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Viral Skin Warts: Clinical and Histopathological Characterization.

Maha k Al- Malak ¹, Hayder Abdulhuseein Al-Hmudi ¹, and Khalil I. Al- Hamdi ²

¹College of Science, University of Basra, Iraq.

²College of Medicine, University of Basra, Iraq.

ABSTRACT

Skin warts are considered the main manifestation of the cutaneous Human papilloma viruses (HPV) types. The aims of this study were to clarify the main Clinical and histopathological changes of skin warts that associated with HPV infection of Basra patients. Histopathologically, skin specimens from lesions were collected, fixed, washed, dehydrated, cleared, embedded then sectioned and stained to examine with light microscopy and photographed. The results showed hyperkeratosis, papillomatosis with dead, flattened squamous cells on epithelial surface layer. Deposition of collagenous fibers, also keratinocytes with keratin granules appeared dense coarse and clumped. Cell pyknotic nucleus and perinuclear space (koilocyte) were observed. The solid masses located on surface layer while masses with blood vessels found on deep layer. Addition features referred to keratin deposition with mitotic activity of some keratinocytes, and basilar hyperplasia. So this study diagnosed HPV infection based on clinical and histopathological examinations.

**Corresponding author*

INTRODUCTION

Warts have been recognized since Greek and Roman times, since when and in many cultures, warts have been associated with magic and a multitude of folk cures, due to the sudden appearance and disappearance of warts [1]. Human papilloma viruses (HPV) are small double-stranded DNA viruses that comprise a family of more than 130 types [2]. Skin warts are considered the main manifestation of the cutaneous HPV types, with HPV 1, 2, 3, 4, 27, and 57 being detected most frequently [3]. HPV can be classified into mucosal and cutaneous types. Cutaneous types infect the squamous epithelium of the skin and produce common warts (*Verruca vulgaris*), plantar warts (*Verruca plantaris*), and flat warts (*Verruca plana*), which occur commonly on the hands, face, and feet [4]. Common warts represent 70% of skin warts and occur primarily in children, whereas plantar and flat warts occur in a slightly older population [5]. Skin warts are estimated to occur in up to 10% of children and young adults, with the greatest incidence between 12 and 16 years of age [3]. Warts typically continue to increase in size and distribution and may become more resistant to treatment over time [6]. In addition, warts can be painful depending on their location (e.g. soles of the feet and near the nails) and viewed as socially unacceptable when located on visible areas (e.g. hands and face) [7]. Infections with HPV types causing skin warts are usually acquired through micro-injuries. Transmission occurs either directly from one person to another, or indirectly via contaminated objects or surfaces. Auto-inoculation (by scratching) from one site of the body to another is also possible [8]. Therefore, the objective of the present study was to evaluate the clinical and histopathological characteristics features of common warts that caused by Human Papillomavirus infection.

MATERIAL AND METHODS

Macroscopically and clinical included viral skin warts appearance, color, size, consistency and distribution were recorded. Skin specimens regarded to different patients from both sex and different age were collected randomly from infection locations on feet, arms and hands fingers. All samples fixed with formalin 10 % for processing according to Luna (1968) to evaluated histopathological changes. The samples washed, then dehydrated with gradual series of alcohol, after that all the samples cleared with pure xyelene, infiltrated and embedded with paraffin wax, then sectioned to suitable sections, stained with Cole's haematoxylin – eosin, examined and photographed with light microscopy.

RESULTS

Macroscopically and clinical observations

The present study showed that *Verruca vulgaris* were the most common types of cutaneous warts, appeared like nodules or papules with rough surfaces, firm consistency, mostly skin colored with hyperkeratotic border, some of them showed black dotes on their surfaces.

Most of the warts were located at the dorsum of the hands and fingers, and sometimes these warts restricted were peri or subungual.

The study also showed that *verruca plana* warts as small papules which were slightly raised, flat topped, rounded or polygonal shape which was either skin colored or slightly brownish, their diameter less 5 mm, distributed mainly on the face and back of hands.

Furthermore the study showed that *Verruca plantaris* was either endophytic or exophytic. Endophytic plantar warts were almost seen single, deep, and painful, and appeared as a keratinous lesions, with a black pointed central area, the border covered by a thick keratinous ring. while exophytic plantar verruca or mosaic plantar verruca were generally more than one, located superficial, painless, and were manifested by a slightly raised lesions consist from circumscriptive keratinous lesions grouped together into a mosaic pattern, these almost shown on heels.

Histological examinations

The results of microscopic investigation of skin sections have shown multiple lesions on surface epithelial layer with accumulation of inclusions on it, also proliferative foci of epithelial keratinocytes arranged in circular masses, hypergranulosis with dense coarse granules deposit in it (Figure, 1, 2). Result referred that the inner layer of papillomavirus appeared denser, active and we may be seen more than one papilloma grouped together, sometimes single large papillomavirus formed by more than one primary masse with sheath of connective tissue surrounded it (Figure, 3, 4). Histological changes clarified solid papilloma near the surface with dense collagen fibers deposit, also elongation of dermal papilla and abnormal keratin in the superficial layers of the epidermis which be very clear that the vacuolization on epidermis layer (Figure, 5, 6). The histology study also showed vascularized masses on the epidermis layer with elongated papillary ridge extend to penetrate the epidermal layer with dense granules of keratohyaline deposit in active keratinocytes, the hyperkeratosis very clear (Figure, 7, 8).

Also cellular infiltrates of an inflammatory cells most of lymphocytes and mononuclear phagocytes at the destruction epidermal layers with hyperkeratosis on surface layer (Figure, 9).

The present study indicated that large numbers of mature and immature skin papilloma masses distributed at the same region and cover all the surface area (Figure, 10).

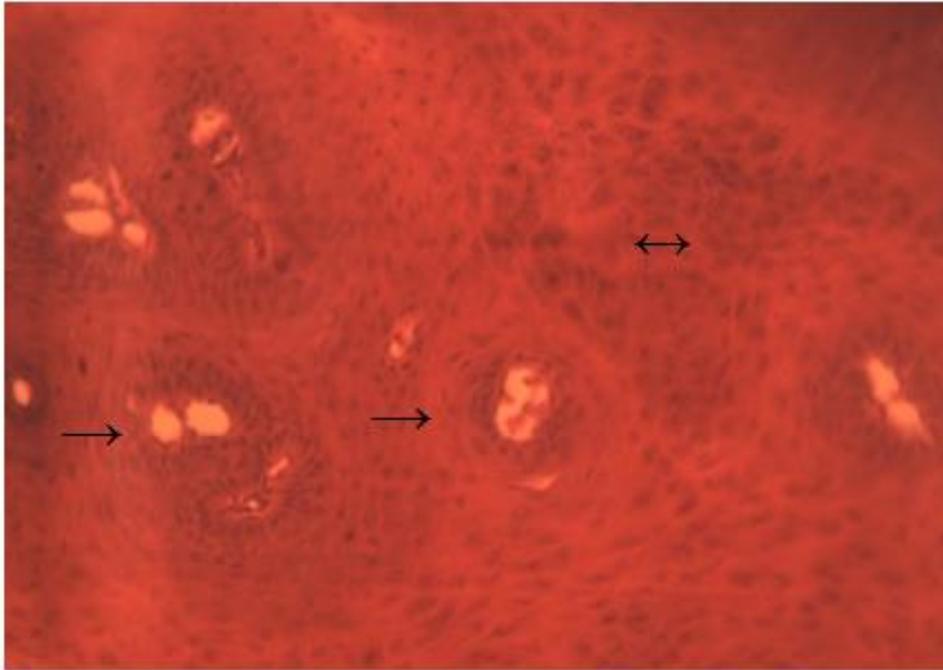


Figure (1) Section in skin showed more than one papillomavirus lesion (→) and proliferation of keratinocytes(↔), (H&E) stain. X (10)

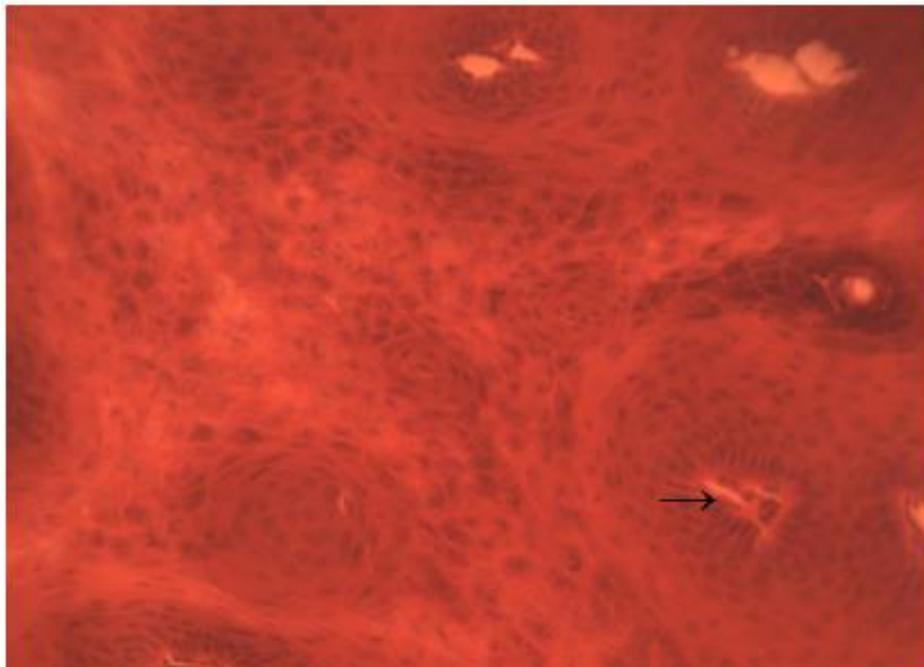


Figure (2) Section showed primary papillomavirus with accumulation of inclusions on it (→), (H&E) stain. X (10)

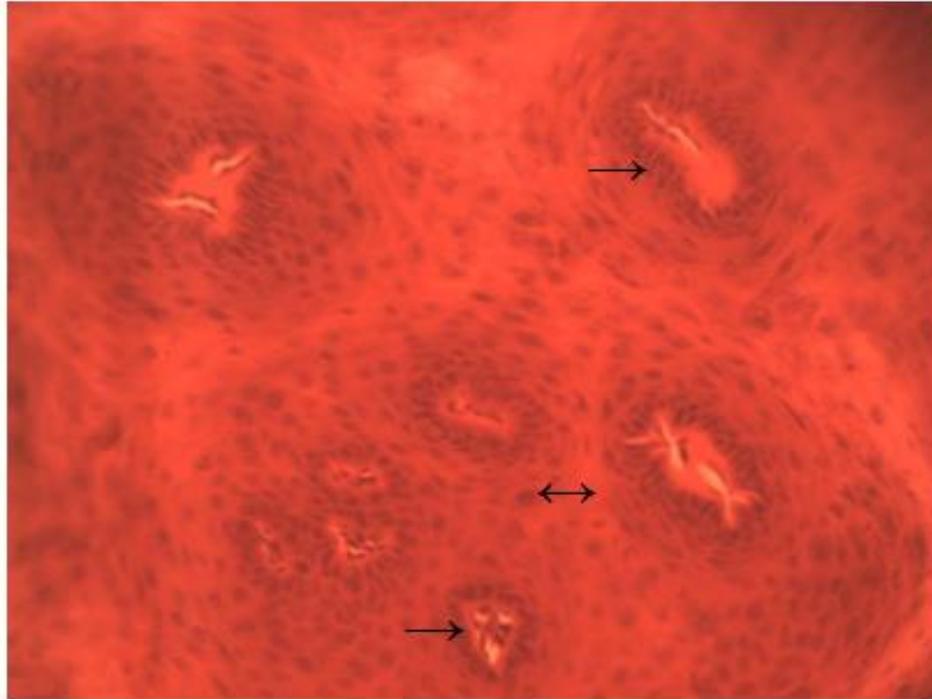


Figure (3) Section in skin papillomatosis showed denser, active an inner layer of each mass (→), also more than one masses grouped together (↔), (H&E) stain. X (40)

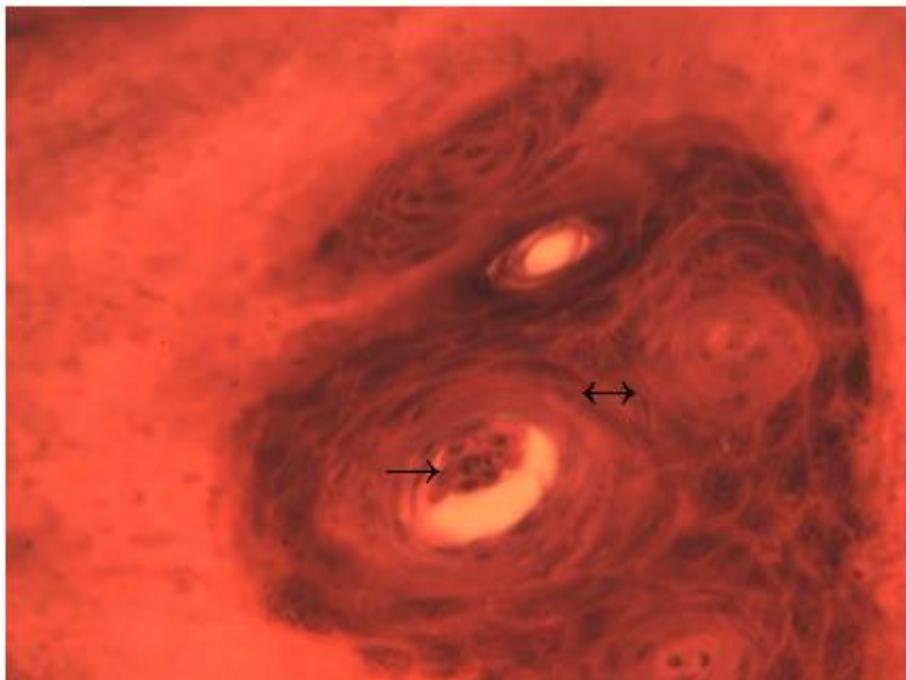
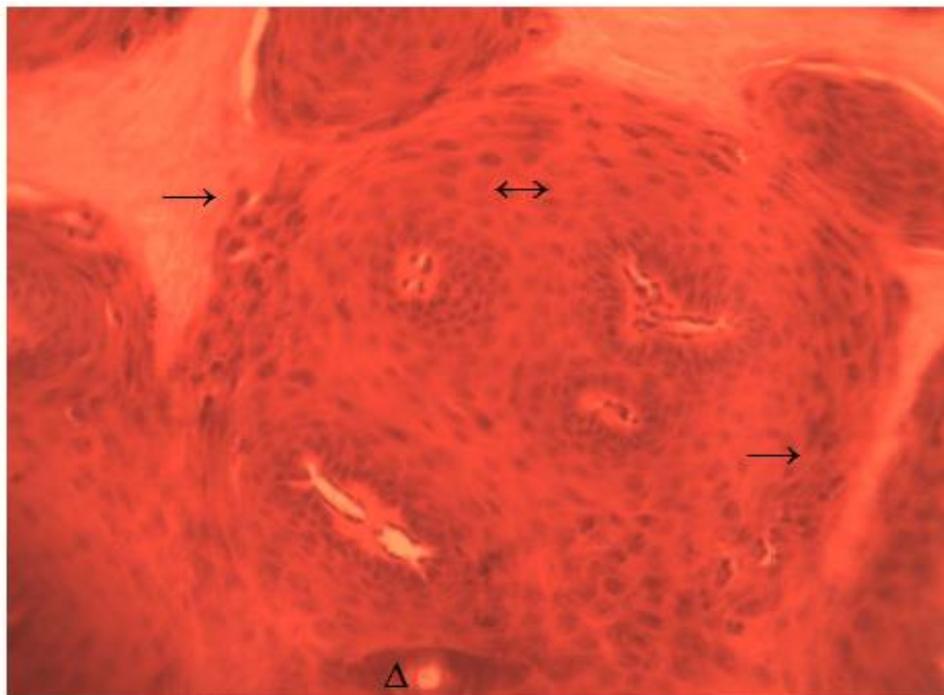


Figure (4) Section showed large single mass, formed from more than one papilloma grouped together by dense connective tissue (↔), also an inclusions appear (→), (H&E) stain. X (40)



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Figure (5) Section on skin showed large papillomavirus elongation of dermal papilla (→), abnormal keratinocytes(↔), tortuous vessels (Δ), (H&E) stain. X (10)

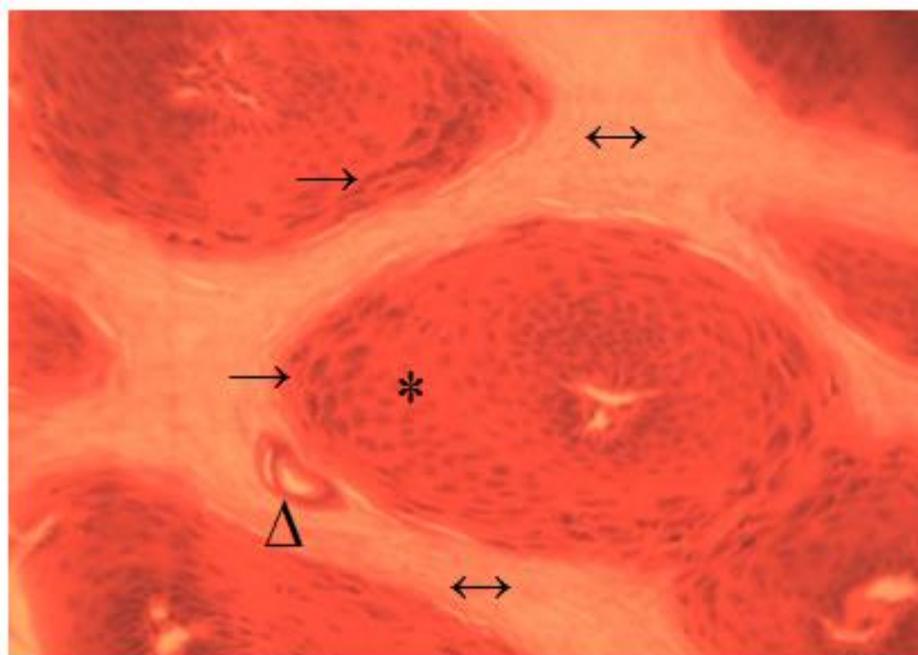


Figure (6) Section showed skin clarified solid masses (*), active keratinocytes(→) and collagen deposition(↔) ,tortuous capillary (Δ), (H&E) stain. X (10)

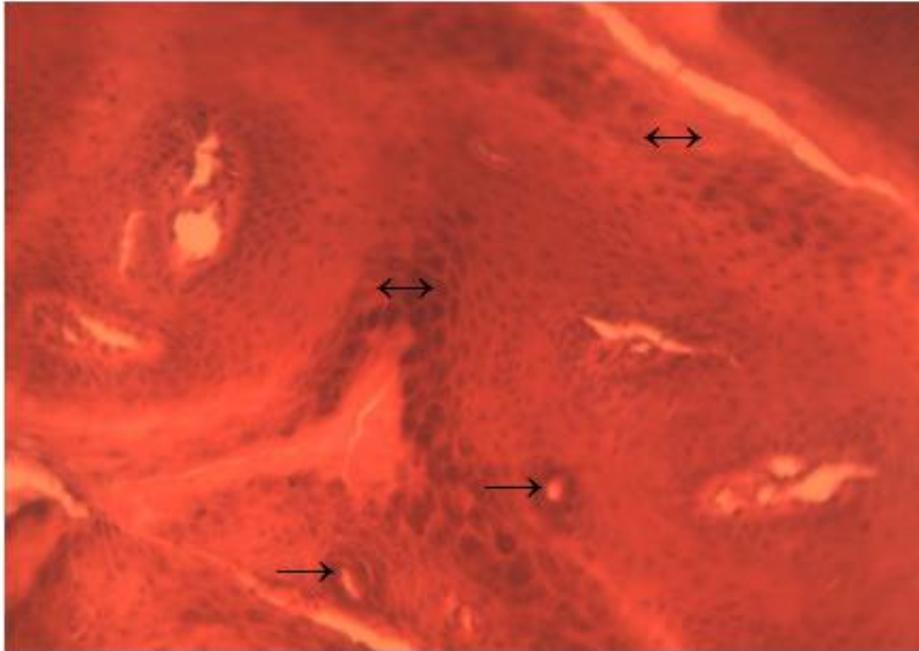


Figure (7) Section showed dermal papillary (↔), with active keratinocyte (→); (H&E) stain. X (40)

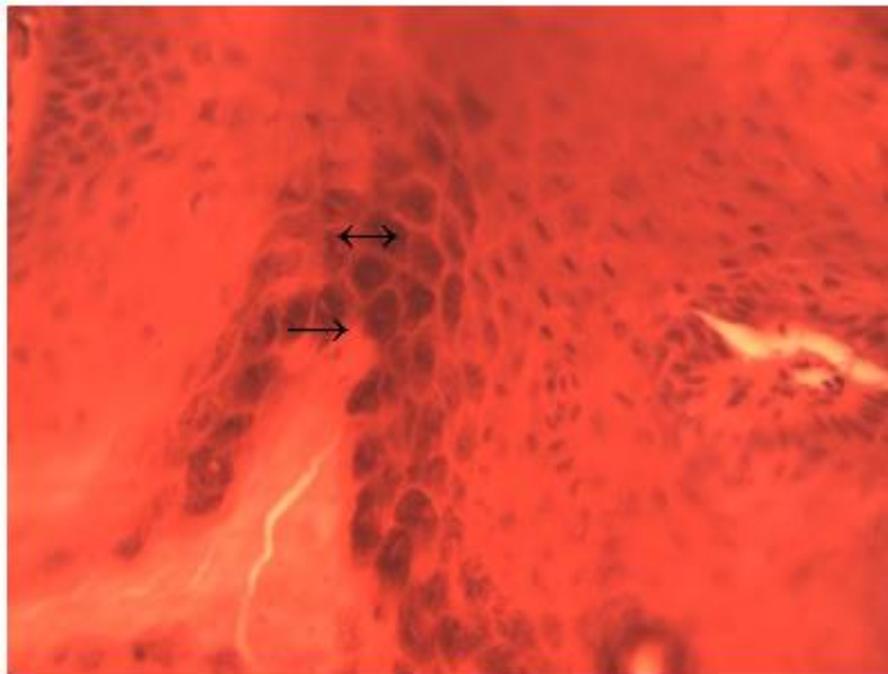


Figure (8) Section showed dermal papillae (→) with active keratinocyte(↔); (H&E) stain. X (40)

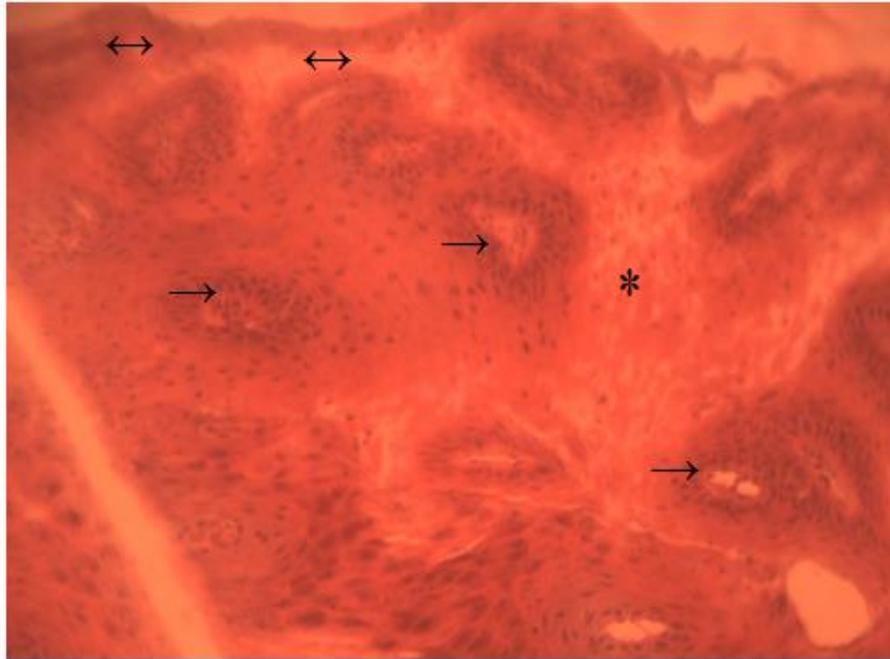


Figure (9) Section in skin showed multiple papillomavirus (→) with keratin deposition (↔) on surface layer and inflammatory cells (*); (H&E) stain. X (10)

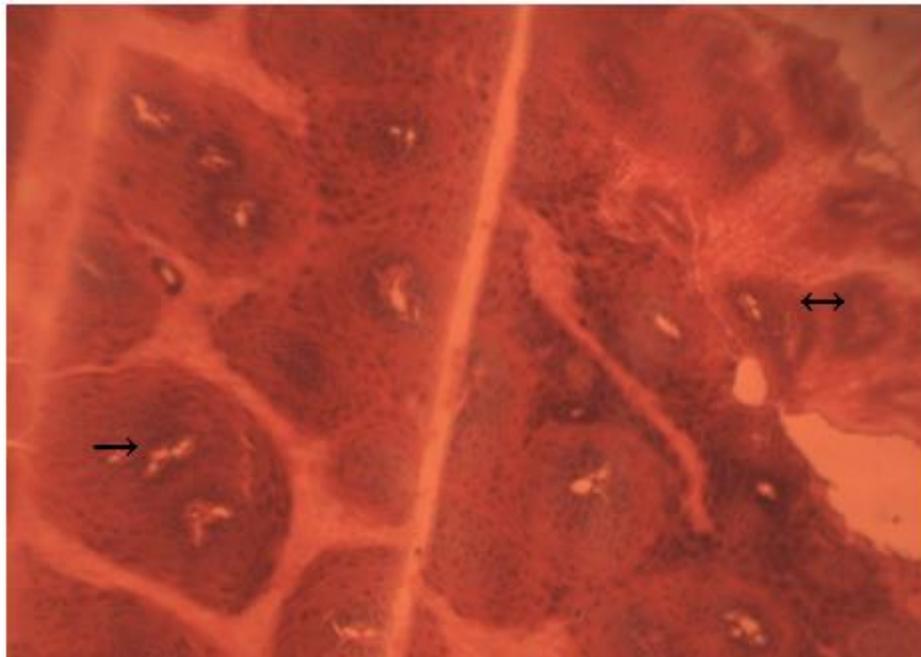


Figure (10) Section in skin showed mature (→) and immature (↔) skin papilloma; (H&E) stain. X (10)

Results also illustrated keratinocyte hyperplasia with pyknotic nuclei and other keratinocyte showed mild mitotic activity with dense, coarse keratin granules and most of the papilloma was vascularized (Figure, 11, 12, and 13). The present study clarified also an increase with keratin deposition as granules (hyperkeratosis and hypergranulosis), some of epithelial

cells with perinuclear space(koliocyte) and others formed like network around the skin papilloma (Figure, 14, 15). Histopathological changes referred to keratinocytes around papillomavirus masses that extrude their nuclei and just full with karatohyaline granules especially at the surface of epidermis (Figure, 16). Also the vascularized papilloma appeared deeply on skin layers while the surface layer showed more keratin deposition and as a result the skin surface epidermis lost the arrangement of its layers with dead surface and more cellular debris (Figure, 17, 18, and 19). Moreover damaged of smooth surface of the keratin beneath the colonies (Figure, 20, 21)

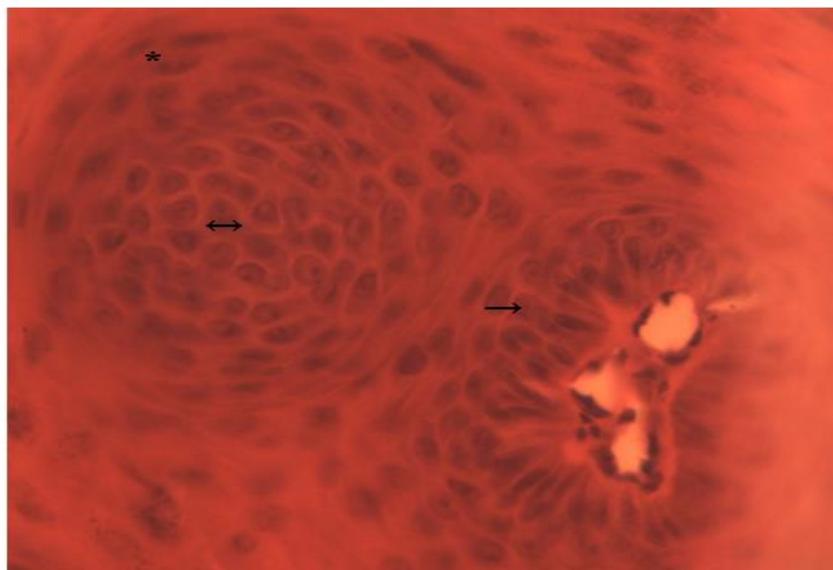


Figure (11) Section showed active keratinocyte with mitosis (↔), vascularized papilloma mass (→),koilocyte very clear (*); (H&E) stain. X (40)

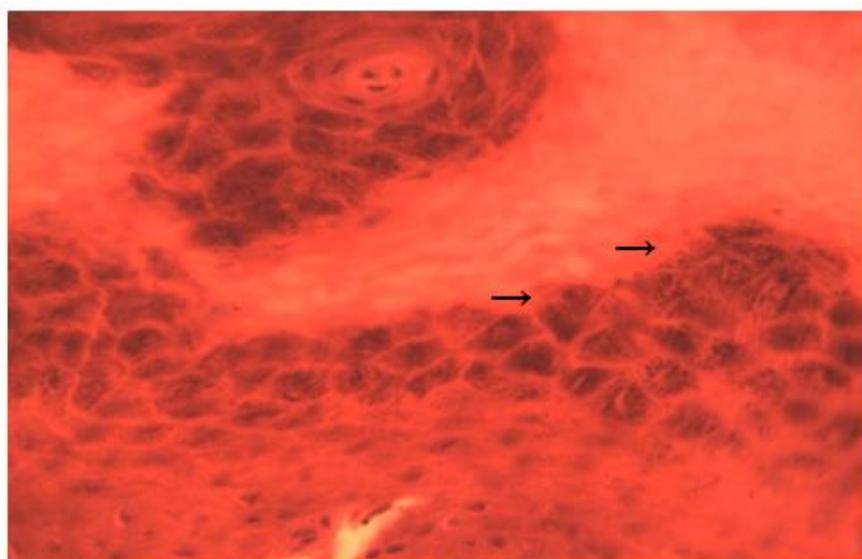


Figure (12) High power magnification on skin masses showed hyperplasia of keratinocyte with coarse granules (→); (H&E) stain. X (40)

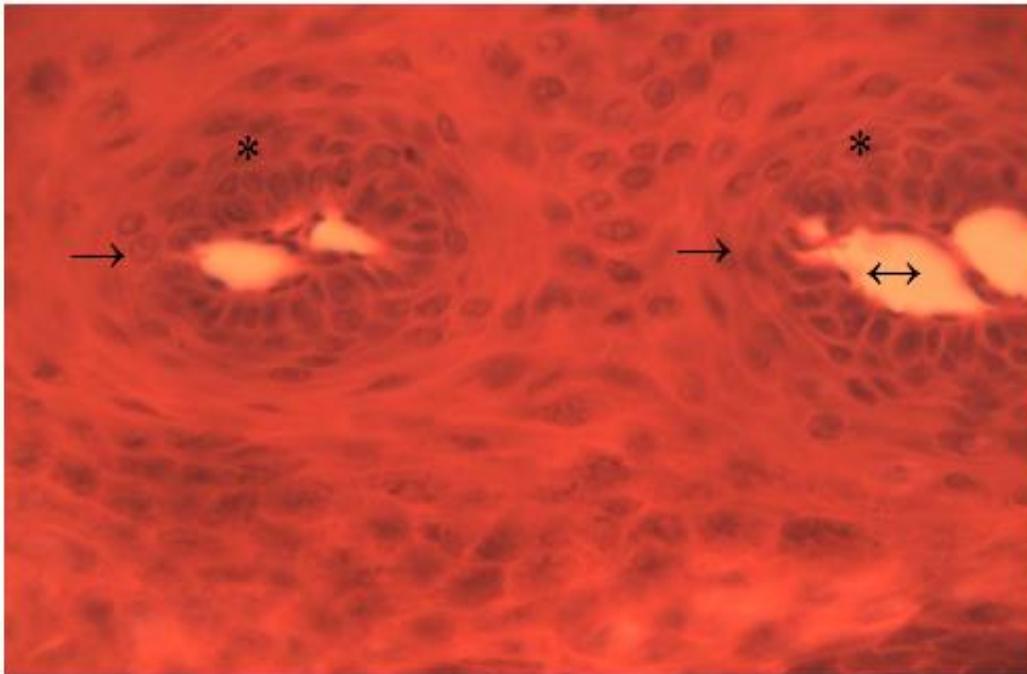


Figure (13) photomicrograph showed vascularized papillomavirus (→) with capillaries(↔)and keratinocyte arranged circularly (*); (H&E) stain. X (10)

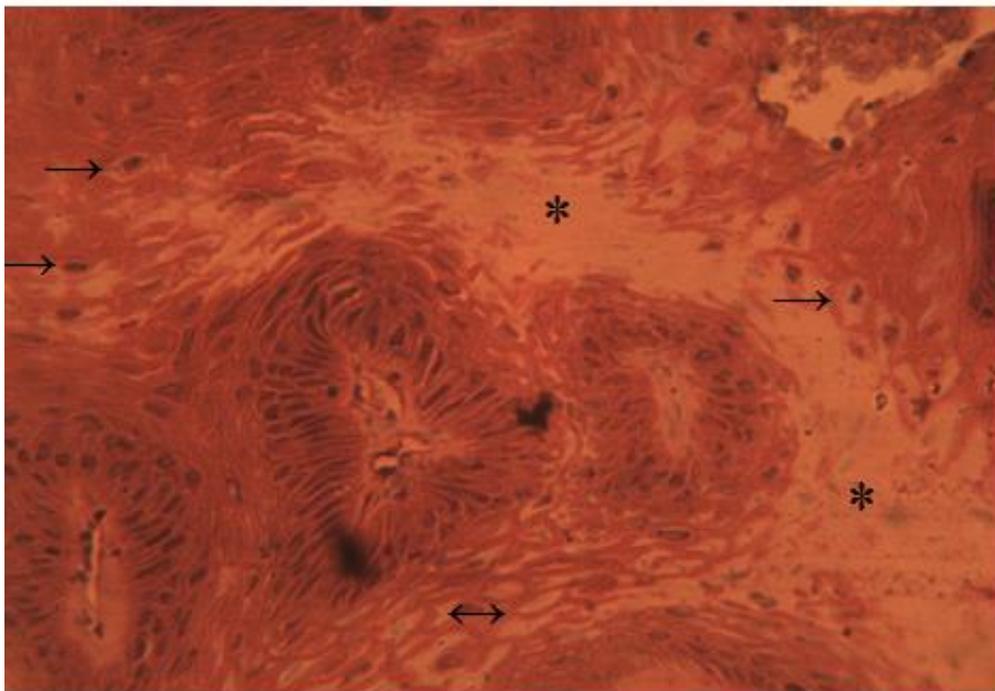


Figure (14) Section showed some keratinocytes with perinuclear space filled with keratin (→), and others formed like network (↔) with collagen deposition (*); (H&E) stain. X (10)

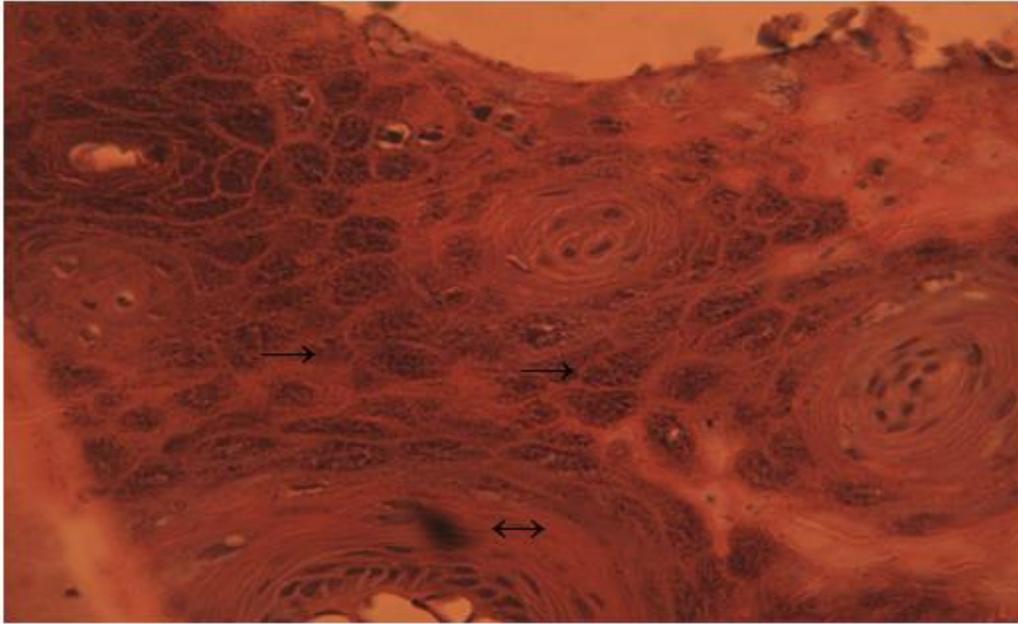


Figure (15) Section in skin papillomavirus clarified dense, coarse keratin granules (→) deposit in keratinocytes with collagen fibers arranged circularly around the masses (↔); (H&E) stain. X (40)

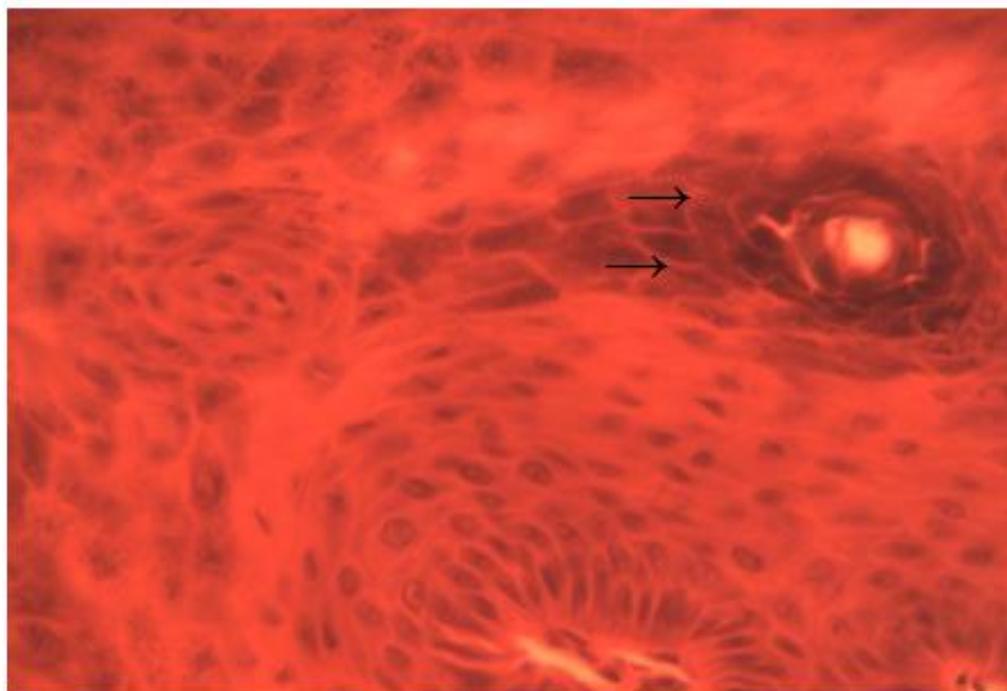


Figure (16) photomicrography showed keratinocytes full with keratin (→); (H&E) stain. X (40)

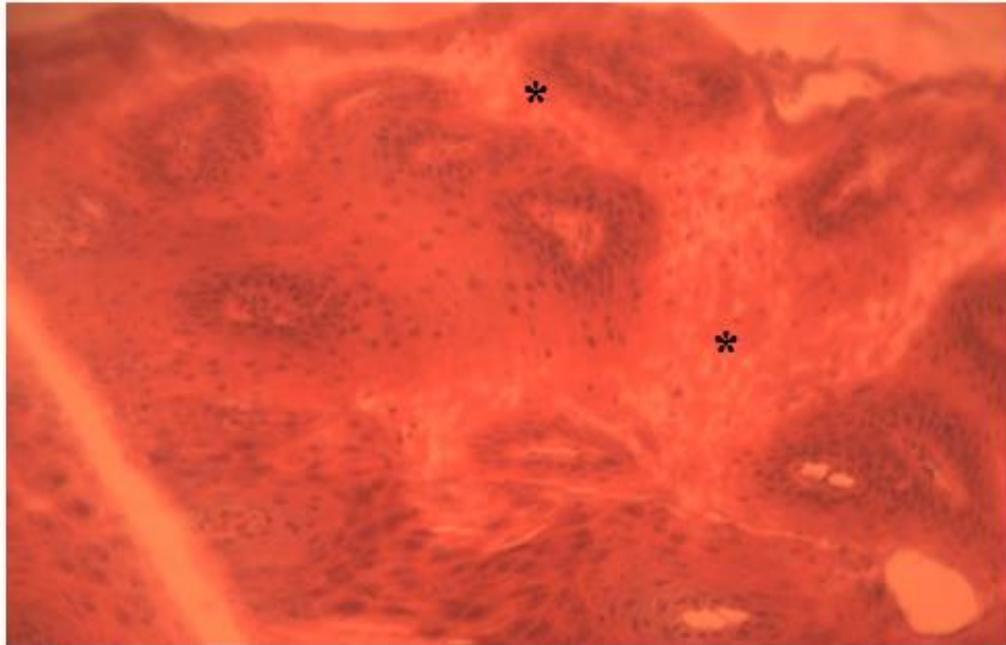


Figure (17) Section in skin illustrated complete damaged of epidermis, no visible layers and cellular debris was seen (*); (H&E) stain. X (10)

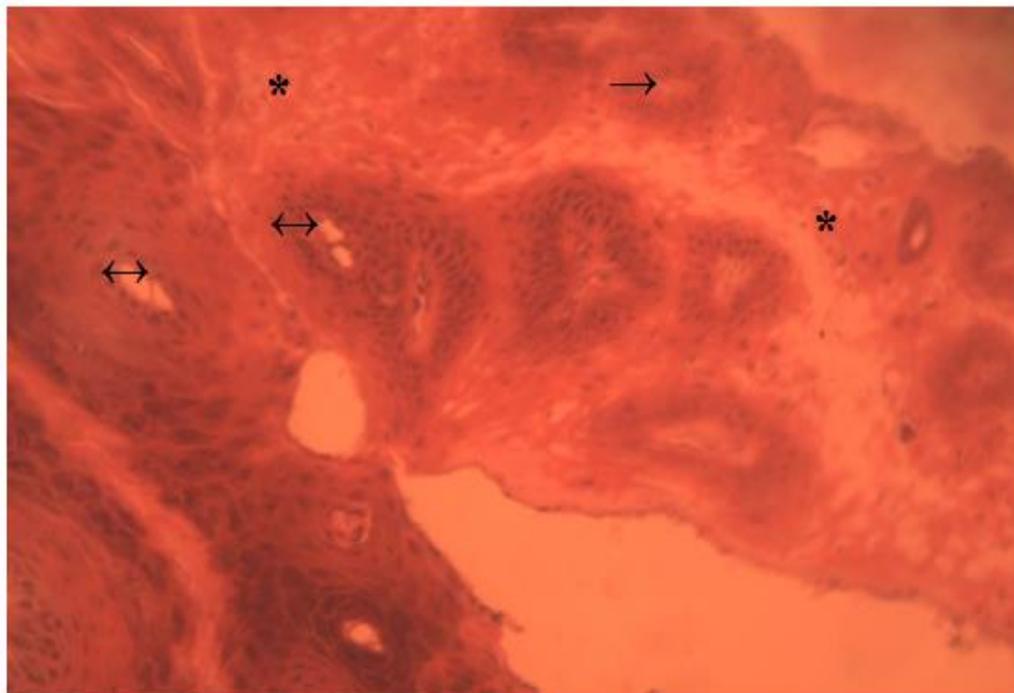


Figure (18) Section showed skin papilloma on the surface layer (→) and vascularized papilloma on the deep layer (↔), large number of koilocyte(*); (H&E) stain. X (10)

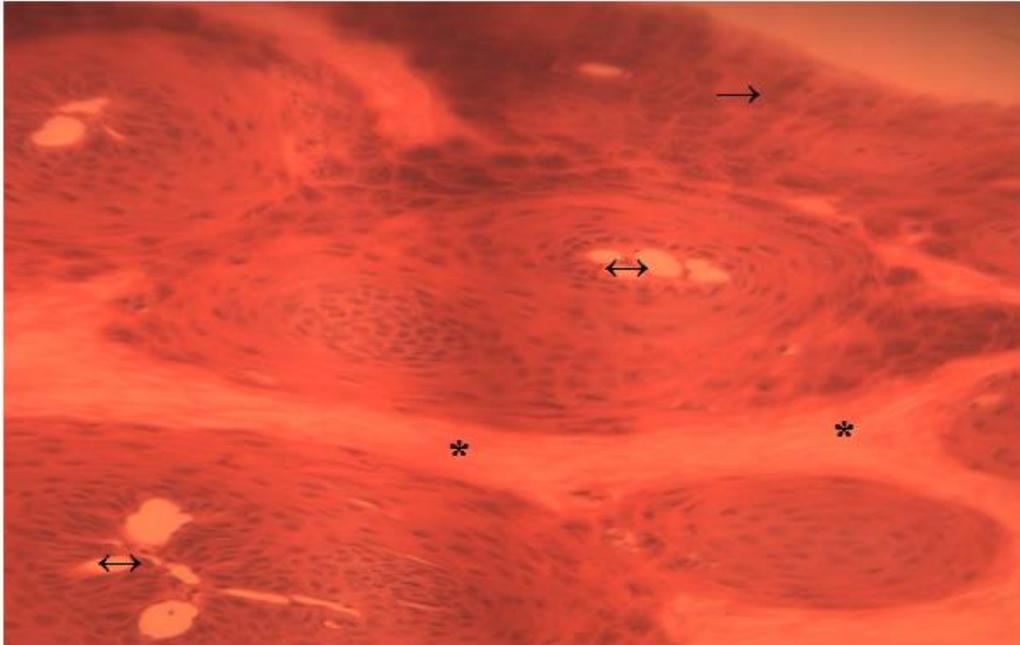


Figure (19) Section in skin showed thick surface layer (→), more than papilloma, some of them vascularized (↔) and bundles of collagen fibers deposit (*); (H&E) stain. X (40)

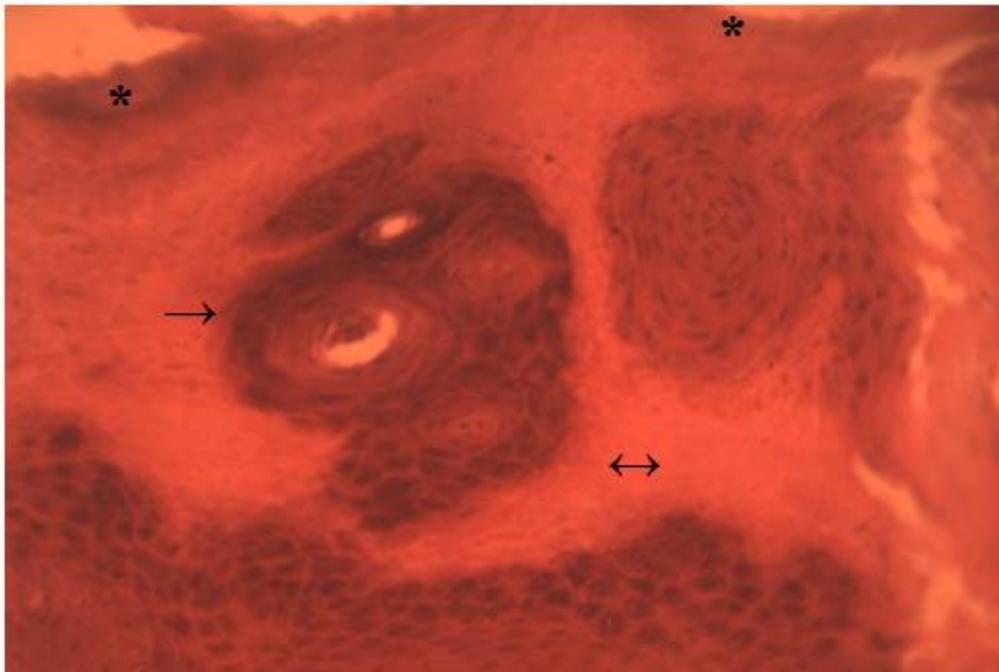


Figure (20) Section showed colonies of papillomavirus masses (→), keratin deposition (↔) and active keratinocytes layer (*); (H&E) stain. X (10)

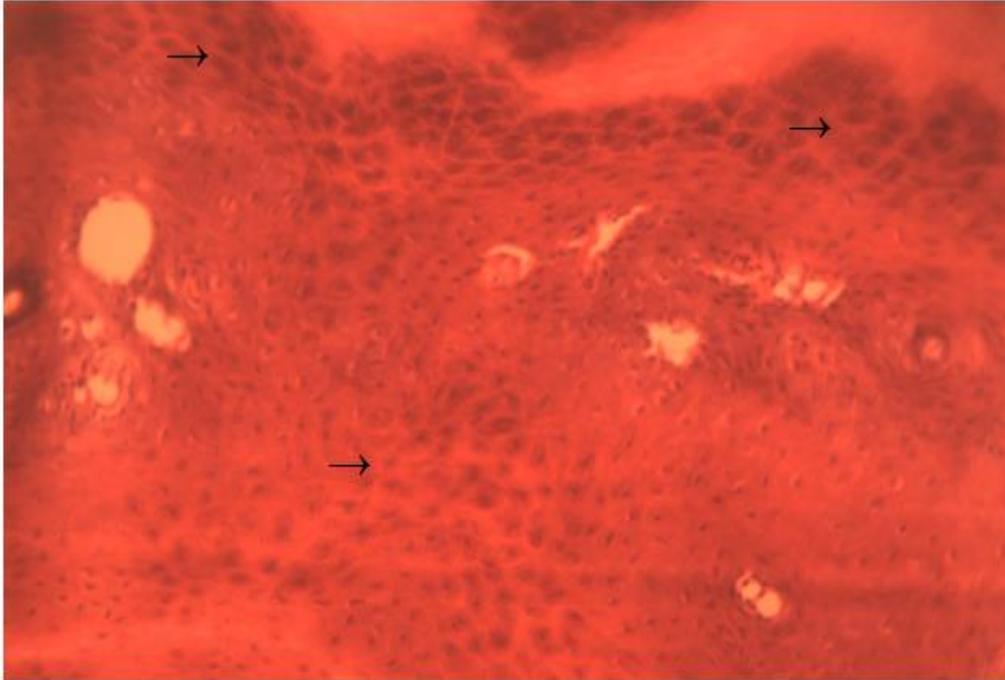


Figure (21) Section clarified destruction of most layers and keratinocytes with pyknotic nuclei extrude deeply (→); (H&E) stain. X (40)

DISCUSSION

The papillomaviridae are ancient and ubiquitous viruses, with over 200 types of species-specific viruses classified into 16 genera. PVs preferentially infect differentiating squamous epithelium and in humans, almost every part of human skin can be infected. HPV was the first known human tumor virus, associated with benign, epithelial proliferations or papillomas and there are now 120 different HPV types officially recognized with others pending classification [9]. Cutaneous types infect the squamous epithelium of the skin and produce common warts (*Verruca vulgaris*), plantar warts (*Verruca plantaris*), and flat warts (*Verruca plana*), which occur commonly on the hands, face, and feet [4].

Histologically, warts (often called verruca from the Latin meaning 'little hill') are benign lesions, with hypertrophy of all layers of the dermis, resulting in acanthosis (thickening), papillomatosis (folding) and hyperkeratosis (increase in the horny layer) often with abnormal keratohyaline granules. Vacuolation of cells occurs in the upper layers and inclusion bodies are sometimes observed [10].

Results demonstrated that the papillomavirus lesions caused by human papillomavirus specialized infection in hands, feet and other parts of body which exposure to an external environment and more contact with surface and objects this agreed with [4]. Cardoso and Calonje showed that infections are limited in scope to skin and mucosal surface exposed to the environment. Some of infections with HPV types causing skin warts are usually acquired

through micro-injuries. Transmission occurs either directly from one person to another or indirectly via contaminated surfaces [11].

The study referred to masses of proliferative keratinocytes with hypergranulosis of keratin granules deposit in it and these may be related to the active role of viruses which inhibited an epithelial layer and replicate inside these cells and this was similar to the findings of (8), they found that HPV can induce hyperplastic, papillomatous and verrucous squamous cells lesions in the skin and various mucosal sites.

As shown the koiocytes and abnormal keratin in the superficial layers of an epidermis with elongation of dermal papillae another diagnostic features which characterized this viral infection, this may be explained that the most effect of viruses limited to keratinocytes and epithelial layer of epidermis like spinosum and granulosum layers and this illustrated by other studies which clarified that most of Cutaneous warts occur more frequently on the surface layer as small dome-shaped papules with a keratotic and verrucous surface, the active virus replication found in the superficial spinous layer and that resemble viral particles occur in the granular cell layer [11,12].

Some of virus proteins interfere directly with the structure and function of keratinocytes for example (E6) proteins disrupt the keratinocytes network leading to the phenomenon of koiocytes, some others interfere with cell cycle and apoptosis mechanism, potentially leading to proliferation and transformation [13, 14]. Also koiocytes are the hallmark of the cytopathogenic effect of HPV [11].

Another result may be attributed to the activation of viruses that histologically the lesions characterized by papillomatosis and hyperkeratosis with parakeratosis seen on the upper surface, this may be regarded to the effect of virus and the role of keratinocytes at the stratum corneum, these findings agreed with researches showed that parakeratosis overlying the tips of the papillomatous projections alternating with orthokeratosis overlying the cavities [11,15]. The present study indicated to cellular infiltrates of an inflammatory cells and destruction of smooth surface beneath the papillomavirus colonies, this may be explained that an infection caused skin damaged and histological response against the virus included infiltration of lymphocytes and phagocytes and these findings reported by other studies which clarified that cellular infiltrates in regressing warts were most of lymphocytes and mononuclear phagocytes, these cells found at the region of damaged epidermis and damaged keratinocytes [13,16]. regarded that the cells mediated immune response to the virus are probably the most important factor in host resistance and infiltrated of T-cell and other satellite cells necrosis indicative of cell-mediated keratinocytes death which shown in regressing warts supports this concept [14, 17]. HPV infections are normally controlled by intact cell-mediated and humoral immune systems. Regression was shown to be largely driven by cytotoxic T cells and NK cells many years ago, while protection from subsequent infection with the same HPV type results from stimulation of the adaptive response and production of antibodies. However, sequestration of HPV in epithelial cells provides protection for the virus, resulting in inefficient

activation of innate immunity, poor priming of the adaptive response and persistence of infection [18].

Also the recent study indicated to the vacuolization of keratinocytes at an upper surface, most of vacuolized papillomavirus appeared deeply and supplied with tortuous capillaries this may be possible explained as a characteristic features of keratinocytes which loss their most structures and full with keratin granules and the vacuolized papilloma formed near to the dermis layer, this finding in agreement with studies referred to the vacuolization which it is diagnostic features of viral infection principally in an upper layers of epidermis [19].

Approximately 70% of HPV infections resolve spontaneously in 1 year and 90% in 2 years, while HPV persistence develops in the remainder [20]. For most children, cutaneous HPV infections are simply an inconvenience with spontaneous resolution occurring in almost 80% within 2 years [7]. Warts usually disappears spontaneously but occasionally may be resistant to treatment. Regrowth of lesions after treatment is frequently due to persistence of the virus in the skin surrounding the original wart. Topical salicylic acid solutions used at home are effective for most cutaneous warts and should generally be the first line therapy, reserving the application of cryotherapy with dry ice or liquid nitrogen for recalcitrant warts [10].

It was concluded that HPV infection caused many manifestations which associated with abnormalities features morphologically and histopathologically in patients of different age in Basrah city. So this study diagnosed HPV infection based on clinical and histopathological examinations.

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