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ORIGINAL ARTICLE

Molecular Detection of *Molluscum contagiosum virus* (MCV) from Patients of Basra Province / Iraq

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ABSTRACT

Objectives: To detect of *Molluscum cotagiosum virus* genetically using PCR technique and DNA sequencing.

Methods: The present study was conducted during the period from October 2017 to April 2018 in outpatient clinic of Basrah Teaching hospital, Al-Fayha hospital, and Al-Muanaa hospital in Basrah city/Iraq. 102 samples from patients were detected and identified genetically by Polymerase chain reaction technique and confirmed by DNA sequencing. The potential MCV lesion samples of 102 patient were taken and detected genetically by PCR technique and confirmed using DNA sequencing.

Results: The total number of the examined samples were 102, including 62 male and 40 female. The results showed that 79 (77.4%) patients of age group (1-11years), of which 46 (58.2%) were males and 33 (44.8%) females, 23(22.6%) of age group (twelve years or more) including 17 (73.9 %) male and 6 (26%) female. The patients age in current study ranged from 1-65 years. PCR results revealed that the size of the gene after the electrophoresis of the PCR product was 393bp and this band size also reported by other studies.

Conclusion: The *Molluscum contagiosum* was predominantly in males and the age group 1-10 y was more infected than other age groups. Molluscum virus infections were leaded to in important histological changes in the skin, and Molecular detection of Molluscum virus was the best way to diagnose the infection.

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INTRODUCTION

Molluscum contagiosum is a benign and self-limiting viral skin infection that generally affects young children, young adults, and immunocompromised individuals, but can occur at any age. It can affect any part of body surface and characterized by smooth, dome-shaped discrete papules or lesions called "mollusca", usually 2-5 mm in diameter[1]. Although rare, the biggest lesions (up to 15 mm) have been reported in patients with HIV. The incubation period can vary from 14 days to 6 months[2]. Molluscum contagiosum (MC) is a skin and mucous membrane infection caused by a DNA viruses called *Molluscum virus* belongs to the family *Poxviridae* subgenus *Molluscipoxvirus*, the virus belongs to the pox group, it measures 300 nm in greatest dimension[3], which

comprises 4 genetically subdivided but clinically indistinguishable MC viral types[4]. However, virus type I is responsible for the majority (76%-97%) of MC infection[5]. In contrast, MC virus type 2 causes the majority (60%) of infection in HIV patients[6]. Clinically, MC lesions can be confused with other lesions of viral skin infections such as herpes simplex virus, varicella zoster virus, and human papillomavirus (HPV) infection, particularly, in immunecompromised patients such as HIV patients[7], therefore, the laboratory diagnosis of MCV is important[8]. MCV can affect some tissue culture cell lines causing cytopathic effect (CPE)[8]. The diagnosis of MCV is usually done clinically and the clinical diagnosis can be confirmed by a histopathology. The requirement for laboratory diagnosis MCV is contemplative, since a spontaneous healing is perceived in cases where no underlying immune defect is present. The disease is deliberated as a self-limiting situation, which deserves no more medical attention than an aesthetic nuisance[9,10]. *Molluscum contagiosum* infects the epidermal layer of the skin producing umbilicated lesions especially in children. In adults, *Molluscum contagiosum* has also been recognized during the last two decades as a sexually transmitted disease (STD. Duration of the individual lesion is varies. Although most cases of infection resolve without treatment within 6-9 months, some are lasted for 3-4 years . In USA, this virus is responsible for approximately 1% of all diagnosed dermatologic condition[11]. The MCV infections occurs worldwide. Although the MC disease is common, but the incidence rate in most areas is not exactly known yet. The main route for transmission is a direct contact with infected persons or contaminated objects such as towels, clothes or toys, but the importance of epidermal injury is unknown[2]. MCV is transmitted directly by skin contact to produce the typical cutaneous, and rarely mucosal lesions, it also transmitted via fomites on bath sponges and bath towels, in beauty parlors and school swimming pools[12]. In adults, Molluscum is often spread sexually[13].

MATERIALS AND METHODS

In current study, 102 samples were collected from the patients admitted to three main hospitals in Basrah (Basrah Teaching Hospital, Al Fayhaa Hospital and Al-Muanaa Hospital) during the period between 1 October 2017 and 30 April 2018.

The samples were immediately transferred to the virology laboratory at Biology Department, University of Basrah, Faculty of Science. The DNA was extracted from the samples using DNA extraction kit (Viral Nucleic Acid Extraction Kit II \ Geneaid / Taiwan) . The gene was then amplified by PCR technique according to a special primers (Table 1).

Table1: **Primers sequence.**

Primers	Sequence	Molecular weight
F	(5-GGCGCGTAGCCGAGCGG-3)	393-bp
R	(5'-GCTTCCGGGCTTGCCGCCGGGCAG-3')	

The PCR technique was applied by adding 5 µl from DNA extracted to the PCR tube containing 5 µl of the master mix and 1 µl of the F and R primers was then added to this PCR tube. In the end, 13 µl of the NFW were added to the tube to get 25 µl as a final size. The mixture then transfer to PCR system , the conditions of PCR reaction are listed in (Table 2).

Table 2: **The PCR amplification Condition.**

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

Histological preparation of the MCV was according to (Bancroft and steven, 2012).

RESULTS

The total numbers of the examined samples were 102, including 62 (60.8 %) infected males and 40 (39.2%) infected females. The results showed that the 79 (77.4%) patients of age group (1-11years), of which 46 (58.2%) were males and 33 (41.7 %) females, 23 (22.6%) of age group (twelve years or more) including 17 (73.9 %) male and 6 (26%) female. (Figure 1).

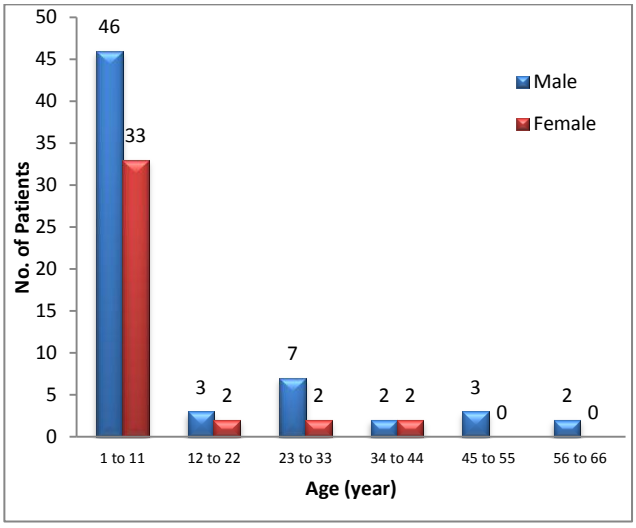

Figure 1. **MCV positive clinical specimen based on patient gender and age.**

Regarding to lesion distribution, the present study was mainly reported that the lesions of virus infection were more occurrence in head area 76.5 % and less sightseeing in trunk area 16.6%, while the infection were very few in limbs area 6.9%. Molecular detection of MCV virus has been done by extraction of the viral DNA by using Tissue Genomic Viral Nucleic Acid Extraction Kit II (Geneaid / Taiwan). MCV DNA fragment with the designed primer yielded band corresponding to their molecular size of approximately 393 bp.

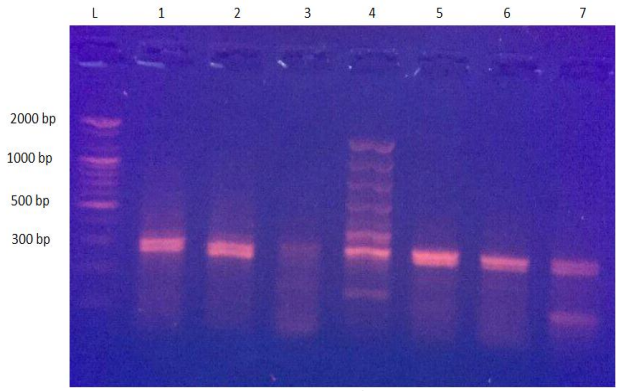

Figure 2. **Agarose gel electrophoresis of PCR products L = DNA ladder Lane 1,2,4,5,6,7 = positive to (393bp)**.

Sequencing of DNA for confirming virus Species identification: Table 3 showed that the amplicons from three isolates of MCV were submitted to sequencing , This isolates were successfully sequenced, and identified to species level as *Molluscum contagiosum* , the alignments of the virus isolates were identified and the molecular identity of the samples performed by multiple alignment of each sample sequence with NCBI database using basic local alignment search tool (BLAST) software (Appendix, 1). The best references were selected to show the results, (Figure 3, 4, 5, 6, 7 and 8).

Table 3: **Nucleotides Sequencing Data for Isolates.**

Isolate No.	Compatible with	Identity %	Query cover %	Strand
1 – R	KY040277.1	85%	80%	Plus/Minus
1 – F	KY040277.1	89%	85%	Plus/Plus
2 – R	KY040277.1	88%	79%	Plus/Minus
2 – F	KY040277.1	91%	94%	Plus/Plus
3 – R	KY040275.1	83%	31%	Plus/Minus
3 - F	KY040277.1	91%	82%	Plus/Plus

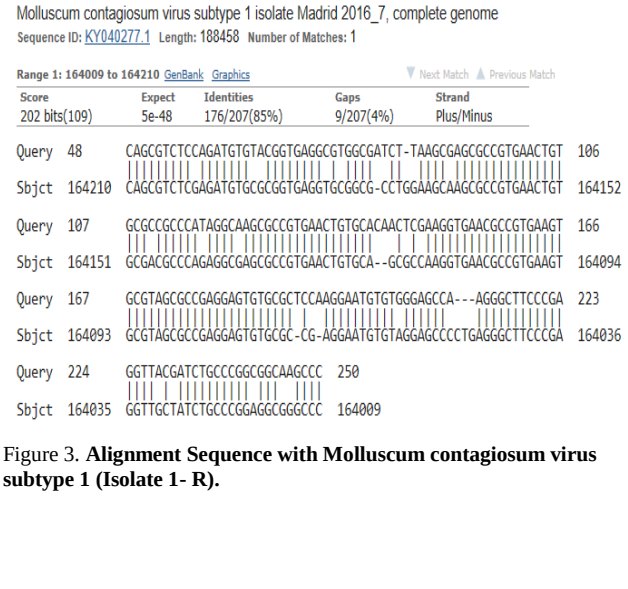

Figure 3. **Alignment Sequence with Molluscum contagiosum virus subtype 1 (Isolate 1- R).**

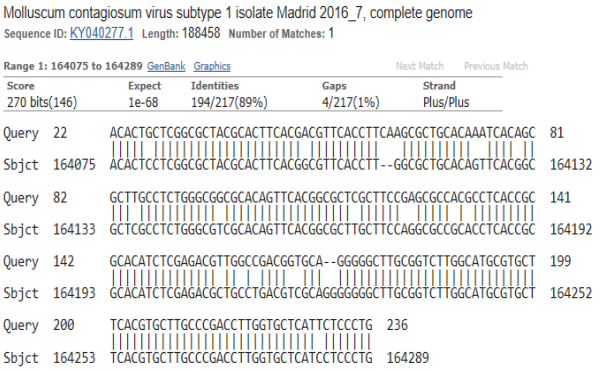

Figure 4. **Alignment Sequence with Molluscum contagiosum virus subtype 1 (Isolate 1- F).**

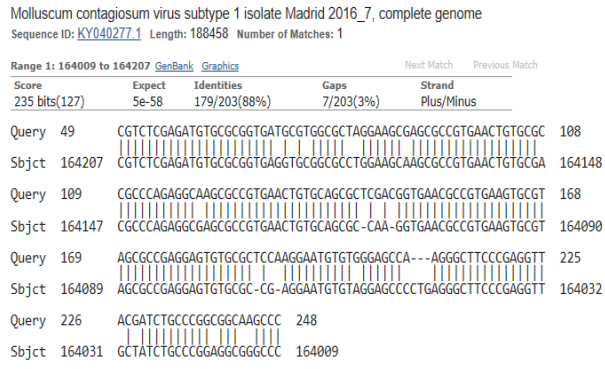

Figure 5. **Alignment Sequence with Molluscum contagiosum virus subtype 1 (Isolate 2 –R).**

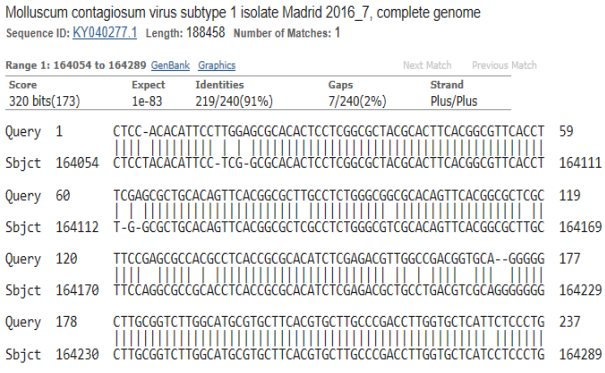

Figure 6. **Alignment Sequence with Molluscum contagiosum virus subtype 1 (Isolate 2 –F).**

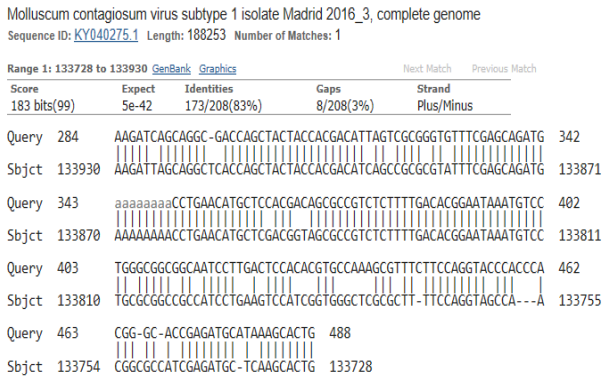

Figure 7. **Alignment Sequence with Molluscum contagiosum virus subtype 1 (Isolate 3 –R).**

Molluscum contagiosum virus subtype 1 isolate Madrid 2016_7, complete genome
Sequence ID: KY040277.1 Length: 188458 Number of Matches: 1

Range 1: 164052 to 164289 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
318 bits(172)	6e-83	220/242(91%)	8/242(3%)	Plus/Plus

```
Query  33      GGCT-C-ACAGATTCCTTGGAGCGCACACTCCTCGGCGCTACGCACTTCACGGCGTTCAC  90
               |||| | ||| ||||| | | ||||||||||||||||||||||||||||||||||||
Sbjct  164052  GGCTCCTACACATTCC-TCG-GCGCACACTCCTCGGCGCTACGCACTTCACGGCGTTCAC  164109

Query  91      CTTCGAGCGCTGCACAGTTCACGGCGCTTGCCTCTGGGCGGCGCACAGTTCACGGCGCTC  150
               ||| | ||||||||||||||||||||||| |||||||||| ||||||||||||||||||
Sbjct  164110  CTT-G-GCGCTGCACAGTTCACGGCGCTCGCCTCTGGGCGTCGCACAGTTCACGGCGCTT  164167

Query  151     GCTTCCAAGCGCCACGCCTCACCGCGCACATCTCGAGACGTTGGCCGACGGTGCA--GGG  208
               ||||||| ||||| | |||||||||||||||||||||||| || | ||||  |||  |||
Sbjct  164168  GCTTCCAGGCGCCGCACCTCACCGCGCACATCTCGAGACGCTGCCTGACGTCGCAGGGGG  164227

Query  209     GGCTTGCGGTCTTGGCATGCGTGCTTCACGTGCTTGCCCGACCTTGGTGCTCATTCTCCC  268
               ||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  164228  GGCTTGCGGTCTTGGCATGCGTGCTTCACGTGCTTGCCCGACCTTGGTGCTCATCCTCCC  164287

Query  269     TG  270
               ||
Sbjct  164288  TG  164289
```

Figure 8. **Alignment Sequence with Molluscum contagiosum virus subtype 1 (Isolate 3 –F).**

Histological study: Results on skin sections related to patients in infected with MCV showed variable changes , masses of lesions composed of lobules ,each lobule of hyperplastic epidermal tissue that growth down .Ward into the dermis layer, each mass surrounded with dens connective tissue and the lobules separated from each other with strand of fibers (Figure 9 ,10) .

The surface epithelial layer invaded by these masses and extend to the deep layer (dermis) and hypodermis , clear hyperkeratosis the keratinocytes showed active with mitotic figures but near the surface most cells was hypertrophied and showed keratiningation with cellular debris (Figure 11, 12).

These Koliocytes appeared with picnotic halo , surrounded by obvious oustid halo which noticed higher than that of cytoplasm, within these cells forming bodies inclusion caused by virus (MC) and push the nuclei to peripheral region ,some kerationcytes showed with large size and more number of this inclusion bodies (Figure 13, 14). Figure revealed to keratin deposition and form horn at the warts apex and the corneum layer with hyperkeratosis, more over active granulosum stratum and deposition of dense granules (Figure 15), the epidermal layer formed center region surrounded with shoulder most of koliocytes , the lobules meet at this region and some of degenerated keratinocytes noticed (Figure 16, 17). Histological observation indicated to vacuolated cells that known Koliocytes at the surface epithelial layer , no boundaries between epidermis and deep dermis layer which showed vascularized and strands of connective tissue extend , mild inflammatory cells within the hypodermis layer (Figure 18).

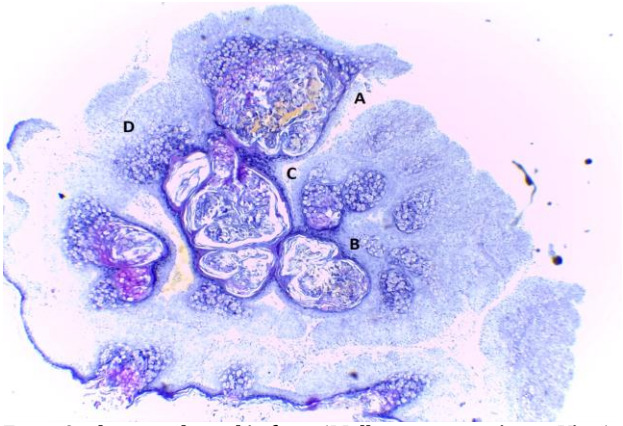

Figure 9. **photograph on skin from (*Molluscum comtagiosum Virus*) patient showed masses(A) of lesion , each composed Of lobules(B) hyperplastic epidermis(C) , dense connective tissue. (D)Separated the lobules . (H & E) stain (4 x).**

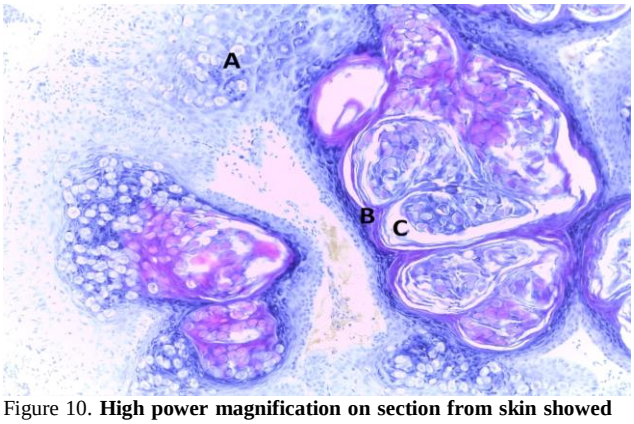

Figure 10. **High power magnification on section from skin showed hyperplastic epidermis(A), The mass composed of variable lobules(B) , active stratum granuiosum(C). (H & E) stain (10 x).**

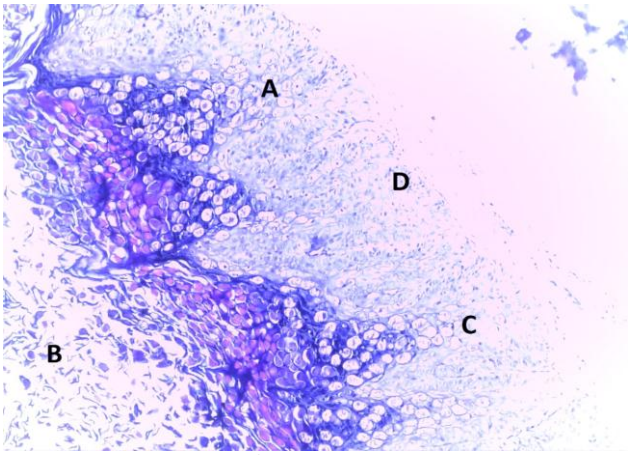

Figure 11. **Section in skin from patients with (MCV) showed no boundaries between epidermis(A) and dermis layer (B) , the epithelial layer formed horns(C) , hyperkeratosis layer (D) most of with mitotic figures. (H & E) stain (10 x).**

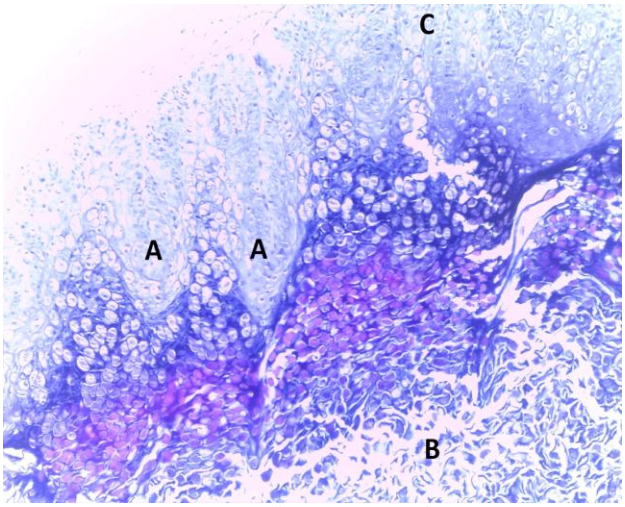

Figure 12. **High power magnification in skin from (MCV) patient showed the horns separated by layers of keratinocytes (A) , heavy deposition of fibers (B) within the reticular is layer , hypertrophied cells (C) with flat nuclei at the surface layer . (H & E) stain (10x).**

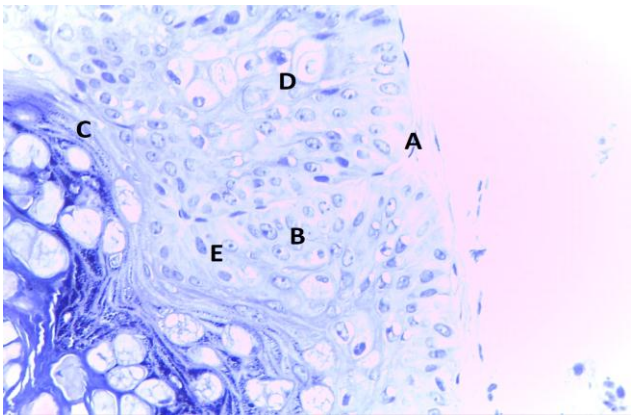

Figure 13. **Section in skin from patients with (MCV) showed heavy proliferation of keratinocytes(A) , most with peripheral nuclei(B), strands of fibers(C) separated the surface layer frame the underlying layer , halo(D) obvious and some keratinocytes with inclusions(E) . (H & E) stain.(40 x**).

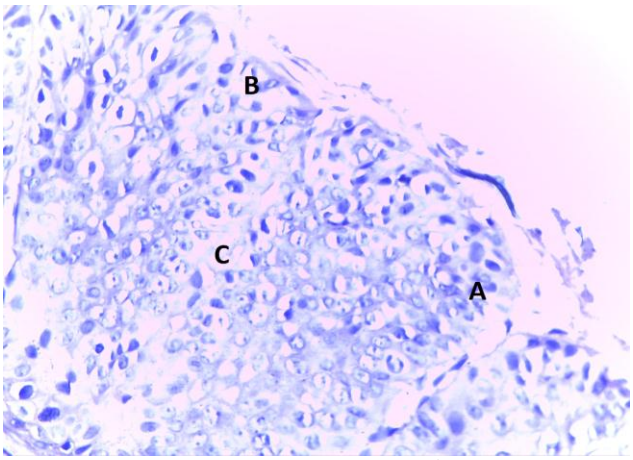

Figure 14. **Photomicrograph on skin section from patients with (MCV) showed destruction and desquamation epidermis(A) , most keratinocytes with peripheral flat nuclei(B) , some halo(C) with inclusions. (H & E) stain . (40x).**

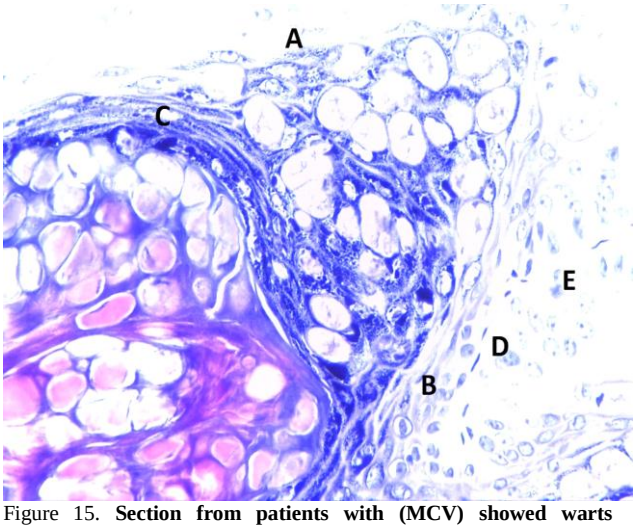

Figure 15. **Section from patients with (MCV) showed warts apex(A), hyperkeratosis(B) , active granulosum (C) with deposition of dense granules , desquamated cells(D) and cellular debris(E) . (H & E) stain (40 x).**

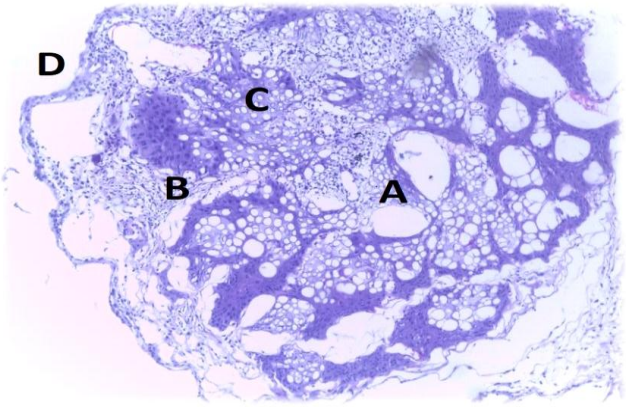

Figure 16. **Photograph on skin section from patients with (MCV) showed central region(A) surrounded with lobules(B) of hyperkeratosis, number of Koliocytes(C), Keratinization obvious(D) on surface layer. (H & E) stain. (10 x).**

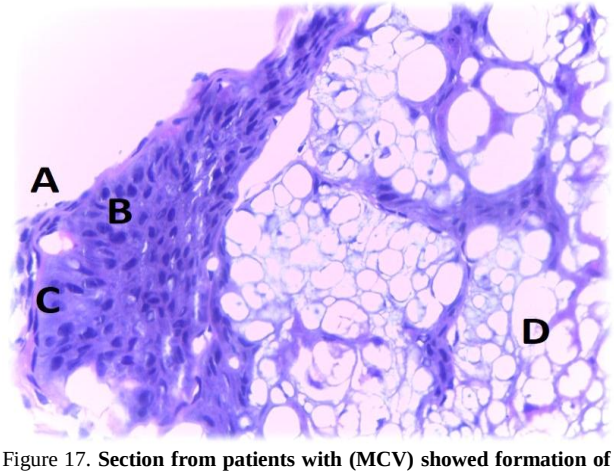

Figure 17. **Section from patients with (MCV) showed formation of epithelial horn(A) , most Keratinocytes with mitotic figure(B) , the surface cells was dead with flat nuclei(C), the underlying layer composed of large number of Koliocytes(D). (H & E) stain. (40 x).**

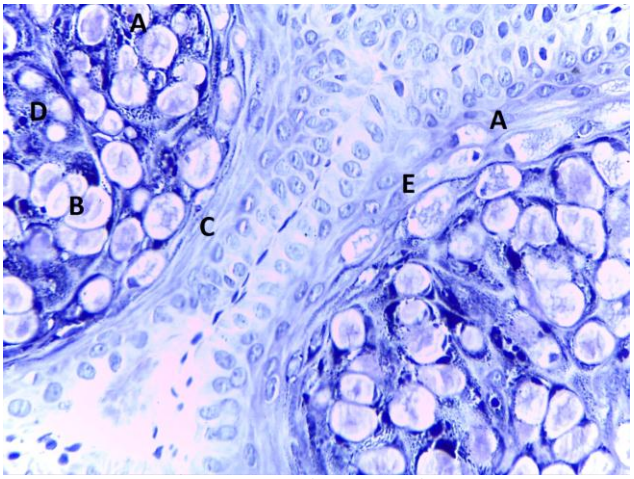

Figure 18. **Photograph on lesion from skin of patient showed two distinct lobules(A) from Koliocytes(B) , each lobule surrounded with fibers(C) , Keratinocytes with dense keratohyalin granules(D) obvious , some Koliocytes with inclusion(E). (H & E) stain. (40 x).**

Discussion

Molluscum contagiosum (MC) is a self-limited skin viral infection (Molluscipox virus) and is commonly transmitted via direct contact[14]. The infection usually appear in children but also infect the young adult. Lesions are typically confined to the epidermal layer of the skin and appear as umbilicated, popular or nodular lesions[15].

The detection of MCV infection is primarily dependent on clinical feature. It is characterized by smooth, dome-shaped discrete papules. The incubation period is variously estimated at 14 days to 6 months[2]. MCV detection confirming by using molecular techniques and histopathological studies[16].

Present study aims to detect the MCV infection in Basra city using clinical, molecular and histopathological techniques.

This study revealed that most infection were found in patients of age group (1-10 years) which was disagreement with the study done in Iraq by Al-Kayalli[17]. In which Molluscum contagiosum was most commonly seen in the age group (31-40 years),and disagree with[18] who reported that 62%-64% of patients belonged to age 11- 30 years. Current study agreement with study in the USA which reported that approximately 80% of the patients were younger than 8 years[19]. One of the main causes of dermatitis among children is due to the lack of tissue immunity in the body as well as the mixing of children in schools and swimming pools.

The prevalence of MCV dependent on gender data revealed that the males were 60.8 %, while percentage of infection in the females was 39.2% and there results was agree with Turkish report[15], which recorded that the MCV infection was associated with 67.2% of males and 32.8% of females but disagreement with two studies one of them reported in Iran[20] that revealed around 45% of girls have MCV lesion compared with 55 % of boys and other study in Egypt in which males represented 42.9% and females 57.1%, [21]. The incidence of infection in males is higher than that of females as a result of the frequent mixing between males in society in terms of work this difference in results may be due to the differences in the educational level and the lack of attention to health, especially when the emergence of such diseases is not just of interest because elitist little knowledge of health matters.

Regarding to lesion distribution, the present study was mainly reported that the lesions of virus infection were more occurrence in head area 76.5% and less sightseeing in trunk area 16.6%, while the infection were very few in limbs area 6.9%. Current results agreement with[22] and [23] study that show the MC lesions in adults were located mainly on the face, whereas in older children they are located on the trunk. On the other side, the present data disagreement with study reported by Stulberg and Talia in which documented that the most commonly location of MC were on the arms, legs, trunk and less common on face[24]. Another study is also reported different result compared with present study which the most MC lesion 64% were found on the trunk and extremities[25].

As the previous studies, this study found that the lesions caused by MCV typically appear as white, pink, or flesh-colored, umbilicated, raised papules (1 to 5 mm in diameter) or nodules (6 to 10 mm in diameter)[26].

The results of the molecular analysis to detect 102 isolates of MCV by using primers showed all band corresponding to a 393bp. Also, the results of sequencing of PCR amplicons of six isolates of MCV were identified to species level, the alignments of the virus isolates were identified and compatible and identified as MCV.

Molecular epidemiological study of MCV infection indicates the prevalence of MCV was observed whatever the age, in contrast to the reported differences in the distribution of MCV subtypes among patients of different age groups[27].

In histological examinations of hypertrophied and hyperplastic masses, large cells close to the surface and containing ‘‘molluscum bodies’’ in their cytoplasm are demonstrated. Large cells are underlined by mitotically active germinal layer cells[16].

In current study the histopathological results on skin sections related to patients infected with MCV showed masses of lesions composed of lobules, each lobule of hyperplastic epidermal tissue that growth down this occurred may due to replicated harmful effect of MCV in epidermis, that agreed with[28], who documented that the MC virus is an intra-cytoplasmic replicating virus in which infected cells grow in size while internal organelles are dislocated and eventually obliterated by large inclusion bodies, each mass surrounded with dense connective tissue and the lobules separated from each other with strand of fibers.

Also The surface epithelial layer due to invaded by masses and extend to the deep layer (dermis) and hypodermis, clear hyperkeratosis the keratinocytes showed active with mitotic figures but near the surface most cells was hypertrophied and showed

keratiningation with cellular debris which agreed with[29]. Which mention that MCV infects epidermal keratinocytes leading to the formation of epithelial down growing lobules containing Molluscum bodies and Infection of keratinocytes of hair follicle infundibulum, may give rise to comedones or abscesses.

Histological observation indicated to vacuolated cells that known Koliocytes at the surface epithelial layer , no boundaries between epidermis and deep dermis layer which showed vascularized and strands of connective tissue extend may due to forming inclusion bodies caused by virus (MC) which pushed the nuclei to peripheral region and led to forming halo like vacuolated cells that known Koliocytes which agreed with[30] whose mention these lobules consist of enlarged keratinocytes having an abundant cytoplasm containing viral inclusions (also called Henderson Paterson bodies) and a peripheral nucleus , also agreed with Gupta *et al*., (2003) whose documented the characteristic cytological feature of MC is presence of Molluscum bodies or Henderson–Patterson bodies. They appear as large ,round intracytoplasmic inclusions in epidermal cells which push the nucleus to the periphery.

Conclusions

The Molluscum contagiosum was predominantly in males and the age group 1-10 y was more infected than other age groups. Molluscum virus infections were leaded to in important histological changes in the skin, and Molecular detection of Molluscum virus was the best way to diagnose the infection.

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