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Pathogenesis of COVID-19; Acute Auto-inflammatory Disease (Endotheliopathica & Leukocytoclastica COVIDicus)

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Abstract
Background: The pathogenesis of the COVID19 pandemic, that has killed one million nine hundred people and infected more the 90 million until end of 2020, has been studied by many researchers. Here, we try to explain its biological behavior based on our recent autopsy information and review of literature.
Methods: In this study, patients with a positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) result were considered eligible for enrollment. Histopathological examinations were done on 13 people who were hospitalized in Afzalipour hospital, Kerman, Iran. Clinical and laboratory data were reviewed. Tissue examination was done by light microscopy, immunohistochemistry and electron microscopy.
Results: The most frequent co-morbidity in the patients was cardiovascular disease. The common initial symptoms of COVID-19 infection were dyspnea and cough. In all cases, the number of white blood cells was higher than the normal range. Common histopathological findings were variable degrees of vasculitis as degenerative to necrotic changes of endothelium and trafficking of inflammatory cells in the vessel wall with fibrinoid necrosis. Tissue damage included interstitial acute inflammatory cells reaction with degenerative to necrotic changes of the parenchymal cells. CD34 and Factor VIII immunohistochemistry staining showed endothelial cell degeneration to necrosis at the vessel wall and infiltration by inflammatory cells. Electron microscopic features confirmed the degenerative damages in the endothelial cells.
Conclusion: Our histopathological studies suggest that the main focus of the viral damage is the endothelial cells (endotheliopathica) in involved organs. Also, our findings suggest that degeneration of leukocytes occurs at the site of inflammation and release of cytokines (leukocytoclastica) resulting in a cytokine storm.
Keywords: Coronavirus, COVID-19, Endothelial cells, Leukocytoclastic, Pathology, Pneumonia
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Introduction
Influenza is an acute, highly contagious viral infection. Viral proliferation occurs in the upper respiratory tract and trachea. In most patients, the illness is benign, but it may be complicated by (a) primary influenza viral pneumonia; (b) secondary bacterial pneumonia; (c) combined influenza viral pneumonia and bacterial pneumonia. In several pandemic years of emergence (1918, 1947 and 1968), the virus emerged as a mutant zoonotic variant most likely acquired from animals (swine, birds).[1] COVID-19 is a different virus that often produces an influenza-like illness. The COVID-19 RNA coronavirus is thought to be a mutated form of a similar virus acquired from bats.[2] The earliest cases of novel coronavirus (2019-nCoV)-infected pneumonia (NCIP) occurred in Wuhan, Hubei Province, China and were linked demographically to Huanan Seafood Wholesale Market.[2] Five years prior to this pandemic, another group identified a severe acute respiratory syndrome (SARS)-like virus, SHC014-CoV circulating in Chinese horseshoe bat populations as a potential risk for SARS-CoV human outbreak.[3] These two viruses were characterized to be very similar to the whole-genome level of the bat coronavirus relying on the same cell entry receptor-angiotensin converting enzyme II (ACE2).[4,5]

Clinically, COVID-19-induced viral pneumonia

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resembles that of the other coronaviruses that produced Middle East respiratory syndrome (MERS) and SARS.[2] COVID-19 infection causes dry cough, fever, and myalgia. In complicated cases, after several days, the patients may develop severe dyspnea and rarely, respiratory failure.[6] Knowledge of the pathological findings of cases with coronavirus-19 is relatively limited. The first case report came from two cancer patients in China.[7] These tumor resections were completed prior to any knowledge of the COVID-19 superimposed infection. Histopathological findings from these two cases were non-specific and included edema, focal inflammation, proteinaceous exudate with granules, and hyperplastic pneumocytes with suspected viral inclusions.[7]

It is important to note that when the cause of death is the result of COVID-19 infection, the current recommendation for autopsy dissection is a staged technique limited to only retrieving samples to verify COVID-19 infection.[8] This restriction is one of the main contributing factors to limited publications of COVID-19 related histopathological findings. Here, we report the pathological findings in ten patients who died of COVID-19 infection in Iran and two patients who developed skin rashes after recovery from COVID-19 and one case with pericarditis.

Materials and Methods

The study population consisted of thirteen patients hospitalized in the intensive care unit (ICU) of Afzalipour hospital, Kerman, south-eastern Iran, between April and June 2020. Patients were included in the study after confirmation of COVID-19 by reverse transcription-polymerase chain reaction (RT-PCR) assay for virus detection. Clinical parameters were recorded including age, sex, personal history of previous diseases, hematological and biochemical parameters, and also radiographic findings. After the declaration of death in eleven cases, autopsies were done by utilizing an automatic 14-gauge core biopsy needle. Visceral organs including the lungs and livers were preserved in 10% neutral buffered formalin solution and the multiple serial sections were stained with hematoxylin and eosin. Histopathological assessment was done separately by two pathologists under a light microscope. Skin rashes were also biopsied from two patients with COVID-19 who recovered after one month. To recognize endothelial cells, immunohistochemical stains were performed for CD34 (Diagnostic BioSystems, USA, Lot number: E122 and ready to use) and Factor VIII (ScyTek Laboratories, USA, Lot number: 49570 and ready to use) according to manufacturers' protocols. Also, for identifying macrophages in tissue sections, CD68 (Diagnostic BioSystems, USA, Lot number: N371-QD and ready to use) was used. CD4 (Lot number: 503-17680), CD8 (Lot number: BRB036), and CD20 (Lot number: BMS003) (ZYTOMED, Germany, and ready to use) IHC staining was used to detect the immune cells.

Tissue slices were fixed in 2.5% glutaraldehyde, 4% paraformaldehyde in 90Mm sodium cacodylate buffer with 0.02 mM $CaCl_2$ added and pH adjusted to 7.2–7.4. The specimens were put in the fixative at 5°C for 24 hours. After washing with sodium cacodylate buffer, the slices were washed in distilled water for 20 min. The slices were then dehydrated through a graded series of acetone and embedded. Ultrathin sections (50–60 nm) were cut with a Leica Ultramicrotome, mounted on copper grids and examined with a transmission electron microscope ZIESS EM900 based on standard protocols.

Results

Patient characteristics, comorbidities, initial symptoms and radiographic findings are listed in Table 1. Seven patients were men and six were women. The median age of patients was 54 years (range 43–83). All thirteen patients suffered from a comorbidity. The most frequent initial symptom in those with cardiovascular disease was dyspnea. Weakness and cough were seen in some patients. Except for three cases, all other patients showed severe damage in lung radiographic findings (Table 1). Changes in hematological and biochemical parameters in the study group are shown in Table 2. All laboratory analytes were interpreted according to age and gender reference range. In all cases, the number of white blood cells was higher than the normal range. Elevated urea was seen in most of the patients and some of the patients suffered from lower platelets and hemoglobin.

Histopathological findings of the lung were emphysematous lung parenchyma along with interstitial pneumonitis and filling of alveolar spaces by collections of desquamated cells and inflammatory cells. Emphysematous alveoli along with inflammatory cells present in the interstitial alveolar septa, interstitial edema and early fibrosis were seen (Figure 1A). Degenerated alveolar epithelial cells, alveolar macrophages, and endothelial cells of vessels were noted. Degenerated and necrotic alveolar epithelial cells of type II and alveolar macrophages were identified in exfoliated floating cells within the alveolar spaces (Figure 1B). Degenerated alveolar epithelial cells with possible viral intracytoplasmic inclusion bodies were noted together with necrotic exfoliated epithelial cells and loss of cohesion and detachment of the epithelial lining of alveolar septa, leaving just a few fibroblasts. The inflammatory cell infiltrate was within interstitial spaces confirmed by CD68 IHC staining (Figure 1C) and also around amorphous collections of eosinophilic material.

In some areas, no remnants of alveolar epithelial cells were seen, and the alveolar septa were occupied by inflammatory cells only. Hyaline material deposits lining the alveoli were noted. Focally, there was eruption of surfactant eosinophilic material associated with hydropic degeneration of alveolar epithelial cells and variable collapse or ecstatic changes of the lymphovascular tree. The vascular changes could be seen upon IHC staining for Factor VIII and CD34. There were degenerative changes and disintegration of the endothelial cells (Figures 1D and

Table 1. Patient Characteristics, Comorbidities, Symptoms, Radiographic Findings, and COVID19 RT PCR

	Age (y)	Sex	Comorbidities	Initial Symptoms	Radiographic Findings	COVID19 RT PCR
1	70	Male	Parkinson	Weakness, dyspnea, flu like symptoms	Negative	RNA detected
2	48	Male	Heart failure, gastrointestinal malignancy, methadone addict	Weakness, vomiting, loss of consciousness, abdominal pain, lower limb edema	Left hemi thorax pleural effusion	RNA detected
3	43	Male	Hypertension	Dyspnea, cough	Ground glass opacity	RNA detected
4	45	Female	Hypertension, breast cancer	Fever, dyspnea, cough	Ground glass opacity	RNA detected
5	83	Female	Smoker, air way disease	Dyspnea, loss of consciousness	Ground glass opacity	RNA detected
6	52	Female	End stage renal disease	Fever, headache	Ground glass bilateral opacity	RNA detected
7	72	Male	Diabetes mellitus, ischemic heart disease, chronic kidney disease	Dyspnea, generalized weakness	Ground glass opacity	RNA detected
8	83	Female	Hypertension, diabetes mellitus, core pulmonary disease	Dyspnea, cough	Ground glass opacity	RNA detected
9	61	Female	Diabetes mellitus	Dyspnea, myalgia	Ground glass opacity	RNA detected
10	48	Male	Chronic kidney disease, Hypertension, Epilepsy, burger disease, methadone and heroin addiction	Dyspnea	Ground glass opacity	RNA detected
11	52	Male	Diabetes mellitus, Hypertension, Left bundle branch block	Dyspnea	Ground glass opacity	RNA detected
12	61	Male	Hypertension, core pulmonary disease	Lower limb edema	—	RNA detected
13	54	Female	Diabetes mellitus	Pustular skin lesion on the lower limb	—	RNA detected

Table 2. Hematological and Biochemical Parameters in the Study Group

	Elevated WBC	Lower RBC	Lower Hb	Lower Platelet	Elevated Urea	Elevated Creatinine
1	Yes	No	No	No	Yes	No
2	Yes	Yes	Yes	Yes	Yes	No
3	Yes	Yes	Yes	Yes	No	No
4	Yes	No	Yes	No	Yes	Yes
5	Yes	Yes	Yes	No	Yes	Yes
6	Yes	Yes	Yes	No	Yes	Yes
7	Yes	No	No	Yes	No	No
8	Yes	No	No	Yes	Yes	Yes
9	Yes	Yes	Yes	Yes	Yes	No
10	Yes	No	No	Yes	Yes	Yes
11	Yes	No	Yes	No	Yes	Yes
12	Yes	Yes	No	Yes	Yes	No
13	Yes	No	Yes	No	No	No

WBC, White blood cell; RBC, Red blood cell; Hb, hemoglobin.

1E) and even collapse of the vessels and neovascularization (Figure 1F). They might induce chemotactic effects for fibroplasia growth factor and interstitial fibrosis. Early reparative changes including fibroplasia with plump reactive fibroblasts were also present.

The liver showed fatty and/or hydropic degeneration along with congestion of sinusoids and/or congestion of space of Disse, Kupffer cell hyperplasia and prominence of endothelial cells (Figures 2A and 2B). This was also confirmed by electron microscopy findings (Figure 2C).

Clinically, the skin lesions were bullous lichen planus and vasculitis involving the lower limbs. Microscopic findings showed leukocytoclastic vasculitis and swelling of endothelial cells and pericytes together with prominence of nucleoli (Figures 3A and 3B).

The patient with COVID pneumonia and pericarditis had a biopsy of pericardial lesion and showed the same pattern in other biopsies such as: edematous, congested stroma with plumped degenerated to necrotic endothelial cells with leukocytoclastic changes in the stroma and
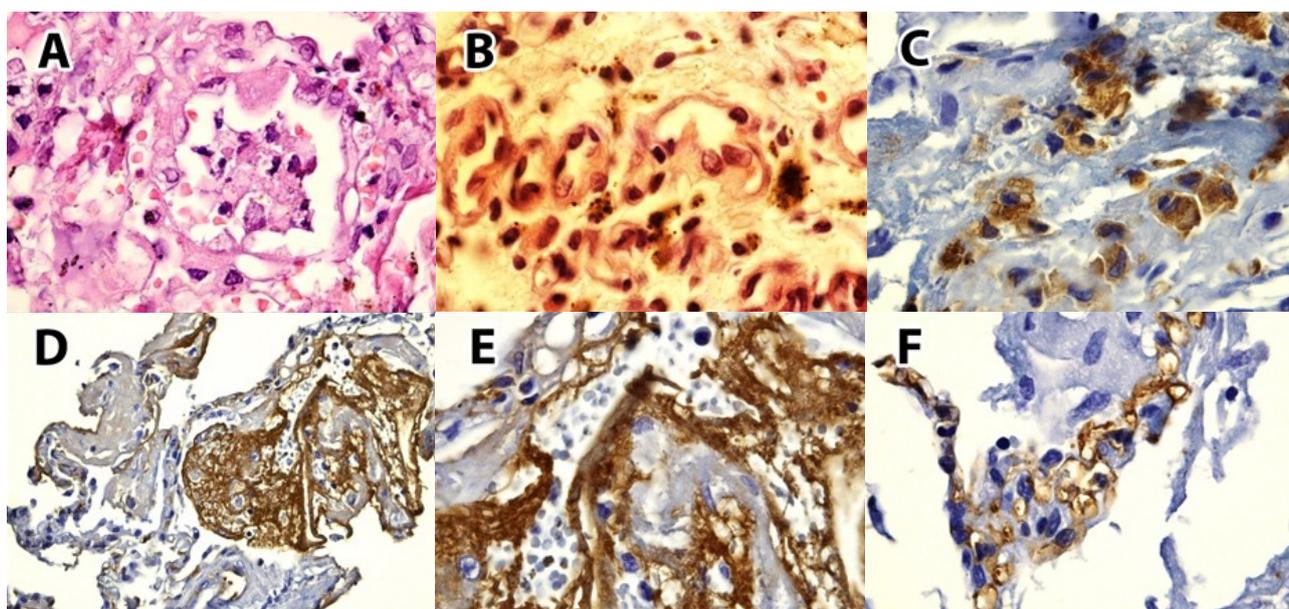
Figure 1. Light Micrographs of Lung Sections. **(A)** Emphysematous alveoli with degenerated to necrotic alveolar cells and macrophages, congestion of vessels, degenerated endothelial cells and acute inflammatory cells infiltrate; Hematoxylin & Eosin, magnification ×400. **(B)** Collapse of alveoli and plumped endothelial cells and trafficking of neutrophils and macrophages; Hematoxylin & Eosin, magnification ×1000. **(C)** Proliferation and aggregation of the macrophages at the site of inflammation by CD68 immunohistochemistry staining, magnification ×1000. **(D & E)** Endothelial cell degeneration to necrosis at the vessels wall and infiltration by inflammatory cells, Factor VIII immunohistochemistry staining, magnification ×100 and ×400, respectively. **(F)** Spectrum of vascular collapse to others with plumped endothelial cells; CD34 immunohistochemistry staining, magnification ×200.

around the blood vessels.

Tissue stains negative for the T cell marker CD4 and B cell marker CD20 and also CD8 were scattered rarely positive.

Discussion

Since COVID-19 appeared in Wuhan, China in December 2019, the virus has spread rapidly to many countries around the world, and increased global mortality. Various studies have shown that severe SARS-CoV-2 infection is more commonly observed in people of any age with certain underlying medical conditions, including cancer, chronic kidney disease, immunocompromised state, obesity, heart failure, coronary artery disease and type 2 diabetes mellitus, yet the exact mechanism of these co morbidities and cause of severe disease in patients remains unclear.[9,10] Most infected people will develop mild to moderate sickness with some clinical symptoms such as fever, cough, headache, tiredness, and sore throat and recover with home isolation, while some patients with underlying comorbidities such as hypertension, diabetes and cardiovascular diseases will die.[11]

Similar to other coronaviruses, SARS-COV-2 enters the body through the upper respiratory tract and infects epithelial cells within the lung. Also, other potential sites of damage, including kidney, heart and endothelial cells, have been proposed.[12]

The postulated mechanism of damage is thought to be mediated via ACE2, a transmembrane receptor that has an extracellular domain to which the SARS-CoV-2 binds

and infects the host cell. The ACE2 protein is expressed in alveolar epithelial cells, small intestine enterocytes, heart and kidneys.[13-15]

At first, SARS-CoV-2 binds to ACE2 receptor and enters the epithelial and endothelial cells, then neutrophils accumulate and undergo degranulation. Next, platelets are activated by the extracellular DNA of neutrophils and finally, thrombus formation will occur by activated platelets and erythrocytes.[16]

Sialic acid as a family of derivatives of neuraminic acid is found widely distributed in human cells. Brain cells, endothelial cells in the blood vessels, epithelial cells of the lungs and respiratory tract heavily express sialic acid residues.[17] Huertas et al showed that SARS-COV-2 interact with the cell surface attachment factors such as sialic acid residues, transmembrane serine protease 2 (TMPRSS2) and extracellular matrix metalloproteinase inducer (CD147) and causes cell swelling, disruption of intercellular junctions, increased vascular permeability and finally, pulmonary endothelial cell dysfunction.[18] Increased pro-inflammatory responses, complement activation, thrombin production and reduced nitric oxide production can lead to development of endotheliitis.[19,20]

The histological changes in COVID-19 infection have been little studied. The autopsy of a man in his 50s who died with severe COVID-19 infection revealed specific histopathological findings.[21] These include microscopic findings of diffuse alveolar damage with fibrinous exudate. However, there was no definitive testing for viral inclusions. Many of the histopathological findings of this
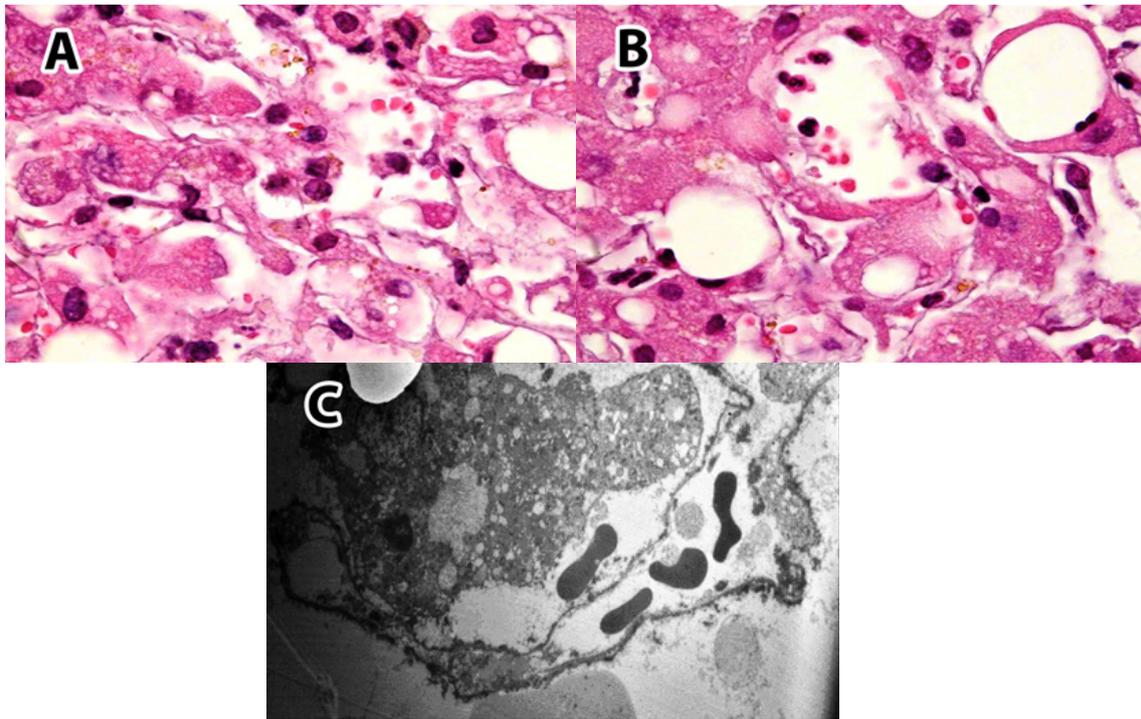
Figure 2. Liver Biopsies. **(A & B)** Degenerated hepatocytes along with degenerated to necrotic endothelial cells, congestion of space of Disse, and neutrophil and macrophage activity; Hematoxylin & Eosin, magnification ×1000 and ×400, respectively. **(C)** Congested space of Disse along with degenerated endothelial cells and also the hepatocytes by electron microscopic findings, magnification ×1250.

case were similar to those seen in patients suffering from SARS and MERS coronavirus infection.

Zhao et al described the pathology of SARS pneumonia as inflammatory reparative changes in terminal bronchioles and alveoli with desquamation of the alveolar cells, and immune-mediated injury cellular responses.[22] Our histopathological findings are similar to those of Yao et al as alveolar damage was noted together with formation of hyaline membranes.[23]

Hyaline membrane structures were also found in SARS pneumonia in an earlier study by Lai et al.[24] In their study, they also noted virus-like inclusions in the cytoplasm of the alveolar epithelium and mononuclear macrophages, confirmed by IHC staining. We also found probable intracytoplasmic viral inclusions in the alveolar macrophages and/or alveolar epithelial cells. Dan et al mentioned in their recent, very sophisticated and interesting paper on immune response in patients who recovered of acute COVID after 3-6 months, that cellular and humoral immune response interact with the virus components in very details and strange ways to activate the immune cells and their products.[25] In this regard, we mentioned that immune activation was not so obvious in the acute phase of this disease.

We found the collapse of lymphatic and/or ecstatic changes of lymphatics vessels, that together with the alveolar exudates, may explain the radiographic findings of "ground glass" changes in the peripheral, basilar and/ or peri-pleural areas of the lungs. Yoon et al described the radiographic findings of early COVID-19 pneumonia in
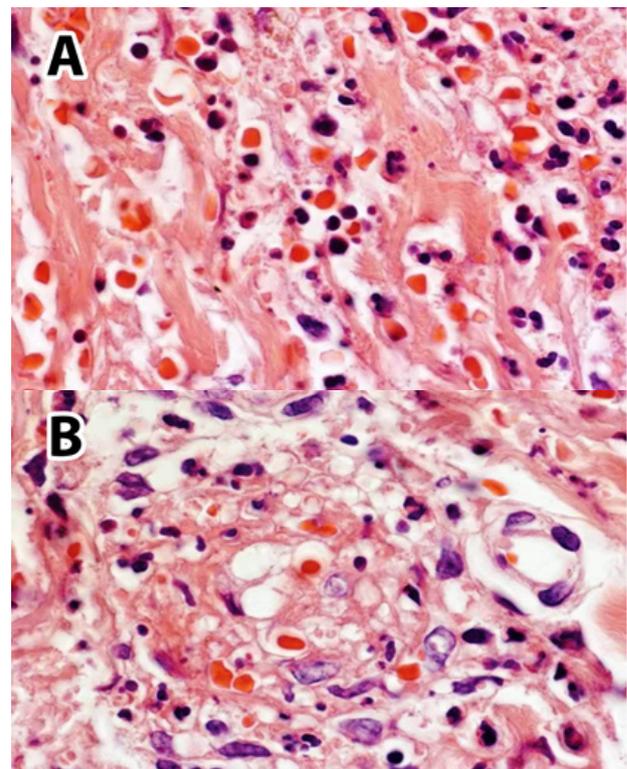

Figure 3. Skin Biopsies. **(A)** Edema, congested dermal stroma and diffuse neutrophils and macrophages infiltrate by karyorrhexis in the vessel wall and stroma (Leukocytoclastic vasculitis); Hematoxylin & Eosin, magnification ×1000. **(B)**. Endothelial cells plumping and degeneration was noted; Hematoxylin & Eosin, magnification ×400.

Korea.[26] CT-scans showed pure to mixed ground-glass opacities with a patchy to confluent or nodular shape in the bilateral peripheral posterior lungs.

Based on the findings of the present study and review of the literature, we could confirm the spectrum of histopathological changes in lung injury which include epithelial, vascular and fibrotic patterns in autopsy specimen in patients who died after SARS-CoV-2 (COVID-19) infection. The results of the present study, in accordance with other studies[27-29] show that endothelial dysfunction, disrupting vascular homeostasis and endotheliitis which cause thrombus formation, likely plays a central role in the development of COVID-19 related pulmonary injury. However, direct viral damage to the alveolar lining cells most likely also occurs.

The major histopathological features of the COVID-19 infection in the lung include diffuse alveolar damage, pulmonary edema, alveolar septal thickening, pulmonary hemorrhage, and aggregation of neutrophils and lymphocytes. These findings are similar to those seen in SARS and MERS coronavirus infection. The histopathological findings differ between patients according to the severity of the disease and the interval between the onset of symptoms and death.

Based on our findings in the lung, liver and skin, damage to the endothelial cells may also be the primary insult outside of the lung that triggers a cascade of other cell injuries. Screening patients at the time of admission and therapeutic management of endothelial injury causing microthrombi particularly with anti-inflammatory anti-cytokine drugs, ACE inhibitors, and statins can be vital for the patients.

Key Messages/Learning Points

From the histogenesis point of view, our case report findings suggest the following learning points: (1) It appears that even in young patients with limited comorbidity, the virus can travel from the upper airways protective barriers of ciliated respiratory to the alveolar epithelial cells where it can replicate. (2) At the peripheral and base of the lungs, the virus appears to be present in the alveoli and/or macrophages. (3) The COVID-19 infection can destroy lung parenchyma and create severe respiratory distress syndrome while using both innate and adaptive immunity to new stranger COVID19 viruses. (4) COVID-19 also results in the collapse of blind loop pulmonary vascular and lymphatic vessels. (5) Finally, viremia causes disseminated endotheliopathy in involved organs. This causes acute inflammation and leukocytoclastic changes and creates a cytokine storm (Figure 4).
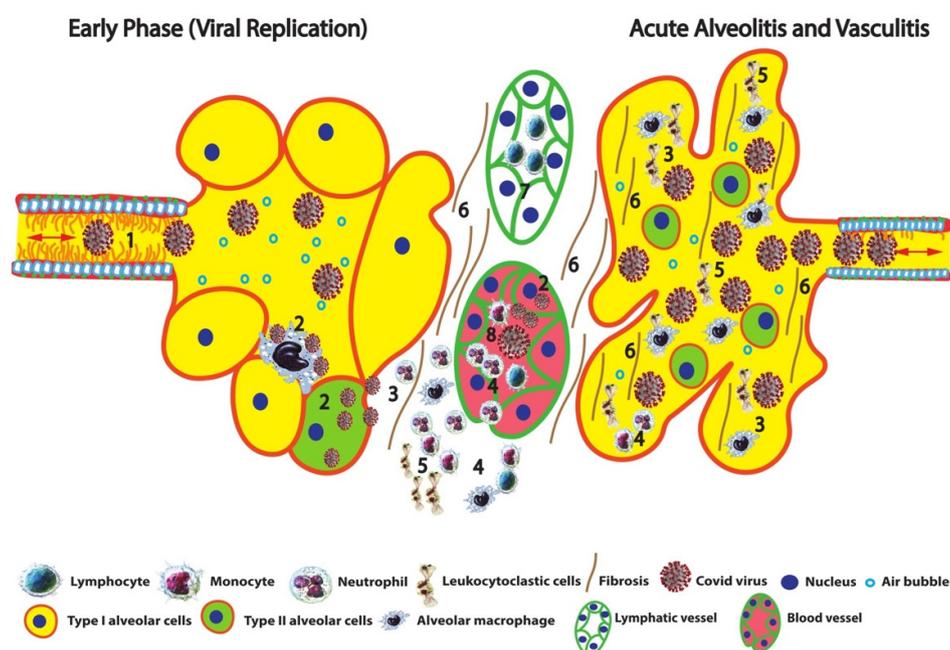

Figure 4. Algorithm of pathogenesis of the COVID19 disease in the lung.
1-Virus passes the damaged and non-ciliated respiratory epithelial cells.
2-Virus replicates in type II alveolar cells, alveolar macrophages and endothelial cells of the blood vessels.
3-Virus attracts neutrophils and macrophages in the alveolar spaces and even the interstitial spaces upon damage to the alveolar septa.
4-Neutrophils and monocytes pass the damaged endothelial cells (homing of viral replication) and aggregate at the alveolar spaces and inter-alveolar interstitial areas.
5-Leukocytoclastic changes and release of cytokine storm complicate the viral alveolitis.
6- Release of fibroblastic growth factors of cytokine storm makes the condition worse by forming scar at the alveolar surfactant membrane as hyaline material deposition and even interstitial fibrosis.
7- Lymphatic vessels collapse and block the passage of the lymphocytes along with restrictive lung disease for the patient.
8- Disseminated viremia reaching other organs with endothelial cells damage and leukocytoclastic changes at the new implantation site (kidneys, liver, heart, brain, skin, etc.)

Authors' Contribution

MB, ShD, AJ, SShM, SM and MShM: Project leaders and responsible for the study conception, design and critically revising the manuscript. PKh, MF, MY, HR, FS, AF and HA: Involved in the acquisition of data. SSh, and BD: Drafting the manuscript. MR, AM, NM, and SR: Involved in the electron microscopy and immunohistochemistry. All the authors provided their final approval for the completed manuscript.

Conflict of Interest Disclosures

There are no conflicts of interest.

Ethical Statement

This study was conducted in agreement with the Declaration of Helsinki for research involving human subjects, and was approved by the ethics committees of Kerman University of Medical Sciences (IR KMU Rec 99000656). Written consent was taken from all participant.

Acknowledgements

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