Iraqi Journal of Science, 2024, Vol. 65, No. 7, pp: 3668-3679
DOI: 10.24996/ijs.2024.65.7.9

ISSN: 0067-2904

Immunopathogenesis of Lung in Fatal COVID-19 Cases in Erbil, Iraq

Taban Kamal Rasheed [1*], Zahra Abdulqader Amin[2], Tuqa Yousif Sharef [3], Lana Sardar Saleh[1]

[1]*Department of Biology, College of Science, Salahaddin University, Erbil 44001, Iraq*
[2]*Department of Clinical Analysis, College of `Pharmacy, Hawler Medical University, Erbil 44001, Iraq*
[3]*Department of Basic Science, College of Dentistry, Hawler Medical University, Erbil 44001, Iraq*

Received: 3/4/2023 Accepted: 19/6/2023 Published: 30/7/2024

Abstract

Coronavirus disease 2019 (COVID-19) infection is caused by severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2). It is characterized by respiratory distress, multiorgan dysfunction and death in some cases. The host immune response to SARS-CoV-2 appears to play a critical role in disease pathogenesis and clinical manifestation. However the pathological mechanism underling the disease has not been fully defined. Lung autopsy samples from 3 patients with fatal COVID-19 were evaluated using hematoxylin and eosin stain to analyze the histopathological changes. While immunohistochemical (IHC) staining was performed for detecting CD4 in helper T-cells, CD8 in cytotoxic T-cells, CD56 in NK-cells and CD45RO in memory T-cells. Histopathological examination revealed features of diffuse alveolar damage (DAD) with exudate in alveolar space and infiltration of inflammatory cells. Immunohistochemistry staining for CD4 showed weak to positive staining, CD8 showed weak positive staining, CD56 showed negative staining, while CD45RO showed a positive staining. It can be concluded that SARS-CoV-2 virus impaired innate immune response through a decrease in NK cells and adaptive immune response through a decrease in CD8 T cells which is one of the explanations of the destructive nature of SARS-CoV-2 virus.

Keywords: Lung Autopsy Sample, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), Immunohistochemical staining (IHC).

ألامراضية المناعية للرئة في حالات كوفيد-19 المميتة

تابان كمال رشيد[1*], زهرة عبد القادر امين[2], تقى يوسف شريف[3], لانه سردار صالح[1]

[1]قسم علوم الحياة, كلية العلوم, جامعة صلاح الدين, اربيل, العراق
[2]قسم التحليلات المرضية, كلية الصيدلة, جامعة هولير الطبية, اربيل, العراق
[3]قسم العلوم الاساسية, كلية طب الاسنان, جامعة هولير الطبية, اربيل, العراق

الخلاصة

تحدث الاصابة بمرض كوفيد-19 بواسطة فايروس كورونا-2 (SARS-CoV-2) والذي يتسبب بضيق

* Email: taban.rasheed@su.edu.krd

التنفس والاختلال الوظيفي للاعضاء المتعددة والوفاة في بعض الاحالات. ويبدو ان الاستجابة المناعية للمضيف لفايروس SARS−CoV−2 تلعب دورا مهما في التسبب في المرض والمظاهر السريرية له. ولكن لم يتم تحديد الآلية المرضية الكامنة وراء المرض بشكل كامل. وقد تم تقييم عينات تشريحية للرئة من 3 مرضى مصابين ب كوفيد−19 المميت باستخدام صبغة الهيماتوكسلين والأيوسين لتحليل التغيرات النسيجية المرضية بينما تم أجراء التصبيغ الكميائي المناعي (IHC) للكشف عن CD4 في الخلايا التائية المساعدة، CD8 في الخلايا التائية السامة للخلايا، CD56 في الخلايا القاتلة الطبيعية و CD45RO في خلايا الذاكرة التائية. كشف فحص الأنسجة المرضية عن ملامح الضرر النسخي المنتشر (DAD) مع إفرازات في الفراغ النسخي وتسلل الخلايا الالتهابية. وأظهر التصبيغ الكميائي المناعي ل CD4 تصبيغا ضعيفا الى ايجابيا، واظهر CD8 تصبيغا ايجابيا ضعيفا، واظهر CD56 تصبيغا سلبيا، بينما أظهر CD45RO تصبيغا ايجابيا. ومن ذالك نستنتج بأن SARS− CoV−2 يضعف الاستجابة المناعية الغير المتخصصة، عن طريق تقليل الخلايا القاتلة الطبيعية (NK) وكذلك يضعف الاستجابة المناعية المتخصصة عن طريق تقليل الخلايا التائية السامة (CD8− T cells) . وهذا هو احد التفسيرات للطبيعة المدمرة لفايروس ال SARS−CoV−2 .

Introduction

Three years have passed since coronavirus disease 2019 (COVID-19) was first identified in Wuhan-Hubei Provence in China [1] and was recognized as a pandemic by World Health Organization in March 2020 [2]. As of 28 February 2023, there have been nearly 758,390,564 million cases confirmed worldwide and over 6,859,093 deaths [3]. Severe acute respiratory syndrome COVID-2 (SARS-CoV-2) is a coronavirus that binds with angiotensin converting enzyme2 (ACE-2) as a receptor for intracellular binding and entry, as other coronavirus agents like SARS and middle east respiratory syndrome (MERS) did [4, 5]. This receptor is expressed on the surface of lung epithelial and alveolar cells, vascular endothelium, pericytes and bronchial epithelium cells [6]. Organizing pneumonia, chronic interstitial pneumonia and diffuse alveolar damage were the major reported of lung pathologies in severe COVID-19 [7]. Lung histopathology of rapidly died COVID-19 patients with mechanical ventilation showed organizing diffuse alveolar damage (DAD). On the other hand, acute DAD with acute changes appeared in five patients who did not receive mechanical ventilation. Interestingly, inflammatory pattern in the lung changes according to DAD stage, from neutrophils in the acute phase to chronic inflammation in DAD tissue. This finding raises the question that lymphocytes, plasma cells and the lung macrophages may have a pathogenic role, possibly in immune response and virus clearance. [8]. Lot of studies have showed a close relation between SARS-CoV-2 disease progression and the immune system [9]. A decrease in peripheral T-cell subset has been shown as a unique characteristic of SARS and recovered patients showed a rapid restoration of peripheral T-cell subset. Thus, peripheral T-cells can serve as an accurate diagnostics tool for SARS [10]. Pro inflammatory cytokines have been found to elevate in a group of hospitalized non-vaccinated SARS patients and it has been shown that six cytokines were involved in the inflammation process of lung, including IL-6, IL-1β, IL-17A, TGF-β, TNF-α and IFN- γ [11]. Most SARS-CoV-2 studies were limited to peripheral blood markers, and only few gross lung anatomical analysis and immune-morphological analysis have been conducted in this field. The aim of this study was to provide an evaluation for infiltrations of immunological cells of lung autopsy specimen from different autopsy samples to give an insight into immunopathological changes of lung in SARA-CoV-2 patients and their effects on the disease outcome, and their implications for potential treatment.

Patients and Methods

Cases and Ethical Issues

Salahaddin University, Erbil-Iraq, College of Science's human ethics committee authorized and approved the current study (Approval No.45/431 Date 28/06/2022) and the General

Directorate of Health–Erbil (Approval No.24538 in 20/9/2022) for the use of autopsy samples. The cases were one female and two males, aged 67, 55 and 50 years respectively. The three autopsy samples were taken from histopathological department in Medico Legal Institute-Erbil Hospital that were confirmed for SARS-CoV-2 infection by real-time polymerase chain reactions (RT-PCR) at the time of hospital admission and died from respiratory failure. The three samples analysis was carried out in accordance with the principles of the medical committee of the hospital.

Lung Tissue Sampling, Processing and Analysis

All lung lobes were sampled, including central and peripheral area. Tissue specimens were fixed in 10% formalin solution for 48 hrs., then processed and embedded in paraffin according to Medico Legal Institute-Erbil Hospital-Histopathology department protocols. The samples were then transported to PAR private hospital for slide preparation and staining. Tissue sections were stained with hematoxylin and eosin stain (Thermo Scientific USA). The sections were viewed under a microscope (Olympus BX40, Japan) and photographed to analyze the histopathological changes in all autopsy samples. Immunohistochemical (IHC) staining performed on the most representative areas of selected cases included staining with antibodies against CD4 (clone 4B12, Fisher MA5-12259) for identification of helper T-cells, CD8 (clone SP16, Fisher MA5-14548) for identification of cytotoxic T-cells, CD56 (clone 56C04, Fisher MA5-11563) for identification of natural killer (NK) cells, and CD45RO (clone UCHL-1 Fisher MA5-11532) for identification of memory T-cells. Control samples for IHC was provided by Par Hospital. Two specialized pathologists, for each slide, did histopathological evaluation and approval. The analysis of immunological markers and the features of immune cells responses was done on the basis of staining intensity on cell membrane. Staining intensity was assessed for mild (Weak positive), moderate (Positive), intense (Strong positive), or no expression (Negative). All antibodies were ready-to-use monoclonal antibodies (Thermo Fisher Scientific, Tudor Road, Manor Park, Runcom, Cheshire WA7 1TA, UK). The sections obtained were analyzed under magnified power (X200 and X400) with high-resolution color microscope camera.

Results

Postmortem lung autopsy samples were collected from a case series of confirmed SARS-CoV-2 positive patients who died between 2020-2021. All patients had clinical and radiological features of severe pneumonia with high D-dimer levels and had respiratory failure, and mechanical ventilator support was provided for all cases.

1. Histopathological Findings

Upon macroscopic examination, the lungs of all patients were found to be heavy, congested with patchy involvement. And histological examination revealed features of diffuse alveolar damage (DAD) with exudate in alveolar space and infiltration of inflammatory cells specially monocytes in alveolar space, interstitial infiltration of mononuclear inflammatory cells with deposition of collagenous fibers. These features were also associated with desquamation of epithelial cells in the alveolar lumen with hyperplasia of fibrocytes, infiltration of lymphocytes cells (Figures 1 & 2).

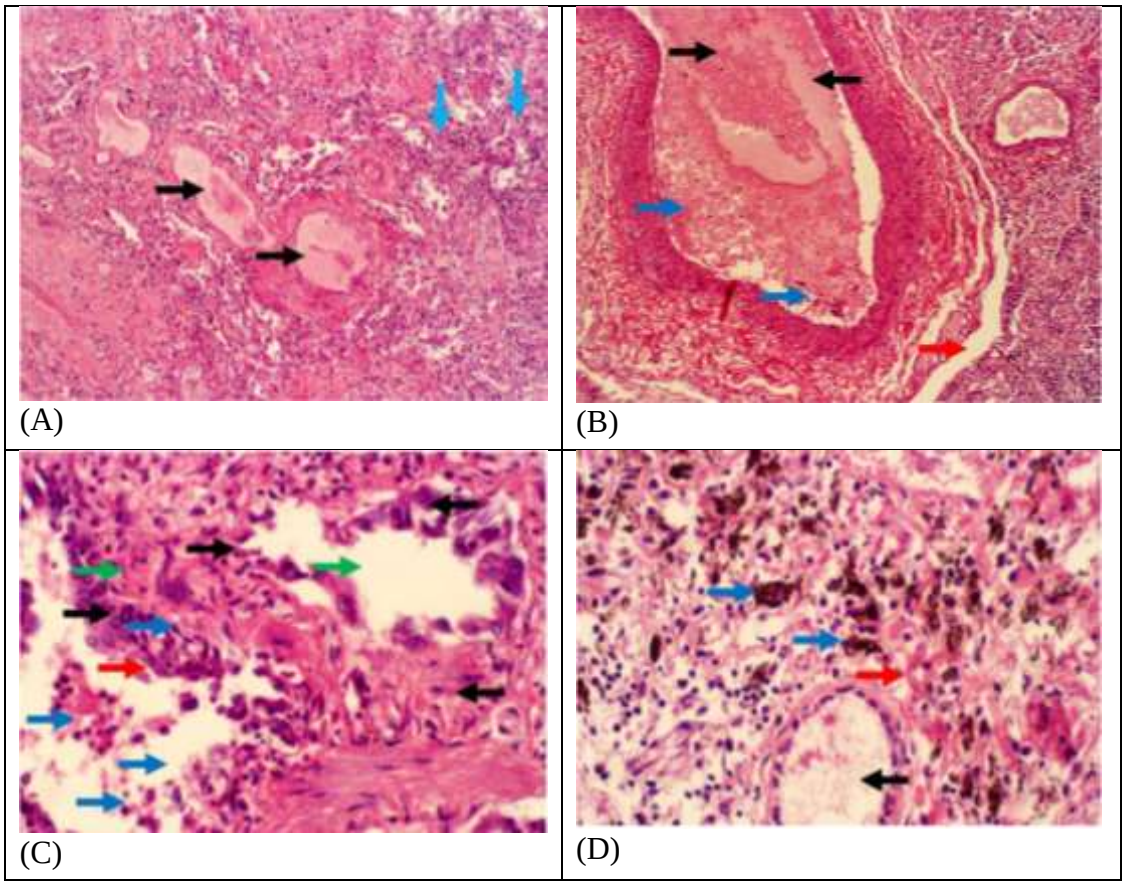

Figure 1: Lung autopsy samples showed intravascular fibrin clot (black arrow), hyperplasia of pneumocytes type II (blue arrow). H&E. 100x (A). Intravascular clot (black arrow), few vascular intraluminal inflammatory cells (blue arrow), perivascular edema (red arrow). H&E. 100x (B). Hyperplasia of pneumocytes type II (black arrow), sloughing and desquamination of necrotic epithelial cells (blue arrow). H&E. 400x (C). Organized vascular thrombi (black arrow), anthracosis lesions (blue arrow), collagen fibers deposition (red arrow). H&E. 400x (D).

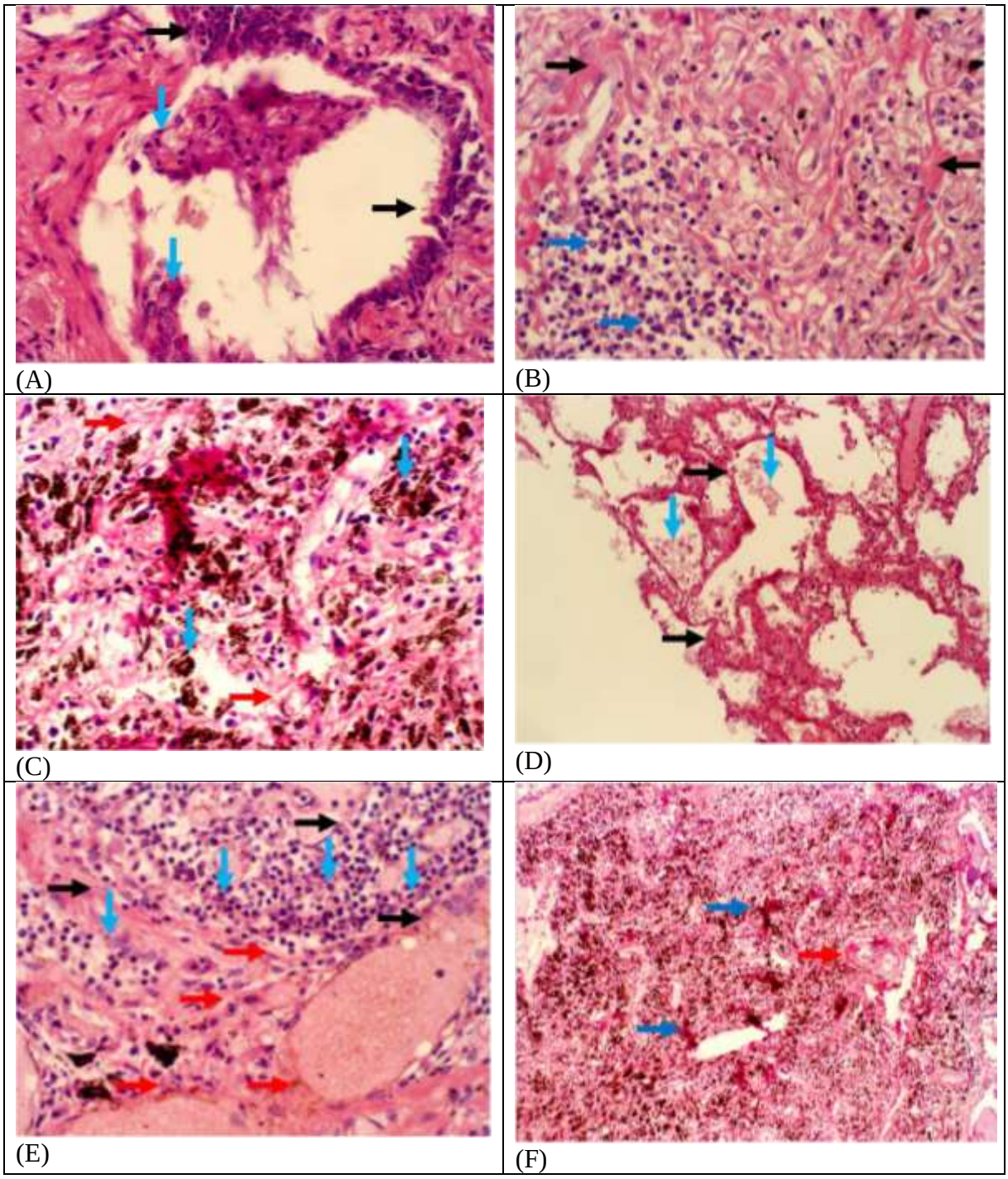

Figure 2: Lung autopsy samples showed hyperplasia of pneumocytes type II (black arrow), sloughing and desquamination of necrotic epithelial cells (blue arrow). H&E. 400x (A). Deposition of fibrous tissue (black arrow), infiltration of inflammatory cells (blue arrow). H&E. 400x (B). multiple anthracosis lesions (blue arrow), collagen fibers deposition (red arrow). H&E. 400x (C) Case 55. Wide spread of necrotic changes in the affected alveoli (Black arrow), with desquamination and sloughing of necrotic cells (Blue arrow). H&E. 100x (D). Deposition of collagen fibers (Black arrow), infiltration of mononuclear inflammatory cells (Blue arrow), hemosiderin pigment (Red arrow). H&E. 400x (E). Showed multiple anthracosis lesions (Blue arrow), collagen fibers deposition (Red arrow). H&E. 100x (F).

2. Immunopathological Findings

The representative immunohistochemistry staining for CD4 T-helper cells, CD8 Cytotoxic T-cells, CD56 in natural killer cells, and CD45RO in memory T-cells are summarized in Figures 3, 4, 5 and 6.

2.1 CD4 Helper T-cells: Lung sections were immuno-stained for CD4 antibody staining, a member of the cluster of differentiation which is mainly expressed on the surface of thymocytes and mature helper T-cells (Figure 3).

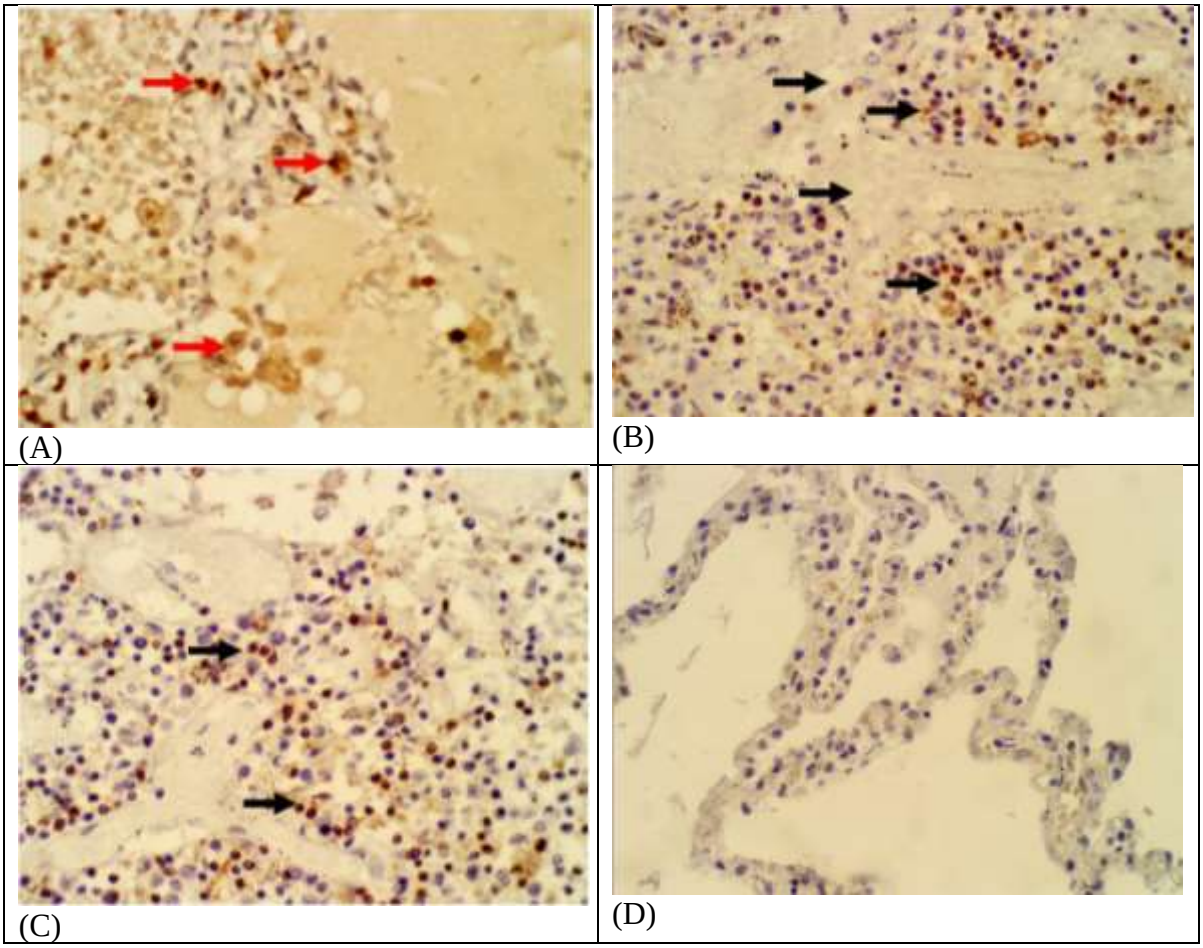

Figure 3: Lung sections were immuno-stained for CD4 antibody staining (400x). The first autopsy sample showed positive staining (Figure 3A), while the second autopsy sample showed weak positive staining (Figure 3B), and the third autopsy sample showed positive staining (Figure 3C) compared to normal tissue (Figure 3D).

2.2 CD8 Cytotoxic T-cells: Lung sections were immuno-stained for CD8 antibody staining (400x), a member of the cluster of differentiation which is mainly expressed on the surface of cytotoxic T-cells (Figure 4).

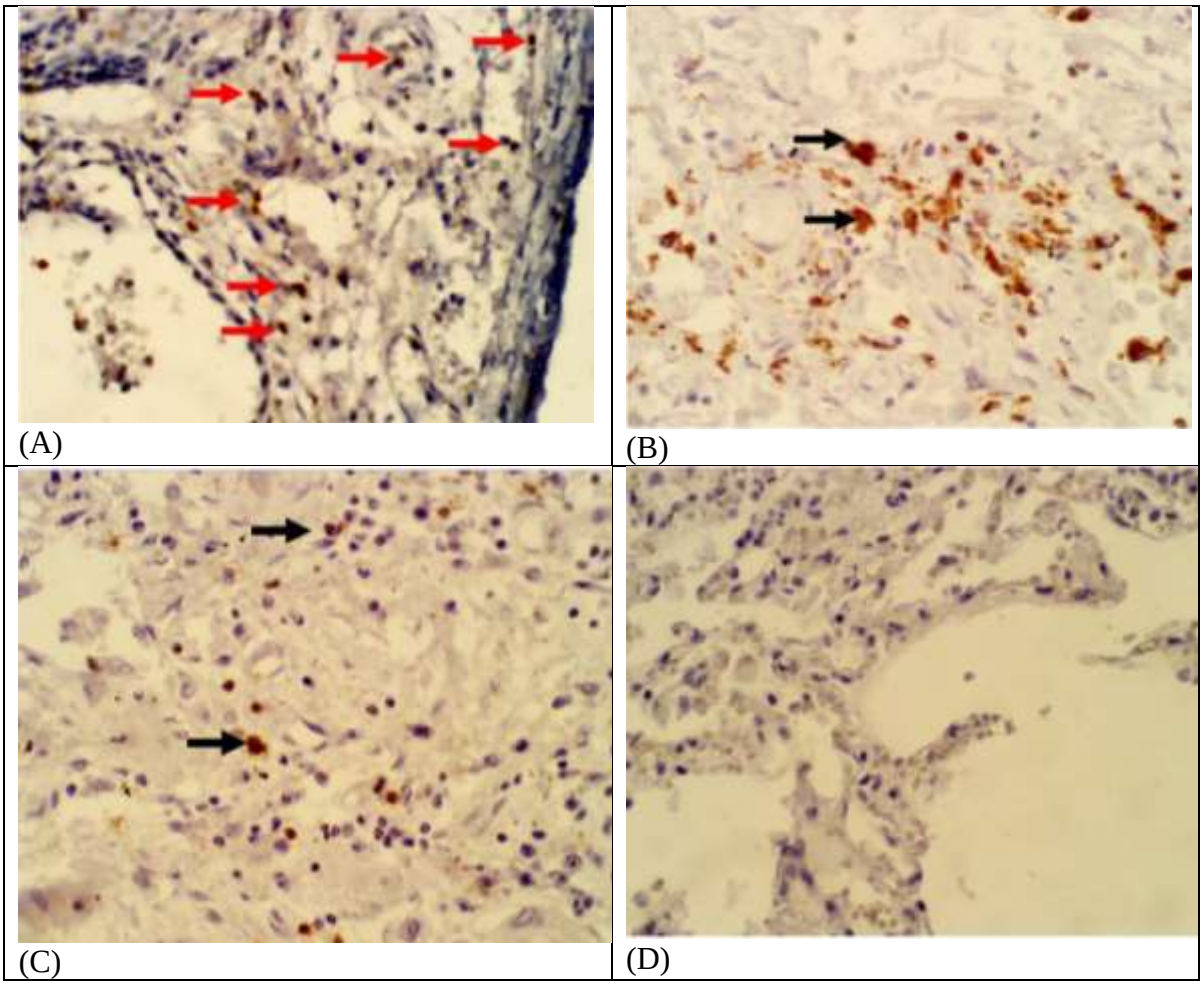

Figure 4: Lung sections were immuno-stained for CD8 antibody staining (400x). The first autopsy sample showed positive intense staining (Figure 4A), while the second autopsy sample showed weak positive intense staining (Figure 4B), and the third case autopsy sample showed weak positive intense staining (Figure 4C) compared to normal tissue (Figure 4D).

2.3 CD56 Natural Killer (NK) Cells: Lung sections were immuno-stained for CD56 antibody staining, a member of the cluster of differentiation which is mainly expressed on the surface of Natural Killer (NK) cells (Figure 5).

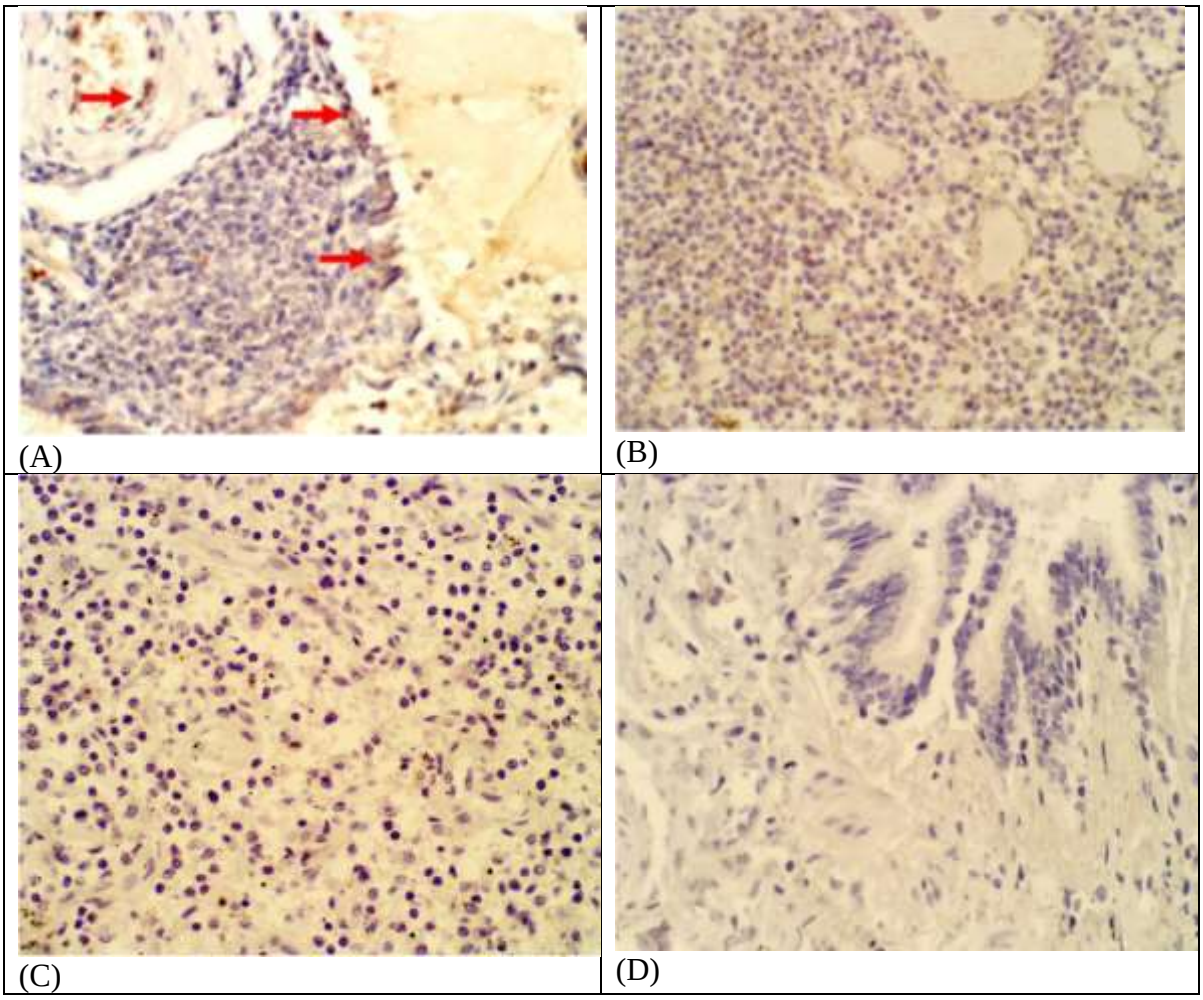

Figure 5: Lung sections were immuno-stained for CD56 antibody staining (400x). The first autopsy sample showed a weak positive intense staining (Figure 5A), while the second and the third autopsy sample showed negative staining (Figure 5B & 5C) compared to normal tissue that showed a negative staining (Figure 5D).

2.4 CD45RO Memory T-cells: Lung sections were immuno-stained for CD45RO antibody staining, a member of the cluster of differentiation which is mainly expressed on the surface of memory T-cells (Figure 6).

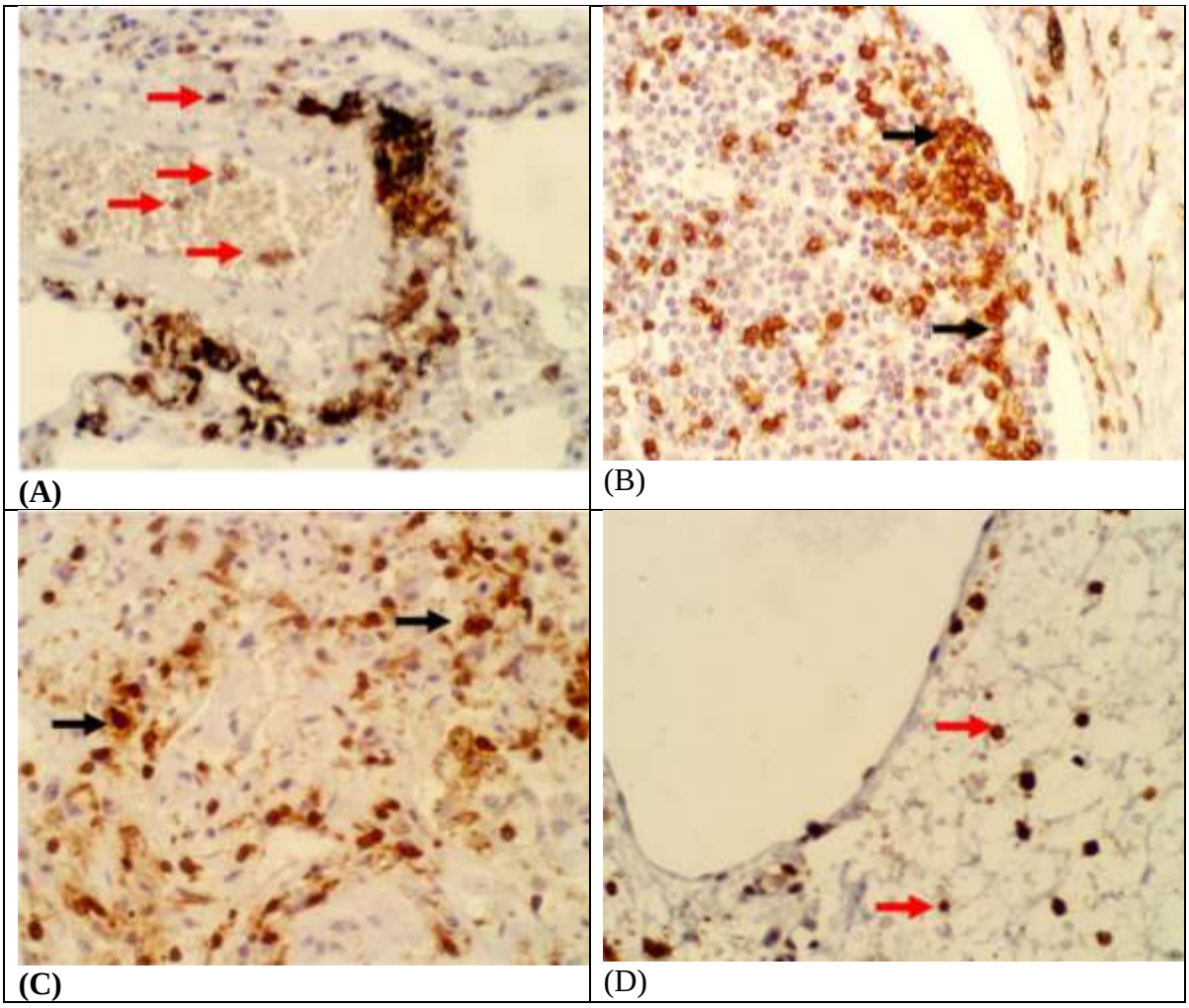

Figure 6: Lung sections were immuno-stained for CD45RO antibody staining (400x). The first autopsy sample showed weak positive staining (Figure 6A), while the second autopsy sample showed strong positive staining (Figure 6B), and the third case autopsy sample showed positive staining (Figure 6C) compared to normal tissue that showed a weak positive staining (Figure 6D).

Discussion

The postmortem autopsy samples from patients with fatal COVID-19 in the few published research has showed differences in pulmonary responses to the infection that were associated with viral load, different immune response and different duration for the clinical illness before death [12]. Histopathological observation indicated that the COVID-19 virus has a devastative cytopathic effect (Figures 1 and 2) which results from the immune responses, including progressive diffuse alveolar damage with sloughing and desquamination of the necrotic epithelial cells, wide spread necrotic changes in the affected alveoli, deposition of fibrous tissues and collagen fibers with infiltration of inflammatory cells with organized vascular thrombi and intra vascular fibrin clot. All these findings are comparable to different studies done by different researchers [8, 13, 14].

SARS-CoV-2 stimulates a severe antiviral immune response and, therefore, we analyzed presence of specific immune cells in the lung autopsy samples. In our study, T-cells ($CD3^+$) of the $CD4^+$ lineages showed positive staining compared with the control section (Figure 3). Enriched infiltration of $CD4^+$ in the lung of autopsy lung samples was reported by Valaebenito

and his team [15]. Also, Carsana and his colleagues reported increased infiltration of $CD4^+$ to the lung [16].

T-cells ($CD3^+$) of the $CD8^+$ lineages lymphocyte showed a weak positivity in 2 cases (Figure 4) which is comparable to the study done by Yang Li and his colleague in 2020 that showed that lymphocytes particularly CD8+ T-cells significantly decreased in severe cases compared with mild cases [17]. Accumulation of CD8 cells in the lung gradually leads to an increase in the disease outcome in other lung viral infections [18]. CD4 helper T-cells are stimulated to secrete Th1 cytokine Interleukin-12 (IL-12) and IFN- interferon-γ (IFN-γ), and this activation subsequently leads to $CD8^+$ cytotoxic T-cells activation which may also get activated through the direct binding with MHC class I on the infected cells. The absence of a robust T-cell response (CD3 and CD8) in all patients under analysis is crucial since it points to a possible systemic immunological malfunction. In agreement, a T-cell lymphopenia has been seen in the blood in certain severe COVID-19 cases compared to those who are not infected [19, 20]. Within the first few days of viral infection, $CD3^-CD56^+$ cells, which are commonly referred to as human NK cells, rapidly increase in the lungs [18]. By the generation of IFN-γ, the activation of adaptive immune cells, antibody dependent cell cytotoxicity (ADCC), and cytotoxic lysis, activated lung NK cells which then aid in the removal of the virus. However, many research [21] indicate a production of IFN-γ at high-dose induce immunopathology in lungs infected with virus [22]. $CD3^-CD56^+$ cells in the lung autopsy samples showed a weak to negative infiltration in the lung section compared to the control section (Figure. 5). NK cells are triggered during viral infections by infected cells through contact-dependent processes [21] and by cytokines such as IL-15, IL-12, IL-2, and type I IFN which are produced by infected cells and perhaps other cell types [22, 23]. CD45RO is a memory T-cells that plays a role in the immunopathogenesis of lung. Our results showed a positive to strong positive staining compared with the weak positive control section (Figure 6). The first set of COVID-19 research on systemic blood cells indicated low percentage of CD45RO+ memory cells compared with CD45RA+ naïve T-cells in severe cases [12] and these results are comparable to our results, although our findings were in the lung tissues itself and not systemic, as we couldn't find any similar research regarding the CD45RO+ memory cells in lung tissue [23]. It can be concluded that following infection with virus monocyte, macrophage, DS, fibroblast, epithelial cell and other cells secrete interleukins, in particular IL-15, which stimulates CD8 T-cell and natural killer (NK) cell growth, proliferation, and cytolytic activity and increases the expression of anti-apoptotic and decreases the expression of pro-apoptotic factors to prevent apoptosis [24]. This cytokine keeps the balance between better immune response and modulation in the disease conditions. When the concentrations of IL-15 increased with a normal level this probably decreased the danger of infection, but when the concentration increased dramatically the function of this cytokine reversed and the proliferation and the cytolytic activity of CD8 and NK cells decreased, and apoptosis of these cell increased which is probably the reason behind a decrease in tissue CD8 T cells and tissue NK cells in our study. What approves our conclusions is a research done by Perpiñan and his colleague who concluded that SARS CoV-2 patients with high levels of IL-15 were at a high risk (increased 2.7 times $p = 0.048$, IC 95% = 1.008–7.710) for suffering more severe COVID-19 symptoms and would more probably require invasive ventilation [25]. The removal of smooth muscle cells, endothelial cells and pneumocytes, as well as increased fibroblast growth, highlight the destructive nature of this virus. Moreover, our data showed severe alveolar wall destruction and loss of lung function unlike any other known disease.

Limitations: The main limitation of our research study was the small sample size. All COVID-19 related deaths, according to the Iraq-Kurdistan Region MOH policy, were to be disposed

immediately without further investigations except the 3 cases that we could obtain as they went under further forensic investigations as required by the medico-Legal institution of Erbil.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

[1] H. Y. Fadhil and T. A. Abed Alhussien, "Analysis of Mutations in Conserved and Susceptible Regions Across the Whole Genome Sequencing Analysis for SARS-CoV-2 in Iraqi Patients " *Iraqi Journal of Science*, vol. 64, no. 1, pp. pp: 56-64, Jan, 2023.

[2] M. Vanelli, D. Vanelli, and M. Vanelli, "WHO declares COVID–19 a pandemic," *Acta Biomed: Atenei Parmensis*, vol. 91, pp. 157-60, Mar. 2020.

[3] W. H. Organization, ""“WHO Corona virus (COVID-19) Dashboard” Retrieved from https://covid19.who.int/?mapFilter=deaths " [Accessed Feb, 2023].

[4] A. C. Borczuk *et al.*, "COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Italy and New York City," *Modern Pathology*, vol. 33, no. 11, pp. 2156-2168, Nov. 2020.

[5] M. K. Ismael, A. H. Mohammed, M. A. H. Aldabagh, and L. M. Rasuol, "Matrix Metalloproteinase-3 and Tissue inhibitor of metalloproteinase-2 as Diagnostic Markers for COVID-19 Infection " *Iraqi Journal of Science* vol. 63, no. 9, pp. 3679-3687, Sep. 2022.

[6] H. Zhang, J. M. Penninger, Y. Li, N. Zhong, and A. S. Slutsky, "Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target," *Intensive care medicine*, vol. 46, pp. 586-590, Apr. 2020.

[7] J. L. Sauter *et al.*, "Insights into pathogenesis of fatal COVID-19 pneumonia from histopathology with immunohistochemical and viral RNA studies," *Histopathology*, vol. 77, no. 6, pp. 915-925, Dec. 2020.

[8] F. D’Agnillo *et al.*, "Lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19," *Science Translational Medicine*, vol. 13, no. 620, p. eabj7790, Nov. 2021.

[9] A. M. H. Al-Bayati, A. H. Alwan, and H. Y. Fadhil, "Potential Role of TLR3 and RIG-I Genes Expression in Surviving COVID- 19 Patients with Different Severity of Infection " *Iraqi Journal of Science*, vol. 63, no. 7, pp. 2873-2883, July. 2022.

[10] N. Chen *et al.*, "Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study," *The lancet*, vol. 395, no. 10223, pp. 507-513, Feb. 2020.

[11] A. Romano *et al.*, "In-vitro NET-osis induced by COVID-19 sera is associated to severe clinical course in not vaccinated patients and immune-dysregulation in breakthrough infection," *Scientific Reports* vol. 12, no. 1, p. 7237, May. 2022.

[12] L. M. Khosroshahi, M. Rokni, T. Mokhtari, and F. Noorbakhsh, "Immunology, immunopathogenesis and immunotherapeutics of COVID-19; an overview," *International immunopharmacology*, vol. 93, p. 107364, Apr. 2021.

[13] A. Gheware *et al.*, "ACE2 protein expression in lung tissues of severe COVID-19 infection," *Scientific Reports*, vol. 12, no. 1, p. 4058, Mar. 2022.

[14] R. Nienhold *et al.*, "Two distinct immunopathological profiles in autopsy lungs of COVID-19," *Nature communications* vol. 11, no. 1, p. 5086, Oct. 2020.

[15] S. Valdebenito *et al.*, "COVID-19 lung pathogenesis in SARS-CoV-2 autopsy cases," *Frontiers in immunology*, p. 3900, Oct. 2021.

[16] L. Carsana *et al.*, "Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study," *The Lancet infectious diseases* vol. 20, no. 10, pp. 1135-1140, Oct. 2020.

[17] L. Yang *et al.*, "COVID-19: immunopathogenesis and Immunotherapeutics," *Signal transduction and targeted therapy*, vol. 5, no. 1, p. 128, July. 2020.

[18] N. P. Goplen *et al.*, "Tissue-resident CD8+ T cells drive age-associated chronic lung sequelae after viral pneumonia," *Science immunology*, vol. 5, no. 53, p. eabc4557, Nov. 2020.

[19] L.-l. Cheng *et al.*, "Effect of recombinant human granulocyte colony–stimulating factor for patients with coronavirus disease 2019 (COVID-19) and lymphopenia: a randomized clinical trial," *JAMA internal medicine*, vol. 181, no. 1, pp. 71-78, Jan. 2021.

[20] Y. Zhang *et al.*, "Potential contribution of increased soluble IL-2R to lymphopenia in COVID-19 patients," *Cellular & molecular immunology*, vol. 17, no. 8, pp. 878-880, Aug. 2020.
[21] X. Xu *et al.*, "Conventional NK cells can produce IL-22 and promote host defense in Klebsiella pneumoniae pneumonia," *The Journal of Immunology*, vol. 192, no. 4, pp. 1778-1786, Feb. 2014.
[22] J. Cong and H. Wei, "Natural killer cells in the lungs," *Frontiers in immunology*, vol. 10, p. 1416, June. 2019.
[23] C. Qin, M. P. L. Z. M. Ziwei, S. Y. M. Y. Tao, P. C. X. M. P. Ke, and M. M. P. K. Shang, "Dysregulation of immune response in patients with COVID-19 in Wuhan, China; clinical infectious diseases; Oxford academic," *Clinical Infectious Diseases*, 71(15): 762-768, Jul. 2020.
[24] M. Patidar, N. Yadav, S. K. J. C. Dalai, and g. f. reviews, "Interleukin 15: A key cytokine for immunotherapy," *Cytokine & growth factor reviews* vol. 31, pp. 49-59, Oct. 2016.
[25] C. Perpiñan *et al.*, "Resistin and IL-15 as Predictors of Invasive Mechanical Ventilation in COVID-19 Pneumonia Irrespective of the Presence of Obesity and Metabolic Syndrome," *Journal of Personalized Medicine*, vol. 12, no. 3, p. 391, Mar. 2022.