Pathomorphological Changes In The Lungs Of COVID-19 Patients

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Introduction In December 2019, a novel virus was discovered in China causing severe acute respiratory distress syndrome and the virus was designated as SARS-CoV-2. On 11th March 2020, the CoronaVirus (SARS-CoV-2) was declared a pandemic by the World Health Organization (WHO). Millions of people worldwide have been affected by this virus. Most of the patients show mild symptoms. Severe cases lead to the death of patients due to severe respiratory failure. Multiple organ failure has been seen in many patients suffering from COVID-19.

The main mechanism of the virus is not yet completely understood. In this article, we have tried to describe the pathomorphological changes that we observed in a sequence, along with two unique observations.

Selection Criteria We have chosen only those patients autopsies who died because of lung complications, as the lungs are the first organ to damage, among approximately 200 autopsies, 58 were from such patients.

Autopsies were performed after the concern of their families and by the order of the government without any ethical issues at Republican Pathoanatomical Bureau Bishkek for patients who died, with a polymerase chain reaction confirmed diagnosis of COVID-19.

The benefit of studying autopsy over other studies is that it gives incredibly valuable information especially when we still know so little about COVID-19 and especially when it presents in different ways.

Design Descriptive Study
**Purpose** The purpose of the research was to study the pathomorphological changes found in autopsies of the lung lesions with COVID-19 on autopsy materials, which can allow us to understand what could be done to reduce COVID-19 lung damage and mortality.

**Methods** After selecting lung autopsies only, the autopsy materials of 58 lethal cases were carried out by using H&E staining under the light microscope at Republican Pathoanatomical Bureau Bishkek. Macro- and microscopic changes in the lungs were studied for this research.

**Results** We have observed many pathomorphological changes in the lungs as a result but diffuse alveolar damage (DAD), which is synonymous with the clinical concept of "acute respiratory distress syndrome" (ARDS) was the main cause of death. Diffuse alveolar damage (DAD) in its development, has “exudative” and “proliferative” stages, furthermore, four cases demonstrated a discrepancy between the classical phases of diffuse alveolar damage and the duration of the disease where the characteristic changes of the early exudative phase were found after 10 days from the onset of disease in the absence of characteristic changes of the late proliferative while in two cases changes that were found, showed characteristics of both, the exudative phase as well as, proliferative phase.

**Conclusion** Our study concluded that Diffuse alveolar damage of the lungs tissue in COVID-19 patients is the main findings and that leads to the development of respiratory failure, which is the main cause of death.

**Keywords** Coronavirus, Autopsy, COVID-19, Acute Respiratory Distress Syndrome, Diffuse Alveolar Damage, SARS-CoV-2
Introduction
Throughout history, the world of medicine has faced many obstacles and challenges and even now, it continues to do so. In December 2019, a novel virus was discovered in Wuhan city of China causing severe acute respiratory distress syndrome and was termed as SARS-CoV-2. It is believed that the virus is zoonotic and has similar properties as SARS-CoV and MERS-CoV and is fatal. This virus has spread throughout the globe in a very short period. On March 11, 2020, this novel virus was declared as a pandemic. As of 5 Oct 2020, approximately 35 million cases of COVID-19 have been noted, in 215 countries, resulting in approximately 1 million deaths. Among all countries, the USA is on the top, with 7.6 million cases, and with 214,693 deaths. In Kyrgyzstan, there are 47,635 active cases and so far, 1,066 deaths have been reported. Based on studies, it is believed that the SARS-CoV-2 enters into the host cell by using ACE-2 (Angiotensin Converting Enzyme-2) receptor, which is usually present on the surface of alveolar cells of the epithelium of the lung, it causes diffuse damage to the alveoli leading to severe respiratory distress syndrome. In the early stages, the virus causes upper respiratory tract symptoms, which further progresses to lower respiratory symptoms. Most of the patients show mild respiratory symptoms but in severe cases, it leads to the death of the patient. Old aged patients and those with other comorbidities such as cardiovascular diseases, hypertension, diabetes mellitus, cancer, immunosuppression, and chronic disease are at high risk of viral infection with severe complications, causing an increase in death and has been reported in ages >60 and patients with underlying diseases. It has been suggested that severe disease progression might result in patient deaths due to massive pulmonary injury. Pathomorphological studies have far more beneficial evidence of lung injuries and it helps in providing incredibly valuable information when we have still less information about COVID-19. Autopsies provide real and live visual evidence.

Purpose
We hypothesized that the severe COVID-19 manifestation leadings to mortality occurs due to diffuse alveolar damage, therefore the study aims to evaluate the pathomorphological changes within the lung tissues of COVID-19 patients that have demised due to severe COVID-19 disease manifestation. This study will allow us to understand the disease mechanism behind COVID-19 related severe lung damage enabling the physicians to better treat or prevent patients from COVID-19 related severe lung damage and death. To prove the hypothesis, patient’s autopsies were taken without any ethical issues, who died due to COVID-19 in Kyrgyzstan, and macro and microscopical analyses were carried out at Republican Pathoanatomical Bureau Bishkek Kyrgyzstan.

Materials and Methods
Study Materials
Patients who died during COVID-19 pandemic in different hospitals of Kyrgyzstan, the special team leaves for the
hospital and in a means of special transport, the corpse was brought to the morgue of the pathological bureau with the concern of their families and by the order of health ministries.

The pathologists examined the medical history in great detail before going to the autopsy. Autopsies were carried out within 2 to 12 hours after the death of patients were ascertained to exclude possible post-mortem changes in the organs. All organs and systems were examined macroscopically.

In total, approximately 200 autopsies were there, but we included in the study only those cases in which the main cause of death was because of lung complications like respiratory failure and 58 autopsies were such.

Sectional materials were then obtained after the autopsy of 58 deceased patients, confirmation of COVID-19 was done by the polymerase chain reaction method both during life, as well as posthumously.

Methods
The autopsies were carried out in compliance with all the rules specified in the temporary guideline for pathologists for the examination of a COVID-19 positive corpse and its parts in cases of intravital establishment of COVID-19. Lungs autopsy material were taken, and the specimens were fixed in formalin, embedded in paraffin. 5µm sections were made, and were then stained with hematoxylin and eosin and Van Gieson’s Picro-fuchsin method, according to the standard technique. The hematoxylin and eosin stain provided a comprehensive picture of the microanatomy of lung tissues. Hematoxylin precisely stains nuclear components including heterochromatin and nucleoli, while eosin stains cytoplasmic components including collagen and elastic fibers, muscle fibers, and red blood cells. The specimens were then studied under a light microscope.

Results
Comprehensive When the specimens taken from the deceased patients were studied using the H&E staining method under the microscope, it was found that many morphological changes had occurred in the lungs during the two phases of the DAD.

Out of 58 specimens taken, 32 (55.2%) were male, and 26 (44.8%) were from female patients. The male to female ratio found was 1.2:1. The patients were aged between 28 to 86. The average age of those who died was 58.4±12.63 years, (52.4±11.32 years for men and 64.8±13.4 years for women). Autopsies of the lung lesions revealed pathomorphological changes of varying severity and prevalence. The study showed that the main pathomorphological manifestation in the lungs is diffuse alveolar damage, which is synonymous with the clinical concept of acute respiratory distress syndrome (ARDS).

- On gross examination, an increase in the lungs volume and the mass, predominantly posterior basal sections were affected, as well as acute swelling was observed in the upper anterior sections. Lung changes macroscopically corresponded to the concept of the shock lung, on a cut section, the lungs appeared dark cherry or red-
brown, with areas of atelectasis, often extensive confluent hemorrhages and hemorrhagic infarctions of various sizes, also multiple obstructing thrombi in the branches of pulmonary arteries and veins of various sizes are characteristic. On the pleura in some deceased patients, characteristic fibrin deposits were found (focal and widespread fibrinous pleurisy, usually without significant effusion in the pleural cavities).

Histological examinations revealed changes corresponding to two phases of diffuse alveolar damage.

**Exudative Phase and Proliferative Phase**

- In the early phase (corresponding to the first 10 days of disease), 28 specimens- 48.3% revealed proteinaceous exudative changes along with the following characteristics from the onset were found: Intra alveolar edema with the admixture of erythrocytes, neutrophils, monocytes, and macrophages in the edematous fluid, desquamated alveolocytes, lymphocytes, and plasma cells also found (Fig. 1&2). Later, marked pink amorphous hyaline membranes of different thicknesses over the alveolar wall, which is the hallmark of viral pneumonia was seen (Fig. 3&4.). Atypically large nuclei and hyperplasia with papillary appearance were observed in Type II pneumocytes (Fig. 5). Additionally, infiltration of lymphocytes and macrophages was determined around the vessels and bronchioles. (Fig. 6)

Also, reactive changes, with large nuclei and few areas of metaplasia were noted in the bronchial epithelial linings. A pronounced plethora of intra-alveolar septa and capillaries of septal branches of pulmonary arteries and veins with sludge fresh fibrinous and organizing blood clots with destructive-productive thrombovasculitis were observed (Fig.7&8). In eight cases (28.6%, perivascular, interalveolar, and intra alveolar hemorrhages were found (Fig.9&10). Three cases (10.7%) revealed foci of hemorrhagic infarction, the red infarct being wedge-shaped, with its based-on pleura, though the pulmonary artery carrying most of the blood and oxygen is cut off (Fig 11 & 12). In cases where the patients were connected to a ventilator (Six observations (37.5%) out of 16), foci of emphysema with rupture of intra alveolar septa were found (Fig 13 &14).

- The later phase or the proliferative (corresponding to the 10 days after the onset of disease) (24 observation-41.4%), revealed Diffuse Alveolar Damage, and proliferative pathologies. It was characterized by the accumulation of fibrin of varying degrees of maturity in the alveolar
lumens and in some cases, by polypoid growth of fibroblasts (granulation tissue) were detected (Fig. 15).

Furthermore, in nine cases (37.5 %), pronounced interstitial thickening of the intra alveolar septa with, myxomatosis edema of the perivascular stroma was found (Fig. 16). In four cases (16.4 %), focal bacterial pneumonia was found confluent with viral pneumonia (Fig 17).

In the later stage of the disease in the lungs (more often in the lower lobes), moderately expressed areas of organizing pneumonia with the proliferation of fibrous tissues are found.

In four (6.8 %) cases, there was a discrepancy between the classical phases of diffuse alveolar damage and the duration of the disease where the characteristic changes of the early exudative phase were found after 10 days from the onset of disease in the absence of characteristic changes of the late proliferative.

In two (3.4 %) cases changes that were found, showed characteristics of both, the exudative phase as well as, proliferative phase.

In conclusion, all specimens showed DAD, 52 (89.7%) cases followed the main mechanism and timing of DAD while 6 (10.2%) cases with discrepancies. Thus, our study revealed that diffuse alveolar damage is the main morphological manifestation in the lungs of patients with COVID-19, which is synonymous with the clinical concept of ARDS. Diffuse alveolar damage in its development goes through two stages- exudative and proliferative.

Discussion
Comprehensive Few reports were done about the morphological findings of COVID-19-associated with respiratory distress syndrome. Xu et al. reported bilateral diffuse alveolar damage with proteinaceous exudates, pneumocytes desquamation, and amorphous hyaline membrane in addition to atypical enlarged pneumocytes in post-mortem lung biopsy samples of 50-years old man who died after 2 weeks of illness with previous medical treatment. Tian et al. reported two cases of lung lobectomies specimen for adenocarcinoma, which retrospectively were found to have had COVID-19 at the time of the operation: the pathologic phases of acute lung injury. Tian et al. did post-mortem needle core biopsies of the lung, the liver, and the heart in four patients who died of COVID-19 pneumonia and showed advanced diffuse alveolar damage as the main pathologic finding and secondary bacterial pneumonia in some patients.
Histopathological features of Lungs of a COVID-19 patient.

Figure 1: Extensive pulmonary proteinaceous exudates in alveolar spaces, highlighted by arrows (H&E, ×100)

Figure 2: Intra alveolar infiltration and proliferation of inflammatory cells, highlighted by arrows (H&E, ×100)

Figures 3&4: Homogenous pink hyaline membrane (thick and thin layer) lining the alveolar wall (H&E × 100)
Figure 5: Atypical Type-II Pneumocyte with hyperplasia in the alveolar spaces; highlighted by arrows (H&E, × 400)

Figure 6: Perivascular infiltration of lymphocytes and macrophages. (H&E, × 400)

Figures 7&8: Endothelial cell damage, inflammatory cell infiltration into the vessel wall (pulmonary vasculitis), which leads to the formation of microthrombi in the vessel that causes pulmonary hemorrhagic infarction. (H&E, × 400)
Figure 9 Massive pulmonary hemorrhage.
Figure 10: Alveolar spaces filled with blood, hyaline membrane, collapsed alveoli, and interstitial proliferations. (H & E, × 100)

Figures 11&12: Red area is the pulmonary hemorrhagic infarction and the border between hemorrhagic and non-hemorrhagic area can be seen. (H&E, ×100 and 40)
Figure 13: Marked dilation of alveoli due to destruction of elastic tissues and alveolar wall

Figure 14: Wall become weakened and ruptured (emphysematous changes or emphysema) (H&E, × 400)

Figure 15: Alveolar infiltration containing inflammatory cells, fibroblasts & collagen fibers in alveolar space. (H&E, × 400).

Figure 16: This figure demonstrates a large number of morphological changes including alveolar infiltration, interstitial fibrosis and thickening, and also alveolar damage and collapse of alveoli as shown by arrows (hematoxylin and eosin stain; original magnification × 100)
Christine Suess and Roland Hausmann also reported the presence of diffuse alveolar damage and atypical enlarged type II pneumocytes without obvious viral inclusions as major histopathologic findings in the lung parenchyma. Furthermore, superimposed bacterial pneumonia was suspected because of the detection of some neutrophilic infiltrates in the lower lobes. In addition, the evidence of lymphocytic infiltrate in the pericardium suggests the spread to heart tissue.

In our cases, the specimens revealed many such changes during the two phases (exudative and proliferative phases) including proteinaceous exudates, intraalveolar edema with the admixture of erythrocytes, neutrophils, monocytes and macrophages in the edematous fluid, desquamated alveolocytes, lymphocytes, and plasma cells also found, pulmonary hemorrhagic infarcts and hyaline membrane over the alveolar walls, atypical type II pneumocytes with hyperplasia, fibrocytes proliferation with collagen fibers of varying degree of maturity, thickened interstitium but the main histopathological finding was diffuse alveolar damage. Some specimens demonstrated superimposed bacterial pneumonia.

But there was a discrepancy between the classical phases and the duration of the disease where the characteristic changes of the early exudative phase were found after 10 days from the onset of disease and two cases revealed characteristics of both, the exudative phase as well as, proliferative phase, which is unique in our case.

**Limitation**
The presence of viruses in the specimens was not detected because of the lack of validated tests available for immunohistochemistry.

**Conclusion**
As various scientists have researched the structure of coronavirus, its mechanism of infection, transmission, resistance, virulence, and variability in surface proteins, as well as studies, have been done to confirm that the COVID-19 virus mainly attacks our respiratory system causing SARS. Our case study on a COVID-19 patient confirmed our hypothesis and showed the destructive changes in lung morphology. These changes included diffuse alveolar damage, atypical type II pneumocytes with hyperplasia, thickened interstitium,
pulmonary hemorrhagic infarctions, thrombus in pulmonary vessels, and many dilated alveoli (honey-comb appearance). These destructive changes could have contributed to decreasing gaseous exchange between alveoli and blood vessels, stiffness of lungs, pulmonary congestion, and severe hypoxia, which may have led to the death of patients. Six samples showed discrepancies in the sequence of the pathomorphological changes/phases of diffuse alveolar damage that were unique in our studies. These microscopic morphological findings help in better understanding the mechanism of action of COVID-19 virus in lung and to determine the cause of death and hope soon for better control and recovery.

References


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