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Environmental Evaluation and Molecular characterization of *Molluscum contagiosum virus* (MCV) in Misan province/Iraq

A thesis

Submitted to the Council of the College of Science / University of Misan as partial Fulfillment of the Requirements for the Master Degree in Biology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اللَّهُ نَزَّلَ أَحْسَنَ الْحَدِيثِ كِتَابًا مُّتَشَابِهًا مَّثَانِيَ تَقْشَعِرُّ مِنْهُ جُلُودُ الَّذِينَ
يَخْشَوْنَ رَبَّهُمْ ثُمَّ تَلِينُ جُلُودُهُمْ وَقُلُوبُهُمْ إِلَى ذِكْرِ اللَّهِ ذَلِكَ هُدَى اللَّهِ يَهْدِي
بِهِ مَن يَشَاءُ وَمَن يُضْلِلْ اللَّهُ فَمَا لَهُ مِنْ هَادٍ

صَدَقَ اللهُ العَلِيُّ العَظِيمْ

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Dedication

I dedicate the fruits of my efforts and the achievement of my goal to God Almighty

I dedicate the fruits of my efforts to the Imam of the Age and Time, Imam Mahdi (may God hasten his reappearance).

I dedicate this effort to my dear Assist. Prof. Dr. Niran, out of respect and appreciation for her.

To the light that illumine

ted my path, to those who were the first support to achieve my ambition, to those who were my refuge and my right hand in this stage, to those who gave me my life and my self-esteem, to those whose prayers surrounded me,

My father and mother

To the love of my heart, to

My brothers and sisters.

To those who gave me their effort and time

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Summary

The current study investigated the p43K gene of the *Molluscum contagiosum virus*, which causes pearl disease in Maysan Province. This gene was detected in environmental samples of swimming pool water and clinical samples taken from patients with pearl disease, which were diagnosed clinically, using molecular and histopathological methods, and examination by transmission electron microscopy. Thus, the study addressed two aspects: studying environmental samples of swimming pool water and studying clinical samples taken from patients with pearl disease. The environmental study focused on examining pool water samples in Maysan Provence using molecular methods, using PCR technology, to detect the *Molluscum contagiosum virus*. Twenty water samples were collected from swimming pools in Maysan Province, and the results were negative. We did not find any link between its transmission through swimming pools and the use of correct sterilization methods and the continuous treatment of swimming pools in Maysan Province by pool workers. The pH and temperature of the pools in Maysan were also measured, with pH levels ranging from 6.5 to 7.4. The results of measuring the temperature of the swimming pools ranged from 24 to 28 degrees Celsius. As for the study of clinical samples isolated from people infected with pearly disease, 140 patients infected with pearly disease, which is primarily caused by the *Molluscum contagiosum virus*, were collected from Al-Sadr Teaching Hospital between November 2023 and April 2024. In Maysan Province, males and females aged (1-60) years. The study focused on the infection rate, the highest rate was in the age group of (1-10) years at 45% and was less common in the age group of (51-60) years at 1%. The study also revealed that the infection rate was higher in males at 51% than in females at 49%. Also, urban areas had a higher infection rate of 77% than rural areas at 23%. Also, the infection sites were more severe in the face and neck at 90% higher than in the genital area at 10%. Thus, the study showed continuous mixing, lack of health awareness, failure to use correct sterilization methods, weak immunity, other skin diseases associated with the disease and other external factors are among the causes of infection with the *Molluscum contagiosum virus*. The studied gene sequences were placed in the National Center for Biotechnology Information (NCBI) and accession numbers were taken for all sequences that were studied and analyzed. Ten GenBank accession numbers of the P43K amplicons of PQ816764 to PQ816773 were deposited in NBCI to represent the A1 to A10

samples, respectively. To clarify the evolutionary relationship between the samples we studied and the reference strains that are related to the amplified molluscum contagiosum virus samples, a genetic tree was constructed based on the DNA sequences of the p43k gene, which included the amplified samples and the samples related to the virus strains.A rectangular cladogram was generated to illustrate the Molluscum contagiosum virus sequences with their corresponding sequences .The total number of aligned nucleic acid sequences in the generated tree was twenty-six. The evolutionary relationships of the taxa studied were inferred by methods: Neighbor--Joining . Evolutionary tree with all branch lengths = 1.32550160. The percentage of similar trees that group related taxa together in a reassignment test (1000 replicates)is shown next to the branches. The genetic tree was drawn to scale, and the branch lengths were plotted using units of evolutionary distance. These distances were calculated using the maximum composite method, which is the number of base substitutions per site.Based on the deposited annotation of the P43K gene in the GenBank OQ401160.2, the third reading frame of the codon positions is included. There were a total of 41 positions in the final dataset. The appearance of the *Molluscum contagiosum virus* under the electron microscope was significantly larger than in other studies. The presence of Henderson-Paterson bodies under the light microscope is indicative of *Molluscum contagiosum* infection.

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List of Abbreviations

The word	Abbreviate
Acquired Immune Deficiency Syndrome	AIDS
base pair	bp
DPX	Distyrene, plasticizer and xylene
Extracellular (envelope) virion	EV
Glycosaminoglycans	GAGs
Intracellular mature virus	IMV
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
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
Bacillus amyloliquefaciens	Bam.H1
Nuclear facter kappa B cells	NF-KB
VB	Veronal Buffer
GST	Glutathione s Transferase

Chapter one

Introduction

1.Introduction

Water pollution is one of the most serious environmental problems, directly impacting the quality and accessibility of water resources, threatening the sustainability of ecosystems and human health. It occurs due to the introduction of polluting and harmful substances into water bodies as a result of various human practices, most notably industrial activities and random and improper waste disposal (Lam and Li, 2019).Water pollutants vary in nature; they may be chemical, biological, or physical. These pollutants lead to numerous harmful effects, including the death of aquatic organisms and the spread of waterborne diseases(Omar and Noguchi, 2020).

Bacteria, protozoa, fungi, and viruses are among the most important infectious agents that pose a health threat to pool users. Viruses are the primary cause of most waterborne infections in pools, lakes, ponds, thermal spas, rivers, and hot springs. These contaminants often enter water bodies through the inadvertent release of human waste or body fluids—such as saliva and mucus—or through the shedding of skin cells, whether from asymptomatic or symptomatic individuals (Bonadonna and La Rosa , 2019).

There are studies that have praised the swimming pool as an effective role in the transmission of infections in swimming pools. Water pollution occurs through the feces that swimmers accidentally or through the use of polluted water (Barna and Kadar, 2012). 48% of virus spreads occurred in swimming pools, between 40% in lakes or ponds, and the remaining 12%, in fountains, hot springs, and rivers, was 4% (Sinclair *et al.,* 2009).

There are evidences that the skin is the main source of virus infection, so swimming pools are also a pathogen, so it is necessary to pay attention to the quality of swimming pools to ensure the safety of swimmers (Lakind *et al.,* 2012). The main categories of which are human pollutants and reduce their occurrence

by showering, as well as human pollutants that are accidentally released while swimming (Keuten *et al.,* 2012; Keuten *et al.,* 2014).

Infectious molluscum (MC) is a common skin viral infection that affects the skin and sometimes the oral mucosa, caused by the infectious molluscum (MCV), a dual-chain DNA virus that belongs to the *Poxviridae* family. Infection usually occurs through direct contact with the affected skin, by self-pollination, swimming pools, or contact of contaminated surfaces and tools (Chaurasia *et al.,* 2024). Infectious molluscum, also known as Water warts is a benign skin condition that causes characteristic lesions known as slurred skin (Burrell *et al.,* 2017; Badri *et al.,* 2021).

There are four main Genotypes of MCV-1, MCV-2, MCV-3, and MCV-4 infections. In general, all of these types cause the same clinical symptoms that have skin lesions. Which is limited to the skin, and the first type is more prevalent than other types, and it constitutes about 96.6% of infections, while the second type is limited to patients with weak immunity and constitutes about 60% (Kaufman *et al.,* 2018; Hadlow *et al.,* 2017).

Ultimately, the infectious molluscum virus (MCV) is one of the viruses that are spread globally, with about 122 million cases of infection in 2010. Despite its prevalence in the world, it is spread especially in areas with a warm and humid climate. It is common in children under the age of ten years, and also affects sexually active and immune adults (Leung *et al.,* 2017).

1.1 Aim of the study:

The aim of the study of isolated MCV is to achieve:

- Study of general characteristics and molecular investigation of *Molluscum contagisoum virus* (MCV) from environmental and clinical specimens.
- **Objective of the study**

1. An environmental study of *Molluscum contagiosum virus* (MCV) in swimming pools water samples.
2. A microscopic depiction of the *Molluscum contagiosum virus* (MCV) that infects the skin using light and Transmission electron microscopy (TEM).
3. Study of the molecular characteristics of (MCV) isolated through the phylogenetic tree and bioinformatics of *Molluscum contagiosum virus*.

Chapter Two

Literature Review

2. Literature Review

2.1.Water pollution:

Water has been considered one of the most important natural resources in supporting the life processes of living organisms (Vanloon and Duffy, 2005). Water is important for human existence, when it is polluted it poses a harmful threat, being a means of transmitting diseases (Okoro *et al*., 2012). Pollution is generally defined as the introduction of materials or energy into the environment, whether directly or indirectly, thereby causing damage to living organisms or ecosystems, and it is any physical or chemical change in the quality of water. (Rittmann and McCarty, 2001).

2.1.1.Sources of water pollution:

Pollutants of the aquatic environment can be classified into physical, chemical and biological pollutants:

1. Physical pollutants: include those substances that cause a change in the color and taste of water Thermal pollution is one of the physical sources of water pollution. The high temperature resulting from the release of cooling water from electrical power generation plants and factory waste causes an increase in the temperature .The temperature of the water thus affects the pH and the solubility of the gasses (Al-Shammari, 2005).
2. Chemical pollutants: are produced by both agricultural and industrial activities Close to water, many pollutants accumulate, which often reach sediment riverbeds without treatment and then into groundwater, posing a threat to the environment and health. Pesticides , heavy metal , hydrocarbon component , ect. Are among the main pollutants of water (Barakat, 2007).
3. Biological pollutants: Biological pollutants are found in food residues and waste Human waste is a container that contains a large number of pathogenic

and non pathogenic microorganisms, which are represented by viruses, fungi, protozoa, and bacteria (Al-Fatlawi, 2007).

2.1.2 Physical and chemical specifications:

2.1.2.1Temperature:

Temperature is one of the determining factors for the growth of microorganisms because the temperature tends to be moderate. Microorganisms are stimulated to grow and diversify, while when the temperature tends to rise or fall, this determines the growth and diversity of microorganisms. As the temperature increases, some species prevail over others and this leads to This is due to the imbalance in the natural environmental balance, which is reflected in the numbers of microorganisms, which are used as biological indicators to detect water pollution (Abdel Razzaq ,2017).

2.1.2.2 Potential of Hydrogen (PH):

The pH value reflects and affects life and chemical processes,in the distribution of living organisms, as it is an indicator of the balance of carbons, bicarbonates, and carbon dioxide (Alibi *et al*. 2020). It is considered one of the factors determining bacterial content and growth by influencing the chlorination process. The efficiency of disinfection operations decreases with increasing pH value, because it reduces HCIO, which plays an effective role in the chlorination process (Al-Hashimi ,2012)

2.2 Swimming pool :

Swimming is a popular sport for people of all ages and social and economic statuses, but providing clean and healthy water for swimming constitutes an important source of health. Human contaminants introduced by swimmers into swimming pool water are classified as suspended and colloidal substances, microorganisms, and soluble chemicals. Microorganisms are introduced into the

pool water through several channels, in addition organic and inorganic compounds can be soluble. Fecal-derived and non-fecal-derived pathogens produced by swimmers (viruses, bacteria, and protozoa) have previously been associated with several pool-borne epidemics. (WHO,2006 ; Attallah *et al*., 2024)

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2.2.1 Swimming pools pollution:

Large concrete basins and tanks contain swimming water. This water meets the standards of potable water in terms of taste, color and transparency. Its boiling point is 100 degrees Celsius and its freezing point is 0 degrees Celsius (Cairns and Dickson, 1973).

Environmental pollutants that directly affect outdoor swimming pools are dirt carried by the wind, unhealthy water from unknown sources, and the waste of most animals. Thus, the cause of swimming pool pollution is from the surrounding environment and also swimmers .As for indoor swimming pools, they are less polluted. The direct cause of contamination of indoor swimming pools with microorganisms is the infected person who enters from feces, skin cells, urine, saliva, and cosmetics. The rate of urine urine in the swimming pool has been estimated at 30-80 ml per person (Arnaud, 2016). In addition, chloramine is considered a chemical substance that is dangerous to human health and is produced from the interaction of disinfectants and swimming pool pollutants. Urine and sweat interact with chlorine in swimming pools and produce harmful and dangerous substances to human health, which are both cyanogen chloride and trichloramine (WHO, 2006).

Swimming is one of the widely spread water recreational activities that is in direct contact with water and the entire body (WHO, 2021). Swimming was a healthy and recreational activity, and through it there was social communication between swimmers (Kamioka, *et al* .,2010). If swimming pools are not managed properly,

swimmers are exposed to many microbial infections, the causes of which are from fecal and non-fecal sources (Masoud *et al*.,2016). Swimmers can also be exposed to several chemical, physical and microbiological factors (Fantuzzi *et al*.,2013). Water contamination with feces is a microbiological damage in swimming pools that can occur due to not bathing before swimming, personal hygiene, or not cleansing well after defecation due to the presence of residue on the skin of children and the elderly, as well as accidental release unintentionally or intentionally. Therefore, swimming in poor water constitutes a health risk. On human health, it causes inflammation of the stomach and intestines due to a viral, bacterial, or parasitic infection (Bwire *et al*., 2016).

The environment in swimming pools causes skin conditions for swimmers. Swimming is considered a sport different from other sports in terms of the lack of injuries, which are caused by friction with opponents, surfaces, or equipment. Swimming pools are considered a source of infection with microorganisms that cause skin diseases in the field of sports medicine related to swimming pool activity (Basley *et al*., 2000).

Molluscum contagiosum and warts are two common viral infections of the skin. The MCV is a member the pox viruses , which is self-transmitting and affects the skin. It infects children, sexually active people, and people with weak immunity. It is transmitted from one person to another through direct contact with equipment. In addition, the incubation period for the *Molluscum contagiosum* virus was 2-8 weeks, and the lesions remained for a period of 2-4 months (Kyriakis *et al*.,2007). Several epidemiological studies of the *Molluscum contagiosum virus* among most of the population have confirmed that there is a relationship between the use of swimming pools and the *Molluscum contagiosum* virus (Al Aboud and Nigam, 2021).

2.3 Molluscum contagiosum virus (MCV):

Molluscum contagiosum (MC) is a common viral in skin infection that is classified within, the family of (*Poxviridae*). It affects children. Approximately 7% of children who suffer from immunodeficiency suffer from this condition and are more exposed to viral infections, which are constantly observed, that affects mucosal surfaces (Chen *et al*., 2013). The infection occurs in people between the ages of 1-14 years, as it is uncommon under the age of one (Eichenfield *et al*., 2021).

There are four types of MCV: MCV-1, MCV-2, MCV-3, and MCV-4. MCV only infects humans and does not infect animals. The most common MCVs are MCV-1, which most commonly affects children (with a prevalence ranging from 75% to 96%), and MCV-2, which also infects adults and is sexually transmitted. MCV-3 and MCV-4 are extremely rare (Trčko *et al*., 2018).

The mode of transmission is through direct-contact with the skin, which occurs during sexual or non-sexual. In addition, contaminated object as towels or bath sponges can serveas vectorsfor spreading the infection (Bugert, 2007). There is a historicalrelationship between MCV and swimmingpool use.

2.3.1 *Poxviridae*:

A large family of viruses that is characterized by its complex viral shape, it is has a dsDNA genome, and its multiplication site in the cytoplasm. *Poxviridae* is a tile-like or oval shape with a complex internal structure characterized by a envelope and a biconcave core surrounded by lateral bodies. The structure and morphology of poxviruses is distinct from other viruses. *Poxviridae* do not have the common symmetry features of spiral capsids or nucleocapsids. Virions are assembled in an elegant manner, which it contains a mother membrane of immature virions that develop into mature ones in a process that is unparalleled

in virology. The causative agent of smallpox is the most famous member of the poxvirus family, which is the smallpox virus (Condit *et al*., 2006).

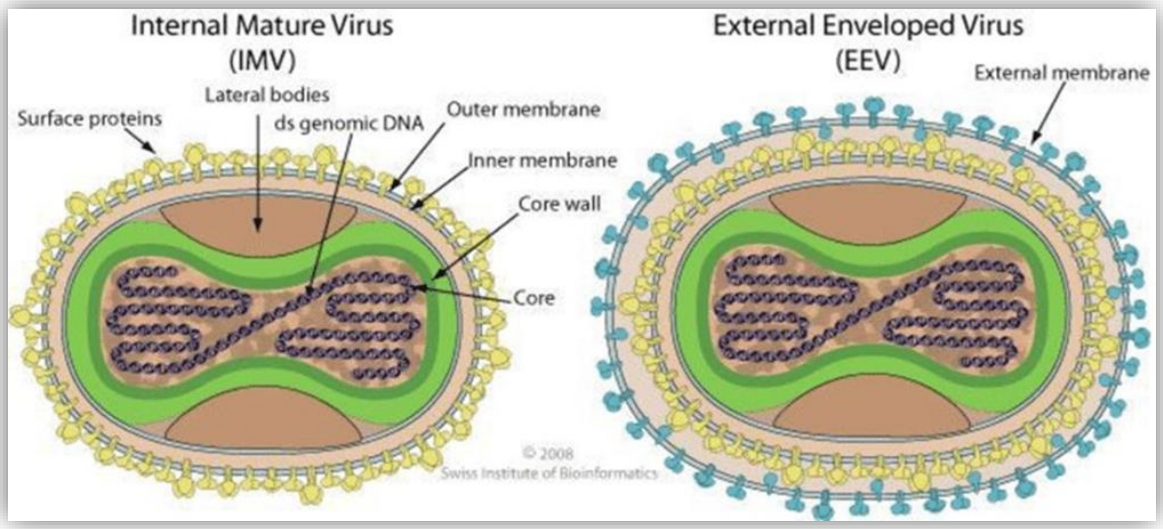
Poxviruses infect vertebrate and invertebrate animals, including humans, although the notorious smallpox has historically been eradicated, making it a major health concern and a serious endemic disease. (Yang *et al.,* 2021) .

2.3.2General properties of Poxviruses:

The virion It is in the form of a complex with protrusions on the outer surface with a diameter of 230 nanometers and a length of 300-400 nanometers, including the main and lateral bodies. Composition of 90% protein, 3% DNA, and 5% fat, The genome It is a double-stranded DNA with a filamentous structure, with the exception of the Parapox virus, 63% in size, 130-375 kilobases at both ends of the loopsand alow concentration of C+G (30-40%). Also the protein in the center, as the viruses contain about more than 100 peptides. To assemble the virus, the development of many membranes is required. As well as replication cytoplasmic factories play an essential role in replication. Furthermore distinctive features Large, complex viruses with high inactivation resistance are their most prominent features. Viruses encoded proteins help to evade the host's immune system (Riddle *et al*., 2019).

2.3.3 Morphology:

Poxviridae are a family of oval, or square, viruses, ranging in length from 220 to 450 nm, in width from 140 to 260 nm, and in thickness from 140 to 260 nm. The genome is a single linear DNA. The double-stranded DNA (dsDNA) molecule is 128 to 375 kb long, with a covalently linked portion. Its ends are closed. The family includes two subfamilies (McInnes *et al*., 2023). Poxviruses (POXV) are oval in shape, 250 mm in diameter and 360 mm in length. Poxviruses have two infection forms: the(MV), which has a single outer membrane, and the (EV), which is essentially the mature virion but also has an additional outer lipid membrane containing membrane proteins unique to the extracellular enveloped virionThe mature virion is the most common and infectious form, but the extracellular enveloped virion induces efficient cell-to-cell spread (Blasco & Moss, 1992) Figuer 1-2.



Figuer(1-2): *Poxviridae* Morphological structure (Hu *et al*., 2010).

2.3.4 Classiffication:

The family *Poxviridae* (order *Chitovirales*, phylum *Nucleocytoviricota*) includes a select number of genetically diverse dsDNA viruses that replicate in the cytoplasm. Members of this virus family infect a wide range of hosts, including insects, birds, reptiles, and mammals (Gyuranecz *et al*., 2013; Moss, 2013).

Depending on their phylogenetic relationships and host association, the Chordopoxviruses are arranged into two subfamilies, *Chordopoxvirinae* and *Entomopoxvirinae.* For viruses that infect vertebrates and invertebrates, Chordopoxviruses are also classified into several genera (McInnes *et al*., 2023).

The genus *Orthopoxvirus* includes viruses of great medical importance, such as variola virusVaccine virus (VACV, which is used in smallpox eradication campaign) and *Monkeypox virus* (MPV), that responsible for outbreaks of Smallpox as itspread globally recently (Fenner *et al*., 1988; Kraemer *et al*., 2022).

Humans are infected with Orthopox viruses, which are transmitted by animals (Silva *et al*., 2020). Most rope poxviruses infect wild and domestic animals causing significant disease and economic losses (e.g. Fowl pox virus, Lumpy skin disease virus, or Orff virus) (McVey *et al*., 2022). Other genera from *Chordopoxvirinae* also infect humans, the best example being *Molluscum contagiosum virus* (MCV), an endemic human pathogen, and the only representative of the genus *Molluscipoxvirus* (Chen *et al*., 2013). The subfamily *Entomopoxvirinae* is divided into four genera and includes viruses that establish parasitic or symbiotic relationships with their insect hosts (Takatsuka *et al*., 2017). The subfamily *Chordopoxvirinae*consists of 9 genera, *Avipoxvirus*ten species *Capripoxvirus*3 speciesCervidopox- virus one1 species), *Leporipoxvirus* four species: *Molluscipoxvirus* one1 species), *Orthopoxvirus* (9 species), *Parapoxvirus* (4 species), *Suipoxvirus* one1 species), and *Yatapoxvirus* two

speciesand 2 unassigned specie, here as the *Entomo-poxvirinae* has three recognized genera:

1.*Alphaentomopoxvirus*,2-*Betaentomopoxvirus,*3-*Gammaentomopoxvirus*
(Burell *et al*., 2017)

2.4 Poxvirus life cycle :

It is a typical virus of the genus Orthopoxvirus and the family Poxviridae. The vast majority of information about the life cycles of poxviruses (POXVs) and virus-host interactions has been obtained from studies using variola virus (VACV) (Moss, 2013). The existence of two infectious forms of poxviruses, EV and MV, leads to the hypothesis of different entry routes (Figure 2). Viral vesicle binding is mediated by four viral proteins that bind to glycosaminoglycans and laminin on the cell surface (Carter *et al*., 2005; Moss, 2012), with two possible cell entry mechanisms: either the outer membrane of the viral vesicles fuses immediately with the plasma membrane, or the virion is more efficiently internalized by actin-dependent macropinocytosis (Carter *et al*., 2005; Mercer & Helenius, 2008). The proteins involved in viral vesicle binding have not yet been described. It is known that the entry and fusion complex, which appears to consist of 12 proteins, is involved in virus-cell fusion (Senkevich *et a*l., 2005; Laliberte *et al*., 2011). Because this complex is located in the viral vesicle membrane, removal of the outer viral vesicle membrane was suspected to precede viral vesicle-cell fusion. Electron microscopy images of ruptured extracellular vesicle envelopes and fusion of the inner membrane with the cell plasma membrane have confirmed this (Law *et al*., 2006). Although many proteins involved in virion entry have been identified, specific protein receptors have yet to be identified. Figure 2-2.

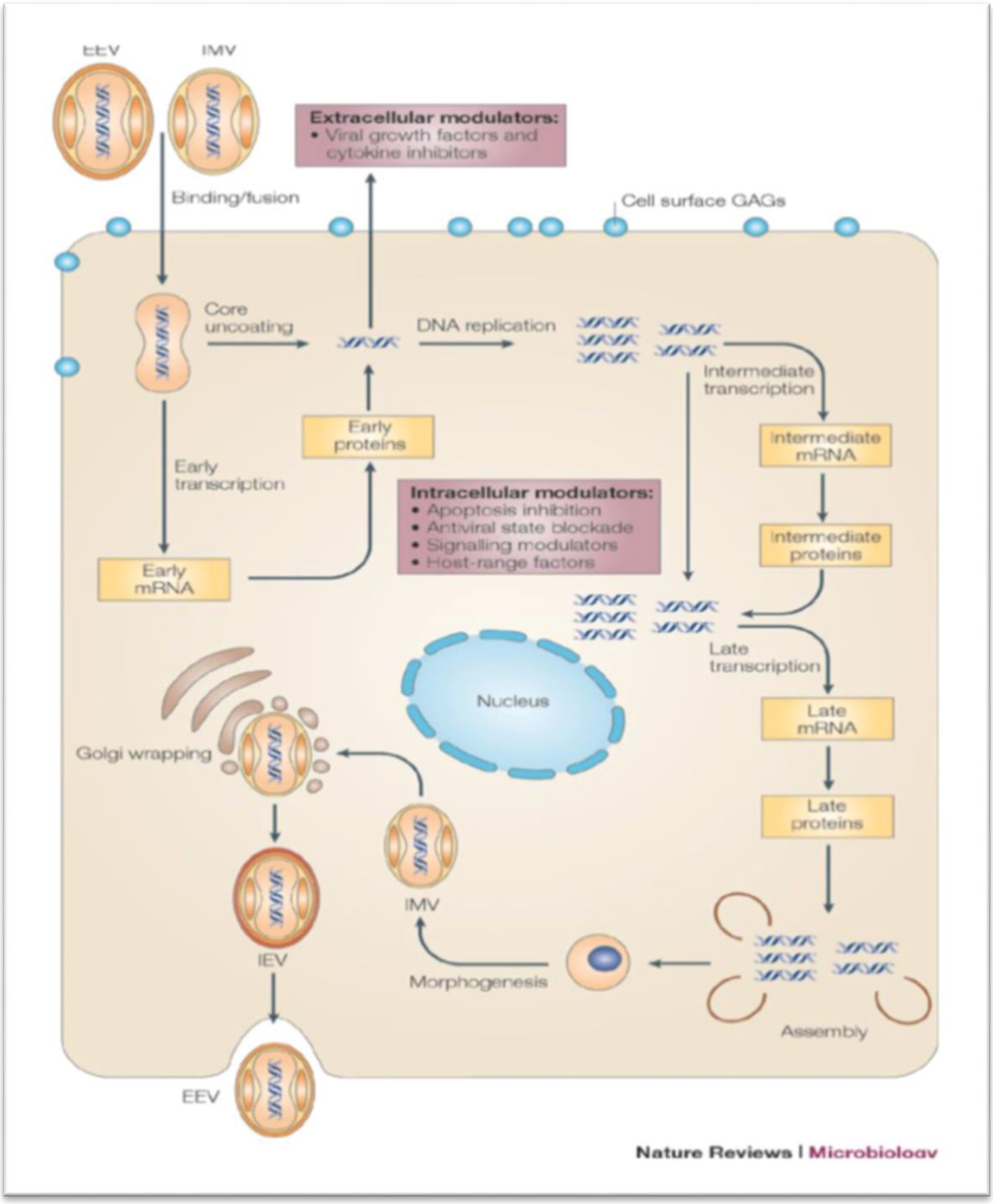


Figure (2-2): Cycle of poxvirus infection (Grant, 2005).

2.5 Pathogenesis of MCV:

This infection is characterized by the appearance of a cluster of pinkish-red papules, as well as the presence of a central, secretive viral nucleus. In severe cases, up to 100 lesions can form.

The *Molluscum contagiosum virus* multiplies in the skin's cytoplasm, and microscopic proliferation of keratinocytes appears as aggregated hyaline particles (Henderson-Patterson bodies).) (Meza-Romero *et al*., 2019).

It is distinguished from keratinocytes that detach from the stratum basale, as the *Molluscum contagiosum virus* (MCV) may be present in this layer. When keratinocytes differentiate and migrate from the stratum basale to the stratum corneum, the virus is released onto the skin's surface. Receptors then regulate epidermal growth factor, after which cell division occurs(Chen *et al*., 2013).

After cell division, these lesions spread to other parts of the body through a cycle of scratching and self-inoculation. The infection can persist for months to years and remain contagious until the lesions heal spontaneously or are removed through physical or chemical destruction by experienced healthcare providers. (Kimberlin *et al*., 2021).

The *Molluscum contagiosum* virus replicates extensively in the horn, the squamous epithelial nodes, and is not transmitted by conjugation (Leung *et al.,*2017).

The *Molluscum contagiosum* virus remains stable and does not persist until the latest developments. The duration of the papules varies from one to six weeks. By affecting the body over a period of time after their disappearance, it consumes new quantities. The papules are described as red, but they have a central umbilicus (Gofur *et al.,*2022).

Small lesions without a central umbilicus appear in the form of aspiration. Large lesions have a large central umbilicus, and are typically of a distinct size, corresponding to lesions on different areas of the body (Cribier *et al.,*2001). In healthy individuals, the papules are common on the groin, buttocks, and anterior abdominal wall, creating a lesion (Brown *et al.,* 2006).

Molluscum contagiosum is not painful, although the surrounding skin becomes red, inflamed, and itchy. There is variation in the distribution of the papules compared to monkeypox. Infectious *Molluscum contagiosum*, the papules are fragmented and less than 20 in number, while in monkeypox, they are found in chronic, well-known areas and are painful (Schaffer and Berger, 2016).

2.6 Characteristics of the MCV genome:

The characteristics of the genome of the variola virus (MCV) are closely related to the cause of infection. Variola viruses contain linear-double-stranded -DNA genome in size from 130-300 kilobases. Their core is dumbbell-shaped to protect the GM. Consisting of membranes and derived from the Golgi apparatus, surrounds the virion core. A DNA-dependent RNA polymerase is also present in the virion, which is essential for variola virus replication in the cytoplasm. (Moss, 2013).

Virus has four known (MCV1, -2, -3, -4), identified by limited-length polymorphisms in their genomes. (Nakamura *et al.,* 1995).

MCV-1 is the most common type in healthy humans, while MCV-2 is common in humans infected with human immunodeficiency virus (HIV). The skin is affected by MCV. Furthermore, as the mutation rate increases, the incidence of mutations in the MCV genome may decrease. Sequence analyses have also revealed that the *Molluscum contagiosum virus* encodes 182 proteins. Comparisons between the genomes of the *Molluscum contagiosum virus* and orthopoxviruses have revealed a close relationship. This comparison was made based on the physiology of VACV genes, and it was expected to identify genes conserved between MCV and VACV (Moss, 2013).

2.6.1 Genomic organization of MCV

Comparative analyses revealed that 105 of the 182 predicted MCV proteins contain homologs of VACV (Senkevich *et al*., 1997). These homologs are known to be important for virus structure, replication, and transcription. The MCV and VACV genes with the highest levels of conservation in viral gene transcription (e.g., polymerase subunits and genes encoding early and late transcription factors) dominate, demonstrating that the MCV life cycle resembles that of VACV. As in other pox virus genomes, the MCV genes are located the center (Moss, 2013).Phylogenetic analysis of five highly conserved poxvirus genes (DNA polymerase, DNA uracil glycosylase, early transcription factor subunit NTPase

1, and rifampicin sensitivity factor) suggests that poxvirus and members of the *Orthopoxvirus* and *Leporopoxvirus* genera evolved from a common ancestor. Recently, the genome of poxvirus was compared to that of Orf virus, a member of the Parapoxvirus genus. Orf virus causes cutaneous infections in sheep and humans. Sequence analysis of the ORF virus has revealed many similarities to the variola virus, although they belong to different genera, ORF and ORF. These homologues have a high GC content in the genome, three putative immune paralogues, and the absence of viral genes involved in nucleotide (Delhon *et al*., 2004).

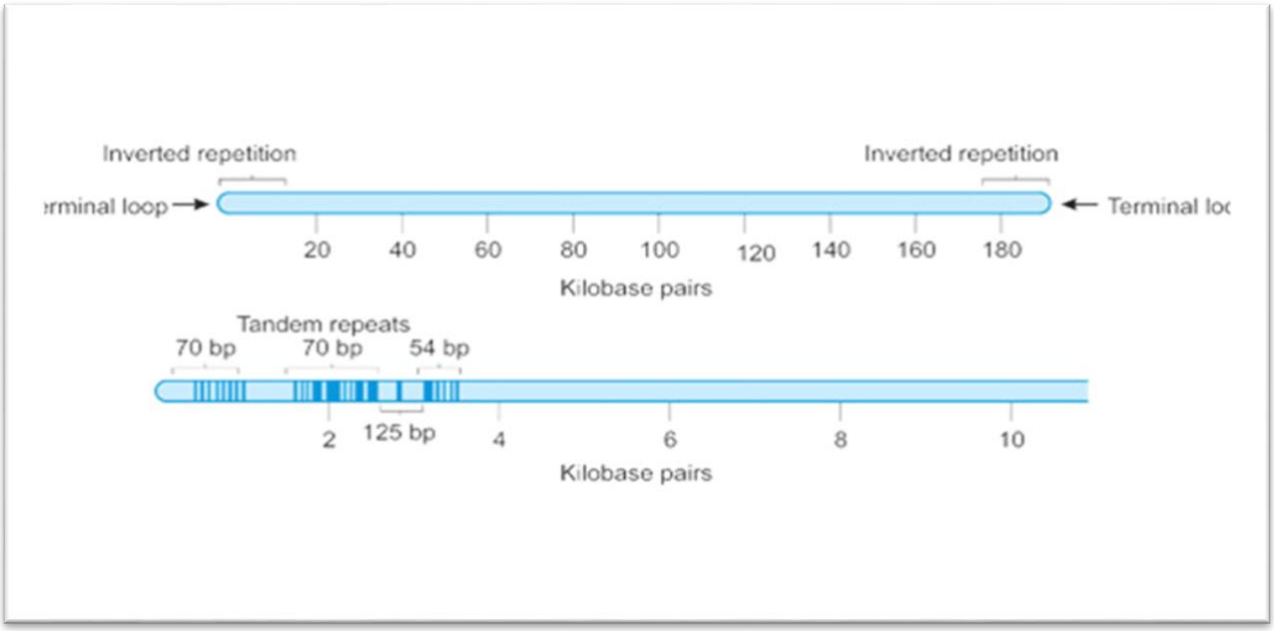


Figure (2-3): The structure of Poxvirus genomic DNA(cann, 2016).

2.7 Etiology and epidemiology:

Molluscum contagiosum is a skin disease by *the Molluscum contagiosum virus* (MCV), adouble-stranded DNA (dsDNA) virus belonging to the *Poxviridae* family. Human the only known host this virus, which includes four genotypes: MCV1, MCV2, MCV3, and MCV4. MCV1 is the most common genotype, accounting for between 75% and 96% of cases, followed by MCV2, while MCV3 and MCV4 are extremely rare. A study conducted in Slovenia showed that children are most commonly infected with MCV1, while MCV2 is more adult women, (Trcko *et al*., 2018).

MCV invades the skin and replicates within the cytoplasm of cells during an incubation period typically ranging from two to six weeks (Braue et al., 2005). Whole-genome sequencing studies of this virus have been developed to identify genes potentially responsible for immune evasion, a finding suggested based on the absence of inflammatory markers in infected skin tissue samples (Zorec *et* al., 2018).

MCV is transmitted through direct skin-to-skin contact, whether sexual or non-sexual, or through self-inoculation. It can also be transmitted via contaminated items such astowels (Leung *et al*., 2017), and infection has been linked to the using of swimming pool.. MCV is widespread globally and predominantly affects children, although it can also infect adolescents and adults. Infection is particularly prevalent among children between the ages of 2 and 5 years, while the incidence is lower in children under 1 year. Studies indicate that the sex of the child does not significantly affect the likelihood of infection (Braue *et al*., 2005).

2.8 Diagnosis of MCV:

2.8.1 Clinically:

Molluscum contagiosum virus (MCV) is diagnosed clinically as shiny papules that are pink or skin-colored or red, inflamed, and solid. There is a difference between the lesions in their location, size, and shape. The duration of the lesions is variable, and in most cases their presence is limited to 6-9 months. They are more common in patients with weak immunity, which causes eczema and bacterial infections. These papules are caused the MCV*,* which is from the *Poxviridae*family and infects people with weak immunity, children, and sexually active adults. *Molluscum contagiosum virus* (MCV) primarily meanwhile direct contact with an infected person, can be sexual nonsexual(Meza-Romero *et al*., 2019).

2.8.2.Laboratory

2.8.2.1 Polymerase chain reaction (PCR):

PCR is a technology used in biological sciences, forensic sciences, and diagnosis, and it is considered one of the most important and widely used technologies (Zhu *et al*., 2020). Polymerase chain reaction (PCR) is used to amplify specific DNA sequences and is a very sensitive method in which to diagnose a specific disease.

The first discovery of th (PCR)was made in 1983 by Dr. Carey Mullisa biochemist who won the Nobel Prize. Dr. Carey works for cetus biotechnology company in California (Mullis *et al*., 1986).

He won the Nobel Prize in Chemistry in 1993 for his discovery of using the target gene as a template. The polymerase chain reaction (PCR) technique synthesizes DNA in the laboratory. Double-stranded DNA is heated to a high TM of approximately 95–98°C to the two strands. The TM. lowered to about 55–65°C, allowing specific primers (short -DNA-sequences) to the ends of the target sequence. The temp.is raised about 72–75°C, allowing the polymerase enzyme extend primer, forming new DNA strands complementary to target sequences. This process is repeated for several cycles, and the target DNA is duplicated each time. The amount of sequence increases over a few cycles. Primers complementary to both ends of the target sequence are used (Suraka *et al*., 2022). The main amplification area are the conserve gene for the penton base, hexamer, and fiber, which serves as the target sequence (Wu *et al.,* 2022; Shieh, 2022).

2.8.2.2 Histopathology Finding:

In most symptomatic cases, histopathological examination by biopsy can be atypical. The diameter of the raised papules is about 1-5 mm in diameter, or the nodules are about 6-10 mm in diameter, which are white or skin-colored and are clustered or solitary in their presence. *Molluscum contagiosum virus* lesions do not heal easily in people infected with HIV and spread easily to different parts of the body (Haque and Coury, 2018).

Separated foci of endoepidermal hyperplasiaform pearshaped lobes inthe superficialdermis, which contain large intracytoplasmic inclusions found in the keratinocytes, which are called molluscum bodies. Through the sloughing of the keratinocytes, after which the stratum corneum is formed and the central umbilicus is formed, in cytology we know on mollusk particles in which the dermis is not inflamed (Jahnke *et al*., 2018).

On histological examination, enlarged epidermis was found, and enlarged cells could be seen large intra-cytoplasmic inclusions identified as (Henderson-Patterson-bodies) (Silverberg, 2018). *Molluscum contagiosum virus* has a very limited range of tissue-directed characteristics of poxviruses. *Molluscum contagiosum virus* infection is limited to the skin only, and it cannot spread deeply even in immunocompromised patients. *Molluscum contagiosum virus* is found in

areas containing hair follicles and is rarely found in the palms of the hands, soles of the feet, and mucous membranes. *Molluscum contagiosum virus* coexists in the skin, especially in superficial tissues such as the epidermis, and is proliferative in the cyst surrounding the hair follicle or through self-inoculation when there is friction in the skin that can be transmitted from one place to another. There is diversity in the multiplicity of forms of *Molluscum contagiosum virus* lesions (Haddock *et al*., 2017).

Cyst change can occur in lesion cause by the MCV on surface of the skin. These changes can appear as bumps on the surface of the skin and contain a small opening that facilitates the transmission of the virus, which enhances the spread of the infection. The smallpox virus can determine the persistent infection and the infection remains for a long time inside the cells. People who have previously been infected with AIDS are more susceptible to infection with the *Molluscum contagiosum virus*. This occurs due to immunosuppression, which leads to the virus appearing in a subclinical form. Genital lesions have also been discovered in sexually active adults (Manti *et al*., 2017).

One study suggests that the immune system responds quickly to infection caused by the *Molluscum contagiosum virus*, and resolves inflammatory lesions quickly. Also, not all people with *Molluscum contagiosum virus* lesions develop preclinical inflammation, meaning that some lesions may develop without any noticeable inflammation. The virus tends to mimic the follicular pattern of growth in hair follicles, so the basal cells are similar to the lobular stromal cells found in hair root cells. Through growth, the virus can secrete holocrin bodies and trigger an immune response that is the cause of the lesions. The infection originates in the hair follicles, and the virus releases growth factors that cause the cells to multiply rapidly and stimulate the appearance of skin lesions (Olsen *et al.,* 2016 ; Fisher *et al.*, 2019).

Molluscum contagiosum virus-infected cells exhibit features similar to those that occur during hair follicle growth. This causes viral replication similar to follicular growth. The growth phase ends with keratinocyte lysis, which leads to the destruction of dead or infected cells. *Molluscum contagiosum* lesions are in the form of cup-like internal lobules of squamous epithelium. Cells infected with *Molluscum contagiosum virus* accumulate acidic cytoplasmic impurities. These impurities are abnormal substances that accumulate in the cytoplasm due to viral infection. These cause swelling and replace the entire cell. After the impurities accumulate inside the cells, they push the infected cells outward and appear on

the surface of the skin. In most cases, the lesions do not show inflammation, but some of them show a dense red lymphoid sequence (Jahnke *et al.,* 2018).

2.9 Previousstudies of *Molluscum contagiosum virus*

2.9.1 Localstudies of *Molluscum contagiosum virus*

Various studies have reported differing prevalence rates of *Molluscum contagiosum virus* (MCV) across multiple regions in Iraq and neighboring countries. In Diyala province, which is the city in east middle of Iraq a study by Mohammad (2020) recorded a prevalence of 53.3% among children aged 1 to 10 years. The virus was detected using PCR techniques, with skin lesions serving as the diagnostic model. Similarly, research conducted in Basra province, a city in south of Iraq by Gaeta *et al*. (2019) reported a higher prevalence of 77.4% among children aged 1 to 11 years, using the same diagnostic approach.

In Najaf province, a city in west middle of Iraq, Shubbar *et al*. (2019) found a 27.9% infection rate among children under the age of six, based on histopathological examination. Other study conducted in Diyala by AlAzawi (2013) showed-that 45.1% of individuals aged 30 to 41 years tested positive for MCV using PCR technique. Additionally, Ahmed (2013) reported that 78.4% of MCV-related skin lesions were located on the head and neck regions.

2.9.2 Global studies of *Molluscum contagiosum virus*

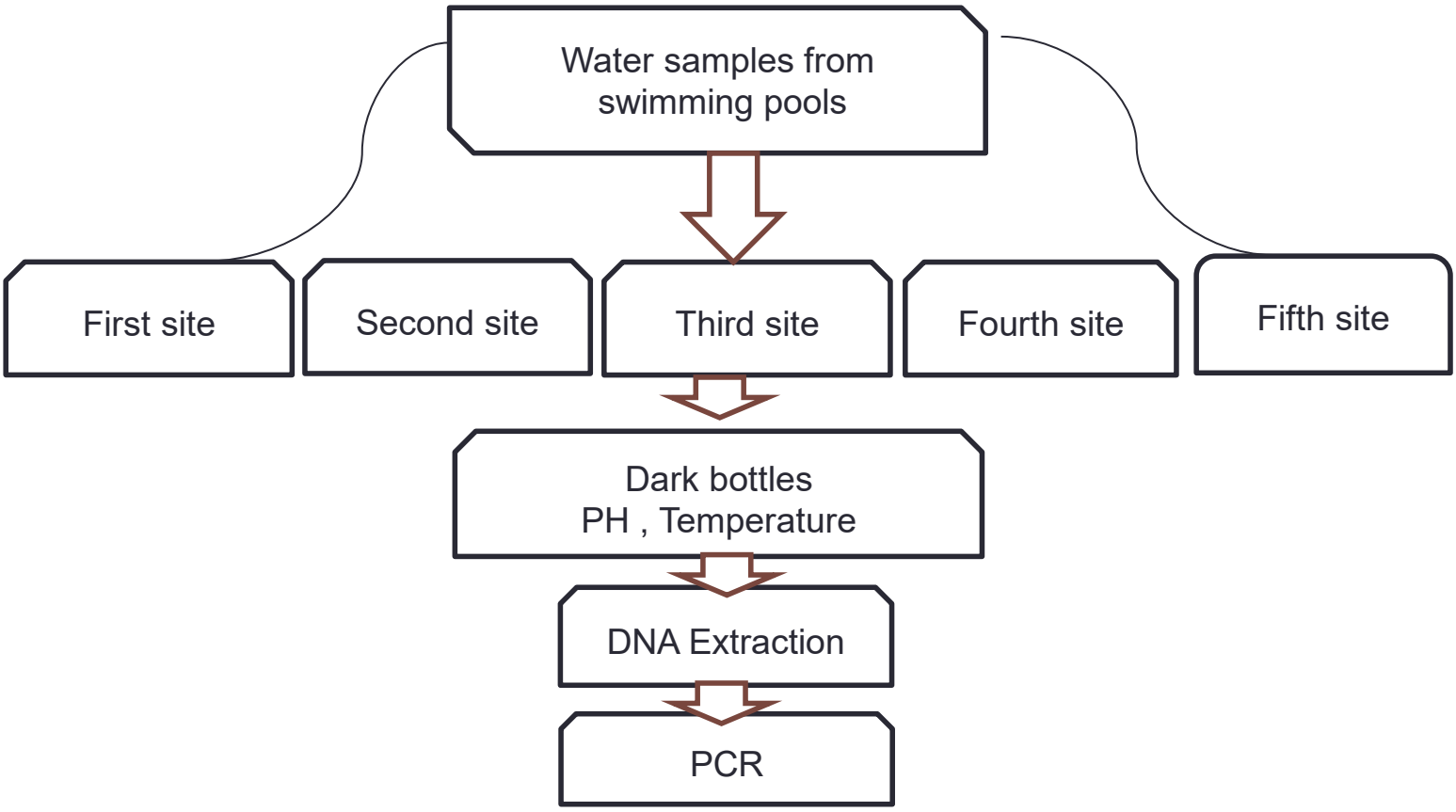
MCV spreads regionally and globally, A 2018 study from Iran found that 114 out of 1,470 specimens (7.75%) tested positive for MCV. Among the positive specimens, 71.05% were identified as MCV1, while 28.95% were MCV2 (Taghinezhad *et al*., 2018). In Slovenia, a study involving 188 patients found that 72% of the cases occurred in adults. The findings also revealed that MCV1 infection was more common in children, whereas MCV2 was more frequently observed in adult women (Trčko *et al*., 2018). In 2006 Approximately 80% of the patients were younger than 8 years old. The majority of patients (63%) had more than 15 lesions(Dohil, 2006). In Iran in 1999, the percentage of girls infected with *Molluscum contagiosum virus* was about 45% compared to 55% of males (Zandi *et al*., 1999).

Chapter Three

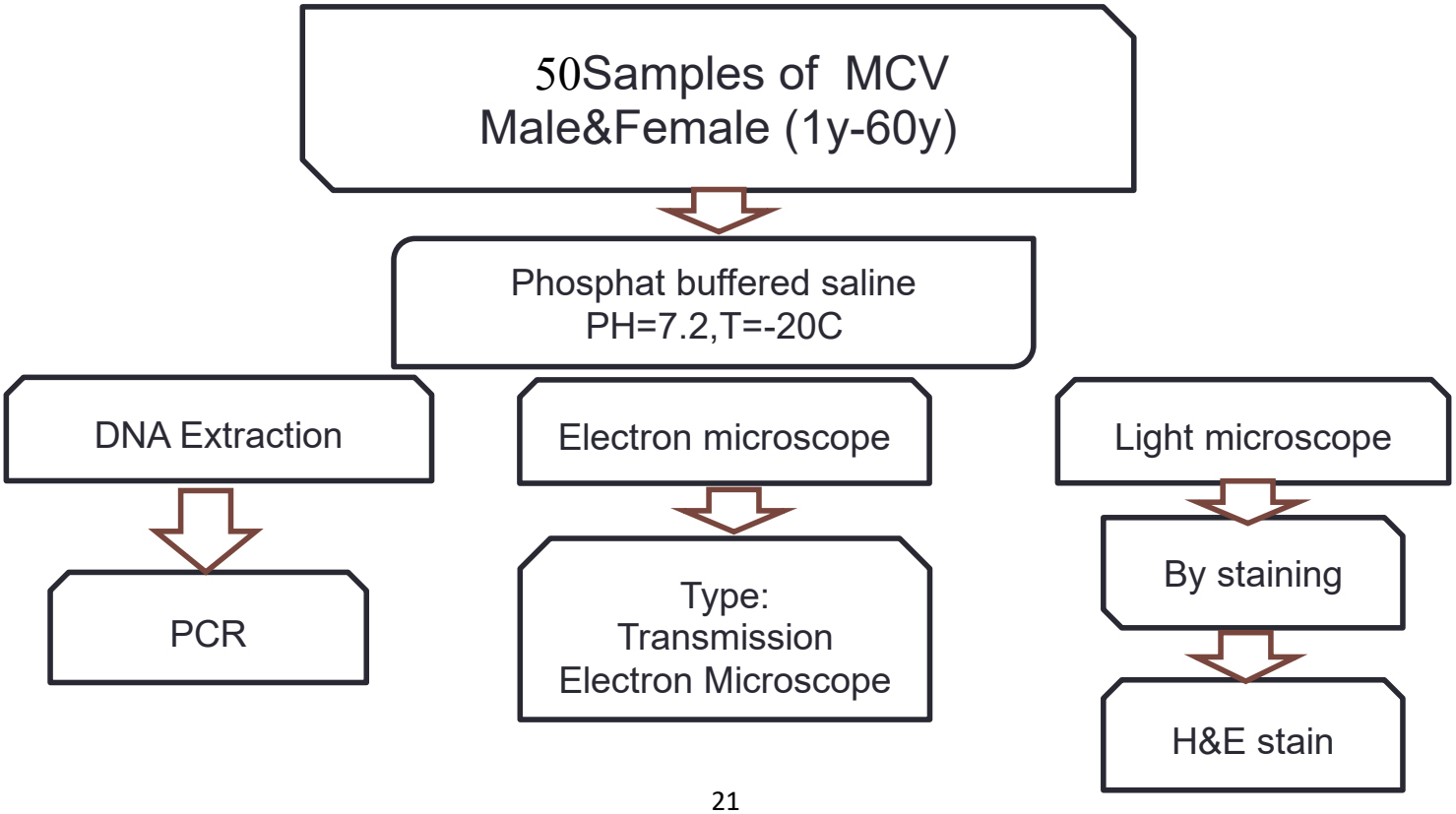
Materials and Method

The scheme used in the current study:

Experimental design 1:



Experimental **design 2:**



3.Materials and Methods

3.1.subject

A cross-sectional study was conducted on 140 male and female patients with pearly white disease, aged 1–60 years, diagnosed by a dermatologist at Al-Sadr Teaching Hospital, Department of Dermatology, Maysan Province, during the period from November 2023 to April 2024 . Samples were taken after approval from the Research Ethics Committee. Verbal consent was obtained from the patients, and information was recorded using a questionnaire (Appendix 1). The disease is attributed to family history, friction, and failure to follow the necessary preventive measures to protect against infection. The disease occurs on most parts of the body and spreads easily to the rest of the body upon contact. The lesions were located in various locations on the body. The lesions were small, pointed, and sometimes large, inflamed, red or pink papules with a central navel and containing a white, cheesy substance. Most patients suffered from skin infections, such as dermatitis and eczema, and some were frequent swimmers in swimming pool..

3.1.1. Swimming pool

Twenty water samples from swimming pool were taken from swimming pools in Maysan Province after obtaining verbal consent from the owners during the period from June 2024 to July 2024. The sample was taken at a depth of 30 cm while swimming. The pool dimensions were approximately 25 meters long, 12.5-25 meters wide, and 1.2-2 meters deep. The design and dimensions of swimming pools are critical factors affecting water circulation, disinfection efficiency, and microbial load, all of which directly impact water quality and safety for users. The pools were located in the governorate center and were filled with sterilized water tanks.

3.2. Materials:

Table (3-1): The equipment that was used in the current research experiments.

Equipment	Company	Origin
Centrifuge	Hettich	Germany
Bench microcentrifuge Eppendroff	Hamburg	Germany
Deionized water	Bioneer	Korea
Electrophoresis	Bioneer	Korea
Exispin vortex centrifuge	Bioneer	Korea
Gel Documentation	Atto	Japan
Labconco	Kansas	USA
Micropipettes(different volumes)	Eppendorf	Germany
Microwave	Shownic	China
Nanodrop	Thermo Scientific	UK
HANNA multimeter PH	model HI 9633	
HANNA multimeter	model HI 9633	
PCR	Bioneer	Korea
Light microscope	Olympus	Japan
Water Path	Memmert	Germany
Universal Pipette Tips	Globe scientific	Germany
Slides	TRUSTLAB	China
Eppendorf tubes	Bioneer	Korea
Cover Slides	Superstar	India
Beaker	General	USA
Flask	Iso Lab	Germany
Cylinder (50,100,250)	HBG	Engeland

Table (3-2):The Chemical materials that were used in experiments.

Chemical	Company	Origin
DNA extraction Kit	Geneaid	Germany
Nuclease free water	Bioneer	Korea
Absolute ethanol	Scharlan	Spain
TBE 10X	Bioneer	Korea
DNA Ladder	Bioneer	Korea
Ethidium Bromide	Bio Basic	Canada
Phosphate Buffer Saline(PBS)		
Xylene	Sigma-Aldrich	Germany
Haematoxyline stain solution	BDH	England
DPX	Sigma-Aldrich	Germany
Primers		
AccuPower® PCR PreMix	Bioneer	Korea
Agarose	Bioneer	Korea

.33.Primer design:

The primer was manufactured by Bioneer Company and was designed based on all types of *Molluscum contagiosum virus* because it is universal. P43K was used in several studies for different sequences and regions. As for the primer that designed, it has a sequence of 21 genetic bases and 337bp. That is, the design is specific to the current study.

p43K gene primer: Amplicon size 337 bp

Table (3.3): Primer Sequence

Primer name	Sequence	No. b p	Company
P43K	**F: 3′GCGCSCGCGCGGACGCGGGCG5′** **R: 5′GCGTGGCATCCGCGTCCCGGC3′**	**337bp**	**Bioneer**

3.4. Sample collection

3.4.1. Clinical specimens :

One hundred and forty samples were collected from patients whose infected with MCV during the period from November 2023 to April 2024 at Al-Sadr Teaching Hospital in Maysan Province. The samples were obtained from patients after diagnosis by a dermatologist ,Tissue specimens were stored in phosphate buffered saline (PBS) pH = 7.2 at temperature -20°C.

3.4.2. Swimming pool water specimens:

Twenty samples of water were collected from swimming pools (Al-Amara Grand Pool, Happy Dream Pool, Water City Pool, Crane Land Pool, Four Seasons Pool) in Maysan Province during the period from June 2024 to July 2024.Water samples were stored in 5 ml sterile tubes at standard conditions, temperature and pH were measured for each pool.

The Steps for measuring temperature and pH:

The temperature was measured using a HANNA multimeter (model HI 9633). Samples were examined immediately after collection. (Jawad *et al*., 2022) The pH was measured directly using a HANNA multimeter (model HI 9633) using standard solutions with different pH values (APHA, 2017).

3.5. Methods

3.5.1. DNA extraction from clinical samples:

DNA was extracted from 50samples using tissue DNA-extraction kit g SYNCTM DNA Extraction Kit, The procedure were carried out as shown below:

1. Tissue dissociation: MCV was transferred to a 1.5 ml microcentrifuge tube. Then, 200 µl GST Buffer and 20 µl Proteinase K were added and mixed well. Finally, the mixture was incubated at 60°C overnight.
2. Cell lysis: After incubation if insoluble material remains, the samples were centrifuged for 2 min at 14-16,000 x g. Then carefully transfer the upper fluid to a new 1.5 ml microcentrifuge tube. At least, add 200 µl GSB buffer and shaked vigorously for 10 second.
3. DNA binding: 200 µl of ethanol was added to the sample and next, the GS column was placed in a 2 ml collection tube and then transfer all of the mixture to the G-S column. centrifuged at 14-16,000 x gfor 1 min. After centrifugation, if the does not flux through the membrane of the G-S, increase the centrifugation time until it has completely passed. Finally, discard the 2 ml collection tube contain the effluent and transfer GS to a new 2 ml tube.
4. DNA washing : 400 µl of solution W-1was added to the G-S column. Then, the centri-fuge was run at 14-16,000 x g for30 second and discard the effluent. Eventually, GS column was placed back in the 2 ml collection tube. 600 µl of wash solution (using absolute ethanol) was added to the GS column. Centrifuge at 14-16,000 x g for 30 seconds and discard the effluent. The GS column was put back in the 2 ml collection tube. Finally, the GS column was centrifuged for 3 minutes at 14-16,000 x g.
5. Elution: The standard elution volume is 100 µl. If fewer samples are to be used, reduce the elution volume (30-50 µl) to increase the DNA

concentration. If a higher yield of DNA is required, repeat the DNA elution step to increase the DNA recovery and total elution volume to approximately 200 µl. Transfer the dried GS column to a clean 1.5 ml micro centrifuge tube. Add 100 µl of elution buffer 1, TE buffer 2, or preheated water 3 to the center of the column matrix.Then leave for at least 3 minutes to allow the elution buffer, TE buffer, or water to be completely absorbed. Centrifuge at 14-16,000 × g for 30 seconds to elution the purified DNA.

3.5.2 Swimming pool water specimens:

DNA was extracted from20 sample use theViral Nucleic -Acid Extraction Kit II as shown below:

1. Lysis:

• Two hundred µl of the sample is transfer to a 1.5 ml micro centrifuge tube.

• Four hundred µl was added of VB lysis buffer to the sample and mix by vortex.

• Then the samples was incubated at room temperature for 10 minutes.

2. DNA Binding:

four hundred and fifty µl were added of AD solution (make sure to add ethanol) to the samples solution, and then the samples were.

• Shacked vigorously.

• Next VB columns is Place in a 2 ml collection tube.

• six hundred µl of the sample was transferred to the VB column and

• centrifuged at 14-16,000 × g for 1 min.

• The effluent was discard placed the VB column back in a 2 ml collection tube.

• Transfer the remaining was transferred to the VB column.

• The samples were centrifuged at 14-16,000 × g for 1 min.

• The 2 ml collection tubes were discard containing the effluent.

• 2 ml of the sample was transferred the VB to a new tube.

3. Wash:

Four hundred µl was added of W1 buffer solution to the VB column and at 14-16,000 × g for 30 seconds.

• The solution was placed the VB back into the 2 ml collection tube.

• six hundred µl was added of wash solution (make sure to add ethanol) to the VB column.

• The samples were centrifuged at 14-16,000 × g for 30 seconds.

• Discard the solution and placed the VB back into the 2 ml collection tube.

• The samples was centrifuged at 14-16,000 × g for 3 minutes dry the column matrix.

4. DNA eluting:

Dried VB colum placed n into a clean 1.5 ml microcentrifuge .

• fifty µl was added of RNase-free and Dnase water to the center of the VB column matrix and left for at least 3 minutes to ensure that the matrix absorbs the RNase-free water.

• The samples were centrifuged at 14-16,000 x g for 1 minute to extract pure DNA

3.6. The Steps of PCR Amplification :

PCR amplifications of p43K gene were performed in 25 µl volumes in total The PCR technique was applied by adding 5µl from DNA extraction to the PCR tube

containing 5µl of the master mix and 1 µl of each for word and reverse of universal primers, 13µl of the Nuclease Free Water were added to this tube to get 25 µl as a final volume. The mixture then put in PCR system (Bioneer/Korea), the PCR was run according to the conditions listed in (Table 3-5). PCR cycling was performed using PCR Express (Thermal Cycler, Bioneer , Korea).

Table (3.4):PCR conditions used for P43K gene amplification .

STEPS	TEMP.	TIME	NO. of Cycles
Initial Denaturation	95 C	5 MIN	1 cycle
Denaturation	95 C	30 SEC.	35 cycles
Annealing	60C	30 SEC.	
Extension	72 C	30 sec.	
Final extension	72 C	5 MIN	1 cycle

3.7.Gel electrophoresis:

1- 2 gm of agarose gel was weighted and dissolved in 100 ml of TBE buffer (1X).

2- After the mixture become clear in oven , 2ul of ethidium bromide stain was added.

3- Then pour the mixture was poured in tray ,to solidify.

4- DNA ladder was loaded in one of the wells, and then 5µl of the samples were loaded in the other wells.

5- The electrophoresis device was run for 30 mint ,at 135 V The DNA would move from the cathode to the anode.

6- Finally, the gel was visualized by UV device Gel Documentation device.

3.8. Gel extraction :

The amplicons were extracted from the gel using gel extraction kit (Bioneer). The protocol run according to industrial instruction. The extracted amplicons were sent to the company for further analysis. Just clear chromatograms obtained from-ABI (Applied--Biosystem) sequences file further analyze the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Also, to ensure that comments and discrepancies were not due to PCR or sequencing residues, the observed nucleic-acid sequence the samples were compared with the recovered nucleic sequences, and the putative of the recovered PCR fragments were determined. The sample sequence ,of the P43K,gene.

3.9. Gene Sequence Analysis:

3.9.1. Nucleic acids sequencing of PCR amplicons

Extracted viralDNA were commercially sequence the forward-directions, following the instru-ction manual of the sequenc company (Macrogen Inc. Geumchen, Seoul, South Korea). Only cleared chromatograph from ABI (Applied Biosystem) sequence files were further analyzed, ensuring that the annotation and variations, notbecause of PCR or sequencing artifacts. By comparing the observed nucleic acid sequence of local samples with nucleic acid sequence, and other detailsof the retrieved PCR fragments was identified.

3.9.2. Interpretation of sequencing data

Sequencing results of the PCRproducts of that target sample is edited, aligned, analyzed as long as with the respective sequences in the reference database using Bio-Edit Sequence Align-ment Editor Software Version 7.1 (DNASTAR,

Madison, WI, USA). The observed variations in sequence sample is number in PCR amplicons as well as in their corresponding position within the referring genome. The observe nucleic acids were alsonumbered in PCR amplicon as in their corresponding position with referring

genome. The sequences within *Molluscum contagiosum virus* sequences is annotated by Snap Gene-Viewer ver. 4.0.4 (https://www.snapg ene.com).

3.9.3. Translation of nucleic acid variations intoamino acidresidues

Amino acid sequence of the target protein were retriev from the protein databank .To assess the impact of the identified nucleic acid variations located in the coding regions on the encoded protein, all nucleic acid sequences of the investigated samples were translated to their corresponding amino acid sequences using the Expasy translate suite. at https://web.expasy.org/translate/.

3.9.4. Comprehensive phylogenetic tree construction

Before generating phylogenetic tree, the observed variants were compared with their neighbor homologous reference sequences using the NCBI-BLASTn server (Zhang *et al*.,2000). Sequences that of less than 93% of coverage with the investigated PCR amplicons were omitted from downstream phylogenetic analysis. Repeated Gene Bank accession numbers with identical homology were omitted. As one of the *Poxviridae* family, Rousettus bat poxvirus was included as an out group species in the generated tree due to its relatedness to the P43K sequences. The ClustalW tool was used to align the retrieved nucleic acid sequences. The aligned sequences were used to generate a phylogenetic tree using MEGA7 software ((Kumar *et al.,* 2016). Based on the neighbor -joining protocol (Saitou and Nei, 1987), a bootstrapped tree was generated and its robustness was tested using 1000 replications (Felsenstein, 1985). All positions containing gaps and missing data were eliminated in the generated tree, and the evolutionary distances of the generated tree were computed using the Maximum Composite

Likelihood method (Tamura and Kumar, 2004). The generated tree was visualized as an original tree and annotated for each specific clade.

3.10. Histopathological specimens:

Preparation of formalin-fixed, paraffin-embedded (FFPE) tissues:

• Tissue fixation:

• Tissue sections were placed in formalin (10%) and fixed for 48 hours at room temperature.

After fixation, the tissues were treated with gentle agitation, placed in 70% ethanol for 2 h, then in 80% ethanol for 2 h, then in 90% ethanol for 2 h, then in absolute ethanol for 2 h, then in absolute ethanol for 2 h, then in xylene for 2 h, then in xylene for 2 h, then in a first paraffin embedding at 58°C for 2 h, then in a second paraffin embedding at 58°C for 2 h, then the tissues were embed in blocks:

1. A small amount of paraffin was placed in the mold and dispensed into a paraffin reservoir. Warm forceps were used to transfer tissue to the mold, cut side down, and placed it in the cassette.
2. The mold was transfer to a cold plate; the paraffin solidified into a thin layer that held the tissue in place. When the tissue was in the a tissue strip was added to the top of the mold as a support. This was then pressed firmly.
3. Hot paraffin was then added to the mold from a paraffin distribution until it covered the surface of the plastic strip.
4. The strip was filled with paraffin through cooling, keeping the mold full until it solidified. The paraffin then frozen for 30 minutes. When the wax had completely cooled and solid (30 minutes), the paraffin blocks were easily removed from the mold.

3.10.1. Tissue sectioning and slide preparation:

Serial tissue sections (3-5 μm) 1 μm thick were obtained using a microtome, and one slide was prepared from each paraffin block. The sections were mounted on plain slides (hematoxylin and eosin stained) use 45°C water bath to prevent folding of the tissue sections during the mounting process. Using a pencil, each slides was mark with the same no. paraffin block.

3.10.2.Hematoxylin and Eosin (H&E) staining of paraffin sections:

The hematoxy eosin staining system were used for histopathological examination as follows: Paraffin-embedded sections were prepared for hematoxylin and isopropylene staining by:

1. Mounting on plain slides, with the edges frozen on both sides.
2. The paraffin was removed, using an oven at 65°C for 30 minutes.
3. The slides were immersed in three sets of Xylene for 10 m each remove paraffin from the tissue

4.slide were immersed in three sets of ascend ethanol concentrations starting from 100%, 90%,80%,70%for10 mins each.

5.The slides were rinsed with tap water. for 5 minutes.

6.The slides were soaked the slides in hematoxylin stain for 5 minutes.

7.The slides were rinsed them thoroughly under tap water for approximately 4-5

mins to remove excess hematoxylin.

8.Residual hematoxylin remov byadding 1%acid alcohol (1% hydrochloric acid in 70% (v/v)) for5seconds, followed by washing with tap water.

9.hematoxylin stain was turned blue by adding Scott tap water for approximately 10seconds until the turned blue.

10.Slide were rinsed with tap water before staining with eosin (1% (w/v)) for 5 minutes followed by washing with run tap water for1-5minutes.

11.Section were then dehydrated by soaking in decreasing concentrations of ethanol (50%, 70%, 80%, 90%, and absolute alcohol) for 5 minutes each.

12.2 washes with xylene for10 minutes each before mounting, leaving the slides clean and ready for mounting with DPX.

13.DPX was mounted and the slides were then wrapped with coverslips. (Chong et al., 2012).

3.11. Transmission electron microscope specimens:

Two samples were taken from two patients infected with *Molluscum contagiosum* virus and were preserved in fixative consisting of Glutaraldehyde and Phosphate buffered saline .The specimens were examined with Zeiss Supea 55vp with STEM detector (Germany) under Transmission electron microscope (TEM).

3.12. Statistical Analysis

Descriptive statistics were used to summarize the laboratory findings, including frequencies and percentages for categorical variables such as PCR results (positive vs. negative). In addition, two representative samples were examined under scanning transmission electron microscopy (STEM), which confirmed the presence of viral particles consistent with MCV morphology.

Chapter Four

Results and Discussion

4. Results and Discussion

4.1.Environmental study:

4.1.1.Water specimens of swimming pool:

In our current study, physical and chemical parameters such as pH and temperature were determined, where the pH in the large building pool was between 6.5-7.3, the pH in the Happy Dream City pool was between 7.0-7.5, the pH in the Green Land pool was between 6.9-7.4, the pH in the Water City pool was between 7.0-7.5, and the pH in the Four Seasons women's pool was between 6.9-7.2, as almost all pools in Maysan Province have similar pH levels (Table 4-1), and the World Health Organization recommends that the pH be between 7.2-7.8. Because when the acidity is very low or very high, swimming pool users will suffer from eye irritation and skin inflammation due to the direct effect of water on them (WHO, 2003). It also plays another role in the effectiveness of disinfection and coagulation, as well as preventing damage to the pool fabric (WHO, 2005). Therefore, the pH of swimming pool water must be monitored to ensure that swimmers are not exposed to any harmful factors.

As for measuring the temperature of swimming pools, it is very necessary to control the growth of microorganisms and has an important impact on swimming pool users, as shown in our current study in Maysan Province swimming pools. The temperature in the large Al-Amara swimming pool was between 24-27°C the temperature in the Happy Dream City swimming pool was between 24-26°C and the temperature in the Green Land swimming pool was between 25-28 °C. The temperature in the water city pool was between 25-27 °C and the temperature in the Four Seasons women's pool was between 24-28°C as the temperature in the pools of Maysan Province (Table 4-1) was close and within the conditions recommended by the World Health Organization, as the recommended rates were between 21-32°C.The percentage of difference in temperatures is due to the

difference in the temperature of the users of the swimming pools, which has an effect, as well as the difference in the season of taking the samples and also the location of the pool, all of which have effects on the temperature of the pools, and also the turbidity of the swimming pool water has an effect on the users of the swimming pool, some of which are the reasons for dust, microorganisms, and the uncleanliness of some users of the swimming pool, all of which have effects on the turbidity of the swimming pool water.

Table(4-1): Describes the temperature and pH of swimming pools in Maysan Province.

Swimming pool	PH	Temperature
Al-Amara Grand Pool	**6.5-7.3**	**24-27 ℃**
Happy Dream Pool	**7.0-7.5**	**24-26 ℃**
Water City Pool	**7.0-7.5**	**25-27 ℃**
Green Land Pool	**6.9-7.4**	**25-28 ℃**
Four Seasons Pool	**6.9-7.2**	**24-28 ℃**

In addition ,20 samples of water were collected from swimming pools (Al-Amara Grand Pool, Happy Dream Pool, Water City Pool, Crane Land Pool, Four Seasons Pool) in Maysan Province during the period from June 2024 to July 2024.The samples were taken from each pool four times at different times. In addition, the non-clinical results of the examination of swimming pool water samples in Maysan Province , Iraq, using the PCR technique, indicate that there is no relationship between swimming pools and the *Molluscum contagiosum virus*, and also the presence of correct sterilization methods in swimming pools in Maysan Province (Table 4-2).

Table (4-2) :Detection of MCV in swimming pool water samples by PCR technique.

Result	Frequency	Percentage%
Positive	0	0%
Negative	20	100%
Total	20	100%

Figure (4-1): Displayed the results of conventional PCR of MCV from swimming pools water. Sample 1-20.

4.1.2 Distribution of MCV according to the age of patients:

The total number of samples was 140 patients (72 males and 68 females). The results showed that the age group (1-10) years represented 45%, the age group (11-20) years represented 25%, the age group (21-30) years represented 15%, the age group (31-40) years represented 11%, the age group (41-50) years

represented 3%, and the age group (51-60) years represented 1%, as shown in Table 4-3.

Table (4-3): Distribution of MCV according to the age patients.

Age	Frequency	Percentage%
1-1 0	64	45%
11-20	35	5%
21-30	21	15%
31-40	15	11%
41-50	4	3%
51-60	1	1%
Total	140	100%

the results showed a higher infection rate in the ages of 1-10 years compared to the ages of 51-60 years. The our study showed most cases of infection *Molluscum contagiosum virus* are in the age group 1-10 years, which is consistent with the Gatea,(2019) in Iraq at a rate of 77.4%. These results were consistent with Jameel, (2020). Also, Humoud and Gatea, (2019) agreed The cases of infection were in group (1-10) years, and this does not agree with the study conducted by Al-Kayalli in Iraq, where the cases of infection with *Molluscum contagiosum* were in the group (31-40) years (Al-Kayalli *et al*.,2015).

The incidence of *Molluscum contagiosum virus* infection ranged between the ages of 1-10 due to the frequent mixing between this age group and the lack of health awareness and educational level, as well as playing and direct contact between them in schools, swimming pools and entertainment cities. Therefore, the incidence in this age group is more than in the age group of 10-20 years. It as well as agrees with a study in the United States of America that showed that 80% of patients infected with the *Molluscum contagiosum virus* were under the age of eight (Dohil, 2006).

Also, one of the reasons that children between the ages of one and ten years are infected is due to their low physical immunity and may also be due to different in the social standard of live (Saleh, 2016). The infection rate was different from what was published in the United States of America, which proved that the increase in infection with the *Molluscum contagiosum virus* is parallel to infection with sexually transmitted diseases, but the infection rate children is less than 5% (Dohil, 2006).

4.1.3 Distribution of MCV according to the Sex of patients:

As for sex, the current study had a higher percentage of males than females, with males at 51% and females at 49% Table 4-4 , which is consistent with the study by Gatea, (2019), where 60.8% were males and 39.2% were females. These results were consistent with the study by Al-Azawi, (2013) in the age group 31-40 years, which recorded that the infection with *Molluscum contagiosum virus* in males was 66.6%, while it was 33.3% in females, which is consistent with the Turkish report of 2006, where males infected with *Molluscum contagiosum virus* were 67.2% and females infected were 32.8%. In contrast, in Iran in 1999, the percentage of girls infected with *Molluscum contagiosum virus* was about 45% compared to 55% of males (Zandi *et al.*, 1999). The infection rates are higher among males than females due to the increased mixing between males compared to females, and also due to the lack of health awareness, low educational level, and playing among themselves in the streets, schools, and gatherings in parks, swimming pools, and schools. as shown in Table 4-7. In addition, a study in Egypt contradicted our current study, where the percentage of males was 42.9% and the percentage of females was 57%. This is attributed to the fact that males are a more mixed group in society than females, and they interact in all places, from entertainment cities, gatherings, and swimming pools. (Mostafa *et al*., 2012) . other study conduct research in Iraq the no. of 25male and 95 females out of a total of 120 samples (Al-Maliki *et al.,* 2019) .

Table (4-4) :Distribution of MCV according to the Sex of patients.

Sex	Frequency	percentage %
Male	72	51%
Female	68	49%
Total	140	100%

4.1.3.Distribution of MCV according to the regions of patients:

In addition, in our current study, we found that urban have a increase in infection than rural , where urban areas were 77% and rural areas were 23% Table 4-5. Our study did not agree with previous studies, which concluded that the infection rate was higher in rural areas than urban areas. The results were consistent with a survey by Al-Azawi, (2013) in Iraq, which found that rural areas were 29.4% and urban areas were 70.6% of the population. The current study agrees with several studies such as by (Al-Kayalli *et al*., 2015) in Iraq, where the results were 29.6% in rural areas and 70.4% in urban areas. It also agreed with another study (Mohammad, 2020) in Iraq, which see the infection rate in rural at 20% and in urban areas at 80%. Which our current study does not agree with the study conducted by (Saleh, 2016) in Iraqi which found that rural areas were 62.7% and urban areas at 37.3%. These studies show that the residents of urban areas are exaggeratedly exposed to environmental pollutants, the proximity of factories to cities, car smoke, and the abundance of environmental pollutants from the poor quality of water for washing and drinking, and this is a negative indicator of the poor quality of cleanliness.

Table(4-5): Distribution of MCV according to the regions of patients.

Region	Frequency	Percentage%
Rural	32	23%
Urban	108	77%
Total	140	100%

4.1.4.Distribution of MCV according to the site of lesion of patients:

As for the location of the lesion in the body, the results of the study showed that it was in the face and neck, i.e. the upper part, at a rate of 90%, and the lower genital part at a rate of 10% Table 4-6. This was consistent with the study conducted by Gatea, (2019), in which the infection was more common in the head area, i.e. the face and neck, at a rate of 76.5%, and less in the genital area at a rate of 16.6%. This is due to the high incidence of infection in children and the color of the upper part, which is exposed to direct contact, making it more susceptible to infection with the contagious *Molluscum contagiosum virus* compared to the genital part, in which people are infected at the age of 30 or 20 years due to sexual intercourse and several factors that negatively affect it, and due to weak immunity and lack of personal hygiene. The infection was in the face and neck by 90% more than the genital area by 10%, which agreed with Al-Kayali, (2015). The current study agreed with study , by (Mohammad, 2020) in Iraq the infection, in the neck and face is more than the genitals. Also, a study (Maytham and Abbas, 2012) in Iraq showed that the infection is more in the face and neck by 78.18%. Also, a study conducted that 64% of lesions in the trunk and extremities. Also, a study (Maytham and Abbas, 2012) in Iraq on the number, of infections, as it is one, two or three infections that affect the body, which is caused by the difference in body tissues from one person to another, as well as the immune system and methods of prevention, and also the person being infected with other skin diseases that help in infection with the *Molluscum contagiosum virus*.

Table (4-6): Distribution of MCV according to the site of lesion of patients.

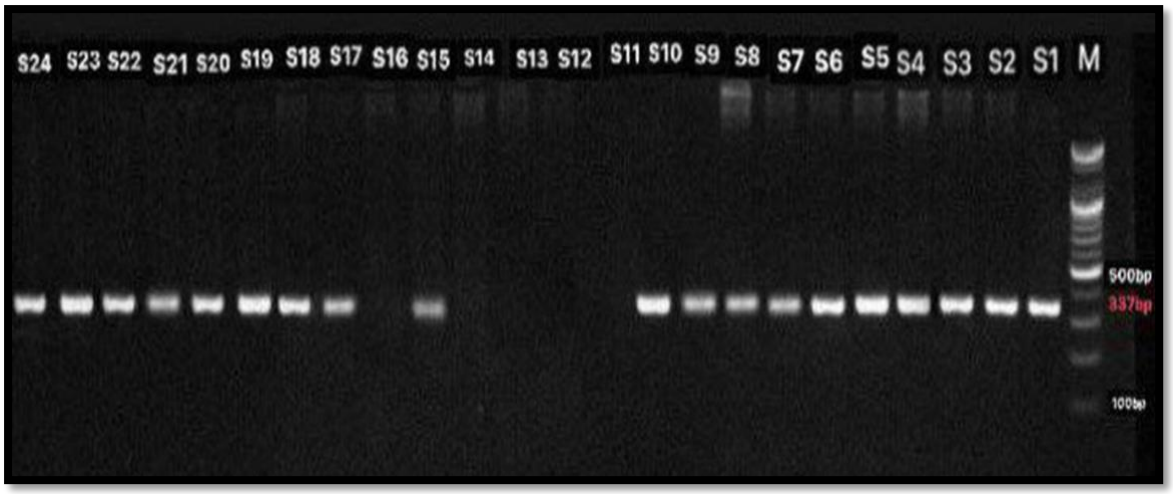
Site of lesion	Frequency	Percentage %
Face/Neck	126	90%
Genitals	14	10%
Total	140	100%

4. 2 PCR detection :

In our current study, clinical samples were diagnosed by PCR using the universal primer p43k with a size of 337 bp. 50 samples were examined by PCR and the results showed that 82% were positive and were infected with Molluscum contagiosum virus and 18% were negative Table 4-7.

Table (4-7) :Detection of MCV in samples of the patients by PCR technique.

Result	Frequency	Percentage%
Positive	41	82%
Negative	9	18%
Total	50	100%



Figure(4-2): Agarose gel electrophoresis images of PCR results show that the amplified fragment was expressed p43k gene in 337 bp samples

Fragment sequences were analyzed at Macrogen Corporation in Korea. DNA sequencing results showed that to identify MCV species, amplicons from ten isolates were sent and the isolates were successfully sequenced and identified as *Molluscum contagiosum virus*. The *Molluscum contagiosum virus* isolates and molecular identity were determined through the NCBI database using BLAST software. All the investigated genetic sequences were deposited in the NCBI web server, and unique accession numbers were obtained for all analyzed sequences. Ten GenBank accession numbers of the P43K amplicons of PQ816764 to PQ816773 were deposited in NBCI to represent the A1 to A10 samples, respectively as shown in Table (4-8)

Table (4-8): Nucleotide Sequencing Data for Isolates.

Isolate No.	Accession numbers	Identity %	Query cover %	Strand
A1-F	PQ816764	100%	100%	Plus/Plus
A2-F	PQ816765	100%	100%	Plus/Plus
A3-F	PQ816766	100%	100%	Plus/Plus
A4-F	PQ816767	99%	100%	Plus/Plus
A5-F	PQ816768	99%	100%	Plus/Plus
A6-F	PQ816769	99%	100%	Plus/Plus
A7-F	PQ816770	99%	100%	Plus/Plus
A8-F	PQ816771	99%	100%	Plus/Plus
A9-F	PQ816772	99%	100%	Plus/Plus
A10-F	PQ816773	99%	100%	Plus/Plus

4.2.1 Phylogenetic tree and Bioinformatic of *Molluscum contagiosum virus*:

Sequencing Analysis of P43K gene Sequences of *Molluscum contagiosum virus*: within the targeted locus, ten samples were placed (assigned A1 to A10) in the current study. Samples were tested to amplify the p43e gene for the studied virus

types. The NCBI BLASTn engine show the highest sequence similarities, the A1 – A10 samples and the reference P43K gene sequences of *Molluscum contagiosum virus* (GenBank acc. EF138622.1).

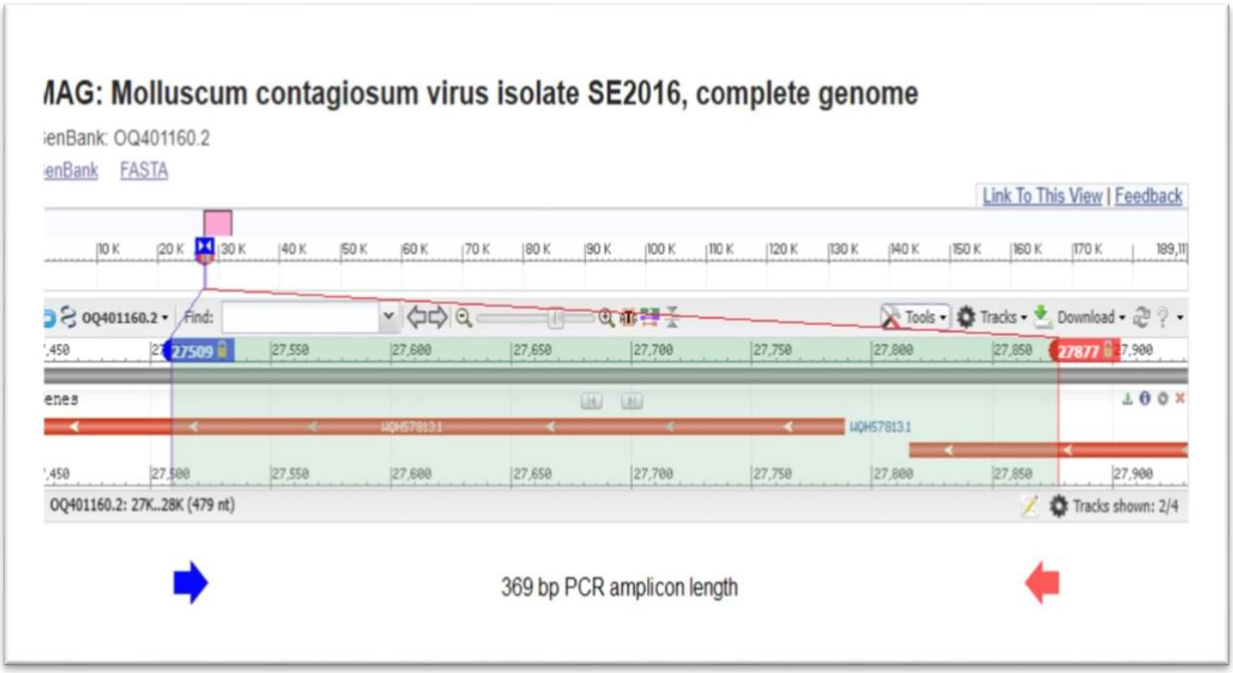


Figure (4-3): showed the locus of the part of p43k gene of MCV which the blue and red arrows refer to the start and end of the amplified PCR fragment.

After positioning the P43K gene amplicons' sequences within the genomic sequences of the amplified Molluscum contagiosum virus sequences, the details of its sequences were highlighted, and the total length of the amplified amplicons was also determined (**Table** 4-9).

Table(4-9). The position and length of the PCR amplicons that are used to partially amplify the P43K sequences with in the amplified *MCV* genomic sequence.

Amplicon	Reference locus sequences (5′ - 3′)	length
1) Molluscum contagiosum viral sequences	*GCGCGTGCGCGCACGCGGGCGCTCGCGAGTACGCGTGTGCGCGTGCGCGCGCA GGTGCGTAACATCGGTGCGTTTTTTGCGGCCTTAAAATGGGAAACCTCACCTCT GAGCCCGCGAACTGCAAGATTGTGGAAACGTTGCCTGCGACGCTGCCGCTGGCA CTGCCTACTGGCAGCATGCTCACGTACGACTGCTTTGACACGCTCATCTCGCAG ACGCGGAAAGAGCTGTGCATCGCGTCGTACTGCTGCAACCTGCGCTCCACGCCC GAGGGCGGACACGTGCTGCTGCGGCTGCTGGAGCTAGCGCGCGCCGACGTGCGC GTAACCATCATCGTGGACGAGCAGAGCCGGGACGCGGACGCTACGC**	369 bp

* refers to the position of the forward primer

** refers to the position of the reverse primer

Results of the alignment of the P43K gene sequences for two nucleic-acid variations in the samples, compared to the most similar reference nucleic acid sequences of *Molluscum contagiosum virus* (GenBank accession number EF138622.1.1) (Figure 9). These variations include 110G>T, detected in samples A1–A3, and 117A>C, detected in samples A1–A4.figuer 4-4.

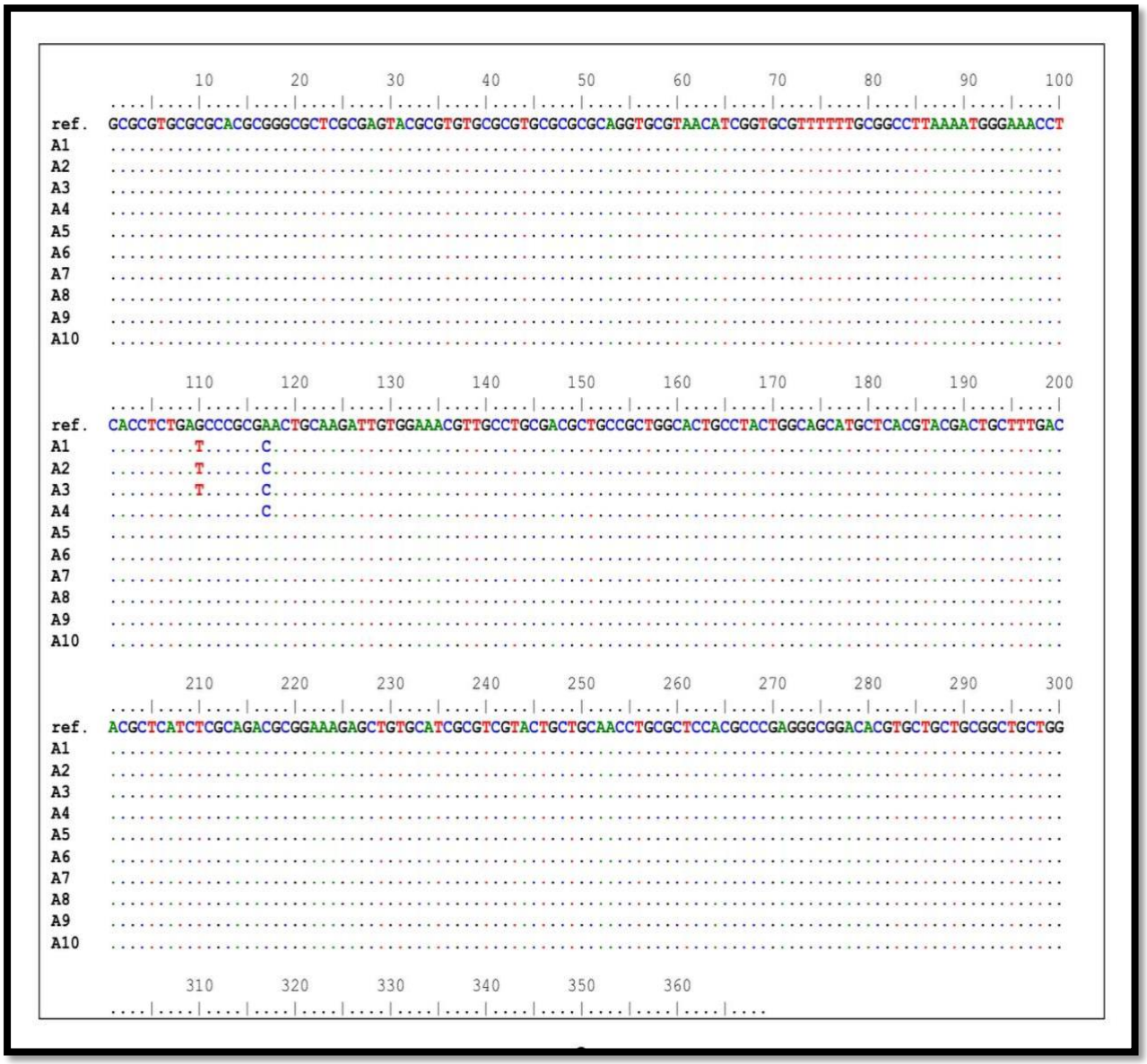
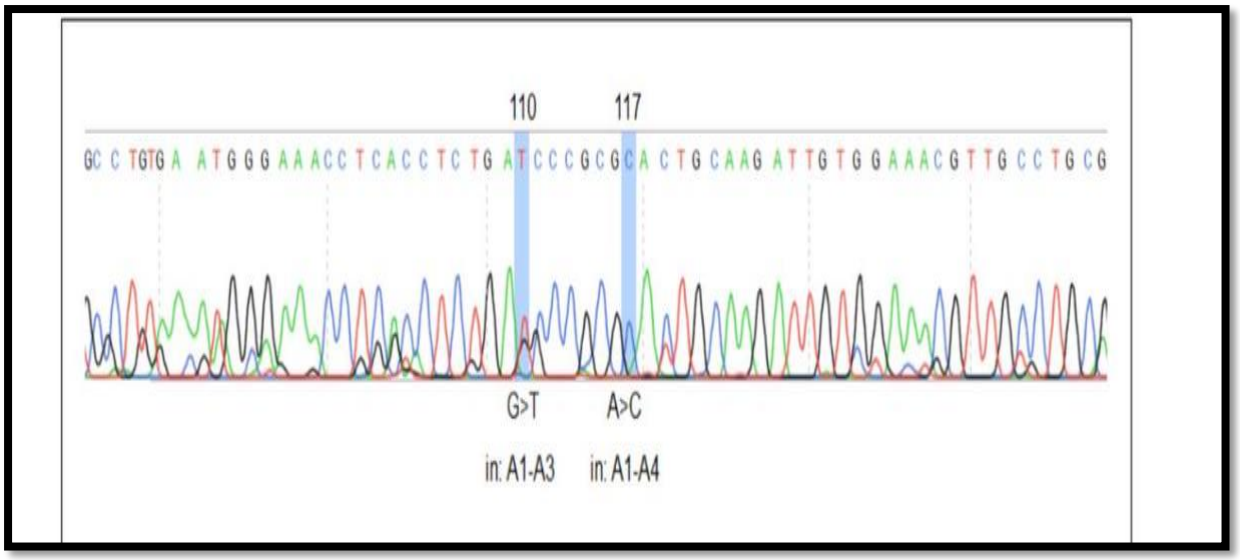


Figure (4-4): Nucleic acid sequences of ten Molluscum contagiosum virus samples with their reference- sequences p43K gene genomic sequences. The symbol "ref" refers to the NCBI referring sequence, while the letter "A#" refers to the sample code..

The presence of the two observed nucleic acid variations was thoroughly validated through a detailed manual inspection of the corresponding DNA chromatograms. This process ensured the accuracy of the detected mutations and ruled out any potential technical errors during sequence analysis (Figure 4-5).



Figure(4-5):. The chromatogram of the *Molluscum contagiosum virus* sequences in the amplified PCR products in study. The symbol ">" refers to the substitution mutation.

The observed nucleic acid variations were further analyzed to identify whether such substitutions induce possible alteration in their corresponding positions in the translated products of protein of 43 kilodaltons (p43k) protein. Accordingly, the amplified nucleic acid sequences of the A1 – A10 samples were translated to their corresponding amino acid sequences using the Expasy translate server. As a result of these translations, it was found that both of the observed nucleic acid variations were found to exhibit amino acid substitutions (7E>D and 10N>H) (Figure 4-6) (Table 4-10).

Table (4-10): Nucleic acid variations to exhibit amino acid substitutions.

Gene	VanSNP	a.a.change	Type	Genetic code
p43K	110G>T	7E>D	Missinse	GAG>GAU
P43K	117A>C	10N>H	Missinse	AAC>CAC

Table(4-11): Amino acid abbreviations.

Abbrevia.a.acid	Type of a.acid
E	Glutimine aid
D	Aspartic acid
N	Asparagine
H	Histidine

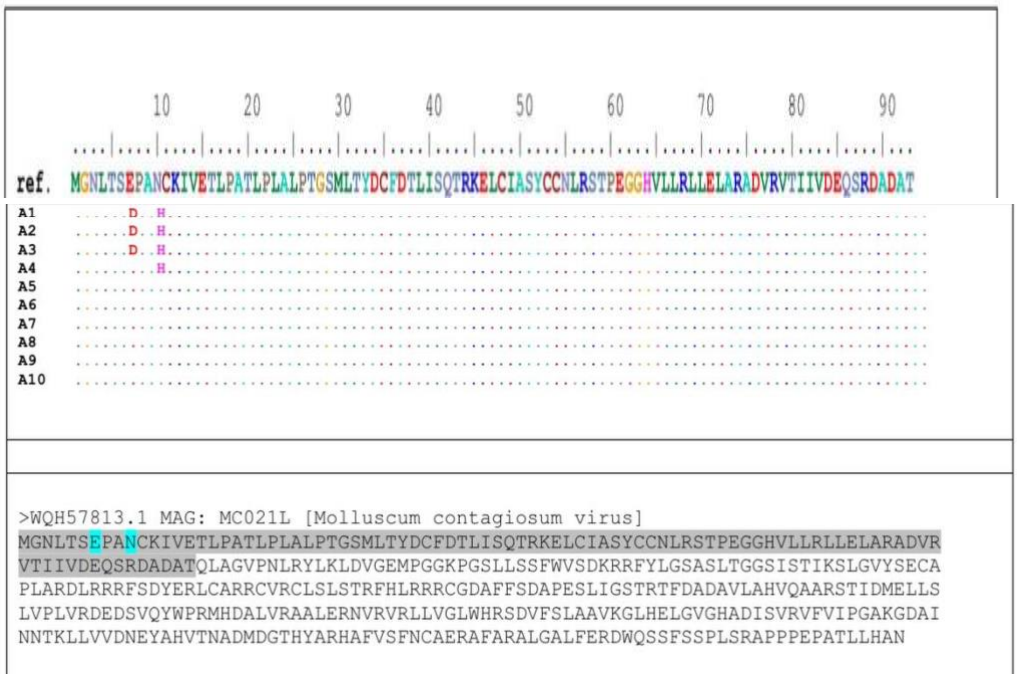


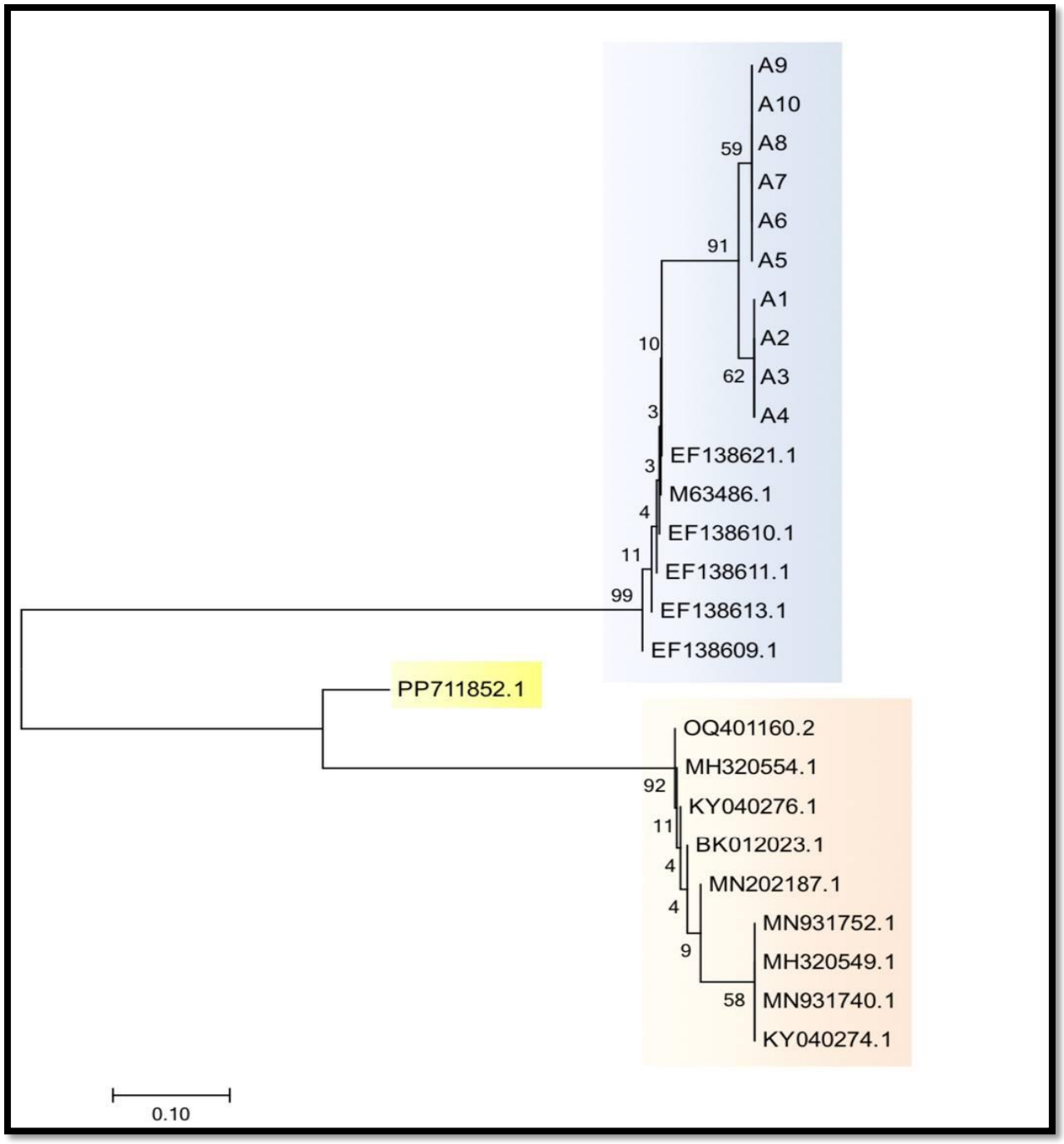
Figure (4-6):Amino acid residues alignment of the detected variations within the amplified products in the study. P43K sequences are translated to their corresponding sequences in the protein of 43kilodaltons.

The studied gene sequences were placed in the National Center for Biotechnology Information (NCBI) and accession numbers were taken for all sequences that were studied and analyzed. Ten GenBank accession numbers of the P43K amplicons of PQ816764 to PQ816773 were deposited in NBCI to represent the A1 to A10 samples, respectively. To clarify the evolutionary relationship between the samples we studied and the reference strains that are related to the amplified *Molluscum contagiosum virus* samples, a genetic tree was constructed based on the DNA sequences of the p43k gene, which included the amplified samples and the samples related to the virus strains.A rectangular cladogram was generated to illustrate the *Molluscum contagiosum virus* sequences with their corresponding sequences (Figure 4-7).

The total number of aligned nucleic acid sequences in the generated tree was twenty-six. The evolutionary relationships of the taxa studied were inferred by methods: Neighbor--Joining . Evolutionary tree with all branch lengths = 1.32550160. The percentage of similar trees that group related taxa together in a reassignment test (1000 replicates)is shown next to the branches. The genetic tree was drawn to scale, and the branch lengths were plotted using units of evolutionary distance. These distances were calculated using the maximum composite method, which is the number of base substitutions per site.

Based on the deposited annotation of the P43K gene in the GenBank OQ401160.2, the third reading frame of the codon positions is included. There were a total of 41 positions in the final dataset. Within the generated phylogenetic tree, three distinct viral clades were identified: clade-1, clade-2, and clade-3. In the major clade-1 (blue clade), our analyzed samples were distributed into two separate clusters. The first cluster includes samples A1–A4, while the second comprises samples A5–A10. This separation is attributed to the presence of the SNPs 110G>T and 117A>C, which were exclusively detected in the A1–A4 samples. Despite this
division, both clusters are distinctly positioned within the overarching clade-1. Interestingly, GenBank accession EF138621.1 was identified as the closest relative to our samples. This sequence corresponds to *Molluscum contagiosum virus* subtype 1, which was originally isolated from Thai children. This finding suggests that our samples may have originated from an Asian lineage. Clade-2 (yellow clade) serves as outgroup, represented by Rousettus bat poxvirus due to its genetic relatedness to the P43K sequences of *Molluscum contagiosum virus*. This clade is positioned distinctly apart from both clade-1 and clade-3, highlighting significant evolutionary divergence from the other two clades of *Molluscum contagiosum virus*. This separation underscores the broad biological diversity of the virus.

Clade-3 (pink clade), which is also distant from clade-1, further emphasizes this diversity.The clade-3 is consist of two positions, the first one include viral particles of the subtype-1, such as MH320554.1. Whereas the second position is represented by the viral particles of the subtype-2, such as MH320549.1 and KY040274.1. However, the distant and diverse evolutionary positioning of this clade underscores the high sensitivity of the P43K fragment used in this analysis to describe viral diversity.The phylogenetic tree was constructed based on an alignment comprising 393 nucleotide sites. Among these, 146 sites were conserved, reflecting regions of genetic stability, while 241 sites exhibited variability. Of the variable sites, 219 were parsimony-informative, providing critical information for reconstructing evolutionary relationships, and 22 were singleton sites, representing unique variations. The alignment also revealed codon degeneracy, with 292 zero-fold degenerate sites indicating positions with no synonymous substitution potential, 14 two-fold degenerate sites allowing limited synonymous changes, and 57 four-fold degenerate sites permitting complete synonymous substitution. These features underscore the alignment's utility for robust phylogenetic analysis.



Figure(4-7):.The rectangular phylogenetic tree of p43K gene sequences of the *Molluscum contagiosum virus*-based tree. All the mentioned numbers referred to the GenBank accession number of each referring species. The number at the bottom portion of the tree refers to the degree of scale range among the comprehensive tree-categorized organisms. The letter "A#" refers to the code of the investigated samples

4.3 Histopathological study of MCV:

Histopathology of *Molluscum contagiosum virus*(MCV) lesion shows proliferation stratum spinosum cells in the form of lobules containing central cellular and viral debris and complex changes occur in the level of the skin tissues, especially in the layers of the epidermis. The lobules are separated within the epidermis by septa in the connective tissue and the presence of molluscan deposit particles in the lobules that are in the form of round or oval cells undergoing keratohyalin degeneration. Also in the basal layer there are cell divisions and enlarged basal nuclei (Hayashida *et al*., 2010 ; Giner-Soriano *et al*., 2019) vacuolization process can be found and eosinophilic globies are obtained as shown in (figure 4-8). In histological examination of the enlarged masses, clear molluscum bodies were found in the cytoplasm. The histological findings of patients infected with *Molluscum contagiosum virus* were found to be lobulated, with each of these lobes causing *Molluscum contagiosum virus* to replicate in the skin. This study is consistent with (Chakrabarti *et al*., 2016) and also with (Gatea *et al.*, 2019). *Molluscum contagiosum virus* causes blisters on the surface of the skin, which appear through the infection of the hair follicles with molluscum bodies, which in turn leads to the appearance of blisters or abscesses. There are also cells known as Koliocytes, which are found in the superficial epithelial layer. There is no barrier between the epidermis and the dermis, which leads to the extension of blood vessels and connective tissue threads due to the *Molluscum contagiosum virus* bodies, which push their nuclei to the peripheral area, thus forming cells known as Koliocytes. This is consistent with what was agreed upon by (Badri *et al.*, 2018).The virus is spherical in shape and abundantly distributed in the cytoplasm, which contains inclusions called Henderson-Patterson bodies, which have a peripheral nucleus. This is consistent with (Gupta *et al.,* 2003), who explained that the cell contains molluscum particles, also known as Henderson-Patterson bodies.

A unique characteristic of the MCV is the pathological changes, it causes. The lesion caused by the *Molluscum contagiosum virus* is surrounded by an enlarged epidermis and surrounded by lobules filled with lysed *Molluscum contagiosum* bodies or keratin. In the stratum basale, the nucleus and cytoplasm of the keratinocytes will be enlarged and there will be an increase in equal divisions, leading to abnormal multiplication and spread of the infected cells. In the stratum spinosum, the cells begin to show cytoplasmic vacuoles and the infected cells enlarge and begin to undergo structural changes and are replaced by eosinophils.

The Molluscum contagiosum bodies are well-defined cysts and suppression of the nucleus of the peripheral cells may occur. In the stratum granulosum, the Molluscum contagiosum bodies are more homogeneous, begin to lose internal parts and structural features and then peel off and appear as lobular cystic changes. The dermis is also commonly affected compared to the epidermis, which is limited to stem cell proliferation. There is inflammation in 20% of clinical lesions with epithelial infiltration by lymphocytes, histiocytes and neutrophils. Histological lesions are atypical in tissues hyperkeratosis (Berger *et a*l., 2012).

And the lesions grow to 10-15 mm, which leads to the production of a giant soft tumor. The *Molluscum contagiosum* is flesh-colored, transparent, dome-shaped, and has a distinct central umbilicus. It is more common on the face, neck and thighs, and is minimum common on t-he genitals , anus (DiBiagio *et al*., 2018). Adults: Lesions are more common in the genitals. Adults with genital infections rarely develop lesions outside the genitals, compared with 10-50% of children with genital infections. Single lesions sometimes disappear within two months (Hayashida *et al*., 2010).

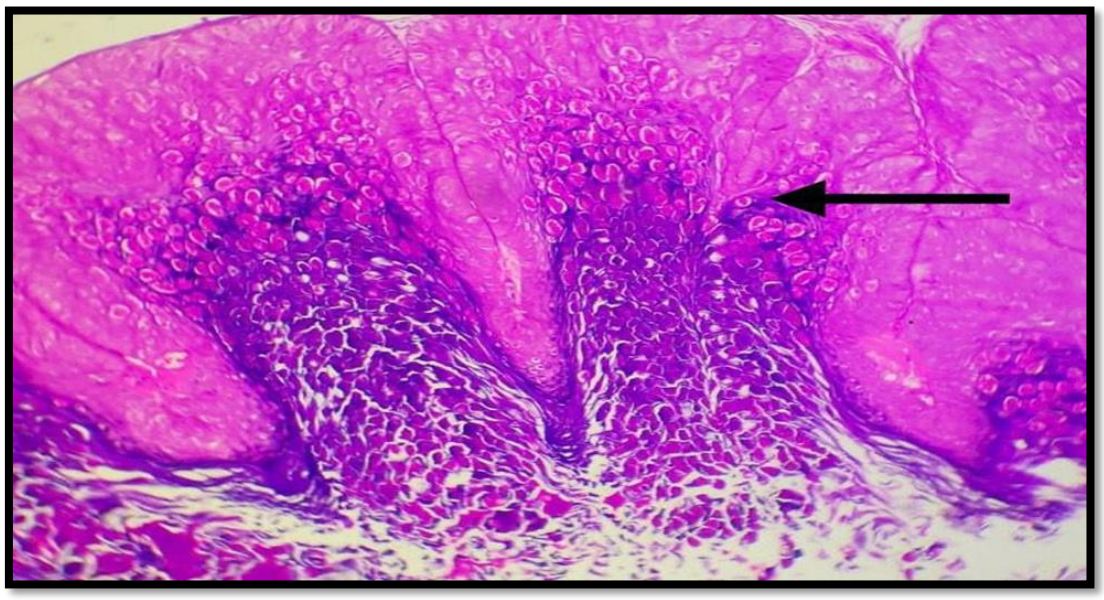


Figure (4-8): Lobular hyperplasia of epidermis resulting in a cup shaped invagination into the dermis (Hand E, ×4).

The results of the histological aspect of the samples of patients with *Molluscum contagiosum virus* showed cellular effects in the keratinocytes and the formation of Henderson-Paterson bodies, which indicates that the person is infected with the *Molluscum contagiosum virus*, as shown in the(Figure 4-9).

The primary diagnosis for patients with *Molluscum contagiosum* was a clinical examination. It can be difficult in certain cases, so the diagnosis must be made with an accurate diagnosis after taking a biopsy from the patient and fixing it with 10% formalin and staining it with hematoxylin and eosin. It began with the onset of pathological tissue that occurred inside the skin, the presence of Henderson-Patterson bodies, *Molluscum contagiosum* bodies, as shown in the (Figure4-9).

In addition, results were obtained on skin strips from patients with, the *Molluscum contagiosum virus*, which showed tissue changes in the form of masses of varying sizes, and each mass is surrounded by connective fibers and lobes separated from each other by threads of fibers that grow downward towards the deep layer of the dermis. Among the skin layers, we note that the superficial epithelium has large, abundant masses and invaginations into the subcutaneous dermis, extending to the deep layer. Keratinization is evident and excessive, and as for the keratinocytes, they show activity in many forms of mitosis. Keratinization appears with cellular debris close to the surface and most of the cells are enlarged, which is consistent with (kanilakis ,2011).

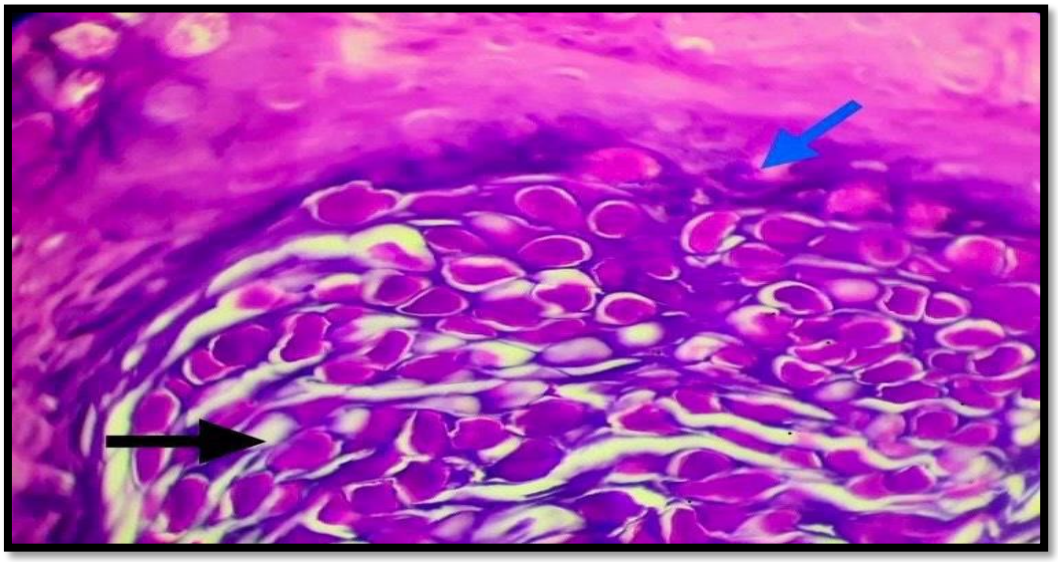


Figure (4-9):Henderson-Paterson/molluscum bodies appearing eosinophilic in the spinous layer black arrow and basophilic in the granular layer bule arrow (H and E, ×40).

4.4 Transmission electron microscope study of *Molluscum contagiosum virus*:

Finally, the results of the current study obtained through the transmission electron microscope showed that the mosaic structure conforms to the cross-sectional shape of the *Molluscum contagiosum virus* particles observed by the light microscope, which are in the form of large cells with an acidic cytoplasm, granular and a small peripheral nucleus, which is considered a distinctive sign, as shown in Figure 4-10. The shape of the *Molluscum contagiosum virus* particles under the transmission electron microscope was that the infected keratinocytes were a viral colony cyst that is found in the cytoplasm and its shape is also spherical, oval or brick-shaped, and we also found a keratin passage below the central navel and viral particles at the end of the passage and on the surface of the lesion, which explains its diffuse shape. It shows the proliferation of epithelial cells that begins with the keratinocytes of the outer root sheath, which we conclude from it its skin localization, in contrast to the human papillomavirus infection that occurs in the skin. This biopsy was taken from sample No. 4 and sample No. 35, which were showed positive PCR results.

The results of the samples examined by electron microscopy revealed a relationship about the structure of the molluscan cells and there are also two reports on the use of transmission and electron microscopy (Smith *et al*., 1992). The results of our present study revealed an epidermal-like layer surrounding the proliferation caused by the *Molluscum contagiosum virus*. The mosaic structure found in the cross-section indicates that the infected cells that appear under the light microscope as molluscum bodies are large in size with acidic cytoplasm and the nucleus is small and peripheral in location that is considered as a distinctive sign of molluscum. (Jain *et al*., 2000).

In previous reports, using scanning electron microscopy, keratinocytes infected with viral cyst colonies were described (Shelley and Burmeister, 1986). This means that the virus replicates intensively inside these cells. Transmission electron microscopy showed that there were large amounts of viral clumps in the cytoplasm with the cell nucleus shifted to the side .Also, replication *Molluscum contagiosum virus* inside the cell leads to pressure on the nucleus, which leads to its destruction, as it a characteristic sign of the effect of viruses on infected cells (Fonseca *et al*., 1987).

In previous studies, *Molluscum contagiosum virus* has been described as having a spherical, oval, or brick-like shape (i.e., it refers to a rectangular or multi-faceted shape).It was observed that only the viruses studied had a spherical shape. This suggests that the spherical shape is the most common or dominant shape in the samples studied.

It was noted that the size of the virus was quite similar to what the data obtained support the information in the scientific literature about the size of MCV (Mihara, 1991).

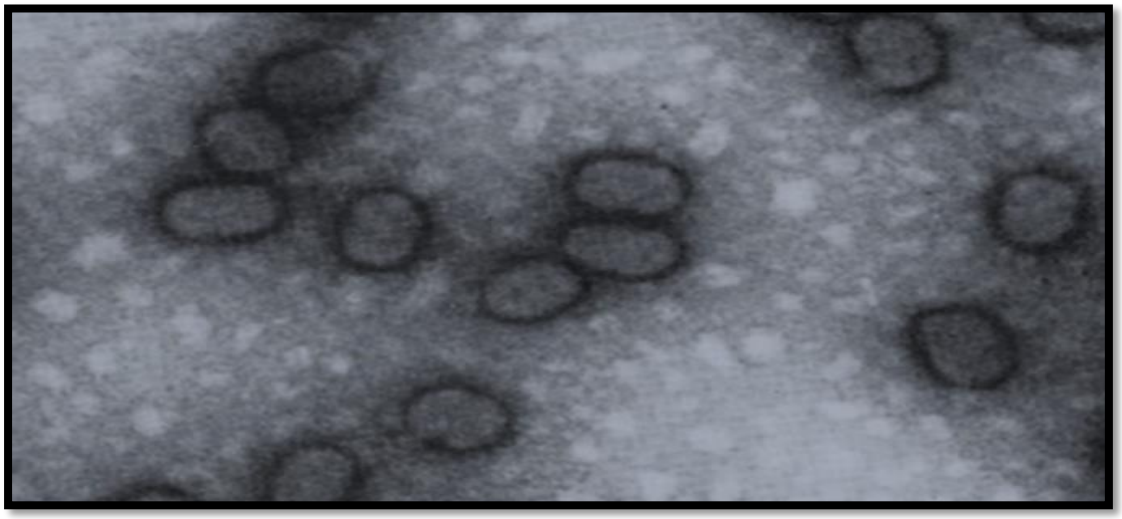


Figure (4-10):Electron microscopy (TEM) examination of clinical samples by Zeiss Supea 55vp with STEM detector

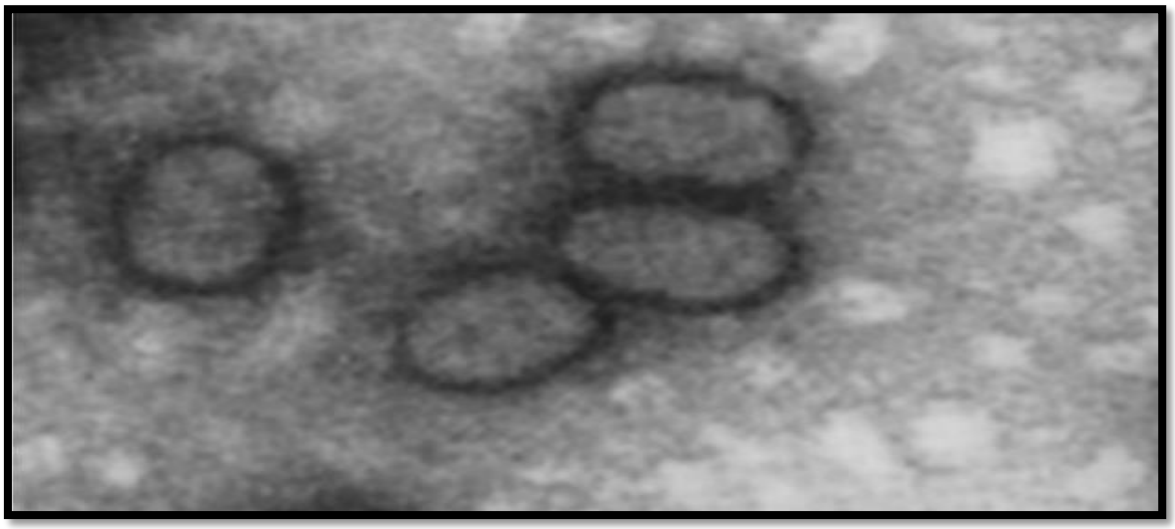


Figure (4-11): Electron microscopy (TEM) examination of clinical samples by Zeiss Supea 55vp with STEM detector .

Chapter Five

Conclusions and Recommendations

5.Conclusions and Recommendations

5.1. Conclusions:

1. In the current study, no association was found between swimming pools and *Molluscum contagiosum virus.*

2. The prevalence of *Molluscum contagiosum virus* is higher in males than in females in Maysan Governorate.

3. The P43k gene was strongly associated with infection in the 1-10 year age group, with a lesser association with the site of infection and medical history.

4. The severity of infection was associated with other skin diseases or immunodeficiency in the patient.

5. The appearance of the *Molluscum contagiosum virus u*nder the electron microscope was significantly larger than in other studies.

6. The presence of Henderson-Paterson bodies under the light microscope is indicative of *Molluscum contagiosum* infection.

7. The impact of geographical factors on the incidence of *Molluscum contagiosum,* which is higher in urban areas than in rural areas.

8.The prevalence of *Molluscum contagiosum* was higher on the face and neck (90%) than on the genitals (10%).

5.2.Recommedations:

1. It is recommended to study smallpox viruses, including monkeypox, using molecular PCR methods.
2. A comprehensive study of rural and urban pools should be conducted to identify the viruses transmitted from them and their impact on society.
3. A health awareness program should be developed regarding the transmission of *Molluscum contagiosum* within families and society at large, as well as the factors that promote it.
4. It is recommended to conduct a comprehensive study of swimming pool users and the equipment used in swimming pools.
5. A detailed study of the *Molluscum contagiosum virus* genome is recommended.
6. study the relationship between immunity and infection with *Molluscum contagiousm virus* is recommended.
7. It is recommended to study and detect rotavirus using molecular methods.

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Appendixces

Appendix 1:

Patient Questionnaire

Age:

Gender:

Place of Residence:

Site of Lesions:

History of Infection:

Diseases Associated with Molluscum Contagiosum virus (MCV):

Duration of Infection:

Season of Infection:

Recurrence of Lesions:

Marital Status:

Educational Attainment:

Appendix 2:

SUBMITTED SEQUENCES TO NCBI PORTAL

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Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae;
Molluscipoxvirus.
REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
REFERENCE 2 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Direct Submission
JOURNAL Submitted (27-DEC-2024) Department of Biology, College of Science,
University of Misan, University complex, Misan, El Emara 62001,
Iraq
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ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota;
Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae;
Molluscipoxvirus.
REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
REFERENCE 2 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Direct Submission
JOURNAL Submitted (27-DEC-2024) Department of Biology, College of Science,
University of Misan, University complex, Misan, El Emara 62001,
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ACCESSION Seq3
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ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota;
Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae;
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REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
REFERENCE 2 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Direct Submission
JOURNAL Submitted (27-DEC-2024) Department of Biology, College of Science,
University of Misan, University complex, Misan, El Emara 62001,
Iraq
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REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
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AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
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University of Misan, University complex, Misan, El Emara 62001,
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AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
REFERENCE 2 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.

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JOURNAL Submitted (27-DEC-2024) Department of Biology, College of Science,
University of Misan, University complex, Misan, El Emara 62001,
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Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota;
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Molluscipoxvirus.
REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
REFERENCE 2 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Direct Submission
JOURNAL Submitted (27-DEC-2024) Department of Biology, College of Science,
University of Misan, University complex, Misan, El Emara 62001,
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Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae;
Molluscipoxvirus.
REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
REFERENCE 2 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Direct Submission
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ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota;
Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae;
Molluscipoxvirus.
REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province
JOURNAL unpublished
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TITLE Direct Submission
JOURNAL Submitted (27-DEC-2024) Department of Biology, College of Science,
University of Misan, University complex, Misan, El Emara 62001,
Iraq
COMMENT Bankit Comment: ALT EMAIL:baquralhilly_79@yahoo.com
Bankit Comment: TOTAL # OF SEQS:10
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121 acgtacgact gctttgacac gctcatctcg cagacgcgga aagagctgtg catcgcgtcg
181 tactgctgca acctgcgctc cacgcccgag ggcggacacg tgctgctgcg gctgctggag
241 ctagcgcgcg ccgacgtgcg cgtaaccatc atcgtggacg agcagagccg ggacgcggac
301 gctacgc

LOCUS Seq9 307 bp DNA linear VRL 27-DEC-2024

DEFINITION Molluscum contagiosum virus strain Jamali-A9.
ACCESSION Seq9
VERSION
KEYWORDS .
SOURCE Molluscum contagiosum virus
ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota;
Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae;
Molluscipoxvirus.
REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
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Bankit Comment: TOTAL # OF SEQS:10
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Sequencing Technology :: Sanger dideoxy sequencing
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121 acgtacgact gctttgacac gctcatctcg cagacgcgga aagagctgtg catcgcgtcg
181 tactgctgca acctgcgctc cacgcccgag ggcggacacg tgctgctgcg gctgctggag
241 ctagcgcgcg ccgacgtgcg cgtaaccatc atcgtggacg agcagagccg ggacgcggac
301 gctacgc

LOCUS Seq10 307 bp DNA linear VRL 27-DEC-2024
DEFINITION Molluscum contagiosum virus strain Jamali-A10.
ACCESSION Seq10
VERSION
KEYWORDS .
SOURCE Molluscum contagiosum virus
ORGANISM Molluscum contagiosum virus
Viruses; Varidnaviria; Bamfordvirae; Nucleocytoviricota;
Pokkesviricetes; Chitovirales; Poxviridae; Chordopoxvirinae;
Molluscipoxvirus.
REFERENCE 1 (bases 1 to 307)
AUTHORS Al-Jamali,Z.A., Al-dby,S.H. and Alrashedi,H.S.
TITLE Ecological and Diagnostic Study of Molluscum contagiousm virus in Maysan Province

JOURNAL unpublished
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COMMENT Bankit Comment: ALT EMAIL:baquralhilly_79@yahoo.com
Bankit Comment: TOTAL # OF SEQS:10
##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
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BASE COUNT 57 a 98 c 95 g 57 t
ORIGIN
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181 tactgctgca acctgcgctc cacgcccgag ggcggacacg tgctgctgcg gctgctggag
241 ctagcgcgcg ccgacgtgcg cgtaaccatc atcgtggacg agcagagccg ggacgcggac
301 gctacgc

Appendix 3:

Chromatography of the studied Molluscum contagiosum virus samples. The letter "AF 1" refers to the sample code used in this study.

File: A1_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:149 A:215 C:431 T:208 Sample: A1_AF Lane: 11 Base spacing: 16.123238 2093 bases in 25283 scans

File: A2_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:171 A:236 C:489 T:235 Sample: A2_AF Lane: 9 Base spacing: 16.17097 1574 bases in 19442 scans

File: A3_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:536 A:931 C:1914 T:846 Sample: A3_AF Lane: 7 Base spacing: 16.057402 1843 bases in 24020 scans

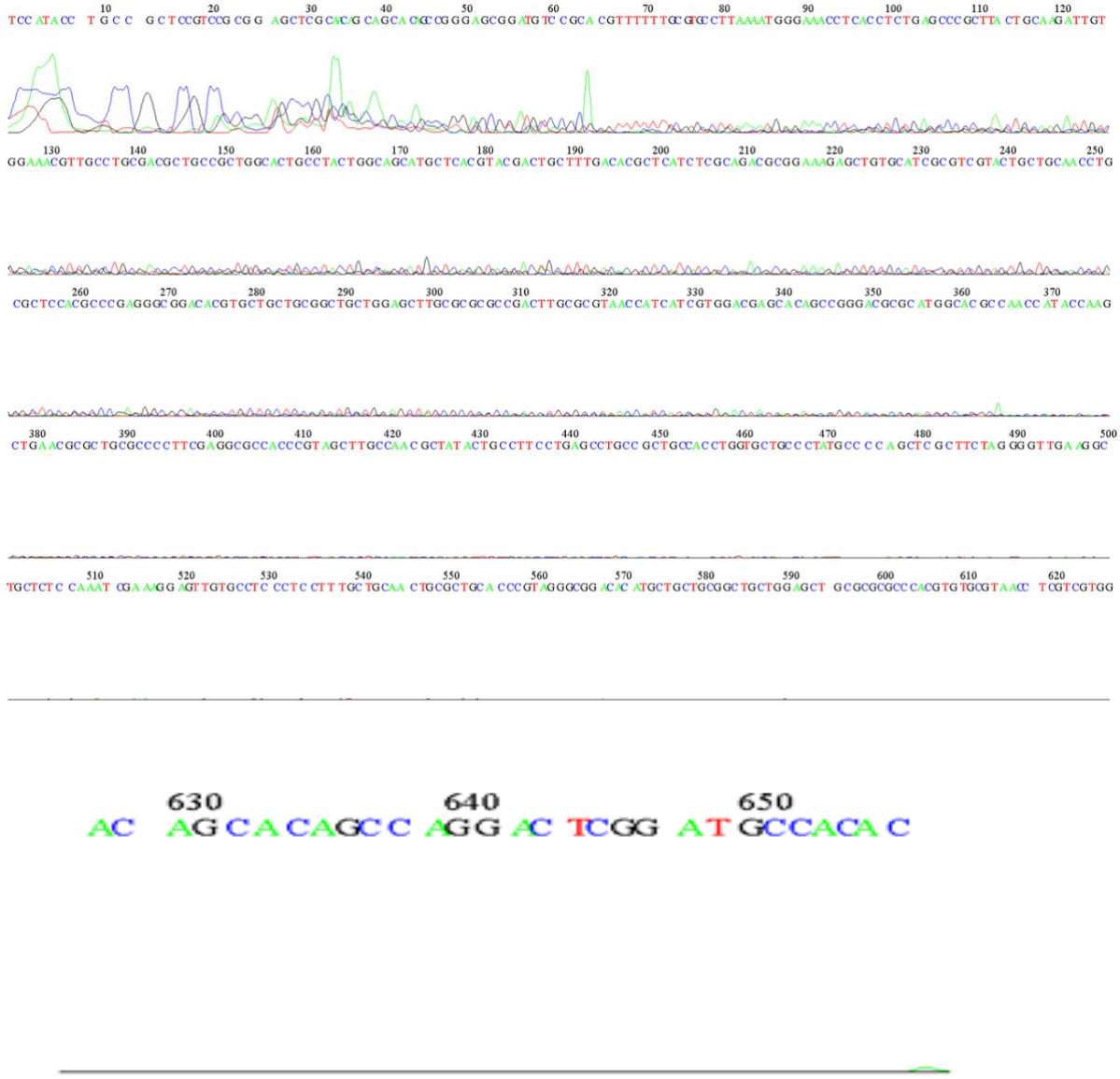
File: A4_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:295 A:452 C:1129 T:490 Sample: A4_AF Lane: 5 Base spacing: 16.243118 1838 bases in 25868 scans

File: A5_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:568 A:561 C:5800 T:727 Sample: A5_AF Lane: 3 Base spacing: -16.163063 1810 bases in 21401 scans

File: A6_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:1456 A:1621 C:3511 T:1740 Sample: A6_AF Lane: 1 Base spacing: 16.327736 2122 bases in 23444 scans

File: A7_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:183 A:298 C:438 T:232 Sample: A7_AF Lane: 16 Base spacing: 16.265802 2042 bases in 25238 scans

File: A8_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:970 A:1217 C:2333 T:1054 Sample: A8_AF Lane: 14 Base spacing: 16.392082 656 bases in 7863 scans

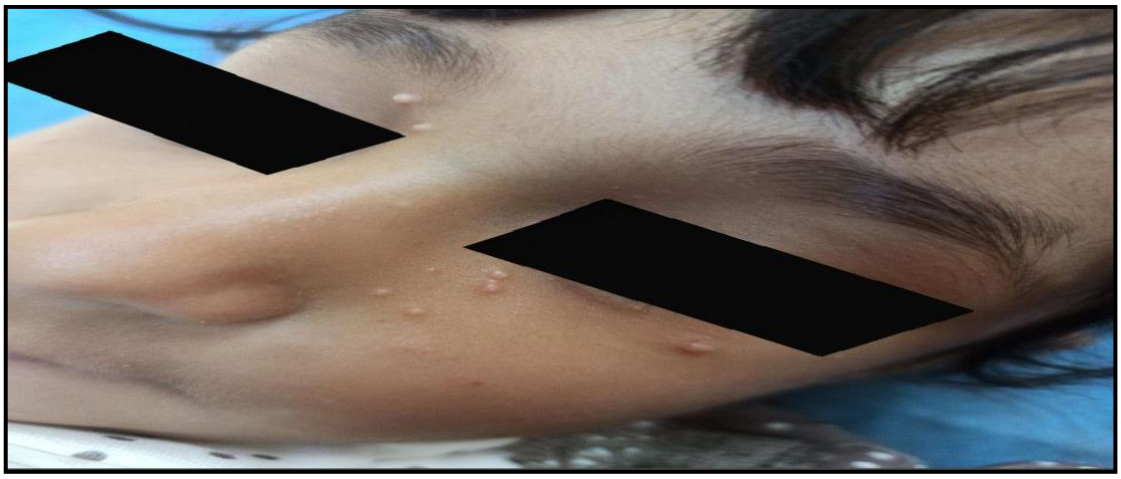


File: A9_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:627 A:643 C:1407 T:539 Sample: A9_AF Lane: 12 Base spacing: 15.845614 2175 bases in 23857 scans

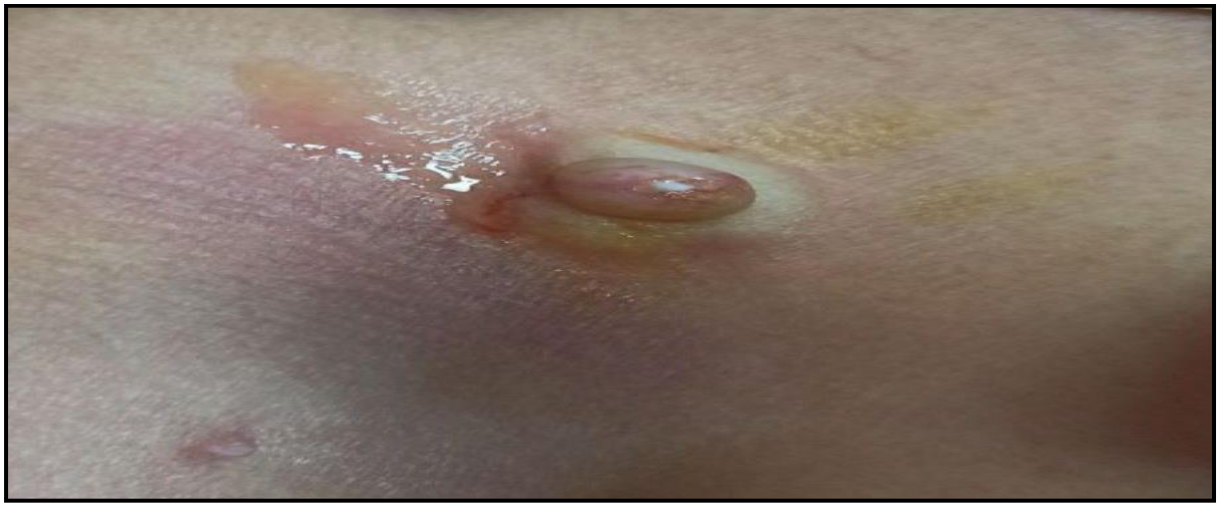
File: A10_AF.ab1 Run Ended: 2024/7/16 11:57:59 Signal G:576 A:832 C:1584 T:709 Sample: A10_AF Lane: 10 Base spacing: 16.302717 878 bases in 10549 scans

Appendix 4:

A seven-year-old male is infected with the *Molluscum contagiosum virus*, The first infection.



A 40-year-old female is infected with the *Molluscum contagiosum virus* in the neck,The first infection. showing a central area with white and/or yellow.



الخلاصة

بحثت الدراسة الحالية عن جين p43K لفايروس المليساء المعدية المسبب لمرض داء لؤلؤ في محافظه ميسان الذي تم الكشف عنه في العنيات البيئيه لمياة احواض السباحه والعينات السريريه المأخوذه من المصابين بداء اللؤلؤ والذي تم تشخيصه سريريًا و بالطرق الجزيئيه ووالطرق النسيجيه المرضيه والفحص بلمجهر الالكتروني النافذ وبذلك تناولت الدراسة جانبين دراسة العينات البيئيه لمياة احواض السباحه ودراسة العينات السريرية المأخوذة من المصابين بمرض داء اللؤلؤ .

الدراسة البيئيه تمحورت حول فحص عينات مياه المسابح في محافظة ميسان بالطرق الجزيئيه بااستخدام تقنيه تفاعل البوليميريز المتسلسل للكشف عن فيروس المليساء المعديه الذي تم جمع عشرون عينه مياه من احواض سباحة محافظه ميسان وكانت النتائج سلبيه لم اجد اي علاقه لانتقاله عن طريق احواض السباحه لاستخدام طرق التعقيم الصحيحه والمعالجه المستمره لاحواض السباحه في محافظه ميسان من قبل العاملين في أحواض السباحه وايضًا تم قياس الاس الهيدروجيني ودرجه الحراره للمسابح في محافظه ميسان وكانت النسب بالنسبة لأس الهيدروجيني تتراوح من (6.5_7.4) وكانت نتائج قياس درجات الحراره لاحواض السباحه تتراوح من (24_28) درجه سيليزية.

اما بالنسبة لدراسة العينات السريريه التي تم عزلها من الاشخاص المصابين بداء اللؤلؤ تم تم جمع 140 مريضًا مصابًا بداء اللؤلؤ، والذي سببه فيروس المليساء المعدية بشكل رئيسي، خلال الفترة من تشرين الثاني 2023 إلى نيسان 2024 في مستشفى الصدر التعليمي بمحافظة ميسان من ذكور واناث والاعمار تتراوح من (1_60) سنه. كانت الدراسة تتمحور حول نسبة الاصابة كانت اعلى نسبة تصيب الاعمار من (1_10) سنه بنسبة 45%وكانت اقل شيوعًا في الاعمار من (51_60) سنه بنسبة 1%.وكشفت الدراسه ايضًا ان نسبة الاصابه كانت في الذكور اعلى بنسبة 51% مما في الاناث كانت 49%. وايضًا كانت المناطق الحضريه اعلى نسبة إصابة 77% من المناطق الريفيه بنسبة23٪. وكانت ايضا مواقع الاصابه كانت اشد نسبه

في الوجه والرقبه اعلى بنسبة 90% مما هو عليه في المنطقه التناسليه بنسبة 10%وبذلك اظهرت الدراسه الاختلاط المستمر وعدم التوعيه الصحيه وعدم أستخدم طرق تعقيم الصحيحه وايضًا ضعف المناعه والامراض الجلدية الاخرى المصاحبه للمرض والعوامل الخارجيه الاخرى من مسببات العدوى بفيروس المليساء المعدية .

أُودعت جميع التسلسلات الجينية المدروسة في خادم الويب التابع للمركز الوطني لمعلومات التكنولوجيا الحيوية(Ncbi) ، وحصلنا على أرقام وصول فريدة لجميع التسلسلات التي تم تحليلها. كما أُودعت عشرة أرقام وصول من genbank لمضخمات p43k من pq816764 إلى pq816773في nbci لتمثيل العينات من a1 إلىa10 ، على التوالي. ولتعزيز فهمنا للعلاقات التطورية بين عيناتنا المدروسة والسلالات المرجعية الأكثر صلة بين عينات فيروس المليساء المعدية المُضخّمة، أنشأت هذه الدراسة شجرة تطورية بناءً على تسلسلات الأحماض النووية المحددة في مضخمات جين p43k المُضخّمة. وتضمنت الشجرة التطورية العينات المُضخّمة وتسلسلات الأحماض النووية الأخرى ذات الصلة من تسلسلاتها الخاصة. تم إنشاء مخطط فرعي مستطيل الشكل لتوضيح تسلسلات فيروس المليساء المعدية مع تسلسلاتها المقابلة. بلغ العدد الإجمالي لتسلسلات الأحماض النووية المتراصة في الشجرة المُولَّدة ستة وعشرين. تم استنتاج العلاقات التطورية للأصناف المدروسة باستخدام طريقة الجار–الربط. الشجرة المثالية بمجموع أطوال الفروع = 1.32550160. تظهر النسبة المئوية للأشجار المتماثلة التي تجمعت فيها الأصناف المرتبطة معًا في اختبار إعادة التشغيل (1000 تكرار) بجوار الفروع. رُسمت الشجرة وفقًا لمقياس الرسم، مع أطوال الفروع بنفس وحدات المسافات التطورية المستخدمة لاستنتاج الشجرة التطورية. حُسبت المسافات التطورية باستخدام طريقة الاحتمالية المركبة القصوى، وهي بوحدات عدد الاستبدالات القاعدية لكل موقع. بناءً على الشرح المُرسَّب لجين p43k في بنك الجيناتoq401160.2 ، أُدرج إطار القراءة الثالث لمواضع الكودون. بلغ إجمالي المواضع في مجموعة البيانات النهائية 41 موضعًا. كان ظهور فيروس المليساء المعدية تحت المجهر الإلكتروني أكبر بكثير مما هو عليه في دراسات أخرى. ويُشير وجود أجسام هندرسون–باترسون تحت المجهر الضوئي إلى الإصابة بالمليساء المعدية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعـــة ميسان
كليـــــة العلوم
علـــوم الحياة

التقييم البيئي والتوصيف الجزيئي لفيروس المليساء المعدية في محافظة ميسان /العراق

رسالة مقدمة
إلى مجلس كلية العلوم / جامعة ميسان
وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة
من قبل

زهراء علي فنجان
بكالوريوس في علوم الحياة / جامعة ميسان
(2020-2021)

بإشراف

ا.د. صالح حسن جازع
ا.م.د. حسن سلام حسون

أغسطس ٢٠٢٥ م صفر ١٤٤٧هـ