Republic of Iraq
Ministry of Higher Education
and Scientific Research
Diyala University
College of Science
Department of Biology

Phylogenetic analysis of Molluscum contagiosum virus isolated from different human's body regions

A thesis

Submitted to the Council of College of Science/Diyala University In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

By

Sameer Mohammad Deyab

B.Sc.Biology/ University of Baghdad (2004)

Supervised by

Assist.Prof. Zahraa Jaafar Jameel

2021 A.D **1443 A.H**

بسم الله الرحمن الرحيم

﴿ يَٰٓأَيُّهَا ٱلَّذِينَ ءَامَنُوٓاْ إِذَا قِيلَ لَكُمۡ تَفَسَّحُواْ فِي ٱلۡمَجَٰلِسِ فَٱفۡسَحُواْ يَفۡسَحِ ٱللَّهُ لَكُمۡۖ وَإِذَا قِيلَ ٱنشُزُواْ فَٱنشُزُواْ يَرۡفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمۡ وَٱلَّذِينَ أُوتُواْ ٱلۡعِلۡمَ دَرَجَٰتٖۚ وَٱللَّهُ بِمَا تَعۡمَلُونَ خَبِيرٞ ١١ ﴾

صدق الله العظيم

سورة المجادلة: الآية (11)

DEDICATION

To my compassionate mother, whose prayers were the secret of my success.

To my wife, the mother of my sons, who was my support and help

To my brothers and sisters and to all my friends who wished me success.

,Respected professors, I offer you the fruits of my effort, in what my Lord has helped me ..

Sameer Mohammad

Acknowledgements

I swear I thank God Almighty for helping me and me to complete this work.

Then my heartfelt thanks to the Dean of the College of Science and Head of the Department ofbiology and all the teaching staff of biology / Diyala University for giving me the opportunity to complete my higher education,

And with all my respect and appreciation to my supervisor on my research Assist.Professor (Dr. Zahraa Jaafar Jameel) for his guidance, assistance and encouragement, which was the main motivation to complete this research I ask God Almighty to make this in the balance of her good deeds.

Also, my heartfelt thanks to (Dr. Ataa Akram Kadory)for assisting me in diagnosing and collecting samples.

I also thank my colleague Manaf Thamer for his continuous help and support during my studies, and finally my sincere prayer to my family for their support and assistance while working on this thesis.

Sameer Mohammad

Abstract

The Molluscum contagiosum virus (MCV) is a member of the poxviridae family that infects human skin and is highly contagious in all ages, but it is more prevalent in ages from (1-10) years, as well as patients with weak immunity such as HIV infected persons. The aims of study are molecular detection of the MCV using Conventional polymerase chain reaction (PCR) technique, followed by viral nucleotide sequence and phylogenetic analysis. Also to explore the association between MCV infection and certain socio-demographic and clinical risk factors. The present study is cross-sectional study conducted in Diyala province for the period extended from 1 October 2020 until 1 April 2021. A total of 101 patients with clinically suspected as having MCV lesion in different parts of their bodies were included. This were from Baquba Teaching Hospital, Consultancy Department, and some Primary Healthcare Centers belong to Diyala Directory of Health. The age ranged from 5month to 60 years old from both sexes. Skin lesion tissue biopsies were collected using tissue curette was done under the direct super vision of specialized dermatology doctoer's . Conventional PCR was done using special primers. Genetic sequencing was done in Korea and the provisional phylogenetic analysis was done. statistical analysis was done using SPSS program (Version 27) and P values less than 0.05 were considered significant.The results showed that MC133L, MC021L and MC002L gene swas among the of skin specimens was detected in 2.0%, 18.8% and 84.2% respectively. Analysis of genomic sequence showed that MC021L gene with 979 pb was detected in six isolated of MCV and two isolated of MCV showed the presence of MC133L with 575 pb . When comparing each of these isolates with the NCBI. Regarding the MC021L gene locus, the results indicated that there were 51 DNA differences distributed in almost all the samples examined. The

majority of specific differences in the MC021L gene showed a nearly equal distribution among the examined viral samples. The results from the comprehensive tree indicated that all the examined viral samples belonged to a new subtype located between both subtypes I and subtype II. This new distribution occupied by these isolates was not assessed by any other related sequences. It was confirmed that the screened isolates were not found among any of these known subtypes while the genetic variation based on MC133L showed only one DNA variant indicated.Five isolates were recorded in the NCBI, one isolate from the MC133L gene, and the serial number of isolates was LC629161, and four isolates from the MC021L gene, and the serial number of the isolates was LC629162, LC629163, LC629164, LC629165. The current reslts show there is no significantly association between with age, sex, educational levels, clinical features and some lesion characteristics. However , it is significantly higher among rurals (P=0.037), winter season (P= 0.001), lesion frequency of 1-4 (P= 0.035).

Based on the locus MC021L, the present study was concluded that new investigated sequences that is confirmed doesn't belong to any known subtype. Thus, the present study documented the emergence of a new subtype of MCV that can be placed between subtype I and type II, leaving the door opened for further studies.

List of Contents

List of Table

List of Figure

List of Appendix

List of Abbreviations

The word	Abbreviate
Acquired Immune Deficiency Syndrome	AIDS
Adenine + Thymine	A+T
base pair	bp
Deoxyribonucleic acid	DNA
Kilobate	Kb
Extracellular (envelope) virion	EV
Glycosaminoglycans	GAGs
Guanine + Cytosine	G+C
Human immunodeficiency virus	HIV
Intracellular mature virus	IMV
Orthropoxvirus	Orf
Kilobase pairs	Kbp
Mature virion	MV
MolluscumContagiosum	MC
Molluscum Contagiosum Virus	MCV
Reflectauce confocal microscopy	RCM
National Center for Biotechnology Information	NCBI
Phosphate Buffered Saline	PBS
Polymerase chain reaction	PCR
43 kilodalton protein	p43k
37 kilodalton protein	P37k
Vaccinia virus	VACV
Variola	VAR

Molluscum contagiosum type 1 versus a	MCV -1va
Molluscum contagiosum type 1 versus b	MCV -1vb
Molluscum contagiosum type 1 versus c	MCV -1vc
Molluscum contagiosum virus type 1	MCV1
Molluscum contagiosum virus type 2	MCV2
Molluscum contagiosum virus type 3	MCV3
Molluscum contagiosum virus type 4	MCV4
Molluscum contagiosum 7Leftward	MC007L
Molluscum contagiosum 13Leftward	MC013L
Molluscum contagiosum 066Leftward	MC066L
Molluscum contagiosum 148Rightward	MC148R1
Bacillus amyloliquefaciens	Bam.H1
Nuclear facter kappa B cells	NF-KB

Chapter one

Introduction

Introduction

Molluscum contagiosum, often known as water warts, caused a benign skin disease. Molluscum contagiosumskin lesions are known as Mollusca. The most common type of lesion is dome-shaped, spherical, and pinkish purple in hue, multiple distinct nodules 2 to 5 mm in diameter, appear on the epidermis, and appearing everywhere on the body excluding the soles and palms, define infection (Burrell *et al.*,2017; Badri *et al.*,2021). The Molluscum contagiosum virus (MCV), a double-strand DNA virus belonging to the Poxviridae family, causes Pearl disease(Leung *et al*., 2017). Bateman was the first to describe it in 1817, and Paterson showed its contagiousness in 1841(Bateman, 1953).

Molluscum contagiosum is a kind of Molluscum that heals without leaving scars in the majority of individuals. The condition is benign, and spontaneous remission is the most common outcome, but it can take anywhere from 12 to 24 months. The lesions can last for 3-5 years in some people and can be disfiguring. One-third of patients had recurrences. The lesions are widespread in HIV patients or those who are immunocompromised, and they appear when CD4 levels are low. The lesions seldom resolve spontaneously in these people (vander Wouden *et al.*,2017).

The MCV has four subtypes: MCV-1 (which accounts for 98% of infections) is primarily seen in youngsters, whereas MCV-2 is predominantly responsible for skin lesions in Asia and Australia, MCV-3 and MCV-4 are also found (Peterson *et al.*,2019). Cultures of the MCV are currently unavailable. MC lesions are spread via direct contact or indirect contact through towels, underwear, toys, razors, tattoo and other fomites. skin-to-skin contact MCV can also spread to normal skin by autoinoculation

when patients scrape the molluscum. Transmission may occur in shared swimming pools and other moist situations. In utero and prepartum transmissions have been recorded, resulting in congenital Molluscum contagiosum or skin lesions in the first few months of life (Rosner *et al* ., 2018 ; Peterson *et al.*, 2019).

The MCV has a worldwide distribution. There were around 122 million cases in 2010. It can be found all throughout the world, although it appears to be more common in hot, humid climates. MC is most commonly found in children aged two to five years, although it can also be found in sexually active adolescents and adults, as well as immune compromised people (Leung *et al.*, 2017).

The viral DNA is similar to that of the vaccinia virus. Its total G+Ccontents around 60% (Riedel *et al.*, 2019). MC lesions were divided into six categories based on their clinical appearance:umbilicated nodular, big/giant, conglomerated, erythematous, inflamed, and ped unculated (Zloto&Rosner, 2015). It's possible that the incubation period last up to 6 months. Itchy lesions may lead to autoinoculation. The lesions may last up to two years before spontaneously regressing. The virus is a weak immunogen, with only approximately a third of patients developing antibodies against it (Riedel *et al.*, 2019).

In most situations, the clinical appearance of molluscum lesions is sufficiently distinctive to allow clinical diagnosis. If the cheesy material expressed from the lesion is studied using negative-stain transmission electron microscopy, brick-shaped virions are generally seen in huge numbers (Longnecker *et al.*, 2013). For a definitive diagnosis of MCV, PCR-based tests are the best option. The results of molecular diagnostics

also offer information on the genotyping of the infected MCstrain (Trama *et al.*, 2007).

Aims of the study:

The present study was conducted to achieve the following goals:

1- Molecular detection of MC133L, MC021L and MC002L genes of MCV by conventional PCR test from skin lesions of clinically suspected patients.

2- Exploration of the nucleotide sequence of MC133L and MC021L genes.

3- Investigation of the genetic variation among detected MCV strains with global isolated in NCBI.

4- Draw the phylogenetic tree for detected MCV isolates comparing with globalstrains in NCBI to figure out the relatedness of local isolates of MCV .

5-Determination the associated socio-demographic and clinical features risk factoer.

Chapter two Literature Review

2. Literature review

2.1.Poxviridae:

Poxviruses are large brick-shaped or ovoid viruses with dimensions of 220–450 nm in length, 140–260 nm in width, and 140–260 nm in thickness. With a lipid-containing envelope, the particle structure is complicated.This family has double-strand DNA virus (Leung *et al.,* 2017; Riedel *et al.,* 2019).

About 100 proteins are found in poxvirus particles, many of which have enzymatic functions, such as a DNA-dependent RNA polymerase. Replication takes place exclusively within the cytoplasm of the cell. Variola (smallpox), vaccinia, and Molluscum contagiosum are harmful for humans; others that are pathogenic for animals can infect people (cowpox, monkeypox) , Most poxvirus infections result in a rash , however lesions caused by some members of the family are notably proliferative. The etiologic agent of smallpox, a viral illness that has afflicted humans since recorded history, is among the members of this group, (Riedel *et al.,*2019).

The virion's internal body is made up of a dumbbell-shaped nucleus and lateral bodies made up of heterogeneous material aggregates. The inner layer was continuous except for a few lipid channels, and the core wall was two layered with a total thickness of 18-19 nm (Longnecker *et al.,* 2013).

2.1.1. General Properties of Poxviruses:

The follwoing are the important properties

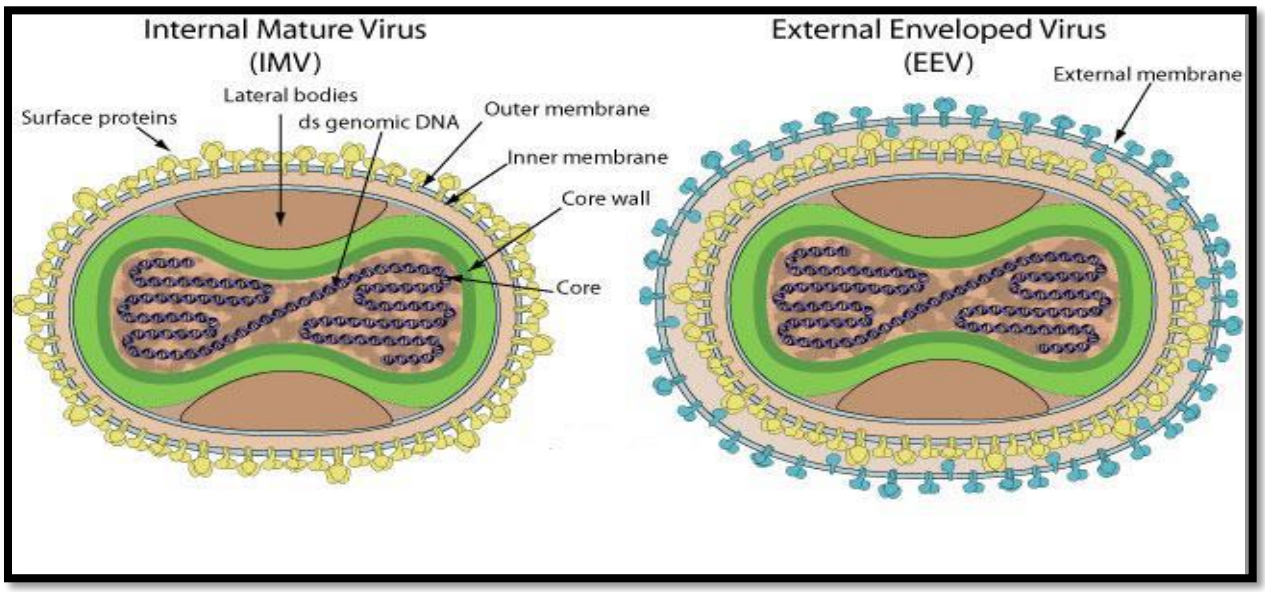
1- Virion: Complex structure with ridges on the exterior surface, 300–400 nm in length and 230 nm in diameter; includes core and lateral bodies.
2- Composition: DNA (3%) is present, followed by protein (90%) and lipids (5%).
3- Genome: Except for Parapoxvirus(63 %) , the genome is double-

stranded DNA with a linear structure, a size of 130–375 kbp, terminal loops, and a low G + C concentration (30–40%).

4- Proteins: Virions contain about more than 100 polypeptides; the center contains numerous enzymes, including the transcriptional machinery.
5- Virion Envelope: The development of numerous membranes is required for virion assembly.
6- Replication: Cytoplasmic factories are responsible for replication.
7- Outstanding traits: Large and complex viruses with high resistance to inactivation are outstanding traits. Virus-encoded proteins aid in evading the host's immune system. Smallpox was the world's first viral illness to be eliminated (Riedel *et al.,* 2019).

2.1.2.Morphology

Poxvirus virions are enormus, barrel-shaped particles with a diameter of 400 - 230 nm. The outer layer is 5-6 nm thick, which, together with the density, is compatible with a single lipid membrane bilayer . The outer membrane features irregular protrusions ranging from 3 to 5 nm in length. The virion's inner structure is made up of a dumbbell-shaped core and lateral bodies, which are heterogeneous material aggregates. The inner layer is continuous except for a few lipid channels, and the core wall is two layered with an overall thickness of 18-19 nm (Longnecker *et al.* ,2013), figure (2.1).



(Figure 2.1)Poxvirus virions are There are two types of infectious viral particles: intracellular mature virus (IMV) and extracellular enveloped virus (EVV)(http:/ /www. poxvirus. org., 2021) (Hu, 2010)

2.1.3.Classiffication

The family's name, Poxviridae, comes from the initial grouping of viruses linked to illnesses that caused skin poxes. The modern categorization of viruses is based on phenotypic features such as morphology, nucleic acid type, mechanism of replication, host species, and the illness they cause. The smallpox virus continues to be the most well-known member of the family (Afonso *et al.*, 2012).

Based on their vertebrate and insect host ranges, Poxviridae are classified into two subfamilies: Chordopoxvirinae and Entomopoxvirinae . Poxviruses are distantly linked to Asfarviridae, Ascoviridae, Iridoviridae, Phycodnaviridae, Mimiviridae, and Marseilllevirus, according to DNA sequencing and bioinformatics study, There are nine genera in the Chordopoxvirinae subfamily. Avipoxvirus (1species), Capripoxvirus (3 species), Cervidopoxvirus (1 species), Leporipoxvirus (4 species),

Molluscipoxvirus (1 species) (table 2.1), Orthopoxvirus (9 species), Parapoxvirus (4 species), Suipoxvirus (1 species), and Yatapoxvirus (2 species).whereas the Entomopoxvirinae has three recognized genera: 1.Alphaentomopoxvirus , *2*- Betaentomopoxvirus *3* - Gammaentomopoxvirus (Burell *et al.*, 2017).

Table 2.1: Classification of human MCV

Virus classification	
Group :	**Group I (dsDNA)**
Family :	***Poxviridae***
Subfamily :	***Chordopoxvirinae***
Genus :	***Molluscipoxvirus***
Species :	***Molluscum contagiosum virus***

There are a number of unidentified species in both subfamilies for which new genera may be formed in the future. *Cotia virus* is a strange virus that might represent the start of a new genus (Afonso *et al.,* 2012). MCV was the sole member of the Molluscipox genus that has been discovered. It was the only poxvirus capable of causing long-term infection in humans, and it was the only poxvirus with a host range restricted to humans since smallpox was eradicated (Shisler, 2015).

2.2. Molluscum contagiosum virus:

Molluscum contagiosum virus (MCV), the type member of the genus Molluscipoxvirus, is the only 18 surviving poxvirus with a substantial prevalence in the human population after the elimination of variola virus. MCV produces benign and self-limited cutaneous tumors, particularly in youngsters and immune compromised people. According to a meta–analysis ,

the prevalence of MC in children aged 0 to 16 years is between 5.1 and 11.5 % (Olsen *et al.*,2013). The Molluscum Contagiosum virus (MCV), a double-strand DNA virus belonging to the Poxviridae family, causes MC. (Leung *et al.*, 2017) .

The Molluscum contagiosum virus (MCV) is the only poxvirus genus that causes disease in humans. The arrangement of the virions was complex and close to that of the poxvirus family: an envelope, surface membrane, core, and lateral bodies (Büchen- osmod *et al.*, 2003). The virus hasn't been passed on to animals or developed in tissue culture. Electron microscopy was used to look at it in a human lesion (Riedel *et al.*, 2019). The Molluscum contagiosum virus replicates in epithelial cells' cytoplasm, causing cytoplasmic inclusions and cell expansion. Only the epidermis is infected by this virus. Infection occurs after contact with infected people or contaminated things, but the degree of epidermal damage required is unclear (Coutu *et al.*, 2017).

2.2.1. MCV variation and Genotyping:

There are four genotypes of MCV: MCV 1, MCV 2, MCV 3, and MCV 4. MCV 1 is the most prevalent genotype (75–96%), followed by MCV 2 and MCV 3, with MCV 3 and 4 being exceedingly rare. (Leung *et al.*, 2017; Trčko *et al.*, 2018).

Genomic DNA nucleotide composition was extremely variable. This heterogeneity did not only affect non-coding DNA or codon usage; it also affected the amino acid composition of proteins, with a Molluscipoxvirus having 35% A+T. MCV-1 had variation in its genome and showed a common difference of genome, this MCV-1 prototype (MCV -1p), reported to be most prevalent in Europe. The common markers of the variants of MCV-1were 24

kb fusion fragments generated by the loss of a Bam.HI site. MCV has been observed in other species including chickens, sparrows, pigeons, chimpanzees, kangaroos, dogs and horses, was thought to be identical or closely related to human MCV. The common of the variant's of MCV-1 were 24kbp fusion fragments generated by the loss of a Bam.HI site between D2 and F fragments of MCV -1P. These varaits of MCV-P were classificated into three groups (MCV-1va, MCV-1vb, MCV-1vc). MCV-1 and 1v are fully homologous, but MCV 1/1v and MCV-2 have small nucleotide differences (Jason *et al.*, 2007).

A disease that is passed on from one species to the next. There was still very little evidence of horse-to-human transmission. MCV-4's restriction map was created and compared to that of the other groups (Thiemann *et al.*, 2012). Both forms of MCV have similar clinical appearance and did not localize to a single area of the body, which indicates that types and their variants develop lesions that are similar in number, duration, and there was no association between type and the sex of the patients (Scholz *et al.*,1989; Trama *et al.*, 2007).

MCV-1 and MCV-2 can be identified using restriction endonuclease and PCR analysis of MCV DNA. Among MCV-1 and MCV-2, there was a lot of genetic organization that was conserved. However, MCV-1 had a 12 kbp zone that was special to it, and MCV-2 had a 2-3 kbp deletion. MCV-1 had a G+C content of 67 % in 200 bp, while MCV-2 had a G+C content of 69 % in 173 bp.The sequences downstream of the MCV genes were also compared, and there was 75 % homology between the MCV-1 and MCV-2 sequences within the first 70bp downstream of the termination codon (Jason *et al.*, 2007).

MCV-1 was the most prevalent cause of illness in children aged one to ten years, accounting for 76% to 97% of all infections (Hanson *et al* .,2003; Dixit *et al* 2009). Molluscum contagiosum was most frequently doesn't transmitted by sexually route in both the genital and non-genital regions, and the disease was unusual in children under the age of one year, likely due to maternal immune transmission and the long incubation time of 2 to 7 weeks, with a range of 6 months. The genome of MCV type 1 was discovered to be 185.5 kb (Nanhai *et al.*, 2000; Silvey, 2000).

It is characterized by benign self-limited eruption of single or multiple cutaneous spherical and pearly papules in adults who are immune compromised (AIDS) or immunosuppressed. Transmission is typically through direct interaction with infected hosts or through the use of a tool such as a tattoo tool, but it may also be sexually transmitted. In HIV-infected molluscum, MCV-2 is responsible for the bulk of infections (60 %). MCV type 2 genome is roughly (195)Kb in HIV infection-associated molluscum (Odom *et al.*, 2000; Molina *et al.*, 2011).

2.2.2.Genomic Organization of MCV:

The genomes of poxviruses are enormous, ranging from 130 to 380 kbp (Longnecker *et al.*, 2013). The Molluscum contagiosum virus has four subtypes: MCV-1 (which accounts for 98 % of infections) is primarily seen in youngsters, whereas MCV-2 is predominantly responsible for skin lesions, In Asia and Australia, MCV-3 and MCV-4 are also found (Peterson *et al.*, 2019). The most conserved genes between MCV and VACV are found in the middle of the genome and encode proteins that are required for structure, replication, and transcription. MCV genes are found at the ends of its DNA genome, and

these genes are thought to code for proteins that function as host-range and immunomodulatory factors (Senkevich *et al* .,1996; Senkevich *et al* ., 1997). As a result, the MCV genome is organized similarly to those of other poxviruses (Moss B *et al.,* 2000) .

The only poxviruses known to infect people are Molluscum contagiosum virus (MCV) andvariola virus (Shchelkunov *et al.,* 2003). Despite the fact that MCV and Orf belong to distinct genera, the Orf virus's genome has numerous remarkable similarities to MCV. The genome has a high C- G content, three potential immune evasion orf virus, and no viral genes involved in nucleotide metabolism, among other commonalities. The authors conclude that Orf and MCV are different from other proteins based on their findings (Delhon *et al.,*2004).

MCV's 190-kilobase pair genome has now been sequenced, revealing that the virus may encode 163 proteins, 103 of which are similar to smallpox virus homologs. MCV lacks smallpox viral equivalents for 83 genes, including those involved in suppressing host immune responses, nucleotide biosynthesis, and cell proliferation. MCV has 59 genes expected to encode hitherto uncharacterized proteins, including homologs of the major histocompatibility complex class I, chemokine, and glutathione peroxidase, implying that MCV has its own coexistence tactics with its human host (Senkevich *et al.,*1996).

Variola virus is eliminated by immunizing people with live vaccinia virus. MCV is the only virus in the molluscipoxvirus genus. MCV contains a big double-strand DNA genome with a high G C content (63%) compared to just 34% in VARV and VACV (Senkevich *et al.*,1996), as well as four

different genotypes (Dohil *et al.,* 2006). The genome of MCV type I (figure 2.2) has been sequenced and is 190289 bp long, with the accession number U60315 in GenBank (Senkevich *et al.*,1997).

MCV is expected to have 182 open reading frames (ORFs) that might encode for functional proteins. When comparing the MCV genome to that of the orthopoxviruses (OPV), 105 genes are homologous to OPV proteins, with 55 of these genes being more extensively conserved poxvirus genes.The conserved gene set is involved in viral genome replication or expression, or is a structural component of the virion. All of the VACV genes required for viral development in cell culture are retained in MCV, implying that the mechanisms involved in productive MCV replication are comparable to those involved in OPV replication. The MCV genes known to regulate the host immunological response, such as MC066L, MC080R, MC148R, and MC033L, do not have homologues in the OPV (Senkevich *et al.*,1996; Senkevich *et al.*,1997).

MCV proteins had cellular homologs, and conserved functional motifs shared with cellular proteins were found in a number of other proteins, including the poly(A) polymerase catalytic subunit and putative Adenine triphosphatase (ATPases). MCV, which encodes the antioxidant protein MC066L, is one of these proteins (Molluscum contaiosum 066 Liftward). Since it contains the protein MC080R (Molluscum contagiosum 080 rightward) and MCV encodes two chemokine-like proteins, MC148R1 (Molluscum contagiosum 148Rightward) and MC148R2 (Molluscum contagiosum 148Rightward) (Hans *et al.*,2000; Qingwen *et al.*, 2011). Different studies have been developed to sequence the genome of this virus and determine possible genes involved in the evasion of the host immune response, a hypothesis that arose based on the absence of inflammation

observed in histopathological samples of infected skin. (Brady *et al.*, 2017; Zorec *et al.*, 2018).

MC159, MC160, MC132, and MC005 are the four viral genes that code for proteins that affect the activation of the nuclear factor kB (NF-kB.) (Brady *et al.*, 2017; Brady *et al.*, 2015). NF-kB is a nuclear protein complex found in dendritic cells that regulates DNA transcription and promotes the production of pro-inflammatory cytokines (TNF, IL-1, and IL-6, among others) as well as the activation of the innate and acquired immune systems, (Gurtler *et al.* , 2013) .

Have discovered that the proteins MC132 and MC005 affect NF-kB activity by blocking pattern recognition receptors (PRRs). MC132 would bind to the p65 subunit of NF-kB and induce its degradation, whereas MC005 would block the activation of the IKK complex (IkB kinase) attaching to active NEMO subunit (essential modulator of NF-kB), (Brady *et al.*, 2017; Brady *et al.*, 2015).

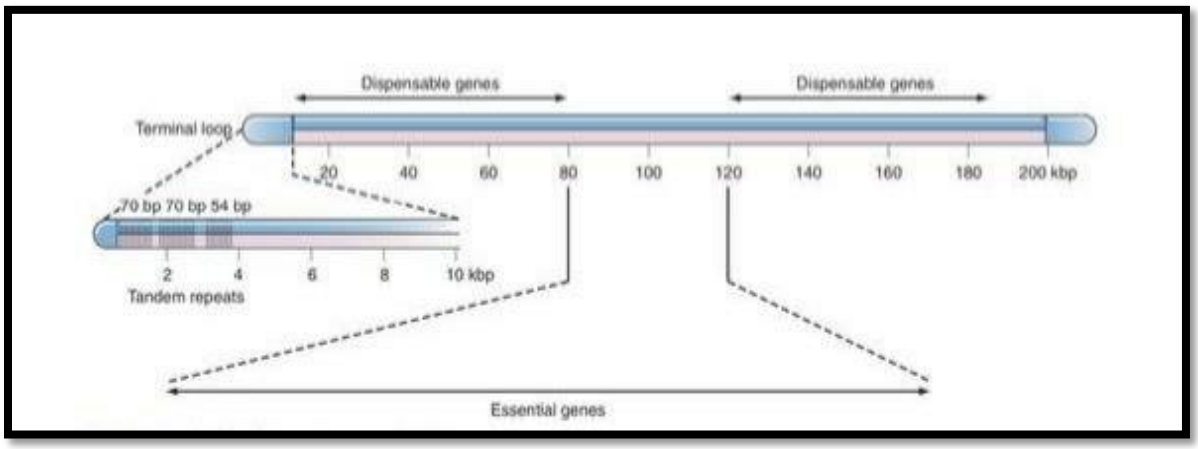


Figure 2.2: The structure of the MCV genome (Cann, 2016)

MC021L: MCV's MC021L gene, The single nucleotide polymorphism A27451G has a nucleotide sequence that distinguishes MCV1 from MCV2(Trama *at al.,* 2007). Molluscum contagiosum virus contenta homolog of F13L termed MC021L gene (Monticilli *at al*., 2020). Vaccinia

Virus MC021L, a Molluscum contagiosum virus Homolog of vaccinia virus F13L (Bryk and Ward, 2018)MC021, the homolog of VACV F13, is encoded by the MCV gene MC021L, and the proteins have a 40 % amino acid sequence similarity. The proteins F13 and B5 are required for the formation of wrap virues (WV) in VACV infection, and deletions of their encoding genes, F13L and B5R, result in the loss of wrapped forms of the virus and a significant reduction in cell-to-cell transmission (Schmidt *et al* ., 2011).

MC133L:MC133Lwas a special MCV type I (70 kD protein: MC133) (Watanabe,1998). The A-typeinclusion (ATI) proteins MC130,MC131, and MC133 belong to a multigene family (Senkevich *et al.*,1997). These proteins are most likely glycosylated and can be found on the surface of the cell. MCV virions' surfaces, where antibodies can attach and immunological electron microscopy may identify them (Sherwani, 2013).

To enter cells , Molluscum contagiosum virus employs homologs of a cell fusion complex seen in vaccinia virus; these are the MCV genes MC084 and MC133, which I have primarily dealt with. Because these proteins are found on the surface of the virion particle and play a role in entrance, most human antibodies against MCV are produced against MC084 and MC133(Sherwani, 2013). IMVs have the genes MC084 and MC133 linked with their surface. The trans-Golgi and endosomal cistrnae wrap these MVs, resulting in wrapped virions that are delivered to the cell's periphery through microtubules. Extracellular encapsulated virions are released after fusion with the plasma membrane (EV) (Knipe and Hawley, 2013).

MC002L : MC002L Genome Location bp Begins with 4849 ends 6201 Protein Volume 451 human homolog singling molecule (Gene Bank HSU33017 0-1) MC162L, MC161L (Senkevich *et al.*,1996).

MC002L was the 167-bp primer gene; this gene, which began with the nucleotide 4846 and ended with 6201, is homologous to the MC161R and MC162R genes, which were discovered by Senkevich and colleagues in the 1996 genome sequencing project and assigned to a gene family (Senkevich *et al.*,1996).

Because they share positional amino acid similarity between the N terminal regions of the predicted proteins and are produced at an early stage during infection, the MCV ORF MC002L, MC161R, and MC162L were clustered into a family of MCV genes.All members of this family have predicted signal peptides, a carboxyterminal transmembrane domain, and a marginal amino acid homology to hum`an SLAM 14 proteins, a cellular membrane protein with a truncated form lacking the transmembrane domain that can act as a selfligand and induce T lymphocyte activation (Bugert and Darai, 2000). As a result, this viral gene family may have played a dual role in the virus life cycle as well as virus-directed immune evasion (Sherwani, 2013). MC002L was an early transcription gen for MCV (Bugert ,1999)

The proteins are produced as type I membrane proteins early in life, and MC002 and MC162 have PY motifs in their cytoplasmic domains, allowing them to interact with the cellular ubiquitin ligases AIP4 and NEDD4 as well as the Hrs endosomal switching protein. In human and mouse fibroblasts, over expression of MC162 induces vesicle abnormalities. MCV-induced over-recycling of surface receptors MC162, including EGFR, as shown in situ, competitive binding of cellular SLAM through an extracellular SLAM homology domain MC002, and T lymphocyte activation are among the hypotheses now being explored (Bugert, 2008). As a result, this viral gene

family might play a dual function in the virus life cycle as well as virus-directed immune evasion (Sherwani, 2013).

2.3.Envelope Antigen p43k protein :

The MCV segments sequenced have a nucleotide composition of 66 % G or C. The codon use inside the p43K gene reflected this high G + C content, with G or C being prevalent at position 3 of codons (82 and 87 percent for MCVI and MCVII, respectively). Immediately upstream of the MCV p43K-encoding gene are motifs that are comparable to those necessary for vaccinia virus late gene expression. The position and direction of transcription of the MCV p43K-encoding gene were identical to those of the vaccinia virus p37K gene, indicating that MCV and vaccinia virus had comparable genetic structureIn both MCVI and MCVII, another incomplete ORF was discovered downstream of the p43K-encoding gene. The region immediately upstream of this ORF overlapped the p43K-encoding gene's termination codon and featured a pattern that was homologous to the vaccinia virus early gene promoter's derived consensus sequence (Blake *et al* ., 1991).

The 5' region of the MCV gene for p43K is very G+C rich (72%) overall, but within this region, the A+T-rich sequence TAAAATG encompassed the initiation codon. At position -13 bp from theP initiation codon, there was a string of four thymidine residues. The presence of these two motifs (TAAAATG and TTTT) was more obvious in the G+C-rich MCV genome than in the A+T-rich vaccinia virus genome up stream of the gene for p43K differs from the TAAAT motif associated with vaccinia virus late genes by the presence of an additional adenine residue (Neil *et al.*,1991).

2.4. Previous Molluscum Contagiosum Virus studies:

2.4.1.Prior MCV studies in the field:

In 2020 study conducted in Diyala by Mohammad the prevalence of MCV in children aged to 1 to 10 years was 53.3%. MCV was detected using PCR, and the study's model was a lesion (Mohammed, 2020). According to a Gaeta survey conducted in Basra in 2019, the prevalence of MCV in children aged 1 to 11 years was 77.4 %. MCV was detected using PCR, and the study's model was a lesion (Gaeta *et al.*,2019). In 2013 a study conducted in Najaf Province showed that the prevalence of MCV was (27.9%)among patient with (0-5). MCV was detected using histopathology (Shubbar *et al*., 2019).

In 2013 study conducted in Diyala by Al-Azawi, the prevalence of MCV was found to be 45.1 % in people aged 30-41 years. MCV was detected using PCR, and the study's model was a lesion (Al-Azawi, 2013).in 2013 study conducted in Diyala province , the prevalence of MCV was found to be 40(78.4%) of lesions located on the head and neck, MCV was detected using PCR, and the study's model was a lesion (Ahmed, 2013).

2.4.2.Worldwide studies:

In 2018 study conducted in Iran of 1470 samples, 114 (7.75%) samples were positive for the MCV. From MCV-positive samples, (71.05%) sequences were found to be related to the MCV1 and (28.95%)to the MCV2(Taghinezhad*et al.,* 2018). According to studies conducted in Slovenia there were 188 patients in the research, with 121 (64%) being men and 67 (36%) being women. Adults made up a total of 135 (72%) of the cases of MCV , MCV 1 infection is more common in children than in adults, and MCV 2 infection is more common in adult women than MCV (Trčko *et al.*, 2018).

The bulk of Molluscum contagiosum infections occurred in children aged 1 to 15 years (41.5%), which is close to the findings of a review of MCV infection in American Indians and Alaskan aboriginal peoples, which showed that most MCV infections occur in children aged 1 to 15 years (Reynolds *et al.,* 2009; Shisler, 2015).

Sherwani *et al.,* (2013) showed that the infection with MCV among male and female was 31(58%) and 31(42%) respectively (Sherwani *et al.*,2013). According to studies conducted in Israel, Romania, New Guinea, Mali, Japan, and Turkey, the prevalence of MC in children ranged from 0.27 % in 6-12 year olds in Romania (Popescu *et al.*,1999) to 34 %in (2-9) year olds in Israel (Oren *et al.*,1991). to 21.8% in (0-10) years olds in New Guinea **(**Sturt *et al.*,1971). to 6.9% in (4-11) years olds in Japan (Niizeka *et al.*,1984), to 3.6% in (0-12) years olds in Mali(Mahe *et al.*,1995), to 0.3% in (6-12) years olds in Romania (Popescu *et al.*,1999), to 1.2% in (14 -16) in Turky (Tuncel *et al.*, 2005), to 2.1% in (1-5) years olds in Iran (Tabari and Shakerian, 2007).

Japan was the population sample that was most close to Western Europe and North America in terms of economic growth, with prevalence rates of MCV infection was 6.9% (Niizeki *et al.*,1984) and 19.7% (Niizeki *et al.*,1984). (Hayashida *et al.,* 2010).

2.5. MCV replication

In order for a virus to cause infection, it must first find a suitable host with sensitive cells. The skin can operate as a gateway of entrance, however this may need a breach of the integument's barrier function or direct inoculation (Rook, 2012). After binding to glycosaminoglycans (GAGs) on the surface of the target cell, the fusion event between the virion and the host cell membranes is still poorly understood, but at least one conserved virion

protein has been linked to this fusion/entry event that eventually releases the virion core structure into the cytoplasm (Senkevich, 2004). The virion was then uncoated, which displays the DNA to commence replication (Burrell *et al.,* 2016).

1- Viral proteins bind to glycosaminoglycans (GAGs) on the host cell membrane, and virions are endocytosed into the host cell. Fusion with plasma membranes, on the other hand, can release cores into the host cytoplasm.

2- Early phase: Viral DNA depending RNA polymerase transcribes early genes in the cytoplasm. At 30 minutes after infection, early expression occurs.

3- As early expression stops, the core is entirely uncoated, and the viral DNA is now free in the cytoplasm.

4- Intermediate phase: At around 100 minutes after infection, intermediate genes are expressed, starting genomic DNA replication.

5- Late phase: From 140 minutes to 48 hours after infection, late genes are expressed, generating all structural proteins.

6- Progeny virions are assembled in cytoplasmic viral factories, resulting in a spherical immature particle (IV). This viral particle develops into an intracellular mature virion with a brick-like structure.

7- Intracellular MVs can be discharged following cell lysis, or they can acquire a second double membrane from the trans-Golgi and blossom as enclosed exterior virions (EV)(Burral *et al.*,2017), figure (2.3).

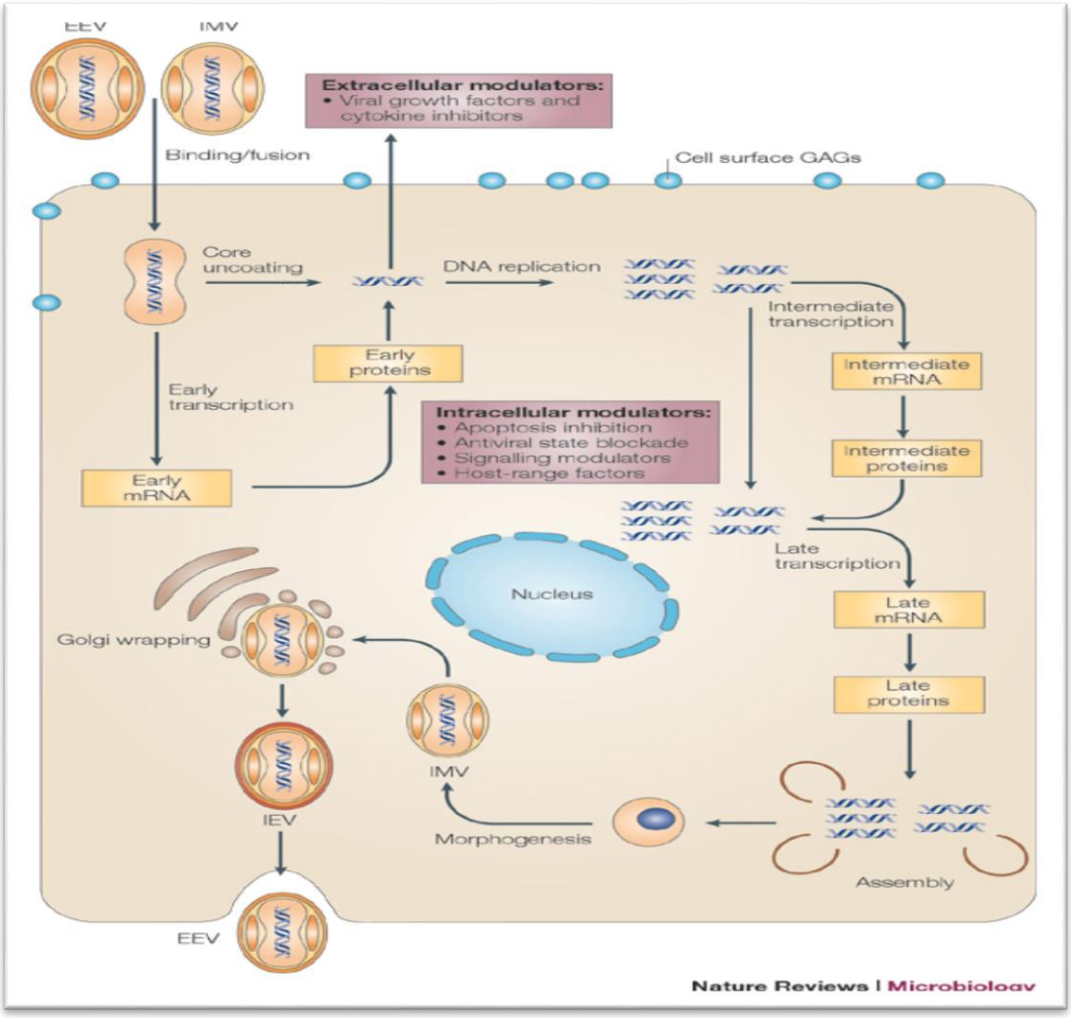


Figure (2.3) The replication cycle of the poxvirus (Grant, 2005).

2.6. Infection of MCV:

Molluscum contagiosum is a mollusk that does not induce systemic illness and stays localized at the injection site. Molluscum contagiosum lesions have an unique pathophysiology that has been recognized for a long time. Henderson and Patterson described the distinctive Molluscum bodies, sometimes known as Henderson-Patterson bodies, in 1841.Infection begins when the virus replicates in the bottom layers of the epidermis and spreads upward. The asymptomatic incubation period varies, although it might last a

long time.The epidermis hypertrophies and spreads down into the underlying dermal layers as the illness develops.In the prickle cell layer, characteristic inclusions (Molluscum bodies) develop, which increasingly increase as the cells mature and move to the surface. The basal cell layer hyperplasia replaces these cells, and the stratum basale has few or no viral components(Riedel *et al.,* 2019).

The papillae are compacted by cellular proliferation, which generates lobulated epidermal growths that form a fibrous barrier between the lobules, which were pear-shaped with the pinnacle upward. The base layer is unaffected. The basal layer stays intact,There are a lot of developing virions in these molluscum bodies. This was found within a collagen-rich lipids sac-like structure that was thought to be used to assess the host's immunological acceptance. At the center of the lesion, the contagious virus-packed cells break and empty. The Molluscum contagiosum virus induces hyperplasia and hypertrophy of the epidermis, resulting in a benign tumor, rather from the typical necrotic pox lesion. Free viral cores were found in all layers of the epidermis. So-called viral factories have been identified in the malpighian and granular cell layers (Tony *et al.,* 2010).

Large hyaline bodies (ie, Molluscum bodies, Henderson-Paterson bodies) containing cytoplasmic masses of virus material form at the core of the lesion and are eventually destroyed, resulting in large hyaline bodies (ie, Molluscum bodies, Henderson-Paterson bodies) containing cytoplasmic masses of virus material. These entities show as a white depression in the middle of fully formed lesions and are seen in vast numbers. The lesions can occasionally proceed beyond local cellular proliferation and become inflammatory, resulting in edema, increased vascularity, and neutrophil, lymphocyte, and

monocyte infiltration. Apoptosis induced by mitochondria is inhibited by viral-derived proteins, (Coutu *et al.*, 2017).

Lesions caused by the Molluscum contagiosum virus, parapoxviruses, and yatapoxvirus, on the other hand, are prolific. Leukocyte-associated viremia is a stage of generalized poxvirus infection that results in virus localisation in the skin and to a lesser extent in internal organs. The immunity to such illnesses lasts a long time (Burrell *et al.*, 2017).

2.7. Diagnosis

2.7.1.Clinical

Clinical examination is used to diagnose Molluscum contagiosum. Lesions are pearly papules that are solid, white to flesh-colored, dome-shaped, and have a central umbilication from which a cheesy substance can be released. Mollusca have a diameter of one millimeter to one centimeter (Rayala and Morrell, 2017; Lacarrubba *et al.*,2015). Lesions can appear everywhere on the body, including the palmar and plantar skin.Because of self-inoculation by microtrauma or scratching, the distribution is highly varied, and they are frequently clustered in clusters or dispersed linearly.The majority of patients are youngsters between the ages of 3 and 10, and they are usually limited to the vaginal region in adults, however they do not necessarily arise from sexual transmission.A history of atopic dermatitis or moderate lesions are common in younger individuals, and lesions preferentially form on eczematous skin, leading to a diagnosis of eczema Molluscatum. Single Molluscum contagiosum lesions in the inguinal folds or perianal area might be misinterpreted as fibroma molle. Molluscum contagiosum is linked to immunodeficiency disorders.In clinically challenging

situations, such as those with inflammation or perilesional inflammation and tiny lesions, dermoscopy and in vivo confocal microscopy may be highly effective in aiding diagnosis and excluding the differential diagnosis of other forms of skin lesion. Dermoscopy is more sensitive than eye inspection in detecting orifices, arteries, and particular vascular patterns (crown, radial, floral, and punctiform) (Ianhez *et al.,* 2011).

Confocal microscopy in *vivo* reveals a circular, well-circum scribed lesion with center round cystic regions filled with brilliantly refractile material, which corresponds to the histopathologically identified Molluscum bodies. (Scope *et al.*,2008). It can grow to be larger than 1 centimeter in diameter (giant Molluscum contagiosum) at times, (Vardhan *et al.,* 2010). Reflectance confocal microscopy is another new diagnostic modality that may help in the diagnosis of MC (RCM). They show as circular, well-circumscribed lesions with brilliant refractile material in center round-to-cystic regions under RCM (Lacarrubba *et al.,*2017). The lesions may often develop in odd ways and have unusual appearances, such as hypertrophic and verrucous lesions (Vora *et al.,* 2015). The lesions are usually fast-progressing, disseminated, resistant to therapy, and recurrent (Ajithkumar *et al.*,2017). The nipples are an example of a common spot (Ives *et al.,* 2017), areolae (Hoyt *et al.,* 2013), conjunctiva (Falzon *et al.,* 2015), eyelids (Nair *et al.,* 2016), oral mucosa (Fernando *et al.,* 2015), lips (Ma *et al.,* 2015), scalp (Kim *et al.,*2013) and soles (Bahalı *et al.,* 2016).

2.7.2.Laboratory

The PCR concepts, which were first identified in 1986 by Kary Mullis, revolutionized the field of molecular biology. Dr. Mullis, the inventor of PCR, was awarded the Nobel Prize in Chemistry in 1993(Domingues, 2017) .

Has completely transformed viral diagnostics. Because it simply requires one instrument (a thermocycler) and a small number of chemicals, PCR is a very useful technique. PCR also enables for the diagnosis of viral infection at very low titers (eg,1.6 particle per mL). PCR, for example, can be used to providea clinical diagnosis of HIV infection in its early stages, when neither viralantigens nor antibodies are detected. DNA melting and amplification by enzymes. The DNA serves as a template for amplification as the PCR process continues causing a chain reaction in which the DNA gets exponentially multiplied Primers with complementary sequences to the target area allow for precise and repeatable amplification.Another important component for repeated amplification is a heat-stable *Taq* polymerase. One drawback is that, because PCR requires amplification, the result is only semi quantitative (Ryu , 2017).

Strip containing the sequence that needs to be amplified and containing tens of thousands of nucleotides, as well as a thermally stable Polymerase *Taq* DNA and a pair of primers, as well as a buffer solution that constitutes a medium in which the process will take place, the reaction in which the four nitrogenous bases are present (Aljawasim, 2014).

2.8. DNA Sequencing

The technique of identifying the nucleic acid sequence — the order of nucleotides in DNA – is known as DNA sequencing. Any method or technology for determining the order of the four bases: adenine, guanine, cytosine, and thymine is included. Rapid DNA sequencing technologies have significantly advanced biological and medical research and discoveries (Behjati, 2013).

DNA sequence knowledge is currently required for fundamental biological research as well as a variety of applied disciplines such as medical diagnostics, biotechnology, forensic biology, virology, and biological systematics. Different illnesses, including malignancies, can be diagnosed by comparing healthy and mutant DNA sequences(Chmieleckiand Meyerson, 2014). Identify the antibody repertoire (Abate *et al.*, 2013), and can be utilized to help patients get the care they need.(Pekin *et al.*, 2011). Having a rapid technique to sequence DNA enables for more species to be recognized and cataloged, as well as speedier and more customized medical care. (Abate *et al.*, 2013).

Modern DNA sequencing technology has aided in the sequencing of full DNA sequences, or genomes, of many types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species,Academic researchers used arduous two-dimensional chromatography procedures to get the first DNA sequences in the early 1970s. Fluorescence-based sequencing techniques with a DNA sequencer were developed after the development of fluorescence-based sequencing methods with a DNA sequencer, (Olsvik *et al.*,1993).

Because most viruses are too tiny to be seen with a light microscope, one of the most important tools in virology is sequencing (Castro *et al.*, 2019),Viral genomes can be DNA or RNA-based. Because RNA viruses decay quicker in clinical samples, they are more time-sensitive for genome sequencing (Wohl *et al* ., 2016), In scientific and clinical research, as well as for the detection of new viral illnesses, viral epidemiology, and drug resistance testing, traditional Sanger sequencing and next-generation sequencing are used to sequence viruses. In the GenBank database, there are

about 2.3 million distinct viral sequences NGS has recently eclipsed conventional Sanger as the most widely used method for producing viral genomes(Castro *et al.,* 2020**).**

2.9. Epidemiology

MC lesions are spread via direct contact or indirect contact through towels, underwear, toys, razors, tattoo and other fomites. skin-to-skin contact MCV can also spread to normal skin by autoinoculation when patients scrape the molluscum. Transmission may occur in shared swimming pools and other moist situations. In utero and prepartum transmissions have been recorded, resulting in congenital Molluscum contagiosumor skin lesions in the first few months of life (Rosner *et al* ., 2018 ; Peterson *et al.*, 2019).

A common medical issue is Molluscum contagiosum. There were around 122 million cases in 2010. It can be found all throughout the world, although it appears to be more common in hot, humid climates.Molluscum contagiosum is most commonly found in children aged two to five years, although it can also be found in sexually active adolescents and adults, as well as immunocompromised people. Because it affects the skin barrier and immunity, atopic dermatitis may raise the chance of developing Molluscumcontagiosum.The clinical prevalence of Molluscum contagiosumin persons living with HIV (human immunodeficiency virus) can reach up to 18 %. There is no evidence of a gender imbalance, (Leung *et al.*,2017).

In the biggest clinical research of MC in Central Europe to date, MC was shown to be more frequent in adult men than adult women, but in children, the age distribution was reversed. The anogenital area was the most prevalent site of MC lesions in adults in our study, whereas the torso and

extremities were the most common sites in children. MCV infection was not shown to be more common in people with atopic dermatitis.The cure rate after curettage therapy was reasonably high (63%) and recurrences were not related to the number of lesions or the presence of atopic dermatitis (Trčko *et al.*, 2018).

The Molluscum contagiosum virus is found all throughout the world, and its prevalence in most places is unknown. In tropical places, it is more common. Molluscum contagiosum is one of the most common dermatoses in Mali, with a prevalence of 3.6 % (Mahe *et al* .,1995). In Australia, the total seropositivity rate is reported to be 23% (Konya *et al.*,1999).possibly due to maternal immunity and a long incubation period; otherwise, the prevalence appears to follow exposure to others. Children under the age of five and young adults have the highest rates.Casual contact is associated with the peak in the childhood age range, whereas sexual contact is associated with the peak in young adults (Laxmisha *et al.*,2003; Dohil *et al* .,2006). According to a Slovenian study, MCV 1 infection is more common in children than in adults, and MCV 2 infection is more common in adult women than MCV (Trčko *et al.*,2018).

2.10. Prevention and control of MCV:

To deter the MCV transmission, avoid close contact with skin lesions and avoid sex action. To reduce disease transmission in children, parents should be advised to refrain from taking their children to public bathing or swimming pools, particularly during documented outbreaks of attendees. Kids with Molluscum contagiosum should also be bathed separately from their siblings and not sharing towels or bath sponges. In fact, any towel used by an infected child should be washed right away to protect the child from being

infected or spreading the disease to other family members (Cohen *et al* ., 2004).

It is also important to Keeping your hands clean will help keep the infection from spreading, as will protecting your bruises with a bandage if there's a chance that another human will come into contact with infected tissue. It's unclear if the Molluscum contagiosum viruswill spread in chlorinated swimming pool water (Pickering *et al.*, 2009).

Topical compounds that induce skin damage, physical interventions to debride the lesion, systemic therapies such as immunomodulatory agents, or waiting for natural resolution were the four primary treatment choices (Gould, 2008). Physical treatments can be uncomfortable and leave you with the possibility of scarring (Ghura *et al.*, 2001).

Mechanical procedures (such as liquid nitrogen cryotherapy and curettage) were largely successful, but they exposed children to a potentially unpleasant and stressful experience (Al-Mutairi *et al.* ,2010).

2.10.Responses of the immune system to MCV:

MCV was a pox virus that triggers tumor-like skin lesions that periodically become inflamed and revert on their own, an occurrence likely triggered by plasmacytoid dendritic cell-mediated immune rejection of the lesion, resulting in type 1 interferon formation. These characteristics describe the inflammatory response in lesions that will undoubtedly spontaneously resolve, but it ultimately entails the invasion of epidermal tissue to open the Molluscum bodies to the dermis' tissue fluids. It has been suggested that the Molluscum bodies induce inflammation by a mechanism similar to that involved in ruptured epidermal cysts or in acne (Melissa *et al.*, 2011).

Chapter Three

Materials

and

Methods

3. Materials and Methods

3.1. Materials:

3.1.1. Laboratory Equipments and Instruments:

Laboratory equipments and instruments used throughout this study were summarized in table (3.1).

Table (3.1): - Laboratory equipments and Instrument used in the study.

NO	Devices	Origin	Company
12	Eppenddrof tube rack	USA	Biobasic
16	Big pestle	Korea	Bioneer
18	Pipette tips blue, white, yellow	Korea	Bioneer
15	Mineral curette	USA	Blinder
21	Rack tube	Korea	Blinder
1	Centrifuge	USA	Fisher Scientific
17	Micropipettes different size 100,200,50,10,2.5,0.5)	Human	Germany
6	Microwave Oven	China	GOSONIC
5	Micropipette	Germany	Human
22	1.5ml, 0.5ml and 0.2ml Tube	Singapore	JET BIOFIL
14	Graduated cylinders	India	Jlasssco
3	Gel Imaging System	Taiwan	Major Science
20	Plastic disposable syringes 3ml	China	Meheco
4	Micro spin Centrifuge	China	My Fugene,
10	Vortex	England	Quality Lab System,
13	Flask	Germany	Scott
19	Plain tube	Turkey	Superestar
8	Refrigerator	Spain	TEKA,
11	Water bath	China	Termax
9	Thermal Cycler	USA	Thermo Fisher Scientific,
7	OWL Electrophoresis System	USA	Thermo,

3.1.2. Chemicals and solutions:

The following chemicals and solutions used in this study were shown in table (3.2).

Table (3.2): Chemicals and solutions used in the current study.

No.	Chemical Methods	Origin	Company
1	Absolute Ethanol	USA	ROMIL pure chemistry
2	DNA Ladder 100bp/1500	USA	Promega
3	QuantyFlour Dye	USA	Promega
4	PBS pH(7.2)	USA	Reagent World

3.1.3. Viral DNA Extraction Kit:

ReliaPrep™ Blood gDNA Miniprep System(Promega , USA), used for extraction of DNA of MCV from skin lesions pecimens of this Kit listed in table **(3.3)**.

Table(3.3) Component of DNA virus extraction Kit used in this study

No.	component	Origon	Company
1	TAE Buffer (40X)	USA	Promega
2	Proteinase K solution(20mg/ml)	USA	Promega
3	Cell lysis Buffer 200 µl	USA	Promega
4	Binding Buffer 200 µl	USA	Promega
5	Column Wash Solution500µl	USA	Promega

Table (3.4):Molecular Weight Marker

DNA marker	Description	Source
Quick-Load 100bp DNA Ladder	100-1517 base pairs. The ladder consists of 12 double strand DNA fragment with size of 100, 200, 300, 400, **500**, 600, 700, 800, 900, **1000**, 1200 and 1517bp. The 500bp and 1000bp bands have high intensity to serve as reference bands. All other fragments appear with equal intensity on gel.	BioLabs (New England)

Table (3.5) component of the Master Mix

NO	component
1	PCR Buffer
2	*Taq* . polymerase
3	MgCl2
4	dnTPS(A,C,G,T)
5	Ioading dye

3.1.4. Components of the reaction medium

Components of the reaction medium used to multiply the DNA are described in Table (3.6)

Table(3.6) :Components of the reaction medium.

N0	components	Volume (µl)
1	Master Mix	**10**
2	Forward primer	**1**
3	Reverse primer	**1**
4	Nuclease Free Water	**0**
5	DNA	**8**
Total volume		**20**

3.1.5 . Primers:

Macrogen, Company in a lyophilized form. provided the primer as a lyophilized substance. Previous research (Meitte *et al.*,2017; ; Taghinezhad *et al.*,2018;Gatea *et al.*, 2019) used the MC133L ,MC021L and M002L genes Respectively as seen in table (3.7).

Table (3.7): lists the primers

NO	Primer Name	Sequence	Annealing Temp. (˚C)	Product size (bp)	Ref
1	MC002 L-F	5`-CCGATCTTTGCGAGCGTTCTTAA- 3`	60	167	**Meite, 2017**
	MC002 L-R	5`-TCCCATACAGCGAGGACAGCATA- 3`			
2	MC021 L-F	5`-CAAGATTGTAGAGACGCTGC-3`	55	979	**Taghinezhad 2018**
	MC021 L-R	5`-GTAGTGCGTGCCGTCCATGT-3`			
3	MC133 L-F	5`-GGAGGAGTGCCCATCAAGAAT-3`	58	575	**Gatea2019**
	MC133 L-R	5`-GCTTTTCAGTTTTTGTGCGA-3`			

3.1.6. Preparation of materials in the laboratory:

3.1.6.1. Primers Preparation:

These primers as in table (3-7) were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 ^{0}C) to 90µl of nuclease free water to obtain a working primer solution of 10 pmo l/µl.

3.2. Methods and Workflow

3-2-1- Collection of samples

Skin specimens of this study were collected in Baquba Teaching Hospital / Consulting Clinic and some Primary healthcare Centers within Diyala Directory of Health. The collection of samples were extended from October 2020 to April 2021 in the College of Science as well as Diyala Directory of Health. The study included 101 skin specimens taken from lesions. The age of the patients was ranged from 5 month to 60 years. The skin lesionswere diagnosed by dermatologists all patients on a clinical basis. Special questionnaire form was preconstructed for this study (Appendix 1).

It include socio-demographic and clinical information for each patient. Human privacy was respected by obtaining verbal consent from all included patients. The lesions were curetted from each patient and placed in sterile tubcontain 2mls of sterile phosphate buffer saline, pH 7.2 and the samples were stored at -20 °C until DNA extraction.

3.2.2. DNA Extraction:

Genomic DNA was isolated from tissue specimens according to the protocol Relia Prep™ Blood g DNA Mini prep System, Pro mega as the following steps:

1- For each 1.5ml microcentrifuge tube 20µl of Proteinase K Solution (20 mg/ml)were dispensed then small part of tissue was added to protein digestion.
2- For Cells lysis 200µls of Cell Lysis Buffer (CLB) were added to the tube and mixed by vortex for 10 seconds.
3- All mixes were incubated in water bath at 56°C for 30 minutes.
4- While the sample was incubated, a ReliaPrep™ Binding Column was placed into an empty collection tube.
5- After incubation the tube was removed from water bath and 250µls of Binding Buffer (BBA) were added and mixed by vortex for 10 seconds.
6- All the tube contents were transferred to the ReliaPrep™ Binding Column and centrifuged for 3 minutes at 12000rpm.
7- The collection tube containing flow through was removed and discarded.
8- The binding column was placed into a fresh collection tube.
9- For column washing 500µl of Column Wash Solution (CWS) were added to the column and centrifuged for 3 minutes at maximum speed, the flow through was discarded and this step was repeated two time.
10- After washing step, the column was placed in a clean 1.5ml microcentrifuge tube and 50µls of Nuclease-Free Water was added to the column.

11- After 5 minutes, the 1.5ml tube with column centrifuge for 5 minutes at 9000rpm.

12- After centrifugation, the ReliaPrep™ Binding Column was discarded and eluate saved and stored at -20 C until required.

3.2.3. Preparation of agarose:

1- One handerd ml of 1X TAE were poured into a flask.

2- One and half grams (of 1.5%) agarose were added to the buffer.

3- The solution was heated to boiling (using microwave) until all the gel particles were dissolved.

4- One microgram of Ethidium Bromide (10mg/ml) was added to the agarose.

5- The agarose was stirred in order to get mixed and to avoid bubbles.

6- The solution was left to cool down to 50-60°C.

3.2.4. Casting of the horizontal agarose gel

The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was allowed to solidify at room temperature for 30 minutes. The comb was carefully removed, and the gel was placed in the gel electrophoresis tank. The tank was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of thegel.

3.2.5. Primers optemazation

The PCR amplifications were performed with 20µl volume containing 10µls GoTaq Green Master Mix (2X); 1µl for each primers (10pmol); 6µls nuclease free water and 2µl of template DNA.PCR cycling were performed

with PCR Express (Thermal Cycler, thermo Fisher Scientific, USA) with the following temperature program: denatured at 94ºC for 4 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds;annealing at 55, 58 and 60°C for 30 seconds; andextension at 72°C for 30 seconds.A final extension incubation of 7 minutes at 72ºC was included, followed by a 10 minutes incubation at 4ºC to stop the reactions.

3.2.6. Thermal Cycler condition of conventional PCR:

PCRcycling were performed with PCR Express (Thermal Cycler, thermo Fisher Scientific, USA) with the following temperature program, as follows illustrated in table (3.8).

Table(3.8) Thermal Cycler condition of conventional PCR for detection the genes ander study

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	40
Annealing	55 , 58 and 60*	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

*55°Ctemperature for MC133L(575)bp.
* 58°C temperature for MC021L(979)bp.
* 60°C temperature for MC002L (167)bp.

3.2. 7. Agarose Gel Electrophoresis:

Amplified PCR products were detected by 1.5 % agarose gel electrophorses, DNA moves from Cathode to plus Anode poles and visualized by staining with Ethidium bromide. PCR products were loaded into agarose gel wells in the following order: 5 µl from a single product to a single well in a known layout, then 100 bp ladder to one or two wells in each

row. In the electrophoresis chamber, the gel tray was fixed Electrical power was turned on at 100 volt/50mAmp for 90minutes(Sambrook and Rusell, 2006).

3.2.8. Gel documentation:

For PCR product, 5µlwere directly loaded to well. Electrical power was turned on at 100 volt/50mAmp for 60minutes. DNA moves from Cathode to plus Anode. The Ethidium bromide-stained bands in gel were visualized using Gel imaging system(Thermal Cycler, thermo Fisher Scientific, USA).

Sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Biosystem) sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, the virtual positions, and other details of the retrieved PCR fragments were identified8 samples were sequences to Gene MC021L and MC133L.

3-2-9- Results sequences

3.2.9.1 Interpretation of sequencing data:

The sequencing results of the PCR products of the targeted samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA).

The observed variationsin each sequenced sample were numbered in PCR amplicons as well as in its corresponding position within the referring

genome.The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome.Each detected variant within the viralsequences was annotated by SnapGene Viewer ver. 4.0.4 (https://www.snapg ene.com).

3.2.9.1. Translation of nucleic acid variations into amino acid residues:

The amino acid sequences of the targetedMC021Land MC133Lprotein were retrieved online from the protein data bank (http://www.ncbi.nlm.nih.gov). The retrieved GenBank accession number of the MCO21L-encoded palmytilated EEV membrane glycoprotein was AYO89042.1, while the retrieved GenBank accession number of the MC133L-encoded protein was AYO88982.1.

Theobserved nucleic acid variants in the coding portionswere translated into a reading frame corresponds to the referring amino acid residuesin the encoded protein using the Expasy online program (http://web.expasy.org/translate/). Multiple amino acid sequence alignment was conducted between the referring amino acid sequences and their observed mutated counterpart using the "align" script of the BioEdit server.

3.2.9.2. Comprehensive phylogenetic tree construction:

A specific comprehensive tree was constructed in this study according to the neighbor-joining protocol described by(Al-Dabbagh *et al.*, 2019). The observed variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server (Zhang *et al*.2000). Then, a full inclusive tree, including the observed variant, was built by the neighbor-joining method and visualized as a circular (cladogram) tree using

I TOL suit (Letunic and Bork, 2019). The sequences of each classified phylogenetic group in the comprehensive tree were colored appropriately.

3.2.10. Statistical analysis:

Analysis of data was carried out using the available statistical package of SPSS-27 (Statistical Packages for Social Sciences- version 27). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (χ2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05 (Daniel *et al.*,2013).

Chapter Four Results and Discussion

4. Results and Discussion

4-1- Results of PCR to detect MCV by specific primers.

Using the polymerase chain reaction technique and three genes, MC021L,MC133L and MC002L genes deoxyribonucleic acid was amplified in a specimens**.** Where the presence of DNA was observed in the electrophoresis of reaction products on an agarose gel as A total of 101 tissue specimens were subjected for detection of three genes ; MC021L , MC133L and MC002L genes using PCR technique.

Regarding for the MC021L gene, 19 (18.8%) were positive, 51 (50.5%) were negative and 31 (30.7%) showed non-specific result ,as shown in table(4.1)and figure(4.1).

Table (4.1):MC021L Gene detection rates among the study samples

Genes		No.	%
MC021L*(*979)pb	Positive	19	18.8
	Negative	51	50.5
	Non specific	31	30.7
	Total	101	100

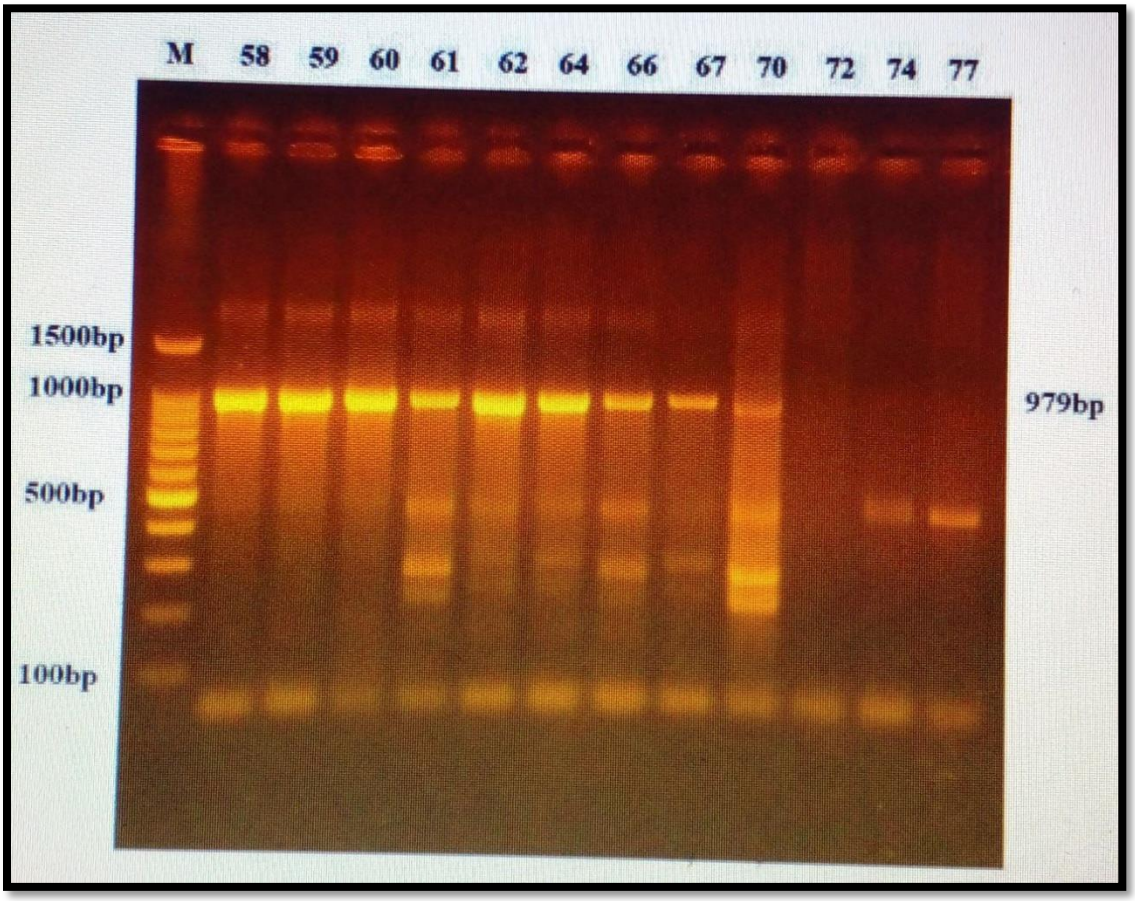


Figure (4.1)Results of the amplification of MC021L primerof Human samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 58-70 resemble 979bp PCR products possitive.

The current study showed a significant difference compared to a study conducted in Iran by Taghinezahad, where it reached (7.7%) 114 out of 1470 samples by Taghinezahad, for same gene 979 bp (Taghinezahad *et al.*, 2018).

This discrepancy might be due to a mutation in this gene area, or it could be a novel strain, or it could be owing to the techniques used by various authors, genotype, or conditions (environment, climate, according to the isolation).

While for the MC133L gene, 2(2.0%) of the samples were positive against 99 (98.0%) were negative ,as shown in table(4.2) and figure(4.2).

Table (4.2): *MC133L*Gene detection rates among the study samples

Genes		No.	%
MC133L	Positive	2	2.0
	Negative	99	98.0
	Total	101	100

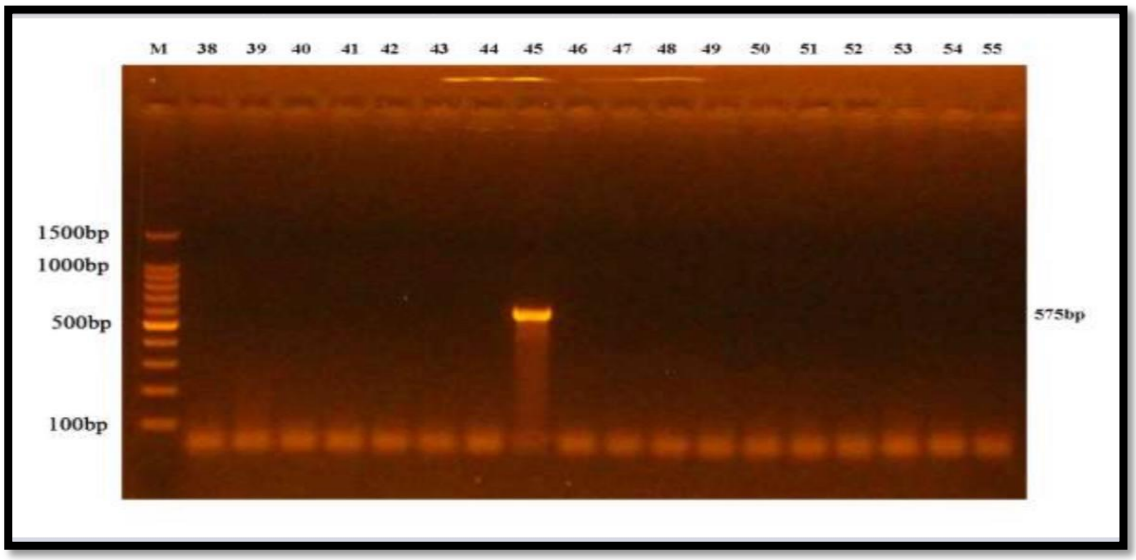


Figure (4.2);Results of the amplification of MC133L primerof Human samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 45 sample resemble 575bp PCR products possitive.

The current study showed disagreement with the study conducted in Iraq, Diyala province, conducted by Al-Azzawi, where 85% of all MCV infections in 2013 showed the same gene size 575bp (Al-Azzawi, 2013). Another work contradicts Saral's study in Turkey, found that all samples carry this gene in 100% of cases same gene size 575bp (Saral *et al.*, 2006).

The reason for the difference in the results of the current study with previous studies may be due to the difference in the strategies of collection, preservation and investigation of infection .

And lastly for the MC002L gene, 85 (84.2%) of the sample were positive versus 16 (15.8%) were negative. As in (table4.6), (figure 4.5).

Table (4.3):MC002L Gene detection rates among the study samples

Genes		No.	%
MC002L	Positive	85	84.2
	Negative	16	15.8
	Total	101	100

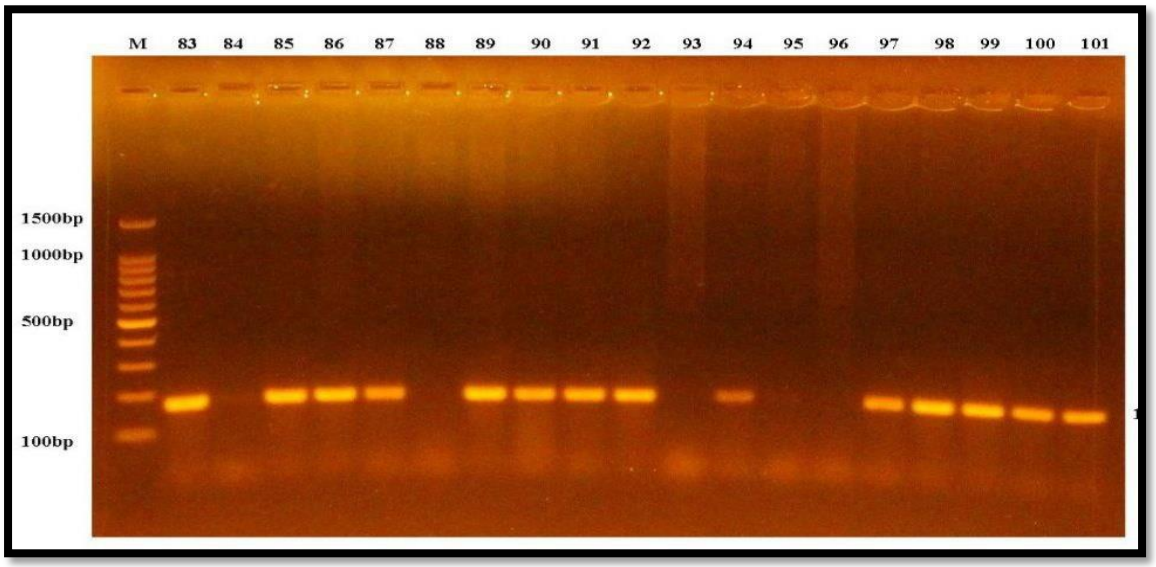


Figure (4.3);Results of the amplification of MC002L primerof Human samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 85 samples resemble 167bp PCR products possitive.

The current study fully agreed with a study conducted in Iraq Wasit province by Meite, which gave 85.71% of the same gene (Meite *et al.*, 2017). Another study conducted in Iraq showed Diyala province by

Mohammad, where the percentage of 100% was given slightly higher than the current study with the same gene(Mohammad, 2020). Perhaps the reason for the low percentage in the current study with the study carried out by Mohammad in Diyala province is due to the difference in the Samples and Number of sample so the different isolates which differ in genomic characteristics.

4.2. Detection of nucleotides sequence of the MC021L and MC133L genes.

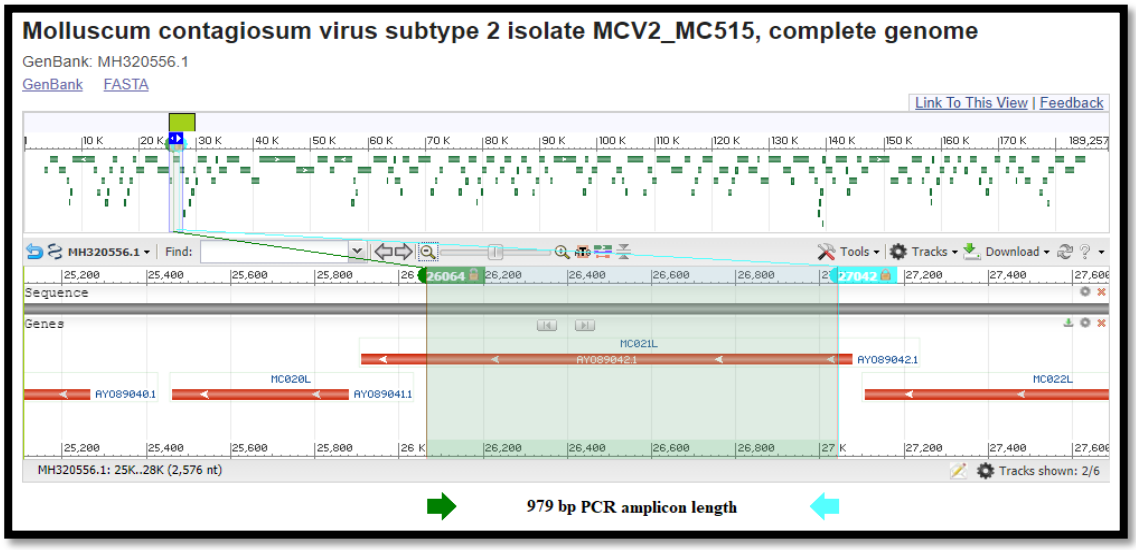
To confirm the molecular diagnosis resulting from the conventional PCR reaction, the sequence of the multiplexed piece of the MC021L and MC133L genes of the MCV was analyzed using a genious software program and the results were compared with the sequence of genes in the database using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) website.

4.2.1. Sequencing Results

4.2.1.1. MC021L gene

Within this locus, six samples were included in the present study. These samples were screened to amplify MC021L gene sequences of Molluscum contagiosum virus. Thus, the variation of the MC021L gene can be used for Molluscum contagiosum virus genotyping due to its possible ability to adapt to variable genetic diversity as it was seen in different viral types. The sequencing reactions indicated the exact identity after performing NCBI Blastn for these PCR amplicons (Zhang *et al.*,2000). Concerning the 979 bp amplicons, the NCBI BLASTn engine shown about 94.7% sequences

similarities between the sequenced samples and the intended reference target sequences. By comparing the observed nucleic acid sequences of these investigated samples with the retrieved nucleic acid sequences (GenBank acc.MH320556.1), the accurate positions and other details of the retrieved PCR fragments were identified. The total length of the targeted locus was localized in the NCBI server, and the positions of the start and end of the targeted locus were also confirmed , figuer (4.4).



Figuer(4.4) The exact position of the retrieved 979bp amplicon that partially covered a portion of the MC021L gene within Molluscum contagiosum virus genomic sequences(GenBank acc. no. MH320556.1). The green arrow refers to the starting point of this amplicon while the cyan arrow refers to its endpoint.

After positioning the 979 bp amplicons' sequences within the genomic sequences of the Molluscum contagiosum virus, the details of its sequences were highlighted, in terms of the positioning of both forward and reverse primers of the 979 bp amplified amplicons(table 4.4).

Table (4.4).The position and length of the 979bp PCR amplicons that used to amplify a portion of the MC021L gene within Molluscum contagiosum virus genomic sequences (GenBank acc. no. MH320556.1). The gray-colored sequences referred to the position of the reverse and forward primers, respectively.

Amplicon	Reference locus sequences (5′ - 3′)	length
Nucleic acidsequences withinthe viral MC021L gene	*GTAGTGCGTGCCGTCCATGTCGGCGCTGGTCACATGCACGTACTCGTCGT CCACCACGAGGAGCTTGGTGTTATTGACGGCGTCGCCCTTAGCTCCCGGGA TCGCGAAGACGCGCACGCTAATGTCGGCGTGGCCCACGCCCAGCTCGTGCA GCCCCTTGACGGCGGCCAGCGAGAACACGTCGCTGCGGTGCCACAGCCCT ACCAGCAGGCGCACGCGCACGTTGCGTTCCAGTGCCGCGCGCACCAACGC GTCGTGCATGCGCGGCCAGTACTGTACAGAGTCCTCGTCGCGCACCAGTGG CACCAGCGAGAGTAGCTCCATGTCGATAGTACTGCGAGCGGCCTGCACGTG CGCGAGCACAGCGTCGGCATCAAAGGTGCGTGTGCTGCCGATAAGACTTTC CGGCGCGTCGGAAAAGAAGGCGTTTTCGCAGTGGCGGCGCAAATGGAAGC GCGTGCTAAGCGAGAGGCAGCGCACGCAGCGGCGCGCGCACAGGCGCTCG TAGTCGCGGAAGCGGCGACGCAGGTCACGCGCCAGCGGCTCGCACTCGGA GTACACGCCCAGGCTCTTGATGGTCGAAATGGAGCCCCCGGTAAGCGAGG CGCTGCCCAGGTAAAAGCGGCGCTTGTCCGATACCCAGAAGCTGCTGAGC AAGCTTCCGGGCTTGCCGCCGGGCAGTTCGCCCACGTCCAGCTTCAGGTAG CGTAGGTTGGGCACGCCCGCCAGCTGCGTGGCATCCGCGTCCCGGCTCTGC TCGTCCACGATAATCGTCACGCGCACGTCGGCGCGCGCTAGCTCCAGCAGC CGCAGCAGCACGTGCCCGCCCTCGGGCGTGGAGCGCAGATTGCAGCAGTA AGACGCAATGCACAGCTCGCGCTGCGTCTGCGAGATGAGCGTGTCAAAGC AGTCGTACGTGAGCATGCTGCCGGTAGGTAGCGCCAGCGGCAGCGTTGCC GGCAGCGTCTCTACAATCTTG**	979 bp

* Refers to the reverse primer sequences (placed in a forward direction)
**Refers to the forward primer sequences (placed in a reverse complement direction).

Interestingly, the alignment results of the 979 bp samples revealed the presence of fifty-one mutations in the analyzed samples in comparison with the most similar referring reference a nucleic acid sequences (Appendix 2).

These differences observed in the currently observed nucleic acid sequences in the analyzed samples were not found in the reference sequences. To confirm these extremely high number of variations, the sequencing chromatograms of the investigated sample, as well as its detailed annotations, were verified and documented, and the chromatograms of its sequences were shown according to their positions in the PCR amplicons.

The observed mutations were further analyzed to identify whether such substitutions induce possible alteration in their corresponding positions in the encoded MC021L protein. All nucleic acid sequences were translated to their corresponding amino acid sequences using the Expasy translate suite. The total number of the detected nucleic acid substitutions was fifty one. Amino acid alignment of these amino acid sequences with their references showed that some of them exhibited asilent effect on the protein, while the other samples exerted a missense effect on the same protein (Appendix 3 a).

These variations were exemplified in the entire protein sequences and showed that the majority of the detected nucleic acid variations were silent in the investigated samples, namelyp.V321=, p.D322=, p.A308=, p.S281=, p.F280=, p.V279=, p.L270=, p.R266=, p.L256=, p.H253=, p.D242=, p.L233=, p.S227=, p.A225=, p.A220=, p.V218=, p.G208=, p.P203=, p.S200=, p.D196=, p.L190=, p.R161=, p.S154=, p.G141=, p.E111=, p.G98=, p.Q95=, p.A93=¸ p.I82=, p.T81=, p.V80=, p.E72=, p.L71=, p.G64=¸ p.N56=, p.A51=, and p.F37= (Appendix 3 b)Whereas the other nucleic acid variations were found to exertmissense effects on the resulting altered MC021L protein, namely p.329S>T, p.326V>A, p.323D>N, p.304A>V, p.247K>Q, p.179L>V, p.168R>S, p.163L>Q, p.112L>M¸ p.107M>L, p.77N>D, p.46S>K, and p.45Q>R. These amino acid substitutions observed in the MC021L-encoded glycoprotein seem to induce remarkable alterations in this protein in such a way the altered amino acid residues force this protein to exhibit another behavior against the host hostile

immunity. However, this alteration may be developed by the invading organism as an adaptation to drugs that are directed toward its targeted glycoprotein (Topalis *et al.*, 2016; Sarhan *et al.*, 2019).

To summarize all the results obtained from the sequenced 979 bp fragments, the precise positions and annotations of the observed nucleic acid substitution mutation nwere described in the NCBI reference sequences(table 4.5).

Table (4.5). The pattern of the observed SNPs in the 979 bp amplicons of the MC021L gene in comparison with the NCBI referring sequences (GenBank acc. no. MH320556.1). The symbol "S" followed by a number refers to thenumbers of theinvestigated viral sample.

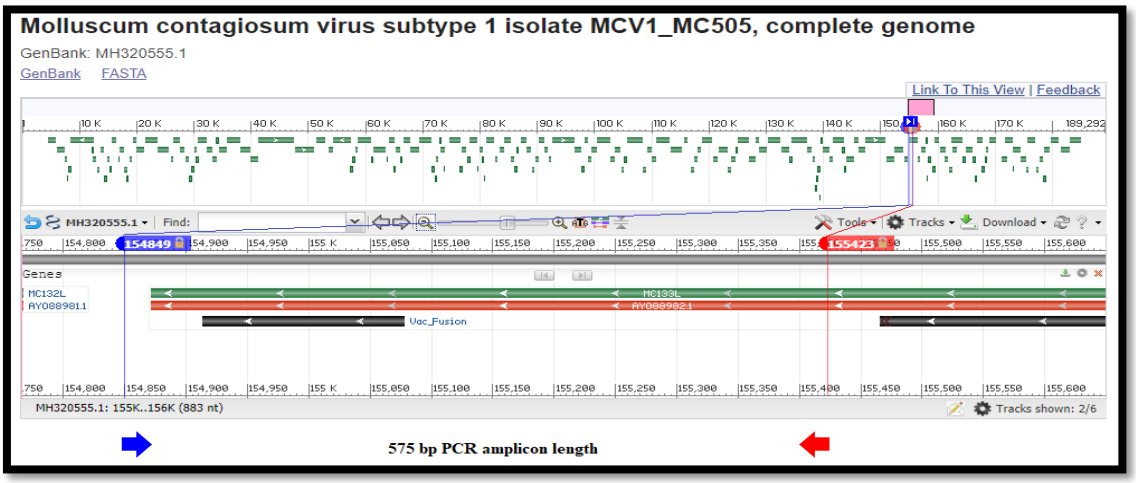
Sample No.	Native	Allele	Position of nucleic acid in the PCR fragment	Position of nucleic acid in the reference genome	Position in the reference amino acid of the entire protein	Type of mutation
S1, S2, S3, S4, S5, S6	C	G	29	26092	S329	Missense (p.329S>T)
S1, S2, S3, S4, S5, S6	C	G	38	26101	V326	Missense (p.326V>A)
S1, S2, S3, S4, S5, S6	C	T	48	26111	D323	Missense (p.323D>N)
S1, S2, S3, S4, S5, S6	A	C	52	26115	V321	Silent (p.V321=)
S1, S2, S3, S4, S5, S6	G	A	70	26113	D322	Silent (p.D322=)
S1, S2, S3, S4, S5, S6	G	A	91	26154	A308	Silent (p.A308=)
S1, S2, S3, S4, S5, S6	G	A	104	26167	A304	Missense (p.304A>V)
S1, S2, S3, S4, S5, S6	C	T	172	26235	S281	Silent (p.S281=)
S1, S2, S3, S4, S5, S6	G	A	175	26238	F280	Silent (p.F280=)
S1, S2, S3, S4, S5, S6	C	A	178	26241	V279	Silent (p.V279=)
S1, S2, S3, S4, S5, S6	G	A	207	26270	L270	Silent (p.L270=)
S1, S2, S3, S4, S5, S6	G	A	217	26280	R266	Silent (p.R266=)
S1, S2, S3, S4, S5, S6	A	G	249	26312	L256	Silent (p.L256=)
S1, S2, S3, S4, S5, S6	G	A	256	26319	H253	Silent (p.H253=)
S1, S2, S3, S4, S5, S6	T	G	276	26339	K247	Missense (p.247K>Q)
S1, S2, S3, S4, S5, S6	A	G	289	26352	D242	Silent (p.D242=)
S1, S2, S3, S4, S5, S6	C	T	316	26379	L233	Silent (p.L233=)
S1, S2, S3, S4, S5, S6	A	G	334	26397	S227	Silent (p.S227=)

S1, S2, S3, S4, S5, S6	G	A	340	26403	A225	Silent (p.A225=)
S1, S2, S3, S4, S5, S6	A	C	355	26418	A220	Silent (p.A220=)
S1, S2, S3, S4, S5, S6	T	C	361	26424	V218	Silent (p.V218=)
S1, S2, S3, S4, S5, S6	G	A	391	26454	G208	Silent (p.G208=)
S1, S2, S3, S4, S5, S6	G	T	406	26469	P203	Silent (p.P203=)
S1, S2, S3, S4, S5, S6	G	A	415	26478	S200	Silent (p.S200=)
S1, S2, S3, S4, S5, S6	G	A	427	26490	D196	Silent (p.D196=)
S1, S2, S3, S4, S5, S6	A	G	447	26510	L190	Silent (p.L190=)
S1, S2, S3, S4, S5, S6	G	C	480	26543	L179	Missense (p.179L>V)
S1, S2, S3, S4, S5, S6	G	T	513	26576	R168	Missense (p.168R>S)
S5, S6	A	T	527	26590	L163	Missense (p.163L>Q)
S1, S2, S3, S4, S5, S6	A	G	532	26595	R161	Silent (p.R161=)
S1, S2, S3, S4, S5, S6	G	T	553	26616	S154	Silent (p.S154=)
S1, S2, S3, S4, S5, S6	T	C	592	26655	G141	Silent (p.G141=)
S1, S2, S3, S4, S5, S6	G	T	681	26744	L112	Missense (p.112L>M)
S1, S2, S3, S4, S5, S6	C	T	682	26745	E111	Silent (p.E111=)
S1, S2, S3, S4, S5, S6	T	G	696	26759	M107	Missense (p.107M>L)
S1, S2, S3, S4, S5, S6	A	G	721	26784	G98	Silent (p.G98=)
S1, S2, S3, S4, S5, S6	C	T	730	26793	Q95	Silent (p.Q95=)
S1, S2, S3, S4, S5, S6	G	A	736	26799	A93	Silent (p.A93=)
S1, S2, S3, S4, S5, S6	A	G	769	26832	I82	Silent (p.I82=)
S1, S2, S3, S4, S5, S6	A	G	772	26835	T81	Silent (p.T81=)
S1, S2, S3, S4, S5, S6	C	T	775	26838	V80	Silent (p.V80=)
S1, S2, S3, S4, S5, S6	T	C	786	26849	N77	Missense (p.77N>D)
S1, S2, S3, S4, S5, S6	T	C	799	26862	E72	Silent (p.E72=)
S1, S2, S3, S4, S5, S6	T	C	802	26865	L71	Silent (p.L71=)
S1, S2, S3, S4, S5, S6	C	T	823	26886	G64	Silent (p.G64=)
S1, S2, S3, S4, S5, S6	A	G	847	26910	N56	Silent (p.N56=)
S1, S2, S3, S4, S5, S6	G	C	862	26925	A51	Silent (p.A51=)
S1, S2, S3, S4, S5, S6	G	T	877	26940	S46	Missense (p.46S>K)
S1, S2, S3, S4, S5, S6	C	T	878	26941	S46	Missense (p.46S>K)
S1, S2, S3, S4, S5, S6	T	C	881	26944	Q45	Missense (p.45Q>R)
S1, S2, S3, S4, S5, S6	G	A	904	26967	F37	Silent (p.F37=)

4.2.2. MC133L gene

Within this locus, two samples were included in the present study. Both samples were screened to amplify MC133L gene sequences of

Molluscum contagiosum virus. As in the case of the MC21L gene, the variation of the MC133L gene was also used for Molluscum contagiosum virus genotyping to explore the possibility of its using as a genotyping tool in different viral subtypes. The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons. Concerning the 575 bp amplicons, the NCBI BLASTn engine shown about 99.9% sequences similarities between the sequenced samples and the intended reference target sequences. By comparing the observed nucleic acid sequences of these investigated samples with the retrieved nucleic acid sequences (GenBank acc.MH320555.1), the accurate positions and other details of the retrieved PCR fragments were identified. The total length of the targeted locus was localized in the NCBI server, and the positions of the start and end of the targeted locus were also confirmed (figuer 4.5).



Figuer(4.5).The exact position of the retrieved 575 bp amplicon that partially covered a portion of the MC133L gene within Molluscum contagiosum virus genomic sequences (GenBank acc. no. MH320555.1). The cyan arrow refers to the starting point of this amplicon while the red arrow refers to its end point.

After positioning the 575 bp amplicons' sequences within the genomic sequences of the Molluscum contagiosum virus, the details of its sequences were highlighted, in terms of the positioning of both forward and reverse primers of the 575 bp amplified amplicons(table 4.6).

Table (4.6).The position and length of the 575bp PCR amplicons that used to amplify a portion of the MC133L gene within Molluscum contagiosum virus genomic sequences (GenBank acc. no. MH320555.1). The gray-colored sequences referred to the position of the reverse and forward primers, respectively.

Amplicon	Reference locus sequences (5′ - 3′)	length
Nucleic acidsequences within the viral MC133L gene	*GCTTTTCAGTTTTTGTGCGAAACTTACTCGTATACCATGTCGTCTC TGTAGCCTGCAGGCACGCGCCCTGTCTGGACGTTAATCTTGCGGAC TAGCGCCAGCATAGTCTTGCGCAGTGTTTCCGCGTGGCGCTCCAGT CGAGAAAGGCCCTCGGACACGGTACTGCAGCAGTTCACAACATCT GTGTGGTCCTTAGCTAGCGTGGTTACCCTGTCCTCGAGCTCCCGAA TCAGCCTCACCGAGTTTGCGAGAGAACCTAGATCTCCACGACGACG AGCAATGTCTGCTCCGGGATCCTCGTCTTGAAGCCCTACGGGGATC GTGTTTTCTGTCTGATCGGCGACGGCGCCAGGCTCGTCCTGTCCTTC GGAGAGCTTTGTATCTTTGCTCTCGTCACGTGCCGCGGGAGTAAGG TCTTCATCGGTTAGCTGGGTCCCGCACTCCTCCGGAGTGATGAGCT TTGCCAGGGACAGGTACGGGTCTGGCGTGCCTGTCGCTGCTTCTGA AGTCGGGGGGATCACCAACAGCGCGCTAATGTCGCTACCATAGTTC ATATTCTTGATGGGCACTCCTCC**	575 bp

* Refers to the reverse primer sequences (placed in a forward direction)
**Refers to the forward primer sequences (placed in a reverse complement direction)

The current results study, the alignment results of the 575 bp samples revaled the presence of only one mutation in one of the analyzed samples in comparison with the most similar referring reference nucleic acid sequence (Appendix 4).

These differences observed in the currently observed nucleic acid sequences in the analyzed samples were not found in the reference sequences. To confirm this obsesved variations, the sequencing chromatograms of the investigated sample, as well as its detailed

annotations, were verified and documented, and the chromatograms of its sequences were shown according to their positions in the PCR amplicons (Appendix 5).

The observed mutation was further analyzed to assess whether such substitution induces a possible alteration in its corresponding position in the encoded MC133L-encoded protein. As in the case of the MC021L locus, the nucleic acid sequences of the MC133L locus were translated to their corresponding amino acid sequences using the Expasy translate suite. The total number of the detected nucleic acid substitutions in the MC133L locus was only one. Amino acid alignment of these amino acid sequences with its reference sequences showed that investigated S8 exhibited a missense effect on the MC133L-encoded protein (Appendix 6 a). This observed variant was exemplified in the entire protein sequences and showed that the detected nucleic acid variation was p.524E>K (Appendix 6 b). As in the case of the MC021L locus, the observed amino acid substitution of p.524E>K seems to induce a particular alteration in this protein in such a way the altered amino acid residues force this protein to exhibit another behavior against the host hostile immunity. Alternatively, it is maybe a minor polymorphism conducted by the same protein to perform the same scheduled role in the response to the same host immunity.

4.3. Registeration of samples in NCBI

An isolate of the MC133L gene-specific primer and four isolates of the MC021L gene-specific primer were sent to NCBI, and the isolates were registered in 5/7/2021.The serial number of the recorded isolates was

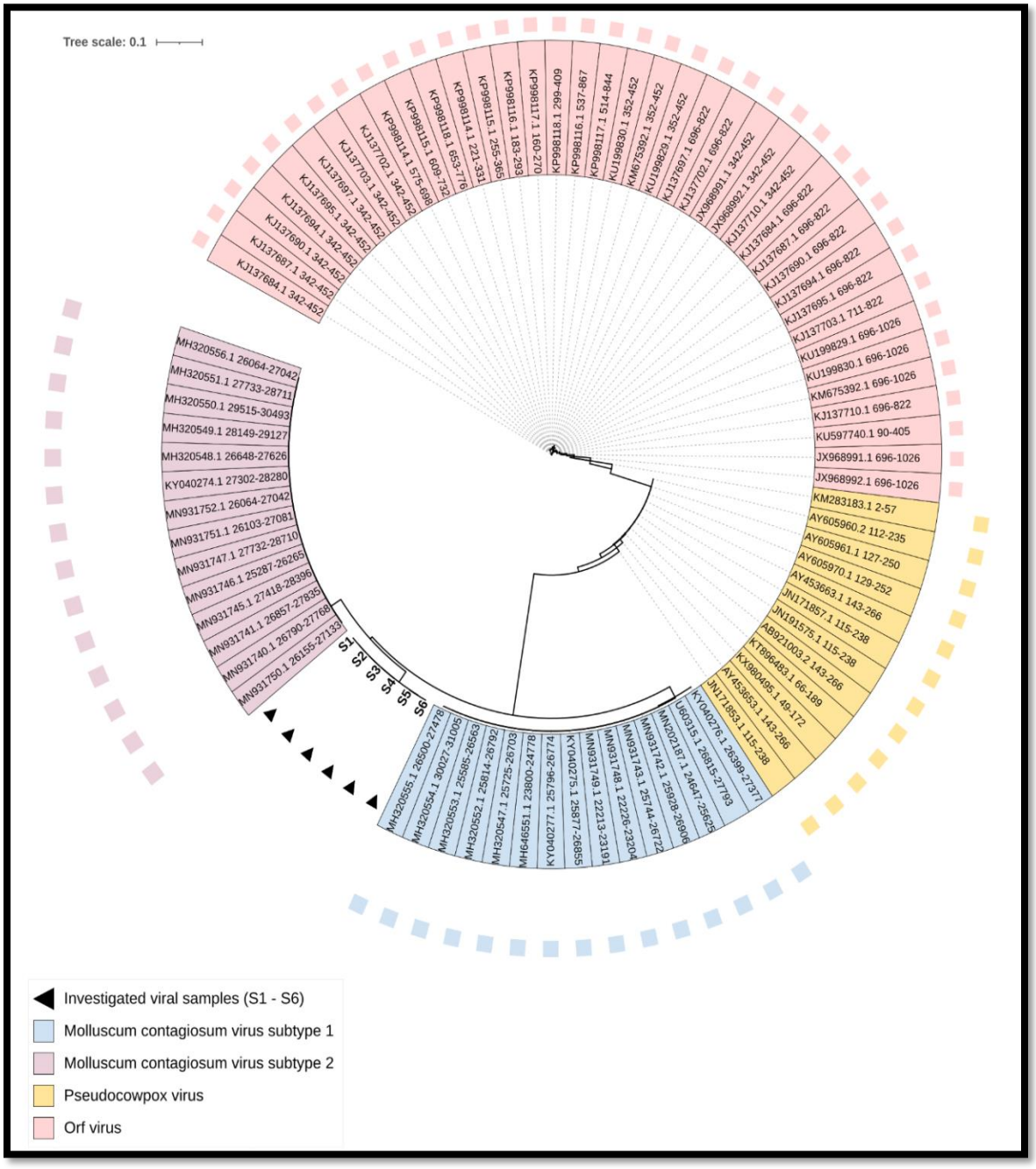
obtained **,** LC629161,for MC133L , LC629162 , LC629163 , LC629164 and LC629165, for MC021L. as in (Appendix 7,8,9,10 and 11).

4.4. phylogenetic tree

4.4.1. phylogenetic tree for MC021L

A comprehensive phylogenetic tree was generated in the present study according to nucleic acid variations observed in the amplified 979 bp of the MC021L amplicons. This phylogenetic tree was contained S1 to S6 samples alongside other relative nucleic acid sequences of Molluscum contagiosum virus sequences. As well, two types of viral organisms were also incorporated into the tree to serve as outgroup sequences within the same tree. These incorporated outgroups belonged to two types of Parapoxviruses; Pseudocowpoxviruses and Orfviruses. Both pathogenic outgroups viruses shared relatively similar nucleic acid sequences with our investigated samples of Molluscum contagiosum.

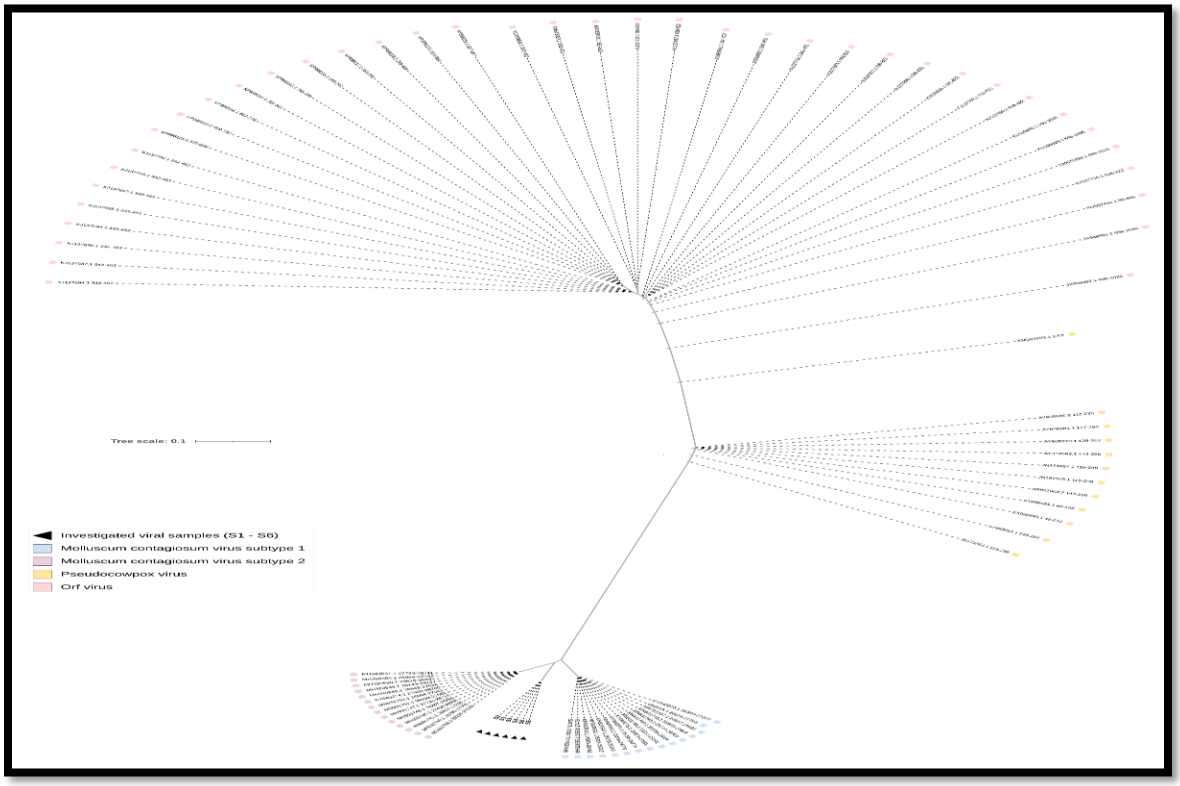
The total number of the aligned nucleic acid sequences in this comprehensive tree was eighty-sixed. In the constructed cladogram, the investigated samples were clustered into only one clade within the region of the Molluscum contagiosum virus sequences (figure 4.6).



Figure(4.6) The comprehensive cladogram phylogenetic tree of genetic variants of the MC021L fragment of six Molluscum contagiosum samples. The black-colored triangle refers to the analyzed viral variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0.1" at the top portion of the tree refers to the degree of scale range among the comprehensive tree categorized organisms. The letter "S#" refersto the code of the investigated samples.

The most interesting fact observed in our investigated viral isolates is correlated with their unique positioning between both Molluscum contagiosum Subtype-I and Subtype-II. This fact was obviously clarified using the unrooted form of the same constructed tree.

Based on the unrootedtree, it was found that this sort of positioning could not be directed toward any one of these two Subtypes. Instead, our neighbour-joining cladograms revealed that such sequences could not be belonged to any known Subtype, whether Subtype-I or II (figure 4.7)



Figure(4.7). The comprehensive eunrooted phylog enetic tree of genetic variants of the MC021L fragment of six Molluscum contagiosum virus local samples. The black-colored triangle refers to the analyzed viral variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0. 1" of the tree refers to the degree of scale range among the comprehensive tree categorized organisms. The letter "S#" refersto the code of the investigated samples.

According to the high number of the detected mutations in these samples, all the investigated S1 – S6 samples were clustered beside each other in one unique clade. Within this clade, no other isolates of the Molluscum contagiosum virus were incorporated, which provided unique genetic data for these viral samples. Within the incorporated clades of both Subtype-I and II, all our samples were clustered together in only one clade, or S1 – S6 clade in a phylogenetic position resided between both of them. This data indicated that all the observed nucleic acid variations were major polymorphisms within the main Molluscum contagiosum virus clade. Furthermore, the aggregation of all investigated viral samples with each other may refer to the presence of only one pattern of the phylogenetic distribution of these sequences. This major deviation of the observed S1 – S6 clade is attributed to the presence of these fifty-one detected mutations in these samples, which are not detected in the other viral sequences in the other two Subtypes.

The current observation of this tree has added another layer of confirmation of sequencing reactions because it explained the presence of a high ratio of nucleic acid substitutions with such known Subtypes. This finding was strongly suggested that these S1 – S6 sequences represent a new Subtype of Molluscum contagiosum sequences. Noteworthy, the utilization of the MC021L gene sequences in this study has given further indication for our suggestion of the presence of a new Subtype in this viral organism. This is due to the presence of considerable amino acid substitutions in the MC021L gene-encoded glycoprotein of Molluscum contagiosum. This fact entails that such altered protein may take a considerable difference from

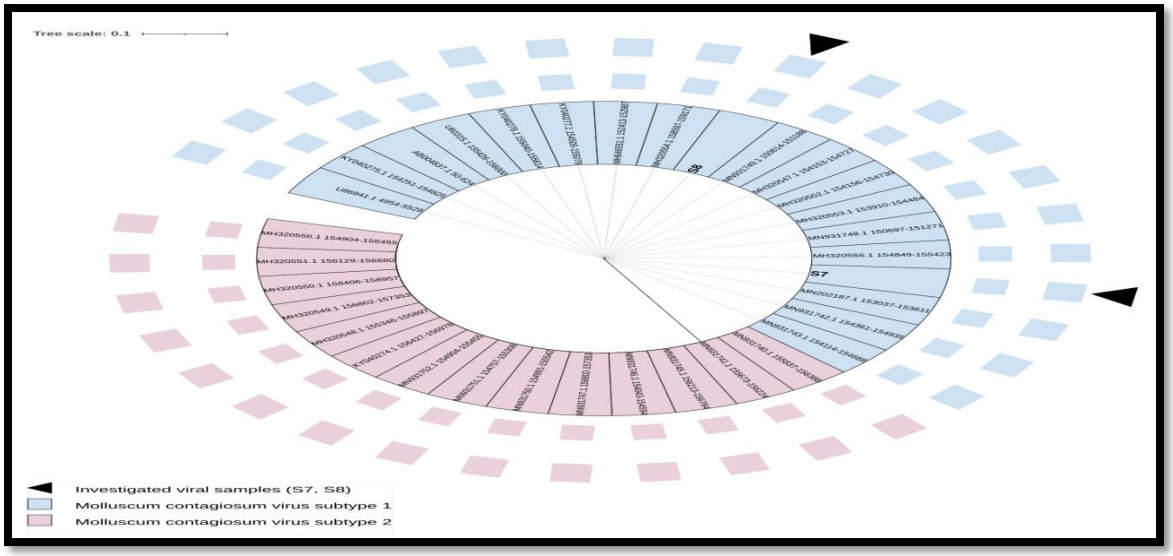
their normal counterpart. Thus, due to these amino acid changes, the altered glycoprotein would participate in different metabolic pathways than those found in the wild-type glycoprotein. Consequently, these pieces of pieces of evidence are inline with each other to support our finding of the presence of a new Subtype for this viral pathogenic DNA sequences.

This MC021L gene-based comprehensive tree has provided an extremely inclusive tool about the high ability of such genetic fragment to efficiently identify MC021L samples using this genetic fragment. This, in turn, gives a further indication of the ability of the currently utilized MC021L specific primers to describe the currently investigated Molluscum contagiosum viruses and their unique phylogenetic positions. Thus, according to the observed nucleic acid substitutions, as shown in the constructed phylogenetic tree, all these samples were positioned within only one phylogenetic position. These observations suggested the potential of the detected mutations in inducing a noticeable evolutionary alteration of the currently investigated viral samples in the Molluscum contagiosum phylogenetic positioning.

This unique positioning can also be explained by the ability of the MC021L fragment to exhibit altered nucleic acid variations as a response to host immunity. Therefore, it is rational to conclude that the investigated S1 – S6 viral samples have developed considerable alterations in their nucleic acid sequences to face the challenges of host immunity. These new remarkable alterations that are notified in the MC021L genetic fragment are in need to further studies via applying large-scale explorations to unmask more data for this possible new viral Subtype.

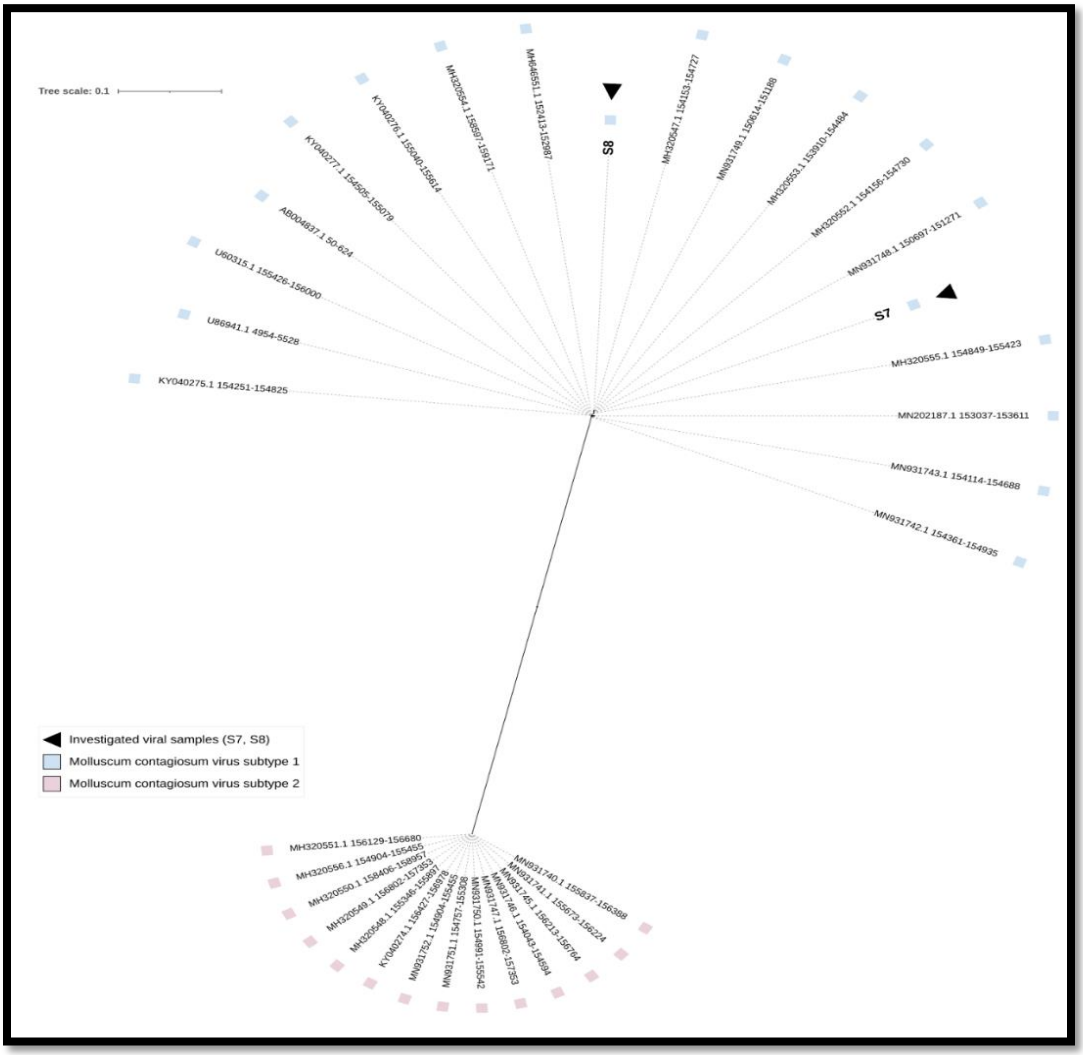
4.4.2.phylogenetic tree for MC133L

A comprehensive phylogenetic tree was generated in the present study according to nucleic acid variations observed in the amplified 575 bp of the MC133L amplicons. This phylogenetic tree was contained both S7 and S8 samples alongside other relative nucleic acid sequences of Molluscum contagiosum virus sequences. In contrast to the MC021L-based tree, no outgroup sequences were incorporated in the MC133L tree. This is due to the low level of nucleic acid variations observed in this genetic locus. However, the total number of the aligned nucleic acid sequences in this comprehensive tree was thirty-three. In the constructed cladogram, the investigated samples were clustered into only one clade within the region of the Molluscum contagiosum virus sequences (figure 4.8).



Figure(4.8) The comprehensive cladogram phylogenetic tree of genetic variants of the MC133L fragment of two Molluscum contagiosum samples. The black-colored triangle refers to the analyzed viral variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0. 1" at the top portion of the tree refers to the degree of scale range among the comprehensive tree categorized organisms. The letter "S#" refersto the code of the investigated samples.

The most noticeable observation in our investigated viral isolates is correlated with their positioning within the Molluscum contagiosum Subtype-I. This fact was obviously clarified using the unrooted form of the same constructed tree. Based on the unrooted tree, it was found that this sort of positioning was obviously suited in the Subtype-I .figure(4.9)



Figure(4.9) The comprehensive unrooted phylogenetic tree of genetic variants of the MC133L fragment of two Molluscum contagiosum samples. The black-colored triangle refers to the analyzed viral variants. All the mentioned numbers referred to GenBank accession number of each referring species. The number "0. 1" of the tree refers to the degree of scale range among the comprehensive tree categorized organisms. The letter "S#" refersto the code of the investigated samples.

Due to the detected MC133-based mutations in the investigated S8 sample, a slight tilt was identified in this sample with respect to the other wild-type (S7) sample. However, both samples were clustered in the same clade of the Molluscum contagiosum Subtype-I. Accordingly, a little effect was revealed for the observed p.524E>K on the evolutionary positioning of the S8 with regard to its counterpart S7 sample. Within this clade, no other subtype of the Molluscum contagiosum virus were incorporated, which may provide genetic data for these viral samples to be cultured to the Subtype-I. According to the MC133-based data, it was found thatboth S7 and S8 belonged to the same Subtype-I. However, this data was in contrast with the data provided by the MC021L variations. This contradicting data may be attributed to the limited capacity of the MC133L in detecting the real subtype of the investigated viral samples compared with the MC021L locus.

The current observation of this tree has shown a confined capacity of the MC133L whether in terms of the absence of enough variations of MC133L-based locus or terms of the presence of a low level of nucleic acid substitutions. This finding was strongly suggested Thus, the MC021L locus showed an extremely high ability to detect a possible new subtype of this virus. Whereas the MC133-based locus was not recommended to observe such a new suggested Subtype. for this reason, it is extremely recommended to utilize the MC021L-based genetic variations studies to assess the possibility of the occurrence of this new Subtype in the larger population sizes.

4.5.Comparison of the study isolates with global isolates in the Phylogenetic tree:

For the MC133L gene,it was observed that the isolates match the global isolates as they were closer to the isolation (MH320554) from Slovenia ,2018 that showed, that the isolates (MN931749) from Slovenia 2020 , that the isolates (AB004837) from Japan 1998,The isolates of the study showed identical with these isolates, as they fell within the isolates belonging to MCV type I.

As for MC021L gene,The study samples were in the site of the evolutionary tree and their comparison with global isolates was independent between isolates of MCV type I and MCV type II,that the isolation (MN931750) from Slovenia 2020, that the isolation (MH320548) from Slovenia 2018, that the isolation (KY040274) from USA 2012, associated with MCV type 2 and that the isolation (U60315) from USA 2002, that the isolation (MH320555) from Slovenia 2018 that, that the isolation (KY040277) from SpaIn 2016, that the isolation (MN931748) from Slovenia 2020, associated with MCV type 2.

4.6. Study groups MC002L gene association:

4.6.1. Socio-demographic variables:

Regarding the MC002L gene detection, the results found that 85 out of 101 samples (84.15%) were positive. 48 (85.7%) of them were less than 10 years old, 18(94.7%) were 10-29 years old and the remaining 19 (73.1%) were more 30 years and more. The difference among the age groups was statistically insignificant (P= 0.129). Regarding the gender, 45(84.9%) of the positive patients were males and 40 (83.3%) were

females, so, the difference was statistically insignificant (P= 0.829).The results of educational levels showed that the highest detection rate of MC002L gene was among those with secondary school education (100%) followed by those Illiterate (87.8%). However, the difference among the educational levels was statistically insignificant (P= 0.552).Concerning the residence, 25 (73.5%) of the positive patients were reside in urban areas versus 60 (89.6%) patients who were reside in rural areas with statistically significant difference (P= 0.037).All these association were illustrated in (table 4.7).

Table (4.7): Association ofMC002L gene detection rate with socio-demographic variables.

Variables		*MC002L* gene				P value
		Positive		Negative		
		No	%	No	%	
Age (Ys)	< 10	48	85.7	8	14.3	**0.129**
	10---29	18	94.7	1	5.3	
	≥ 30	19	73.1	7	26.9	
Gender	Male	45	84.9	8	15.1	**0.829**
	Female	40	83.3	8	16.7	
Educational level	Illiterate	43	87.8	6	12.2	**0.552**
	Primary	18	85.7	3	14.3	
	Secondary	4	100.0	-	-	
	Institute	7	70.0	3	30.0	
	College	12	80.0	3	20.0	
Residence	Urban	25	73.5	9	26.5	**0.037***
	Rural	60	89.6	7	10.4	

*Significant difference between percentages using Pearson Chi-square test (χ2-test) at 0.05 level.

The current study showed that the highest incidence of MCV was in the age group of less 10 years. The findings are consistent with Mohammad 2020 research in Iraqi which found that of(1-10)years 48% of the patients, (Mohammad, 2020).

This finding matched the findings of a Gatea *et al.*,(2019) study in Iraq, which found that MCV was most common in children aged 1 to 10 years (77.4%) (Gatea *et al* ., 2019). The findings are also consistent with Dohil's 2006 research in the United States, which found that nearly 80% of the patients were under the age of eight (Dohil, 2006).

This affection rate vary from what has been published in the united state in one study which indicate that the increase in Molluscum appears to be parallel the overall rise in sexually transmitted disease while the prevalence rate in children is of less than 5 %.(Dohil, 2006). The current findings contradicted a study conducted in Iraq by AlKayalli in 2015, which found that approximately (44.5 %) of MCV patients in the age group (31-40 years) were male (AlKayalli *et al* ., 2015). The loss of tissue immunity in the body, as well as the combination of children in schools and swimming pools, is two of the major factors (Gatea *et al.*,2019). According to another study, this may be attributed to differences in social living levels (Saleh, 2016).

Or the cause of the spread of the virus in a group less than 10 years old is a lack of awareness and immunity, a lack of attention to hygiene and exams in schools, kindergartens and public parks, and the use of games without cleaning or sterilization, so the percentage of pollution increases and results in infection with the virus.

Regarding the gender, 45(84.9%) of the positive patients were males and 40 (83.3%) were females, the difference was statistically insignificant,The current study agreed with a study conducted in Iraq, Diyala Governorate by Mohammad 2020, where the proportion of males reached

50.7% and 49.3% of females (Mohammad, 2020).also agreed with a study conducted in Basra by Gatea2019 that MCV infections are prevalent in males and females, reaching 60.8% of males and 39.2% of females(Gatea *et al.*, 2019). And agree with Al- Kayalli's analysis in Iraq 2015, which found that males made up 70.4 % of the population and females made up 29.6% (Al-Kayalli *et al.*,2015). And also agree with were consistent with a survey conducted in Iraq by Al-Azawi 2013 found MCV in 66.6%t of males and 33.3% of females (Al-Azawi, 2013) . The current study agreed with a study conducted in Iraq by Maytham and Abbas in 2012, where the percentage of females was 180 (54.54%) and 150 (45.46%), (Maytham and Abbas, 2012). Another study conducted in Iraq by Al-Maliki, the number of males was 25 and 95 females out of a total of 120 samples (Al-Maliki *et al.*, 2019).

The reason for the spread of the disease in males and females is due to the constant mixing between females and males and participation in work .the prevalence of infection in males is higher than in females. This disparity in outcomes may be attributed to disparities in educational levels and a lack of attention to health, particularly when the occurrence of such diseases is not just of concern because elitist little awareness of health matters.

As for the educational level, where the study showed a significant increase in infection among the secretary, the reason is due to the lack of health awareness among this class of society, as well as the lack of hygiene and continuous neglect in terms of health and behavior.

The current study did not agree with previous studies, as it showed that rural areas have a higher incidence of MCV than urban areas.The findings were not agree with a survey conducted in Iraq by Al-Azawi in

2013, which found that urban areas accounted for 70.6 % of the population, while rural areas accounted for 29.4% (Al-Azawi, 2013). Another result did not agree with the findings of an Iraqi survey undertaken by Al-Kayalli in 2015, which showed that urban areas accounted for 70.4 % of the population, while rural areas accounted for 29.6 % (Al-Kayalli *et al.*, 2015). The findings not matched those of a survey conducted in Iraq by Sleh in 2015, which found that 62.7 %of people live in rural areas, compared to 37.3 percent in urban areas (Saleh, 2016). The study also did not agree with a study conducted by Mohammad in Iraq 2020, which showed that the rate of infection in urban areas (80%) to the rate of infection in rural areas was 20%. (Mohammad, 2020). The reason is attributed to the higher incidence of infection in rural areas than in urban areas, because most of the samples were taken from rural areas in this study, as well as the lack of sanitation, awareness and hygiene.

4.6.2. Clinical variables:

The results in table (4.8) found that 16 (80.0%) patients who were positive for MC002L gene had previous infection with MCV against 69 (85.2%) who were positive for MC002L gene had no previous infection, the difference between the two groups was statistically unsignificant (P= 0.570). On the other hand, 49 (89.1%) of patients who were positive for MC002L gene had concomitant diseases versus 36 (78.3%) of patients who were positive for MC002L gene, but had no concomitant diseases with astatistically unsignificant (P= 0.138).

Table (4.8): Association of MC002L gene detection rate with clinical variables.

Variables		*MC002L* gene				P value
		Positive		Negative		
		No	%	No	%	
Previous infection by MCV	Yes	16	80.0	4	20.0	**0.570**
	No	69	85.2	12	14.8	
Associated diseases	Yes	49	89.1	6	10.9	**0.138**
	No	36	78.3	10	21.7	

*Significant difference between percentages using Pearson Chi-square test (χ2-test) at 0.05 level.

The current study showed that it is possible to repeat infection with Molluscum contagiosum again, and the reason may be due to weak immunity for some people.As well as in terms of its association with diseases, as it showed that it is associated with some diseases such as eczema, influenza and other diseases that may increase the period of infection with the virus.

4.6.3. Lesion characteristics:

The association of MC002L gene detection rate with lesion characteristics was shown in table (4.9). Regarding the duration of lesion, the results found that the MC002L gene positivity was highest among those lesions with 1 month duration (90.4%) and the lowest positivity rate was among those lesions with 3 months or more (75.0%). However, the difference was statistically insignificant (P= 0.202). In connection with the season, the results showed that there was a significant association between MC002L gene positivity and the development of lesions during winter season 39 (100%) compared to that developed during the spring season 46 (74.2%),(P= **0.001).**

Concerning the site of the lesion, it was found that the MC002L gene positivity was highest among lesions in anal and genitalia region (100%) while the lowest positive was found among those lesions appeared on upper or lower extremities (63.3%), but the difference was statistically insignificant (P= 0.227). The results also revealed that there was insignificant (P= 0.537) association of MC002L gene positivity with the nature of the lesion since single lesion had (85.1%) positivity versus (78.6%) for the cluster lesion. Concerning the frequency of MCVlesions, the results revealed that 53 (88.3%) of those specimens which were positive for MC002L gene had (1-4) frequency, while 22(88.0%) of the specimens positive for MC002L gene had (5-9) frequency, and lastly Those lesions with 10 frequency or more constitute 62.5% of the MC002L gene positive lesions. Therefore, the difference among these categories was statistically significant **(0.035).** Regarding the size of the lesion, the results found that all lesion (100%) with 1millimeter, 3 millimeter and 6 millimeter had positive MC002L gene positivity followed by those with 5 millimeter (88.9%) with insignificant difference among these categories (P = 0.465).

Table (4.9): Association of MC002L gene detection rate with lesion characteristics.

Variables		MC002Lgene				P value
		Positive		Negative		
		No	%	No	%	
Duration of infection	1 month	47	90.4	5	9.6	0.202
	2 months	29	78.4	8	21.6	
	≥ 3 months	9	75.0	3	25.0	
Season of infection	Winter	39	100.0	-	-	0.001*
	Spring	46	74.2	16	25.8	
Site of lesion	Face, Nose, Ear, Eye & Lips	50	83.3	10	16.7	0.227
	Head & Neck	9	90.0	1	10.0	
	Chest, Back & Abdominal wall	14	93.3	1	6.7	
	Hand, Armpit, Leg & Feet	7	63.6	4	36.4	
	Anal & Genitalia	5	100.0	-	-	
Nature of lesion	Single	74	85.1	13	14.9	0.537
	Cluster	11	78.6	3	21.4	
Frequency oflesion	1-- 4	53	88.3	7	11.7	0.035*
	5--9	22	88.0	3	12.0	
	≥ 10	10	62.5	6	37.5	
Size of lesion (mm)	1	8	100.0	-	-	0.465
	2	41	78.8	11	21.2	
	3	1	100.0	-	-	
	4	12	80.0	3	20.0	
	5	16	88.9	2	11.1	
	6	7	100.0	-	-	

***Significant difference between percentages using Pearson Chi-square test (χ2-test) at** 0.05 level.

The current study agreed with a study conducted in Iraq by Maitham and Abbas in 2012, where the duration of infection ranged from between 2 week and seven months(Maytham and Abbas, 2012).Another study in the USA showed that MC lesions can last anywhere from six months to five years (Damon, 2007).

As for the season, the spread of infection was in the winter and spring seasons, and the number of infections was more in the spring than in the winter, and the reason is that the spread of the virus is faster in hot and humid areas, or it may be the cause of climate change, which leads to

irritation of some allergic diseases to the body. The current study agreed with a study conducted in Iraq by Maytham and Abbas, where the results showed the highest rate of injury in the neck and head site, reaching 258 (78.18%), (Maytham and Abbas, 2012). This finding matched the findings of a study conducted in Iraq by Gatea 2019, which found that the largest incidence of MCV lesions occurred in the head (76.5%), followed by the trunk (16.6%), and the limbs (6.9%). (Gatea *et al.*, 2019). As well as another study in agreement with the current study, which was conducted in Iraq by Mohammad 2020, where the study showed that most of the injuries were in the upper part of the body,(Mohammad, 2020).

Another survey conducted in North Carolina by Molino (2004) found different results from the current study since the majority of MC lesions (64%) were found on the trunk and extremities (Molino *et al.*,2004).The reason for the difference in the sites of infection with the virus may be due to social customs or the nature of clothing and uniforms that are customary for each region.

Frequency of lesion and With regard to the nature of the lesion, the study showed that the lesion may be single or in the form of groups,The study agreed with a study conducted by Maitham and Abbas in Iraq in 2012, which showed that the number of lesions may be one, from two to three, or more than three lesions in the body (Maitham and Abbas, 2012). and the reason is due to this diversity due to the different nature of tissues from one person to another, as well as the effectiveness of immunization against infection may be weak or accompanying one of the skin diseases and

allergies, which leads to the transmission of infection spontaneously from the area to others in the form of groups or singly.

Finally, with regard to the sample size, the study results showed that the lesion may be 1mm to 6 mm. The study agreed with a study conducted by Maitham and Abbas in Iraq in 2012, In 310 individuals (93.93 %), the lesions were the same size or larger than 6mm. While Molluscum of 6mm or more was found on the face in 23 (6.9%) of patients (Maitham and Abbas , 2012). Other study shown in USA have a diameter of one millimeter to one centimeter (Rayala and Morrell; 2017 Lacarrubba *et al.*,2017).

Conclusions and Recommendations

Conclusions

1. The rate of molecular detection of MC133L gene was very low among clinically diagnosed patients with Molluscum contagiosum.
2. The phylogenetic tree analysis showed that the MC133L gene is located within the region specific to MCV type I, when compared with global isolates.
3. The MC021L gene polymorphism is one of the main key factors in the subtyping of the pathogenic strains of Molluscum contagiosum.
4. According to investigations of the MC021L locus, it was confirmed that the currently detected sequences were not attributed to any known subtype deposited previously, suggesting the emergence of a new subtype of the Molluscum contagiosum virus that can be positioned between both subtype-I and Subtype-II sequences.
5. The molecular detection rate of the MC002L gene was 84.2% among clinically suspected patients, indicating high infection rate of MCVin Diyala province.
6. The MC002L genewas significantly associated with 10-29 years age group, and rural residence. Whileit is insignificantly associated with site of lesion, previous history,previous infection by MCV,associated diseases and MC021L gene detection rate.
7. Correlation of the severity of the infection with its association with other skin diseases or the presence of an immune weakness in the patient.

Recommedations

1. The results of this study should be informed to the Diyala Directory of Health to make benefits of its contents especially the Dermatological Department.
2. The study suggests a possible employment of the MC021L amplicons as a promising tool for exploring further details within the newly identified subtype.
3. A future studies on the attribution of other diseases in the pathogenesis and severity of MCV infection are recommended.
4. MC133L A special area for MCV type 1.
5. Similar comprehensive molecular study on MCV genes using multiplex PCR technique is recommended.
6. Further immunological studies on the CMV infection are recommended.
7. Adoption of health education program regards about the intrafamilial and intra-community transmission of the CMV and its promoting factors.

References

References

Abate AR, Hung T, Sperling RA, Mary P, Rotem A, Agresti JJ, et al. (2013). "DNA sequence analysis with droplet-based microfluidics". *Lab on a Chip*. **13** (24): 4864–9.

Afonso, P. P.; Silva, P. M.; Schnellrath, L. C.; Jesus, D. M.; Hu, J.; Yang, Y. and Damaso, C. R. (2012). Biological characterization and next-generation genome sequencing of the unclassified Cotia virus SPAn232 (Poxviridae). Journal of virology, 86(9): 5039-5054.

Ahmed R.(2013).Polymerase Chain Reaction For Detection And Genotyping Of *Molluscum Contagiosum* Virus In Diyala Province (thesis), 66.

Ajithkumar, V. T.; Sasidharanpillai, S.; Muhammed, K.; Sreejayan, M. P.; Simin, M.; Ashraf, F. and Dhanyasree, T. (2017). Disseminated molluscum contagiosum following chemotherapy: A therapeutic challenge. Indian Journal of Dermatology, Venereology, and Leprology, 83(4): 516.

AL–Azawi, M.K., 2013. Polymerase chain reaction for detection and genotyping of molluscum contagiosum virus in diyala province. *Diyala journal of medicine*, *4*(1), pp.33-43.

Al-Dabbagh, N.N., Al-Janabi, W.H. and Al-Shuhaib, M.B.S., 2019. Identification of Candida Species Using 26S Ribosomal RNA Gene Sequencing in Patients with Periodontitis. Journal of Bacteriology and Virology, 49(4), pp.212-220.

Al-Kayalli, K. K.; Numman, N. K. and Al-Qaisy, M. H. (2015). Prevalence of Viral Skin Infections among Patients in Diyala Provence-Iraq. Diyala Journal of Medicine, 6(1): 92-99.

Al-Malkey, M. K.; Al-Obaidi, M. J.; Mohammed, S. W.; Nayyef, H. J.; Jabbar, F. and Al-Deeri, M. M. (2019). Pearl Skin Disease Comprehension among University of Baghdad Students. Age, 25(97): 81.

Al-Mutairi, N.; Al-Doukhi, A.; Al-Farag, S. and Al-Haddad, A. (2010). Comparative study on the efficacy, safety, and acceptability of imiquimod 5% cream versus cryotherapy for molluscum contagiosum in children. Pediatric Dermatology, 27(4): 388-394.

Badri T, Gandhi GR. Molluscum Contagiosum. (2021). Mar 21. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID:28722927 .

Bahalı, A. G.; Su, O.; Ozkaya, D. B.; Sallahoglu, K.; Yıldız, P.; Demirkesen, C. and Onsun, N. (2016). Plantar Molluscum contagiosum in an adult patient. Journal of the American Podiatric Medical Association, 106(3): 235-236.

Bateman, F. (1953). Molluscum contagiosum. Classics in Dermatology. SpringfieldIL .

Behjati, S. and Tarpey, P.S., 2013. What is next generation sequencing?. Archives of Disease in Childhood-Education and Practice, 98(6), pp.236-238.

Blake, N.W., Porter, C.D. and Archard, L.C., 1991. Characterization of a molluscum contagiosum virus homolog of the vaccinia virus p37K major envelope antigen. *Journal of virology*, *65*(7), pp.3583-3589.

Brady, G., Haas, D.A., Farrell, P.J., Pichlmair, A. and Bowie, A.G., 2017. Molluscum contagiosum virus protein MC005 inhibits NF-κB activation by targeting NEMO-regulated IκB kinase activation. *Journal of Virology*, *91*(15), pp.e00545-17.

Brady, G., Haas, D.A., Farrell, P.J., Pichlmair, A. and Bowie, A.G., 2015. Poxvirus protein MC132 from molluscum contagiosum virus inhibits NF-κB activation by targeting p65 for degradation. *Journal of virology*, *89*(16), pp.8406-8415.

Bryk, P.J., 2018. Investigation of the Roles of Vaccinia Virus Protein F13 and its Molluscum Contagiosum Virus Homolog MC021 at Steps Post-Envelopment (Doctoral dissertation, University of Rochester School of Medicine and Dentistry).

Büchen-Osmond, C., 2003. ICTVdB: the Universal Virus Database of the International Committee on Taxonomy of Viruses, version 3, 03.019. 0.1 (Coronaviridae). Biomedical Informatics Core, Northeast Biodefense Center, Columbia University, New York, NY.

Bugert, J.J. and Darai, G., 2000. Poxvirus homologues of cellular genes. Virus genes, 21(1), pp.111-133.

Bugert, J.J., Lohmüller, C. and Darai, G., 1999. Characterization of early gene transcripts of molluscum contagiosum virus. *Virology*, *257*(1), pp.119-129.

Burrell, C.J., Howard, C.R. and Murphy, F.A., 2016. Fenner and White's medical virology. Academic Press.

Bugert, J.J.(2008) . in Encyclopedia of Virology (Third Edition), 111–133 .

Burrell, C. J.; Howard, C. R. and Murphy, F. A. (2017). In: Poxviruses. Fenner and White's Medical Virology.5th. London,UK. Academic Press.

Cann,A.J.; (2016). Principle of molecular virology.6 Edition.UK:University of Leicester,308.

Castro, C.J., Marine, R.L., Ramos, E. and Ng, T.F.F., 2020. The effect of variant interference on de novo assembly for viral deep sequencing. BMC genomics, 21(1), pp.1-12.

Chmielecki, J. and Meyerson, M., 2014. DNA sequencing of cancer: what have we learned?. *Annual review of medicine*, *65*, pp.63-79.

Cohen J, Powderly WG. (2004). Infectious Diseases. 2nd ed. New York, NY: Elsevier;:2053-2056 .

Coutu, J., Ryerson, M.R., Bugert, J. and Nichols, D.B., 2017. The molluscum contagiosum virus protein MC163 localizes to the mitochondria and dampens mitochondrial mediated apoptotic responses. Virology, 505, pp.91-101.

Daniel, W.W., 1974. Biostatistics; a foundation for analysis in the health sciences (No. 574.0182 D3).

Damon IK. (2013). Poxviruses. In: Fields BN, Knipe DM and Howley PM(editors). Fields Virology. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; pp. 2160–2184.

Delhon, G.U.S.T.A.V.O., Tulman, E.R., Afonso, C.L., Lu, Z., De La Concha-Bermejillo, A., Lehmkuhl, H.D., Piccone, M.E., Kutish, G.F. and Rock, D.L., 2004. Genomes of the parapoxviruses ORF virus and bovine papular stomatitis virus. Journal of virology, 78(1), pp.168-177.

Dixit, S., Chaudhary, M. and Singh, A., 2009. Molluscum contagiosum and dental caries: A pertinent combination. Journal of Indian Society of Pedodontics and Preventive Dentistry, 27(4), p.197.

Dohil, M.A., Lin, P., Lee, J., Lucky, A.W., Paller, A.S. and Eichenfield, L.F., 2006. The epidemiology of molluscum contagiosum in children. Journal of the american academy of dermatology, 54(1), pp.47-54.

Domingues, L. (2017). PCR-methods and protocols. New York: 283. Springer. Humana Press.

Falzon, K., Scotcher, S. and Parulekar, M., 2015. Primary epibulbar molluscum contagiosum in an immunocompetent child. The Journal of pediatrics, 167(4), p.936.

Fernando, I., Pritchard, J., Edwards, S.K. and Grover, D., 2015. UK national guideline for the management of Genital Molluscum in adults, 2014 Clinical Effectiveness Group, British Association for Sexual Health and HIV. International journal of STD & AIDS, 26(10), pp.687-695.

Gatea, M.A., Humoud, M.N. and Al-Hmudi, H.A., 2019. Molecular Detection of Molluscum contagiosum virus (MCV) from Patients of Basra Province/Iraq. Sci. J. Med. Res, 3(9), pp.39-46.

Ghura, H.S. and Camp, R.D.R., 2001. Scarring molluscum contagiosum in patients with severe atopic dermatitis: report of two cases. British Journal of Dermatology, 144(5), pp.1094-1095.

Gould, D., 2008. An overview of molluscum contagiosum: a viral skin condition. *Nursing Standard*, *22*(41).

Grant Mc (2005),Poxvirus tropism. Journal of microbiology ,Nature Reviews Microbiology 3, 201-213.

Gürtler, C. and Bowie, A.G., 2013. Innate immune detection of microbial nucleic acids. Trends in microbiology, 21(8), pp.413-420.

Hans R, Johnny S ,Thomas P. ,Anders H, Johnsen D, Jan G, *et al*. S (2000). A Highly Selective CC Chemokine Receptor (CCR)8 antagonist encoded by the poxvirus molluscum contagiosum from the laboratory for molecular pharmacology, Journal of Experim- ental Medicine (impact factor: 13.85); 191(1):171-80.

Hanson, D. and Diven, D.G., 2003. Molluscum contagiosum. Dermatology online journal, 9(2).

Hayashida, S., Furusho, N., Uchi, H., Miyazaki, S., Eiraku, K., Gondo, C., Tsuji, G., Hachisuka, J., Fukagawa, S., Kido, M. and Nakahara, T., 2010. Are lifetime prevalence of impetigo, molluscum and herpes infection really increased in children having atopic dermatitis?. Journal of dermatological science, 60(3), pp.173-178.

Hoyt, B.S., Tschen, J.A. and Cohen, P.R., 2013. Molluscum contagiosum of the areola and nipple: case report and literature review. Dermatology online journal, 19(7).

Hu, N.C., 2010. The development of penguinpox virus (PEPV) as a vaccine vector: transfer vector construction and rescue of virus growth in rabbit kidney cells (RK-13) by vaccinia virus K1 (Master's thesis, University of Cape Town)."(net).http://www. poxvirus. org.,2021.

Ianhez, M., Cestari, S.D.C.P., Enokihara, M.Y. and Seize, M.B.D.P.M., 2011. Dermoscopic patterns of molluscum contagiosum: a study of 211 lesions confirmed by histopathology. Anais brasileiros de dermatologia, 86, pp.74-79.

Ives, C., Green, M. and Wright, T., 2017. Molluscum contagiosum: a rare nipple lesion. The breast journal, 23(1), pp.107-108.

Jason P, Trama M, Mordechai E. (2007). Identification and genotyping of molluscum contagiosum virus from genital swab samples by real-time PCR and pyrosequencing. Journal of Clinical Virology 40 325–329.

Kim, H.K., Jang, W.S., Kim, B.J. and Kim, M.N., 2013. Rare manifestation of giant molluscum contagiosum on the scalp in old age. Annals of dermatology, 25(1), pp.109-110.

Longnecker, R., Kieff, E. and Cohen, J.I., 2013. Fields virology. Epstein-Barr virus (Chapter 61), 2, pp.1898-1859.

Konya, J. and Thompson, C.H., 1999. Molluscum contagiosum virus: antibody responses in persons with clinical lesions and seroepidemiology in a representative Australian population. The Journal of infectious diseases, 179(3), pp.701-704.

Lacarrubba, F., Verzì, A.E., Ardigò, M. and Micali, G., 2017. Handheld reflectance confocal microscopy for the diagnosis of molluscum contagiosum: histopathology and dermoscopy correlation. Australasian Journal of Dermatology, 58(3), pp.e123-e125.

Laxmisha, C., Thappa, D.M. and Jaisankar, T.J., 2003. Clinical profile of molluscum contagiosum in children versus adults. Dermatology online journal, 9(5).

Letunicand

BorkP.(2019).Interactive TreeOf Life (iTOL) v4: recent updates an d new developments. Nucleic Acids Res 2;47(W1): W256-W259.

Leung, A. K.; Barankin, B. and Hon, K. L. (2017). Molluscum contagiosum: an update. Recent patents on inflammation & allergy drug discovery, 11(1): 22-31.

Ma, H., Yang, H., Zhou, Y. and Jiang, L., 2015. Molluscum contagiosum on the lip. Journal of Craniofacial Surgery, 26(7), pp.e681-e682.

Mahé, A., Prual, A., Konaté, M. and Bobin, P., 1995. Skin diseases of children in Mali: a public health problem. Transactions of the Royal Society of Tropical Medicine and Hygiene, 89(5), pp.467-470.

Maytham, M. and Abbas, M.Y., 2012. A typical clinical presentation of molluscum. Al-Kindy College Medical Journal, 8(2).

Meite, S., Coulibaly, N.D., Boni-Cissé, C., Koffi, K.S., Sylla, A., Kouassi, K.S., Mlan, A.P., Kouame, S.M., Zaba, F.S., Ngazoa, K.S. and Faye-Ketté, H., 2017. Molecular diagnostics by PCR of poxviruses (Orthopoxvirus (OPV) and Molluscum contagiosum virus (MCV)) in Cote d'Ivoire West Africa. African Journal of Clinical and Experimental Microbiology, 18(2), pp.86-91.

Melissa Swiecki And Marco Colonna.(2011). Disparate Antiviral responses in molluscum contagiosum virus–induced skin lesions . Journal Of Investigative Dermatology Volume 131.

Mohammed, S.(2020).Molecular study of Molluscum contagiosum virus in Diyala province.MSc.thesis,- Biology. Unversity of Diyala, Iraqi,66.

Molina, L. and Romiti, R. (2011). Molluscum contagiosum on tattoo. An 'Bras' Dermatol, 86(2), pp.352-4.

Molino, A.C., Fleischer Jr, A.B. and Feldman, S.R., 2004. Patient demographics and utilization of health care services for molluscum contagiosum. Pediatric dermatology, 21(6), pp.628-632..

Monticelli S,Bryk,Pand Ward.(2020)The molluscum contagiosum gene MC021L partially compensates for the loss of It is vaccinia virus homologus F13L.Journal of virology.94.20

Moss, B., Shisler, J.L., Xiang, Y. and Senkevich, T.G., 2000. Immune-defense molecules of molluscum contagiosum virus, a human poxvirus. Trends in microbiology, 8(10), pp.473-477.

Nair, A.G., Desai, R.J. and Gopinathan, I., 2016. Giant eyelid Molluscum contagiosum in a child with AIDS. Ophthalmology, 123(9), p.1925.

Nanhai C, Troy B, Paul NM, Michael G, William L. , Joseph B, *et al* (2000). Rapid Communication Selective Inhibition Of Nuclear Stroid Receptor By A Protein A Human Tumorigenic Poxvirus Virology 274, 17-25.

Neil W. B, Colin D. P,T And Leonard C. A. (1991) . Characterization Of A Molluscum Contagiosum Virus Homolog Of The Vaccinia Virus P37k Major Envelope Antigen. Journal of virology, july, p. 3583-3589.

Niizeki, K., Kano, O. and Kondo, Y., 1984. An epidemic study of molluscum contagiosum. Dermatology, 169(4), pp.197-198.

Odom, R.B., James, W.D. and Berger, T.G., 2000. Andrew, s diseases of the skin: clinical dermatology.

Olsen, J.R., Gallacher, J., Piguet, V. and Francis, N.A., 2014. Epidemiology of molluscum contagiosum in children: a systematic review. Family practice, 31(2), pp.130-136.

Olsvik, Ø., Wahlberg, J., Petterson, B., Uhlen, M., Popovic, T., Wachsmuth, I.K. and Fields, P.I., 1993. Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of cholera toxin subunit B in Vibrio cholerae O1 strains. Journal of clinical microbiology, 31(1), pp.22-25.

Oren, B. and Wende, S.O., 1991. An outbreak of molluscum contagiosum in a kibbutz. Infection, 19(3), pp.159-161.

Pekin, D., Skhiri, Y., Baret, J.C., Le Corre, D., Mazutis, L., Salem, C.B., Millot, F., El Harrak, A., Hutchison, J.B., Larson, J.W. and Link, D.R., 2011. Quantitative and sensitive detection of rare mutations using droplet-based microfluidics. Lab on a Chip, 11(13), pp.2156-2166.

Peterson, A.R., Nash, E. and Anderson, B.J., 2019. Infectious disease in contact sports. Sports Health, 11(1), pp.47-58.

Pickering LK, Baker CJ, Kimberlin DW, Long SS, Eds. Red Book(2009). Summaries of infectious diseases. in: report of the committee on infectious diseases. 28th ed. Elk. Grove Village, Il: American Academy of Pediatrics;: 466.

Popescu, R., Popescu, C.M., Williams, H.C. and Forsea, D., 1999. The prevalence of skin conditions in Romanian school children. British journal of dermatology, 140(5), pp.891-896.

Qingwen Jin, Jeffrey D. Altenburg, Mohammad M. Hossain, Ghalib Lkhatib.(2011). Role For The Conserved N-Terminal Cysteines In The Anti-Chemokine Activities By The Chemokine-Like Protein MC148R1 Encoded By Molluscum Contagiosum Virus.Virology Volume 417, Issue 2, , Pages 449-456.

Rayala, B.Z. and Morrell, D.S., 2017. Common Skin Conditions in Children: Skin Infections. FP essentials, 453, pp.26-32.

Riedel, S. Hobden, J.A. Miller, S. Morse, S.A. Mietzner, T.A. Detrick, B. Mitchell, T.J. akanari, J.A. Hotez, P. Mejia, R . (2019). Medical Microbiology .28 Edition .page 491-510.New York.

Reynolds, M.G., Holman, R.C., Yorita Christensen, K.L., Cheek, J.E. and Damon, I.K., 2009. The incidence of molluscum contagiosum among American Indians and Alaska Natives. PLoS One, 4(4), p.e5255.

Rook, G.A., 2012. Hygiene hypothesis and autoimmune diseases. Clinical reviews in allergy & immunology, 42(1), pp.5-15.

Rosner, M. and Zloto, O., 2018. Periocular molluscum contagiosum: six different clinical presentations. Acta ophthalmologica, 96(5), pp.e600-e605.

Ryu, W. S. (2017). Molecular virology of human pathogenic viruses. London: Sara Tenney. Academic Press.

Saleh, M.A.D., 2016. Evaluating the Humoral Immunity and Interleukin 18 Receptor 1 in some Patients with Molluscum Contagiosum Infection. Baghdad Science Journal, 13(1).

Sambrook, J. and Russell, D.W., 2006. Purification of nucleic acids by extraction with phenol: chloroform. Cold Spring Harbor Protocols, 2006(1), pp.pdb-prot4455.

Saral, Y., Kalkan, A., Ozdarendeli, A., Bulut, Y. and Doymaz, M.Z., 2006. Detection of Molluscum contagiosum virus (MCV) subtype I as a single dominant virus subtype in Molluscum lesions from a Turkish population. Archives of medical research, 37(3), pp.388-391.

Sarhan, S.R., Hashim, H.O. and Al-Shuhaib, M.B.S., 2019. The Gly152Val mutation possibly confers resistance to beta-lactam antibiotics in ovine Staphylococcus aureus isolates. Open veterinary journal, 9(4), pp.339-348.

Shchelkunov, S.N., 2003. Immunomodulatory proteins of orthopoxviruses. Molecular Biology, 37(1), pp.37-48.

Schaffer JV, Berger EM(2016). Molluscum Contagiosum JAMA Dermatol ;152(9):1072.

Schmidt, F.I., Bleck, C.K.E., Helenius, A. and Mercer, J., 2011. Vaccinia extracellular virions enter cells by macropinocytosis and

acid-activated membrane rupture. *The EMBO journal*, *30*(17), pp.3647-3661.

Scholz, J., Rösen-Wolff, A., Bugert, J., Reisner, H., White, M.I., Darai, G. and Postlethwaite, R., 1989. Epidemiology of molluscum contagiosum using genetic analysis of the viral DNA. Journal of medical virology, 27(2), pp.87-90.

Scope, A., Benvenuto-Andrade, C., Gill, M., Ardigo, M., Gonzalez, S. and Marghoob, A.A., 2008. Reflectance confocal microscopy of molluscum contagiosum. Archives of dermatology, 144(1), pp.134-134.

Senkevich, T.G., Ward, B.M. and Moss, B., 2004. Vaccinia virus entry into cells is dependent on a virion surface protein encoded by the A28L gene. Journal of virology, 78(5), pp.2357-2366.

Senkevich, T.G., Bugert, J.J., Sisler, J.R., Koonin, E.V., Darai, G. and Moss, B., 1996. Genome sequence of a human tumorigenic poxvirus: prediction of specific host response-evasion genes. Science, 273(5276), pp.813-816.

Senkevich, T.G., Koonin, E.V., Bugert, J.J., Darai, G. and Moss, B., 1997. The genome of molluscum contagiosum virus: analysis and comparison with other poxviruses. Virology, 233(1), pp.19-42.

Sherwani, S., 2013. Investigation of the infectious cycle of molluscum contagiosum virus in human skin and the nature of MCV induced immunity (Doctoral dissertation, Cardiff University).

Shisler, J.L., 2015. Immune evasion strategies of molluscum contagiosum virus. Advances in virus research, 92, pp.201-252.

Shubbar, E.E., AL-Khilkhali, H.J. and AL-Barqawi, N.A., 2019. Prevalence and Some Epidemiological Symptoms of Molluscum contagiosum Virus Infection in Al-Najaf Province. Indian Journal of Public Health, 10(10), p.3795.

Silverberg, N.B., Sidbury, R. and Mancini, A.J., 2000. Childhood molluscum contagiosum: experience with cantharidin therapy in 300 patients. *Journal of the American Academy of Dermatology*, *43*(3), pp.503-507.

Sturt, R.J., Muller, H.K. and Francis, G.D., 1971. Molluscum contagiosum in villages of the West Sepik District of New Guinea. Medical Journal of Australia, 2(15), pp.751-754.

Tabari, S.T. and Shakerian, M.A., 2007. Prevalence of molluscum contagiosum disease in day care centers in Babol, Northern Iran. Journal of Pediatric Infectious Diseases, 2(3), pp.153-155.

Taghinezhad-S, S., Mohseni, A.H., Keyvani, H. and Ghobadi, N., 2018. Molecular screening and single nucleotide polymorphism typing of Molluscum contagiosum virus (MCV) from genital specimens, between 2012 and 2015. Iranian biomedical journal, 22(2), p.129.

Thiemann, D. Everest, F. Steinbach, A. Dastjerdi, C. Finnegan, (2012) . Molluscum contagiosum in two donkeys. Journal of the British Veterinary Association.;170:649

Tony, B.; Stephen, B; Neil, C. and Christopher, G. (2010) .Rook's Textbook Of Dermatology,. Eighth Edition. chapter 33.P:13

Topalis, D., Gillemot, S., Snoeck, R. and Andrei, G., 2016. Distribution and effects of amino acid changes in drug-resistant α and β herpesviruses DNA polymerase. Nucleic acids research, 44(20), pp.9530-9554.

Trama, J.P., Adelson, M.E. and Mordechai, E., 2007. Identification and genotyping of molluscum contagiosum virus from genital swab samples by real-time PCR and Pyrosequencing. Journal of clinical virology, 40(4), pp.325-329.

Trčko, K., Hošnjak; L., Kušar, B.; Zorec, T. M.; Kocjan, B. J.; Križmarić, M. and Poljak, M. (2018). Clinical, histopathological, and virological evaluation of 203 patients with a clinical diagnosis of molluscum contagiosum. In Open forum infectious diseases, 5(11): 298. US: Oxford University Press.

Tuncel, A.A. and Erbagci, Z., 2005. Prevalence of skin diseases among male adolescent and post-adolescent boarding school students in Turkey. The Journal of dermatology, 32(7), pp.557-564.

Van Der Wouden, J.C., Koning, S. and Katz, K.A., 2018. Interventions for nongenital molluscum contagiosum in persons without immune deficiency. Jama dermatology, 154(2), pp.203-204.

Vardhan, P., Goel, S., Goyal, G. and Kumar, N., 2010. Solitary giant molluscum contagiosum presenting as lid tumor in an

immunocompetent child. Indian journal of ophthalmology, 58(3), p.236.

Vora, R.V., Pilani, A.P. and Kota, R.K., 2015. Extensive giant molluscum contagiosum in a HIV positive patient. Journal of clinical and diagnostic research: JCDR, 9(11), p.WD01.

Watanabe, T., Morikawa, S., Suzuki, K., Miyamura, T., Tamaki, K. and Ueda, Y., 1998. Two major antigenic polypeptides of molluscum contagiosum virus. Journal of Infectious Diseases, 177(2), pp.284-292.

Wohl, S., Schaffner, S.F. and Sabeti, P.C., 2016. Genomic analysis of viral outbreaks. *Annual review of virology*, *3*, pp.173-195.

Zhang, Z., Schwartz, S., Wagner, L. and Miller, W., 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational biology*, *7*(1-2), pp.203-214.

Zloto, O. and Rosner, M., 2015. The Different Presentations of Periocular Molluscum Contagiosum. *Harefuah*, *154*(11), pp.703-7.

Zorec, T.M., Kutnjak, D., Hošnjak, L., Kušar, B., Trčko, K., Kocjan, B.J., Li, Y., Križmarić, M., Miljković, J., Ravnikar, M. and Poljak, M., 2018. New Insights into the evolutionary and genomic landscape of Molluscum Contagiosum Virus (MCV) based on nine MCV1 and six MCV2 complete genome sequences. *Viruses*, *10*(11), p.586.

Appendix

Appendix 1

Patients Questionaire

Age :

Gendr:

Residence:

Site of lesions in the body :

Molluscum contagiosum virus concomitant diseases:

Previous infection with Molluscum contagiosum virus :

Size of lesion:

Duration of infection:

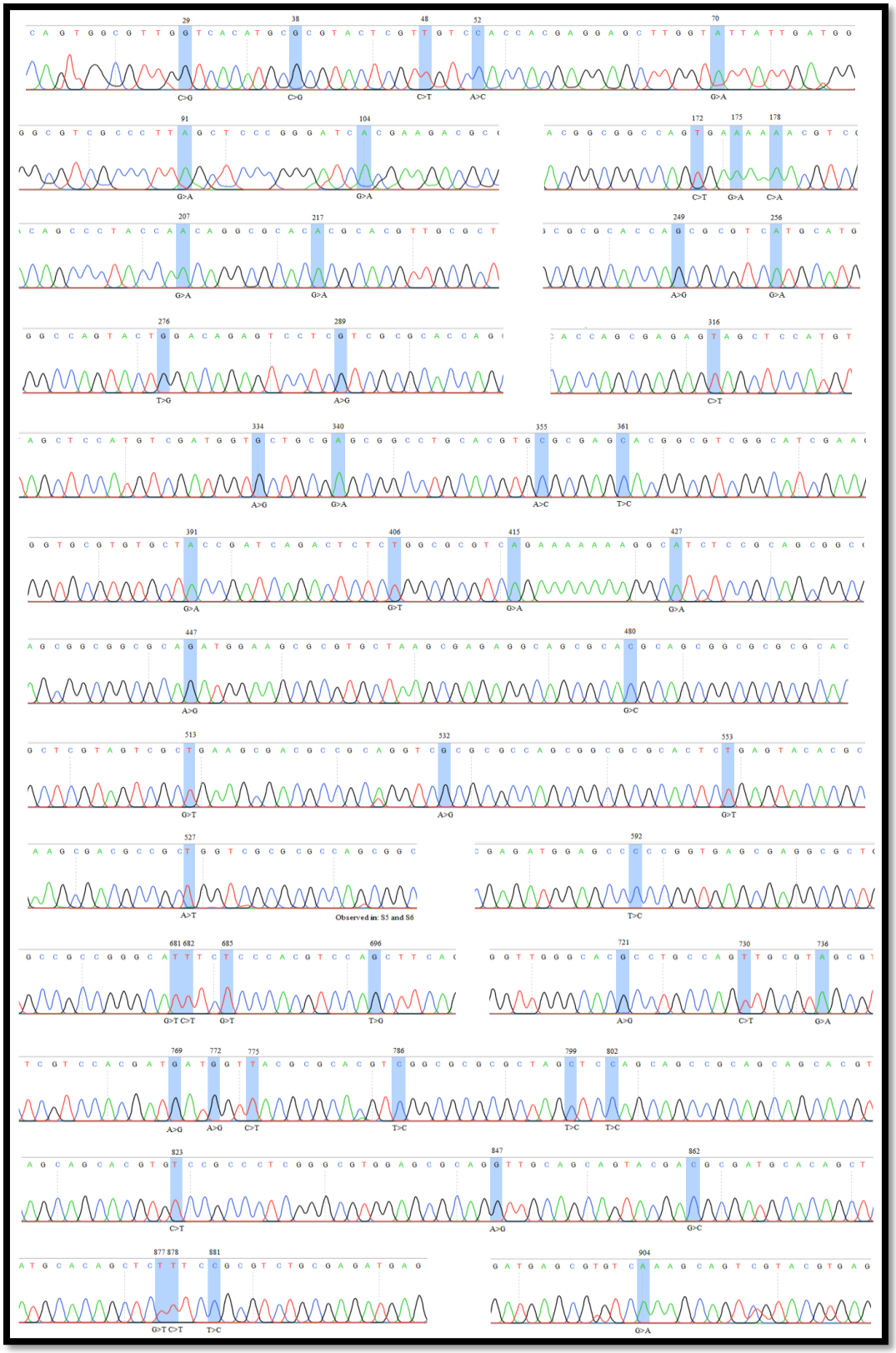
Season of infection:

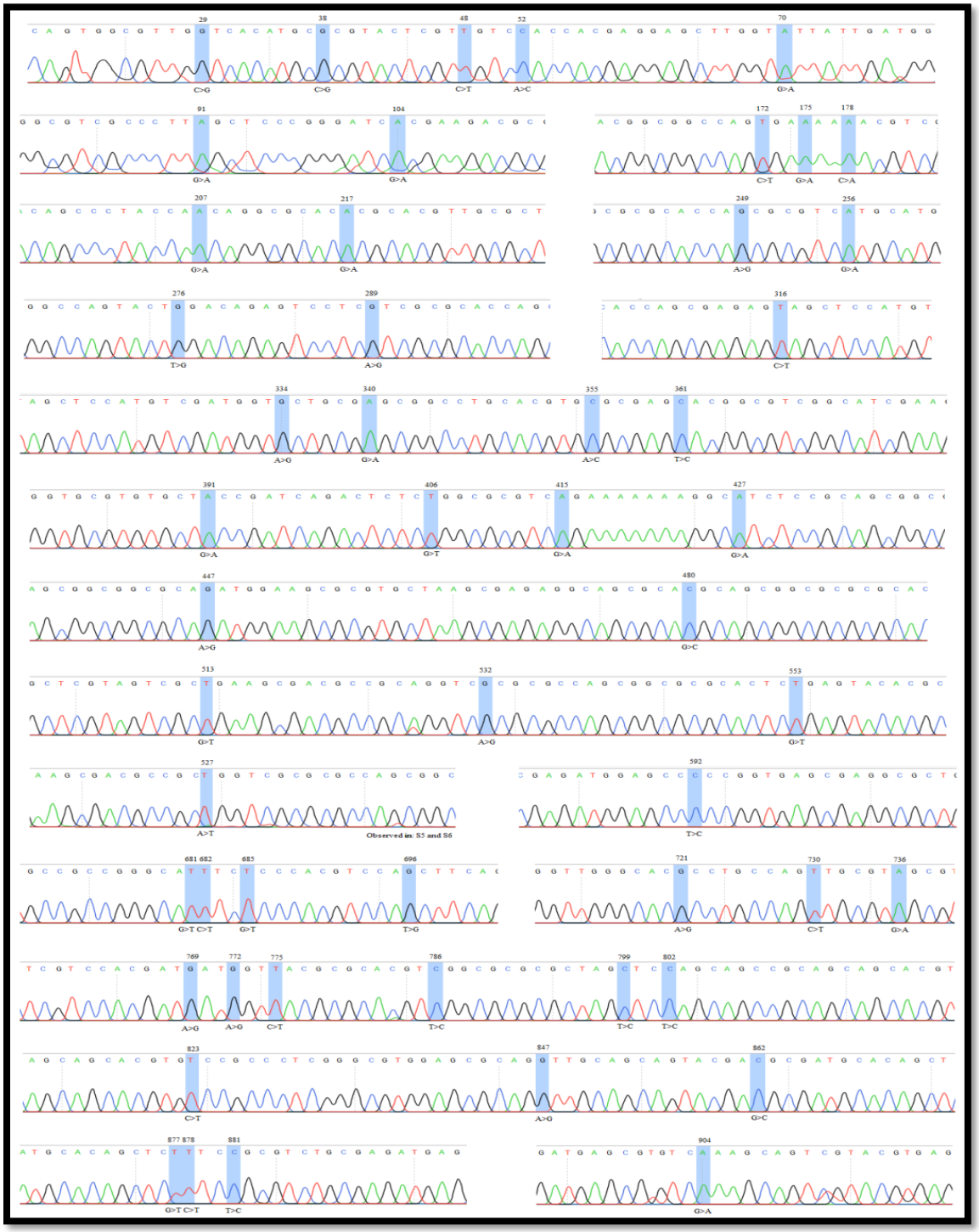
Nature of lesions:

Frequency of lesions:

Gene detection rate MC002L with MCV liesion:

Appendix 2 :


29 38 48 52 70
C>G C>G C>T A>C G>A
91 104
G>A G>A
172 175 178
C>T G>A C>A
207 217
G>A G>A
249 256
A>G G>A
276 289
T>G A>G
316
C>T
334 340 355 361
A>G G>A A>C T>C
391 406 415 427
G>A G>T G>A G>A
447 480
A>G G>C
513 532 553
G>T A>G G>T
527 592
A>T T>C
Observed in: S5 and S6
681 682 685 696
G>T C>T G>T T>G
721 730 736
A>G C>T G>A
769 772 775 786 799 802
A>G A>G C>T T>C T>C T>C
823 847 862
C>T A>G G>C
877 878 881 904
G>T C>T T>C G>A



The chromatogram of the investigated samples of Molluscum contagiosum virus samples. The clear peaks of each nucleotide refer to the strict contamination-free technical parameters followed to validate each variant in the present samples. The letter "S" refers to the code of the investigated samples in this study. All variations were observed in all (S1 – S6) samples unless otherwise indicated.

Appendix 3a,b :

A)sequences of amino acid residues within PCR amplicons

```
          10      20      30      40      50      60      70      80      90      100
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.KIVETLPATLPLALPAGSMLTYDCFDTLISQTQSELCIASYCCNLRSTPEGGHVLLRLLELARANVRVTIIVDEQSRDADATQLAGVPNLRYLK
MDVGEL
S1  ..............................RK.............................D.............................L....M
S2  ..............................RK.............................D.............................L....M
S3  ..............................RK.............................D.............................L....M
S4  ..............................RK.............................D.............................L....M
S5  ..............................RK.............................D.............................L....M
S6  ..............................RK.............................D.............................L....M

          110     120     130     140     150     160     170     180     190     200
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.PGGKPGSLLSSFWVSDKRRFYLGSASLTGGSISTIKSLGVYSECAPLARDLRRRFRDYERLCARRCLRCLSLSTRFHLRRRCGDAFFSDAPESL
IGSTRT
S1  ..................................................S.........V.....................
S2  ..................................................S.........V.....................
S3  ..................................................S.........V.....................
S4  ..................................................S.........V.....................
S5  ..................................................Q....S.........V.....................
S6  ..................................................Q....S.........V.....................

          210     220     230     240     250     260     270     280     290     300
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.FDADAVLAHVQAARSTIDMELLSLVPLVRDEDSVKYWPRMHDALVRAALERNVRVRLLVGLWHRSDVFSLAAVKGLHELGVGHADISVR
VFAIPGAKGDA
S1  ..............................Q.....................................V.......
S2  ..............................Q.....................................V.......
S3  ..............................Q.....................................V.......
S4  ..............................Q.....................................V.......
S5  ..............................Q.....................................V.......
```

B)sequences of amino acid residues within the entire protein

The entire sequence of the reference of MC021L gene-encoded protein

MGNLTSAQPAGCKIVETLPATLPLALPAGSMLTYDCFDTLISQTQSELCIASYCCNLRSTPEGGHVLLRLLELARAN
VRVTIIVDEQSRDADATQLAGVPNLRYLKMDVGELPGGKPGSLLSSFWVSDKRRFYLGSASLTGGSISTIKSLGVYS
ECAPLARDLRRRFRDYERLCARRCLRCLSLSTRFHLRRRCGDAFFSDAPESLIGSTRTFDADAVLAHVQAARSTIDM
ELLSLVPLVRDEDSVKYWPRMHDALVRAALERNVRVRLLVGLWHRSDVFSLAAVKGLHELGVGHADISVRVFAIP
GAKGDAINNTKLLVVDDEYVHVSNADIDGTHYARHAFVSFNCAERTFARALGALFERDWQSSFSSPLPRALPPEPAT
LLSVN

The entire sequence of the mutant samples (S1, S2, S3, S4) of MC021L gene-encoded protein

MGNLTSAQPAGCKIVETLPATLPLALPAGSMLTYDCFDTLISQTRKELCIASYCCNLRSTPEGGHVLLRLLELARAD
VRVTIIVDEQSRDADATQLAGVPNLRYLKLDVGEMPGGKPGSLLSSFWVSDKRRFYLGSASLTGGSISTIKSLGVYS
ECAPLARDLRRRFSDYERLCARRCVRCLSLSTRFHLRRRCGDAFFSDAPESLIGSTRTFDADAVLAHVQAARSTIDM
ELLSLVPLVRDEDSVQYWPRMHDALVRAALERNVRVRLLVGLWHRSDVFSLAAVKGLHELGVGHADISVRVFVIP
GAKGDAINNTKLLVVDDEYVHVSNADIDGTHYARHAFVSFNCAERTFARALGALFERDWQSSFSSPLPRALPPEPAT
LLSVN

The entire sequence of the mutant samples (S5, S6) of MC021L gene-encoded protein

MGNLTSAQPAGCKIVETLPATLPLALPAGSMLTYDCFDTLISQTRKELCIASYCCNLRSTPEGGHVLLRLLELARAD
VRVTIIVDEQSRDADATQLAGVPNLRYLKLDVGEMPGGKPGSLLSSFWVSDKRRFYLGSASLTGGSISTIKSLGVYS
ECAPLARDQRRRFSDYERLCARRCVRCLSLSTRFHLRRRCGDAFFSDAPESLIGSTRTFDADAVLAHVQAARSTIDM
ELLSLVPLVRDEDSVQYWPRMHDALVRAALERNVRVRLLVGLWHRSDVFSLAAVKGLHELGVGHADISVRVFVIP
GAKGDAINNTKLLVVDDEYVHVSNADIDGTHYARHAFVSFNCAERTFARALGALFERDWQSSFSSPLPRALPPEPAT
LLSVN

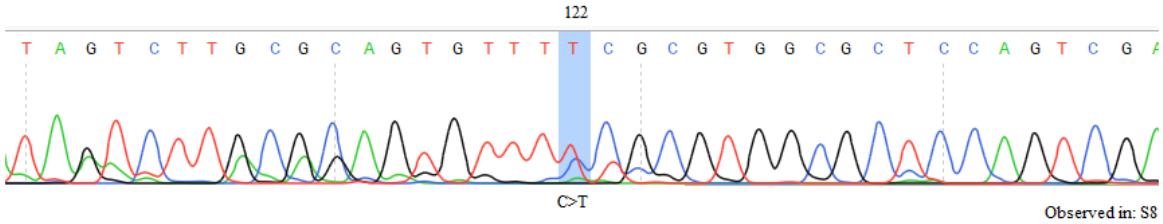
Amino acid residues alignment of the detected variations of the -encoded palmytilated EEV membrane glycoprotein within the investigated samples of Molluscum contagiosum virus samples. A) The amino acid substitutions are highlighted according to their corresponding positions within the amplified 979 bp locus. B) The amino acid substitutions are highlighted according to their corresponding positions within the entire protein. The grey highlights refer to the amplified region of the MCO21L-encoded glycoprotein. The cyan colors refer to the amino acid substitutions in the alignment chart.

Appendix 4:

```
10      20      30      40      50      60      70      80      90      100
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.GCTTTTCAGTTTTTGTGCGAAACTTACTCGTATACCATGTCGTCTCTGTAGCCTGCAGGCACGCGCCCTGTCTGGACGTTAATC
TTGCGGACTAGCGCCA
S7  ..........................................................................................
S8  ..........................................................................................

          110     120     130     140     150     160     170     180     190     200
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.GCATAGTCTTGCGCAGTGTTTCCGCGTGGCGCTCCAGTCGAGAAAGGCCCTCGGACACGGTACTGCAGCAGTTCACAACATCT
GTGTGGTCCTTAGCTAG
S7  ..........................................................................................
S8  .....................T..........................................................................

          210     220     230     240     250     260     270     280     290     300
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.CGTGGTTACCCTGTCCTCGAGCTCCCGAATCAGCCTCACCGAGTTTGCGAGAGAACCTAGATCTCCACGACGACGAGCAATGT
CTGCTCCGGGATCCTCG
S7  ..........................................................................................
S8  ..........................................................................................

          310     320     330     340     350     360     370     380     390     400
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.TCTTGAAGCCCTACGGGGATCGTGTTTTCTGTCTGATCGGCGACGGCGCCAGGCTCGTCCTGTCCTTCGGAGAGCTTTGTATC
TTTGCTCTCGTCACGTG
S7  ..........................................................................................
S8  ..........................................................................................

          410     420     430     440     450     460     470     480     490     500
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.CCGCGGGAGTAAGGTCTTCATCGGTTAGCTGGGTCCCGCACTCCTCCGGAGTGATGAGCTTTGCCAGGGACAGGTACGGGTC
TGGCGTGCCTGTCGCTGC
S7  ..........................................................................................
S8  ..........................................................................................
          510     520     530     540     550     560     570
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.TTCTGAAGTCGGGGGGATCACCAACAGCGCGCTAATGTCGCTACCATAGTTCATATTCTTGATGGGCACTCCTCC
S7  ...........................................................................
S8  ...........................................................................
```

Nucleic acid sequences alignment of two samples with their corresponding reference sequences of the 575 bp amplicons of the MC133L genetic sequences. The symbol "ref" refers to the NCBI referring sequence, letters "S", followed by a number refers to the sample number

Appendix 5:



The chromatogram of the investigated Molluscum contagiosum viral samples.The letter "S" refers to the code of the investigated samples in this study. All variations were observed in both (S7, S8) samples unless otherwise indicated. The presence of a double-peaks may refer to the occurrence of a double infection possibility.

Appendix 6,a,b

A) sequences of amino acid residues within PCR amplicons

```
          10        20        30        40        50        60        70        80        90       100
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.MADSEAVDPTPSGDTSSAGNTGGTSSAGNTGDTSSAGNTGDTSSAGNTGETTGSSSAGSTDATTAHAAGTTPTPVPVPEPAVGVPSSIVD
FAKLVNNTWN
S7  ..........................................................................................
S8  ..........................................................................................

          110       120       130       140       150       160       170       180       190       200
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.TALTSTICLHRQQRDAIRNVLRGYMSKNNTNNNESGVSASVPAAATSQDVAPADILSYPYMLGNSYYRQLMSYDQMQALVEMVPRSNT
VDTVGKYVLYMV
S7  ..........................................................................................
S8  ..........................................................................................

          210       220       230       240       250       260       270       280       290       300
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.NYYMTVGQEQVSLRTASDPNYGPSVLTIMMHKILNRVYEIHTRTQCKYMFVGIPSYYWHGVSVSALQDWGAGLLRESSMKLYAAYLR
FIAQNPRDIPSAR
S7  ..........................................................................................
S8  ..........................................................................................

          310       320       330       340       350       360       370       380       390       400
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.LKVEYMGESWNFRRSVPEFAMEALCFRADDELTRYDLSTVRVYVDKVATDSYSYYEYPVLDSSAGAAGVTSKGGVPIKNMNYGSDISA
LLVIPPTSEAAT
S7  ..........................................................................................
S8  ..........................................................................................

          410       420       430       440       450       460       470       480       490       500
    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.GTPDPYLSLAKLITPEECGTQLTDEDLTPAARDESKDTKLSEGQDEPGAVADQTENTIPVGLQDEDPGADIARRRGDLGSLANSVRLIRE
LEDRVTTLAK
S7  ..........................................................................................
S8  ..........................................................................................

          510       520       530       540       550
    ....|....|....|....|....|....|....|....|....|....|
ref.DHTDVVNCCSTVSEGLSRLERHAETLRKTMLALVRKINVQTGRVPAGYRDDMVYE
S7  ...................................................
S8  ..................K............................
```

B) sequences of amino acid residues within the entire protein

The entire sequence of the reference of MC133L gene-encoded protein

MADSEAVDPTPSGDTSSAGNTGGTSSAGNTGDTSSAGNTGDTSSAGNTGETTGSSSAGSTDATTAHAAGTTPTP
VPVPEPAVGVPSSIVDFAKLVNNTWNTALTSTICLHRQQRDAIRNVLRGYMSKNNTNNNESGVSASVPAAATSQ
DVAPADILSYPYMLGNSYYRQLMSYDQMQALVEMVPRSNTVDTVGKYVLYMVNYYMTVGQEQVSLRTASDP
NYGPSVLTIMMHKILNRVYEIHTRTQCKYMFVGIPSYYWHGVSVSALQDWGAGLLRESSMKLYAAYLRFIAQN
PRDIPSARLKVEYMGESWNFRRSVPEFAMEALCFRADDELTRYDLSTVRVYVDKVATDSYSYYEYPVLDSSAG
AAGVTSKGGVPIKNMNYGSDISALLVIPPTSEAATGTPDPYLSLAKLITPEECGTQLTDEDLTPAARDESKDTKLS
EGQDEPGAVADQTENTIPVGLQDEDPGADIARRRGDLGSLANSVRLIRELEDRVTTLAKDHTDVVNCCSTVSEG
LSRLERHAETLRKTMLALVRKINVQTGRVPAGYRDDMVYE

The entire sequence of the mutant samples (S7) of MC133L gene-encoded protein

MADSEAVDPTPSGDTSSAGNTGGTSSAGNTGDTSSAGNTGDTSSAGNTGETTGSSSAGSTDATTAHAAGTTPTP
VPVPEPAVGVPSSIVDFAKLVNNTWNTALTSTICLHRQQRDAIRNVLRGYMSKNNTNNNESGVSASVPAAATSQ
DVAPADILSYPYMLGNSYYRQLMSYDQMQALVEMVPRSNTVDTVGKYVLYMVNYYMTVGQEQVSLRTASDP
NYGPSVLTIMMHKILNRVYEIHTRTQCKYMFVGIPSYYWHGVSVSALQDWGAGLLRESSMKLYAAYLRFIAQN
PRDIPSARLKVEYMGESWNFRRSVPEFAMEALCFRADDELTRYDLSTVRVYVDKVATDSYSYYEYPVLDSSAG
AAGVTSKGGVPIKNMNYGSDISALLVIPPTSEAATGTPDPYLSLAKLITPEECGTQLTDEDLTPAARDESKDTKLS
EGQDEPGAVADQTENTIPVGLQDEDPGADIARRRGDLGSLANSVRLIRELEDRVTTLAKDHTDVVNCCSTVSEG
LSRLERHAETLRKTMLALVRKINVQTGRVPAGYRDDMVYE

The entire sequence of the mutant samples (S8) of MC133L gene-encoded protein

MADSEAVDPTPSGDTSSAGNTGGTSSAGNTGDTSSAGNTGDTSSAGNTGETTGSSSAGSTDATTAHAAGTTPTP
VPVPEPAVGVPSSIVDFAKLVNNTWNTALTSTICLHRQQRDAIRNVLRGYMSKNNTNNNESGVSASVPAAATSQ

DVAPADILSYPYMLGNSYYRQLMSYDQMQALVEMVPRSNTVDTVGKYVLYMVNYYMTVGQEQVSLRTASDP
NYGPSVLTIMMHKILNRVYEIHTRTQCKYMFVGIPSYYWHGVSVSALQDWGAGLLRESSMKLYAAYLRFIAQN
PRDIPSARLKVEYMGESWNFRRSVPEFAMEALCFRADDELTRYDLSTVRVYVDKVATDSYSYYEYPVLDSSAG
AAGVTSKGGVPIKNMNYGSDISALLVIPPTSEAATGTPDPYLSLAKLITPEECGTQLTDEDLTPAARDESKDTKLS
EGQDEPGAVADQTENTIPVGLQDEDPGADIARRRGDLGSLANSVRLIRELEDRVTTLAKDHTDVVNCCSTVSEG
LSRLERHAKTLRKTMLALVRKINVQTGRVPAGYRDDMVYE

Amino acid residues alignment of the detected variations of the MC133L-encoded protein within the investigated samples of Molluscum contagiosum virus samples. A) The amino acid substitutions are highlighted according to their corresponding positions within the amplified 575 bp locus. B) The amino acid substitutions are highlighted according to their corresponding positions within the entire protein. The grey highlights refer to the amplified region of the MC133L-encoded protein. The cyan colors refer to the amino acid substitutions in the alignment chart.The cyan colors refer to the amino acid substitutions in the alignment chart.

Appendix7:
Registration for isolates L LC629161 on the NCBI website.

LOCUS LC629161 501 bp DNA linear VRL 07-MAY-2021
DEFINITION Molluscum contagiosum virus ST20214 gene for MC133L, partial sequence.
ACCESSION LC629161
VERSION LC629161.1
KEYWORDS .
SOURCE Molluscum contagiosum virus
ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota; Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae; Molluscipoxvirus.
REFERENCE 1
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Phylogenitic analysis of Molluscum contagiosum virus isolated from different from human body
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 501)
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Direct Submission
JOURNAL Submitted (25-APR-2021) Contact:Sameer Mohammed Dheyab Diyala University, College of Science, Department of Biology; Diyala, Diyala, Diyala 32001, Iraq
FEATURES Location/Qualifiers
source 1..501
/organism="Molluscum contagiosum virus"
/mol_type="genomic DNA"
/strain="ST20214"
/isolation_source="The lesion curettage"
/host="Homo sapiens"
/db_xref="taxon:10279"
/country="Iraq"
/collection_date="2020-10-01"
/collected_by="Sameer Mohammed Dheyab"
gene<1..>501
/gene="MC133L"
misc_feature<1..>501
/gene="MC133L"
/note="MC133L; coding region not dermined"
ORIGIN
1 cgacttcaga agcagcgaca ggcacgccag acccgtacct gtccctggca aagctcatca
61 ctccggagga gtgcgggacc cagctaaccg atgaagacct tactcccgcg gcacgtgacg
121 agagcaaaga tacaaagctc tccgaaggac aggacgagcc tggcgccgtc gccgatcaga
181 cagaaaacac gatccccgta gggcttcaag acgaggatcc cggagcagac attgctcgtc
241 gtcgtggaga tctaggttct ctcgcaaact cggtgaggct gattcgggag ctcgaggaca
301 gggtaaccac gctagctaag gaccacacag atgttgtgaa ctgctgcagt accgtgtccg
361 agggcctttc tcgactggag cgccacgcgg aaacactgcg caagactatg ctggcgctag
421 tccgcaagat taacgtccag acagggcgcg tgcctgcagg ctacagagac gacatggtat
481 acgagtaagt ttcgcacaaa a
//

Appendix 8:
Registration for isolates LC629162 on the NCBI website.

LOCUS LC629162 902 bp DNA linear VRL 07-MAY-2021
DEFINITION Molluscum contagiosum virus ST20213 gene for putative major envelope protein, partial sequence.
ACCESSION LC629162
VERSION LC629162.1
KEYWORDS .
SOURCE Molluscum contagiosum virus
ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota; Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae; Molluscipoxvirus.
REFERENCE 1
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Phylogenitic analysis of Molluscum contagiosum virus isolated from different from human body
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 902)
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Direct Submission
JOURNAL Submitted (25-APR-2021) Contact:Sameer Mohammed Dheyab Diyala University, College of Science, Department of Biology; Diyala, Diyala, Diyala 32001, Iraq
FEATURES Location/Qualifiers
source 1..902
/organism="Molluscum contagiosum virus"
/mol_type="genomic DNA"
/strain="ST20213"
/isolation_source="The lesion curettage"
/host="Homo sapiens"
/db_xref="taxon:10279"
/country="Iraq"
/collection_date="2020-10-15"
/collected_by="Sameer Mohammed Dheyab"
gene<1..>902
/gene="MC021L"
misc_feature<1..>902
/gene="MC021L"
/note="putative major envelope protein; coding region not determined"

Appendix9:
Registration for isolates LC629163 on the NCBI website.

LOCUS LC629163 902 bp DNA linear VRL 07-MAY-2021
DEFINITION Molluscum contagiosum virus ST20215 gene for putative major envelope protein, partial sequence.
ACCESSION LC629163
VERSION LC629163.1
KEYWORDS .
SOURCE Molluscum contagiosum virus
ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota; Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae; Molluscipoxvirus.
REFERENCE 1
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Phylogenitic analysis of Molluscum contagiosum virus isolated from different from human body
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 902)
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Direct Submission
JOURNAL Submitted (25-APR-2021) Contact:Sameer Mohammed Dheyab Diyala University, College of Science, Department of Biology; Diyala, Diyala, Diyala 32001, Iraq
FEATURES Location/Qualifiers
source 1..902
/organism="Molluscum contagiosum virus"
/mol_type="genomic DNA"
/strain="ST20215"
/isolation_source="The lesion curettage"
/host="Homo sapiens"
/db_xref="taxon:10279"
/country="Iraq"
/collection_date="2020-11-13"
/collected_by="Sameer Mohammed Dheyab"
gene<1..>902
/gene="MC021L"
misc_feature<1..>902
/gene="MC021L"
/note="putative major envelope protein; coding region not determined"

Appendix10:
Registration for isolates LC629164 on the NCBI website.

LOCUS LC629164 902 bp DNA linear VRL 07-MAY-2021
DEFINITION Molluscum contagiosum virus ST20216 gene for putative major envelope protein, partial sequence.
ACCESSION LC629164
VERSION LC629164.1
KEYWORDS .
SOURCE Molluscum contagiosum virus
ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota; Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae; Molluscipoxvirus.
REFERENCE 1
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Phylogenitic analysis of Molluscum contagiosum virus isolated from different from human body
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 902)
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Direct Submission
JOURNAL Submitted (25-APR-2021) Contact:Sameer Mohammed Dheyab Diyala University, College of Science, Department of Biology; Diyala, Diyala, Diyala 32001, Iraq
FEATURES Location/Qualifiers
source 1..902
/organism="Molluscum contagiosum virus"
/mol_type="genomic DNA"
/strain="ST20216"
/isolation_source="The lesion curettage"
/host="Homo sapiens"
/db_xref="taxon:10279"
/country="Iraq"
/collection_date="2021-01-08"
/collected_by="Sameer Mohammed Dheyab"
gene<1..>902
/gene="MC021L"
misc_feature<1..>902
/gene="MC021L"
/note="putative major envelope protein; coding region not determined"

Apendix 11:

Registration for isolates LC629165 on the NCBI website.

LOCUS LC629165 902 bp DNA linear VRL 07-MAY-2021
DEFINITION Molluscum contagiosum virus ST20217 gene for putative major envelope protein, partial sequence.
ACCESSION LC629165
VERSION LC629165.1
KEYWORDS .
SOURCE Molluscum contagiosum virus
ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota; Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae; Molluscipoxvirus.
REFERENCE 1
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Phylogenitic analysis of Molluscum contagiosum virus isolated from different from human body
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 902)
AUTHORS Dheyab,S.M. and Jameel,Z.J.
TITLE Direct Submission
JOURNAL Submitted (25-APR-2021) Contact:Sameer Mohammed Dheyab Diyala University, College of Science, Department of Biology; Diyala, Diyala, Diyala 32001, Iraq
FEATURES Location/Qualifiers
source 1..902
/organism="Molluscum contagiosum virus"
/mol_type="genomic DNA"
/strain="ST20217"
/isolation_source="The lesion curettage"
/host="Homo sapiens"
/db_xref="taxon:10279"
/country="Iraq"
/collection_date="2021-04-01"
/collected_by="Sameer Mohammed Dheyab"
gene<1..>902
/gene="MC021L"
misc_feature<1..>902
/gene="MC021L"
/note="putative major envelope protein; coding region not determined"

.

الخلاصة

فيروس المليساء المعدية (MCV) هو أحد أفراد عائلة الجدري الذي يصيب جلد الإنسان وهو شديد العدوى في جميع الأعمار ، ولكنه أكثر انتشارًا في الأعمار من (1-10) سنوات ، وكذلك المرضى الذين يعانون من ضعف المناعة مثل الأشخاص المصابون بفيروس نقص المناعة البشرية. تهدف الدراسة إلى الكشف الجزيئي عن MCV باستخدام تقنية تفاعل البلمرة المتسلسل (PCR) ، متبوعًا بتسلسل النوكليوتيدات الفيروسية وتحليل النشوء والتطور. أيضًا لاستكشاف العلاقة بين عدوى MCV وبعض عوامل الخطر الاجتماعية والديموغرافية والسريرية.

اجريت هذه الدراسة المقطعية لمحافظة ديالى للمدة الممتدة من 1 تشرين الاول2020 حتى 1 نيسان 2021. تمت الموافقة على الدراسة من قبل اللجنة العلمية في كلية العلوم واللجنة العلمية لدليل ديالى الصحي. تم تضمين ما مجموعه 101 مريض يشتبه سريريًا في إصابتهم بآفة MCV في أجزاء مختلفة من أجسامهم. تم تخصيصها من مستشفى بعقوبة التعليمي ، قسم الاستشارات ، وبعض مراكز الرعاية الصحية الأولية تابعة لدليل ديالى الصحي. تراوحت الأعمار من 5 شهور إلى 60 سنة من كلا الجنسين. تم جمع خزعات نسيج الآفة الجلدية باستخدام مكشطة الأنسجة بواسطة أطباء الأمراض الجلدية. تم إجراء تفاعل البوليميراز المتسلسل التقليدي باستخدام بادئات خاصة. تم إجراء التسلسل الجيني في كوريا وتم إجراء تحليل النشوء والتطور المؤقت. تم إجراء التحليل الإحصائي المؤقت باستخدام برنامج SPSS (الإصدار 27) واعتبرت قيم P أقل من 0.05 ذات دلالة.

كان معدل الكشف عن الجينات MC133L و MC021L و MC002L بين عينات الجلد 2.0% و 18.8% و 84.2% على التوالي.

اظهر تحليل التسلسل الزوج الأساسي 979 لجين MC021L في 6عزلات من MCV ، وعزلتان من MCV لجين MCI 3L مع 575 نقطة أساس عند مقارنة كل من هذه العزلات مع NCBI. فيما يتعلق بموضع MC021L gene ، أشارت النتائج إلى وجود 51 اختلافًا في الحمض النووي موزعة في جميع العينات التي تم فحصها تقريبًا. أظهرت غالبية الاختلافات المحددة في جين MC021L توزيعًا متساويًا تقريبًا بين العينات الفيروسية التي تم فحصها. أشارت نتائج الشجرة الشاملة إلى أن جميع العينات الفيروسية التي تم فحصها تنتمي إلى نوع فرعي جديد يقع بين كلا النوعين الفرعيين الأول والنوع الثاني. لم يتم تقييم هذا التوزيع الجديد الذي تشغله هذه العزلات

من خلال أي متواليات أخرى ذات صلة. تم التأكد من عدم وجود العزلات المفحوصة بين أي من هذه الأنواع الفرعية المعروفة بينما أظهر التباين الجيني القائم على MC133L متغير واحد فقط من الحمض النووي المشار إليه.

تم تسجيل خمس عزلات في NCBI ، عزلة واحدة من جين MC133L ، وكان الرقم التسلسلي للعزلاتLC629161 ، وأربع عزلات من جين MC021L ، والرقم التسلسلي للعزلات كان LC629162، LC629163، LC629164، LC629165.

ارتبط جين MC002L بشكل غير معنوي بالعمر والجنس والمستويات التعليمية والسمات السريرية وبعض خصائص الآفة. ومع ذلك ، فهو أعلى بشكل ملحوظ بين المناطق الريفية (P-0.037) ، وموسم الشتاء (P = 0.001) ، وتواتر الآفات 1-4 (P = 0.035).

بناءً على الموضع MC021L ، خلصت الدراسة الحالية إلى أن التسلسلات الجديدة التي تم فحصها والتي تم تأكيدها لا تنتمي إلى أي نوع فرعي معروف. وهكذا ، وثقت الدراسة الحالية ظهور نوع فرعي جديد من MCV يمكن وضعه بين النوعين الفرعيين الأول والنوع الثاني ، تاركًا الباب مفتوحًا لمزيد من الدراسات.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى/كلية العلوم

قسم علوم الحياة

التحليل التطوري الوراثي لفيروس المليساء المعدية المعزولة من مناطق مختلفة من جسم الإنسان

رسالة مقدمة الى

مجلس كلية العلوم – جامعة ديالى

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل الطالب

سمير محمـد ذياب

بكالوريوس علوم حياة / جامعة بغداد (2004)

بإشراف

أ.م.د. زهراء جعفر جميل

1443هـ 2021 م