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SARS-CoV-2 , MERS-CoV and SARS-CoV, the Emerging Coronaviruses: An insight into the Pathological features

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Abstract

The zoonoses as causes of human infections have been progressively reported. Many of these zoonotic diseases are viruses and cause severe pulmonary infections. The recent outbreak of severe acute respiratory syndrome (SARS) coronavirus-2 in Wuhan/China has now circulated worldwide with an elevation of a death rate. This paper focuses on the pathology of three zoonotic coronaviruses SARS-CoV, MERS-CoV, and SARS-CoV-2 that have been emerged in the last two decades and caused severe lower respiratory infection and fatal pneumonia worldwide. However, scarce publications on pathological and ultrastructural features have been reported because of hardly accessible biopsy or autopsy due to cultural and religious intentions, additionally to avoid environmental contagion with consequent infection of health- care staff. Pathological findings have a vital role in improving the understanding of diseases, although it is rarely considered as a diagnostic tool for these types of infections. Additionally, histopathological features raise the suspicion of these diseases and help toward prompt control of viral spreading between populations. Similar pathological findings were reported in human infections with SARS-CoV, MERS-CoV and SARS-CoV-2 comprise bilateral diffuse alveolar damage (DAD), pulmonary edema, desquamation of pneumocytes and formation of hyaline membrane, indicative of acute respiratory distress syndrome (ARDS), presence of cellular fibromyxoid exudate accompanied by marked cytopathic effects, multinucleated syncytial cells along with atypical enlarged pneumocytes and interstitial mononuclear inflammatory infiltration dominated by lymphocytes in the affected lungs. However, in particular, the MERS-CoV mainly infects type II pneumocytes, while both SARS-CoV and SARS-CoV-2 also infect type 1 pneumocytes. In conclusion, COVID-19 macroscopic features are found in the chest and depend on the stage of the disease. While, the histopathological features are like those seen in SARS and MERS-coronavirus infections. Moreover, the nature of coronaviruses outbreaks, specially COVID-19 in many more countries, a greater awareness of SARS-CoV-2 pandemic infection is essential.

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Introduction

The coronaviruses involve a group of zoonotic viruses that cause a grave human disease comprising Severe Acute Respiratory Syndrome (SARS) /2002 and Middle East Respiratory Syndrome (MERS) / 2012 (Biscayart *et al.,* 2020; Zaki *et al.,* 2012; Peiris *et al.,*2003). In December 2019 a new highly pathogenic coronavirus SARS-CoV-2 has emerged in Wuhan / China, which causes fatal outbreaks in humans termed as coronavirus disease-2019 (COVID-2019) by WHO (https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020). COVID-19 is quickly circulated around the globe and pose a worldwide threat to public health (Zhu *et al.,* 2020). Coronaviruses are a single-strand RNA viruses that belong to subfamily Orthocoronavirinae in the family Coronaviridae, order Nidovirales and realm Riboviria (Perlman & Netland, 2009; Weiss & Leibowitz, 2011; Masters & Perlman, 2013; Fehr & Perlman, 2015; Weiss & Navas-Martin, 2005; Lai *et al.,* 2007; Lai & Cavanagh, 1997; Langereis *et al.,* 2010). The coronaviruses of the zoonotic origin are highly pathogenic, crossing the species barrier and causing high morbidity and mortality in human populations (Li *et al.,* 2005; Kupferschmidt, 2013; Cui *et al.,* 2019; who/cds/csr/gar/2003.11; Hijawi *et al.,* 2013; Drosten *et al.,* 2014; El-Kafrawy et al., 2019; https://www.who.int/emergencies/mers-cov/en/ ; Zumla *et al.,* 2015; Kim *et al.,* 2017; Oh *et al.,* 2018). Histopathological features and ultrastructural tissue examination have enabled the diagnosis of previous coronaviruses epidemics of SARS-CoV and MERS-CoV. Tse *et al.,* (2004) approved that post-mortem tissues were essential for the isolation of viable SARS-CoV. The autopsy investigation permits tissue to be collected for virological and ultrastructural examination. Besides, as combined with the proper lung morphological features, it is valuable to approve the diagnosis of SARS- CoV, particularly in clinically unapparent or suspicious but unconfirmed cases (Tse *et al.,* 2004). Ng *et al.,* (2016) mentioned that the histopathological investigations provided an important and valuable vision into the histopathologic changes that provide critical insights into the pathogenesis of MERS-CoV in humans. This review article intends to focus on the pathological and ultrastructural findings of three emerging zoonotic coronaviruses diseases SARS-CoV, MERS-CoV, and SARS-CoV-2.

Severe Acute Respiratory Syndrome (SARS-CoV)

SARS is a rapidly fatal viral pulmonary infection caused by a coronavirus (SARS-CoV). The outbreak was first reported in China between 2002 -2004. Later on, the disease was circulated in Europe and North America due to international travelers. The total reported cases were 8096 from 29 countries involving 774 fatalities (9.6%). The virus genetic map indicates the insertion of SARS-CoV into the human population from civets cat or other mammals in the live-animal markets of China (Guan *et al.,* 2003). Later on, SARS coronavirus was recognized genetically from the horseshoe bats population, indicating that bats were the origin of the virus before circulating into the civet cat in the live-animal markets of China (Hu *et al.,* 2017). The virus was so-called super spreaders because of its transmission between humans via respiratory droplets and close interactions with some individuals (Leung *et al.,* 2004), and clinical signs appeared within 2-12 days after infection. The deaths were occurred in elderly and immunosuppression individuals but were limited in youngers and 12 years of age (Chan *et al.,* 2007). The common symptoms were non-specific, including malaise and myalgia

associated with lymphopenia, thrombocytopenia, and elevation in the Lactate dehydrogenase (LDH) and C-Reactive protein (Nicholls *et al.,* 2005). SARS clinical severe cases termed "atypical pneumonia," and the post-mortem examination of the dead patients has been principally used to determine the existing histopathological data for SARS-CoV. According to Nicholls *et al.,* (2003) and Gu *et al.,* (2005), the upper respiratory tract revealed mucopurulent materials and the lungs of the dead patients were edematous, congested, and heavily weighted up to 2100 gm accompanied by irregular patchy areas of consolidation and absence of pathognomic features (Figure. 1). The histological features SARS were non-specific and depended on the stage of diseases (Ding *et al.,* 2003; Franks *et al.,* 2003) , where acute diffuse alveolar damage was usually occurred in the early stage of infection (<11 days), accompanied with a mixture of acute fibrinous, organization pneumonia in the later stages of the disease (Figure. 2). Additionally, intravascular microthrombi, squamous metaplasia, multinuclear giant cell formation with intracytoplasmic viral inclusions also have been described (Figure. 3) (Ding *et al.,* 2003). Affected lungs also showed a mild increase of the alveolar macrophages with hyaline membrane formation accompanied by a slight increase in interstitial lymphocytes. Nicholls *et al.,* (2003) also described the presence of occasional pneumocytes revealing viral cytopathic-like changes, including cytomegaly with nuclear enlargement and prominent nucleoli. Additionally, Tse *et al.,* (2004) mentioned that pneumocytes were the primary target site of infection with various degrees of bronchiolitis obliterans organizing pneumonia (BOOP); the presence of multinucleated pneumocytes and diffuse alveolar damage (DAD). Though these features are non-specific, therefore, their combination occurrence, jointly with positive serological/ microbiological investigations and ultrastructural tissue examination, supports SARS diagnosis to be confirmed. Tse *et al.,* (2004) also approved the holding of SARS-CoV by the lung and small intestinal tissue samples supporting the successful isolation of the virus from these tissues. Few viral-like elements enclosing the pneumocytes were observed in the ultrastructural investigation (Figure. 4A) (Tse *et al.,* 2004). The viral particles about 60-90 nm in size were accumulated within dilated cytoplasmic vesicles and reminiscent of endoplasmic reticulum, which showed a club-shaped projection that gave appearance similar to that observed in Vero cell culture (Figure. 4 B, C). Additionally, some well-preserved viral particles revealed a ring closely underneath the envelope; the coronaviruses appeared as typical helical nucleocapsid with typical electron lucent-center in cross-section of these particles. However, neither macrophages nor other cell types in the lung were revealed viral-like particles (Tse *et al.,* 2004).

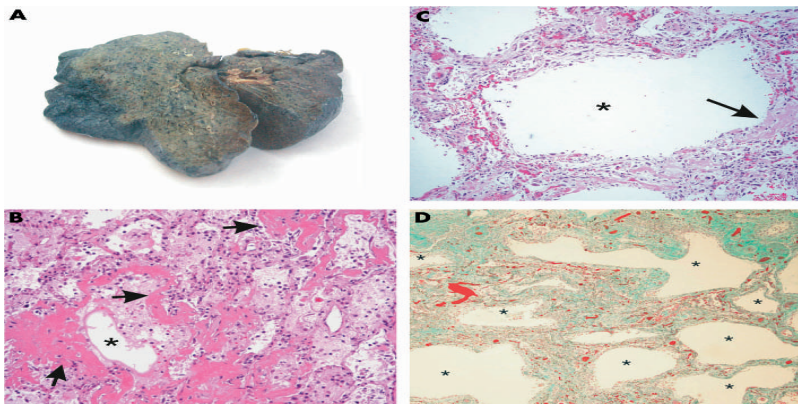

Figure.1: Shows the gross and histopathological features of lungs from patients with severe acute respiratory syndrome (A)Lungs with extensive consolidation accompanied with a greyish cut surface that appeared in the majorities of the patients. (B) Diffuse alveolar damage (airspaces are indicated by asterisks) the features of acute phase accompanied with hyaline membrane formation (arrows) and edema were seen in all patients (haematoxylin and eosin stain; original magnification, 6100). (C) Dilatation of airspace accompanied with interstitial thickening and mild infiltration of inflammatory cells (the asterisk indicates the dilated airspace). Hyaline membrane was evident in small amount as indicated by the arrow (haematoxylin and eosin stain; original magnification, 6100); (D) lungs of patients with marked interstitial fibrosis and honeycombing (asterisks indicated the abnormally dilated airspaces; Masson's trichrome stain; original magnification, 640). (source: Tse *et al.,* 2004).

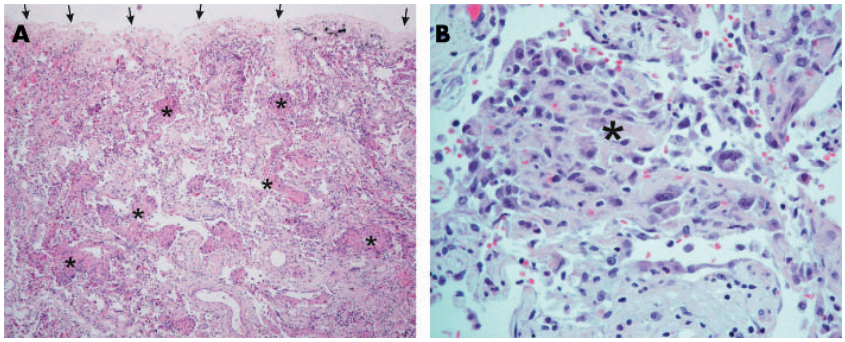

Figure. 2: Shows various pulmonary pathological changes including: Bronchiolitis obliterans organizing pneumonia (BOOP)-like lesion in patients with severe acute respiratory syndrome. (A) A BOOP-like lesion was evident with cellular organizing plugs within the small airways and airspaces (asterisks indicate some of the lesions). Lesions were typically located in the subpleural region (the visceral pleural surface is designated by arrows; haematoxylin and eosin stain; original magnification, 640). (B) Higher power view of cellular organizing plugs (asterisk). The main cellular component consisted of histiocytes, which were CD68 positive (data not shown) (haematoxylin and eosin stain; original magnification, 6200). (source: Tse *et al.,* (2004).

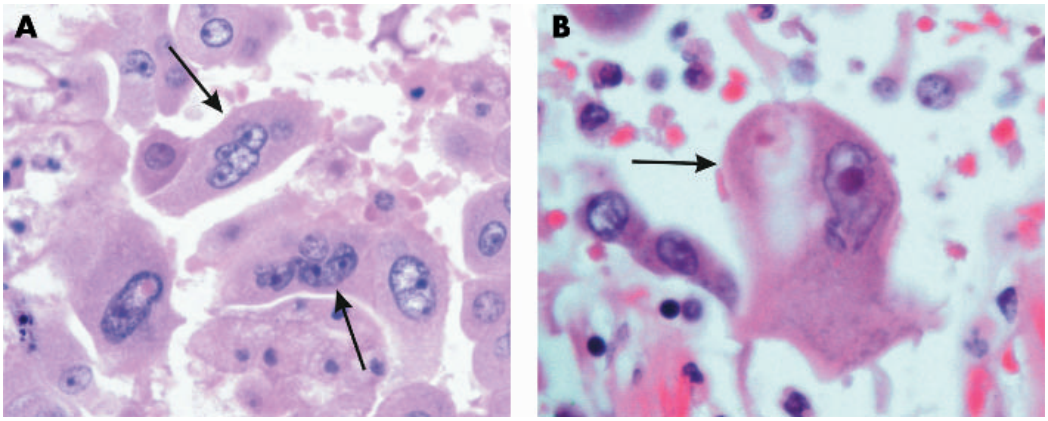

Figure. 3: Lung sections from patients with Atypical pneumocytes with severe acute respiratory syndrome. (A) The multi-nucleated giant pneumocytes with various nuclei distribution (indicated by arrows; haematoxylin and eosin stain; original magnification,

6400). (B) A giant atypical pneumocyte with prominent eosinophilic nucleoli (indicated by arrow; haematoxylin and eosin stain; original magnification, 6400). (source: Tse *et al.,* (2004).

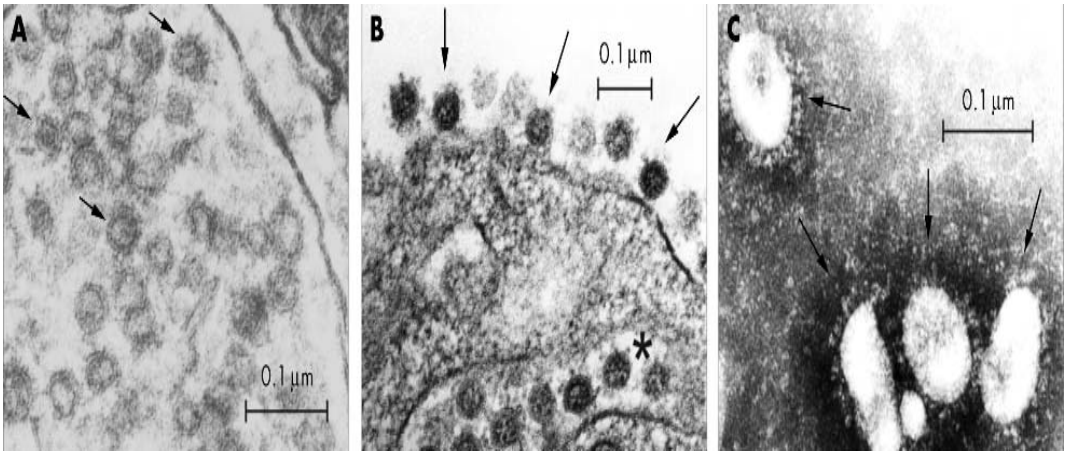

Figure 4. Shows the ultrastructural features of viral particle containing lung cells. (A) Dilated cytoplasmic vesicles contain viral-like particles ranging from 60 to 90 in sizes. Some of the better preserved structures consistent with viral envelopes are indicated by the arrows. The overall appearance of the virus was different from that of multivesicular bodies, which can be seen in some normal cells. Vero cell culture was used for comparison (B and C). (B) Section of an infected Vero cell showing similar features to those seen in the pneumocytes. The viral particles are found within the endoplasmic reticulum (indicated by the asterisk) and on the surface of the cytoplasmic membrane (indicated by the arrows). (C) Typical morphology of coronavirus particles in the supernatant of a Vero cell culture (indicated by the arrows; negative staining with 2% phosphotungstic acid). (source: Tse *et al.,* (2004).

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

MERS-CoV is the cause of acute respiratory syndrome associated with a high case fatality rate (804 fatalities & 35.5% case fatality) from 2266 total confirmed cases (http://www.who.int/emergencies/mers-cov/en/). The first MERS case was reported in 2012 in a patient from King Saudi Arabia, who suffered from respiratory failure and renal failure (Zaki *et al.,* 2012). Unlike the rapid circulation and consequent latency of SARS-CoV, MERS-CoV was continued to spreading and producing sporadic outbreaks within the Arabian Peninsula as well as in countries where the infected patients traveled. MERS is a zoonotic disease, and the dromedary camels were the source of transmission into human populations according to the results of serological and molecular investigations (Azhar *et al.,* 2016). Subsequently, doubt has been raised about the role of camels as an intermediate host or reservoir, and the studies have found a genomic fragments material of MERS-CoV identical to humans in bat populations) (Memish *et al.,* 2013). Human- to the human nosocomial transmission had occurred in the most reported cases, such as the outbreak that occurred in a Korean hospital, when a single patient admitted and led to 186 infections comprised 36 fatal cases (Arabi *et al.,* 2017). Person- to- person spread within the household have been documented as patient respiratory secretions and close nearby bring the high risk of transmission (Arwady *et*

al., 2016). MERS occurs in various clinical presentations from asymptomatic infection about 25% (Oboho *et al.,* 2015), to severe disease in greatest risk groups, including older adults, diabetes, and heart disease patients that are liable for the development of respiratory failure (Arabi *et al.,* 2017). The serological studies reported positive results in 0.15% of patients with a higher probability of positive serology among individuals with a history of camel-exposure, who might act as an asymptomatic source of infection (Müller *et al.,* 205). MERS patients revealed non-specific clinical symptoms, including myalgias, sore throat, and runny nose, with the incubation period from 2-14 days. Additionally, extrapulmonary manifestations include gastrointestinal distress, and neurological sequelae have been reported in some cases accompanied by respiratory symptoms (Arabi *et al.,* 2017). Rarely, postmortem was done for MERS-CoV cases. Therefore, the available pathological findings are limited for MERS cases (Walker, 2016). The first autopsy performed on a fatal MERS-CoV case in the world (from a hospital outbreak in the United Arab Emirates in April 2014), was done by Ng *et al.,* (2016), who determined the histopathological, immunohistochemical, and ultrastructural finding as well. The important autopsy findings were massive effusion in pleural (about 5 Liter), pericardial (150 ml), and abdominal cavities accompanied by generalized congestion and consolidation of the lungs. Diffuse alveolar damage, alveolar fibrin deposits, hyaline membranes formation, type 2 pneumocyte hyperplasia, edema, and various type of inflammatory cells invading alveolar septa, and rare multinucleated syncytial cells, were the common pulmonary histopathological features. Both alveoli and subpleural showed dispersed foci of necrotic debris. However, no viral inclusions were seen, and few anthracosis was found (Figure 5 A, B, C).

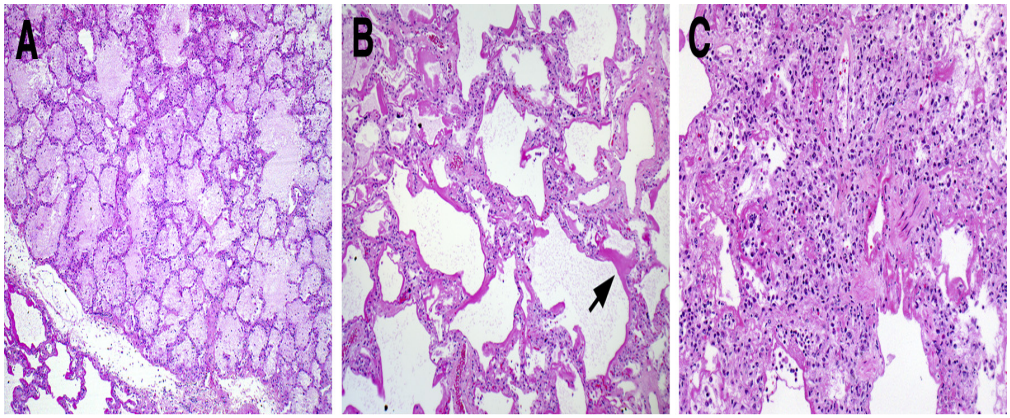

Figure 5: Histopathology of lung from MERS-CoV patient. A: Pulmonary edema. B: Diffuse alveolar damage, including prominent hyaline membrane formation (arrow). C: Alveolar fibrin deposits, type 2 pneumocyte hyperplasia, and thickened alveolar septa involved by edema and a mixed inflammatory infiltrate. Original magnification: x5 (A); x20 (B-C). (Source: Ng *et al.,* 2016).

The tracheal and bronchi sections were also revealed mild to moderate lymphocytic mucosal and submucosal inflammation with infiltration of neutrophils and plasm with focal necrosis in the bronchial submucosal glands (Figure 6 A, B).

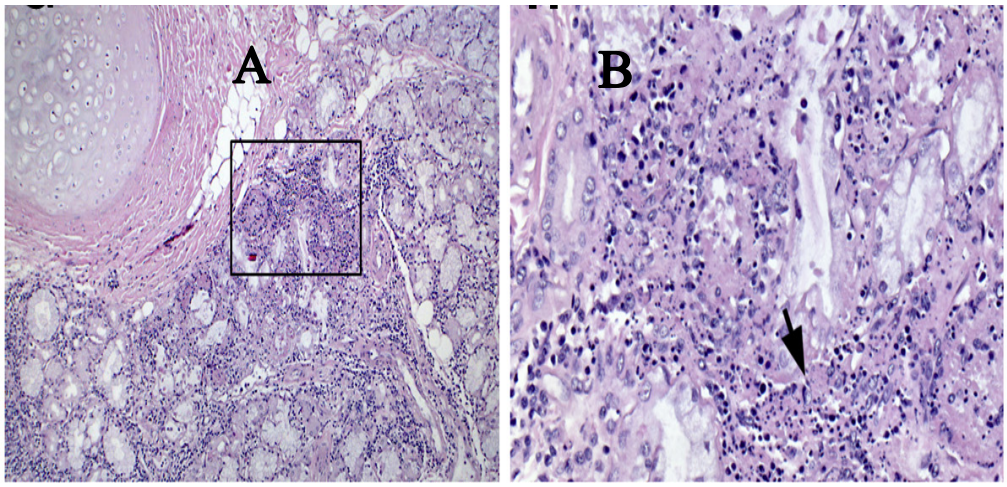

Figure 6: Histopathology of lung from MERS-CoV patient. A: Moderate lymphocytic inflammation of the submucosal glands. B: Magnified from the boxed area in B. Submucosal glands with focal areas of necrosis (arrow). Original magnification: x10 (A); x40 (B) (Ng *et al.,* 2016)

Immunostaining for MERS-CoV antigens was identified in both unremarkable and necrotic bronchial submucosal glands (Ng *et al.,* 2016; Alsaad *et al.,* 2018). Various pathological changes were also seen in the kidney including, thickened Bowman capsules, globally sclerotic glomeruli (5% to 10% of glomeruli) severe atherosclerosis, and hyaline arteriolosclerosis, patchy interstitial inflammation, and intratubular proteinaceous and granular casts. Multiple lymph nodes were revealed a reduction in the lymphoid follicles and a tough interfollicular proliferation of pleomorphic immunoblasts mixed with a polymorphous population of reactive lymphocytes. Extensive myocyte hypertrophy, moderate coronary atherosclerosis, and sparse fibrosis were seen in the heart sections.

The liver sections showed moderate steatosis, scattered calcifications, and mild portal tract and lobular lymphocytic inflammation. The sections of the cerebrum and cerebellum were unremarkable (Alsaad *et al.,* 2018). Pneumocytes and epithelial syncytial cells were identified as essential targets of MERS-CoV antigen by double staining immunoassays that used anti-MERS-CoV antibodies paired with immunohistochemistry for cytokeratin and surfactant. The colonialization of MERS-CoV was seen scattered in cytoplasm of pneumocytes and syncytial cells by immunostaining with dipeptidyl peptidase 4 (PPD-4). However, no evidence of MERS-CoV antigens was identified in the kidney. Immunohistochemistry for MERS-CoV was also negative in numerous specimens from different organs, including kidney, liver, spleen, several lymph nodes, bone marrow, small intestine, and colon (Figure. 7 A-D) (Alsaad *et al.,* 2018).

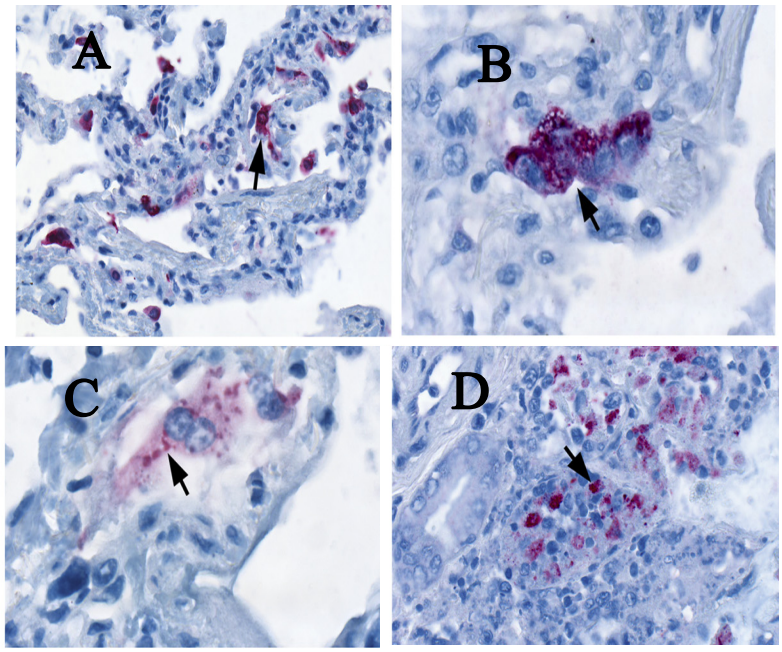

Figure.7: A-D: Immunostaining of MERS-CoV antigen in pneumocytes (Ab 1511; D, arrow), a multinucleated syncytial cell (Ab 1511; E, arrow), and a binucleated cell (Ab 1514; F, arrow). Immunostaining of MERS-CoV antigen in necrotic foci of submucosal glands (arrow; Ab 1512). Ab, antibody; MERS-CoV, Middle East respiratory syndrome coronavirus. Original magnification: x20(A); x 75 (B); x100 (C); x63 (D) (Source: Alsaad *et al.,* 2018).

The electron microscopy observations revealed degenerated and infected pneumocytes that covered by hyaline membrane comprised of the fibrine basement membrane (Fig.8 A). Viral particles were found in the membrane-bound vesicles that appeared as individuals or groups, spherical shaped about 50 to 150 nm in diameter (Figure. 8B) (Source: Alsaad et al., 2018). [43]

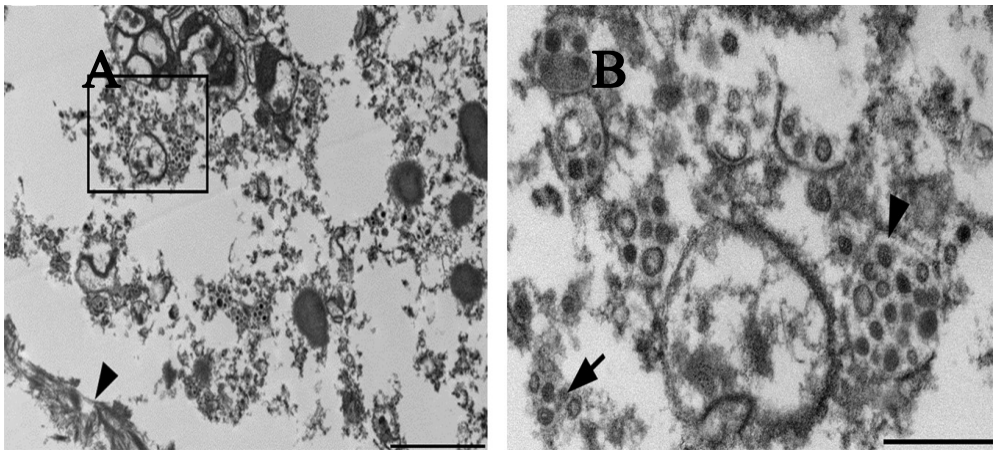

Figure 8: A: Fragmented pneumocyte infected with MERS-CoV, hyaline membrane (arrowhead) present. B: Magnified from the boxed area in A. MERS-CoV virions dispersed as single particles (arrow) or in clusters within membrane-bound vesicles

(arrowhead). Spherical and pleomorphic particles ranged in size from 50 to 150 nm diameter. Scale bars: 2 mm (A); 500 nm (B). (Source: Alsaad *et al.,* 2018). [43]

Alsaad *et al.,* (2018) [43] reported the second MERS-CoV case autopsy, a 33-year-old man with primary cutaneous T cell lymphoma on the face, trunk, scalp, and lower and upper limbs, who was under chemotherapy followed by radiation, he developed a fever and productive cough and was treated as healthcare-associated pneumonia. Sputum was MERS-CoV rRT-PCR positive until his death. Severe acute hematological pneumonia and exudative diffuse alveolar damage (DAD) were recognized in the lungs that are heterogeneous in severity. The lung parenchymal architecture was preserved in less affected areas. However, scattered mononuclear inflammatory cells and pigmented pulmonary macrophages were distributed in the interstitium and alveolar spaces accompanied by different amounts of filamentous fibrin deposits. Sloughing of alveolar cells, sporadic multinucleated syncytial cells, congestion of alveolar walls, and hyaline membrane formation were also reported. However, no granuloma was recognized, and acute and chronic inflammatory cells infiltrate seen in the focal peri-bronchiolar site. Additionally, interstitial lung vasculature was infiltrated by focal subendothelial lymphocytes. Electron microscopy revealed the presence of viral inclusions both in respiratory epithelium and proximal renal tubular epithelial cells (Alsaad *et al.,* 2018). Histopathology of renal biopsy from MERS-CoV infection was reported by Cha *et al.,* (2016) from a man 8 weeks after infection. Acute tubular sclerosis, accompanied by proteinaceous cast formation and acute tubulointerstitial nephritis, was obvious. However, no glomerulosclerosis was recognized. Additionally, the viral component was not seen in renal tissue by electron microscopy and in situ hybridization Cha et al., (2016). The pathogenesis of MERS-CoV in human tissue has been studied in ex vivo and animal models (Memish *et al.,* 2013; Zhou *et al.,* 2017). Similar replication kinetics and cellular tropism were found between camel-isolated to human-isolated MERS-CoV strains. All MERS-CoV strains were infected non-ciliated bronchial epithelium and alveolar epithelial cells comprise type II pneumocytes, though no infection was determined in pulmonary macrophages (Chan *et al.,* 2014). Yeung *et al.,* (2016) reported that MERS-CoV produces infection of multiple cell types, including renal tubular cells, vascular endothelial cells, and podocytes. However, experimental infection of the small intestine explant with MERS-CoV showed limited infection to the enterocytes surface and formation of syncytial cells. Consequently, Corman *et al.,* (2016) found that MERS-CoV patients shed virus in their stool and urine. After the detection of the virus, Li *et al.,* (2017) used a mouse model to adapt MERS-CoV, especially for the DPP-4 receptor. Moreover, understanding the inflammatory pathway and viral localization in the lungs, brain, heart, spleen, and intestine have been supported by these animal models. Another model used to study the pathology of MERS-CoV is a rhesus macaque that showed pulmonary consolidation, edematous lung lesions, and pneumonia, which were identical to the disease phenotypes, as seen in humans. Moreover, histopathological features revealed hyaline membrane formation, acute diffuse alveolar damage, and frequently seen multinucleated giant cells. However, no infectious virus was identified in the blood, upper respiratory tract, and other solid organs, and only was found in the lung (de Wit *et al.,* 2013; Yao *et al.,* 2014).

Severe acute respiratory syndrome- coronavirus 2 (SARS-CoV2/ COVID- 19)

COVID-19 is the third zoonotic human coronavirus disease of the century, which has caused panic and severe fear among the population around the globe. COVID-19 is considered as an acute resolved disease. Still, it can also be deadly, with a 2 % case fatality rate and massive alveolar damage, besides the progressive respiratory failure occurred in severe disease (Huang *et al.,* 2020). It was first recognized on December 12th, 2019, about 27 human cases of viral pneumonia in Wuhan/ Republic of China, furthermore 7 patients were seriously ill. All those patients were exposed to farm animals, bats, and snakes in Huanan Seafood wholesale Market and suggesting possible zoonosis (Chan *et al.,* 2020 ; Zhou *et al.,* 2017 ;Lu *et al.,* 2020). On January 7, 2020, a new coronavirus (SARA-CoV-2) was isolated from those patients. Earlier, the virus termed as novel coronavirus 2019 (2019-nCoV). Nonetheless, on February 11, 2020, the virus was named officially as COVID-19-SARS-CoV-2 by the WHO. More people have infected by this new virus in comparison to its two ancestors Globally, at January 30 th, 2021 about 102, 636,329 cases have been confirmed, over 2,216, 421 deaths and 74,329, 586 recovered comprise 26,086,647 active cases (25,981,318 (99.6%) in mild condition & 109,004 (0,4%) serious or critical) and 76,549,682 closed cases (74,239,586 (97 %) Recovered / Discharged) and 2,216, 421 (3 %) Deaths (https://www.worldometers.info/coronavirus/ 1/30/2021). The polymerase chain reaction was used for confirmation of COVID-19. The infection occurred in a patient with an average age of 55 years. However, cases appear to be sporadic in children (https://www.worldometers.info/coronavirus/).

The phylogenetic investigation and full-genome sequencing revealed that the cause of COVID-19 is a betacoronavirus located in the same subgenus as the severe acute respiratory syndrome virus (SARS-CoV), as well as several bat coronaviruses, but in a dissimilar clade. The bats appear as the primary source of infection. However, the transmission of the COVID-19 virus is still mysterious, whether transmitted directly from bats or through some other mechanism (through an intermediate host) (Perlman, 2020).

A thousand cases with SARS-CoV-2 have already reported in many countries, including European Union, United Kingdom, United States, Middle East, Iran, Africa, New Zealand, and Australia (Yang *et al.,* 2020; Shi *et al.,* 2020; Wang *et al.,* 2020 A). Numerous studies have been published and described the clinical features and distinguishing radiographical findings, especially chest CT scans (Huang *et al.,* 2020; Wang *et al.,* 2020 B). However, scarce studies have been described the postmortem findings and histopathological and ultrastructural features of tissue samples of patients with SARS-CoV-2 in recent months (Zhu *et al.,* 2020 ; Zhe *et al.,* 2020; Tian *et al.,* 2020).

Tian *et al.,* (2020) have reported the several reasons for the scarce autopsies and biopsies, of COVID-19 such as the sudden occurrence of the outbreaks, a massive number of hospitalized infected patients, lack of health care workers, rapidly transmission rate of the virus that leads to reduce the importance of different diagnostic test compare to clinical primacy and avoiding environmental contagion with consequent infection of health- care staff.

Hanley *et al.,* (2020) summarized the interpretation and guidelines released from the Royal College of Pathologists on postmortem examination for mortuary workers in suspected COVID-19 cases according to the understanding the risk of these diseases.

Routinely, the pathogens are classified based on their risk to humans to reduce the risk towards staff in clinical and research-related microbiology laboratories. Nevertheless, the health of mortuary staff and autopsy practice allow the potential hazard due to risk of spreading infectious pathogens during and after postmortem examination Hanley *et al.,* (2020). The hazard groups are accredited HG1-4 depending on the risk of human infection, the probability spread, and approach to treatment or prophylaxis. The SARS-CoV, MERES-CoV, and recently SARS-CoV-2 are approved as HG3 organisms sharing similar criteria of other viruses that categorized in this group like poliovirus, dengue, rabies virus, hepatitis virus B, C, D, and E, and HIV1 and 2. HG3 organisms may lead to severe human disease and pose a significant risk to workers and able to transmit to other humans; however, prophylaxis and treatment are commonly available. Therefore, the autopsy of COVID-19 cases needs appropriate precautions in place, and a slight risk can occur to mortuary workers dealing with these cases. Besides, experienced mortuary staffs must be able to handle any HG3 pathogen cases including COVID-19 cases.

The most important lung pathological features associated with early-phase COVID-2019 pneumonia in two lung cancer patients were described by Tian *et al.,* (2020). The first patient was a female aged 84 suffering from pulmonary cancer with a medical history of hypertension. Her enhanced chest CT scan revealed an irregular solid nodule in the right middle lobe with bilateral ground-glass opacity. Subsequently, she underwent a thoracoscopic resection of the right middle lobe. Postresection, there was a slight wheezing sound on auscultation and experienced some difficulty in breathing, chest tightness, wheezing, and dry cough, and she diagnosed as viral pneumonia. She transferred to a special isolation ward, and her pharyngeal swab test result was positive for SARS-CoV-2 (2019-nCoV). The patient died, although she was under a comprehensive treatment. The clinical information approved that the patient was exposed to another patient in the same room who was infected with SARS-CoV-2. Although the histopathological features of the resected specimen were compatible with typical adenocarcinoma, alveolar damage involving alveolar edema and proteinaceous exudates was reported (Figure. 9A). Moreover, inspissated spherical secretions of globules were also noticed (Figure. 9B) accompanied by vascular congestion with patchy and mild inflammatory infiltration. The airspaces were revealed focal fibrin clusters combined with mononuclear inflammatory cells and multinucleated giant cells (Figure. 9C), while no significant neutrophil infiltration was found in the tissue. Patchy and severe pneumocyte hyperplasia and interstitial thickening were noticed, demonstrating an ongoing reparative process. Viral inclusions were also noted (Figure. 9D).

The second case was for a 73 years aged male with a medical history of hypertension for 20 years, who was also suffering from lung cancer in the right lower lobe. The patient underwent to the right lower lobe lung resection. On 9 days postoperative, the patient developed a fever, dry cough, chest tightness, and muscle pain, and his PCR test for SARS-CoV-2 was positive. However, the patient was discharged after 20 days of treatment. According to the pathological examination, the diagnosis was adenocarcinoma. However, the adjacent area of the lung parenchyma revealed proteinaceous and fibrin exudates (Figure. 10A) accompanied by diffuse thickening of alveolar walls (Figure. 10B), involving type II pneumocyte hyperplasia and proliferating interstitial fibroblasts. The airspaces revealed focal fibroblast mass and multinucleated giant cells (Figure. 10C), demonstrating variable grades of the proliferative phase of

diffuse alveolar damage. Profuse alveolar macrophages and type II pneumocyte hyperplasia (Figure. 10D).

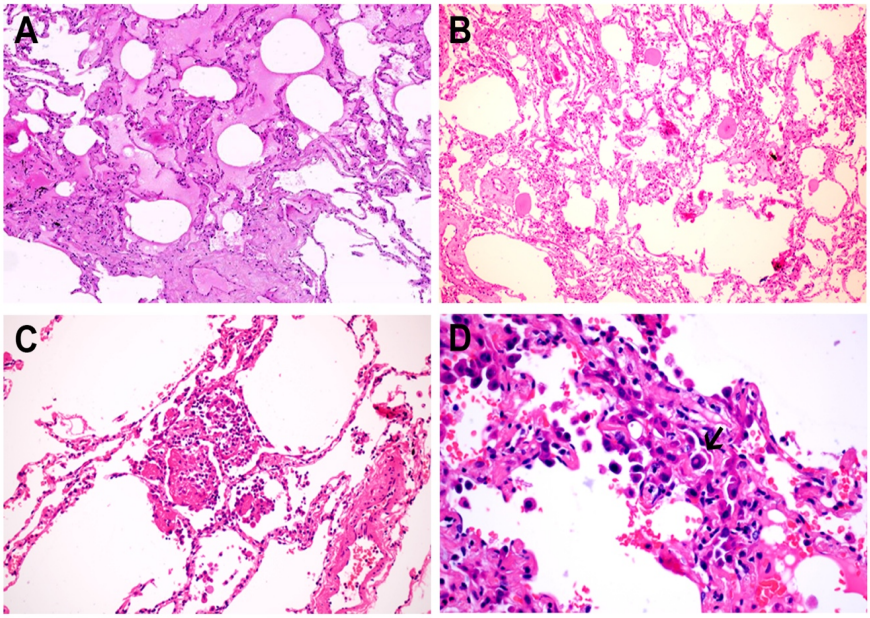

Figure 9. Shows the Histological changes of case 1 described by Tian *et al.,* (2020). Alveolar spaces with focal proteinaceous exudates; B. Scattered protein globules; C. Granuloma-like nodules consisted of fibrin, inflammatory cells and multinucleated giant cells inside the airspaces; D. Hyperplastic pneumocytes, some with suspected viral inclusions (arrow).

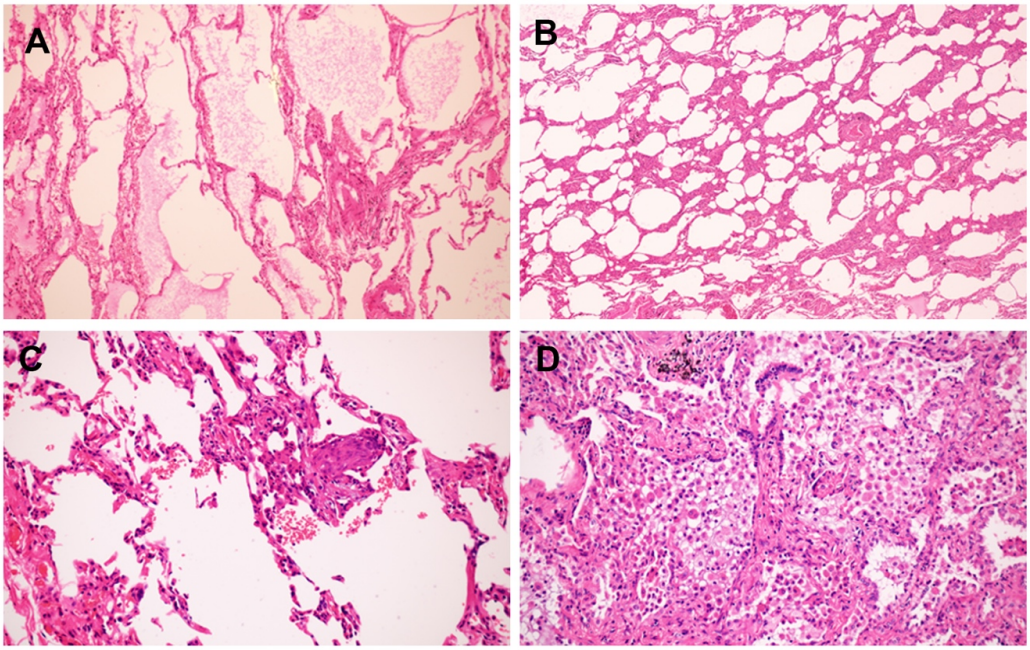

Figure 10. Shows the histological changes of exudative phase and *nonspecific interstitial pneumonia*- like pattern in case 2 described by Tian *et al.,* (2020).[62] A. Evident proteinaceous and fibrin exudate; B. Diffuse thickening and fibrosis of the

alveolar walls and septa without an inflammatory component; C. Fibroblastic foci in the interstitial space (arrow); D. Abundant polymorphonuclear cells and macrophages infiltrating airspaces. (Source: Tian *et al.,* 2020).

Zhe *et al.,* (2020) also reported the pathological findings of COVID-19 in a 50-year-old man who suffered from fever, chills, cough, fatigue, and shortness of breath. On day 14 of illness, his hypoxemia and shortness of breath were worsened, and the patient had a sudden cardiac arrest and was died despite a comprehensive treatment. Biopsy samples from Lung, liver, and heart tissue were collected. A bilateral diffuse alveolar damage with cellular fibromyxoid exudates was noticed in the lung section (Figure. 11A, B). They were accompanied with desquamation of pneumocytes and hyaline membrane formation in the right lung that demonstrating acute respiratory distress syndrome (ARDS; Figure. 11A). Early stages ARDS were also observed in the left lung tissue comprised of pulmonary edema and hyaline membrane formation (Figure. 11B). Moreover, both lungs revealed interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes. Pathological features were also seen in the intra-alveolar spaces, including multinucleated syncytial cells accompanied by atypical enlarged pneumocytes that characterized by large nuclei, amphophilic granular cytoplasm, obvious nucleoli, and viral cytopathic-like changes. However, no intranuclear or intracytoplasmic viral inclusions were recognized. Zhe *et al.,* (2020) also described histopathological features of the liver, which included slight lobular and portal activity and slight, moderate microvesicular steatosis (Figure. 11C) that demonstrating hepatic injury due to SARS-CoV-2 infection or drug-induced liver injury. Moreover, heart tissue revealed a few interstitial mononuclear inflammatory infiltrates, with the absence of other substantial damage (Figure. 11D).

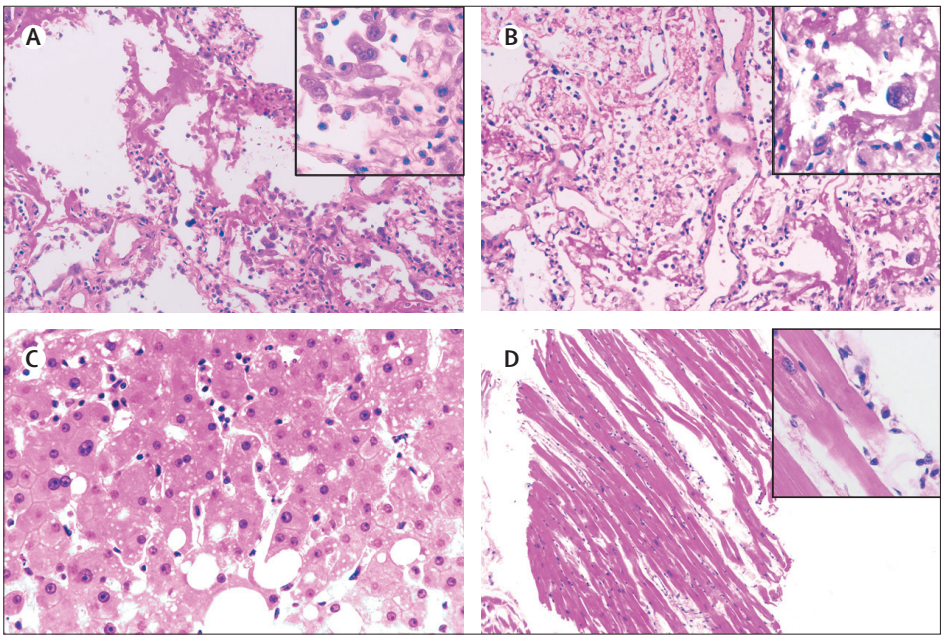

Figure. 11: Shows the pathological manifestations of SARS-CoV-2 described by Zhe *et al.,* (2020) (A) right lung tissue ; (B) left lung tissue; (C) liver tissue ; (D) heart tissue in a patient with severe pneumonia.

The high rate of renal impairment was also observed in COVID-19 patients, indicating the development of kidney dysfunction, accompanied by severe testis damage that

revealed a high expression level of ACE2 (the SARS-CoV-2 receptor) in the testis, kidney and gastrointestinal tract. Severe testis damage also leads to a testicular lesion in males (Li *et al.,* 2020; Fan *et al.,* 2020). The initial description of the SARS-CoV-2 and its specific cytopathic effects and morphology was done by Zhu *et al.,* (2020). The cytopathic effects were seen by a light microscope at 96 hours after inoculation on the surface of human airway epithelial cells accompanied by the absence of cilium beating in the center of the focus (Figure. 12).

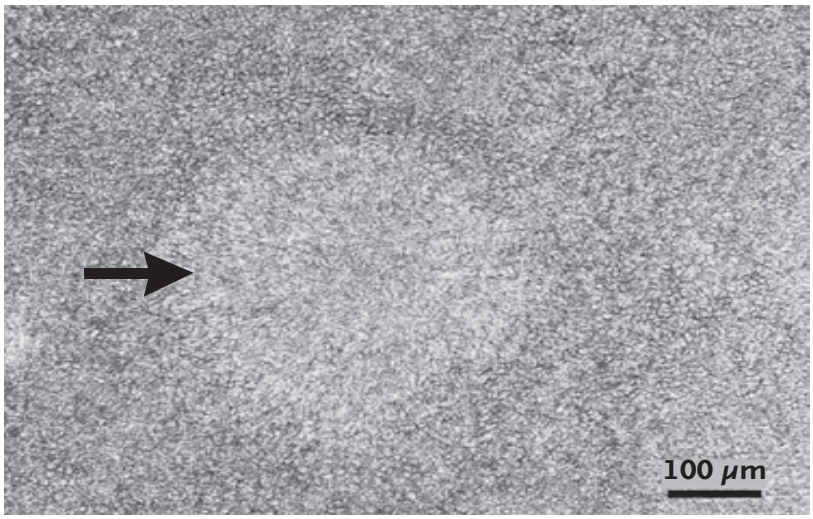

Figure. 12: Shows the Cytopathic effects in human airway epithelial cell cultures after inoculation with SARS-CoV-2 described by Zhu *et al.,* (2020)

The transmission electron microscope observation using negative SARS-CoV-2 generally revealed pleomorphic spherical particles ranged from 60 to 140 nm in diameter (Figure.13). Viral spikes measure about 9 to 12 nm appeared on the virion particle and gave it the typical solar corona appearance. The in vitro SARS-CoV-2 cultured human airway epithelial ultrathin sections revealed inclusion bodies filled with virus particles in membrane-bound vesicles in the cytoplasm and extracellular free virus particles Zhu *et al.,* (2020).

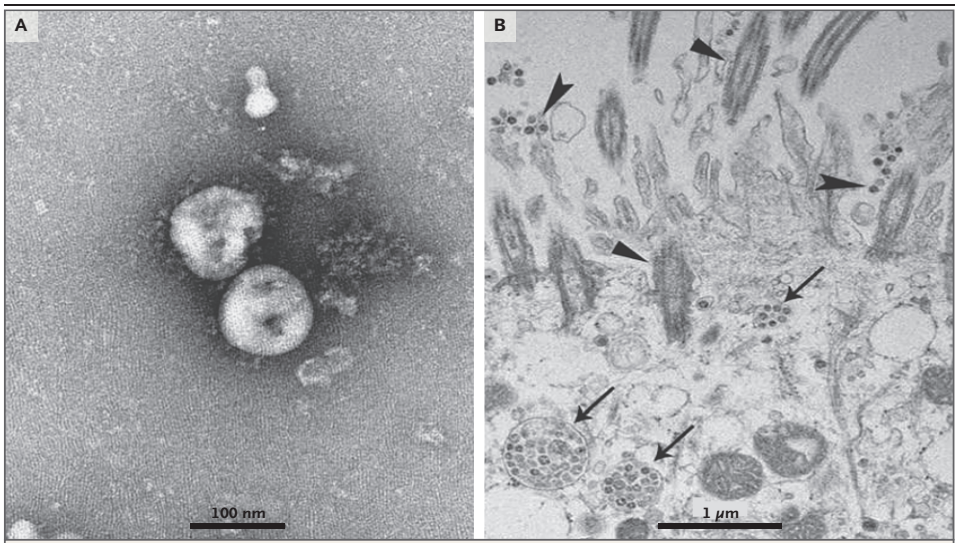

Figure. 13: Shows features of SARS-CoV-2 with transmission electron microscopy described by Zhu *et al.,* (2020). (A) Negative- stained SARS-CoV-2 particles in the human airway epithelial; (B) Cell ultrathin sections , arrowheads indicate extracellular virus particles, arrows indicate inclusion bodies formed by virus components, and triangles indicate cilia.

Lastly, on histopathology of SARS-CoV-2, diagnosis using histopathological investigation needs to submit infected samples to laboratories. Henwood, (2020) mentioned that there is little known neither on how to collect, handle, or transport and the appropriate disinfectants for SARS-CoV-2 nor the safety of histopathological fixation and processing. Therefore, a standard precaution measures and biosafety practice should follow to minimize the possibility of exposure to the pathogen. Nonetheless, authorities have recommended effective disinfectants for other coronaviruses (e.g., SARS and MERS) to inactivate SARS-CoV-2. Formalin and glutaraldehyde found to inactivate SARS-CoV in the temperature-and time-dependent way (Darnell *et al.,* 2004). Accordingly, Henwood, (2020), the appropriate safety precautions to be taken and formalin fixation and paraffin embedding should inactivate SARS-CoV-2.

Conclusions

In conclusion, three emerging zoonotic coronaviruses outbreaks have occurred during two decades included SARS-CoV, MERS-CoV and SARS-CoV-2 (COVID-19). COVID-19 is the most recent pandemic that distributed rapidly worldwide, causes fatal acute respiratory disease and develops panic and health crisis between people and risen extensive social, economic, and health security effects accompanied with severe precautions procedures that apply by majorities of countries to contain this virus transmission. Reports of autopsies or lung tissue samples of patients with SARS-CoV, MERS-CoV, and COVID-19 were limited. Basted on previous published studies, acute respiratory distress syndrome, was appeared on SARS-CoV, MERS-CoV, and COVID-19 patients who showed similar macro and micropathological features. These virus appeared to infect unciliated bronchial epithelial cells and type II pneumocytes. Severe illness of the respiratory tract were occurred due to infection with these viruses accompanied with diffuse alveolar damage, hyaline membrane formation and inflammation of the alveolar walls with desquamation of pneumocytes. Complicated cases with a secondary bacterial pneumonia revealed infiltration of inflammatory cells specially neutrophils in the intra-alveolar area. Moreover, COVID-19 can cause kidney and testis damage.

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