

# Diffuse alveolar damage in the covid-19 pandemic year of 2020 compared to pre-pandemic year: a study from Medico-Legal Directorate in Iraq

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## ABSTRACT

**INTRODUCTION:** Acute lung injury (ALI) and its severe form Acute Respiratory Distress Syndrome (ARDS) is an abrupt onset of hypoxemia and bilateral pulmonary oedema without cardiac failure. Histopathologically, ARDS and ALI show inflammation associated with increased pulmonary vascular permeability, oedema, and epithelial cell death; changes that are pathologically called diffuse alveolar damage (DAD). DAD can be seen in various conditions, and it has been recently reported in Covid -19 infection.

**OBJECTIVE:** This study aims to determine the percentage of diffuse alveolar damage in autopsies of medical suspected death during the covid-19 pandemic year of 2020 and compare it to that reported in the pre-pandemic year of 2019.

**METHODS:** A retrospective study was done in the histopathology division of the Medico-Legal Directorate in Baghdad on medical records registered from 1<sup>st</sup> January 2020 to 31<sup>st</sup> December 2020, the year of the covid-19 pandemic. We enrolled all medically suspected death referred to the MLD for post mortem examination, determined the percentage of DAD among them, and then compared it to that reported in 2019, the pre-pandemic year.

**RESULTS:** Six hundred nineteen (619) out of 3797 (16.3%) autopsies had features of diffuse alveolar damage in 2020 (pandemic year), compared to only 13 out of 2609 (0.5%) in the 2019 (pre-pandemic year). Of those 619, 410 (66.24%) were males, and 209 (33.76 %) were females. While in the pre-pandemic year, the males were slightly more predominant than females; 7 (53.846%) were males, and 6 (46.154%) were females. DAD was reported in all ages, from 1 to 91 years, with the highest percentages in the 5th and 6th decades, 25.52% and 23.90%, respectively. While those in the pre-pandemic year, most of them were children and young adults.

**CONCLUSION:** DAD is remarkably increased as a cause of medically suspected death referred to MLD in 2020, the year of covid-19 pandemic, compared to that reported in 2019. It is more recorded in males and the 5th and 6th decades of life.

**Key words:** Acute lung injury, Acute Respiratory Distress Syndrome, Diffuse Alveolar Damage, Multisystem Organ Diseases, Covid-19.

## INTRODUCTION

Acute lung injury (ALI) is characterised by an abrupt onset of hypoxaemia and bilateral pulmonary oedema in the absence of cardiac failure, i.e. Non-cardiogenic pulmonary oedema. Acute Respiratory Distress Syndrome (ARDS) is a manifestation of severe ALI. Both ARDS and ALI are characterised by inflammation, associated with increased pulmonary vascular permeability, oedema, and epithelial cell death. The histological manifestation of these conditions is scientifically called Diffuse Alveolar Damage

(DAD).<sup>1</sup>

First studies on DAD as a pathological phenomenon in the lung were published over 50 years ago. But the phenomenon is still a mystery; in one of the first studies on this issue, Cederberg et al. in 1965 described hyaline membranes at the autopsy of patients who had received oxygen at a high concentration. [2]

Katzenstein has used diffuse alveolar damage in his review of data from over 400 patients with DAD. He proposed that DAD is a nonspecific reaction of the lung to many damaging

agents, in which endothelial and alveolar cell injury leads to fluid and cellular exudation, with hyaline membranes and oedema being prominent features.<sup>3</sup> A few years later, Askin and Katzenstein reported that pneumocystis infection might masquerade as DAD.<sup>4</sup> In patients with acquired immunodeficiency syndrome (AIDS) who had DAD, cytomegalovirus and/or pneumocystis carinii was the most likely etiology.<sup>5</sup>

ALI is a well-recognised complication of diverse conditions, including pulmonary and systemic disorders. These conditions are infections, physical injury, inhaled irritants, chemical injury, hematological conditions, uremia, pancreatitis, cardiopulmonary bypass, hypersensitivity reaction, and drugs.<sup>1</sup>

Morphologically, DAD has many histopathological Phases, including exudative, proliferative, and fibrotic or organising phases.<sup>6,7</sup>

DAD is consider the pathological correlate of ARDS, but not all patients with ARDS have DAD and not all patients with DAD have ARDS; only about, half of the patients with ARDS displayed typical DAD in postmortem examination, and less than half of patients with postmortem autopsy findings of DAD had been diagnosed clinically as ARDS.<sup>8</sup>

Goodpasture and co-authors described DAD in the autopsy findings of patients who had suffered from influenza in 1918.<sup>9</sup> Moreover, in avian influenza A H5N1 virus infection and severe acute respiratory syndrome (SARS), DAD is a common autopsy finding in the lungs.<sup>10-12</sup> Similarly, DAD was reported in some of the autopsies of patients who died of H1N1-influenza A infection.<sup>13</sup> Studies have shown that DAD, thromboembolism, and non-specific shock injuries in multiple organs are the main histopathological features in patients with covid-19.<sup>14-16</sup>

This study was conducted on records of the first year of the pandemic of covid-19 in Iraq. At the medico-legal directorate, we were re-

ceiving dead persons who died suddenly, unexpectedly or violently. A medical examiner or forensic pathologist performs a postmortem examination to determine the cause of death.

This study aims to determine the percentage of diffuse alveolar damage in lung autopsies of suspected medical death referred to the Medico-legal Directorate (MLD) in Baghdad during 2020, the pandemic year of Covid-19, and compared to that reported in the pre-pandemic year of 2019, distributing the result according to age and gender.

## METHODS

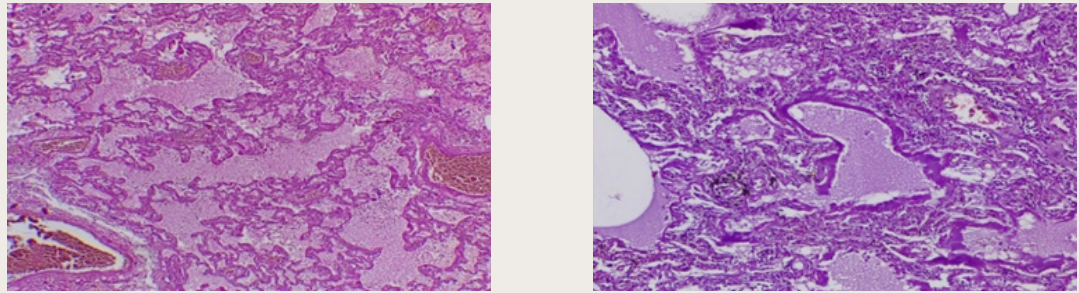
**Setting and Design:** A retrospective descriptive study was done in 2021 at the department of histopathology of Medico-Lega Directorate in Baghdad on data recorded from 1<sup>st</sup> January 2020 to 31<sup>st</sup> December 2020.

**Ethical consideration:** The research ethics committee has approved the proposal for this study, and the administration of the MLD has agreed to use the records to conduct this study. All data were kept confidential during all the stages of this study.

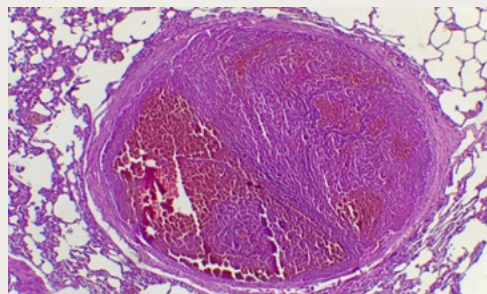
**Definition of the cases and exclusion criteria:** we enrolled the records of any medically suspected death that was received at the department of histopathology in the MLD to perform a histopathological examination of the lung and other organs during the study period. We excluded only cases that had undergone a decomposing process or had missing data.

**Outcomes:** For the sake of this study, we used these histopathological definitions:

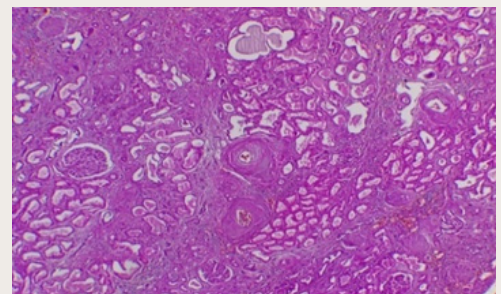
**DAD:** Demonstrating injury to the alveolar lining and endothelial cells with the alveolar influx of fluid and acute inflammatory cells and hyaline membrane formation in the early stage or finding proliferative changes involving alveolar and bronchiolar lining cells and interstitial cells at later stages, as shown in **Figure 1 (A, B)**. **Pulmonary thromboembolism** is defined as arterial or venous occlusion by thrombi, see **figure 2**. **Granulomatous lung disease** is defined as epithelioid



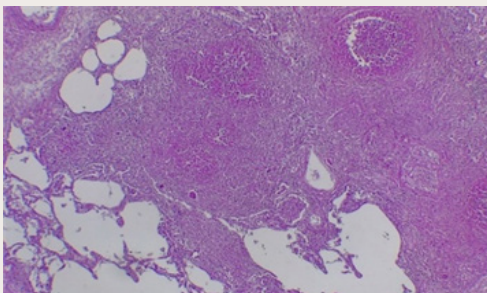
**Figure 1** | A: DAD/ early phase at low magnification, all alveoli are filled with fibrin-rich oedema fluid and inflammatory cells from damage to endothelial and epithelial cells. (H&E, original magnification, X4), B: DAD, At medium magnification (right panel), the alveolar walls are congested and expanded due to inflammation with eosinophilic hyaline membrane on alveolar ducts. (H&E, original magnification, X10)



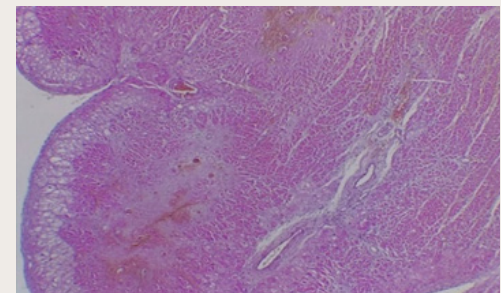
**Figure 2** | Pulmonary thromboembolism: within this pulmonary artery are interdigitating areas of pale pink and red that form the lines of Zahn characteristic of a thrombus. (H&E, original magnification, X4)



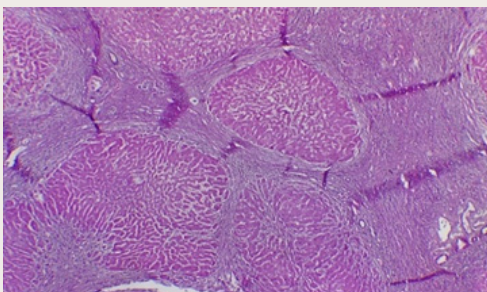
**Figure 5** | Chronic pyelonephritis: Showed renal tubular atrophy with prominent proteinaceous casts (so-called thyroidization) and interstitial fibrosis. (H&E, Original magnification, X4)



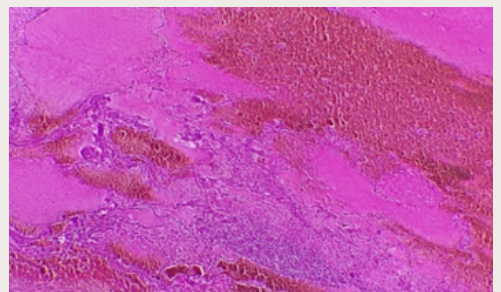
**Figure 3** | Granulomatous lung disease: Well-defined granulomas have rounded outlines with discrete borders, composed of epithelioid cells, along with lymphocytes and Langhans giant cells, central caseous necrosis. (H&E, original magnification, X4)



**Figure 6** | Myocardial infarction: Myocardial fibers are beginning to lose cross-striations and the nuclei are not clearly visible. Acute inflammatory cells start to infiltrate. (H&E, original magnification, X4)



**Figure 4** | Liver cirrhosis: Micro-nodular hepatic cirrhosis at low power, with regenerative nodules of hepatocytes ringed by thick fibrous bands of collagenous fibrosis, within the fibrous bands are lymphocytic infiltrates and a proliferation of bile ductules. (H&E, original Magnification, X4)



**Figure 7** | Viable blood clot in other blood vessels: Showed line of Zahn with interdigitating areas of pale pink and red. These lines represent layers of RBC, platelets, and fibrin that are laid down as the thrombus formed. (H&E, original Magnification, X4)

granuloma with central caseous necrosis surrounded by lymphocytes and multinucleated giant cells, as shown in **figure 3**. **Liver cirrhosis** is defined as the regenerative nodules of hepatocytes surrounded by fibrous connective tissue that bridge between portal tracts; within this collagenous tissue, scattered lymphocytes and proliferation of bile ducts are seen, see **figure 4**. **Chronic pyelonephritis** is defined as tubular thyroidization (filled with colloid casts), tubular atrophy, interstitial fibrosis and inflammation (intense diffuse lympho-plasmacytic inflammatory infiltrate with germinal centres), see **figure 5**. **Myocardial infarction** is defined as necrosis (death) of myocardial tissue, as in **figure 6**. **Viable blood clot** has apparent laminations called lines of Zahn, which are pale platelet and fibrin deposits alternating with darker red cell-rich layers, see **figure 7**.

**Procedure:** we enrolled 3797 autopsies that fulfil the inclusion criteria in this study. We compared the results to 2609 autopsies recorded in 2019, before the pandemic. In our department, we routinely take a sample from the lungs, liver, kidneys, heart and blood clots from the corps of any medically suspected death. The samples are fixed in 10 % formalin solution and dehydrated in different concentrations of alcohol then embedded in paraffin wax. Serial sections of 3 mm thickness are prepared, de-paraffined, and stained with hematoxylin and eosin stains. The slides are examined by an experienced histopathologist who writes the final report. The author has reviewed all the reports enrolled in this study.

**Statistical analysis:** we reported DAD in 2020 and 2019 as percentages of DAD to the total autopsies examined. All categorical variables were shown as ratios. Microsoft excel program has been used to tabulate the result and calculate the ratios.

## RESULTS

We examined 3797 autopsies referred to the histopathology department in MLD in Baghdad between 1<sup>st</sup> January 2020 and 31<sup>st</sup> December 2020 (the first year of a covid-19

**Table 1** | The prevalence of DAD during pandemic and pre-pandemic years.

Year	2020 (pandemic)	2019 (pre-pandemic)
Total autopsies	3797	2609
Cases with DAD	619	13
Ratio of DAD	16.3%	0.5%

**Table 2** | Distribution of DAD reported in pandemic and pre-pandemic according to gender.

Year	2020 ( pandemic)	2019 (pre-pandemic)
Cases with DAD	619	13
Male	410 (66.24%)	7 (53.846%)
Female	209 (33.76%)	6 (46.154%)

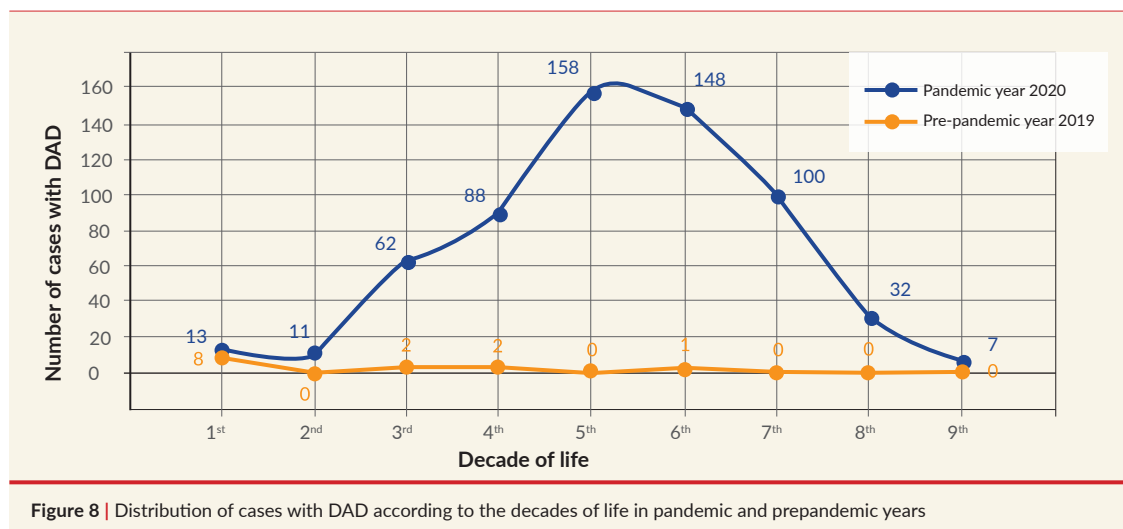
pandemic); 619 had a histopathological diagnosis of DAD, representing 16.3 %. Of those 619, 410 (66.24%) were males, and 209 (33.76%) were females. In 2019 the pre-pandemic year, only 13 out of 2609 (0.5 %) of autopsies were diagnosed with DAD, representing 0.5 %; 7 (53.846%) were males, and 6 (46.154%) were females. See **table 1 and 2**

The age range was between (1 day-91) years, both in pandemic and pre-pandemic years. In the pandemic year 2020, DAD was present mainly in (the 5th and 6th decades of age), 158 (25.52%) and 148 (23.90%), respectively. While in the pre-pandemic year, DAD was mainly present in children and young adults. See **figure 8**.

**Table 3** shown some associated co morbidities seen in histopathological examination of other organs. In 134 cases (21.65%), changes of myocardial infarction have been reported, and in 107 (17.29 %) viable clots in vessels other than pulmonary vessels were detected.

## DISCUSSION

In our study, the diagnosis of DAD is remarkably increased in the covid-19 pandemic year of 2020 compared to the pre-pandemic year of 2019: 619 (16.3%) out of 3797 versus 13 (0.5%) out of 2609, respectively. This finding showed an association between DAD and Covid-19 infection. Many studies have shown that DAD is the most common histopathological manifestation of covid-19.<sup>17-18</sup>



**Figure 8** | Distribution of cases with DAD according to the decades of life in pandemic and pre-pandemic years

**Table 3** | Associated co-morbidities as seen on histopathological examination of other organs in cases diagnosed with DAD in 2020

Co-morbid conditions	Number of cases	%	Male	Female
Myocardial infarction	134	21.65	91	43
Pulmonary thromboembolism	43	6.95	30	13
Viable blood clot in other vessels	107	17.29	63	44
Chronic liver disease (cirrhosis )	27	4.36	19	8
Chronic renal disease	7	1.13	5	2
Granulomatous lung disease	5	0.81	5	0

Of those with DAD reported in 2020, 410 (66.24%) were males, and 209 (33.76%) were females. While in the pre-pandemic year, out of the 13 autopsies with DAD, 7 (53.84%) were males, and 6 (46.15 %) were females. This is clearly shown that there may be an association between the development of DAD during the pandemic year and the gender, more among males than females. Studies have shown that the incidence of ARDS in males is consistent for all age groups. On the other hand, males with covid-19 have a higher rate of respiratory intubation, a longer length of hospital stay than females, and a higher death rate even when compared across age groups.<sup>19-20</sup>

During the pandemic year, we found that all age groups affected by DAD; the higher rate was reported in the 5<sup>th</sup> and 6<sup>th</sup> decade of life; 158/ 619 (25.52%) and 148/619 (23.90%), respectively. While during the pre-pandemic year, DAD was more in infants and early childhood (see **figure 8**).

These findings go with other previous stud-

ies which stated that ARDS is associated with increased morbidity and mortality in the elderly population ( $\geq 65$  years of age). In addition, age is widely reported as a risk factor for developing ARDS and is said to be a higher risk of COVID-19 mortality.<sup>21-22</sup>

Our study has found many chronic comorbidities associated with DAD (See **table 3**). The commonest was old myocardial infarction, 134 (21.65%) and the presence of viable clots in arteries other than the pulmonary, 107 (17.29%). We have also found chronic renal disease, chronic liver disease, granulomatous lung disease, and pulmonary thromboembolism. The study design we chose is unable to discover whether these chronic diseases are risk factors for developing DAD or it is just an accidental finding. The same is applied to pulmonary thromboembolism. Many studies have stated that comorbidities are risk factors for aggravating the mortality rate in infectious diseases, including Covid-19.<sup>23-25]</sup>

We think that prevalent DAD and findings

of comorbidities in 2020 were due to covid-19; however, we could not confirm the diagnosis by molecular tests, especially in the early months of the pandemic, due to the lack of such facilities. Based on the limitations and the initial results of this study, the administration of the MLD recommended receiving the clinical records of any suspected medical death referred to our directorate for postmortem examination, including the radiological and molecular test of covid-19. Also, the directorate has established a Real-Time-Polymerase Chain Reaction (RT-PCR) Laboratory for the diagnosis of covid-19 in June 2020 and issued instructions and the procedures for the proper burying of cadavers of covid-19 suspected death in March 2020.

Limitation of the study: the study faced many limitations; missing clinical and radiological data of the cases and lack of molecular confirmation of covid-19 especially in the first half of 2020. These limitation prevents us from doing a direct link between DAD and covid-19. However, we tried to share the results because we think that they can expand our knowledge, especially in the early days of the pandemic.

## CONCLUSION

DAD is remarkably increased as a cause of medically suspected death referred to MLD in 2020, the year of covid-19 pandemic, compared to that reported in 2019. It is more recorded in males and in the 5<sup>th</sup> and 6<sup>th</sup> decades of life. Many chronic comorbidities have been found in association with DAD; the more frequent were myocardial infarction and viable clots in arteries other than the pulmonary.

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**Abbreviations list:** Acquired immunodeficiency syndrome (AIDS), Acute lung injury (ALI), Acute Respiratory Distress Syndrome (ARDS), Diffuse Alveolar Damage (DAD), Medico-legal Directorate (MLD), Real-Time-Polymerase Chain Reaction (RT-PCR)..

**Conflict of interest:** Authors have nothing to declare.

**Funding:** Authors received no funds to complete this study a part from self funding.