DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN

MEDICAL BIOCHEMISTRY

TITLE

"EVALUATION OF LIVER ENZYMES IN DIAGNOSED COVID-19 SURVIVORS AND CONTROL SUBJECTS"

SUBMITTED

BY

NAGMA IRFAN

2022

DEPARTMENT OF BIOCHEMISTRY

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

FACULTY OF HEALTH & MEDICAL SCIENCES

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SUBMITTED

In partial fulfillment of the requirement for the award of a degree of

Master of Science
In
Medical Biochemistry

By

NAGMA IRFAN

Enrollment No: 1900101573

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CERTIFICATE

This is to certify that **Nagma Irfan** student of **M.Sc. Medical biochemistry**, Integral University has completed her dissertation titled " **Evaluation Of Liver Enzymes In Diagnosed Covid-19 Survivors And Control Subjects** " successfully. She has completed this work in the Department of Biochemistry, Integral Institute of Medical Sciences and Research, Integral University under the guidance of **Dr. Saba Khan**. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future

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Place: Lucknow

Nagma Irfan

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Date: *NAGMA IRFAN*

LIST OF ABBEREVIATIONS

COVID-19	Coronavirus Disease 19
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
RT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
ACE-2	Angiotensin Converting Enzyme 2
WHO	World Health Organisation
ARDS	Acute Respiratory Distress Syndrome
ULM	Upper Limit Of Normal
ALT	Alanine Aminotransfersae
AST	Aspartate Aminotransferse
ALP	Alkaline Phosphatase
GGT	Gamma Glutamyl Transferse

Contents

INTRODUCTION

COVID-19 announced as a widespread -

The ongoing epidemic of severe acute respiratory syndrome caused by coronavirus-2 (SARS-CoV-2) proceed with to present many pharmacogenetic and medicinal challenges. This virus was first reported in December 2019 from Wuhan, China. The World Health Organization officially classified this infection as coronavirus disease 2019 (COVID-2019) on February 11, 2020, and classified the virus as SARS-CoV-2 [1]. The disease was announced as a pandemic on March 11, 2020 [2]. However, in adults, SARS-Co-V-2 commonly causes pneumonia and acute respiratory distress syndrome (ARDS), now identified as a multisystem disease. It affects not only the pulmonary system, but also the heart, liver and gastrointestinal system [3].

Major impact of COVID –

It is widespread in humans and animals. It causes respiratory infections in humans [4]. His ongoing COVID-19 pandemic, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is evolving rapidly [5]. COVID-19 is caused by the virus SARS-COV-2, and the major symptoms are fever, cough, and breathlessness, and the mild symptoms are changes in smell and taste, gastrointestinal symptoms, headache, and skin symptoms [6-8]. SARS-CoV-2 involes into cells through its intended receptor, angiotensin-converting enzyme 2 (ACE2) [9].

Viral host cell invasion -

A noticeable number of analytical studies have been published to explain the clinical manifestations of SARS-CoV-2 infection. In addition to asymptomatic or symptomatic forms and acute viral pneumonia along with respiratory problem, the prognosis of COVID-19 disease is impacted by wondering effects on organs throughout the body through receptor

angiotensin-converting enzyme-2 (ACE-2), which include heart and kidney failure, central nervous system damage & CNS & and gastrointestinal tract [10,11].

ACE-2 has been declared as a functional SARS-CoV receptor and extremely expressed on cells of pulmonary epithelial membrane of lungs[12]. By this host receptor that the S protein initially interact to trigger entry of the virus into the host cell [13-15]. After membrane fusion, the corona virus enters epithelial cells of alveoli of lung, and the content of virus is released inside. After that inside the host cell, the virus start replicating and produce negative-stranded RNA from single-stranded positive RNA which already exist by the action of RNA polymerase (transcription). This newly produced negative-stranded RNA starts producing new positive strands of RNA, which then form new proteins in the cell cytoplasm (translation) [16-18]. The N virus protein binds to the new RNA genome and the M protein fhelps integration into the cellular endoplasmic reticulum. These newly formed nucleocapsids are then enclosed in the ER membrane and carried to the lumen, from lumen they are carried across the Golgi vesicles to the cell membrane, after that through exocytosis to the extracellular space. cell. The novel viral particles are now organised to penetrate the adjoining epithelial cells as well as provide novel infectious contents for community transmission through respiratory droplets [19].

Life cycle of virus is shown in figure 1 [20].

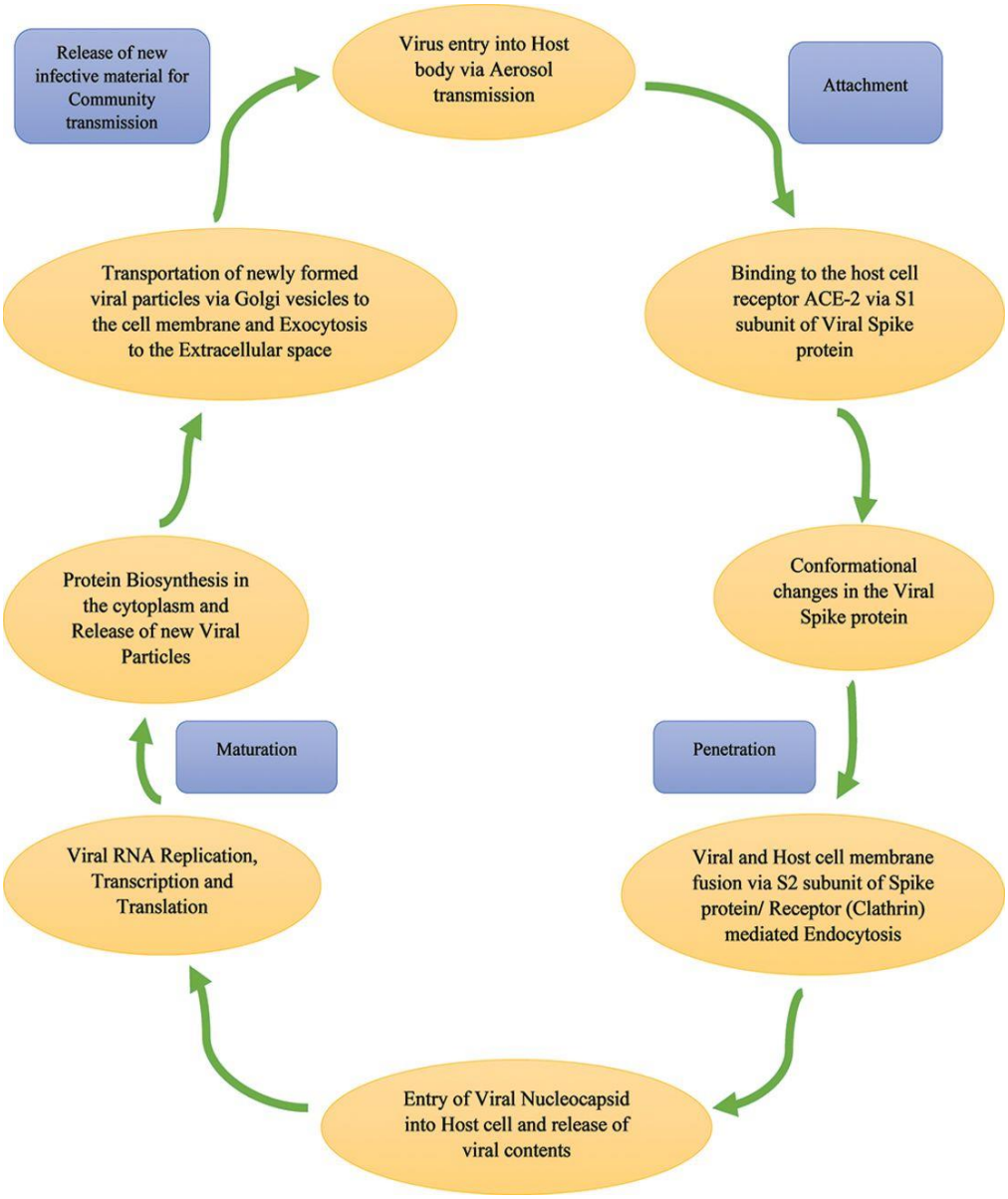


Figure.1 Life cycle of virus and its invasion into host cell. [*Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 2020;55:2000607*]

Morphology of SARS CoV-2 -

The biggest genomes (26–32 kb) among all RNA virus families are found in CoVs, which are RNA viruses. Each viral transcript has a 3-poly(A) tail and a 5-cap structure[21]. The SARS-CoV-2 virus typically has a spherical shape, although it can also take on pleomorphic and oval shapes. The S-spike protein, which creates the peplomers and gives the virus its

distinctive crown shape, the M-membrane protein, and the E-envelope protein, which provides the ring structure, are the three proteins that make up the viral envelope [22,23]. A fourth protein, N-nucleocapsid protein, a phosphoprotein that is a part of the nucleocapsid's structural framework, is also present [24,25,26,27]. The group I fusion glycoproteins include the S protein. Its homotrimeric structure, which has one higher and two lower conformations, is what distinguishes it[28,29-31]. About 75.5 percent of the S protein's amino acid sequence is same in both SARS-CoV-1 and SARS-CoV-2 [32].

New viral particle, which are generally constructed by the components of the host cell, are formed with the help of a structural E protein.

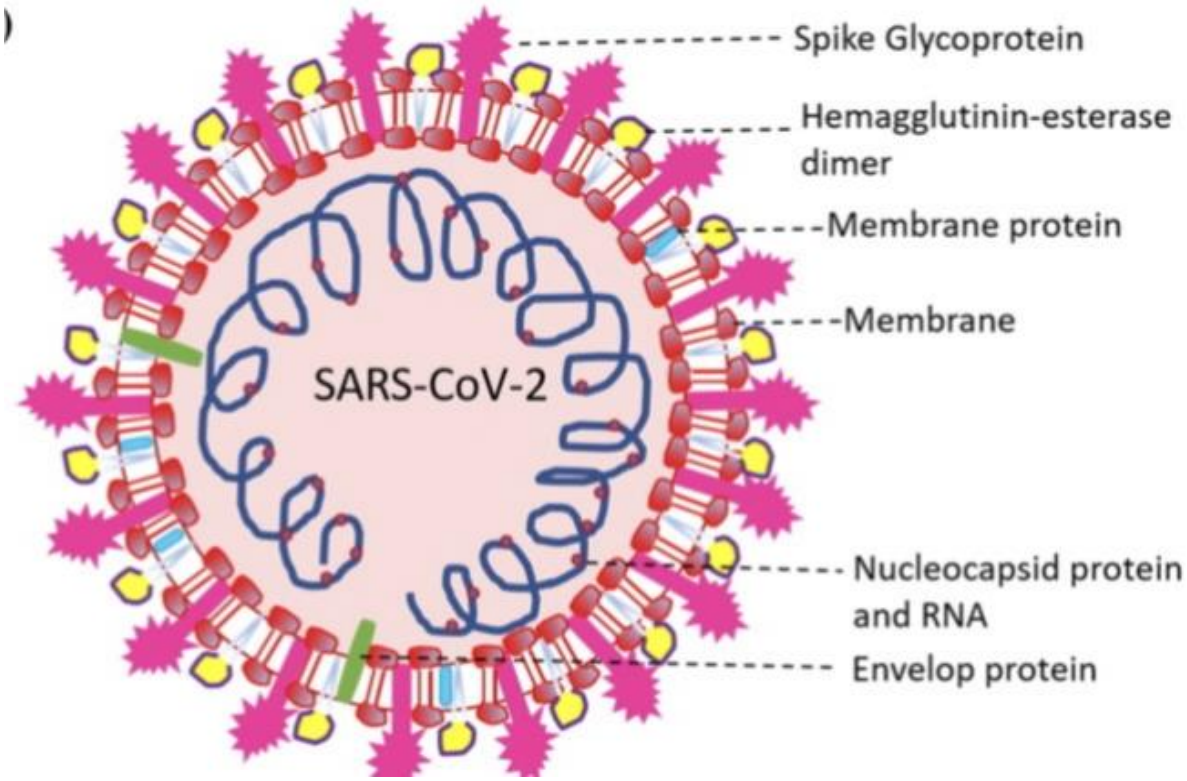


figure.2 Structure of SARS-CoV-2[33]. [*Subramanian Boopathi , Adolfo B. Poma and Ponmalai Kolandaivel. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment, journal of biomolcular structure and dynamics, 2021; 39:9, 3409-3418*]

Impact of COVID-19 on liver -

In particular, the impact of COVID-19 on the liver has been assessed in a small number of studies that concentrated on changes in the liver's histology and biochemistry during the course of infection in patients who died from the virus [34–39].

A number of recent studies have sought to assess variables linked to liver injury in American, Asian, and European cohorts [34,39-42], as well as cohorts from those continents [43-46]. However, little is known about liver illness in the context of SARS-CoV-2 infection, which limits our understanding of aberrant liver function in terms of the overall prognosis.

Additionally, SARS-CoV-2 RNA has been found in the blood and hepatocytes of the infected patients[47–49]. The large number of ACE2 receptors on the surface of liver's cholangiocytes[50,51], bile duct cells[52], probably make it easier for SARS-COV-2 to enter cells where it can reproduce and cause disruption of liver function[53,54].

Additionally, there is evidence to support the idea that the majority of viruses that target the respiratory system can injure liver cells via the CD8+ driven immune response[55]. Regardless of the setting for providing healthcare, findings from several research reveal a link between COVID-19 and abnormal liver function tests (LFTs)[56–59]. The data from more recent epidemiological and clinical research on LFTs in individuals with SARS-CoV-2 infection, however, are essentially contradictory and inconsistent[56, 60-63].

In patients with COVID-19 from China, the UK, and the USA, respectively, the prevalence of the liver damage biomarker alanine transferase (ALT) has been found to be up to 32%, 38%, and 39% [59,64,65]. On the other hand, according to the severity of the diseases, certain studies did not detect any appreciable change in the ALT levels of COVID-19 patients[62,66]. As a result, a number of systematic reviews and meta-analyses have recently

been published, focusing on the incidence of anomalies in the liver biochemistry profile among COVID-19 patients based on clinical severity and disease mortality. This is because there has been revealed discrepancies in the results for the liver chemistries in COVID-19 affected individuals. [58,61,67-70].

The liver enzymes and their function -

The primary liver functions include the detoxification of many metabolites, the production of digestive enzymes, and the synthesis of proteins. The liver also involved a significantly in the regulation of RBCs, glucose synthesis, storage, and metabolism. Mostly, when evaluating LFTs, the), aspartate transaminase (AST), Alanine transferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), serum albumin, serum bilirubin, international normalised ratio (INR), and prothrombin time (PT) are all discussed [71].

These biomarkers typically have

- ALT values of 60U/L for men and 42U/L for women.
- With no discernible sex-related differences, the AST level was 35 U/L.
- ALP for women is 33-98 U/L, and for men it is 43-115 U/L.
- GGT is 70 U/L in men & 40 U/L in women [72].

1) **ALT : Alanine Transaminase – [73]**

- Formed in hepatocytes;
- highly distinct marker of hepatocellular injury;
- proportionately low quantities in additional tissues, making it more distinct than AST;
- levels vary throughout the day;
- Increase may occur while using specific medications or when engaging in vigorous activity.

2) **AST : Aspartate Transaminase – [73]**

- Two isoenzymes of aspartate transaminase (AST) exist and are indistinguishable by means of conventional AST testing.
- The cytosolic isoenzyme is found in skeletal muscle, heart muscle, and kidney tissue. The mitochondrial isoenzyme is formed in hepatocytes and responds to membrane stressors similarly to ALT.
- It generally rises in concert with ALT to show hepatic injury: a hepatitis overview. It must be used with caution to evaluate hepatocellular damage.

3) **ALP: Alkaline Phosphatase [73]**

• A collection of isoenzymes that function to dephosphorylate a number of different compounds all across the body.

• Made in the membranes of the cells lining the canaliculi and bile ducts.

• Released in reaction to cholestasis or the buildup of bile salts.

• Non-hepatic production in the placenta, leukocytes, kidney, gut, and bone.

• Physiological change during pregnancy or in youngsters who are growing.

• A pathological increase in bone metastases, Paget's disease, and renal disease.

4) **GGT: Gamma-glutamyl Transferase [73]**

- Found in hepatocytes and biliary epithelial cells' microsomes.
- Found in the liver, pancreas, kidney, and intestine.
- The cholestatic picture, which includes elevated GGT and elevated ALP, is strongly indicating a biliary tract blockage.

- It is also subject to increase with hepatic enzyme stimulation brought on by prolonged alcohol consumption or medications like rifampicin and phenytoin.

In reality, numerous investigations have claimed that SARS-CoV-2 infection causes an elevation in liver transaminases. The speculative mechanism includes the likelihood of direct liver injury caused by drug toxicity, liver injury due to immunological response and immune-mediated damage, and ischemic hepatitis that may occur in individuals with multiorgan failure, including hemodynamic instability [74]. The dysfunctionality of the LFT in COVID-19 may, theoretically, be caused by a direct virus-induced cytopathic effect. The question of whether liver damage is predictable is more difficult to answer. Due to the large number of patients infected with different HCoVs, and in particular the rapidly rising number of COVID-19 patients worldwide, it is necessary to know how liver injury affects the clinical outcomes of COVID-19 patients and its determinants. This could have a significant impact on many people's outcomes.

Covid-19 has also been shown to have an effect on liver enzymes in long term studies -

Long-Term Effect Of Covid-19 On Liver Enzymes - Post-COVID-19 manifestations following a negative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test for SARS-CoV-2 have been extensively researched in the literature. A significant similarity between the symptoms of post-COVID-19 and post-SARS-CoV was discovered by Kamal et al. [75]. While this was going on, Dana et al. [76] questioned whether patients who had recovered from COVID-19 would be affected by the long-term health effects of the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV). The bulk of earlier studies used extensive medical questionnaires to assess post-COVID-19 symptoms for only a few weeks following recovery. Through epidemiological investigations, they proposed the connection between the severity

of COVID-19 and post-discharge indications. Yvonne et al. [77] conducted a Facebook survey to know the post-COVID-19 symptoms and found that the majority of survivors still had persistent symptoms. Additionally, Carf et al. [78] completed a post-acute COVID-19 thorough questionnaire to identify any lingering character throughout the follow-up period following COVID-19 recovery (14–60 days). Most of them claimed minimum one COVID-19 related symptom persisted.

To the best of our knowledge, however, very few about the pathological remnant that might be responsible for the post-COVID-19 indications that call for extensive medical follow-up treatment. For comparatively lengthy periods of time after recovery, we looked for the main clinical symptoms and biochemical changes in COVID-19 survivors.

REVIEW OF LITERATURE

COVID and its variant -

During adoption to its new human hosts, COVID and its variant, SARS-CoV-2, are capable to genetic evolution with mutations, which results in their mutant variety. It's possible that this new variation differs in some ways from its ancestors' strains. . (World Health Organization)

A recent epidemiological update was provided by the WHO on June 22, 2021. Since the start of the pandemic, SARS-CoV-2 has been found in four different varieties, according to that update:

• Alpha (B.1.1.7), the initial version of concern, was reported in late December in the UK.

• Beta (B.1.351), first describe in December 2020 in South Africa,.

• Gamma (P.1) - first discovered in Brazil at the beginning of January 2021.

Delta (B.1.617.2) was initially discovered in India in December 2020.

Since there is currently no approved treatment for COVID-19, it poses the greatest hazard to public health [79]. Angiotensin converting enzyme 2 (ACE2) ct as receptor for both SARS-CoV and SARS-CoV-2. Human tissues like the lungs, kidneys, testicles, small intestine, thyroid gland, myocardium, pancreas, adipose tissues, blood vessels, blood cells, spleen, bone marrow, brain, liver, urine bladder, and adrenal glands are all diffusely distributed with ACE2 [80].

SARS-CoV-2 has a generally poor tolerance for hot temperatures. It can only endure 56°C for 30 minutes. Additionally, it is sensitive to ultraviolet light, ethanol, ether, both at a concentration of 75%, and preparations including substances like peracetic acid, chloroform, and chlorine [81,82]. Additionally, it shows resistance to chlorhexidine.

Due to their high level of identity, SARS-CoV-2 may share traits with SARS-CoV-1 in terms of their physical and chemical makeup. SARS-CoV-1 may live for around 5 days in an environment with 50% humidity and 22–25°C temperatures. According to studies by Moriyama et al. [83], low humidity and temperature lengthen the time the virus remains in the aerosol. SARS-CoV-2 was extremely stable at 4 C but sensitive to heat, according to Chin et al. [84]. On the fourteenth day at 4 °C, the infectious titeron hardly dropped by about 0.7 log units. If the incubation temperature increased to 70 C, the virus survived for only five minutes. This virus demonstrates stability over a wide pH range(3–10) when kept at room temperature.

Receptor for SARS-CoV-2 Entry and Replication Cycle –

The transmembrane S glycoprotein mediates CoV entrance into host cell [85,86]. The pathogenesis of SARS-CoV-2 is significantly influenced by the receptors and proteases found on the surface of the host cells. The angiotensin-converting enzyme 2 (ACE2) receptor for SARS-CoV-2 is the most well-known [87]. ACE2 is typically a zinc metalloprotease (carboxypeptidase). Although it has a different substrate specificity than dipeptidase angiotensin-converting enzyme (ACE), they are homologous.

In addition to the lungs, Li et al research .'s [88] revealed that ACE2 is expressed in other human tissues as well, suggesting that SARS-CoV-2 may be infected the other tissues also. A transmembrane glycoprotein called CD147, also referred to as basic immunoglobulin, is another entrance receptor for viruses.

The expression of CD147 was found in human and mouse brain cell lines by Qiao et al. [89]. Dipeptidyl peptidase 4 (DPP4), also known as CD26, is the alternative receptor for SARS-

CoV-2. A variety of cells, including those in the immune system, kidneys, lungs, smooth muscle, liver, and capillaries, express this ectopeptidase.

However, only ACE-2 is strongly supported as a functioning receptor protein that is necessary [90].

The ACE2-B0AT1 complex may be able to bind two S proteins concurrently, according to structural modelling, which sheds light on the molecular mechanisms behind coronavirus identification and infection [91]. Men were found to have a higher expression of it than women, which may help to explain why men are more susceptible to SARS-CoV-2 [82,91,92,93,94,95,96].

Replication starts once viral structures have been endocytosed into the cell. This method separates SARS-CoV-2 from other RNA viruses due to enhanced recombination processes [97,98,99]. The S protein is further divided by L cathepsin in an acidic environment. SARS-CoV-2 genetic material is consequently released into the cytoplasm [100]. The next step is a reverse transcription (Figure 3) [101].

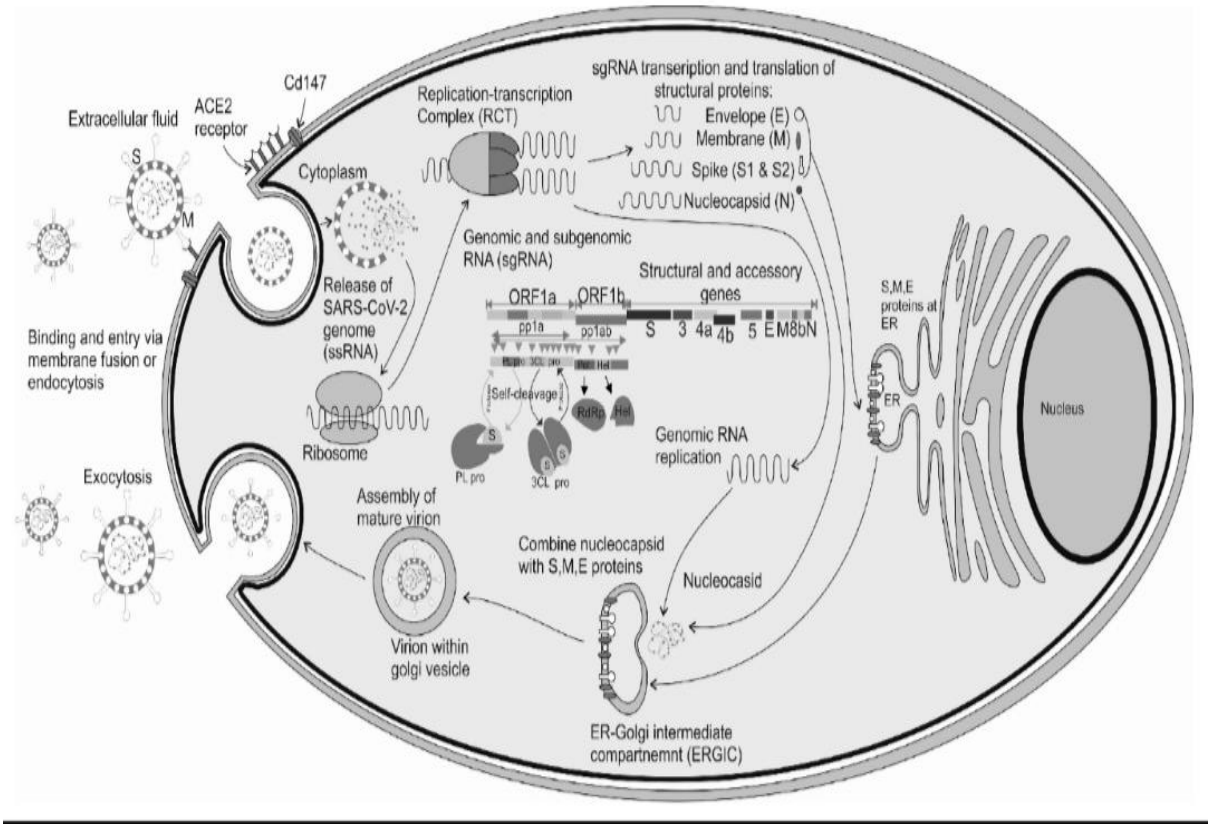


Figure.3 infection mechanism of SARS-CoV-2 into human cells. *[Katarzyna Grudlewska-Buda, Natalia Wiktorczyk-Kapischke, Ewa Wałecka-Zacharska et.al. SARS-CoV-2—Morphology, Transmission and Diagnosis during Pandemic, Review with Element of Meta-Analysis. J. clin. Med. 2021,10,1962]*

The vesicular replication-transcription complex is involved in transcription processes. The host ribosome plays a important role in the formation of an RNA strand that is negatively charged [81].

Mechanism of action –

Coughing, sneezing, and touching an infected surface are the main ways that this dangerous viral infection spreads because it is a viral infection that can be contracted by ingestion or inhalation of virus droplets.

How the virus enters and replicate inside human cells is showed in Figure.4 [102]

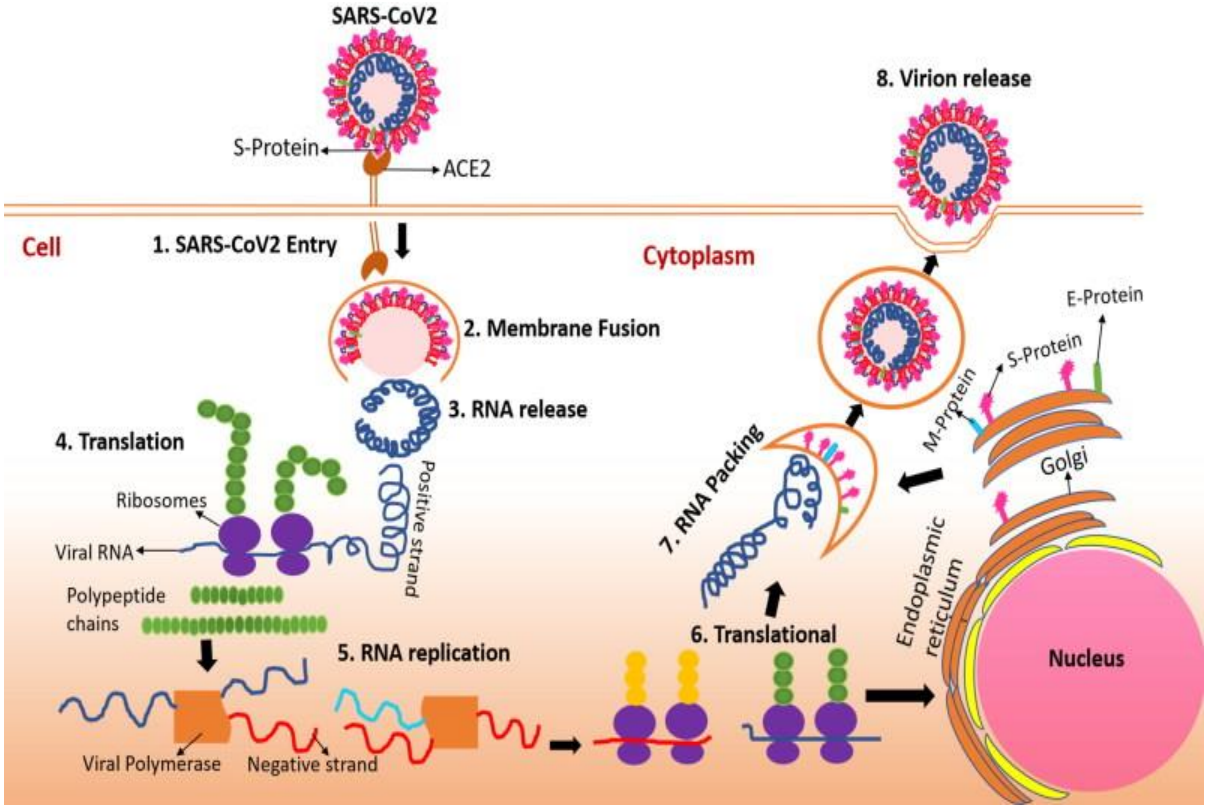


Figure.4 Entry and replication of virus. [*Subramanian Boopathi , Adolfo B. Poma and Ponmalai Kolandaivel. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment, journal of biomolcular structure and dynamics, 2021; 39:9, 3409-3418*]

Pathophysiology of Disease –

Although a lot has been learned about COVID-19's transmission and appearance, less is known about its pathogenesis.. Pathophysiology of the disease has been expressed in figure.5 [103,104,105].

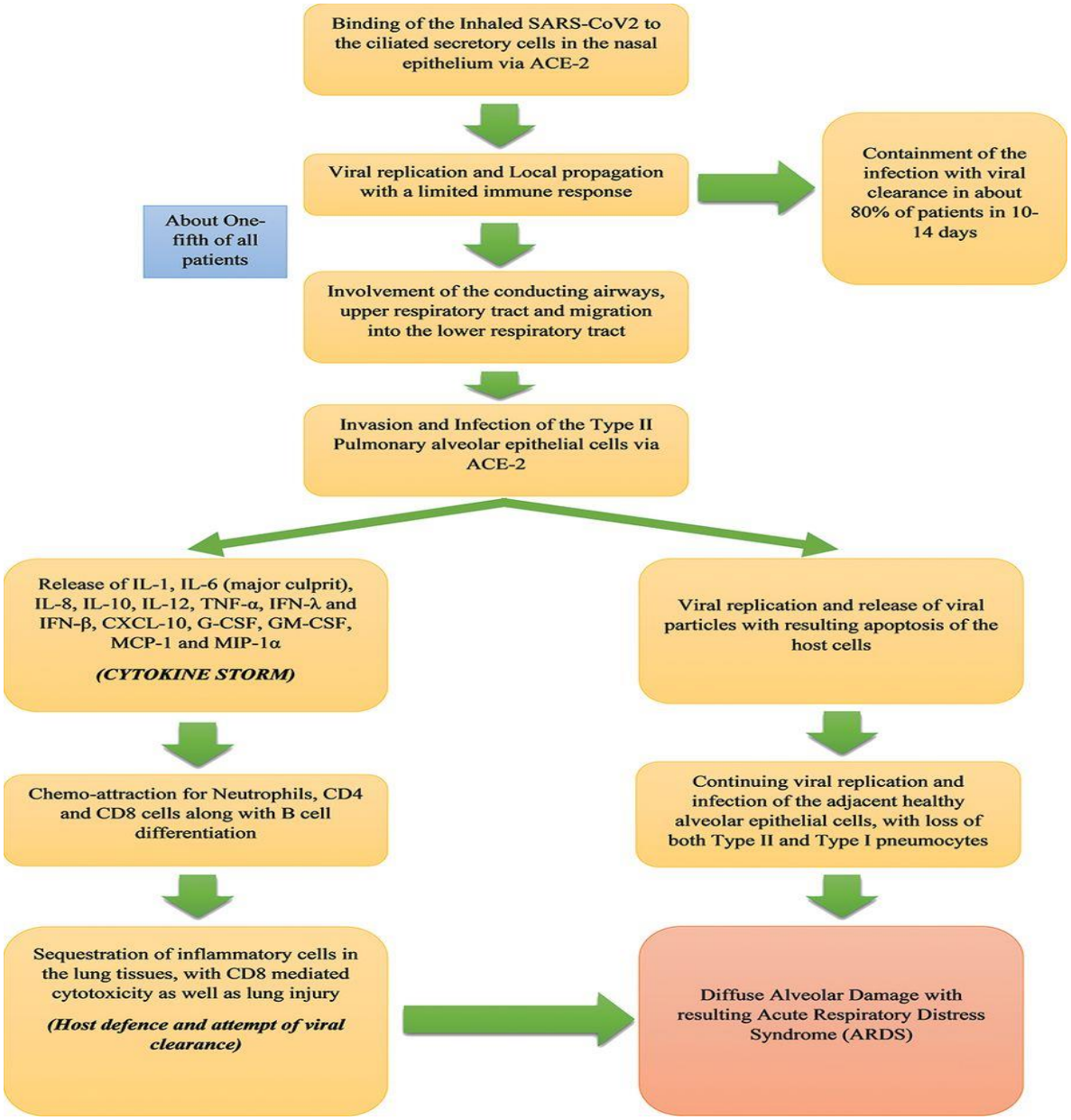


Figure.5 Pathophysiology of COVID-19. *[Cascella M, Rajnik M, Cuomo A, et.al. ,Features, evaluation and treatment coronavirus (COVID-19). Stat pearls [internet]. Treasure Island (FL): Stat Pearls Publishing, Jan 2020], [Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 2020;55:2000607], [Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019(COVID-19) outbreak in China:*

summary of are portof 72314 cases from the Chinese centre for disease control and prevention. JAMA 2020;323:1239].

Asymptomatic Phase –

The upper respiratory tract's nasal epithelial cells are where the SARS-CoV-2, which is acquired through respiratory aerosols, binds. Adult nasal epithelial cells have been found to have significant levels of ACE-2, which is the primary host receptor for viral entrance into cells [106,107]. In addition to infecting ciliated cells in the conducting airways, the virus replicates and spreads locally [108]. The immunological response produced at this stage, which lasts a few days, is only mild. Despite having a less viral load at the moment, the people are quite contagious, and testing with a nose swab can find the virus.

Covid-19 Diagnosis and Imaging - [109,110-115]

1) Lowered WBC count and lymphopenia were found in the basic blood tests.
    - Elevated AST, ALT, LDH, and CRP concentrations
    - A rise in D-dimer
    - A rise in PT/INR

2) RT-PCR molecular testing

- RT-PCR and rRT-PCR are methods used to amplify genetic material of virus collected by a nasal swab.
- inadequate sensitivity
- multi testing is necessary to make sure about viral clearance.

3) Chest X-ray

- Early on in the disease, there were no notable observations.
- In advanced disease, there are bilateral patchy opacities.

4) HRCT chest

- Patchy distribution of multifocal bilateral "ground or ground-glass" areas linked to consolidation areas
- Sign reading "Reverse Halo"
- Lymphadenopathy, cavitation, and calcification
- Significantly sensitive for the diagnosis of COVID-19

5) Tests for antibodies and sera

- There is additional work to be done to develop an accurate and sensitive antibody test.

Hepatotropism of SARS- CoV-2 –

The highest gene expression levels for ACE2 were found in cholangiocytes, which are comparable to alveolar type 2 cells, then sinusoidal endothelial cells, and hepatocytes in healthy livers, according to single-cell RNA sequencing investigations [116,117]. However, in a combined examination of three single-cell RNA sequencing datasets from liver tissue from healthy individuals, only few hepatocytes co-expressed ACE2 and TMPRSS2 [118]. The creation of ACE2- and TMPRSS2-expressing human liver ductal organoids by Zhao et al. that were capable of simulating SARS-CoV-2 infection[119] raises the possibility that the bile duct epithelium could facilitate pseudoparticle entry. It is important to note that the non-cholestatic pattern of liver biochemistry generally identified in COVID-19 is at variance with the ostensibly high SARS-CoV-2 entrance receptor expression and viral permissibility of cholangiocytes; the specific causes of this feature are still umrevealed. But it's feasible that

SARS-CoV-2 can replicate at a decreased level in cholangiocytes in vivo without leading to cell death. This mechanism would be in line with any other long-term viral replication reservoirs, such as those in the small intestine, which can gradually mould memory B cell responses to the virus[120]. It has also been demonstrated that liver organoids made from human pluripotent stem cells, which mostly consist of hepatocytes that express albumin, express ACE2 and allow SARS-CoV-2 pseudoparticle entry[121].

Liver injury due to COVID-19 -

The probable contribution of severe liver injury to rising mortality risk in COVID-19 patients has been highlighted in a number of hospital-based studies conducted globally. Additionally, it has been revealed that hepatic impairment in severe COVID-19 patients is linked to a fatal outcome[122-125, 116-118]. Numerous investigations have shown that patients with severe COVID-19 had a greater occurence of raised value of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and hypoalbuminemia than patients with non-severe COVID-19[126]. There may be a variety of processes at play in these patients' development of impaired liver function. These include inflammation that is immune-mediated, hypoxic injury brought on by acute pneumonia, and inflammation brought on by drugs that results in hepatic injury[127]. Patients with COVID-19, who have high viral loads in their liver cells may also have liver damage[128]. According to some data, the ACE-2 cell surface receptor is present in the liver tissue, however cholangiocytes (59.7%) are more highly expressed than hepatocytes (2.6%). Particularly, evidence suggests that the liver is a possible target organ for SARS-CoV2 since the degree of ACE-2 expression in cholangiocytes was comparable to that of type 2 pneumocytes. Instead, it appears that Kupffer cells express ACE-2 insufficiently [129]. The liver is a potential entry point for SARS-CoV-2 in light of the aforementioned. There may be a number of variables

that contribute to COVID-19-related liver injury. These include direct virus damage to the liver tissue produced by ACE-2, an uncontrolled inflammatory/immune response that results in fibrosis and liver dysfunction, or a liver lesion created by an anti-COVID19 drug therapy [130].

In a retrospective analysis, clinical indications for liver injury were retrieved and examined from 799 patients with COVID-19 confirmation who were hospitalised to a hospital in Madrid, Spain. The relationship between liver damage and the course of the illness was also examined. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and aspartate aminotransferase/alanine aminotransferase ratio were the parameters whose serum levels were increased above the Upper Limit of Normal (ULN) [131]. Liver involvement was observed in two recent pathogenic coronaviruses, SARS-COV and Middle East Respiratory Syndrome coronavirus (MERS-CoV). Hepatic involvement in COVID-19 is not wholly unexpected because these two viruses (especially SARS-COV) had a strong genetic similarity with the new coronavirus SARS-CoV-2 [132].

Few research have examined the effect of COVID-19 on the liver, and those that haveconcentrated on changes in liver biochemistry and histopathology throughout the course of infection in patients who get infected to the virus[133-137]. A number of recent studies have sought to assess variables linked to liver injury in American, Asian, and European cohorts[133,138-141], as well as cohorts from Europe[142-145]. The prevalence of liver illness in SARS-CoV-2 infections is still completely unclear, which limits our understanding of how impaired liver function affects the overall prognosis.

Post COVID-19 effect on liver –

The literature has extensively explored post-COVID-19 symptoms following negative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) tests for SARS-CoV-2. A significant similarity between the symptoms of post-COVID-19 and post-SARS-CoV was discovered by Kamal et al. [146]. The long-term health effects of the Middle East respiratory syndrome coronavirus (MERS-CoV) and the severe acute respiratory syndrome coronavirus (SARS-CoV) on COVID-19 recovery patients were questioned by Dana et al. [147] at the same time.

Our study will take into account how COVID-19 patients' post-recovery liver function effects. Participants in the case group in a case control study would have immaterial medical history prior to COVID-19 or throughout the post-recovery period up until enrollment. To reduce the confounding effects of severe sickness, participants with a history of mild to moderate COVID-19 infection without hospitalisation or oxygen therapy were chosen. Participants in the control group were in good health and had no prior history of COVID-19 or other morbidities. The selection of COVID-19 survivors with mild and moderate diagnoses will follow the World Health Organization's recommendations. Following are WHO recommendations for people with mild, moderate, and severe COVID-19: [148]

WHO Classification based on disease severity (Table 1) **

MILD	Non-specific symptoms like a sore throat, stuffy nose, headache, nausea, and vomiting have also been described, along with loss of taste and smell. The majority of people suffer from fever, cough, tiredness, and myalgias. people who have symptoms but no signs of viral pneumonia or hypoxia.
MODERATE	Cough, fever, dyspnea, and rapid breathing are symptoms of pneumonia, although they are not severe pneumonia, as shown by Sp02 levels greater than 90% on room air.
SEVERE	Along with one of the following conditions: respiratory rate > 30 breaths/min, significant respiratory distress, or Sp02 90% on room air, and the clinical symptoms of pneumonia (fever, cough, dyspnea).

[COVID-19 clinical managements: Living guidance, 25 January 2021].

AIM AND OBJECTIVES

AIM :

To find an association of Liver enzymes (ALT, AST, ALP, GGT) in diagnosed COVID-19 survivors and control subjects.

OBJECTIVES:

1. To estimate levels of serum Alanine transaminase(ALT) in diagnosed COVID-19 survivors and control subjects.
2. To estimate levels of serum Aspartate transaminase(AST) in diagnosed COVID-19 survivors and control subjects.
3. To estimate levels of Alkaline phosphatase(ALP) in diagnosed COVID-19 survivors and control subjects.
4. To estimate levels of serum Gamma-glutamyl transferase(GGT) in diagnosed COVID-19 survivors and control subjects.
5. To find out the correlations between these parameters in COVID-19 survivors and control subjects, if any .

RESEARCH QUESTION

Is there any significant correlation between Liver enzymes in diagnosed COVID-19 survivors and control subjects?

STATISTICAL HYPOTHESIS

NULL HYPOTHESIS (H0) :

There is no significant correlation between liver enzymes in diagnosed COVID-19 survivors and control subjects.

ALTERNATE HYPOTHESIS (H1) :

There is significant correlation between liver enzymes of diagnosed COVID-19 survivors and control subjects .

Materials And Methods

METHODOLOGY

TYPE OF STUDY : A case control study

SAMPLING METHOD: Non-probability purposive sampling

SUBJECT SELECTION

SELECTION OF CASES:

INCLUSION CRITERIA (CASES):

- Age 35-60 years
- Patients diagnosed for COVID-19 with RT-PCR /Rapid Antigen tests without any comorbidities
- Not on hormonal, steroid, diuretics or any other therapies.
- Patients having mild to moderate COVID-19 illness without oxygen therapy at home.
- Patients having mild to moderate COVID-19 illness admitted in isolation ward of hospital without oxygen support

EXCLUSION CRITERIA :

EXCLUSION CRITERIA

1. Diagnosed COVID-19 survivors having comorbidities before COVID-19 will be excluded.
2. Diagnosed COVID-19 survivors who have undergone hospitalization or oxygen therapy.
3. Patients with alcohol consumption, and smoking history.

CONTROL GROUP –

1. Age 35 – 60 years.
2. Age and gender matched.
3. Apparently healthy subjects.

SAMPLE SIZE ESTIMATION

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2(Z_\beta + Z_{\alpha/2})^2}{(\mathrm{difference})^2}$$

Reference: Rosner B Fundamentals of biostatistics 7th ed. Boston, MA: Brooks / cole:2011

r = 1 (control to case ratio)

Z1β = it has desired power (0.84 for 80% power)

Z1α/2 = critical value and a standard value for the corresponding value for confidence (at 95% CI it is 1.96)

σ = 0.52 standard deviation

d = 0.12 effect size (expected difference in the mean)

group 1 = 35

group 2 = 35

Total sample size = 70.

Reference paper: [149]

Gameil, M.A., Marzouk, R.E., Elsebaie, A.H. et al. Long-term clinical and biochemical residue after COVID-19 recovery. Egypt Liver Journal 11, 74 (2021).

COLLECTION OF SAMPLES:

- Under aseptic condition, 4ml of venous blood was collected from the subject in a plain vial.
- 1ml of blood was used for estimation of serum Alanine aminotransferase(ALT).
- 1ml of blood was used for estimation of serum Aspartate aminotransferase(AST).
- 1ml of blood was used for estimation of serum Alkaline phosphatase(ALP).
- 1ml of blood was used for estimation of serum Gamma-glutamyl transferase(GGT).

STORAGE AND TESTING OF SAMPLE: Testing and storage (at -20 °C) of sampling was done in clinical Research Laboratory, Department of Biochemistry, IIMS&R, Integral University, Lucknow.

LABORATORY INVESTIGATION:

1. **Estimation of serum Alanine aminotransferase(ALT): [150]**

Read absorbance at 340 nm using a semi-autoanalyser and the fixed time kinetic technique.

The IFCC's recommendations were the foundation for this ALT reagent, which does not contain pyridoxal phosphate. Following is a list of the reactions that make up the assay system:

1. Pyruvate and L-glutamate are produced as a result of the enzyme SGPT, which is present in the sample, transferring the amino group from alanine to the carbon atom of 2-oxoglutarate.

2. LDH, which is present in the reagent, reduces pyruvate to lactate while simultaneously oxidising NADH to NAD. The rate at which the absorbance at 340 nm decreases as a result of NADH oxidation is used to track the reaction.

3. LDH quickly and fully reduces endogenous sample pyruvate during the initial incubation period to prevent interference during the experiment.

Normal reference value - 60U/L for men and 42U/L for women.

2. Estimation of serum aspartate aminotransferase(AST): [150]

Read the absorbance at 340 nm using a semi-autoanalyser and the fixed time kinetic technique.

Principle: This reagent does not contain pyridoxal phosphate but is based on IFCC recommendations. Following is a list of the reactions that make up the assay system:

1. The sample's SGOT catalyses the transfer of the amino group from L-aspartate to 2-oxoglutarate, resulting in the formation of oxaloacetate and L-glutamate.

2. Oxaloacetate is converted to L-malate in the presence of NADH and malate dehydrogenase (MDH). NADH is converted to NAD during this process. The rate of decrease in absorbance at 340 nm caused by the oxidation of NADH to NAD is used to track the reaction.

3 In order to quickly and completely reduce endogenous pyruvate and prevent it from interfering with the experiment, lactate dehydrogenase (LDH) must be added to the reagent.

Normal reference value - 35 U/L is the normal reference value, and there are no appreciable sex-related variances.

3. Estimation of serum alkaline phosphatase: [151]

Read the absorbance at 405 nm using a semi-autoanalyser and the fixed time kinetic technique.

Principle: Glycerophosphate, phenylphosphate, and p-nitrophenylphosphate are only a few of the key substrates that have been used in alkaline phosphatase activity. The Bessey et al. approach was improved by Bowers and McPomb by using kinetic measurement. This procedure was improved by Tietz et al. to incorporate chelated zinc, magnesium, and HEDTA metal ion buffers. This approach has been modified by the alkaline phosphatase process.

The sample's alkaline phosphatase catalyses the hydrolysis of colourless p-nitrophenylphosphate to produce p-nitrophenol and phosphate at the assay's alkaline pH and p-nitrophenol in the form of yellow phenoxide (17).

Normal reference value - 33-98 U/L for women and 43-115 U/L for men constitute the normal reference range.

4. Estimation of serum Gamma-glutamyl transferase(GGT): [152]

Read the absorbance at 405 nm using a semi-autoanalyser and the fixed time kinetic technique.

L-y-glutamyl-3-carboxy-4 nitroanilide and glycylglycine are converted into L-y-glutamylglycylglycine and 5-amino-2-nitrobenzonate through the action of the enzyme main - GGT. As the GGT activity in the sample increases, an increase in absorbance that is proportional to the rate of 5-amino-2-nitrobenzonate production is observed.

Normal reference value - 40U/L for females and 70U/L for males is the standard reference value.

STATISTICAL ANALYSIS

Statistical analysis was done by using GraphPad and Microsoft- Excel. Data was represented as mean ± SD (standard Deviation). An unpaired t-test was performed to compare the study parameters between cases and controls. Pearson's correlation analysis was employed to determine the relationship between variables. p- value <0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

More than a million COVID-19 patients have recovered from their illness and are resuming their normal social and professional lives. However, COVID-19 may also result in post-traumatic stress disorder, problems and sequelae, as well as the virus's potential reactivation, in these patients [153].

Following up includes keeping knowledge about nucleic acid reactivation as one of its key goals. Although the most recent research indicates that a positive follow-up RT-PCR does not necessarily indicate infectivity [154], typical symptoms are present in a small fraction of recurrence patients and necessitate additional therapy [155-157].

On the other hand, it is crucial to comprehend the potential outcomes of COVID-19 patients who have been released, particularly if they have any other harmful conditions, in order to protect their life in the future [158, 159].

In the present study, we performed and analyzed biochemical parameters (liver enzymes) between COVID-19 survivors and control subjects. The liver enzymes ALT, AST, ALP & GGT are observed to be elevated in 54.28%, 45.71%, 51.42% and 2.8% patients, respectively.

Pearson correlation was done to find out the correlation of liver enzymes as shown in the table no. 6 There is significant positive correlation between ALT&AST, ALT&ALP, ALT&GGT, as depicted in table no. 6 .

ALT and ALP levels are elevated significantly in COVID-19 survivors, while levels of AST and GGT are elevated but not significant.

AGE -

In present study, 35 COVID-19 survivors with verified negative SARS-CoV-2 RT-PCR tests were compared to 35 healthy persons without a history of COVID 19 with mean ages of 44±10.24 and 45±8.64, respectively. Male and female participants make up 17.14% 82.85% and 37.14% 62.85% of the case and control groups, respectively.

ALANINE AMINOTRANSFERASE

After a negative RT-PCR test for SARS-CoV-2, ALT showed significant variations across the study groups, indicating a long-lasting effect residue. Table No. 2 and Fig. 6 demonstrate that COVID-19 survivors had significantly higher ALT values than control subjects (p = 0.0020).

Table no. 2 Comparison of ALT levels between controls and cases:

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
ALT (IU/L)	30.18±5.10	36.63±10.73	0.0020

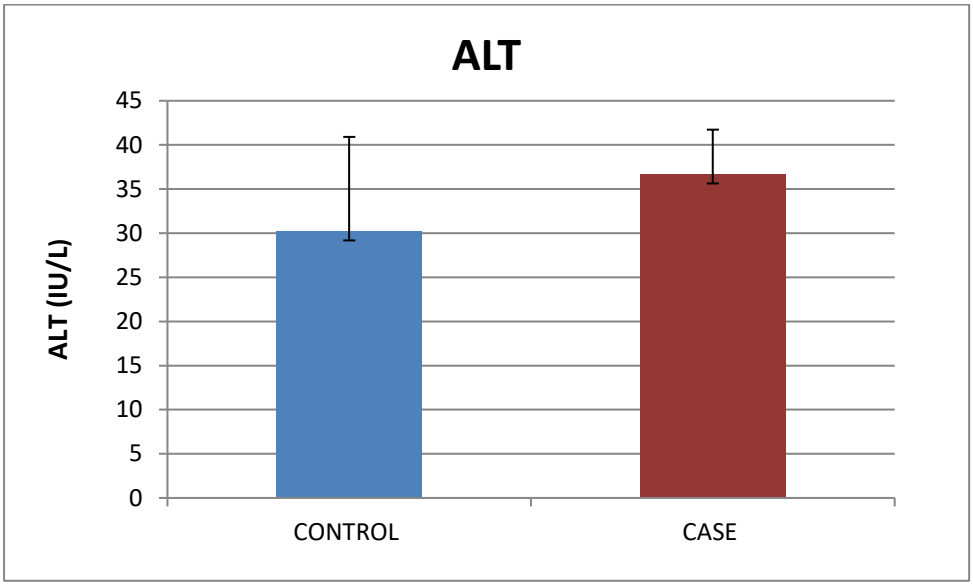


Fig.6 Comparison of ALT levels between controls and cases

Table no. 2 & fig.6 shows the comparison of ALT levels between controls and cases. ALT was significantly (p= 0.0020) higher among cases (36.63(IU/L) ±10.73) than controls (30.18(IU/L) ±5.10).

ASPARTATE AMINOTRANSFERASE

After a negative RT-PCR test for SARS-CoV-2, AST showed variations between the study groups, indicating a long-lasting effect residue, Table No. 3 and fig. 7. demonstrate that COVID-19 survivors had elevated AST values than control subjects, but the result was not statistically significant (p = 0.0738).

Table no. 3 Comparison of AST levels between controls and cases:

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
AST (IU/L)	28.12±6.47	31.10±7.24	0.0738

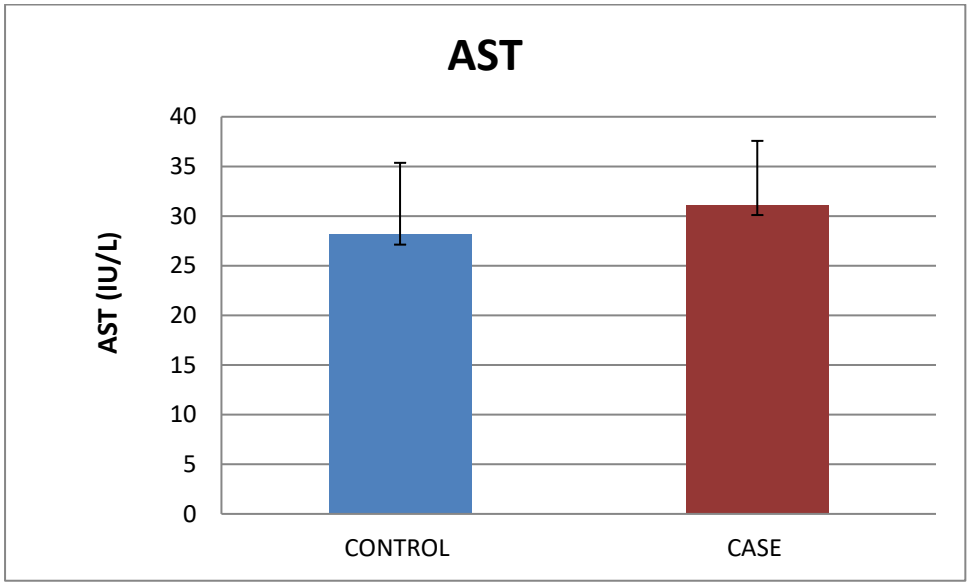


Fig.7 Comparison of AST levels between controls and cases

Table no. 3 & fig.7 shows the comparison of AST levels between controls and cases. AST levels were elevated among cases (31.10(IU/L) ±7.24) than controls (28.12(IU/L) ±6.47). Result was statistically non-significant.

ALKALINE PHOSPHATASE

After a negative RT-PCR test for SARS-CoV-2, ALP showed significant variations across the study groups, indicating a long-lasting effect residue. Table No. 4 and Fig. 8 demonstrate that COVID-19 survivors had significantly higher ALT values than control subjects (p = 0.0001).

Table no. 4 Comparison of ALP levels between controls and cases

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
ALP (IU/L)	88.78±13.13	106.05±15.56	0.0001

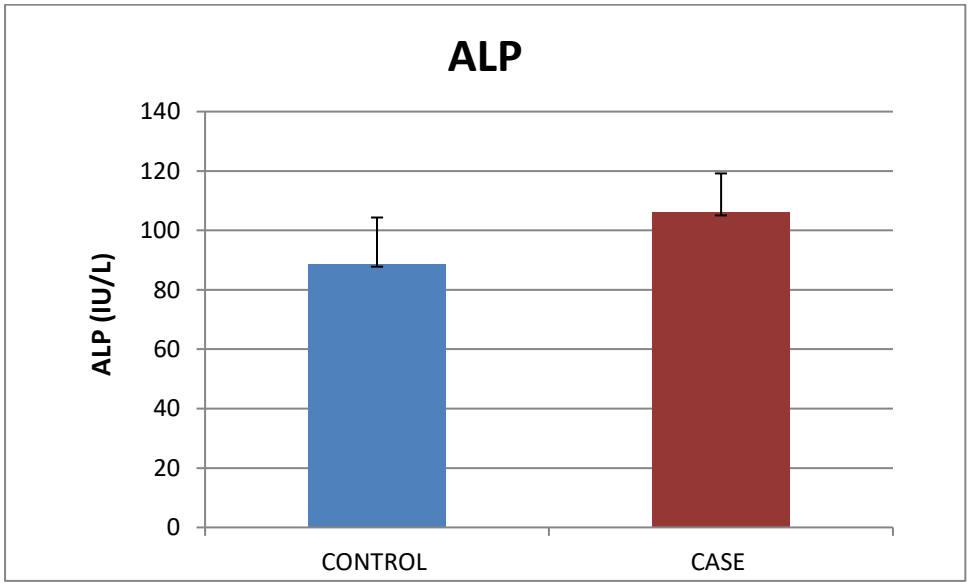


Fig.8 Comparison of ALP levels between controls and cases

Table no. 4 & fig.8 shows the comparison of ALP level between controls and cases. ALP levels were significantly (p= 0.0001) higher among cases (106.05(IU/L) ±15.56) than controls (88.78(IU/L) ±13.13).

GAMMA GLUTAMYL-TRANSFERASE

After a negative RT-PCR test for SARS-CoV-2, GGT showed variations between the study groups, indicating a long-lasting effect residue, Table No. 5 and fig. 9. demonstrate that COVID-19 survivors had elevated GGT values than control subjects, but the result was not statistically significant (p = 0.7778).

Table no. 5 Comparison of GGT levels between controls and cases

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
GGT (IU/L)	13.94±9.02	14.67±12.43	0.7778

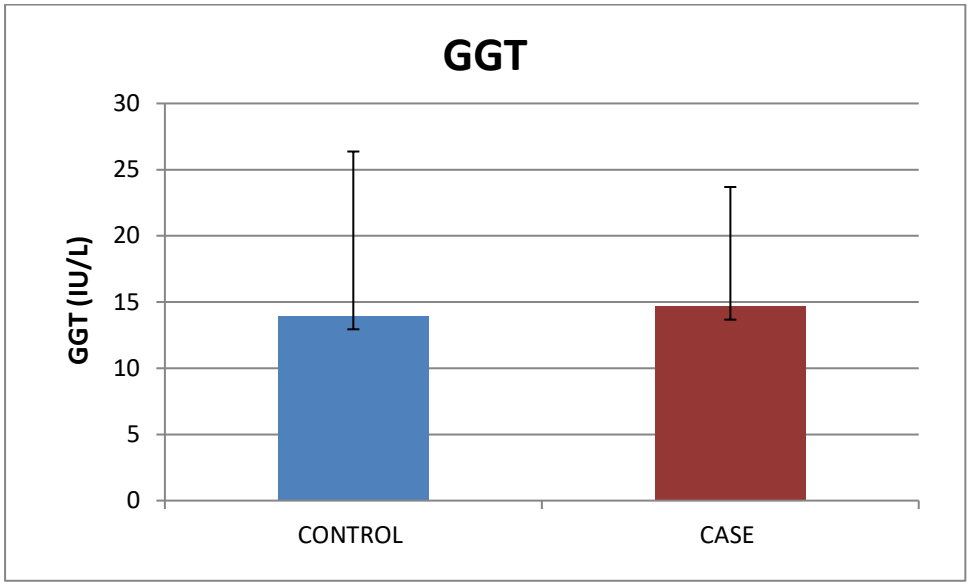


Fig. 9 Comparison of GGT levels between controls and cases

Table no. 5 & fig.9 shows the comparison of GGT levels between controls and cases. GGT levels were elevated among cases (14.67(IU/L) ±12.43) than controls (13.94(IU/L) ±9.02). Result was statistically non-significant.

Table No. 6 Pearson Correlation Coefficient in between the study variables in Cases

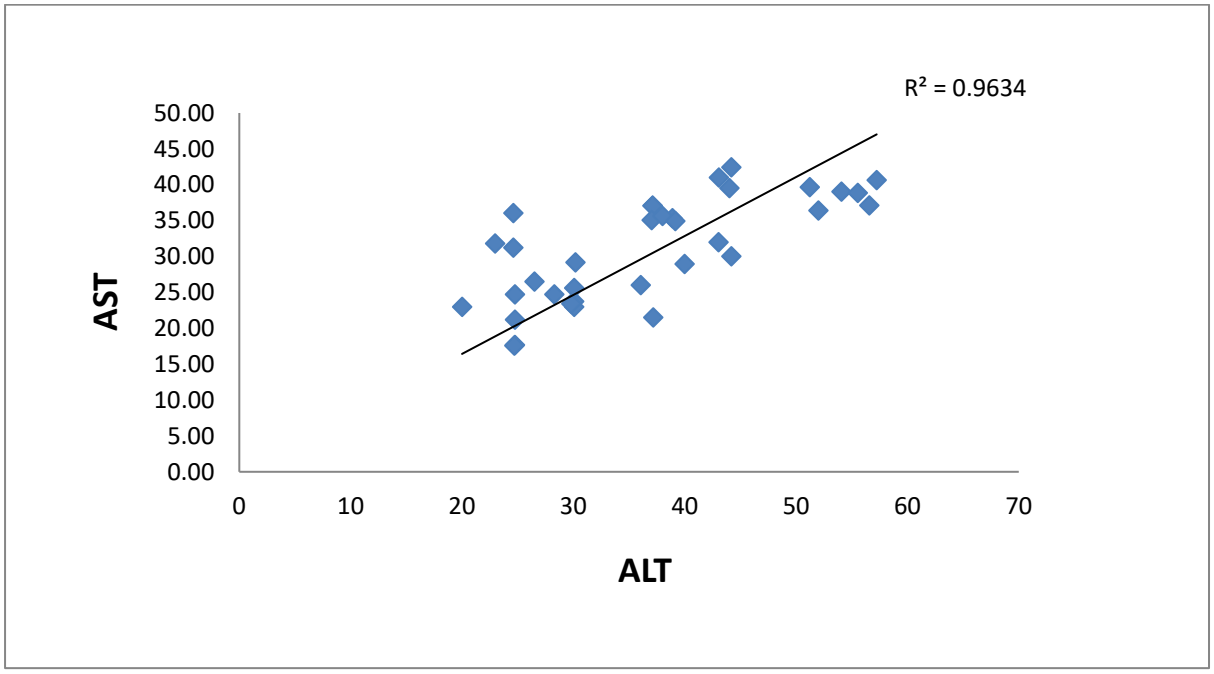
		Age	ALT	AST	ALP	GGT	
Age	**Pearson correlation**	1	-0.07	-0.02	0.011	-0.23	
	Sig. (2-tailed)	>0.05	>0.05	>0.05	>0.05	>0.05	
	N	35	35	35	35	35	
ALT	**Pearson correlation**	-0.07	1	0.7275	0.50	0.625	
	Sig. (2-tailed)	>0.05		<0.001	0.002	<0.001	
	N	35	35	35	35	35	
AST	**Pearson correlation**	-0.02	0.7275	1	0.8845	0.1805	
	Sig. (2-tailed)	>0.05	<0.001		<0.001	0.299	
	N	35	35	35	35	35	
ALP	**Pearson correlation**	0.011	0.50	0.8845	1	0.0237	
	Sig. (2-tailed)	>0.05	0.002	<0.001		0.89	
	N	35	35	35	35	35	
GGT	**Pearson correlation**	-0.23	0.625	0.1805	0.0237	1	
	Sig. (2-tailed)	>0.05	<0.001	0.299	0.89		
	N	35	35	35	35	35	

*.Showed that correlation is significant at the level ($p<0.05$) (2-tailed).

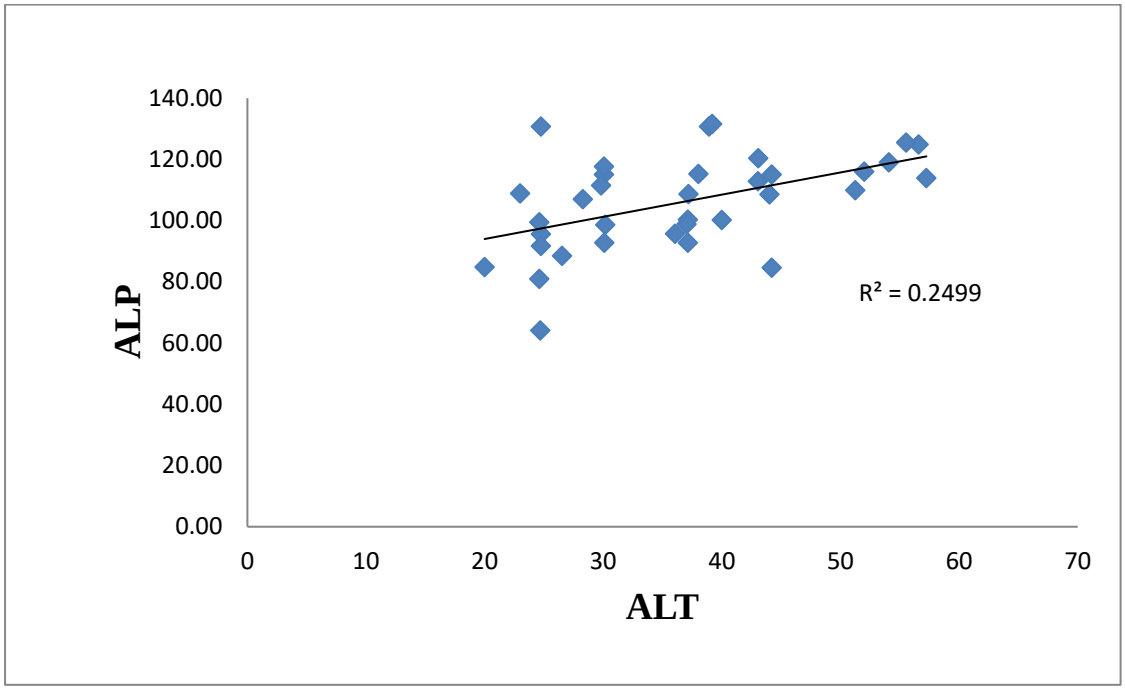
** showed that correlation is significant at the level (<0.001) (2-tailed).

(Table no. 6 & fig.no. 10,11,12,13) shows the correlation matrix which represents the quantitative measurements of degree of relationship among different variables.

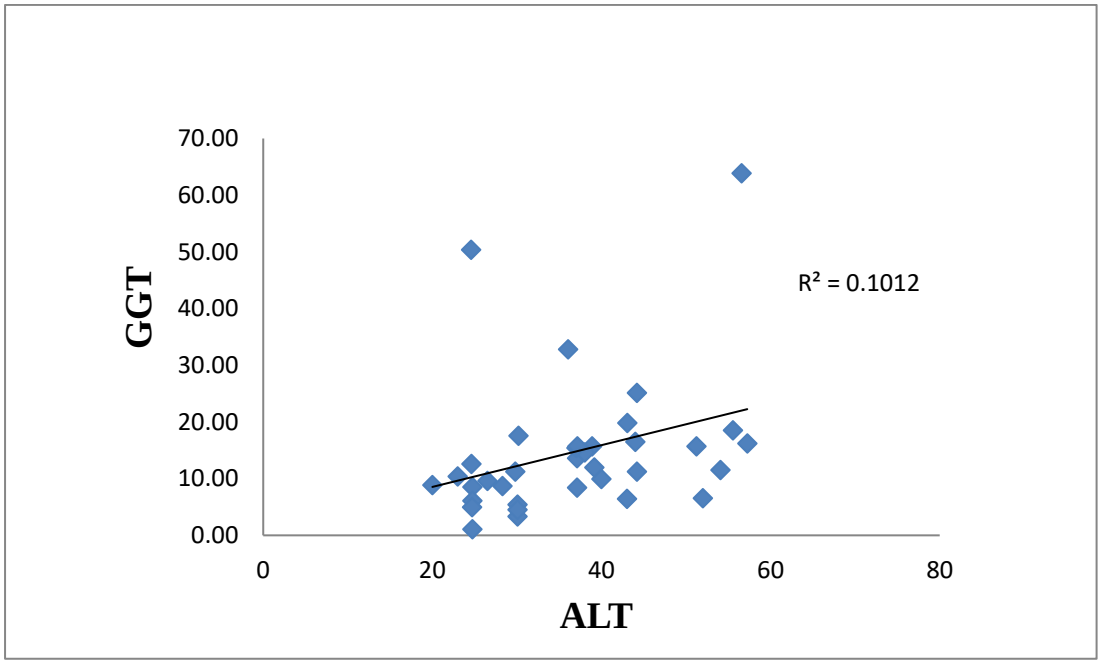
- ALT and AST express a significant positive correlation (r = 0.7275).
- ALT and ALP express a significant positive correlation (r = 0.50).
- ALT and GGT express a significant positive correlation (r = 0.625).
- AST and ALP express a significant positive correlation (r = 0.8845).
- There is no correlation express in between AST and GGT (r = 0.1805).
- There is no correlation express in between ALP and GGT (r = 0.0237).



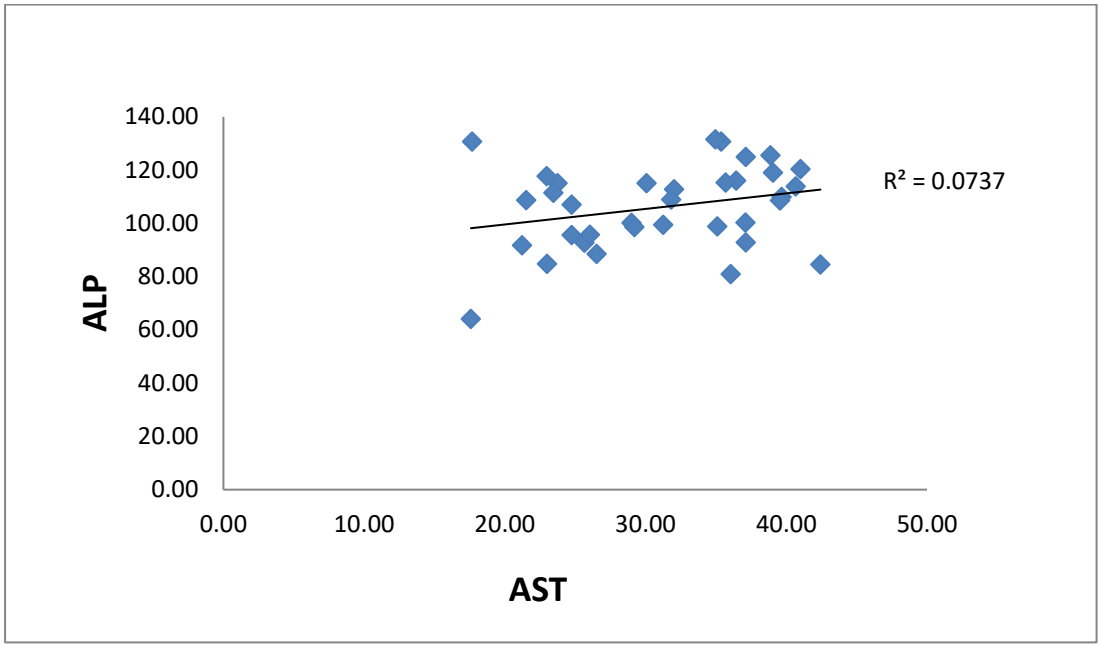
Fig,10: Scatter diagram showing association between ALT and AST in cases.



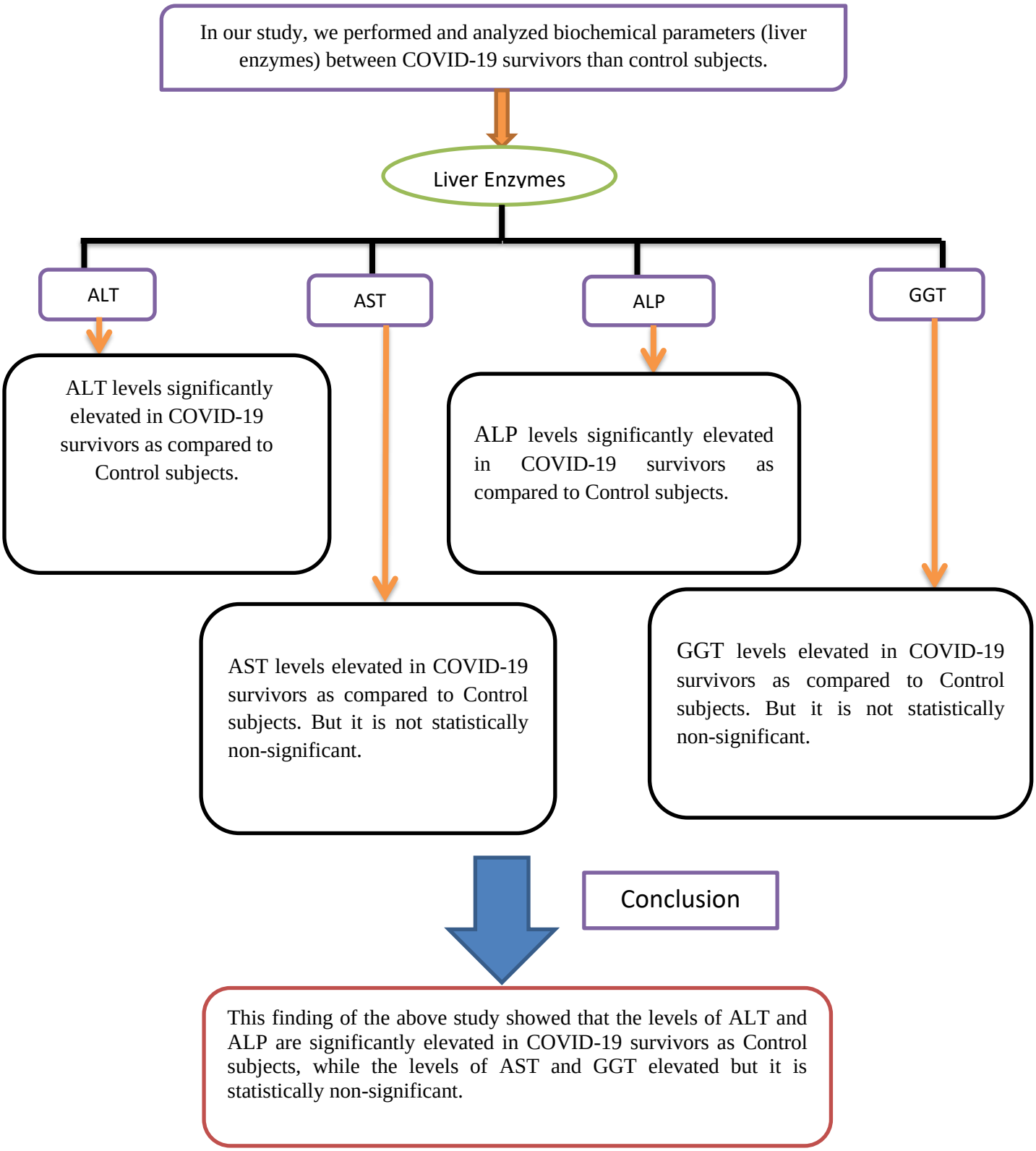
Fig,11: Scatter diagram showing association between ALT and ALP in cases.



Fig,12: Scatter diagram showing association between ALT and GGT in cases.



Fig,13: Scatter diagram showing association between AST and ALP in cases.



Figure;15. Showing the result of the study

DISCUSSION

The pattern of liver damage during acute COVID-19 has received a great deal of attention, but the long-term effects of COVID-19 on the functions of the liver are yet unknown. In the present investigation, liver enzymes differed statistically significantly between COVID-19 survivors and people who had never been exposed to the virus. Significantly higher levels of ALT, AST, GGT, and ALP were found in COVID-19 survivors.

Ya-Wen et. al. reported that for 14 days after discharge, COVID19 survivors had lower serum albumin and higher levels of ALT, GGT, and ALP. But within two months, these values progressively went back to normal. In our study, we found that long-term resolution of COVID-19 caused ALT, AST, GGT, and ALP levels to rise steadily over time. Fan et. al. [161] examination of the disruption of liver function in COVID-19 patients without concurrent elevation of serum total bilirubin, hepatic aminotransferases exhibited reversible mild to moderate rise.

Additionally, Xu et. al. [162] demonstrated non-elevated blood bilirubin in COVID-19 patients despite the high expression of ACE2 in the cholangiocytes rather than the hepatocytes of the hepatic vascular endothelium. They attributed these findings to the enormous systemic inflammatory response rather than a direct viral invasion. The precise pathogenesis of liver injury in COVID-19 patients, however, is still up for discussion.

Numerous theories have been put forth to explain the pathogenesis of hepatic changes, including direct hepatocyte viral invasion mediated by ACE2, immune homeostasis disruption, systemic inflammatory response, concurrent hypotension, pneumonia-associated hypoxia, and cytokine storm with an increase in hepatic stellate cell numbers cytokine storm with an increase in pro-inflammatory cytokines, and drug-induced hepatotoxicity [163, 164].

Tian et al. [165] found isolated macro-vesicular steatosis and mild sinusoidal dilatation without definitive evidence of bile duct damage.

Zsuzsanna et. al. [166] findings further connected these alterations to lymphocytic endothelitis with hepatocyte necrosis brought on by direct vial invasion and immune cell hyper-activation.

The comparatively longer follow-up period following the SARS-CoV-2 RT-PCR test results was very important feature of our study. Specifically, COVID-19 survivors with a history of mild to moderate disease were included in the case group. We made an effort to exclude out potential confounders such severe COVID-19 sickness and a history of acute or chronic morbidities prior to inclusion. Patients who were hospitalised or receiving oxygen therapy are also not included. We had to exclude alcohol abuse because we included the GGT variable in our study.

We deal many difficulties such as the lack of documented data of COVID-19 survivors before and during the acute stage of illness, we relied on the detailed medical history of participants and limited time duration. However, honestly, we cannot rely fully on the story of participants. Single-centre study represented major limitations. In this study, we tried to detect the significant differences between COVID-19 survivors who are expected to be definitely healthy versus healthy subjects without COVID-19 exposure.

Even after all this we finished our work and concluded that there is small or major difference was found between these parameters of COVID-19 survivors and control subjects. ALT & ALP are significantly elevated in COVID-19 survivors. The levels of AST and GGT elevated, but it is statistically non-significant.

In this study we have also shown the correlation matrix which represents the quantitative measurements of degree of relationship among different variables.

Multi-centre trials with larger-scale, multiple ethnicities, longer follow-up period and invasive tools may help more advanced research in the future.

Conclusion

It is still unknown how much liver damage is related to the recent SARS-CoV-2 outbreak. In this work, we described the clinical features of 35 patients who were tested positive for COVID-19 and we include 35 healthy individuals without COVID-19 history. We compared the total outcome of both group to see the change in between these groups.

We employed the levels of the liver enzymes ALT, AST, ALP, and GGT as the main screening tests to determine the liver's functional status.

We made every effort to find any pathological, clinical, and biochemical remnants in our area following the retrieval of COVID-19.

Here we report that, ALT and ALP were significantly elevated in approximately 45-50% of COVID-19-confirmed case history. While AST and GGT were also elevated but, it is statistically non-significant .

Remaining severe clinical and biochemical abnormalities in COVID-19 survivors made longer-term, intensive medical follow-up care necessary.

SUMMARY

The most of earlier studies used detailed medical questionnaires to assess post-COVID-19 symptoms for only a few weeks following recovery. We looked for any potential pathological clinical symptoms and biochemical remnants that might longer after the real-time polymerase chain reaction (RT-PCR) test for SARS-CoV-2 was negative.

Among 35 COVID-19 survivors of mean age 44 and 37.14% male proportion and 62.85% female proportion, and among 35 Control healthy individuals of mean age 45 and 17.14% male proportion and 82.85% female proportion, were involved in this study.

ALT levels were significantly elevated in COVID-19 survivors with mean 36.63(IU/L) ±10.73 (p= 0.0020). The result of AST was elevated in COVID-19 survivors with a mean of 31.10(IU/L) ±7.24 (p= 0.0738) but it is statistically non-significant.. ALP levels were significantly elevated in COVID-19 survivors with a mean of 106.05(IU/L) ±15.56 (p= 0.0001). The result of GGT was slightly elevated in COVID-19 survivors with a mean of 14.67(IU/L) ±12.43 (p= 0.7778), but it is statistically non-significant.

Patients with COVID-19 may experience liver damage that are related to their clinical scores, basic diseases, symptoms, hospitalisation for pharmacological treatment, and consequences. The heart, kidneys, thyroid, lipid and glucose metabolism, immunological index, leukocyte, erythrocyte, haemoglobin, and platelet-related indexes were all connected with indicators of liver function.

Poorer recovery for COVID-19 patients may be indicated by abnormal liver function. When COVID-19 patients are followed for a long time, changes in liver function should be highlighted, along with the importance of using the right therapies to restore liver function.

REFERENCES

REFERENCES

1. World Health Organization. Naming the Coronavirus Disease (COVID-19 and the Virus That Causes it. (2020)
2. World Health Organization. Coronavirus Disease (COVID19) Situation Dashboard. (2020).
3. Cheung KS, Hung IFN, Chan PPY, et al. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. Gastroenterology. 2020;159(1):81-95.
4. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and bio- chemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020;63:364–74.
5. Gorbalenya AE, Baker SC, Baric RS, et al. The species Severe acute respira- tory syndrome related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536–44.
6. Vaira LA, Hopkins C, Salzano G, Petrocelli M, Melis A, Cucurullo M, Ferrari M, Gagliardini L, Pipolo C, Deiana G, et al. Olfactory and gustatory func- tion impairment in COVID-19 patients: Italian objective multicenter-study. Head Neck. 2020;42(7):1560–9.
7. Vaira LA, Deiana G, Fois AG, Pirina P, Madeddu G, De Vito A, Babudieri S, Petrocelli M, Serra A, Bussu F, et al. Objective evaluation of anosmia and ageusia in COVID-19 patients: Single-center experience on 72 cases. Head Neck. 2020;42(6):1252–8.
8. Guarneri C, Rullo EV, Pavone P, Berretta M, Ceccarelli M, Natale A, Nun- nari G. Silent COVID-19: what your skin can reveal. Lancet Infect Dis. 2020;S1473–3099(20):30402–3.
9. Bindom SM, Lazartigues E. The sweeter side of ACE2: Physiological evi- dence for a role in diabetes. Mol Cell Endocrinol. 2008;302(2):193–202
10. Zaim, S.; Chong, J.H.; Sankaranarayanan, V.; Harky, A. Covid-19 and multiorgan response. Curr. Probl. Cardiol. 2020, 45, 100618.
11. Yuki, K.; Fujiogi, M.; Koutsogiannaki, S. Covid-19 pathophysiology: A review. Clin. Immunol. 2020, 215, 108427.
12. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003;426:450–4.
13. Chen Y, Guo Y, Pan Y, et al. Structure analysis of the receptor binding of 2019-nCoV [published online ahead of print, 2020 Feb 17]. Biochem Biophys Res Commun 2020;525:135–40.
14. Walls AC, Park YJ, Tortorici MA, et al. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281–292.e6.

15. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage forSARS-CoV-2 and other lineage beta coronaviruses. Nat Microbiol 2020;5:562–9.
16. Parasher A. COVID-19: Current understanding of its pathophysiology, clinical presentation and treatment Postgrad Med J 2021;97:312–32
17. Lai MM, Cavanagh D. The molecular biology of coronaviruses. Adv Virus Res 1997;48:1–100.
18. Yang N, Shen HM. Targeting the endocytic pathway and autophagy process as a novel therapeutic strategy in COVID-19. Int J Biol Sci 2020;16:1724–31.
19. Cascella M, Rajnik M, Cuomo A, et.al., Features, evaluation and treatment coronavirus (COVID-19). Stat pearls [internet]. Treasure Island (FL): Stat Pearls Publishing, Jan 2020
20. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 2020;55:2000607.
21. Lai,M.M.;Stohlman,S.A.ComparativeanalysisofRNAgenomesofmousehepatitisviruses. J.Virol. 1981,38,661–670.
22. Chen, N.; Zhou, M.; Dong, X.; Qu, J.; Gong, F.; Han, Y.; Qiu, Y.; Wang, I.; Liu, Y.; Wei, Y.; et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet 2020, 395, 507–513.
23. Lu, Q.; Shi, Y. Coronavirus disease (COVID-19) and neonate: What neonatologist need to know. J. Med. Virol. 2020, 92, 564–567.
24. Wu, C.; Liu, Y.; Yang, Y.; Zhang, P.; Zhong, W.; Wang, Y.; Wang, O.; Xu, Y.; Li, M.; Li, X.; et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm. Sin. B 2020, 10, 766–788.
25. Wu, Z.; McGoogan, J.M. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020, 323, 1239.
26. Chatterjee, S. Understanding the nature of variations in structural sequences coding for coronavirus spike, envelope, membrane and nucleocapsid proteins of SARS-CoV-2. SSRN 2020, 1–12.
27. Navratil, V.; Lionnard, L.; Longhi, S.; Combet, C.; Aouacheria, A. The severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) envelope (E) protein harbors a conserved BH3-like motif. bioRxiv 2020.
28. Walls, A.C.; Park, Y.J.; Tortorici, M.A.; Wall, A.; McGuire, A.T.; Veesler, D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020, 181, 281–292.e6.

29. Luan, J.; Lu, Y.; Jin, X.; Zhang, L. Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. Biochem. Biophys. Res. Commun. 2020, 526, 165–169.
30. Ou, X.; Liu, Y.; Lei, X.; Li, P.; Mi, D. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat. Commun. 2020, 11, 1–12.
31. Yan, R.; Zhang, Y.; Li, Y.; Xia, L.; Guo, Y.; Zhou, Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020, 367, 1444–1448.
32. Zheng, M.; Song, L. Novel antibody epitopes dominate the antigenicity of spike glycoprotein in SARS-CoV-2 compared to SARS-CoV. Cell. Mol. Immunol. 2020, 17, 536–538.
33. Subramanian Boopathi , Adolfo B. Poma and Ponmalai Kolandaivel. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment, journal of biomolcular structure and dynamics, 2021; 39:9, 3409-3418.
34. Chen, N.; Zhou, M.; Dong, X.; et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in wuhan, china: A descriptive study. Lancet 2020, 395, 507–513.
35. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020, 395, 497–506.
36. Tian, S.; Hu, N.; Lou, J.; Chen, K.; Kang, X.; Xiang, Z.; Chen, H.; Wang, D.; Liu, N.; Liu, D.; et al. Characteristics of covid-19 infection in beijing. J. Infect. 2020, 80, 401–406.
37. Grein, J.; Ohmagari, N.; Shin, D.; Diaz, G.; Asperges, E.; Castagna, A.; Feldt, T.; Green, G.; Green, M.L.; Lescure, F.X.; et al. Compassionate use of remdesivir for patients with severe covid-19. N. Engl. J. Med. 2020, 382, 2327–2336.
38. Yang, W.; Cao, Q.; Qin, L.; Wang, X.; Cheng, Z.; Pan, A.; Dai, J.; Sun, Q.; Zhao, F.; Qu, J.; et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (covid-19):A multi-center study in Wenzhou city, Zhejiang, China. J. Infect. 2020, 80, 388–393.
39. Cai, Q.; Huang, D.; Yu, H.; Zhu, Z.; Xia, Z.; Su, Y.; Li, Z.; Zhou, G.; Gou, J.; Qu, J.; et al. Covid-19: Abnormal liver function tests. J. Hepatol. 2020, 73, 566–574.
40. Fan, Z.; Chen, L.; Li, J.; Cheng, X.; Yang, J.; Tian, C.; Zhang, Y.; Huang, S.; Liu, Z.; Cheng, J. Clinical features of covid-19-related liver functional abnormality. Clin. Gastroenterol. Hepatol. 2020, 18, 1561–1566.
41. Richardson, S.; Hirsch, J.S.; Narasimhan, M.; Crawford, J.M.; McGinn, T.; Davidson, K.W.; Northwell, C.-R.C.; Barnaby, D.P.; Becker, L.B.; Chelico, J.D.; et al. Presenting

characteristics, comorbidities, and outcomes among 5700 patients hospitalized with covid-19 in the New York city area. JAMA 2020, 323, 2052–2059.

42. Bloom, P.P.; Meyerowitz, E.A.; Reinus, Z.; Daidone, M.; Gustafson, J.; Kim, A.Y.; Schaefer, E.; Chung, R.T. Liver biochemistries in hospitalized patients with covid-19. Hepatology 2020.
43. Piano, S.; Dalbeni, A.; Vettore, E.; Benfaremo, D.; Mattioli, M.; Gambino, C.G.; Framba, V.; Cerruti, L.; Mantovani, A.; Martini, A.; et al. Abnormal liver function tests predict transfer to intensive care unit and death in covid-19. Liver Int. 2020, 40, 2394–2406.
44. Ponziani, F.R.; Del Zompo, F.; Nesci, A.; Santopaolo, F.; Ianiro, G.; Pompili, M.; Gasbarrini, A.; "Gemelli against COVID-19" Group. Liver involvement is not associated with mortality: Results from a large cohort of sars-cov-2-positive patients. Aliment. Pharmacol. Ther. 2020, 52, 1060–1068.
45. Meszaros, M.; Meunier, L.; Morquin, D.; Klouche, K.; Fesler, P.; Malezieux, E.; Makinson, A.; Le Moing, V.; Reynes, J.; Pageaux, G.P. Abnormal liver tests in patients hospitalized with coronavirus disease 2019: Should we worry? Liver Int. 2020, 40, 1860–1864.
46. Effenberger, M.; Grander, C.; Grabherr, F.; Griesmacher, A.; Ploner, T.; Hartig, F.; Bellmann-Weiler, R.; Joannidis, M.; Zoller, H.; Weiss, G.; et al. Systemic inflammation as fuel for acute liver injury in covid-19. Dig. Liver Dis. 2021, 53, 158–165.
47. Zhang, C., Shi, L. & Wang, F. S. Liver injury in COVID-19: Management and challenges. Lancet Gastroenterol. Hepatol.2020, 5, 428–430.
48. Feng, G. et al. COVID-19 and liver dysfunction: Current insights and emergent therapeutic strategies. J. Clin. Transl. Hepatol.2020 8, 18–24.
49. Li, M. Y., Li, L., Zhang, Y. & Wang, X. S. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect.2020, Dis. Poverty 9, 45.
50. Shang, J. et al. Cell entry mechanisms of SARS-CoV-2. Proc Natl Acad Sci USA. 2020 May 26;117(21):11727-11734.
51. Chai, X. et al. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. BioRxiv (2020).
52. Hamming, I. et al. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J. Pathol.2004, 203, 631–637.
53. Zhang, Y. et al. New understanding of the damage of SARS-CoV-2 infection outside the respiratory system. Biomed. Pharmacother.2020, 127, 110195–110195.
54. Xu, L., Liu, J., Lu, M., Yang, D. & Zheng, X. Liver injury during highly pathogenic human coronavirus infections. Liver Int. 40, 998–1004 (2020).
55. Adams, D. H. & Hubscher, S. G. Systemic viral infections and collateral damage in the liver. Am. J. Pathol.2006, 168, 1057–1059.

56. Bertolini, A. et al. Abnormal liver function tests in patients with COVID-19: Relevance and potential pathogenesis. Hepatology (Baltimore, MD) 72, 1864–1872 (2020).
57. Guan, W. J. et al. Clinical characteristics of coronavirus disease 2019 in China. N. Engl. J. Med.2020, 382, 1708–1720.
58. Mao, R. et al. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: A systematic review and meta-analysis. Lancet Gastroenterol. Hepatol.2020
59. Xie, H., Zhao, J. & Lian, N. Clinical characteristics of non-ICU hospitalized patients with coronavirus disease 2019 and liver injury: A retrospective study. Liver Int. 2020 Jun;40(6):1321–1326.
60. Kulkarni, A. V. et al. Systematic review with meta-analysis: Liver manifestations and outcomes in COVID-19. Aliment. Pharmacol. Ther. 52, 584–599 (2020).
61. Kukla, M. et al. COVID-19, MERS and SARS with concomitant liver injury—Systematic review of the existing literature. J. Clin. Med. 9, 1420 (2020).
62. Li, X. et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J. Allergy Clin. Immunol. 146, 110–118 (2020).
63. Yang, X. et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A singlecentered, retrospective, observational study. Lancet Respir. Med.2020, 8, 475–481.
64. Lok, J. & Gess, M. Liver dysfunction in COVID-19: A useful prognostic marker of severe disease? Frontline Gastroenterology (2020).
65. Richardson, S. et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. JAMA 323, 2052–2059 (2020).
66. Wan, S. et al. Clinical features and treatment of COVID-19 patients in northeast Chongqing. J. Med. Virol. 92, 797–806 (2020).
67. Ali, N. & Hossain, K. Liver injury in severe COVID-19 infection: Current insights and challenges. Expert Rev. Gastroenterol. Hepatol. 14, 879–884 (2020).
68. Ali, N. Relationship between COVID-19 infection and liver injury: A review of recent data. Front. Med. 7, 458 (2020).
69. Mantovani, A., Beatrice, G. & Dalbeni, A. Coronavirus disease 2019 and prevalence of chronic liver disease: A meta-analysis. Liver Int. 2020 Jun;40(6):1316-1320.
70. Wang, H., Qiu, P., Liu, J., Wang, F. & Zhao, Q. The liver injury and gastrointestinal symptoms in patients with Coronavirus Disease 19: A systematic review and meta-analysis. Clin. Res. Hepatol. Gastroenterol.2020.

71. Lala V, Goyal A, Bansal P, Minter DA. Liver Function Tests. In: StatPearls. Treasure Island (FL): StatPearls Publishing; August 20, 2021.
72. Nader Rifai; Andrea R Horvath; C Wittwer; Norbert W tietz. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier: St. Louis, Missouri; 2018. Chapter 29, Serum enzyme; p. 404-433.
73. Hall P, Cash J. "What is the Real Function of the Liver 'Function' Tests?." The ulster medical journal vol. 2012,81,1:30-6.
74. Guan GW, Gao L, Wang JW, Wen XJ, Mao TH, Peng SW, Zhang T, Chen XM, Lu FM. [Exploring the mechanism of liver enzyme abnormalities in patients with novel coronavirus-infected pneumonia]. Zhonghua Gan Zang Bing Za Zhi. 2020 Feb 20;28(2):100-106. Chinese
75. Kamal M, Abo Omirah M, Hussein A, Saeed H. Assessment and characterisation of post-COVID19 manifestations. Int J Clin Pract.2021, Mar;75(3): e13746.
76. Dana Yelin, Eytan Wirtheim, Pauline Vetter, et al. Long-term consequences of COVID-19: research needs. Lancet Infect Dis.2020, Oct;20(10): 1115-1117.
77. Yvonne M.J. Goërtz1, Maarten Van Herck, Jeannet M. Delbressine, et al. Persistent symptoms 3 months after a SARS-CoV-2 infection: the post-COVID-19 syndrome?. ERJ Open Res;2020, Oct; 6(4): 00542-02020.
78. Carfì A, Bernabei R, Landi F. Persistent symptoms in patients after acute COVID-19.2020, JAMA 324:603–605.
79. Yang J, Zheng YA, Gou XI, et al. (2020) Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta- analysis. Int J Infect Dis;94:91-95.
80. Li MY, Li L, Zhang Y, Wang XS. (2020) Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect Dis Poverty. Apr 28; 9(1):45.
81. Cascella, M.; Rajnik, M.; Cuomo, A.; Dulebohn, S.C.; Di Napoli, R. Features, evaluation and treatment coronavirus (COVID-19). In Statpearls; Internet; StatPearls Publishing: Treasure Island, FL, USA, 2020.
82. Lu, Q.; Shi, Y. Coronavirus disease (COVID-19) and neonate: What neonatologist need to know. J. Med. Virol. 2020, 92, 564–567.
83. Moriyama, M.; Hugentobler, W.J.; Iwasaki, A. Seasonality of respiratory viral infections. Annu. Rev. Virol. 2020, 7, 2.1–2.
84. Chin, A.W.H.; Chu, J.T.S.; Perera, M.R.A.; Hui, K.P.Y.; Yen, H.L.; Chan, M.C.W.; Peiris, M.; Poon, L.L.M. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 2020, 1, e10.
85. Wang, K.; Chen, W.; Zhou, Y.S.; Lian, J.Q.; Zhang, Z. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. bioRxiv 2020.

86. Yurchenko, V.; Constant, S.; Bukrinsky, M. Dealing with the family: CD147 interactions with cyclophilins. Immunology 2006, 117, 301–309
87. Schatzmann, J.P.S.; Nakaya, H. Susceptibility of the Elderly to SARS-CoV-2 Infection: ACE-2 Overexpression, Shedding and Antibody-dependent Enhancement (ADE). Clinics 2020, 75.
88. Li, M.Y.; Li, L.; Zhang, Y.; Wang, X.S. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect. Dis. Poverty 2020, 9, 45.
89. Qiao, J.; Li, W.; Bao, J.; Peng, Q.; Wen, D.; Wang, J.; Sun, B. The expression of SARS-CoV-2 receptor ACE2 and CD147, and protease TMPRSS2 in human and mouse brain cells and mouse brain tissues. Biochem. Biophys. Res. Commun. 2020, 533, 867–871.
90. Wang, Q.; Zhang, Y.; Wu, L.; Niu, S.; Song, C.; Zhang, Z.; Lu, G.; Qiao, C.; Hu, Y.; Yuen, Y.K.; et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell 2020, 81, 894–904.
91. Yan, R.; Zhang, Y.; Li, Y.; Xia, L.; Guo, Y.; Zhou, Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020, 367, 1444–1448.
92. Shang, J.; Ye, G.; Shi, K.; Wan, Y.; Luo, C.; Aihara, H.; Geng, Q.; Auerbach, A.; Li, F. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020, 581, 221–224.
93. Wrapp, D.; Wang, N.; Corbett, K.S.; Goldsmith, J.A.; Hsieh, C.L.; Abiona, O.; Graham, B.S.; McLellan, J.S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020, 367, 1260–1263.
94. Zhao, Y.; Zhao, Z.; Wang, Y.; Zhou, Y.; Ma, Y.; Zuo, W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. bioRxiv 2020.
95. Zhang, H.; Penninger, J.M.; Li, Y.; Zhong, N.; Slutsky, A.S. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: Molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020, 46, 586–590.
96. Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L.; et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020, 581, 1–9.
97. Chatterjee, S. Understanding the nature of variations in structural sequences coding for coronavirus spike, envelope, membrane and nucleocapsid proteins of SARS-CoV-2. SSRN 2020, 1–12.
98. Gussow, A.B.; Auslander, N.; Faure, G.; Wolf, Y.I.; Zhang, F.; Koonin, E.V. Genomic determinants of pathogenicity in SARS-CoV-2 and other human coronaviruses. Proc. Natl. Acad. Sci. USA 2020, 117, 15193–15199.
99. Ou, X.; Liu, Y.; Lei, X.; Li, P.; Mi, D. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat. Commun. 2020, 11, 1–12.

100. Devaux, C.A.; Rolain, J.M.; Colson, P.; Raoult, D. New insights on the antiviral effects of chloroquine against coronavirus: What to expect for COVID-19? Int. J. Antimicrob. Agents 2020, 105938.

101. Katarzyna Grudlewska-Buda, Natalia Wiktorczyk-Kapischke, Ewa Wałecka-Zacharska et.al. SARS-CoV-2—Morphology, Transmission and Diagnosis during Pandemic, Review with Element of Meta-Analysis. J. clin. Med. 2021;10:1962.

102. Subramanian Boopathi , Adolfo B. Poma and Ponmalai Kolandaivel. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment, journal of biomolcular structure and dynamics, 2021; 39:9, 3409-3418.

103. CascellaM,RajnikM,CuomoA,etal.,Features,evaluationandtreatmentcoronavirus (COVID-19). Stat pearls [internet]. Treasure Island (FL): Stat Pearls Publishing, Jan 2020.

104. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 2020;55:2000607.

105. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019(COVID-19) outbreak in China: summary of are portof 72314 cases from the Chinese centre for disease control and prevention. JAMA 2020;323:1239.

106. Wan Y, Shang J, Graham R, et al. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. J Virol 2020;94: e00127–20.

107. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–280.

108. Sims AC, Baric RS, Yount B, et al. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J Virol 2005;79:15511–24.

109. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: a review [published online ahead of print, 2020 Apr 20]. Clin Immunol. 2020; 215:108427.

110. Donnelly CA, Ghani AC, Leung GM, et al. Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in hong kong. Lancet 2003;361:1761–6.

111. Goyal P, Choi JJ, Pinheiro LC, et al. Clinical characteristics of COVID-19 in New York city. N Engl J Med 2020;382:2372–4.

112. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708–20.

113. Young BE, OngSWX, Kalimuddin S, et.al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 2020;323:1488–94.

114. Cheung KS, Hung IF, Chan PP, et al. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from the hong kong cohort and systematic review and meta-analysis. Gastroenterology 2020;

115. Available https://www.webmd.com/lung/COVID19-digestive-symptoms (accessed 15 Jun 2020).

116. Yang W, Cao Q, Qin L, Wang X, Cheng Z, Pan A, et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19):A multi-center study in Wenzhou city, Zhejiang, China. J Infect 2020;80(4):388–393.

117. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy 2020;75(7):1730–1741.

118. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323(11):1061–1069.

119. Kaushik A, Wani SN, Baba MA, Agarwal AK. Prevalence of abnormal liver function tests in COVID-19 patients at a tertiary care centre. J Assoc Physicians India 2020;68(8):73–75.

120. Clinical management protocol: COVID-19. Available from: https://www. mohfw.gov.in/pdf/ClinicalManagementProtocolforCOVID19.

121. World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Interim guidance.

122. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10223):507–513.

123. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395(10223):497–506.

124. Tian S, Hu N, Lou J, Chen K, Kang X, Xiang Z, et al. Characteristics of COVID-19 infection in Beijing. J Infect 2020;80(4):401–406.

125. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382(18):1708– 1720.

126. Cai Q, Huang D, Yu H, Zhu Z, Xia Z, Su Y, et al. COVID-19: Abnormal liver function tests. J Hepatol 2020;73(3):566–574.

127. Li J, Fan JG. Characteristics and mechanism of liver injury in 2019 coronavirus disease. J Clin Transl Hepatol 2020;8(1):13–17.

128. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and chal lenges. Lancet Gastroenterol Hepatol 2020;5(5):428–430.

129. Chen N, Zhou M (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 395(10223):507–513

130. Chai X, Hu L, Zhang Y, Han W, Lu Z, Ke A (2020) Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection.

131. Chen, N.; Guan GW, Gao L, Wang JW, Wen XJ, Mao TH, Peng SW, Zhang T, Chen XM, Lu FM. Exploring the mechanism of liver enzyme abnormalities in patients with novel coronavirus-infected pneumonia. Zhonghua Gan Zang Bing Za Zhi 2020;28(2):E002

132. Xu L, Liu J, Lu M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. Liver Int. 2020;40(5):998-1004.

133. Zhou, M.; Dong, X.; Qu, J.; Gong, F.; Han, Y.; Qiu, Y.; Wang, J.; Liu, Y.; Wei, Y.; et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in wuhan, china: A descriptive study. Lancet 2020, 395, 507–513.

134. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020, 395, 497–506.

135. Tian, S.; Hu, N.; Lou, J.; Chen, K.; Kang, X.; Xiang, Z.; Chen, H.; Wang, D.; Liu, N.; Liu, D.; et al. Characteristics of covid-19 infection in beijing. J. Infect. 2020, 80, 401–406.

136. Grein, J.; Ohmagari, N.; Shin, D.; Diaz, G.; Asperges, E.; Castagna, A.; Feldt, T.; Green, G.; Green, M.L.; Lescure, F.X.; et al. Compassionate use of remdesivir for patients with severe covid-19. N. Engl. J. Med. 2020, 382, 2327–2336.

137. Yang, W.; Cao, Q.; Qin, L.; Wang, X.; Cheng, Z.; Pan, A.; Dai, J.; Sun, Q.; Zhao, F.; Qu, J.; et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (covid-19):A multi-center study in Wenzhou city, Zhejiang, China. J. Infect. 2020, 80, 388–393.

138. Cai, Q.; Huang, D.; Yu, H.; Zhu, Z.; Xia, Z.; Su, Y.; Li, Z.; Zhou, G.; Gou, J.; Qu, J.; et al. Covid-19: Abnormal liver function tests. J. Hepatol. 2020, 73, 566–574.

139. Fan, Z.; Chen, L.; Li, J.; Cheng, X.; Yang, J.; Tian, C.; Zhang, Y.; Huang, S.; Liu, Z.; Cheng, J. Clinical features of covid-19-related liver functional abnormality. Clin. Gastroenterol. Hepatol. 2020, 18, 1561–1566.

140. Richardson, S.; Hirsch, J.S.; Narasimhan, M.; Crawford, J.M.; McGinn, T.; Davidson, K.W.; Northwell, C.-R.C.; Barnaby, D.P.; Becker, L.B.; Chelico, J.D.; et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with covid-19 in the New York city area. JAMA 2020, 323, 2052–2059.

141. Bloom, P.P.; Meyerowitz, E.A.; Reinus, Z.; Daidone, M.; Gustafson, J.; Kim, A.Y.; Schaefer, E.; Chung, R.T. Liver biochemistries in hospitalized patients with covid-19. Hepatology 2020.

142. Piano, S.; Dalbeni, A.; Vettore, E.; Benfaremo, D.; Mattioli, M.; Gambino, C.G.; Framba, V.; Cerruti, L.; Mantovani, A.; Martini, A.; et al. Abnormal liver function tests predict transfer to intensive care unit and death in covid-19. Liver Int. 2020, 40, 2394–2406.

143. Ponziani, F.R.; Del Zompo, F.; Nesci, A.; Santopaolo, F.; Ianiro, G.; Pompili, M.; Gasbarrini, A.; "Gemelli against COVID-19" Group. Liver involvement is not associated with mortality: Results from a large cohort of sars-cov-2-positive patients. Aliment. Pharmacol. Ther. 2020, 52, 1060–1068.

144. Meszaros, M.; Meunier, L.; Morquin, D.; Klouche, K.; Fesler, P.; Malezieux, E.; Makinson, A.; Le Moing, V.; Reynes, J.; Pageaux, G.P. Abnormal liver tests in patients hospitalized with coronavirus disease 2019: Should we worry? Liver Int. 2020, 40, 1860–1864.

145. Effenberger, M.; Grander, C.; Grabherr, F.; Griesmacher, A.; Ploner, T.; Hartig, F.; Bellmann-Weiler, R.; Joannidis, M.; Zoller, H.; Weiss, G.; et al. Systemic inflammation as fuel for acute liver injury in covid-19. Dig. Liver Dis. 2021, 53, 158–165

146. Kamal M, Abo Omirah M, Hussein A, Saeed H.(2021) Assessment and characterisation of post-COVID19 manifestations. Int J Clin Pract: Mar;75(3): e13746

147. Dana Yelin, Eytan Wirtheim, Pauline Vetter, et al.(2020) Long-term consequences of COVID-19: research needs. Lancet Infect Dis. Oct;20(10): 1115-1117

148. **COVID-19 clinical managements: Living guidance, 25 January 2021.

149. Gameil, M.A., Marzouk, R.E., Elsebaie, A.H. et al. Long-term clinical and biochemical residue after COVID-19 recovery. Egypt Liver Journal 11, 74 (2021).

150. Park GJ, Lin BP, Ngu MC, Jones DB, Katelaris PH. Aspartate aminotransferase: alanine aminotransferase ratio in chronic hepatitis C infection: is it a useful predictor of cirrhosis?. J Gastroenterol Hepatol. 2000;15(4):386-390.

151. Rec. GSCC (DGKC) ; J. Clin. Chem. Clin. Biochem. 1972; 10 : 182

152. Recommendations of the German Society for Clinical Chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions. Z Klin Chem Klin Biochem. 1972;10(6):281-291.

153. Zheng Z, Yao Z, Wu K, Zheng J. Patient follow-up after discharge after COVID-19 pneumonia: Considerations for infectious control. J Med Virol. 2020 Nov;92(11):2412-2419.

154. Suri T, Mittal S, Tiwari P, et al. COVID-19 Real-Time RT-PCR: Does Positivity on Follow-up RT-PCR Always Imply Infectivity? Am J Respir Crit Care Med. 2020; 202: 147.

155. Tian M, Long Y, Hong Y, et al. The treatment and follow-up of 'recurrence' with discharged COVID-19 patients: data from Guizhou, China. Environ Microbiol. 2020; 22: 3588-92.

156. Fan L, Liu S. CT and COVID-19: Chinese experience and recommendations concerning detection, staging and follow-up. Eur Radiol. 2020; 30: 5214-6.

157. Guan CS, Wei LG, Xie RM, et al. CT findings of COVID-19 in follow-up: comparison between progression and recovery. Diagn Interv Radiol. 2020; 26: 301-7.

158. Balachandar V, Mahalaxmi I, Subramaniam M, et al. Follow-up studies in COVID-19 recovered patients - is it mandatory? Sci Total Environ. 2020; 729: 139021.

159. Bikdeli B, Madhavan MV, Jimenez D, et al. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020; 75: 2950-73.

160. Ya-Wen An1, Shuo Song1, Wei-Xin Li, et al.(2021) Liver function recovery of COVID-19 patients after discharge, a follow-up study. Int J Med Sci; 18(1): 176-186.

161. Fan Z, Chen L, Li J, et al.(2020) Clinical features of COVID-19-related liver functional abnormality. Clin Gastroenterol Hepatol;18:1561–1566.

162. Xu L., Liu J., Lu M., Yang D., Zheng X.(2020) Liver injury during highly pathogenic human coronavirus infections. Liver Int;40(5):998–1004.

163. Shi HS, Han XY, Jiang NC, et al.(2020) Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study. Lancet Infect Dis. Apr; 20(4):425-434.

164. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet;395:1033-1034.

165. Tian S., Xiong Y., Liu H., Niu H., Guo J., Liao M.(2020) Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. Mod Pathol;33(6):1007–1014.

166. Zsuzsanna Varga, Andreas J Flammer, Peter Steiger, et al. (2020) Endothelial cell infection and endotheliitis in COVID-19. Lancet;395:1417-1418.

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INCLUSION CRIETERIA (CASES)	**YES**	**NO**
• Age 35-60 years	☐	☐
• Patients diagnosed for COVID-19 with RT-PCR Or Rapid Antigen tests without any comorbidities,	☐	☐
• Not on hormonal, steroid, diuretics or any other therapies.	☐	☐
• Patients having mild to moderate COVID-19 illness without oxygen therapy at home.	☐	☐
• Patients having mild to moderate COVID-19 illness admitted in isolation ward of hospital without oxygen support.	☐	☐

EXCLUSION CRITERIA		
• Diagnosed COVID-19 patients having comorbidities before COVID-19 will be excluded.	☐	☐
• Diagnosed COVID-19 patients who were hospitalized for oxygen therapy	☐	☐
• Patients with alcohol consumption, and smoking history.	☐	☐

The subject is eligible for the study if all **INCLUSION** criteria are **YES** and all **EXCLUSION** criteria are **NO**.

INVESTIGATOR STATEMENT

I have verified the data entered in the case report form and have determined that it is complete, accurate, and compatible with the source documents

Investigator's Name _____ Investigator's Signature _____ Date _____

Mobile number:

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CASE REPORT PROFORMA

Registration No/Date : OPD IPD

Name (in capital) :

Father Name / Husband Name :

Mother Name

Date of birth

Age :

Sex: MALE FEMALE

Marital status :

Permanent Address :

Current Address :

Current status :

Mobile No.

Category : GEN OBC SC ST

Nationality

Mother Tongue

Social / Economical Status : Annual income (approx.)

Education Level : Uneducated / Metric / Graduate / Postgraduate / Ph. D

Vegetarian / Non Vegetarian :

Physical Activity : Sedentary/ Moderate / Active

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DEMOGRAPHICS

1. **AGE** ☐ **years**
2. **SEX** **MALE** ☐ **FEMALE** ☐
3. **RELIGION** ☐
4. **HEIGHT** ☐ **cm**
5. **WEIGHT** ☐ **kg**

	RT – PCR	**RAPID ANTIGEN TEST**
6. COVID -19 REPORT	☐	☐

7. **BODY MASS INDEX (BMI)** ☐ **kg/m^2**
8. **WAIST HIP RATIO :** ☐
9. **SBP (mm/Hg) :**
10. **DBP (mm/Hg):**

MEDICAL HISTORY

1. **DURATION OF COVID - 19** ☐

YES **NO**

2. **COVID -19 COMPLICATION** ☐ ☐

3. **.TREATMENT**
   IF YES , SPECIFY : ⟶

DURATION OF TREATMENT : ⟶

4 . PATIENT COMPLICATIONS: MILD MODERATE SEVERE

☐ ☐ ☐

INFORMED CONSENT FORM (FOR CASE)

- I, NAGMA IRFAN research scholar Medical Biochemistry IIMS&R, Lucknow.
- For this study, I will take your 4ml blood sample for the estimation of Serum ALT, AST, ALP, GGT.
- The blood is only subjected for estimation of Serum ALT, AST, ALP, GGT.
- There will be no charges /fees/any consideration given or taken for the study.
- Your identity will be confidential and information and result of your blood test will not be revealed to any other except you if you desire.
- The study has nothing to do with your treatment and is not going to hamper if you refuse to participate.
- I am not associated with your treating doctor panel.
- The study has nothing to do with your current treatment but may improve the knowledge and understanding of disease process and that knowledge may or not be helpful in future.
- After knowing the all above detail would you like to participate in our study? Yes/ No

CONSENT FORM

I……aged……..W/O,D/O,S/O……. UID No. ……agree to participate in the research work.

My blood sample is used only for this research, Topic " EVALUATION OF LIVER ENZYMES IN DIAGNOSED COVID-19 SURVIVORS AND CONTROL SUBJECTS" and not for any other purpose.

I have known the details of the research work very well and I give my consent for the same.

Date :

Signature/thumb impression of the patients: Name of research scholor

Signature/thumb impression of the witness: signature of research scholor

सूचना पत्र (FOR CASE)

1.मै **नगमा इरफान** मेडिकल बायोकेमिस्ट्री आईआईएमएसआर लखनऊ में शोध छात्र हूँI

2.हमारे अध्यन में नमूने के तौर पर आपका 4 मिली लीटर ब्लड लिया जायेगा

3.मैं आपको आश्वस्त करता हूं,कि नमूने का उपयोग अन्य जाँच के लिए नही किया जायेगा I

4.इस परीक्षण के लिए ना ही शुल्क लिया अथवा दिया जायेगा I

5.इस दौरान आपकेद्वारा दी गयी सारी जानकारी परीक्षण गोपनीय रखा जायेगा I

6.आप अगर चाहेंगे तो उसका परिणाम आपको बताया जायेगा I

7.मै आपके इलाज से सम्बन्धित चिकित्सीय अनुभाग का हिस्सा नहीं हूं I

8.आप इस अध्यन में अपनी स्वेच्छा से शामिल अथवा इंकार कर सकते हैं I

9.इससे आपके इलाज पर कोई दुष्प्रभाव नहीं पड़ेगा I

10.इस समस्त तत्व को समझते हुए क्या अध्यन में योगदान देने की सहमति प्रदान करते है हां/नहींI

स्वीकृति/सहमति पत्र

मै.....उम्र.....पुत्र/पुत्री/पत्नी.....निवासी....

.....

मुझे अधय्यन शीर्षक **"इवेल्यूएशन ऑफ़ लीवर एंजाइम इन डायग्नोज्ड कोविड-19 सर्वाइवर्स एंड कंट्रोल सब्जेक्ट्स"** की संभावनाओ एवं परिणामो के बारे में विधिवत बताया गया है I

अतः मै सूचित करता/ करती हूँ एवं लिखित सहमति देता / देती हूँ, कि मेरे रक्त का नमूना केवल ऊपर कहे गये अधय्यन के लिय एकत्रित किया जाए I

रोगी के हस्ताक्षर/अँगूठे के निशान शोध छात्र के हस्ताक्षर

गवाह के हस्ताक्षर/अंगूठे के निशान

INFORMED CONSENT FORM (FOR CONTROL)

- I, NAGMA IRFAN research scholar Medical Biochemistry IIMS&R, Lucknow.
- You are apparently healthy individual.
- For this study, I will take your 4ml blood sample for the estimation of Serum ALT, AST, ALP, GGT..
- The blood is only subjected for estimation of Serum ALT, AST, ALP, GGT.
- There will be no charges /fees/any consideration given or taken for the study.
- Your identity will be confidential and information and result of your blood test will not be revealed to any other except you if you desire.
- The study may improve the knowledge and understanding of disease process and that knowledge may or not be helpful in future.
- After knowing the all above detail would you like to participate in our study? Yes/ No

CONSENT FORM

I……aged……..W/O,D/O,S/O…… UID No. ……agree to participate in the research work.

My blood sample is used only for this research, Topic " EVALUATION OF LIVER ENZYMES IN DIAGNOSED COVID-19 SURVIVORS AND CONTROL SUBJECTS and not for any other purpose.

I have known the details of the research work very well and I give my consent for the same.

Date :

Signature/thumb impression of the patients: Name of research scholor

Signature/thumb impression of the witness: signature of research scholor

Mobile number: Mobile number:

सूचना पत्र (FOR CONTROL)

1.मै **नगमा इरफान** मेडिकल बायोकेमिस्ट्री आईआईएमएसआर लखनऊ में शोध छात्र हूँI

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4.इस परीक्षण के लिए ना ही शुल्क लिया अथवा दिया जायेगा I

5.इस दौरान आपकेद्वारा दी गयी सारी जानकारी परीक्षण गोपनीय रखा जायेगा I

6.आप अगर चाहेंगे तो उसका परिणाम आपको बताया जायेगा I

7.आप इस अध्यन में अपनी स्वेच्छा से शामिल अथवा इंकार कर सकते हैं I

8.इस समस्त तत्व को समझते हुए क्या अध्यन में योगदान देने की सहमति प्रदान करते है हां/नहींI

स्वीकृति/सहमति पत्र

मै.....उम्र.....पुत्र/पुत्री/पत्नी.....निवासी....

.....

मुझे अधय्यन शीर्षक **" इवेल्यूएशन ऑफ़ लीवर एंजाइम इन डायग्नोज्ड कोविड-19 सर्वाइवर्स एंड कंट्रोल सब्जेक्ट्स"** की संभावनाओ एवं परिणामो के बारे में विधिवत बताया गया है I

अतः मै सूचित करता/ करती हूँ एवं लिखित सहमति देता / देती हूँ, कि मेरे रक्त का नमूना केवल ऊपर कहे गये अधय्यन के लिय एकत्रित किया जाए I

रोगी के हस्ताक्षर/अँगूठे के निशान शोध छात्र के हस्ताक्षर

गवाह के हस्ताक्षर/अंगूठे के निशान

INSTITUTIONAL ETHICS COMMITTEE (IEC)

IIMS&R INTEGRAL UNIVERSITY, LUCKNOW

IEC/IIMS&R/2022/07

CERTIFICATE

This is to certify that research work entitled **"Evaluation of liver enzymes in diagnosed COVID-19 survivors and control subjects"** submitted by **Nagma Irfan, Dr.Saba Khan** for ethical approval before the Institutional Ethics Committee IIMS&R.

The above mentioned research work has been approved by Institutional Ethics Committee, IIMS&R with consensus in the meeting held on **19 May 2022**.

Dr.Deepak Chopra
(Jt.Member Secretary)
IRC/IEC
IIMS &R

Dr.Q.S.Ahmed
(Member Secretary)
IRC/IEC
IIMS &R

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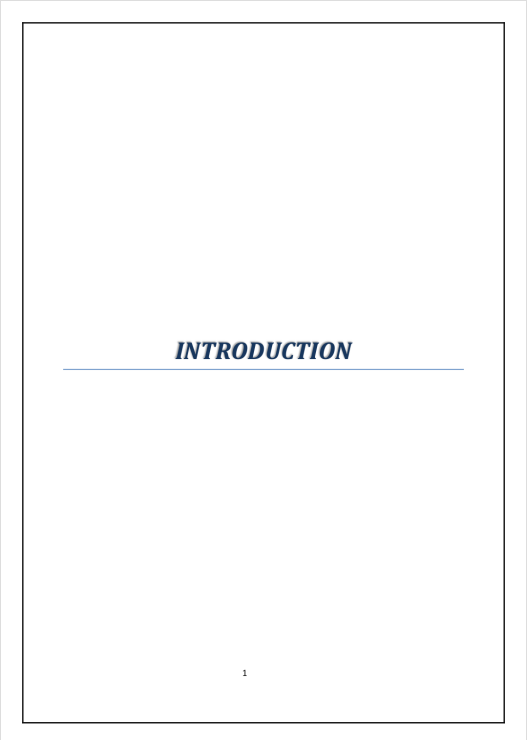


INTRODUCTION

1

INTRODUCTION

COVID-19 announced as a widespread -

The ongoing epidemic of severe acute respiratory syndrome caused by coronavirus-2 (SARS-CoV-2) proceed with to present many pharmacogenetic and medicinal challenges. This virus was first reported in December 2019 from Wuhan, China. The World Health Organization officially classified this infection as coronavirus disease 2019 (COVID-2019) on February 11, 2020, and classified the virus as SARS-CoV-2 [1]. The disease was announced as a pandemic on March 11, 2020 [2]. However, in adults, SARS-Co-V-2 commonly causes pneumonia and acute respiratory distress syndrome (ARDS), now identified as a multisystem disease. It affects not only the pulmonary system, but also the heart, liver and gastrointestinal system [3].

Major impact of COVID –

It is widespread in humans and animals. It causes respiratory infections in humans [4]. His ongoing COVID-19 pandemic, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is evolving rapidly [5]. COVID-19 is caused by the virus SARS-COV-2, and the major symptoms are fever, cough, and breathlessness, and the mild symptoms are changes in smell and taste, gastrointestinal symptoms, headache, and skin symptoms [6-8]. SARS-CoV-2 involes into cells through its intended receptor, angiotensin-converting enzyme 2 (ACE2) [9].

Viral host cell invasion -

A noticeable number of analytical studies have been published to explain the clinical manifestations of SARS-CoV-2 infection. In addition to asymptomatic or symptomatic forms and acute viral pneumonia along with respiratory problem, the prognosis of COVID-19

disease is impacted by wondering effects on organs throughout the body through receptor angiotensin-converting enzyme-2 (ACE-2), which include heart and kidney failure, central nervous system damage & CNS & and gastrointestinal tract [10,11].

ACE-2 has been declared as a functional SARS-CoV receptor and extremely expressed on cells of pulmonary epithelial membrane of lungs[12]. By this host receptor that the S protein initially interact to trigger entry of the virus into the host cell [13-15]. After membrane fusion, the corona virus enters epithelial cells of alveoli of lung, and the content of virus is released inside. After that inside the host cell, the virus start replicating and produce negative-stranded RNA from single-stranded positive RNA which already exist by the action of RNA polymerase (transcription). This newly produced negative-stranded RNA starts producing new positive strands of RNA, which then form new proteins in the cell cytoplasm (translation) [16-18]. The N virus protein binds to the new RNA genome and the M protein fhelps integration into the cellular endoplasmic reticulum. These newly formed nucleocapsids are then enclosed in the ER membrane and carried to the lumen, from lumen they are carried across the Golgi vesicles to the cell membrane, after that through exocytosis to the extracellular space. cell. The novel viral particles are now organised to penetrate the adjoining epithelial cells as well as provide novel infectious contents for community transmission through respiratory droplets [19].

Life cycle of virus is shown in figure 1 [20].

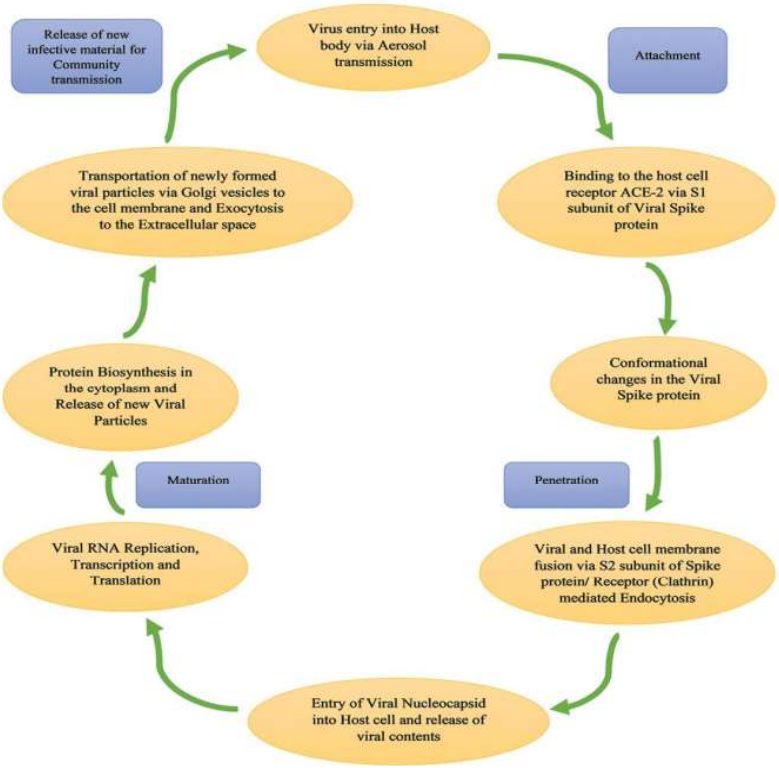


Figure.1 Life cycle of virus and its invasion into host cell. [*Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 2020;55:2000607*]

Morphology of SARS CoV-2 -

The biggest genomes (26–32 kb) among all RNA virus families are found in CoVs, which are RNA viruses. Each viral transcript has a 3-poly(A) tail and a 5-cap structure[21]. The SARS-CoV-2 virus typically has a spherical shape, although it can also take on pleomorphic and oval shapes. The S-spike protein, which creates the peplomers and gives the virus its distinctive crown shape, the M-membrane protein, and the E-envelope protein, which provides the ring structure, are the three proteins that make up the viral envelope [22,23]. A fourth protein, N-nucleocapsid protein, a phosphoprotein that is a part of the nucleocapsid's structural framework, is also present [24,25,26,27]. The group I fusion glycoproteins include the S protein. Its homotrimeric structure, which has one higher and two lower conformations, is what distinguishes it[28,29-31]. About 75.5 percent of the S protein's amino acid sequence is same in both SARS-CoV-1 and SARS-CoV-2 [32].

New viral particle, which are generally constructed by the components of the host cell, are formed with the help of a structural E protein.

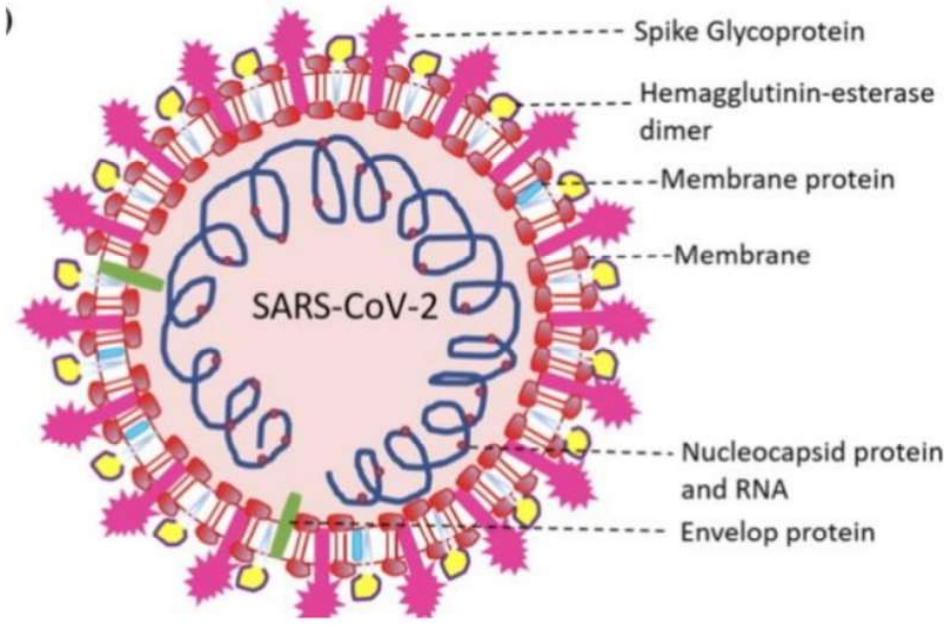


figure.2 Structure of SARS-CoV-2[33]. [*Subramanian Boopathi , Adolfo B. Poma and Ponmalai Kolandaivel. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment, journal of biomolcular structure and dynamics, 2021; 39:9, 3409-3418*]

Impact of COVID-19 on liver -

In particular, the impact of COVID-19 on the liver has been assessed in a small number of studies that concentrated on changes in the liver's histology and biochemistry during the course of infection in patients who died from the virus [34–39].

A number of recent studies have sought to assess variables linked to liver injury in American, Asian, and European cohorts [34,39-42], as well as cohorts from those continents [43-46].

However, little is known about liver illness in the context of SARS-CoV-2 infection, which limits our understanding of aberrant liver function in terms of the overall prognosis.

Additionally, SARS-CoV-2 RNA has been found in the blood and hepatocytes of the infected patients[47–49]. The large number of ACE2 receptors on the surface of liver's cholangiocytes[50,51], bile duct cells[52], probably make it easier for SARS-COV-2 to enter cells where it can reproduce and cause disruption of liver function[53,54].

Additionally, there is evidence to support the idea that the majority of viruses that target the respiratory system can injure liver cells via the CD8+ driven immune response[55]. Regardless of the setting for providing healthcare, findings from several research reveal a link between COVID-19 and abnormal liver function tests (LFTs)[56–59]. The data from more recent epidemiological and clinical research on LFTs in individuals with SARS-CoV-2 infection, however, are essentially contradictory and inconsistent[56, 60-63].

In patients with COVID-19 from China, the UK, and the USA, respectively, the prevalence of the liver damage biomarker alanine transferase (ALT) has been found to be up to 32%, 38%, and 39% [59,64,65]. On the other hand, according to the severity of the diseases, certain studies did not detect any appreciable change in the ALT levels of COVID-19 patients[62,66]. As a result, a number of systematic reviews and meta-analyses have recently been published, focusing on the incidence of anomalies in the liver biochemistry profile among COVID-19 patients based on clinical severity and disease mortality. This is because there has been revealed discrepancies in the results for the liver chemistries in COVID-19 affected individuals. [58,61,67-70].

The liver enzymes and their function -

The primary liver functions include the detoxification of many metabolites, the production of digestive enzymes, and the synthesis of proteins. The liver also involved a significantly in the regulation of RBCs, glucose synthesis, storage, and metabolism. Mostly, when evaluating LFTs, the), aspartate transaminase (AST), Alanine transferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), serum albumin, serum bilirubin, international normalised ratio (INR), and prothrombin time (PT) are all discussed [71].

These biomarkers typically have

- ALT values of 60U/L for men and 42U/L for women.
- With no discernible sex-related differences, the AST level was 35 U/L.
- ALP for women is 33-98 U/L, and for men it is 43-115 U/L.
- GGT is 70 U/L in men & 40 U/L in women [72].

1) **ALT : Alanine Transaminase – [73]**

- Formed in hepatocytes;
- highly distinct marker of hepatocellular injury;
- proportionately low quantities in additional tissues, making it more distinct than AST;
- levels vary throughout the day;
- Increase may occur while using specific medications or when engaging in vigorous activity.

2) **AST : Aspartate Transaminase – [73]**

- Two isoenzymes of aspartate transaminase (AST) exist and are indistinguishable by means of conventional AST testing.

- The cytosolic isoenzyme is found in skeletal muscle, heart muscle, and kidney tissue. The mitochondrial isoenzyme is formed in hepatocytes and responds to membrane stressors similarly to ALT.
- It generally rises in concert with ALT to show hepatic injury: a hepatitis overview. It must be used with caution to evaluate hepatocellular damage.

3) ALP: Alkaline Phosphatase [73]

• A collection of isoenzymes that function to dephosphorylate a number of different compounds all across the body.

• Made in the membranes of the cells lining the canaliculi and bile ducts.

• Released in reaction to cholestasis or the buildup of bile salts.

• Non-hepatic production in the placenta, leukocytes, kidney, gut, and bone.

• Physiological change during pregnancy or in youngsters who are growing.

• A pathological increase in bone metastases, Paget's disease, and renal disease.

4) GGT: Gamma-glutamyl Transferase [73]

- Found in hepatocytes and biliary epithelial cells' microsomes.
- Found in the liver, pancreas, kidney, and intestine.
- The cholestatic picture, which includes elevated GGT and elevated ALP, is strongly indicating a biliary tract blockage.
- It is also subject to increase with hepatic enzyme stimulation brought on by prolonged alcohol consumption or medications like rifampicin and phenytoin.

In reality, numerous investigations have claimed that SARS-CoV-2 infection causes an elevation in liver transaminases. The speculative mechanism includes the likelihood of direct liver injury caused by drug toxicity, liver injury due to immunological response and immune-mediated damage, and ischemic hepatitis that may occur in individuals with multiorgan failure, including hemodynamic instability [74]. The dysfunctionality of the LFT in COVID-19 may, theoretically, be caused by a direct virus-induced cytopathic effect. The question of whether liver damage is predictable is more difficult to answer. Due to the large number of patients infected with different HCoVs, and in particular the rapidly rising number of COVID-19 patients worldwide, it is necessary to know how liver injury affects the clinical outcomes of COVID-19 patients and its determinants. This could have a significant impact on many people's outcomes.

Covid-19 has also been shown to have an effect on liver enzymes in long term studies -

Long-Term Effect Of Covid-19 On Liver Enzymes - Post-COVID-19 manifestations following a negative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test for SARS-CoV-2 have been extensively researched in the literature. A significant similarity between the symptoms of post-COVID-19 and post-SARS-CoV was discovered by Kamal et al. [75]. While this was going on, Dana et al. [76] questioned whether patients who had recovered from COVID-19 would be affected by the long-term health effects of the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV). The bulk of earlier studies used extensive medical questionnaires to assess post-COVID-19 symptoms for only a few weeks following recovery. Through epidemiological investigations, they proposed the connection between the severity of COVID-19 and post-discharge indications. Yvonne et al. [77] conducted a Facebook

survey to know the post-COVID-19 symptoms and found that the majority of survivors still had persistent symptoms. Additionally, Carf et al. [78] completed a post-acute COVID-19 thorough questionnaire to identify any lingering character throughout the follow-up period following COVID-19 recovery (14–60 days). Most of them claimed minimum one COVID-19 related symptom persisted.

To the best of our knowledge, however, very few about the pathological remnant that might be responsible for the post-COVID-19 indications that call for extensive medical follow-up treatment. For comparatively lengthy periods of time after recovery, we looked for the main clinical symptoms and biochemical changes in COVID-19 survivors.

REVIEW OF LITERATURE

COVID and its variant -

During adoption to its new human hosts, COVID and its variant, SARS-CoV-2, are capable to genetic evolution with mutations, which results in their mutant variety. It's possible that this new variation differs in some ways from its ancestors' strains. . (World Health Organization)

A recent epidemiological update was provided by the WHO on June 22, 2021. Since the start of the pandemic, SARS-CoV-2 has been found in four different varieties, according to that update:

• Alpha (B.1.1.7), the initial version of concern, was reported in late December in the UK.

• Beta (B.1.351), first describe in December 2020 in South Africa,.

• Gamma (P.1) - first discovered in Brazil at the beginning of January 2021.

Delta (B.1.617.2) was initially discovered in India in December 2020.

Since there is currently no approved treatment for COVID-19, it poses the greatest hazard to public health [79]. Angiotensin converting enzyme 2 (ACE2) ct as receptor for both SARS-CoV and SARS-CoV-2. Human tissues like the lungs, kidneys, testicles, small intestine, thyroid gland, myocardium, pancreas, adipose tissues, blood vessels, blood cells, spleen, bone marrow, brain, liver, urine bladder, and adrenal glands are all diffusely distributed with ACE2 [80].

SARS-CoV-2 has a generally poor tolerance for hot temperatures. It can only endure 56°C for 30 minutes. Additionally, it is sensitive to ultraviolet light, ethanol, ether, both at a

concentration of 75%, and preparations including substances like peracetic acid, chloroform, and chlorine [81,82]. Additionally, it shows resistance to chlorhexidine.

Due to their high level of identity, SARS-CoV-2 may share traits with SARS-CoV-1 in terms of their physical and chemical makeup. SARS-CoV-1 may live for around 5 days in an environment with 50% humidity and 22–25°C temperatures. According to studies by Moriyama et al. [83], low humidity and temperature lengthen the time the virus remains in the aerosol. SARS-CoV-2 was extremely stable at 4 C but sensitive to heat, according to Chin et al. [84]. On the fourteenth day at 4 °C, the infectious titeron hardly dropped by about 0.7 log units. If the incubation temperature increased to 70 C, the virus survived for only five minutes. This virus demonstrates stability over a wide pH range(3–10) when kept at room temperature.

Receptor for SARS-CoV-2 Entry and Replication Cycle –

The transmembrane S glycoprotein mediates CoV entrance into host cell [85,86]. The pathogenesis of SARS-CoV-2 is significantly influenced by the receptors and proteases found on the surface of the host cells. The angiotensin-converting enzyme 2 (ACE2) receptor for SARS-CoV-2 is the most well-known [87]. ACE2 is typically a zinc metalloprotease (carboxypeptidase). Although it has a different substrate specificity than dipeptidase angiotensin-converting enzyme (ACE), they are homologous.

In addition to the lungs, Li et al research .'s [88] revealed that ACE2 is expressed in other human tissues as well, suggesting that SARS-CoV-2 may be infected the other tissues also. A transmembrane glycoprotein called CD147, also referred to as basic immunoglobulin, is another entrance receptor for viruses.

The expression of CD147 was found in human and mouse brain cell lines by Qiao et al. [89]. Dipeptidyl peptidase 4 (DPP4), also known as CD26, is the alternative receptor for SARS-CoV-2. A variety of cells, including those in the immune system, kidneys, lungs, smooth muscle, liver, and capillaries, express this ectopeptidase.

However, only ACE-2 is strongly supported as a functioning receptor protein that is necessary [90].

The ACE2-B0AT1 complex may be able to bind two S proteins concurrently, according to structural modelling, which sheds light on the molecular mechanisms behind coronavirus identification and infection [91]. Men were found to have a higher expression of it than women, which may help to explain why men are more susceptible to SARS-CoV-2 [82,91,92,93,94,95,96].

Replication starts once viral structures have been endocytosed into the cell. This method separates SARS-CoV-2 from other RNA viruses due to enhanced recombination processes [97,98,99]. The S protein is further divided by L cathepsin in an acidic environment. SARS-CoV-2 genetic material is consequently released into the cytoplasm [100]. The next step is a reverse transcription (Figure 3) [101].

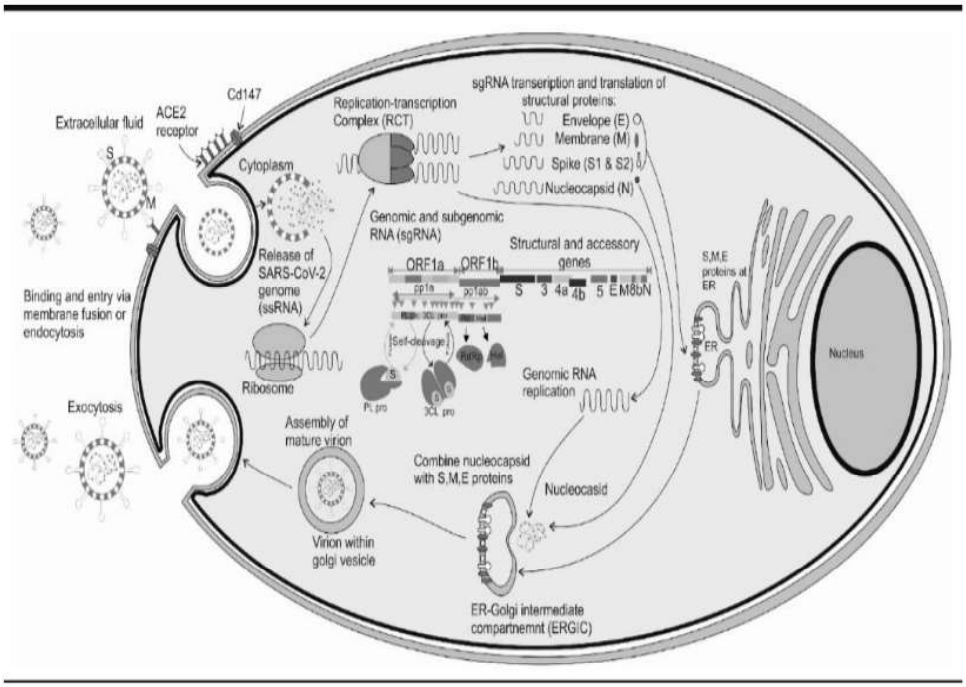


Figure.3 infection mechanism of SARS-CoV-2 into human cells. *[Katarzyna Grudlewska-Buda, Natalia Wiktorczyk-Kapischke, Ewa Wałecka-Zacharska et.al. SARS-CoV-2—Morphology, Transmission and Diagnosis during Pandemic, Review with Element of Meta-Analysis. J. clin. Med. 2021,10,1962]*

The vesicular replication-transcription complex is involved in transcription processes. The host ribosome plays a important role in the formation of an RNA strand that is negatively charged [81].

Mechanism of action –

Coughing, sneezing, and touching an infected surface are the main ways that this dangerous viral infection spreads because it is a viral infection that can be contracted by ingestion or inhalation of virus droplets.

How the virus enters and replicate inside human cells is showed in Figure.4 [102]

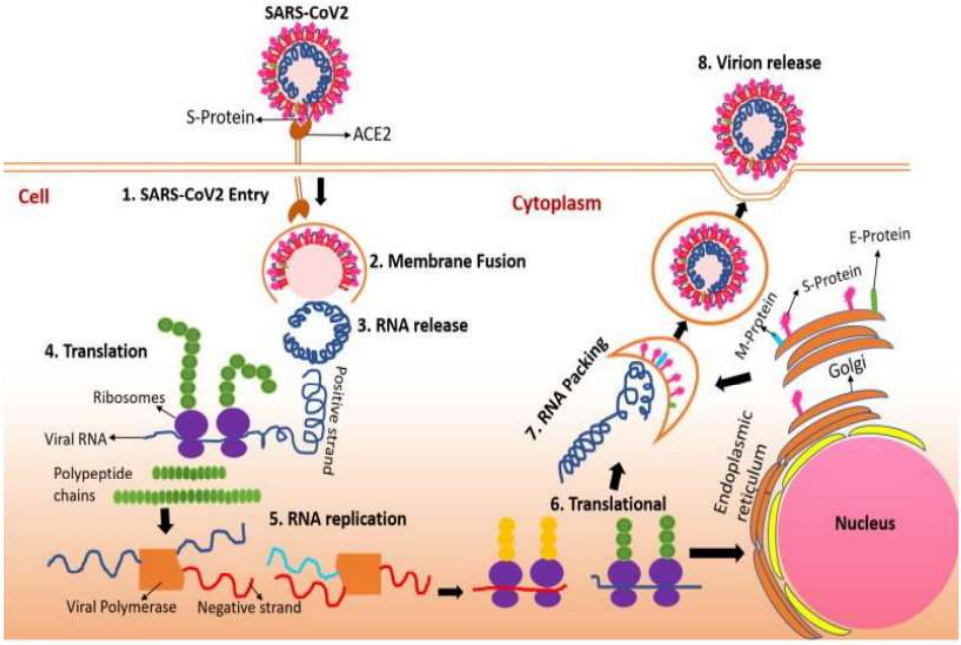


Figure.4 Entry and replication of virus. [*Subramanian Boopathi , Adolfo B. Poma and Ponmalai Kolandaivel. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment, journal of biomolcular structure and dynamics, 2021; 39:9, 3409-3418*]

Pathophysiology of Disease –

Although a lot has been learned about COVID-19's transmission and appearance, less is known about its pathogenesis.. Pathophysiology of the disease has been expressed in figure.5 [103,104,105].

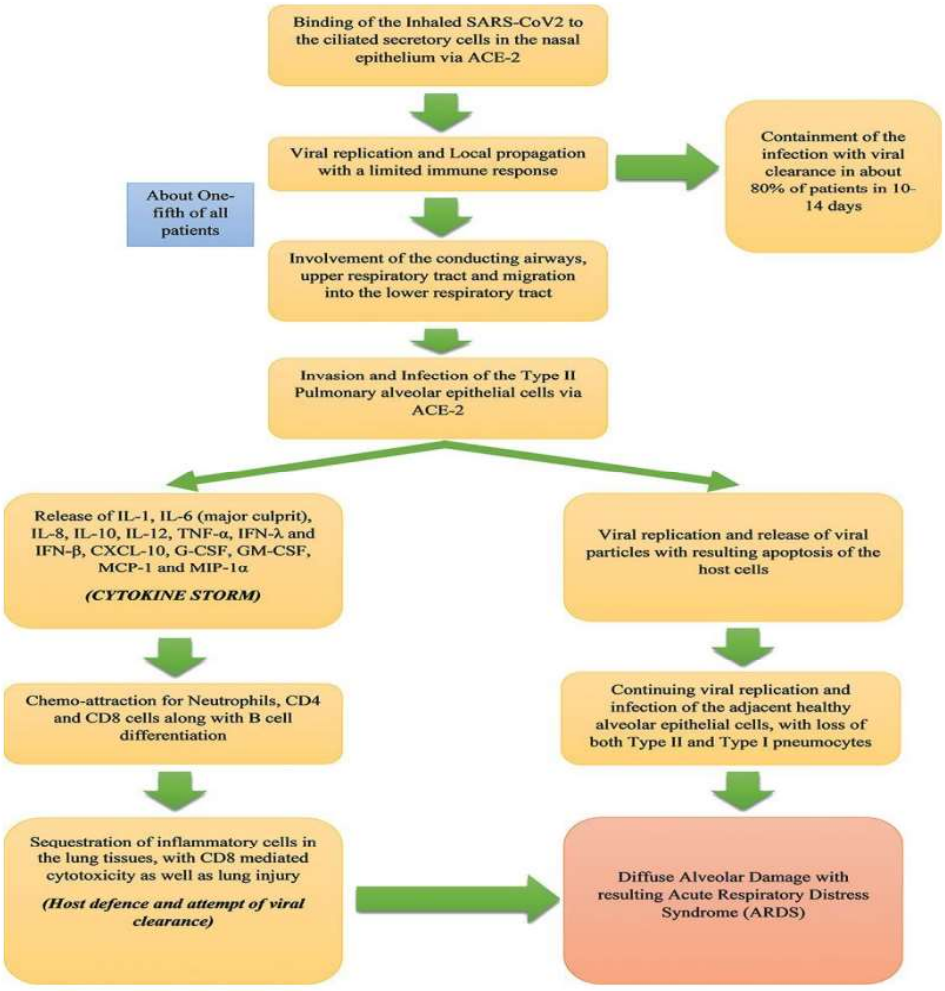


Figure.5 Pathophysiology of COVID-19. *[Cascella M, Rajnik M, Cuomo A, et.al. ,Features, evaluation and treatment coronavirus (COVID-19). Stat pearls [internet]. Treasure Island (FL): Stat Pearls Publishing, Jan 2020], [Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J 2020;55:2000607], [Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019(COVID-19) outbreak in China: summary of are portof 72314 cases from the Chinese centre for disease control and prevention. JAMA 2020;323:1239].*

Asymptomatic Phase –

The upper respiratory tract's nasal epithelial cells are where the SARS-CoV-2, which is acquired through respiratory aerosols, binds. Adult nasal epithelial cells have been found to have significant levels of ACE-2, which is the primary host receptor for viral entrance into cells [106,107]. In addition to infecting ciliated cells in the conducting airways, the virus replicates and spreads locally [108]. The immunological response produced at this stage, which lasts a few days, is only mild. Despite having a less viral load at the moment, the people are quite contagious, and testing with a nose swab can find the virus.

Covid-19 Diagnosis and Imaging - [109,110-115]

1) Lowered WBC count and lymphopenia were found in the basic blood tests.
    - Elevated AST, ALT, LDH, and CRP concentrations
    - A rise in D-dimer
    - A rise in PT/INR

2) RT-PCR molecular testing

- RT-PCR and rRT-PCR are methods used to amplify genetic material of virus collected by a nasal swab.
- inadequate sensitivity
- multi testing is necessary to make sure about viral clearance.

3) Chest X-ray

- Early on in the disease, there were no notable observations.
- In advanced disease, there are bilateral patchy opacities.

4) HRCT chest

- Patchy distribution of multifocal bilateral "ground or ground-glass" areas linked to consolidation areas
- Sign reading "Reverse Halo"
- Lymphadenopathy, cavitation, and calcification
- Significantly sensitive for the diagnosis of COVID-19

5) Tests for antibodies and sera

- There is additional work to be done to develop an accurate and sensitive antibody test.

Hepatotropism of SARS- CoV-2 –

The highest gene expression levels for ACE2 were found in cholangiocytes, which are comparable to alveolar type 2 cells, then sinusoidal endothelial cells, and hepatocytes in healthy livers, according to single-cell RNA sequencing investigations [116,117]. However, in a combined examination of three single-cell RNA sequencing datasets from liver tissue

from healthy individuals, only few hepatocytes co-expressed ACE2 and TMPRSS2 [118]. The creation of ACE2- and TMPRSS2-expressing human liver ductal organoids by Zhao et al. that were capable of simulating SARS-CoV-2 infection[119] raises the possibility that the bile duct epithelium could facilitate pseudoparticle entry. It is important to note that the non-cholestatic pattern of liver biochemistry generally identified in COVID-19 is at variance with the ostensibly high SARS-CoV-2 entrance receptor expression and viral permissibility of cholangiocytes; the specific causes of this feature are still umrevealed. But it's feasible that SARS-CoV-2 can replicate at a decreased level in cholangiocytes in vivo without leading to cell death. This mechanism would be in line with any other long-term viral replication reservoirs, such as those in the small intestine, which can gradually mould memory B cell responses to the virus[120]. It has also been demonstrated that liver organoids made from human pluripotent stem cells, which mostly consist of hepatocytes that express albumin, express ACE2 and allow SARS-CoV-2 pseudoparticle entry[121].

Liver injury due to COVID-19 -

The probable contribution of severe liver injury to rising mortality risk in COVID-19 patients has been highlighted in a number of hospital-based studies conducted globally. Additionally, it has been revealed that hepatic impairment in severe COVID-19 patients is linked to a fatal outcome[122-125, 116-118]. Numerous investigations have shown that patients with severe COVID-19 had a greater occurence of raised value of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and hypoalbuminemia than patients with non-severe COVID-19[126]. There may be a variety of processes at play in these patients' development of impaired liver function. These include inflammation that is immune-mediated, hypoxic injury brought on by acute pneumonia, and

inflammation brought on by drugs that results in hepatic injury[127]. Patients with COVID-19, who have high viral loads in their liver cells may also have liver damage[128]. According to some data, the ACE-2 cell surface receptor is present in the liver tissue, however cholangiocytes (59.7%) are more highly expressed than hepatocytes (2.6%). Particularly, evidence suggests that the liver is a possible target organ for SARS-CoV2 since the degree of ACE-2 expression in cholangiocytes was comparable to that of type 2 pneumocytes. Instead, it appears that Kupffer cells express ACE-2 insufficiently [129]. The liver is a potential entry point for SARS-CoV-2 in light of the aforementioned. There may be a number of variables that contribute to COVID-19-related liver injury. These include direct virus damage to the liver tissue produced by ACE-2, an uncontrolled inflammatory/immune response that results in fibrosis and liver dysfunction, or a liver lesion created by an anti-COVID19 drug therapy [130].

In a retrospective analysis, clinical indications for liver injury were retrieved and examined from 799 patients with COVID-19 confirmation who were hospitalised to a hospital in Madrid, Spain. The relationship between liver damage and the course of the illness was also examined. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and aspartate aminotransferase/alanine aminotransferase ratio were the parameters whose serum levels were increased above the Upper Limit of Normal (ULN) [131]. Liver involvement was observed in two recent pathogenic coronaviruses, SARS-COV and Middle East Respiratory Syndrome coronavirus (MERS-CoV). Hepatic involvement in COVID-19 is not wholly unexpected because these two viruses (especially SARS-COV) had a strong genetic similarity with the new coronavirus SARS-CoV-2 [132].

Few research have examined the effect of COVID-19 on the liver, and those that haveconcentrated on changes in liver biochemistry and histopathology throughout the course of infection in patients who get infected to the virus[133-137]. A number of recent studies have sought to assess variables linked to liver injury in American, Asian, and European cohorts[133,138-141], as well as cohorts from Europe[142-145]. The prevalence of liver illness in SARS-CoV-2 infections is still completely unclear, which limits our understanding of how impaired liver function affects the overall prognosis.

Post COVID-19 effect on liver –

The literature has extensively explored post-COVID-19 symptoms following negative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) tests for SARS-CoV-2. A significant similarity between the symptoms of post-COVID-19 and post-SARS-CoV was discovered by Kamal et al. [146]. The long-term health effects of the Middle East respiratory syndrome coronavirus (MERS-CoV) and the severe acute respiratory syndrome coronavirus (SARS-CoV) on COVID-19 recovery patients were questioned by Dana et al. [147] at the same time.

Our study will take into account how COVID-19 patients' post-recovery liver function effects. Participants in the case group in a case control study would have immaterial medical history prior to COVID-19 or throughout the post-recovery period up until enrollment. To reduce the confounding effects of severe sickness, participants with a history of mild to moderate COVID-19 infection without hospitalisation or oxygen therapy were chosen. Participants in the control group were in good health and had no prior history of COVID-19 or other morbidities. The selection of COVID-19 survivors with mild and moderate diagnoses

will follow the World Health Organization's recommendations. Following are WHO recommendations for people with mild, moderate, and severe COVID-19: [148]

WHO Classification based on disease severity (Table 1) **

MILD	Non-specific symptoms like a sore throat, stuffy nose, headache, nausea, and vomiting have also been described, along with loss of taste and smell. The majority of people suffer from fever, cough, tiredness, and myalgias. people who have symptoms but no signs of viral pneumonia or hypoxia.
MODERATE	Cough, fever, dyspnea, and rapid breathing are symptoms of pneumonia, although they are not severe pneumonia, as shown by Sp02 levels greater than 90% on room air.
SEVERE	Along with one of the following conditions: respiratory rate > 30 breaths/min, significant respiratory distress, or Sp02 90% on room air, and the clinical symptoms of pneumonia (fever, cough, dyspnea).

[COVID-19 clinical managements: Living guidance, 25 January 2021].

OBSERVATIONS AND RESULTS

More than a million COVID-19 patients have recovered from their illness and are resuming their normal social and professional lives. However, COVID-19 may also result in post-traumatic stress disorder, problems and sequelae, as well as the virus's potential reactivation, in these patients [153].

Following up includes keeping knowledge about nucleic acid reactivation as one of its key goals. Although the most recent research indicates that a positive follow-up RT-PCR does not necessarily indicate infectivity [154], typical symptoms are present in a small fraction of recurrence patients and necessitate additional therapy [155-157].

On the other hand, it is crucial to comprehend the potential outcomes of COVID-19 patients who have been released, particularly if they have any other harmful conditions, in order to protect their life in the future [158, 159].

In the present study, we performed and analyzed biochemical parameters (liver enzymes) between COVID-19 survivors and control subjects. The liver enzymes ALT, AST, ALP & GGT are observed to be elevated in 54.28%, 45.71%, 51.42% and 2.8% patients, respectively.

Pearson correlation was done to find out the correlation of liver enzymes as shown in the table no. 6 There is significant positive correlation between ALT&AST, ALT&ALP, ALT&GGT, as depicted in table no. 6 .

ALT and ALP levels are elevated significantly in COVID-19 survivors, while levels of AST and GGT are elevated but not significant.

AGE -

In present study, 35 COVID-19 survivors with verified negative SARS-CoV-2 RT-PCR tests were compared to 35 healthy persons without a history of COVID 19 with mean ages of 44±10.24 and 45±8.64, respectively. Male and female participants make up 17.14% 82.85% and 37.14% 62.85% of the case and control groups, respectively.

ALANINE AMINOTRANSFERASE

After a negative RT-PCR test for SARS-CoV-2, ALT showed significant variations across the study groups, indicating a long-lasting effect residue. Table No. 2 and Fig. 6 demonstrate that COVID-19 survivors had significantly higher ALT values than control subjects (p = 0.0020).

Table no. 2 Comparison of ALT levels between controls and cases:

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
ALT (IU/L)	30.18±5.10	36.63±10.73	0.0020

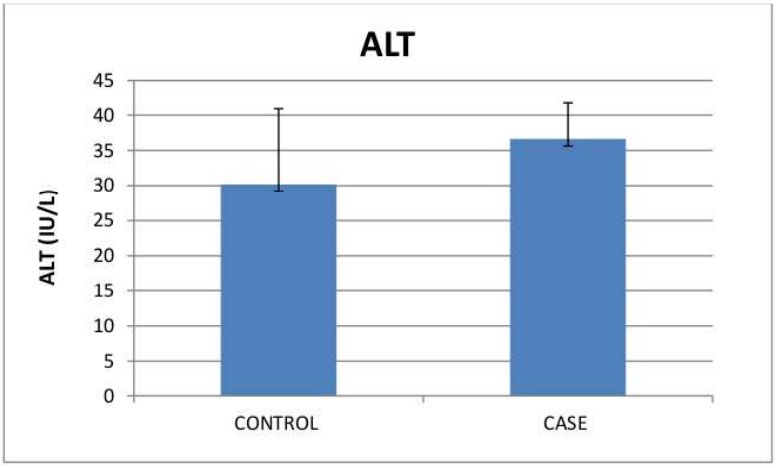


Fig.6 Comparison of ALT levels between controls and cases

Table no. 2 & fig.6 shows the comparison of ALT levels between controls and cases. ALT was significantly (p= 0.0020) higher among cases (36.63(IU/L) ±10.73) than controls (30.18(IU/L) ±5.10).

ASPARTATE AMINOTRANSFERASE

After a negative RT-PCR test for SARS-CoV-2, AST showed variations between the study groups, indicating a long-lasting effect residue, Table No. 3 and fig. 7. demonstrate that COVID-19 survivors had elevated AST values than control subjects, but the result was not statistically significant (p = 0.0738).

Table no. 3 Comparison of AST levels between controls and cases:

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
AST (IU/L)	28.12±6.47	31.10±7.24	0.0738

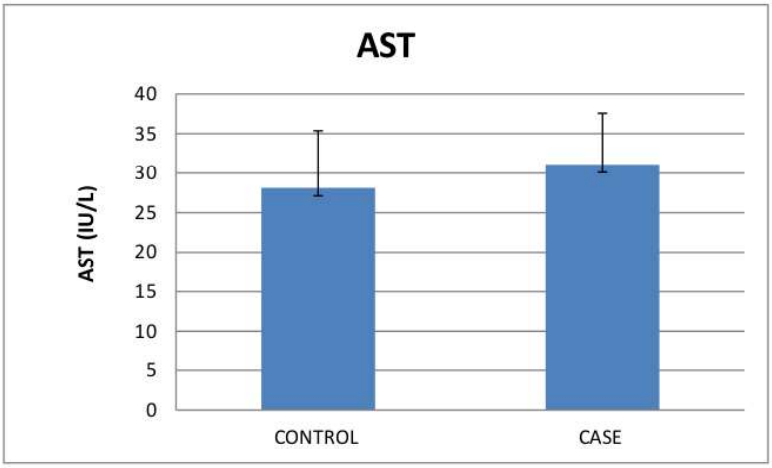


Fig.7 Comparison of AST levels between controls and cases

Table no. 3 & fig.7 shows the comparison of AST levels between controls and cases. AST levels were elevated among cases (31.10(IU/L) ±7.24) than controls (28.12(IU/L) ±6.47). Result was statistically non-significant.

ALKALINE PHOSPHATASE

After a negative RT-PCR test for SARS-CoV-2, ALP showed significant variations across the study groups, indicating a long-lasting effect residue. Table No. 4 and Fig. 8 demonstrate that COVID-19 survivors had significantly higher ALT values than control subjects (p = 0.0001).

Table no. 4 Comparison of ALP levels between controls and cases

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
ALP (IU/L)	88.78±13.13	106.05±15.56	0.0001

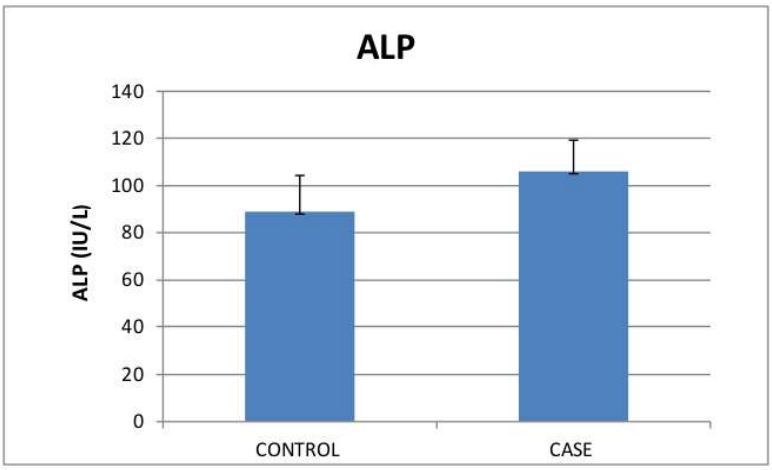


Fig.8 Comparison of ALP levels between controls and cases

Table no. 4 & fig.8 shows the comparison of ALP level between controls and cases. ALP levels were significantly (p= 0.0001) higher among cases (106.05(IU/L) ±15.56) than controls (88.78(IU/L) ±13.13).

GAMMA GLUTAMYL-TRANSFERASE

After a negative RT-PCR test for SARS-CoV-2, GGT showed variations between the study groups, indicating a long-lasting effect residue, Table No. 5 and fig. 9. demonstrate that COVID-19 survivors had elevated GGT values than control subjects, but the result was not statistically significant (p = 0.7778).

Table no. 5 Comparison of GGT levels between controls and cases

Variable	Control (Mean(IU/L)±S.D.) N=35	Case (Mean(IU/L)±S.D.) N=35	p-value
GGT (IU/L)	13.94±9.02	14.67±12.43	0.7778

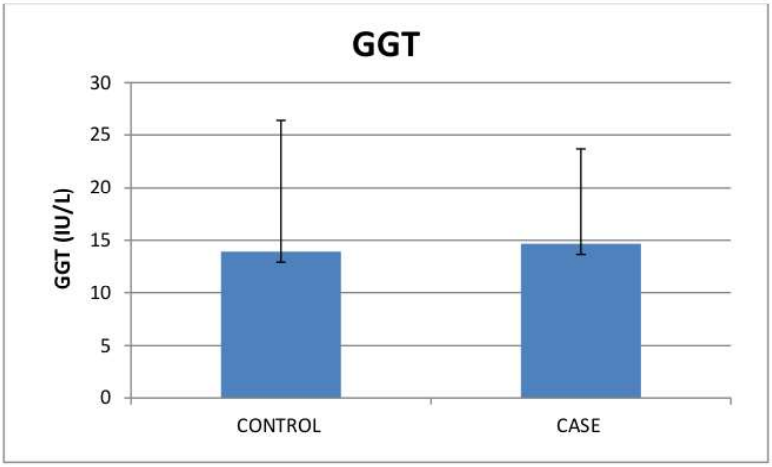


Fig. 9 Comparison of GGT levels between controls and cases

Table no. 5 & fig.9 shows the comparison of GGT levels between controls and cases. GGT levels were elevated among cases (14.67(IU/L) ±12.43) than controls (13.94(IU/L) ±9.02). Result was statistically non-significant.

Table No. 6 Pearson Correlation Coefficient in between the study variables in Cases

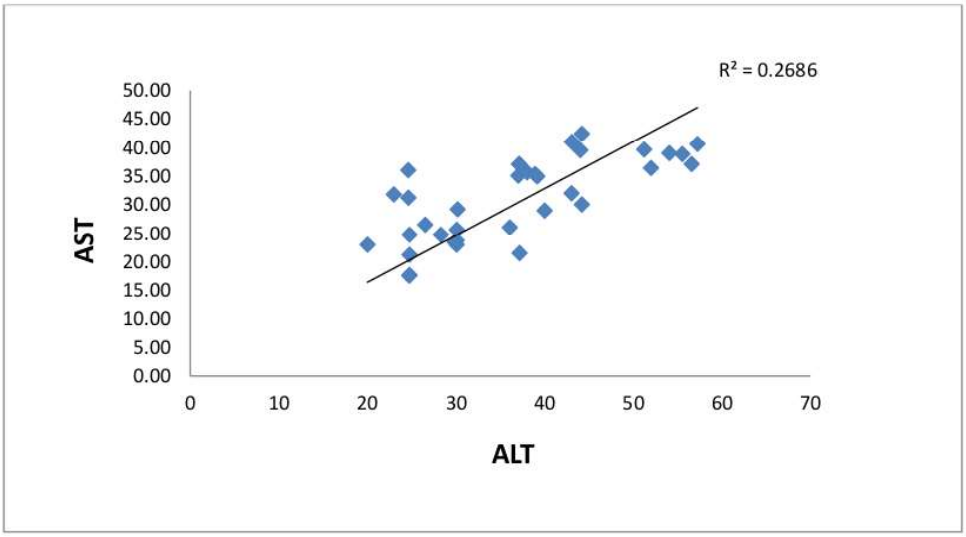
		Age	ALT	AST	ALP	GGT	
Age	**Pearson correlation**	1	-0.07	-0.02	0.011	-0.23	
	Sig. (2-tailed)	>0.05	>0.05	>0.05	>0.05	>0.05	
	N	35	35	35	35	35	
ALT	**Pearson correlation**	-0.07	1	0.7275	0.50	0.625	
	Sig. (2-tailed)	>0.05		<0.001	0.002	<0.001	
	N	35	35	35	35	35	
AST	**Pearson correlation**	-0.02	0.7275	1	0.8845	0.1805	
	Sig. (2-tailed)	>0.05	<0.001		<0.001	0.299	
	N	35	35	35	35	35	
ALP	**Pearson correlation**	0.011	0.50	0.8845	1	0.0237	
	Sig. (2-tailed)	>0.05	0.002	<0.001		0.89	
	N	35	35	35	35	35	
GGT	**Pearson correlation**	-0.23	0.625	0.1805	0.0237	1	
	Sig. (2-tailed)	>0.05	<0.001	0.299	0.89		
	N	35	35	35	35	35	

*.Showed that correlation is significant at the level ($p<0.05$) (2-tailed).

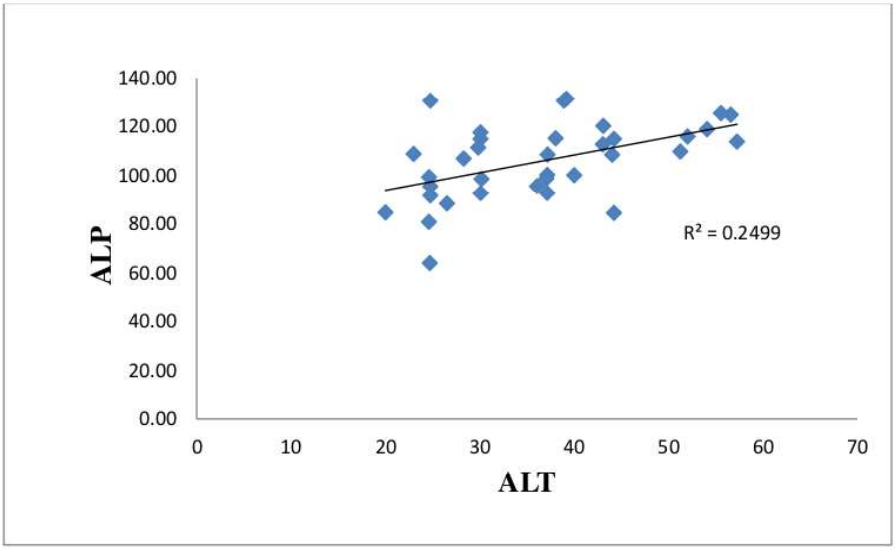
** showed that correlation is significant at the level (<0.001) (2-tailed).

(Table no. 6 & fig.no. 10,11,12,13) shows the correlation matrix which represents the quantitative measurements of degree of relationship among different variables.

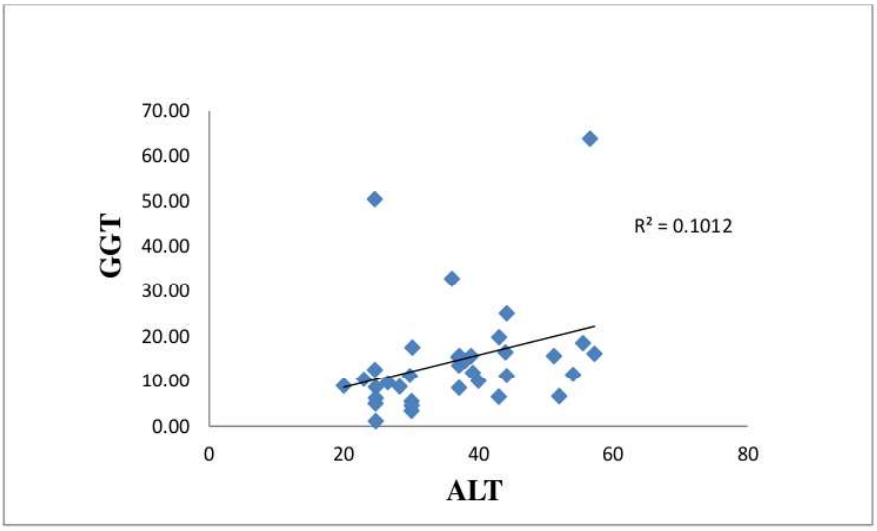
- ALT and AST express a significant positive correlation (r = 0.7275).
- ALT and ALP express a significant positive correlation (r = 0.50).
- ALT and GGT express a significant positive correlation (r = 0.625).
- AST and ALP express a significant positive correlation (r = 0.8845).
- There is no correlation express in between AST and GGT (r = 0.1805).
- There is no correlation express in between ALP and GGT (r = 0.0237).



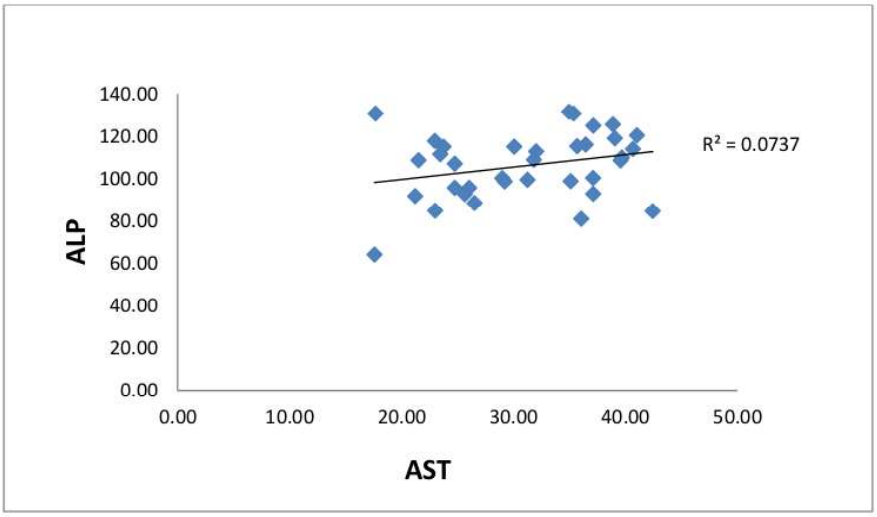
Fig,10: Scatter diagram showing association between ALT and AST in cases.



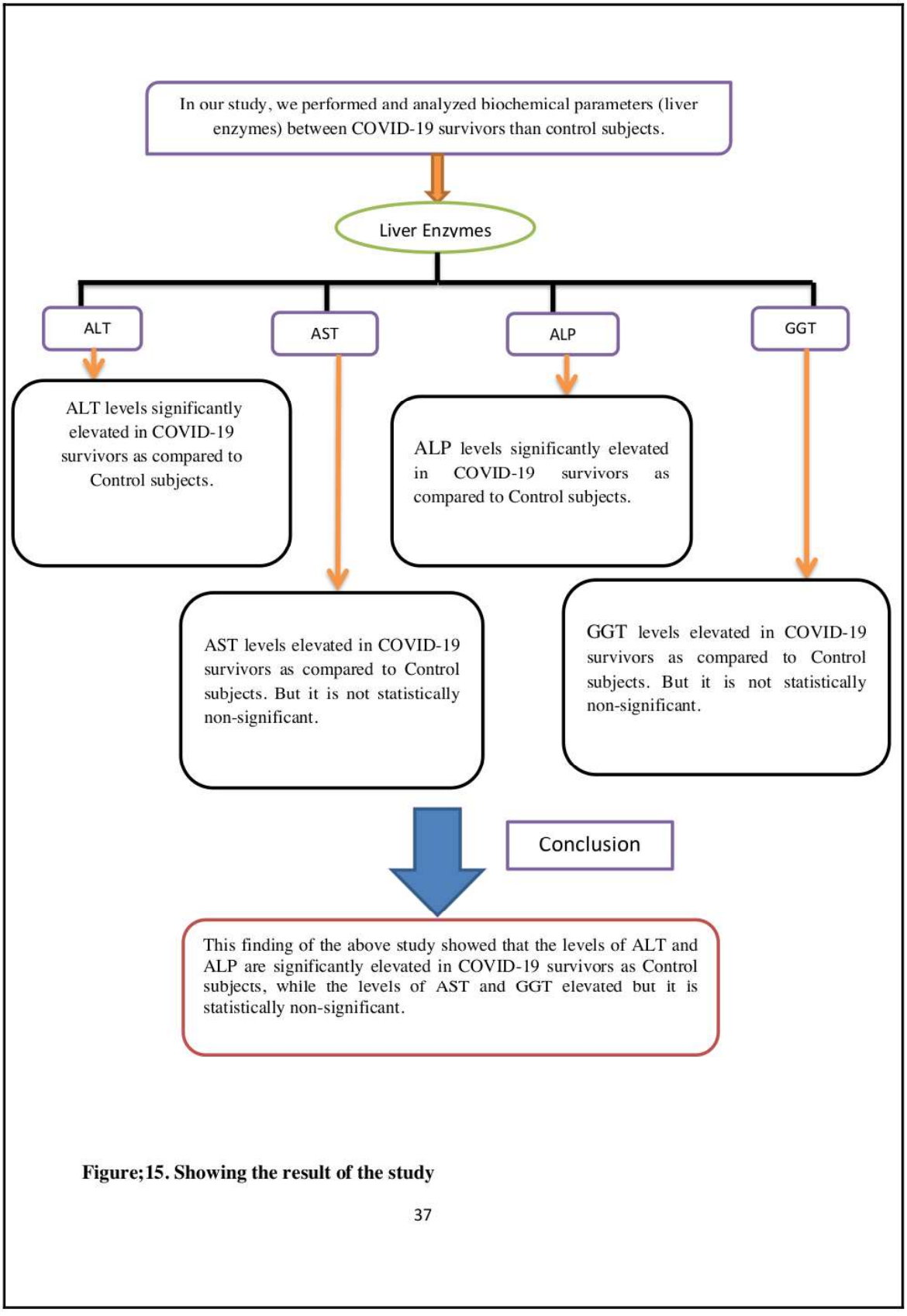
Fig,11: Scatter diagram showing association between ALT and ALP in cases.



Fig,12: Scatter diagram showing association between ALT and GGT in cases.



Fig,13: Scatter diagram showing association between AST and ALP in cases.



Figure;15. Showing the result of the study

DISCUSSION

The pattern of liver damage during acute COVID-19 has received a great deal of attention, but the long-term effects of COVID-19 on the functions of the liver are yet unknown. In the present investigation, liver enzymes differed statistically significantly between COVID-19 survivors and people who had never been exposed to the virus. Significantly higher levels of ALT, AST, GGT, and ALP were found in COVID-19 survivors.

Ya-Wen et. al. reported that for 14 days after discharge, COVID19 survivors had lower serum albumin and higher levels of ALT, GGT, and ALP. But within two months, these values progressively went back to normal. In our study, we found that long-term resolution of COVID-19 caused ALT, AST, GGT, and ALP levels to rise steadily over time. Fan et. al. [161] examination of the disruption of liver function in COVID-19 patients without concurrent elevation of serum total bilirubin, hepatic aminotransferases exhibited reversible mild to moderate rise.

Additionally, Xu et. al. [162] demonstrated non-elevated blood bilirubin in COVID-19 patients despite the high expression of ACE2 in the cholangiocytes rather than the hepatocytes of the hepatic vascular endothelium. They attributed these findings to the enormous systemic inflammatory response rather than a direct viral invasion. The precise pathogenesis of liver injury in COVID-19 patients, however, is still up for discussion.

Numerous theories have been put forth to explain the pathogenesis of hepatic changes, including direct hepatocyte viral invasion mediated by ACE2, immune homeostasis disruption, systemic inflammatory response, concurrent hypotension, pneumonia-associated hypoxia, and cytokine storm with an increase in hepatic stellate cell numbers cytokine storm with an increase in pro-inflammatory cytokines, and drug-induced hepatotoxicity [163, 164].

Tian et al. [165] found isolated macro-vesicular steatosis and mild sinusoidal dilatation without definitive evidence of bile duct damage.

Zsuzsanna et. al. [166] findings further connected these alterations to lymphocytic endothelitis with hepatocyte necrosis brought on by direct vial invasion and immune cell hyper-activation.

The comparatively longer follow-up period following the SARS-CoV-2 RT-PCR test results was very important feature of our study. Specifically, COVID-19 survivors with a history of mild to moderate disease were included in the case group. We made an effort to exclude out potential confounders such severe COVID-19 sickness and a history of acute or chronic morbidities prior to inclusion. Patients who were hospitalised or receiving oxygen therapy are also not included. We had to exclude alcohol abuse because we included the GGT variable in our study.

We deal many difficulties such as the lack of documented data of COVID-19 survivors before and during the acute stage of illness, we relied on the detailed medical history of participants and limited time duration. However, honestly, we cannot rely fully on the story of participants. Single-centre study represented major limitations. In this study, we tried to detect the significant differences between COVID-19 survivors who are expected to be definitely healthy versus healthy subjects without COVID-19 exposure.

Even after all this we finished our work and concluded that there is small or major difference was found between these parameters of COVID-19 survivors and control subjects. ALT & ALP are significantly elevated in COVID-19 survivors. The levels of AST and GGT elevated, but it is statistically non-significant.

In this study we have also shown the correlation matrix which represents the quantitative measurements of degree of relationship among different variables.

Multi-centre trials with larger-scale, multiple ethnicities, longer follow-up period and invasive tools may help more advanced research in the future.

Conclusion

It is still unknown how much liver damage is related to the recent SARS-CoV-2 outbreak. In this work, we described the clinical features of 35 patients who were tested positive for COVID-19 and we include 35 healthy individuals without COVID-19 history. We compared the total outcome of both group to see the change in between these groups.

We employed the levels of the liver enzymes ALT, AST, ALP, and GGT as the main screening tests to determine the liver's functional status.

We made every effort to find any pathological, clinical, and biochemical remnants in our area following the retrieval of COVID-19.

Here we report that, ALT and ALP were significantly elevated in approximately 45-50% of COVID-19-confirmed case history. While AST and GGT were also elevated but, it is statistically non-significant .

Remaining severe clinical and biochemical abnormalities in COVID-19 survivors made longer-term, intensive medical follow-up care necessary.

SUMMARY

The most of earlier studies used detailed medical questionnaires to assess post-COVID-19 symptoms for only a few weeks following recovery. We looked for any potential pathological clinical symptoms and biochemical remnants that might longer after the real-time polymerase chain reaction (RT-PCR) test for SARS-CoV-2 was negative.

Among 35 COVID-19 survivors of mean age 44 and 37.14% male proportion and 62.85% female proportion, and among 35 Control healthy individuals of mean age 45 and 17.14% male proportion and 82.85% female proportion, were involved in this study.

ALT levels were significantly elevated in COVID-19 survivors with mean 36.63(IU/L) ±10.73 (p= 0.0020). The result of AST was elevated in COVID-19 survivors with a mean of 31.10(IU/L) ±7.24 (p= 0.0738) but it is statistically non-significant.. ALP levels were significantly elevated in COVID-19 survivors with a mean of 106.05(IU/L) ±15.56 (p= 0.0001). The result of GGT was slightly elevated in COVID-19 survivors with a mean of 14.67(IU/L) ±12.43 (p= 0.7778), but it is statistically non-significant.

Patients with COVID-19 may experience liver damage that are related to their clinical scores, basic diseases, symptoms, hospitalisation for pharmacological treatment, and consequences. The heart, kidneys, thyroid, lipid and glucose metabolism, immunological index, leukocyte, erythrocyte, haemoglobin, and platelet-related indexes were all connected with indicators of liver function.

Poorer recovery for COVID-19 patients may be indicated by abnormal liver function. When COVID-19 patients are followed for a long time, changes in liver function should be highlighted, along with the importance of using the right therapies to restore liver function.

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