Original Article

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Pathological Features of Lung in COVID-19 Disease Subjects: A Postmortem Study

Others Section

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ABSTRACT

Introduction: The current Coronavirus Disease-19 (COVID-19) pandemic is considered as one of the most serious public health crises which caused more than 1.62 million deaths from October 2020 to November 2020. Acute respiratory failure is leading cause of death followed by sepsis, cardiac failure and haemorrhage. Since the pathological findings are diverse in COVID-19 and majority of studies in literature were by open autopsy; the present study was done using percutaneous core needle biopsy. Postmortem lung biopsies are rather easy and quick to perform and decrease the infective risk caused by full autopsies. This could be an essential tool for diagnosis, surveillance and research.

Aim: To study the pathological features of lung in COVID-19 deceased patients by postmortem.

Materials and Methods: This cross-sectional study was conducted in the Department of Pulmonary Medicine, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Vijayawada, Andhra Pradesh, India from October 2020 to November 2020. In present study, postmortem percutaneous core needle biopsies from lung were performed within two hours of death from eight deceased patients who died of COVID-19. Clinical history, inflammatory markers and treatment details were collected from case sheets, biopsy was done, specimen was collected and sent for pathological examination. Data was presented in the descriptive form for each variable.

Results: Out of eight cases, five were men and three were women with a mean age of 54.12 years. Majority of patients presented with complaints of shortness of breath and fever. Hypertension, type 2 diabetes mellitus, obesity, hypothyroidism, history of pulmonary tuberculosis were the co-morbidities noticed. Four biopsies presented acute lung injury with hyaline membrane changes, Diffuse Alveolar Damage (DAD) with hyaline membrane was seen in two cases, squamous metaplasia was seen in two cases and acute lung injury with organising pneumonia was seen in two cases.

Conclusion: Postmortem lung biopsies are safe, easy to perform and provide insights of possible undergoing pathology of the disease with regard to clinical presentation.

Keywords: Acute lung injury, Core needle biopsy, Coronavirus disease-2019

INTRODUCTION

An outbreak of a novel coronavirus caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) occurred in Wuhan, China in late 2019 [1]. The illness was named COVID-19 by World Health Organisation (WHO) [2]. The outbreak was declared a pandemic in just three months after emerging [3]. SARS-CoV-2 utilises Angiotensin Converting Enzyme 2 (ACE2) as a source of cellular entry. ACE2 is expressed in lung alveolar cells, bronchial epithelium and vascular endothelial cells, therefore the respiratory tract and lung serve as a primary point of viral entry [4]. There was evidence that about 12% of patients presenting with severe symptoms require hospitalisation and the case fatality rate is about 2.3% [5]. COVID-19 has been shown to affect different organ systems, of which respiratory system pathology predominates with mortality primarily due to Acute Respiratory Distress Syndrome (ARDS) [6].

Clinical and laboratory data have identified lung, heart and liver as the three organs involved by the novel coronavirus [7]. Although the disease results in diverse, multiorgan pathology, only a sparse data is obtainable about pathological changes in patients infected with SARS-CoV-2.

Barton LM et al., autopsy reports of the lungs in COVID-19 study has been done and primarily shown DAD or acute lung injury [8]. A study has been done on microscopic evaluation of lungs by Borczuk AC et al., which showed presence of airways inflammation and alveolar zones with hyaline membranes and type 2 pneumocyte (AT2 cell) hyperplasia [9]. Knowledge regarding the underlying pathological variations can lead us to better understanding of the disease and could be of utmost significance in clinical management [10]. Most of the studies were on open autopsy. The present study was done using postmortem percutaneous core needle biopsy with the aim to identify the pathological findings of lung in COVID-19 patients.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pulmonary Medicine, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Gannavaram, Andhra Pradesh, India from October 2020 to November 2020. Informed consent from their legal relatives was taken. Institution Ethical Committee clearance was taken [PG/552/20]. The present study included eight patients that were tested positive for SARS-CoV-2; admitted and subsequently died between October 2020 and November 2020.

With appropriate personal protective equipment as dictated by standard guidelines, percutaneous core needle biopsies were performed from each lung within two hours of death in mid axillary line from 4th or 5th intercostal space. Each specimen was placed into individually labelled specimen bottle. The specimens were fixed in 10% neutral buffered formalin. After 24 hours of fixation, tissue processing was performed by using automated tissue processor. The histopathology was assessed by expert pathologists. Clinical information such as age, gender, co-morbidities, duration of illness, methods of ventilation and treatment (antimicrobials, anticoagulant therapy, and corticosteroid therapy) were collected from the case sheets.

STATISTICAL ANALYSIS

Descriptive form of data was collected for each variable and presented.

RESULTS

Out of eight cases studied, five were men and three were women with mean age of 54.12 years (39-75 years). Majority of patients presented with complaints of shortness of breath and fever. Every patient had at least one co-morbidity like hypertension (5/8), type 2 diabetes mellitus (6/8), obesity (1/8), hypothyroidism (1/8), history of pulmonary

tuberculosis (1/8). Saturation of Peripheral Oxygen (SpO_2) on Room Air (RA) at the time of admission was within the range of 40-85%. The duration of stay ranged from one day to four days. Initially, six patients were receiving ventilation in the form of Non Invasive Ventilation (NIV) with Bilevel Positive Airway Pressure (BIPAP) ventilation; one patient was on High Flow Nasal Oxygen (HFNO) and one patient was on oxygen support with Non Rebreathable Mask (NRBM).

Five out of eight patients had D-dimer value >500 ng/mL (40-2500 ng/mL); four patients had Ferritin >500 ng/mL (30-1300 ng/mL); seven patients had C-Reactive Protein (CRP) >100 mg/L (6 to 200 mg/L). All the patients had bilateral non homogenous opacities (consolidations) on Chest X-ray (CXR). Treatment included antimicrobials, corticosteroid therapy, anticoagulant therapy and details are presented with summarised clinical features in [Table/Fig-1].

DISCUSSION

The present study described the histopathological findings of lung in COVID-19 obtained through postmortem biopsies, which demonstrated diverse pathology. SARS-CoV-2 infected patients start experiencing flu symptoms like fever, cough, nasal congestion and fatigue [11]. As the infection progresses, patients experience dyspnoea and consistent symptoms of viral pneumonitis such as decreased oxygen saturation, lymphopenia and ground glass opacities in chest imaging [12]. Thus, the end result of these patients is a severe condition of acute lung injury named ARDS [13]. In the present study, four among eight patients demonstrated features suggestive of acute lung injury with hyaline membrane changes.

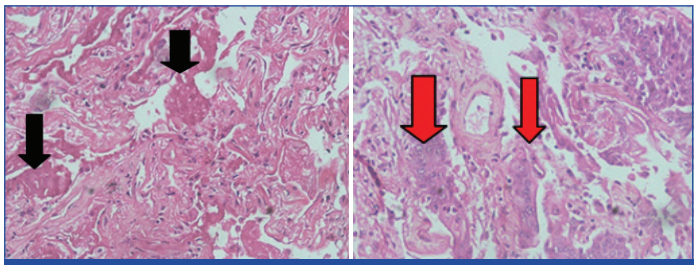
Parameters	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age (years)	50	46	55	75	54	39	42	72
Sex	Male	Female	Male	Male	Male	Male	Female	Female
Co-morbidities	Diabetic, hypertensive	Diabetic, hypertensive	Hypertensive, diabetic	Hypertensive, diabetic	Diabetic	Diabetic, history of pulmonary tuberculosis	Obese	Diabetic, hypertensive and hypothyroid
Duration of symptoms prior to admission (days)	4	10	4	7	3	5	2	4
Symptoms	Shortness of breath	Shortness of breath and fever	Fever, cold and cough	Fever	Shortness of breath, cough	Shortness of breath, cough, cold	Shortness of breath	Fever, shortness of breath
SpO_2 at admission on room air	50%	82%	85%	77%	64%	85%	40%	80%
Duration of stay (days)	1	1	2	3	1	3	1	4
Method of ventilation	NIV-BiPAP	NIV-BiPAP	NRBM-15 Lit oxygen	NIV-BiPAP	NIV-BiPAP	HFNO	NIV-BiPAP	NIV-BiPAP
Antimicrobial use	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Anticoagulation therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Corticosteroid use	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CRP (mg/L)	5.9	108	105	146	119	114	170	196
Ferritin (ng/mL)	709	169	20	106	1360	966	878	56
D-dimer (ng/mL)	2424	927	296	32	1590	900	352	778

[Table/Fig-1]: Clinical features, co-morbidities, treatment, acute phase markers.
SpO_2: Saturation of peripheral oxygen; NIV-BIPAP: Non invasive ventilation-bilevel positive airway pressure; NRBM: Non rebreathable mask; HFNO: High-flow nasal oxygen; CRP: C-reactive protein

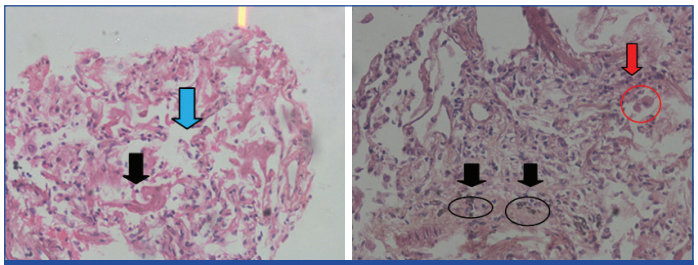
Histology: The histopathological findings are summarised in [Table/Fig-2]. Acute lung injury with hyaline membrane changes were seen in four patients, acute lung injury with organising pneumonia was seen in two cases, inflammatory cell infiltrates seen in all eight cases, hyaline membrane was seen in six cases [Table/Fig-3], squamous metaplasia was seen in two cases [Table/Fig-4], pneumocyte proliferation was seen in five cases, diffuse alveolar damage with hyaline membrane and squamous metaplasia was seen in two patients [Table/Fig-5], organising pneumonia was seen in two cases, fibrin thrombi was seen in three cases and alveolar septal thickening was seen in four cases. Anthracotic pigment laden macrophages were also seen [Table/Fig-6].

Histopathological findings	Number of cases
Acute lung injury with hyaline membrane changes	4
Diffuse alveolar damage with hyaline membrane and squamous metaplasia	2
Acute lung injury with organising pneumonia	2
Inflammatory cell infiltrates	8
Hyaline membrane	6
Squamous metaplasia	2
Pneumocyte proliferation	5
Organising pneumonia	2
Fibrin thrombi	3
Alveolar septal thickening	4

[Table/Fig-2]: Histopathological findings of eight biopsies summarised.



[Table/Fig-3]: Pink, amorphous material suggestive of hyaline membrane formation (Black arrow). **[Table/Fig-4]:** Squamous metaplasia (Red arrow). (Images from left to right)
Haematoxylin and Eosin stain; 40X (H&E)



[Table/Fig-5]: Black arrowhead showing hyaline membrane formation, blue arrowhead showing distorted alveoli. **[Table/Fig-6]:** Black outlined structures are anthracotic pigment, red outlined structure is pigment laden macrophages. (Images from left to right)
Haematoxylin and Eosin stain; 40X

Pathophysiologically ARDS is characterised by acute and diffuse inflammatory damage into alveolar capillary barrier associated with

an increase in vascular permeability, decreased compliance and the size of aerated lung tissue, compromising gas exchange and causing hypoxemia [14]. Histopathologically this clinical picture was named by Katzenstein AL et al., as DAD, corresponding to all cases of SARS-CoV-2 [15]. It consists of permanent damage to capillary endothelial cells, with consequent leakage of protein-rich fluid into interstitial and alveolar space, resulting in hyaline membrane formation and eventually intracapillary thrombi [16]. Two out of eight patients demonstrated DAD. In a study of postmortem lung biopsies from four patients by Bruce-Brand C et al., only one out of four patients reported DAD [17].

The acute phase of DAD is associated with hyaline membrane formation, fibrinoid exudates and alveolar wall oedema [18]. In present study, hyaline membrane formation was reported in six out of eight patients. In a study done by Beigmohammadi MT et al., all the seven deceased SARS-CoV-2 infected patients demonstrated the acute phase of DAD [10]. In a study done by Tian S et al., four postmortem lung pathological reports of COVID-19 patients were presented, referring to hyaline membrane formation, establishing DAD as the pathologic basis of lung involvement [19]. Similarly, DAD was the main finding in a case reported by Xu Z et al., of a patient who died of COVID-19 [20].

Corresponding to first 10 days of viral infection, the first or exudative phase is characterised by intense inflammatory cells infiltration into the intra-alveolar space [21]. Inflammatory cell infiltrates were reported in all the cases in present study. The second or proliferative phase is marked by fibroblast and myofibroblast proliferation which can form organising pneumonia, resulting in parenchymal remodelling, pulmonary fibrosis and squamous metaplasia [22]. Squamous metaplasia has been demonstrated in few cases of COVID-19 particularly with a span of illness beyond 14 days [23]. These stages do not occur sequentially but often occur simultaneously through the lung tissue i.e., while the immune system tries to contain the microorganism (exudative phase) in one region, another lung tissue area begins to organise itself in order to repair the affected areas [24]. In the present study, two out of eight patients revealed squamous metaplasia with span of illness less than 14 days.

In a study done by Nicholls JM et al., histopathological findings of squamous metaplasia were reported in one out of six cases [22]. In a study done by Bruce-Brand C et al., one out of four patients were reported with squamous metaplasia [17].

Pneumocyte proliferation was reported in four out of eight patients in the present study. Similar finding was also observed in studies done by Konopka K et al., and Pei F et al., [25,26].

Evidence suggests that COVID-19 causes an intense inflammatory reaction marked by upregulation of cytokines which result in lung injury [27]. Evidence suggested that corticosteroid therapy can markedly reduce hospital mortality in patients with severe COVID-19 [28]. The presence of organising pneumonia may be explained by the efficacy of corticosteroid therapy. In the present study, two out of eight patients demonstrated organising pneumonia despite all the eight patients received corticosteroid therapy from the time of admission. This may be due to manifestation of far progressed disease by the time of admission.

Similar findings of organising pneumonia were described in a study done by Zhang H et al., [29]. In a study done by Bruce-Brand C et al., three out of four patients were reported with organising pneumonia [17].

Much has been written regarding endothelial damage and coagulopathy caused by COVID-19 [30]. In the present study, three patients demonstrated fibrin thrombi and only one among three had elevated D-dimer levels. All patients were on anticoagulant therapy. In a study done by Bruce-Brand C et al., three out of four had fibrin thrombi with elevated D-dimer levels, despite all being on anticoagulant therapy [17].

Limitation(s)

Sample size being small, limits our ability to draw conclusions and it is not statistically significant. Single centered study results cannot be extrapolated to other population.

CONCLUSION(S)

Most common histopathological findings in COVID-19 affected lung biopsy included acute lung injury, hyaline membrane formation and DAD. Targeted core needle biopsy of lung is guarded, trouble free procedure and effective to study postmortem COVID-19 cases.

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