THE IRAQI JOURNAL OF
VETERINARY MEDICINE
eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Immunohistochemical Detection of Apoptosis in Dermal and Epidermal Cells of the Lips in ORF-Infected Goats

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Received: XX XX 2021
Accepted: XX XX 2021
Published: XX XX 2021

Cite:
Mohammed YJ, Yousif EH. Immunohistochemical detection of apoptosis in dermal and epidermal cells of the lips in ORF-infected goats. Iraqi J. Vet. Med. 2021; 45(2): 60-64.

A B S T R A C T

This paper aimed to investigate the ability of ORF virus to trigger apoptosis in dermal and epidermal cells via immunohistochemical detection of the caspase-3 protein in goat's lip skin: The samples were collected from the goats' lips skin infected with contiguous Ecthyma. The ability of virus to trigger apoptosis was verified using a polyclonal anticaspase-3 antibody to detect apoptotic activity. The results revealed that the ability of virus to induce the apoptosis as indicated by the positive expression of activated caspase-3 in all layers of the epidermis. However, the presence of small areas of apoptotic cells and high percentage of caspase-3 was shown in the affected cells in the epidermis of the lips compared with the control group. The current study concluded the ability of virus to induce apoptosis in dermis and epidermis cells by detected a caspase-3 antibody in tissue.

Keywords: immunohistochemistry, apoptosis, caspase-3

INTRODUCTION

Orf virus (ORFV),which is also called infectious Ecthyma and contagious ecthyma, is one of the most important diseases in the goat farms. It is often regarded as a benign condition, but in several places around the world a malignant type is identified (1). The virus causes an adverse effect on animal health, infected animals are more vulnerable to adventitious bacterial infections (2).

ORFV according to the international committee on virus Taxonomy and belongs to the Poxviridae's genus Parapoxvirus (PPV) (3). The virus transmits directly through direct contact between the infected animals and/or infected environments (4). The disease might also transmit by aerosols and/or near contact with infected animals directly or indirectly (5-7). The typical clinical findings of infection with Contagious Ecthyma in the skin is the erythema, vesicle formation to pustule and then into scab. The characteristic lesion for the disease is the formation of pustular, nodules and crust lesions in the skin around the mouth and nostrils. Lesions may also develop in the other parts of the body (8). ORFV can also be passed on to people

via broken skin, in particular, in close contact with animals, farmers and animal careers (9). Apoptosis, known as programmed cell death (PCD) plays a pivotal role in the body's growth and development process (10). Apoptosis takes by two traditional pathways nominally, include exogenous pathway, which was the FasL, TRAIL, or TNF pathway, and the endogenous pathway that stimuli such as dysregulation of the cell cycle, withdrawal of the survival factor, drugs, DNA injury and pathogens (11). Ultimately, both pathways result in the activation of caspases, subsequently releasing many apoptosis related proteins to cause cell death (12). Caspase-3 forms part of the class of proteins are involved in macromolecular proteolysis of proteins, known as caspase. Caspase-3 in apoptosis plays a role through external signaling pathways (death ligand) and internal signaling systems (mitochondrial) (13, 14).

Apoptosis is an important type of cell death and plays a key role in the disease response to pathogens during development and viral infections. ORFV is the root cause of programmed cell death in late phases and contributes to the release of the virus (15). Caspas-3 is also essential for typical apoptosis characteristics, apoptotic chromatin condensation, and fragmentation of DNA under examination for all cell types. Caspase-3 is therefore important processes related to cell dismantling and the development of apoptotic bodies (16). There are wide range of stimuli involve the pathological and physiological stimuli which lead to apoptosis, Irradiation, or chemotherapy for the treatment of cancer that it causes DNA damage in certain cells, which may lead to apoptotic death via a p53 dependent pathway. Some hormones from the thymocytes may lead to apoptotic or kill in some cells, although other cells are unimpaired or even are stimulated (17). The objective of current study is to investigate the apoptosis is induced by viral infection of ORF by detecting caspase-3 in infected tissues using an immunohistochemistry reaction.

MATERIALS AND METHODS

The immunohistochemistry analysis (Enzymatic and Affinity) 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.

Paraffin embedded lips tissue of goat were sectioned at 4 µm thickness and mounted on charged slides (CrystalCruz™ Adhesive Micro Slides, Santa Cruz, sc-363560, USA). Tissue was deparaffinized into two xylene changes (10 min each), then rehydrated in five ethanol changes of three min each for 100%, 90%, 80%, 70%, and 50%, respectively.

Tissue sections were washed in the distilled water and submerged in the saline bath (pH 9) of Tris (TBS) for five min. The tissue sections were 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 min in a water bath at 97°C. Tissue sections left in a glass container for 20 min at room temperature then were rinsed with distilled water and immersed in a tampon bath for 5 min. 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, covered and incubated the sections in the humidity chamber for a period of 10 min, before rinsing the sections with distilled water and immersing them in a TBS bath pH 9 for 5 min. Excess buffer on the tissue sections was then removed by tapping gently on the slides. The tissue sections were flooded with the normal goat serum (Elabscience, E-IR-R213D, China) as a blocking reagent, covered the sections and incubated 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.

Sections were applied with 0.1 mL of anti-caspase-3 primary antibody and then incubated in a humidity chamber overnight at room temperature, wash by distilled water and dipped in a bath of TBS pH 9 for 5 min. Excessive buffer was applied to tissue sections.

The tissue sections were applied with 0.1 mL Polymer Helper (Elabscience, E-IR-R213B, China), incubated in a humidity room at room temperature for a period of 20 min, then rinsed with distilled water and then immersed in a TBS bath (pH 9) for 5 min.

The tissue sections were then incubated in the humidity at room temperature for 30 min with 0.1 mL of poly-peroxidase-anti-mouse/rabbit IgG secondary antibody (Elabscience, E-IR-R213C, China), rinsed with distilled water, then immersed in a TBS bath (pH 9) for 5 min. 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 min. Then the tissue sections rinsed 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 min each of 50%, 70%, 80%, 90% and 100% ethanol, respectively. Then tissue sections were immersed into two changes for 10 min 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 100×, 200×, 400× and 1000× magnifications.

Goats were taken care according to principle of animal welfare and ethics committee agreement.

RESULTS

The immunohistochemical section of non-infected goat lips skin showed negative expression of caspase-3 in epidermis and dermis layer (Figure 1).

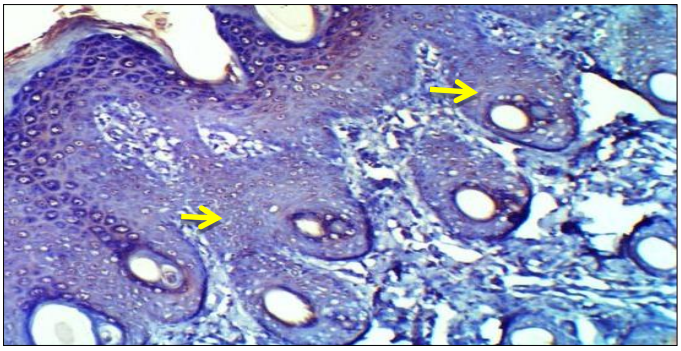

Figure 1. Immunohistochemical detection of caspase-3 protein in lip's skin of non-infected goat represents the negative expression of caspase-3 in dermal and epidermal cells (yellow arrows), 100×

On the other hand, the immunohistochemical section of goat lips skin infected with ORF virus showed positive expression of caspase-3 (brown color) in epidermis of lip. Overexpression of caspase-3 was observed in epidermis layers (Figures 2-6). However, caspase-3 expression was less in expression and intensity comparing with other samples (Figure 2) and with scanty of apoptotic cells, the dermis layer showed a weak expression (Figure 3); while (Figure 4) observed in stratum spinosum layer, note the apoptotic cells in affected area formed a vacuolation in epidermis, where the overexpression of caspase-3 was observed in stratum spinosum layer.

The apoptotic cells in affected areas formed a vacuolation in epidermis (Figure 5). The last epidermis layer of lips is shown in Figure 6 whereas, the overexpression of caspase-3 was observed in stratum spinosum layer especially that was surrounded the apoptosis of affected areas.

As well as Figures 7 and 8 showed the positive expression of caspase-3 (brown color) which was observed in dermis of lip and the overexpression of caspase-3 was observed in dermis of lips. The overexpression of caspase-3 was observed in epithelial cells of hair follicle of dermis layer. In addition to the apoptosis which was observed in these cells to form vacuolation in follicle structure with presence of apoptotic bodies in (Figure 7) as well as the overexpression of caspase-3 was observed in epithelial cells of hair follicle of dermis layer. The apoptosis was also observed in these cells to form vacuolation in follicle structure with presence of apoptotic bodies (Figure 8).

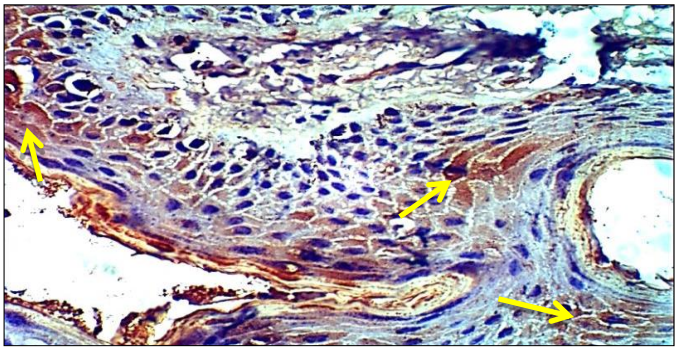

Figure 2. Immunohistochemical detection of caspase 3 protein in lip's skin of a goat infected with ORF virus reveals the positive expression of caspase-3 (brown color) in the epidermal cells The overexpression of caspase-3 (yellow arow) is observed in epidermis layers. However, caspase-3 expression is less in expression and intensity compared with other sample, 400×

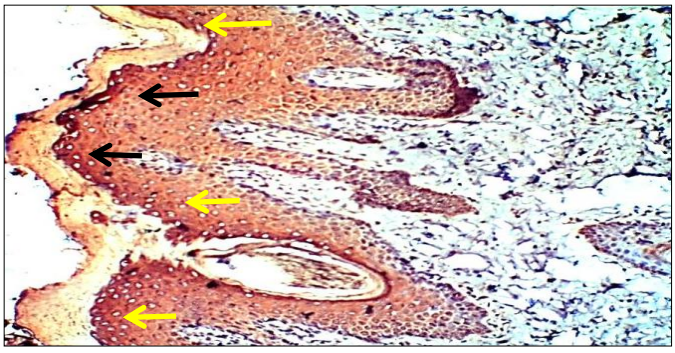

Figure 3. Immunohistochemical section of goat lips skin infected with ORF virus shows the positive expression of caspase-3 (brown color) which is observed in epidermis of lip. The overexpression of caspase-3 (black arrows) is observed in all epidermis layers with presence of small vacuolation of apoptotic cells (yellow arrows), while the dermis layer showed a weak expression, 100×

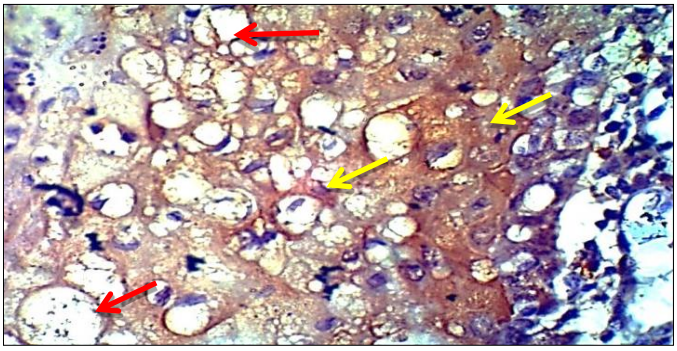

Figure 4. Immunohistochemical section of goat lips skin infected with ORF virus reveals the positive expression of caspase-3 (brown color) which is observed in epidermis of lips, where the overexpression of caspase-3 is observed in stratum spinosum layer (yellow arrows). Note the apoptotic cells in affected area to form the vacuolation in epidermis (red arrows), 400×

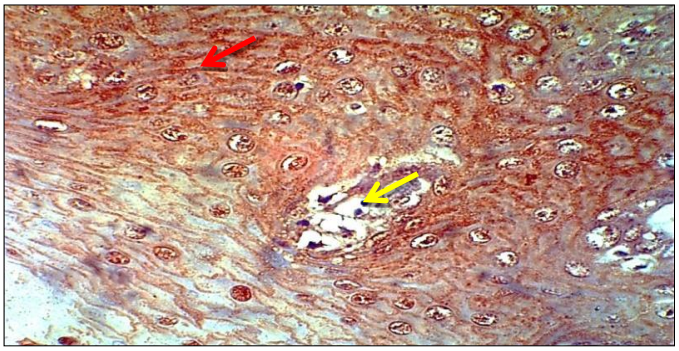

Figure 5. Immunohistochemical section of goat lips skin infected with ORF virus shows the positive expression of caspase-3 (brown color) which is observed in epidermis of lips, where the overexpression of caspase-3 is observed in stratum spinosum layer (red arrows). Note the apoptotic cells in affected area to be form a vacuolation in epidermis (yellow arrows), 400×

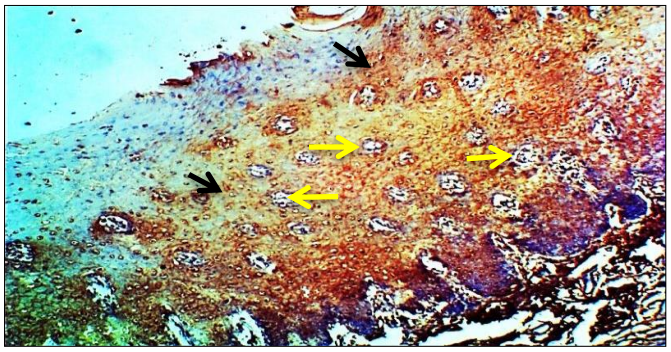

Figure 6. Immunohistochemical section of goat lips skin infected with ORF virus shows the positive expression of caspase-3 (black arrows) which is observed in epidermis of lips, where the overexpression of caspase-3 is observed in stratum spinosum layer (yellow arrows) especially that surrounds the apoptosis affected areas, 400×

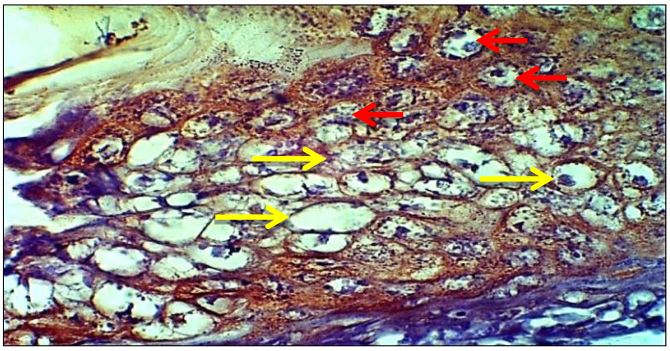

Figure 7. Immunohistochemical section of goat lips skin infected with ORF virus shows the positive expression of caspase-3 (brown color) which is observed in dermis of lip. The overexpression of caspase-3 is observed epithelial cells of hair follicle of dermis layer (red arrows) Note the apoptosis is observed in these cells to form the vacuolation in follicle structure with presence of apoptotic bodies (red arrows) especially that surrounds the apoptosis affected areas, 400×

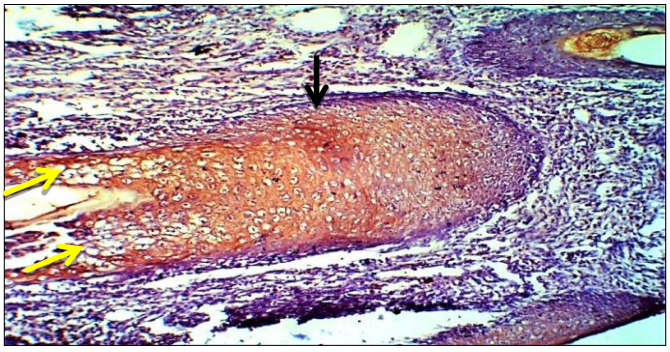

Figure 8. Immunohistochemical section of goat lips skin infected with ORF virus reveals the positive expression of caspase-3 (brown color) which is observed in dermis of lip. The overexpression of caspase-3 (black arrows) is observed (in or as) epithelial cells of hair follicle of dermis layer. Note the apoptosis is observed in these cells to form the vacuolation in follicle structure with presence of apoptotic bodies (yellow arrows), 100×

DISCUSSION

Apoptosis is natural tissue homeostasis its maintenance and physiological functions are to control process (18), for the cellular physiology in addition to its ability to have a suicide program that may be implemented to block replication (19). Microorganisms/viruses that induce and/or inhibit host cell apoptosis may interact between virus and host. Caspase-3 plays a central role in mediating nuclear apoptosis (20). The role of Caspas-3 in apoptosis is important. Caspase-3 is also responsible for the apoptotic characteristics of the immune system (21).

The ability of ORF to trigger apoptosis by caspase-3 expressions in the goat epidermis is clearly detected by IHC method in lips section of goats infected with ORF virus by Immunohistochemistry examination. The results showed positive expression of caspase-3 in cells which indicate the ability of the virus to induce programmed cell death by viral infected in the epidermis and dermis cells. It was found, on the other hand, that the ORF virus had the ability to induce apoptosis as indicated by expression of caspase-3 when it compared with non-infected animals which revealed no expression of caspase-3. The above result was in agreement with Kruse and Weber (22) who referred to the cells in the process of apoptosis and showed their location in the layers of dermal and near hair follicles.

In the current study, it was revealed that over positive expression of caspase-3 to be appeared in dermis cells as (dark brown stain) with small space related to apoptotic cells. The latter data was in correlation to (23). Caspase-3 protein is a major cause of apoptosis because of its role in coordinating cellular destruction, such as DNA fragmentation or cytoskeletal protection degradation. Apoptosis is a systematic cell process that occurs in physiological or pathological conditions of programmed cell death, and caspase-3, which is essential for apoptosis types of chronic chromatin condensation and fragmentation of DNA in all cell types examined which are required. The apoptotic protein is a structural component of the virus itself, which may lead to apoptosis of epidermal cells and dermal structure by inducing expression of caspase-3.

Through the results obtained, it was found that infection with ORF virus causes programmed cell death in the infected cells, which included the cells of the skin layers through the inducing high expression of caspase-3.

In final conclusion, the results of present study indicated that the high ability of ORF virus to induce apoptosis in dermis and epidermis cells by detecting a caspase-3 antibody in tissue.

ACKNOWLEDGEMENTS

My heartfelt thanks go to the pathologists, Prof. Dr. Hafidh Ibraheem Mahmoud Al-Sadi, who helped us and did not hesitate any moment to provide scientific information or direct assistance.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Bharathy N, Akila N. Incidence of contagious ecthyma (ORF) in goats in an organized farm. Ind. J. Vet. & Anim. Sci. Res. 2015; 44(2): 129-131.

2. Gallina L, Dal Pozzo F, Mc Innes CJ, Cardeti G, Guercio A, Battilani M. A real time PCR assay for the detection and quantification of ORF virus. J Viro Meth. 2006; 134(1-2): 140-145.
3. Tedla M, Berhan N, Molla W, Temesgen W, Alemu S. Molecular identification and investigations of contagious ecthyma (ORF virus) in small ruminants, Northwest Ethiopia. BMC Vet Rese. 2018; 14(1): 1-8.
4. Radostits MO, Gay CC, Blood DC, Hinchcliff KW. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th ed. W.B. Saunders co, London. 2000; 1242-1248.
5. Zangana IK, Abdullah MA. Epidemiological, clinical and histopathological studies of lamb and kid pox in Duhok, Iraq. Bulg J Vet Med. 2013; 16(2): 133-138.
6. Manjunathareddy GB, Sumana K, Apsana R, Yogisharadhya R, Prajapati A, Patil SS. Investigation of malignant form of sheep pox outbreak in fattening lambs in Mandya, Karnataka. Indi J Vet Path. 2017; 41(3): 184-188.
7. Abd-Elfatah EB, El-Mekkawi MF, Bastawecy IM, Fawzi EM. Identification and phylogentic analysis of sheep pox during an outbreak of sheep in Sharkia Governorate, Egypt. J Gene Mol Res. 2018; 17(2): gmr16039901.
8. Hosamani M, Scagliarini A, Bhanuprakash V, Mcinnes CJ, Singh RK. ORF: an update on current research and future perspectives. Expert Rev. Anti-Infe. Ther. 2009; 7: 879-893.
9. Fleming SB, Wise LM, Mercer AA. Molecular genetic analysis of orf virus: a poxvirus that has adapted to skin. Viruses. 2015; 7(3): 1505-1539.
10. Meier P, Finch A, Evan G. Apoptosis in development. Nature. 2000; 407: 796-801.
11. Duprez L, Wirawan E, Vanden Berghe T, Vandenabeele P. Major cell death pathways at a glance. Microbes Infect. 2009; 11(13): 1050-1062.
12. Tait SW, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. Nat. Rev. Mol. Cell Biol. 2010; 11: 621-632.
13. Salvesen GS. Caspases: opening the boxes and interpreting the arrows. Cell Death Differ. 2002; 9(1): 3-5.
14. Ghavami S, Hashemi M, Ande SR, Yeganeh B, Xiao W, Eshraghi M, et al. Apoptosis and cancer: mutations within caspase genes. J Med Genet. 2009; 46(8): 497-510.
15. Li W, Chen H, Deng H, Kuang Z, Long M, Chen D, et al. Orf virus encoded protein ORFV119 induces cell apoptosis through the extrinsic and intrinsic pathways. Front Microbiol. 2018; 9: 1056.
16. Porter A, Jänicke R. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 1999; 6: 99-104.
17. Norbury CJ, Hickson ID. Cellular responses to DNA damage. Annu Rev Pharacol Toxicol. 2001; 41: 367-401.
18. Degterev A, Boyce M, Yuan J. A decade of caspases. Oncogene. 2003; 22: 8543-8567.
19. Everett HG, McFadden G. Apoptosis: an innate immune response to virus infection. Trends Microbiol. 1999; 7(4): 160-165.
20. Westphal D, Ledgerwood EC, Hibma MH, Fleming SB, Whelan EM, Mercer AA. Novel Bcl-2-like inhibitor of apoptosis is encoded by the parapoxvirus ORF virus. J Virol. 2007; 81(13): 7178-7188.
21. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol. 2013; 5(4): a008656.
22. Kruse N, Weber O. Selective induction of apoptosis in antigen-presenting cells in mice by Parapoxvirus ovis. J Virol. 2001; 75(10): 4699-4704.
23. Taylor JM, Berry M. Near death experiences: poxvirus regulation of apoptotic death. Virology. 2006; 344(1): 139-150.

الكشف المناعي النسيجي الكيميائي لموت الخلايا المبرمج في الجلد وخلايا البشرة في الشفتين في الماعز المصابة بمرض الأورف

ياسمين جاسم محمد[1] و ايمان هاشم يوسف[2]

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الخلاصة

تم تصميم هذا البحث لاكتشاف قدرة الأكزيما المعدية التي يسببها فيروس ORF على تحفيز موت الخلايا المبرمج في الماعز المصابة بالأكزيما المعدية عن طريق الكشف المناعي النسيجي الكيميائي للكاسبيز (3). تم جمع العينات من جلد الشفاه للحيوانات المصابة بالأكزيما المعدية حيث تم التحقق أيضًا من قدرة الفيروس على إحداث موت الخلايا المبرمج باستخدام جسم مضاد متعدد النسيلة مضاد للكاسبيز (3) للكشف عن نشاط موت الخلايا المبرمج. أظهرت نتائج هذه الدراسة قدرة الفيروس على إحداث موت الخلايا المبرمج من خلال إظهار التعبير الإيجابي للكاسبيز (3)، ووجود فرط في التعبير للكاسبيز (3) في جميع طبقات البشرة، مع وجود مناطق صغيرة من الخلايا الميتة ونسبة عالية من كاسبيز (3) في الخلايا المصابة في بشرة الشفاه مقارنة معمجموعة التحكم. استنتجت الدراسة الحالية القدرة العالية / المعنوية لفيروس ORF على إحداث موت الخلايا المبرمج في خلايا الأدمة والبشرة.

الكلمات المفتاحية: الكيمياء المناعية النسيجية، الكشف عن موت الخلايا المبرمج، كاسبيز 3