Severe Acute Respiratory Syndrome Coronavirus 2 Induces Hepatocyte Cell Death, Active Autophagosome Formation and Caspase 3 Up-Regulation in Postmortem Cases: Stereological and Molecular Study

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Severe acute respiratory syndrome coronavirus 2 induce hepatocyte cell death, active autophagosome formation and Caspase3 up-regulation in postmortem cases: stereological and molecular study

Running title: Severe acute respiratory syndrome coronavirus 2 induce hepatocyte cell death

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Abstract

This research investigated the histopathological changes in the tissue of the lung, heart and liver—hepatocyte cell death, autophagy, and studied the apoptosis inductions in the postmortem cases. Since December 2019, SARS-CoV-2(COVID-19) has become a significant global health concern. Due to the changes in tissues of the lung, heart, and liver, samples taken from five patients who died of COVID-19 and five control cases were performed and the pathological changes in the lung, liver, and heart tissue were studied by X-ray, computed tomography, histological studies, and stereological analysis. The formation of hyaline membranes, alveolar wall edema, and fibrin exudate was seen on histological analysis of the lungs in the COVID-19 group. Stereological analysis illustrated the number of hepatocytes, volume of the sinusoid, and volume of the liver have been decreased, however the changes in the heart tissue were not observed. The ALT, AST, BUN, and ACE significantly increased. Real-time PCR results showed that the Bcl2, Caspase3, ATG5, and LC3 decreased while the Bax increased. COVID-19 causes fibrosis changes in the lung tissue and hepatocyte mortality in the liver tissue. Besides, it induces the level of apoptosis and autophagy markers.

Keywords: Covid-19, Autopsy, Histopathology, Autophagy, Apoptosis.
Statement of Ethics: according to the consent of the patient family, the consent was obtained by the and Iranian Legal Medicine Organization, Tehran, Iran. The protocols of this study are confirmed by the Ethical Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1400.063).
Introduction
Since the end of December 2019, the COVID-19 pandemic outbreak has been detected in Wuhan, China, as a result of COVID-19 infections, and it led to a serious public health threat (Boroujeni et al, 2021). Officially, Iran confirmed the first case of COVID-19 infection on February 19, 2020. Hence, by June 12, 2020, more than 180,000 Iranian cases of the COVID-19 were reported (Moghimi et al, 2021).

Coronavirus infection can result in substantial morbidity and mortality in infected individuals (Van der Hoek et al, 2004). Based on the CT scans, the majority of patients who were suffering from dry cough, fever and dyspnea also had bilateral ground-glass opacity (GGO) (Xu et al 2020, Zhou et al, 2020).

Based on clinical and laboratory studies, the COVID-19 falls into many clinical manifestations according to its spectrum, including the initial, acceleration, and recovery phase. Multiple target organs such as the heart, lungs, and liver were damaged during the acceleration phase, and a cytokine storm was occurred. (Cao et al 2020, Tian et al, 2020).

However, the virus mainly infected the lungs, which may manifest as acute respiratory distress syndrome and mortality due to massive alveolar damage and progressive respiratory failure (Jain et al 2020, Tomaszewski et al, 2000). Besides, Cardiovascular disease was almost common among the COVID-19 patients. Patients who were hospitalized with COVID-19 had been confirmed to have a myocardial injury with elevated troponin levels, which appears to be related to the outcome (Guo et al 2019, Guo et al 2020).

Ischemia caused by thrombotic coronary occlusion can cause myocardial damage. but Note that other factors such as heart failure, pulmonary embolism, and tachycardia can cause it too (Hartikainen et al 2020). The COVID-19 patients with mild to serious illnesses are more likely to experience hepatic injuries (Zhang et al, 2020). According to increasing evidence,
COVID-19 infections affect various phases of autophagy, and autophagy, in turn, may play a critical role in the viral lifecycle (Cottam et al 2014, Cottam et al 2011, Gassen et al 2019).

Autophagy is a complex process, and it necessitates a large number of proteins with significant redundancy. Therefore, it explains why there are contradicting results when it comes to viral multiplication and autophagy inhibition (Mauthe et al 2016). However, the exact mechanisms underlying these interactions between the COVID-19 and autophagy are not entirely clear. Although several studies describe clinical features and characteristic radiographic findings, not enough data is provided about pathological changes and genes involved in autophagy and apoptosis in patients infected by novel coronavirus based on autopsy.

This study examined histopathologic changes in the liver, lungs, heart tissue, hepatocytes number, liver enzyme levels and the expression of genes associated with autophagy and apoptosis of the COVID-19 patients who died. The findings could provide a better insight into the main pathological changes of the disease in the aforementioned organs and improve clinical strategies against the disease.

Methods

Patient selection and patient criteria

Five (3 males and 2 females) COVID-19 samples (Table 1), as well as five control samples (3 males and 2 females) participated in the present study. For the control group, we chose people whose age ranged from 55 to 85 years who did not have any serious illness, and their death causes were accidents, carbon monoxide poisoning, and electric shock and so on. The causes of death were respiratory failure and septic shock due to COVID-19 in Case 1, 2, 4,
and 5, and pneumonia, acute respiratory distress syndrome, and myocardial infarction due to COVID-19 in Case 3.

**Post-mortem analysis and lung, liver, and heart sampling**

Lungs, liver, and heart tissue samples were collected from 5 COVID-19 patients, and 5 healthy individuals as a control group from the Iranian legal organization in Tehran, Iran, between Jun and March 2021. According to the World Health Organization's provisional guidelines, the real-time PCR examination of nasopharyngeal swab samples were taken at the time of hospital admission confirmed the COVID-19 infection in all the patients. Clinical characteristics including medication history, computed tomographic (CT) scans, laboratory reports, and illness duration were all illustrated in Table 1 and Figure 1.

In addition, autopsies were carried out between 8 and 10 hours after the death in a room which was equipped with sufficient ventilation, and all the staffs were using personal protective equipments. A 3 × 3cm² tissue sample was collected by incisive autopsy for histological, molecular, and cellular investigations in five patients and five controls as well. Moreover, the protocols of the present study were confirmed by the Ethical Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1400.063).

**Histological evaluation**

Tissue samples were fixed immediately in 4% paraformaldehyde for 72h before going through routine tissue processing. Following that, histopathological evaluations were performed by sectioning and staining with hematoxylin and eosin (H&E) (Moghimi et al 2021).
Stereological Study

A microtome was utilized to perform serial slices which had 5 µm thickness to estimate the volume and 25 µm thickness to estimate the number by stereological procedures. Systematic Uniform Random Sampling (SURS) was used to select 10 portions of each sample by selecting a random number of 1 to 10.

Estimating the volume of the liver and sinusoid:

To calculate the volume of the liver and sinusoid using the Cavalieri method as an estimator, the following formula was used (Gundersen et al. 1998 a, b):

\[ V_{\text{total}} = \sum P \times t \times \frac{a}{p} \]

In this formulation, \( \Sigma P \) is the total number of points that hit the liver sections. The region associated with a point is \( a/p \), and the distance between sample sections is \( t \).

Estimating the number of cardiomyocytes and hepatocytes:

The total variety of hepatocytes and cardiomyocytes was firm by the optical dissector method. The equation below was used to compute the numerical density (Nv) of various types of cells (Gunderson et al., 1998a):

\[ N_v = \frac{\sum Q}{\sum P \times \frac{a}{f} \times h} \times \frac{t}{BA} \]

In the formula, \( \Sigma P \) is the total number of the microscopic fields, \( a/f \) is the surface per frame, \( h \) is the height of director, \( t \) in the numerator shows the actual thickness of the section, while \( BA \) in the denominator of the 2nd split is the advance of the microtome block. Then, the numerical value of the cell types was determined using the following equation: \( N = N_v \times V_{\text{total}} \).
Real-time PCR (qPCR)

On a Step One TM-Plus method, gene RT-qPCR was performed on samples PE, (Applied Biosystems, CA). For a whole volume of 20 μl, the sample reactions included SYBR green PCR master mixture (TaKaRa), cDNA template, primer forward and reverse), and distilled water. For each primer, the PCR cycle conditions were: 30 denaturation cycles at 94 °C for 30 seconds, annealing temperature for 30 seconds at 60, 62, 59, 65, 58 and 63 °C for the Bax, Bcl2, Caspase 3, GAPDH, ATG5 and LC3, respectively, extension at 72 °C, 30 seconds and the last extension stage at 72°C for 5 minutes. The level of relative gene expression was measured using the ΔΔCq method. GAPDH was used to normalize gene expression. Each sample was examined twice 20. The primers used are listed as follows in Table 2.

Enzyme-linked immunosorbent assay (ELISA)

The enzyme levels of alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), Creatinine, and blood urea nitrogen (BUN) were calculated using the instructions which were provided by the kit company Biorbyt, (Cat #: orb159085, UK). In a brief, the required numeral of blank wells, samples, and standards was designated and arranged on the dish, and 100 ml of samples and calibrators were added to the wells, and the plate was prepared biotin antibody and Stored for 60 minutes at 27 C. The wells were then filled with 100μL of Streptavidin and incubated at normal room temperature for 45 minutes. After incubation for 30 minutes with TMB One-Step Substrate Component, 50μL of Stop Solution was applied to each well and read at 450 nm immediately (Boroujeni et al 2021).

Reactive oxygen species in liver tissue
Subsequently separating cells from liver tissue, they were treated with trypsin-EDTA, the resultant suspension was centrifuged in PBS for five minutes at four °C (1400 g). After the addition of 2, 7-dichlorofluorescein diacetate (DCFDA) to the sample at a concentration of 20 μM in a 100 μl aliquot, it was incubated for 45 minutes a 37 °C. Lastly, the sample was studied by using a flowcytometer at 495 nm (Boroujeni et al. 2021).

**Glutathione disulfide content assessments**

Glutathione peroxidase (GPX) test kit (Zelbio GmbH) was used for detecting GPX in liver tissue samples. In this analysis, the GPX activity was measured based on the total of sample that catalyze the decomposition of 1 μmol of GSH in one minute. Aliquots of the liver cells suspension containing either O-Phthalaldehyde (OPA) and N-Ethylmaleimide (NEM) probes were harvested from the incubation media using centrifuging at 1000 rpm for 1 min (Boroujeni et al. 2021).

**TUNEL assay**

After the fixation of liver tissues, the samples were embedded into paraffin, sectioned, and mounted on glass slides using gelatin. They were preserved by Xylene which dissolved and removed paraffin. Subsequently washing the slices with water, the Tunel staining was done. Image J was also used to measure Tunel-positive cells in normal and Covid groups (Boroujeni et al. 2021).

**Statistical analysis**

9
The mean SD was used to present data that was regularly distributed, and the data, which is not having distribution, was presented as medians. Counts and percentages were used to describe categorical variables. Statistical analysis was completed using Microsoft Excel.

**Result**

**Characteristics of patients**

In the present investigation, five deceased patients with verified COVID-19 virus diagnoses and five control cases were studied. The patients were 3 males and 2 females. The patients' average age was 71.2 years (ranging from 58 to 85 years). The average stay in the hospital was 9.5 days on (average ranging from 4 to 15 days). All of the patients had been intubated for 4.5 days. The main symptoms of these patients were fever, dyspnea and cough, as shown in Table1. The COVID-19 positive individuals' chest X-rays demonstrated undiversified consolidation in peripheral distribution on each lung with obscuration of each CP angle in the middle and lower zones. In all of the patients, CT scan results revealed bilateral peripheral ground-glass opacities that were largely dispersed along basal segments. Other occurrences such as sleek interlobular septal thickening, fibrotic bands, and collapse consolidation in each lung were determined likewise (Figure 1).

**Histopathological findings**

The formation of hyaline membranes, alveolar wall edema, and fibrin exudate was seen on histological analysis of the lungs in the five COVID-19 patients. Interstitial fibroblastic proliferation and type II pneumocyte hyperplasia, as well as the development of bronchial respiratory epithelium desquamation and cytopathic syncytial cells were observed (Figure 2). As seen in figure 3, the COVID-19 autopsy specimens had no clinical symptoms of heart diseases or signs of heart failure. Wholly of cases in this study had indicated congestion and
sinusoidal dilation sinuses in liver slices, which were reached from mild to severe forms. Hepatocytes in all of the cases were mildly expanding. Bile plugs, both focal and dispersed, and hepatocellular regenerative changes were discovered (Figure 4).

Stereological parameters analysis

Figure 5A shows the results of comparing different groups. The volume of the liver and sinusoid was significantly different between COVID-19 and the control group. As shown in Figure 5B, the control group revealed a significant difference in hepatocyte count compared to the COVID-19 group. In heart samples, no significant differences were found concerning the number of cardiomyocytes.

Real time PCR analysis

To evaluate mRNA expression of target genes at the molecular level, the number of transcriptions of two autophagy-related genes (ATG5 and LC3) and three genes which were involved in apoptosis Bcl2, Bax, and Caspase3 were examined in the liver sample. According to the statistical results, Bcl2 expression levels in the COVID-19 group were notably lower than in the control group (P < 0.0001). Finally, the COVID-19 group's Bax and Caspase3 expression levels were higher than the control group (P 0.0001). When the Covid-19 group was compared to the control group, the expression levels of ATG5 and LC3 were significantly higher (P 0.0001) (Figure 6).

ELISA result

The results of liver markers expression, such as ALT, AST, ALKP BUN, and Creatinine, revealed that the Covid-19 group had considerably higher levels of these markers than the control group (Table 3).
Reactive oxygen species (ROS) production

In our study to know oxidative stress result at the COVID-19 samples, the effects of COVID-19 on the ROS formation were investigated by ROS assay. The ROS formation in the COVID-19 group displayed an increase in comparison with the control group (P < 0.5) (Fig. 7A).

GSH analysis

As Fig. 5B showed, the concentration of GSH in the COVID-19 group had significant decreased in comparison to the control group (P < 0.05) (Fig. 7B).

Percentage of apoptotic cells

Tunel-positive cell results showed a considerable increase in the apoptotic cells in the COVID-19 group in comparison with control group (P < 0.001) (Fig. 7C).

Discussion

The results of the present study showed that COVID-19 can induce autophagy and apoptosis. Besides, it causes hepatocyte cell death, and liver tissue damage.

During the COVID-19 pandemic, numerous investigations were conducted to better understand the disease's progression and therapeutic approach (Machhi et al., 2020). The COVID-19 is most commonly associated with the respiratory and immunological systems, although it can also damage the cardiovascular and gastrointestinal systems, particularly in the elderly, and more frequently when comorbidities are present.

In this study, the majority of the pathologic results in autopsy lung, kidney and liver samples of cadaver SARS-CoV-2 diseased persons displayed the severe phase of diffuse alveolar damage (DAD). Hyaline membrane creation, alveolar wall edema, and fibrinoid exudate are
all symptoms of DAD in its acute phase (Kligerman et al, 2013). More advanced stages of DAD, including proliferative and fibrotic phases, have also been identified too. This result is constant by previous pathologic discoveries in patients through SARS and MERS, where DAD was the most protuberant pathological discovery (Beigmohammadi et al, 2021, Ng et al, 2014, Franks et al, 2003).

In their investigation, they mentioned Hyaline membrane formation, desquamated pneumocytes, the attendance of mononuclear leukocytes, and pulmonary edema (Beigmohammadi et al, 2021). Liver dysfunction is widespread in COVID-19 patients, according to lab results serum liver enzyme and BUN and creatinine levels), especially in severe cases.

All of the cases in the present research had elevated levels of liver markers. Although the specific mechanism of liver injury caused by the new coronavirus infection is unknown; medication poisonousness and a general inflammatory reaction have been proposed. All liver autopsy specimens from the dead COVID-19 patients had pathologic characteristics ranging from mild to severe in the current investigation. In a 2003 research of coronavirus-infected individuals, they found numerous mitoses and substantial hepatocytes proliferation (Chau et al, 2004); nevertheless, in this study a decrease in hepatocytes in liver tissue samples from coronavirus-infected individuals was found.

The ischemic injury was highly suggested in the liver, because only a low grade of inflammation was observed in portal tracts and lobules. The results of the present study are consistent with explanations (Beigmohammadi et al, 2021). Micro vesicular steatosis, portal, lobular, sinusoidal inflammation, and multifocal necrosis were seen in COVID-19-patient’s liver biopsies, which were understood as also a COVID viral-induced injury (Beigmohammadi et al, 2021). According to molecular findings, the expression of genes
involved in apoptosis and autophagy increased, which is in line with previous studies (Kudchodkar et al, 2009, Tan et al, 2007).

Apoptosis induction is a hallmark of SARS-CoV-2 infection. Failure to activate apoptosis will not only prevent cell death and tissue damage, but will also slow SARS-CoV-2 clearance from infected cells (Ye et al, 2008). SARS-CoV2 belongs to the beta coronaviruses (b-Cov) family, which has been found to cause an autophagy-related gene 5 (ATG5)-dependent increase of autophagosome production, then afterward impede their maturation (Cottam et al, 2014, Chen et al, 2014).

Upregulation of LC3 during b-Cov infection might suggest viral hijacking of an alternative path, endoplasmic reticulum-associated degradation (Knoops et al, 2008). Understanding how this and other intracellular pathways affect SARS-CoV-2 is crucial in dealing with coronavirus outbreaks.

**Conclusion**

To wrap it up, the COVID-19 promotes fibrosis alterations in lung tissue and hepatocyte mortality in liver tissue, as well as inducing the expression of genes related to autophagy, apoptosis, and increase of ROS.

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**Statement of Ethics:** according to the consent of the patient family, the consent was obtained by the and Iranian Legal Medicine Organization, Tehran, Iran. The protocols of this study are confirmed by the Ethical Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1400.063).
Conflict of Interest Statement: No conflict of interest.

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Author Contributions: HA designed the present research and drafted the manuscript. ASH, GRM, BA and MF provided the clinical information and samples and were involved in the draft of the manuscript. MAA and HAA conducted statistical analyses and were involved in the draft of the manuscript. MAA, MRT and SHA accomplished the histological study and were involved in the draft of the manuscript. Each of the authors read and confirmed the resulting paper.

Data Availability Statement: All data generated or analyzed during this study are included in this article.
References:


**Figure legend**

**Figure 1.** COVID-19 patients' chest X-rays show undiversified consolidation in marginal spreading on each lung, by obscuration of each CP angle (C1-C5). A chest CT scan shown two-sided ground-glass opacities and interlobular septate thickening that resembled crazy paving, as well as areas of enveloping consolidation that resembled reverse paving (C1-CT-C5-CT)

**Figure 2.** H&E stain of pulmonary microscopic findings. A: The pulmonary parenchyma, including the alveolar walls and alveolar sac, appears normal in the control group. B: In the Covid-19 group; development of a hyaline membrane, multinucleated cells, intra-alveolar abnormal expanded cells; fibrinoid material deposition in vessel walls; fibroblast proliferation coordinating diffuse alveolar damage) was seen.

**Figure 3:** (A, B) Hepatic microscopic findings, Focal confluent necrosis; moderate microvesicular alterations; hepatocellular regenerative changes, mild portal tract mixed inflammation was seen; Hepatocytes display glycogenated nuclei and abnormal small lymphocytes densely infiltrating the zone of portal triad. Fibrosis in hepatic nodules, indicating cirrhosis. The liver sinusoids are dilated and full of lymphocytes.

**Figure 4:** (A, B) cardiac microscopic findings, H&E stain not showed pathological sign in heart samples in the control and Covid-19 group.

**Figure 5.** (A) The results of total volume of liver and sinusoid in different group. Values are presented as mean ± standard deviation (Mean ± SD). **p < .01, control group versus COVID-19 group. (B) The results of total number of hepatocyte and cardio myocytes in
different group. result showed that control group revealed a significant difference in hepatocyte count compared to COVID-19 groups, but no significant differences were found with respect to the number of cardiomyocytes. Note: Values are presented as mean ± standard deviation (Mean ± SD). **p < .01, control group versus COVID-19 group.

**Figure 6:** (A) Analysis of Real time PCR. Bax, Bcl2, Caspase3, ATG5 and LC3 mRNA expression levels in the liver tissue. Mean ± SD of the genes expression in the various groups (****P < 0.0001).

**Figure 7:** (A, B) The result showed that ROS production and level of GSH in liver tissue in two groups. Mean ± SD of the ROS production and GPX activity level in control and COVID-19 groups; (*P < 0.05). (C)The percent of formation apoptotic cells in liver tissue in two groups. Mean ± SD of the percent of apoptotic cells of liver in control and COVID-19 groups. (*P < 0.05)
Table 1. Clinical features for COVID-19 patients.

<table>
<thead>
<tr>
<th>Patients (Age)</th>
<th>Sex</th>
<th>Clinical presentation at admission</th>
<th>Past medical history</th>
<th>Drug history</th>
<th>Medications used for COVID-19</th>
<th>Duration of hospital stay (days)</th>
<th>Duration of intubation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (55 years)</td>
<td>M</td>
<td>fever, dyspnea, cough</td>
<td>hypertension</td>
<td>losartan, aspirin</td>
<td>naproxen, remdesivir, dexamethasone</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>C2 (73 years)</td>
<td>M</td>
<td>fever, headache, nausea, vomiting, cough</td>
<td>none</td>
<td>None</td>
<td>dexamethasone, oseltamivir, levofloxacin</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>C3 (78 years)</td>
<td>M</td>
<td>fever, dyspnea, diarrhea, cough</td>
<td>hypertension, diabetes mellitus</td>
<td>losartan, aspirin, insulin</td>
<td>hydroxychlorquine, remdesivir, dexamethasone</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>C4 (85 years)</td>
<td>F</td>
<td>fever, dyspnea, cough</td>
<td>diabetes mellitus</td>
<td>Insulin</td>
<td>hydroxychlorquine, levofloxacin, naproxen</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>C5 (62 years)</td>
<td>F</td>
<td>fever, dyspnea, myalgia, cough</td>
<td>hypertension, rheumatoid arthritis</td>
<td>aspirin, citalopram, sulfasalazine</td>
<td>hydroxychlorquine, remdesivir, oseltamivir, naproxen</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>

M, Male; F, female.
Table 2. Primer sequences of RT-qPCR

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward</th>
<th>Reverse</th>
<th>Annulling temperature</th>
<th>Length</th>
<th>Accession number</th>
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</thead>
<tbody>
<tr>
<td>Bax</td>
<td>CCCGAGAGGCTTTTTTCG</td>
<td>CCAGCCCATGATGGTTCTGAT</td>
<td>60</td>
<td>210bp</td>
<td>NM_004324.3</td>
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<tr>
<td>Bcl2</td>
<td>TACAGGCTGGCTCAGGACTAT</td>
<td>CGCAACATTTTAGCAGCTCTG</td>
<td>62</td>
<td>230bp</td>
<td>BC027258.1</td>
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<tr>
<td>Caspase 3</td>
<td>GTGGAACCTGACGATGATATGCG</td>
<td>GGCAAAGTGACTGGATGAACG</td>
<td>59</td>
<td>190bp</td>
<td>NM_032991.2</td>
</tr>
<tr>
<td>GAPDH</td>
<td>CCACAACCTTTCATTTC</td>
<td>CCAAGATTCAACGGTAGATAC</td>
<td>65</td>
<td>200bp</td>
<td>NM_001289726.1</td>
</tr>
<tr>
<td>ATG5</td>
<td>GCAGATGGACAGTTGCAACACAG</td>
<td>GAGGTGTTTCAACATGGCTCACC</td>
<td>58</td>
<td>180bp</td>
<td>NM_004849.2</td>
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<tr>
<td>LC3</td>
<td>TGTTAGGCTTGTCTTTTGG</td>
<td>GCAGAGGAAATGACCACAGAT</td>
<td>65</td>
<td>195bp</td>
<td>NM_003766.3</td>
</tr>
</tbody>
</table>
Table 3. The laboratory test results of liver (AST, ALT and ALKP) and kidney markers (BUN and creatinine) in the control (N1-N5) and COVID-19 groups (C1-C5).

| Cases | Marker       | C1   | C2   | C3   | C4   | C5   | N1   | N2   | N3   | N4   | N5   |
|-------|--------------|------|------|------|------|------|------|------|------|------|------|------|
|       | AST (U/L)    | 309  | 106  | 172  | 528  | 262  | 20   | 46   | 32   | 20   | 24   |
|       | ALT (U/L)    | 258  | 168  | 217  | 978  | 338  | 24   | 69   | 39   | 24   | 32   |
|       | ALKP (U/L)   | 304  | 226  | 204  | 318  | 374  | 70   | 227  | 250  | 200  | 296  |
|       | BUN (mg/dL)  | 52   | 68   | 107  | 132  | 115  | 17   | 9    | 8    | 17   | 14   |
|       | Creatinine (mg/dL) | 5.56 | 4.5  | 2.93 | 5.26 | 4.88 | 0.8  | 0.6  | 1    | 0.8  | 0.6  |

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; BUN, blood urea nitrogen.
Figure 1
Figure 2

A

B
Figure 3

A

B
Figure 4
Figure 5

A

Volume (cm³)

**

Control  COVID

Volume of Liver

Volume of sinusoid

B

Number × 10⁶

**

Control  COVID

Number of hepatocyte

Number of cardiomyocyte
Figure 6
Figure 7

A. DFS Absorbance (488nm)

B. GPX Activity (µ/ml)

C. Tunel positive cells %

* indicates statistical significance.