ORIGINAL ARTICLE

Morphofunctional Alterations in Peribronchial Lymph Nodes of COVID-19-Related Deaths: A Comparative Study

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Abstract

Background: The coronavirus disease-19 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2, has significantly impacted people worldwide. Reverse transcription polymerase chain reaction is the primary method for validating COVID-19 cases; however, its sensitivity is limited. The lymphatic system, which plays a crucial role in maintaining homeostasis, is also affected by pathological conditions. COVID-19 is characterized by endotheliitis, which involves the blood vessels in the lungs. This study aimed to investigate morpho-functional changes in the peribronchial lymph nodes in COVID-19-related deaths. **Materials and Methods:** This study established methods and specialized techniques for forensic materials and analyzed the lymph nodes of 45 COVID-19 deceased individuals. Cutting 5–6 μm slices using a rotary microtome, the samples were stained with hematoxylin and eosin for general histological examination. **Results:** This study found significant structural changes in the lymph nodes, including a disrupted structure, lack of follicles, unevenly thickened and sometimes absent capsules, random arrangement of cellular elements without forming follicles or reproduction centers, prevalence of mature lymphocytes and a large number of macrophages, large cells, both young and mature plasma cells, frequent observation of large cells with two or three nuclei, and the presence of fibrin and fibrin-like deposits in blood vessels, erythrocytes, and fibrin blood clots in the parenchyma and stroma of the lymph nodes, as well as numerous sharply expanded cavities filled with lymph, resembling cysts, but without a capsule. **Conclusion:** This study suggests that these morphological changes may serve as additional criteria for confirming the cause of death due to COVID-19.

Key words: Coronavirus disease-19, lungs, lymph nodes, morphofunctional changes, severe acute respiratory syndrome coronavirus-2

INTRODUCTION

The coronavirus disease-19 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was the dominant issue that divided the international community between 2019 and 2020. The first cases of the virus were reported in Wuhan, China, in December 2019.[1,2] Unfortunately, the US has also suffered a significant number of deaths, with over 80,000 people losing their lives to the virus.[1]

Reverse transcription polymerase chain reaction (PCR) is the primary method used to validate COVID-19 cases, as stated in various publications. According to these sources, a positive test result can be achieved in 34–62% of presumed COVID-19 cases.[2-4] However, only 15% of adults with COVID-19 may exhibit clinical symptoms, a finding not supported by PCR results. A study in China involving >72,000 patients found that 1%

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of patients had an asymptomatic course of the disease.[3] In addition, only 11% of pediatric cases have been verified using PCR tests because of the limited sensitivity of the methodologies employed.[5] The lymphatic system plays a crucial role in maintaining homeostasis by transporting materials, including metabolic products, from organs to the venous bed. In pathological situations, bacteria and cancerous tumor cells can also move through the lymphatic vessels with lymph nodes. In addition to producing immunological bodies, lymph nodes perform hematopoietic and protective (barrier) functions.

A distinct characteristic of COVID-19 is the involvement of blood vessels in endotheliitis, which has several histological endothelial alterations. Ackermann *et al*. found three key vascular features in lungs affected by COVID-19: vascular thrombosis in alveolar capillaries, endothelial injury with intracellular localization of SARS-CoV-2, and vascular thrombosis leading to distorted vessels.[6] Microthrombi were commonly observed in postcapillary venules, precapillary arteries, and alveolar capillaries. Endotheliitis is not limited to the lungs; it has also been reported in the heart, kidneys, and small intestine vasculature.

An urgent task in modern lymphology is to investigate the morpho-functional state of the lymph nodes when hemolymph microcirculation is disrupted during the pathological process.

Aims and objectives

The aim of this study was to investigate morphofunctional alterations in the peribronchial lymph nodes, which are localized lymph nodes in the lungs, in cases of COVID-19-related deaths.

MATERIALS AND METHODS

This study was conducted using a variety of accepted methods and specialized techniques for practical forensic materials. In the experimental group, the regional lymph nodes of the lungs of 45 (mean age: 44.3 years [standard deviation: 13.2, range: 24–64]; 33 [78.6%] males) cadavers who died from COVID-19 were examined and admitted to the Republican Center of Forensic Medical Examination of the Ministry of Health of the Kyrgyz Republic. As a control group, the regional lymph nodes of the lungs from 42 (mean age: 52.8 years [standard deviation: 14.3, range: 23–83]; 33 [55.2%] males) cadavers that had died from open or severe closed craniocerebral trauma within minutes of the injury were analyzed.

The lymph nodes were removed from the cadavers, subjected to macroscopic analysis, and fixed in 10% formalin solution. The samples were dehydrated in increasing concentrations of alcohol and embedded in paraffin, according to standard procedures. Sections of 5–6 μm were obtained using a rotary microtome, and the samples were stained with hematoxylin and eosin (H and E) staining for general histological analysis. The glass preparations were then examined using a Nikon Eclipse 50i Clinical Microscope (Nikon Corporation, Tokyo, Japan), and microphotography was performed at magnifications of ×280 and ×410.

RESULTS

The morphofunctional characteristics of the lymph nodes in the lungs of the control group were studied. This involved examining the lymph nodes of 42 male and female cadavers who died from open or severe closed craniocerebral trauma. The lymph nodes were selected for study if they showed no macroscopic changes and if there was no evidence of any pathology in the lymph nodes.

The lymph nodes were typically oval or round, measuring between 0.6 × 0.8 cm and 0.8 × 1.0 cm, and had an elastic consistency and a thin translucent capsule. The color of the lymph nodes was bluish-gray, and in most cases, there was a visible deposition of coal dust through the capsule. The influent and efferent lymphatic vessels were visible as thin strands that passed through the node capsule. The capsule of the nodes was transparent and comprised fibrous connective tissue that was poor in cells. The structure of the capsule was visible throughout its entire length, and the trabeculae and stroma of the nodes were clearly stained with Van Gieson stain.

Microscopic observations revealed the presence of cell-free spaces called marginal and medullary sinuses located beneath the capsule or near the lymph node ports. These sinuses remain invisible under low magnification but are identified under high magnification, revealing the endothelium lining. Adjacent to the sinuses, macrophages and lymphocytes were observed in significant numbers [Figure 1]. Lymphatic vessels traversing the capsule were also visible, exhibiting slight dilation [Figure 2].

The lymph nodes in the lungs of individuals who died of COVID-19 were analyzed in this study. Macroscopically, the size of the lymph nodes varied from 1.2 cm to 7 cm. Histological examination revealed that the normal structure of the organ was disrupted, and no follicles were present. The capsule of the organ was thickened in some areas but not in others. The zones were not well defined, and the follicular structure was absent. The cellular elements were arranged randomly and did not form follicles or reproductive centers. The cellular composition was dominated by mature lymphocytes, with diffuse growth of lymphoid cells without follicle formation [Figure 3]. Macrophages were also present in large numbers. Atrophy of the lymphoid base was observed [Figure 4], and both young and mature plasmatic cells were found in greater numbers than normal. The blood vessels were stagnant, and black pigment deposits were observed in the stroma [Figure 5].

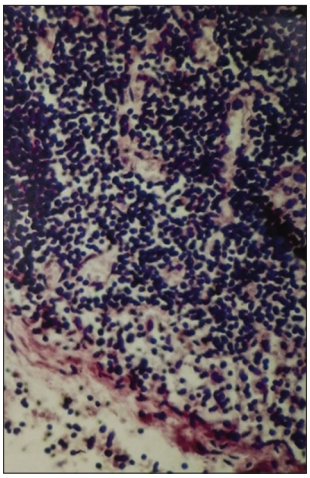

Figure 1: Hematoxylin and eosin staining revealed sinuses with endothelium, macrophages, and lymphocytes in the control group (×280)

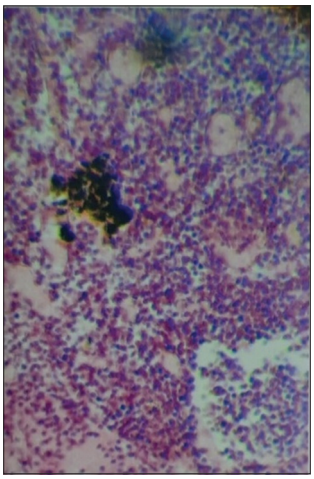

Figure 2: Hematoxylin and eosin staining revealed that lymphatic vessels passed through the capsule in the control group (×280)

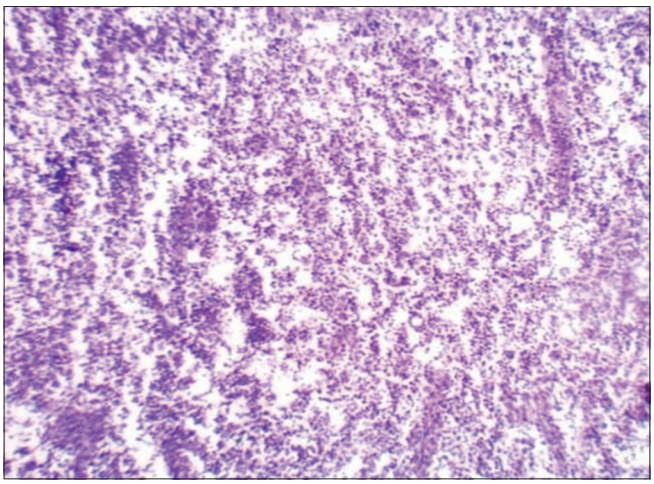

Figure 3: Hematoxylin and eosin staining demonstrated the diffuse growth of lymphoid cells without follicle formation (×280)

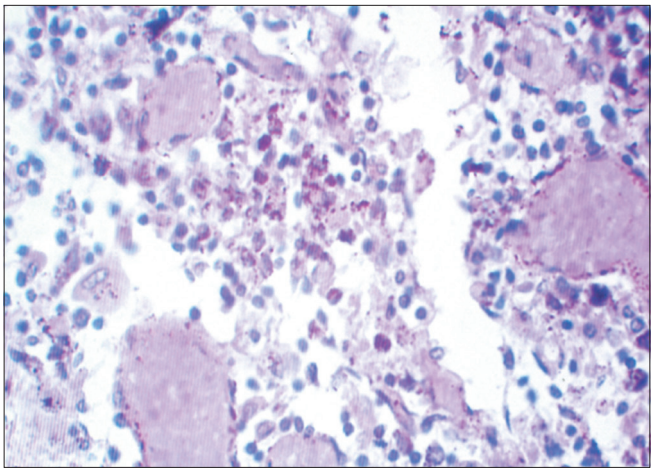

Figure 4: Hematoxylin and eosin staining revealed a large number of macrophages and large cells with atrophy of the lymphoid base (×410)

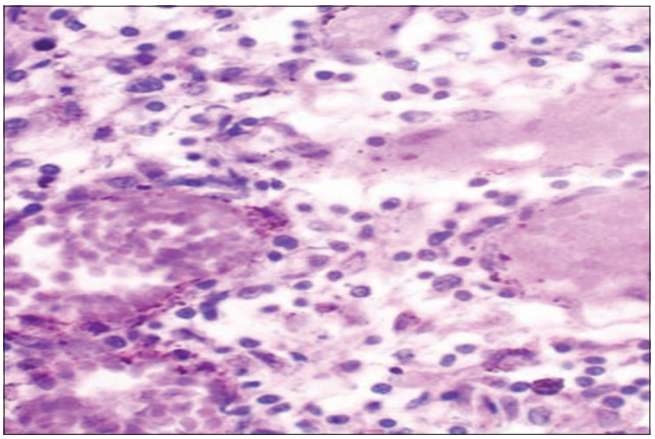

Figure 5: Hematoxylin and eosin staining demonstrated that both young and mature plasma cells were more abundant than normal, and the blood vessels were sharply stagnant and full of blood (×280)

The capsule of the organ is damaged in some areas and is heavily infiltrated with white blood cells, which can be seen as a buildup of fluid in the blood vessels and a sludge-like substance [Figure 5]. In addition, fibrin and fibrin-like deposits were visible within the blood vessels, and large cells with two or three nuclei were often present [Figure 6]. Some blood vessels contained clumps of red blood cells, whereas others had fibrin clumps [Figure 7]. In the organ's parenchyma and stroma, there are numerous cavities filled with lymph, resembling cysts but lacking a capsule, giving the appearance of lakes [Figure 8].

DISCUSSION

This study used immunohistochemical staining and histological examination to investigate modifications in lymph nodes at the regional level, shedding light on the

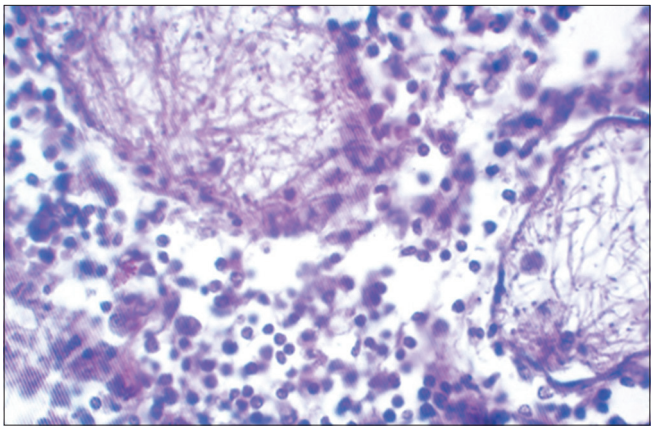

Figure 6: Hematoxylin and eosin staining demonstrated fibrin and fibrin-like deposits in the blood vessels (×410)

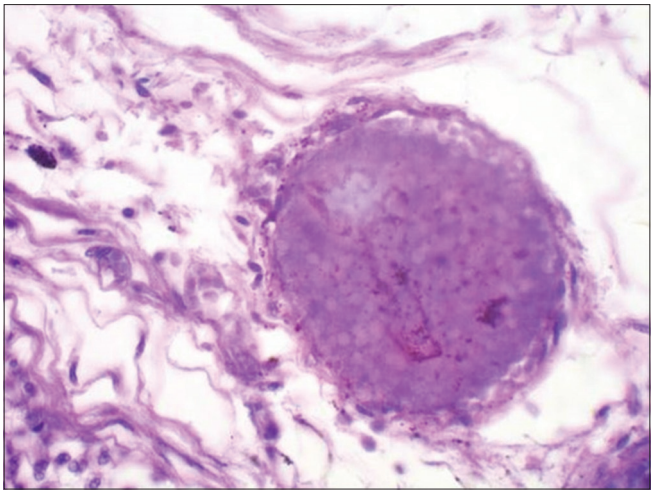

Figure 7: Hematoxylin and eosin staining demonstrated erythrocyte thrombi in the vessels, whereas fibrin thrombi were present in others (×410)

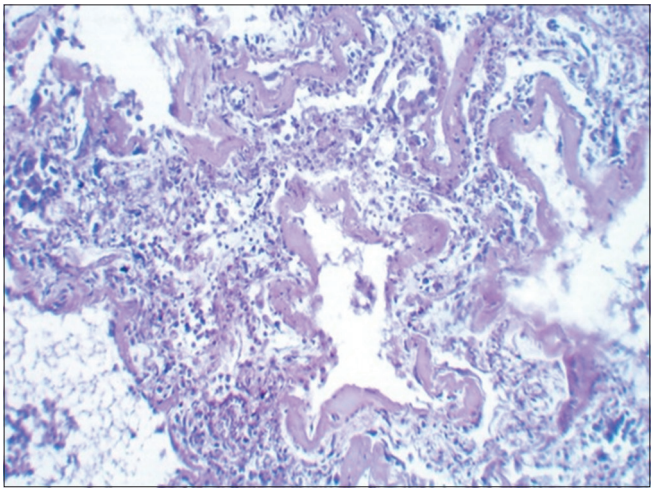

Figure 8: Hematoxylin and eosin staining demonstrated the accumulation of fluid in the sinuses, such as lakes (×280)

impact of the virus on the tissues. Previous studies have shown that SARS-CoV-2 can infiltrate lymph nodes.[7] Due to concerns about aerosolization and contagiousness, only a few autopsies were initially performed. Early autopsy patients with COVID-19 sometimes include only lung samples.[8,9]

Lung damage is the primary cause of death in COVID-19 patients, as established in various studies.[10-12] Angiotensin-converting enzyme-2 is believed to function as a viral receptor, infecting the epithelial lining of the respiratory system and resulting in diffuse alveolar damage, edema, and increased lung weight at autopsy. Notably, lung pathology was diverse and consistent with the radiographic findings of patchy ground-glass opacification. The pulmonary parenchyma exhibited various histological findings, including organizing pneumonia and patchy exudative hyaline membrane disease, although some regions displayed histological normalcy. These features likely indicate the spread of the virus in the lungs; however, further research is needed to fully understand this phenomenon.

An American study reported that many severely affected patients with COVID-19 showed lung pathology during autopsies.[10] In addition, some patients exhibit typical features in their lymph nodes and liver, accompanied by extensive microscopic blood clots.

Autopsies are crucial for understanding the pathophysiology of COVID-19, but they have been overlooked in favor of molecular and clinical research on SARS-CoV-2.[13] The early stages of the pandemic saw limited autopsy data due to recommendations to delay postmortem examinations for suspected COVID-19 cases, posing a significant challenge for researchers seeking to deepen their understanding of the disease. Additional research is needed to shed light on the pathophysiology of COVID-19 and its progression. Moreover, it is essential to establish an autopsy unit equipped with the necessary biosecurity equipment to ensure the safety of personnel conducting these examinations.[14]

Most histological changes occur in the lungs and blood vessels, which seem to have the most significant clinical impact. Although the morphological landscape of COVID-19 in other organs remains poorly understood and sometimes inconsistent, further research on tissue samples is needed to determine the extent to which other organs and tissues are affected by the disease.

CONCLUSION

The morphological characteristics of the regional lymph nodes in the lungs during death from COVID-19 were examined and compared to a control group, revealing several structural changes, including a disrupted structure of the lymph nodes and lack of follicles; unevenly thickened and sometimes absent capsules; random arrangement of cellular elements without forming follicles or reproduction centers; the prevalence of mature lymphocytes and a large number of macrophages, large cells, and both young and mature plasma

cells; frequent observation of large cells with two or three nuclei; and presence of fibrin and fibrin-like deposits in blood vessels, erythrocytes, and fibrin blood clots in the parenchyma and stroma of the lymph nodes, as well as numerous sharply expanded cavities filled with lymph, resembling cysts but without a capsule. These morphological changes may serve as an additional criterion for confirming the cause of death from COVID-19 in conjunction with other signs.

ACKNOWLEDGMENTS

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