Immunohistochemistry Detection Apoptosis Related with ORF Virus Infection in Sheep Based on Caspase 3 Detection from Selected Farms in Basrah

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Abstract

This study was designed for the molecular detect of apoptosis related with ORFV infection in sheep based on detected caspase (3) by immunohistochemistry. The samples were taken from the skin of the lips of animals infected with contiguous ecthema. The ability of the virus to induce apoptosis was also verified using cellular immunohistochemistry and the use of a polyclonal anti-caspase (3) antibody to detect apoptotic activity. The results of the study revealed positive expression of caspase (3), where a high percentage of caspase (3) was observed in the affected cells in the epidermis of the lip, in addition to the presence of overexpression in all layers of the epidermis, with the presence of small areas of apoptotic cells compared with the control group.

Keywords: *immunohistochemistry, detection apoptosis, caspase (3).*

Introduction

Orf is an infectious disease Orf will affect many sheep farmers so that the production rate will fall. Orf is caused by Orf Virus belonging to the family Poxviridae is in the genus Parapoxvirus[1]. The virus can be transmitted Through direct association with an infected animal, or by contact with it infected environments or materials, like during grazing[2]. The virus makes entry into the host through broken and damaged skin and replicates in the skin cells leading to formation of lesions[3]. Orf virus is a major occupational health problem for farmers, butchers, veterinary staff and goat skinners because they are exposed to Orf virus by infected cattle or sheep, or contaminated objects[4].

Medical indicators of the Sheep ORF virus infection include elevated temperatures, decrease appetite , depression, eye and nose mucosal inflammation, respiratory distress, various stages of skin lesions (from erythema to scabs on lips), and lymph node enlargement[5].

Clinically, the skin is slowly progressive development of crusts, development of redness and flares, development of vesicles, and finally forming pustules[6]. Orf is notable for having lesions, inflammations, ulcers, and papules in the lips and nostrils[7]. On histopathological observation of skin tissues, there is epidermal hyperplasia with hyperkeratosis, parakeratosis and acanthosis of the epidermis, showing multilocular vesicles with degenerating cells with phyllodes[8]. Lesions occur as a sequence of erythema, follicular eruption, papules, vesicles, pustules, or crusts located around the muzzle, cheek, tongue, oral mucosa, and on ears or nose, etc. In the same way, ankles, eyelids, and breasts[9]. The ORF virus or ORFV is the causal agent that encodes the 119

protein that induces apoptosis. ORFV is the causative agent that encodes an ORFV119 protein that promotes apoptosis. Orf virus interacts with antigen presenting cells (APC) and epidermal cell and induces apoptosis which is the killing of the cells[10,11]. Orf virus also utilizes ubiquitin proteasome signal pathway This is to protect the virus particles from maturation and then released to the outside[12].

Stimulate Overexpression of ORF-3a, ORF-3b and ORF-7a induce cell death. Prove that overexpression of ORF-6 also causes cell apoptosis. Also, demonstrate that apoptosis can be blocked by inhibitors of the Caspase-3 and NF-κB[13].

The Caspase 3 is part of caspase-like family of proteins which are involved in proteolysis of proteins by macromolecular machines called caspases. Caspase activation is of immense importance in triggering and checking the mechanism of apoptosis programmed cell death[14].

The role of the caspase 3 enzyme in apoptosis is mediated by both external (death ligand) and internal (mitochondrial) signaling pathways[15,16].

Therefore, the zymogen cleavage site of caspase-3 enzyme is important to ensure its regulation. Cleavage of caspase-3 occurs to initiate a caspase-3-dependent signaling cascade that causes cellular events following mitochondrial permeabilization[17]. One signaling event is the introduction of granzyme B, which activated one of the effector for caspases, in cells that target apoptosis by killer T cells. These events trigger the caspase cascade, which culminates in the death of the cellular organelle , Caspase-3 plays a significant role in caspase-3 catalyzed procaspase[18]. In intrinsic activation mechanism, cytochrome c combines with caspase-9, Apaf-1, and ATP, with which the process of procaspase-3 is initiated[19]. It was noticed in vitro study that the molecules have sufficient power to activate caspase-3. However, other regulatory proteins are very essential in vivo.

Apoptosis is a major form of cell death during development and viral infection, and has important roles in the disease response to pathogens. ORFV is the causative agent that induces programmed cell death in the late stages, it contributes to release virus.

Orf virus also exploits the ubiquitin-proteasome system (UPS) signal transduction pathways and then circumvents intracellular signal transduction, thereby activating CD8+ T, in order to protect virus particles toward maturation and release outward[12]. Overexpression of ORF-3a, ORF-3b and ORF-7a leads to apoptosis. This is indicative of demonstrating that overexpression of ORF-6 further induces apoptosis and that Caspase-3 and JNK inhibitor inhibit ORF-6-induced apoptosis[13].

Material and Method

The immunohistochemistry analysis was performed using an immunohistochemistry kit, 2-step plus Poly-HRP Anti Rabbit/Mouse IgG Detection System with DAB Solution (Elabscience, E-IR-R213, China) and as per manufacturer's instruction. The kit contained a 3% H_2O_2 and Normal goat serum as blocking agents, Polymer Helper as linker, Poly-peroxidase-anti-Mouse/Rabbit IgG secondary antibody and diaminobenzidine (DAB). Anti-Caspase 3 primary antibodies were used in this study to detect the apoptosis activity.

Section tissue was washed in distilled water and submerged in a saline bath (pH 9) of Tris (TBS) for five minutes. Tissue Sections The tissue sections are then placed in a glass jar filled with Citrate Buffer Antigen Retrieval pH 6, which has been preheated to 60°C and incubated for 25 minutes in a water bath at 97°C.. Tissue sections were left in a glass container for 20 minutes at room temperature, then rinsed with distilled water and immersed in a tampon bath for 5 minutes. Excessive buffer was tapped into

the tissue section and then gently wiped with tissue paper around the sections. The napkin sections on the glass slides were surrounded by a circle of wax and this was done using a special wax pen (Gene Tech Pen, Elabscience, E-BC-R531, China) Ensure that only the tissue section of the slide was confined to the reactant.. The tissue sections were then immersed in a 3% H_2O_2 block solution (Elabscience, E-IR-R213A, China) As a reagent to block, Cover and incubate the sections in a humidity chamber for a period of 10 minutes, before rinsing the sections with distilled water and immersing them in a TBS bath (pH 9) for 5 minutes. Excess buffer on the tissue sections was then removed by tapping gently on the slides and also gently wiping them with paper towels around the sections. The tissue sections were the flooded with the normal goat serum (Elabscience, E-IR-R213D, China) As a blocking reagent, cover the sections and incubate them in a humidity chamber for 30 min, before rinsing the sections with distilled water and immersing them in a TBS bath (pH 9) for 5 min.

The tissue sections were then applied with 0.1 mL of Poly-peroxidase-anti-Mouse/Rabbit IgG secondary antibody (Elabscience, E-IR-R213C, China), it was incubated in a room with a humidity similar to room temperature for a period of 30 minutes, then rinsed with distilled water and then immersed in a TBS bath (pH 9) for 5 minutes. Excess buffer on the sections was then removed by tapping and wiping gently with a tissue around the sections. Tissue sections were worked and applied with 0.1 ml of DAB. chromogen (Elabscience, E-IR-R213E, China) diluted 20 folds with DAB substrate solution (Elabscience, E-IR-R213F, China), then it is incubated in a humidity room for a period of 5 minutes. Then, we worked on rinsing tissue sections with distilled water before immersing them in a temporary TBS bath for 5 min.

Tissue sections were then stained with Mayer's hematoxylin stain for three minutes, followed by rinsing using tap water. Then tissue sections were dehydrated in five changes of three minutes each of 50%, 70%, 80%, 90% and 100% ethanol, respectively. Then tissue sections were immersed in two changes (ten minutes each) of xylene and then mounted with fixing media (DPX) and covered with cover clips. Finally, tissue sections were examined under a light microscope at 100x, 200x, 400x and 1000x magnifications.

Result and Discussion

Figure (1) represented the immunohistochemical section of non-infected sheep lips skin, showing the negative expression of Caspase3 in epidermis and dermis layer. Figures (2,3,4,5A) represented the immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip, the overexpression of Caspase-3 was observed in dermis layers, also, a spaces of apoptotic cells was observed in positive expressed areas.

As well as figures (5B,6A,6B) showing the positive expression of Caspase-3 (brown color) was observed in epidermis of lip and the overexpression of Caspase-3 (brown color) was observed in all epidermis layers, the overexpression of Caspase-3 was observed in apoptosis affected area of epidermis, also, a spaces of apoptotic cells was observed in positive expressed areas. All this figures above were treated with A&B: X400, anti-Caspase-3 antibody and hematoxylin.

Apoptosis is a multistep process by which the evolutionarily maintained and genetically controlled cell death in response to a variety of different origins, sources and stimuli that can act to send signals from the outside of the cell or from inside the cell. The mechanism of programmed cell death proceeds by a series of morphological changes and transformations that are mediated by the activation and stimulation of a specific cysteine protease called caspases[20].

Apoptosis is a physiological mechanism that controls cell numbers during development and the response to external infection, including the response to viral and bacterial infections[21]. Viruses have developed strategies either by inhibiting or by inducing apoptosis of host cells, depending on specific interactions between the virus and the host [22].

Apoptosis may also be beneficial at later stages of infection by reducing the host's inflammatory response and facilitating virus spread. Caspases are critical mediators of programmed cell death (programmed cell death). Among them, caspase-3 is a frequently activated death protease that stimulates the specific cleavage of several key cellular proteins[19]. caspase-3 plays a central role in mediating nuclear apoptosis[23].

In this paper, we report that ORFV expresses apoptotic activity and identify the caspase-3 in lips skin lesion of sheep. IHC tested for epidermis of infected animals reveal expression of Caspase-3 antibody , these result indicated that orf virus have able to induce Apoptosis in epidermis cells .and orf virus has capacity to activation Caspase-3 by up regulation of pro apoptosis proteins and down regulation of anti-apoptotic proteins compared with control animals that no expression apoptosis this agreed with that detect orf virus (ORFV), the causative agent of orf, began codes for a protein ORFV119 that induces programmed cell death. ORFV119 then targets mitochondria (energy houses) in host cells, thereby inhibiting cell proliferation, and inducing programmed cell death.

Viruses developed various strategies and methods to counter host defenses. Early in the infection, our result dis agreed with[24] Which reveals that viruses prevent programmed cell death in order to facilitate the reproduction that occurs within the cell environment. However, in the late stages of infection, viruses induce apoptosis in order to spread the virus to neighboring cells or tissues.

Caspases-3 was play critical roles in various apoptotic pathways. Apoptosis (programmed cell death), an important form of cell death, is considered to have a major function Defense against virus infection in the host cell.. Both pathways activate caspases, thereby releasing several apoptosis-related proteins that induce cell death[25]. Apoptosis isn't just close associated with the development and occurrence of many cancers or immune disorder but also serves to restrict viral multyplication in host cells[26].

The result of current study reveal positive expression of Caspase-3 was observed in epidermis of lip (dark brown stain). Also to The overexpression of Caspase-3 was observed in all epidermis layers with presence of small spaces of apoptotic cells compared to control groups. These data showed that ORFV could inhibit cell prolife ration and the host needs to remove infected cells to maintain this agreement in a prime condition[27,28].

They reveal occurrence of cell death in late stage of infection, these indicated to promote dissemination into other cells or adjacent tissues by viral particles in late stages of infection by breaking down infected cells.

The study confirmed the presence of cells in the process of programmed cell death and clarified their presence and location in the subcutaneous and dermal layers as well as follicles and sebaceous glands in contagious Ecthyma lesions, this result agreed with [10], who reported the findings of apoptosis in ORF virus cutaneous effected cell by draw attention to the importance of the process of programmed cell death. By at least one viral protein or several proteins that exhibit the modulation, and act as signals and triggers for the purpose of initiating the process of apoptosis.

The downstream caspases (Caspase-3) stimulate the cleavage of protein kinases, DNA repair proteins, cytoskeletal proteins, and finally the "management"

which is the destruction of cellular functions. Caspases also influence and regulate cell cycle, signaling pathways, cytoskeleton architecture, and this ultimately leads to the morphological manifestations of apoptosis, eg membrane hypertrophy, DNA condensation and fragmentation[29]

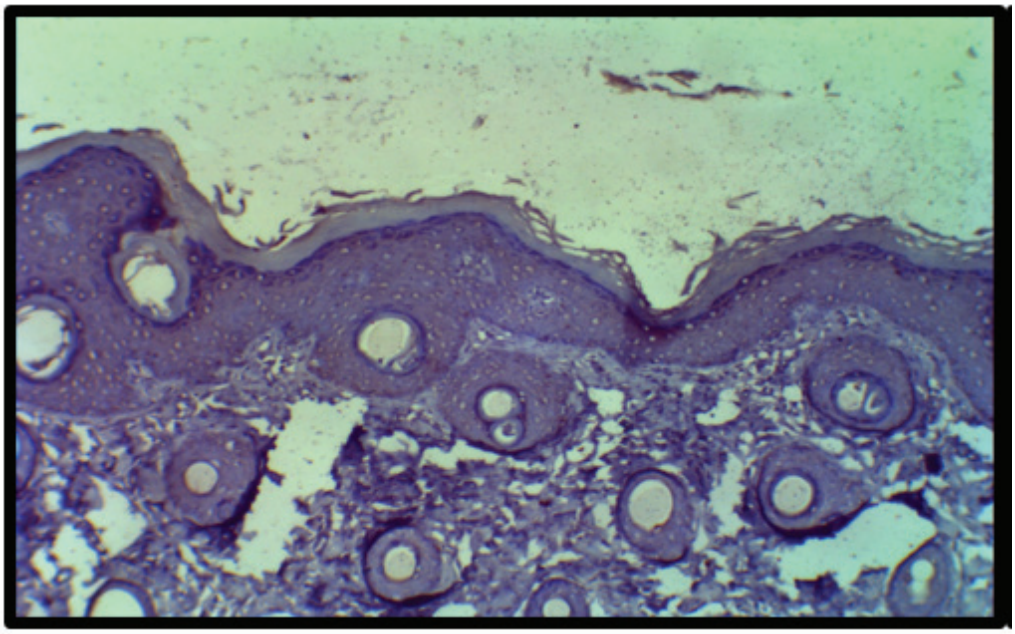

Figure 1. Immunohistochemical section of non-infected sheep lips skin, showing the negative expression of Caspase3 in epidermis and dermis layer. (X100, anti-Caspase-3 antibody & hematoxylin).

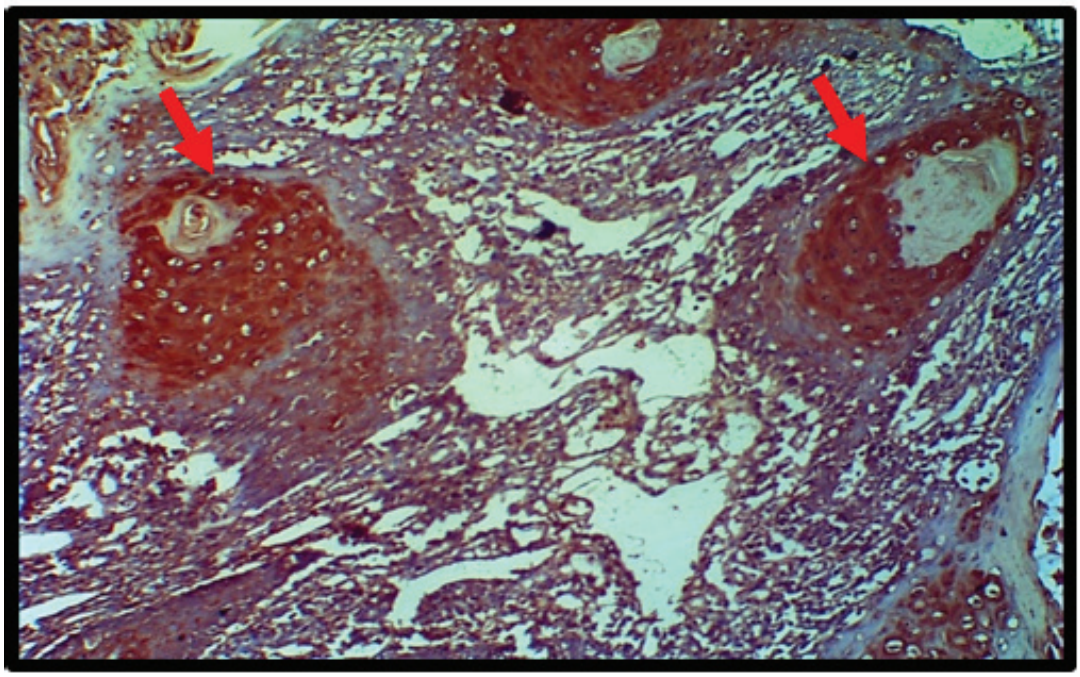

Figure 2. Immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip. The overexpression of Caspase-3 ( ) was observed in dermis layers. (X100, anti-Caspase-3 antibody and hematoxylin).

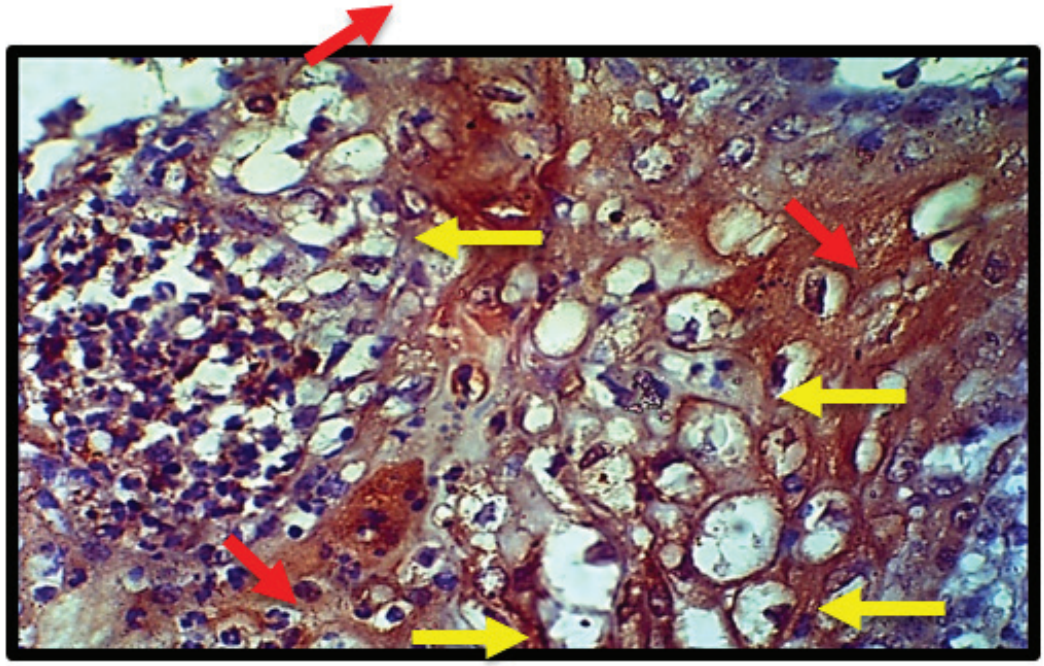

Figure 3. Immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip. The overexpression of Caspase-3 ( ) was observed in dermis layers. Also, a spaces of apoptotic cells was observed in positive expressed areas ( ). (A&B: X100, anti-Caspase-3 antibody and hematoxylin).

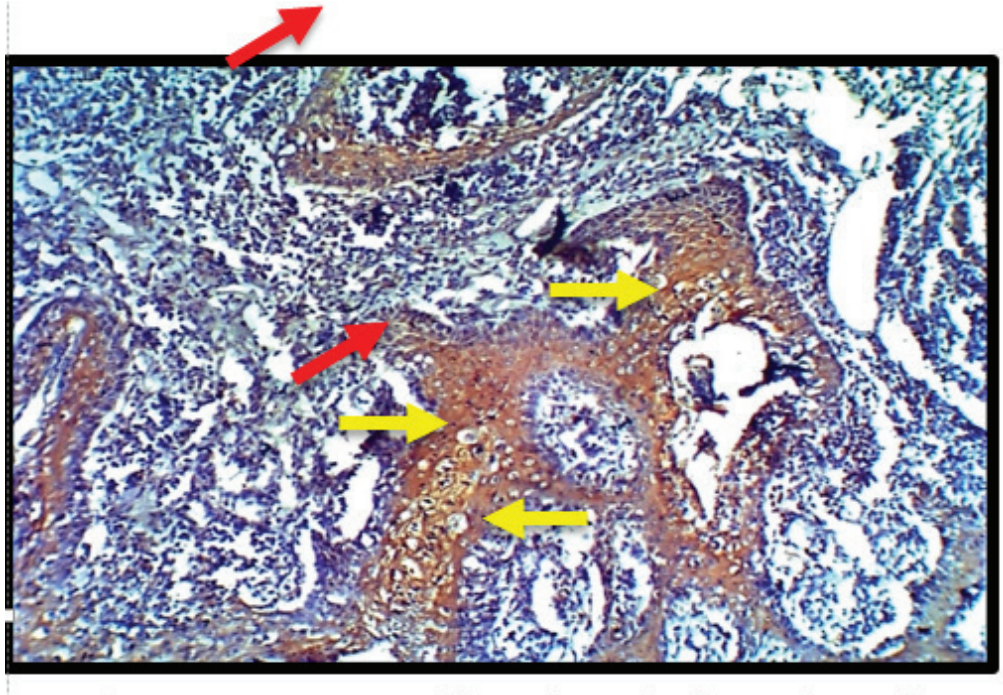

Figure 4. Immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip. The overexpression of Caspase-3 ( ) was observed in dermis layers. Also, a spaces of apoptotic cells was observed in positive expressed areas ( ). (A&B: X100, anti-Caspase-3 antibody and hematoxylin). Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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