



Immunohistochemical Detection of Interleukin-6 Related to Infectious Bronchitis Virus in Broiler in Basrah Province

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Author

Najlaa Hameed Megdad, Yasmeen
Jasim Mohammed, Waleed Majeed
Seger.

Department of Pathology and Poultry
Disease, College of Veterinary Medicine,
University of Basrah, Basrah, Iraq.

Corresponding Author Email Address:
najlaaa.hamed@uobasrah.edu.iq

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Abstract

The gamma coronavirus that causes an extremely contagious disease in chickens is based on the avian infectious bronchitis virus. The respiratory tract, proventriculus, caecal tonsils, oviduct, and kidney are among the virus's preferred host tissues. Young chickens with infections typically experience respiratory distress. The virus causes respiratory distress in broiler chickens, but certain strains can induce interstitial nephritis. This study was performed to analyse and detect the expression of the (Interleukin-6) in the kidney tissue infected with (Infectious Bronchitis Virus) by using the Immunohistochemical detection technique. The samples were taken from a chicken kidney infected with IBV. The results of the study revealed positive expression of (Interleukin – 6), most of this expression was in the epithelial cells of the renal tubules in the cortex area, compared to the negative expression of (Interleukin – 6) in the control group. We concluded that the IBV strain in Basrah province has cell tropism to renal tissues and has nephropathogenic properties, causing nephritis.

Keywords: Immunohistochemistry; Interleukin-6; Infectious Bronchitis Virus

Introduction

Infectious Bronchitis is brought on by the infectious bronchitis virus (IBV), which is a serious and acute disease of poultry. The virus is common in many parts of the world and mostly affects the respiratory system, kidneys, and reproductive system, causing respiratory distress, kidney impairment, and a decrease in egg production. The avian infectious bronchitis virus (IBV) is a coronavirus (CoVs) an enveloped virus belonging to the Coronaviridae family with a positive-sense RNA genome (1). The upper respiratory tract is where IBV replicates most frequently, but it can also spread to other epithelial cells in several host organs (2). The infected chickens may appear depressed with various levels of breathing difficulty and have ruffled feathers (3,4,5,6). Younger chickens show the most severe clinical manifestations (7). Chickens with the infection display respiratory symptoms, kidney and oviduct lesions, reduced egg output, and poor-quality eggs, and potential subsequent problems (8). IBV can spread throughout the epithelial surfaces of the kidney, causing granular degeneration, vacuolation, and desquamation of the tubular epithelium in addition to a considerable infiltration of heterophils into the interstitium (9). Common characteristics of IBV-induced kidney lesions include interstitial nephritis and tubular lesions that are most obvious in the medulla (10,11). Small (15–20 kDa) and transient proteins known as cytokines have a key role in autocrine, paracrine, and endocrine signalling. Cytokines belonging to the interleukin (IL)-6 family are those that use the glycoprotein 130 kDa (gp130) as their common signalling receptor subunit (12).

Numerous mechanisms have been connected to IL-6 family cytokines, including the activation of B cells and the production of the proteins involved in the hepatic acute phase. Furthermore, this category of cytokines has been linked to metabolic and neurotrophic functions. Recently, more than 100 countries approved the use of tocilizumab, a monoclonal antibody that inhibits the IL-6 receptor (IL-6R), to treat autoimmune disorders (13). Furthermore, it was found that blocking IL-6 activity in rheumatoid arthritis patients was at least as effective as blocking tumor necrosis factor α (TNF- α) (14). The goal of this study is to identify the expression of IL-6 in the kidney in relation to renal lesions in broiler chickens infected with an avian infectious bronchitis virus.

Materials and Methods

One hundred tissue samples (kidney tissue) were taken from ten broiler chicken farms located in Al-Qurna, Karmat Ali and Al-Faw areas in Basrah Governorate / southern Iraq, where the ages of the birds ranged from 17 to 28 days, and the capacity of the broiler farms ranged from 5,000 to 9,000 broiler chicks

Histopathological procedure

The collected tissue samples infected with IBV were fixed in 10% natural buffered formalin. The tissue samples were then processed through the routine tissue fixation procedure, and with

haematoxylin and Eosin as the (15) protocol. Kidney samples were taken using sterilized forceps, scissors, and fixatives in 10% formalin; following that, standard procedures for histological identification were carried out on tissues from infected broilers. The slides were created and examined in the pathology department of the Faculty of Veterinary Medicine, University of Basrah, South Iraq, according to (16), the optical microscope Olympus was used to determine the histological properties of tissues using colored slides of haematoxylin and eosin (HE).

Immunohistochemical detection procedure

The Immunohistochemistry procedure was done at the Qiima Veterinary laboratory / Kufah/Najaf by using the Dako EnVision Detection IHC kit (EnVision FLEX, Dako, K8000, Denmark). Anti-Interleukin-6 Primary Antibody (Polyclonal Rabbit Antibody, Mybiosource, USA) was used for the detection expression of IL-6 in the kidney tissue of the current study. The chicken Deparaffinization and rehydration were performed on the slides containing fixed tissue samples. Heat treatment at 60 C° was used to retrieve the antigen using (EnVision FLEX Target Retrieval Solution, High PH, DM828). Peroxidase block (Envision, FLEX, Peroxidase-blocking reagent SM801). Solution was used to prevent endogenous phosphate and non-specific binding protein, thereafter the section was treated with a primary Anti-IL-6 antibody (Ab), and after that the sections were applied with secondary (Ab) labelled to horseradish peroxidase (EnVision FLEX/HRP, SM802). The tissue section was then applied with freshly prepared DAB+ substrate chromogen solution for 10 min, then the sections were rinsed and immersed in two changes of TBS buffer bath (EnVision FLEX Wash. Buffer, SM831) for 5 minutes each. The staining was carried out for 3 minutes, using Mayer Haematoxylin (Bio-Optica, Italy, 05-0600/L). The tissue sections were dehydrated in three changes of 70%, 90%, and 100% of ethanol alcohol for 2 minutes each, respectively. The tissue sections were immersed in two changes (10 minutes each) of xylene and mounted with mounting media (DPX) and covered with cover slips. The tissue sections were examined under a light microscope at 100x and 400x magnifications.

Results

The normal histological section of the kidney tissue of non-infected chicken is shown in the figure (1,A) (control), also there is various change that was noted in the kidney tissue, including the presence of hyperplasia of the renal glomerulus, necrosis of renal tubular epithelium and aggregation of inflammatory cells (1,B), and present of interstitial haemorrhage as shown in figure (1,C).

The represented immunohistochemical section of non-infected chicken kidney (control group) shows negative expression of interleukin-6 in the kidney tissue, as shown in the figure (2,A) . The immunohistochemical section of chicken kidney infected with IBV revealed the Overexpression of anti-IL-6 primary antibody (brown color) was observed in epithelial cells of renal tubules in

cortex area, also noticed the anti-IL-6 primary antibody expression which was observed in the cortex area that suffered from necrosis also, the haemorrhage was observed between renal tubules and inflammatory cells that showed anti-IL-6 primary antibody expression in affected area shown in figure (2,B). Also the overexpression of IL-6 in the epithelial cells of the renal tubules in the cortex area, the expression was observed in the area that suffered from necrosis at the cortex as shown in the figures (2,C), also there is expression of IL-6 with the presence of haemorrhage between renal tubules that showed anti-IL6 primary antibody expression in affected area as shown in the figure (2,D). Overexpression of anti-IL-6 primary antibody was observed in epithelial cells of renal tubules in the cortex area. The anti-IL-6 primary antibody expression was observed in the cortex area that suffered from necrosis, and the haemorrhage was observed between renal tubules that showed anti-IL-6 primary antibody expression in the affected area, as shown in figure (2,E).Anti-IL-6 primary antibody expression was observed in epithelial cells under necrosis and infiltration of inflammatory cells in the figure (2,F).

Discussion

A variety of viruses can infect birds, but poultry is the most vulnerable. The chicken business is directly harmed by these viral infections, which result in large financial losses (17). The disease Infectious Bronchitis has expanded around the world and is incredibly difficult to treat due to its numerous serotypes and variant strains. It is advantageous for scientific study and control measures to detect the Infectious Bronchitis Virus quickly (3). Nephropathogenic strains initially replicate in the trachea and then spread to the kidneys, causing cytopathic changes in the tubular epithelium (18). This generates an interstitial inflammatory response with polymorphonuclear leukocytes progressively infiltrating the medullary and cortical regions of the kidney (19,20). Interleukins, among other cytokines, are thought to play a significant role in the body's defence against inflammatory diseases and cancers. They also play a major role in the development of cancer-related weight loss symptoms.

To identify and determine the existence and production of these factors in tissues using the immunohistochemical technique and gene expression, it is necessary to understand these factors and their effects, as well as their production, particularly the gene expression of interleukin-6 (18). Cytokines are potent polypeptides that are released from inflammatory cells as part of the host response (21). Some IBV strains replicate in the kidney, and due to their nephropathogenic properties, they have the potential to cause severe losses (22). Numerous cytokines, including IL-6, are frequently engaged in escalating or exacerbating the immune response during an acute inflammatory reaction. The acute phase response is mostly induced by IL-6. Our findings matched those of (10, 23, 24), who demonstrated that IBV replicates renal tubular epithelial cells and can cause structural abnormalities in the kidney.

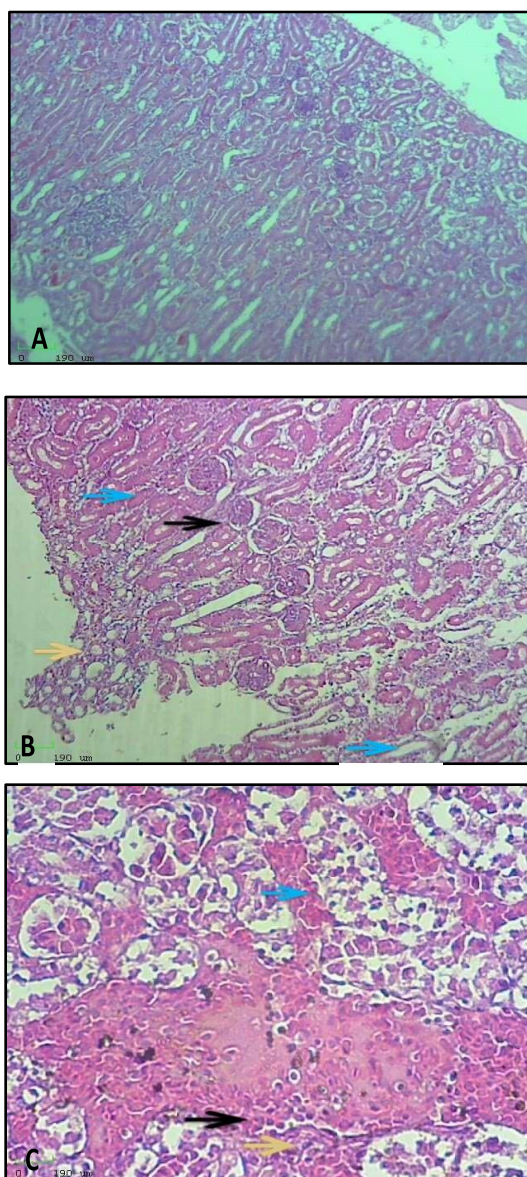








Fig.1:Histopathological section of non-infected and infected chicken kidney showing: non-infected chicken kidney (HE staining100x) as shown in (A), infected chicken kidney with IBV showing the presence of hyperplasia of the glomerulus at the capsular region.(), necrosis of the renal tubular epithelium () and aggregation of inflammatory cells.() (HE staining 100x) as shown in (B), an infected chicken kidney with IBV showing the presence of interstitial haemorrhage.(), necrosis of the renal tubular epithelium () and aggregation of inflammatory cells. () (HE staining 400x) as shown in (C).

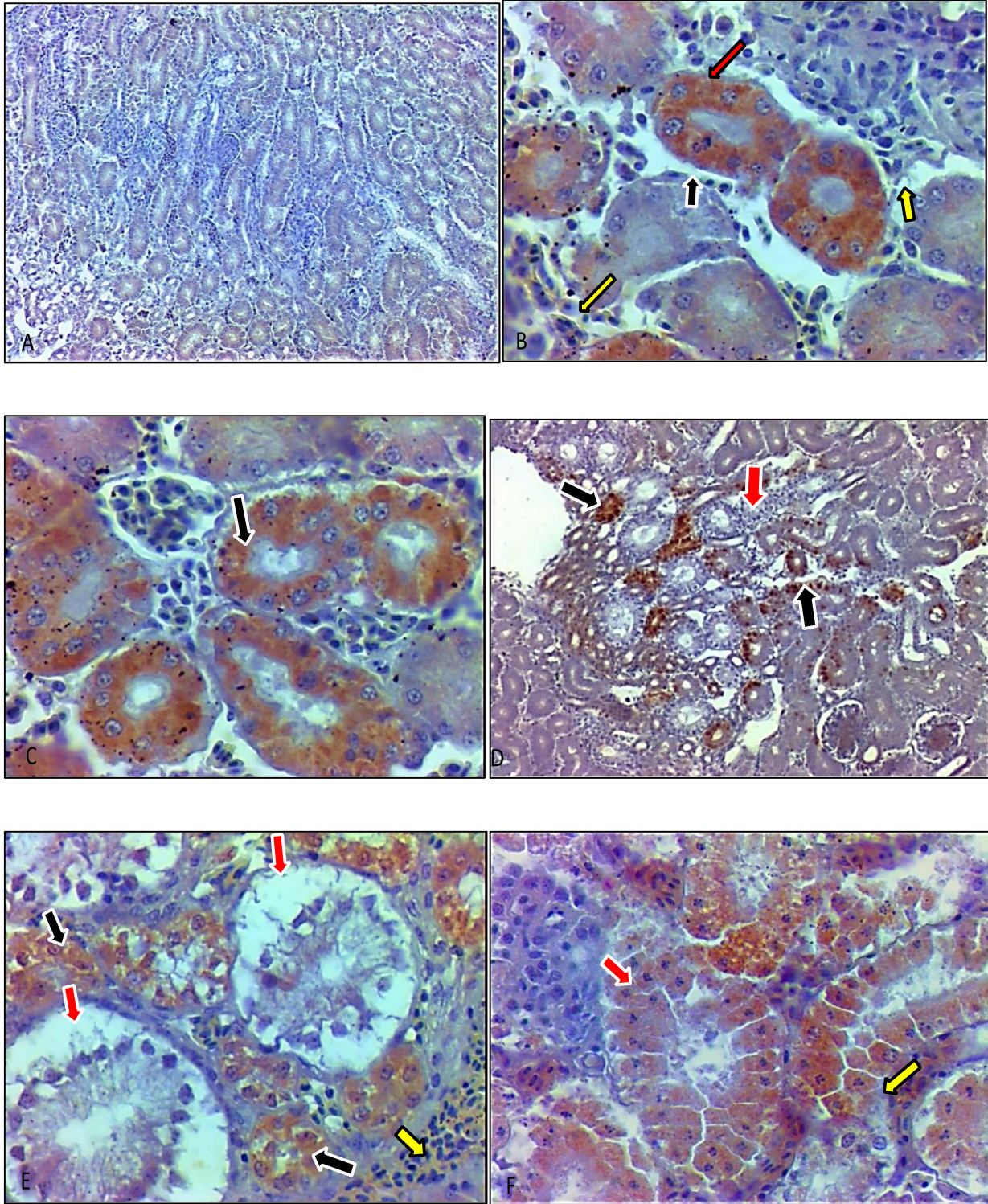


Fig.2: Photomicrograph of kidney control negative chicken 100x(A), examined sections of kidney did not show any expression of anti-IL-6 primary antibody DAB& haematoxylin. Photomicrograph of the kidney of an IBV-infected chicken. Overexpression of anti-IL-6 primary antibody (black arrow) was observed in epithelial cells of renal tubules in cortex area. Note that anti-IL-6 primary antibody expression was observed in the cortex area that suffered from necrosis. Also, the haemorrhage (yellow arrow) was observed between renal tubules, and inflammatory cells (red arrow) that showed anti-IL-6 primary antibody expression in the affected area. DAB& haematoxylin: 400x, (B), Photomicrograph of the kidney of an IBV-infected chicken. Overexpression of anti-IL-6 primary antibody (brown colour) (black arrow) was noticed in renal tubule epithelial cells in the cortical area. Note that anti-IL-6 primary antibody expression was observed in the cortex area that suffered from necrosis. DAB& haematoxylin: 400x, (C) Overexpression of anti-IL-6 primary antibody (brown colour) (black arrow) was noticed in renal tubule epithelial cells in the cortical area. Note that anti-IL-6 primary antibody expression was observed in the cortex area that suffered from necrosis (red arrow). DAB& haematoxylin. 100x (D), Photomicrograph of IBV-infected chicken kidney, Overexpression of anti-IL-6 primary antibody (brown colour) (black arrow) was noticed in the cytoplasm of renal tubule epithelial cells. Note that anti-IL-6 primary antibody expression was observed in areas that suffered from necrosis (red arrow). Also, the haemorrhage (yellow arrow) was observed between renal tubules that showed anti-IL-6 primary antibody expression in the affected area. DAB& haematoxylin. 400x, (E), Photomicrograph of the kidney of IBV-infected chicken. Overexpression of anti-IL-6 primary antibody (brown colour) (black arrow) was noticed in the cytoplasm of renal tubule epithelial cells in the cortical area. Note the cortex area that suffered from necrosis (red arrow). Also, the infiltration of inflammatory cells (yellow arrow) was observed between renal tubules that showed anti-IL-6 primary antibody expression in the affected area. Anti-IL-6 primary antibody expression was observed in epithelial cells under necrosis (red arrow). DAB& haematoxylin: 400x, (F).

This study's findings corroborated those of (25, 26, 27, 28,29 and 30), who observed that birds with IBV infection exhibit increased cytokine expression. Current study results revealed that the presence of large areas of (IL-6) expression in the epithelial cells of the renal tubules. These changes in the kidney may result in the pro-inflammatory response of (IL-6) and its activation in the inflammatory cell against the Infectious Bronchitis Virus. These results agreed with (31), they observed that the development of necrosis and nephritis results from the acute interstitial inflammatory response caused by the presence of IBV in renal epithelial cells, and the result corresponding to (32), they showed an increase of inflammatory cells surrounding the tubular epithelial cells immunohistochemically labelled for antigen detection, and our result also agreed with (33) that related the increased IL-6 expression by IBV-containing epithelial cells causes an upregulation of chemokines molecules and adhesion, which promotes leukocyte migration. The present result showed positive expression of (IL-6) in kidney, most of this expression was in the epithelial cells of the renal tubules;that may be attributed to Massive replication of IBV serotype which caused gross and microscopic lesions in kidney ;these finding in line with that of (34), who

studied IBV (variant 2 (IS/1494-like) in Basrah province that have cell tropism to renal tissues and characterized as nephropathogenic strain. Our results noted increased expression of IL-6 induced by IBV infection led to consequently development of inflammation characterized by nephritis, which observed that interleukin 6 play important role as pro inflammatory cytokine and tissues damage. (IB) pathological severity is dependent on the genetic line of the infected chicken. Furthermore, it has previously been identified that one line of specific-pathogen-free (SPF) Shaver White chicken, designated the S-line, is highly susceptible to Australian T nephropathogenic IBV. Mortality after IBV infection was compared between a susceptible line (S-line) and a resistant line of chickens; mortality reached 90% in S-line birds after low-dose inoculation, whereas the resistant chicks showed no mortality. In addition, the kidneys of S-line chicks showed severe nephritis, tubular necrosis, and less heterophil infiltration throughout the acute phase of infection compared with resistant chicks (35,36,37).

Conclusion

We concluded from current work that during IBV infection, there is an association of IL-6 and induction of nephritis. IBV strain in Basrah province has cell tropism to renal tissues and is characterized as a nephropathogenic strain. IBV infection led to expression of IL-6 in renal tissue, which can induce the development of nephritis in chickens, indicating that Interleukin-6 play important role in the pathogenesis, up-regulation of lesions of nephritis. The findings of this study demonstrate that the Infectious Bronchitis Virus (IBV) strain circulating in Basrah province reveals a strong tropism toward renal tissues in infected chickens. Immunohistochemical detection revealed a significant positive expression of Interleukin-6 in the epithelial cells of renal tubules within the cortex of infected kidneys, while no expression was observed in the control group. This elevated cytokine expression indicates an inflammatory response associated with viral infection. Therefore, the results confirm that the IBV strain present in this region possesses nephropathogenic characteristics and can induce renal inflammation (nephritis) in infected chickens.

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Conflict of Interest

We are declaring that they have no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee

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الكشف الكيميائي المناعي للإنترلوكين-6 المرتبط بفيروس التهاب الشعب الهوائية المعدي في دجاج اللحم في محافظة البصرة

نجلاء حميد مقداد, ياسمين جاسم محمد , وليد مجيد صكر.

فرع الامراض وامراض الدواجن، كلية الطب البيطري، جامعة البصرة، البصرة، العراق.

الخلاصة

فيروس كورونا غاما الذي يسبب مرضًا شديد العدوى في الدجاج يعتمد على فيروس التهاب الشعب الهوائية المعدي الطيري. المسالك التنفسية، القانصة، اللوزات الأوربية، قناة البيض، والكلى هي من الأنسجة المضيفة المفضلة للفيروس. عادة ما يعاني الدجاج الصغير المصاب بضيق تنفسي. يسبب الفيروس ضيقًا تنفسيًا في دجاج اللحم، ولكن بعض السلالات يمكن أن تسبب (الإنترلوكين-6) في أنسجة (IL-6) التهاب الكلية الخلالي. أجريت هذه الدراسة لتحليل وكشف التعبير عن السيتوكين المناعي الكلى المصابة بفيروس التهاب الشعب الهوائية المعدي باستخدام تقنية الكشف الكيميائي المناعي. أخذت العينات من كلى الدجاج وكان معظم هذا (IL-6) المصاب بفيروس التهاب الشعب الهوائية المعدي، وكشفت نتائج الدراسة عن تعبير إيجابي عنفي المجموعة الضابطة. (IL-6) التعبير في الخلايا الظهارية للأنابيب الكلوية في منطقة القشرة مقارنة بالتعبير السلبي عن استنتاجنا أن سلالة فيروس التهاب الشعب الهوائية المعدي في محافظة البصرة لها ميل خلوي إلى أنسجة الكلى ولها خاصية إحداث التهاب الكلية.

الكلمات المفتاحية: الكيمياء المناعية؛ الكشف عن الإنترلوكين-6؛ فيروس التهاب الشعب الهوائية المعدي.