit II (Geneaid / Taiwan). MCV DNA fragment with the designed primer yielded band corresponding to their molecular size of approximately 575 bp.




**(Fig. 4- Agarose gel electrophoresis of PCR products L = DNA ladder Lane 1,2,3,4,5,6,7 = positive to (575bp).**

**4-Discussion**

The study revealed that HPV1 were detected in plantar skin warts and HPV. . Several studies recorded HPV1 most frequently associated with plantar warts[18,19].The results of the molecular analysis to detect 15 isolates of MCV by using primers showed all band corresponding to a 575bp. Molecular epidemiological study of MCV infection indicates the prevalence of MCV was observed whatever the age, in contrast to the reported differences in the distribution of MCV subtypes among patients of different age groups [20].

## 5-Conclusion

The MCV II and HPV I were predominantly in males and the results of this study showed the age group 1-10 y was more infected than other age groups, the distribution of the lesion according to their location revealed that the head was more sensitive to infection than the other body parts, mollecular detection of MCV II and HPV I virus was the best way to diagnose the infection and HPV 57 and 1 genotypes were the most prevalent HPV genotypes that caused skin warts also, the study showed the presence of single and mixed HPV infections and the FAP primers can be dispensed and only use multiplex PCR in diagnosing of HPV genotypes.

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