Changes in the Levels of Biochemical Markers Following Coronavirus Infection in Patients with Liver Disease, Renal Disease and Diabetes Mellitus as Compared to Control Participants: A Cross Sectional Study

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Authors’ contributions
This work was carried out in collaboration among all authors. Authors OJA and EAAW designed the study, wrote the study protocol and performed the recruitment. Authors OJA and EAAW wrote the first draft of the manuscript. Authors DJ and HAAA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information
DOI: 10.9734/JPRI/2021/v33i30B31648

Received 25 March 2021
Accepted 31 May 2021
Published 05 June 2021

ABSTRACT

Background & Aim of the Study: Increased levels of many biomarkers, including liver enzymes, blood urea and serum creatinine as well as glycemic markers have been reported following coronavirus (COVID-19) infection, leading to the development of acute disease. This study aims to
measure and follow-up the following biomarkers (fasting blood glucose, blood urea, serum creatinine, total serum bilirubin, as well as the liver enzymes AST, ALT, and ALP) in otherwise healthy participants and patients with liver disease, renal disease and diabetes following COVID-19 infection.

**Materials and Methods:** This is cross section study, included 144 participants who were infected with COVID-19 and admitted to the Sheikh Zayed Hospital, Baghdad, Iraq. Participants were divided into 4 study groups, Group 1: 46 participants with no pre-existing medical condition (Control), Group 2: 30 patients with existing liver disease. Group 3: 28 patients with existing renal disease and Group 4: 40 patients with diabetes mellitus. Participants were followed up for 14 days following COVID-19 infection to monitor the progression of the biochemical markers.

**Results:** There were significant changes in serum levels of all the markers of this study between the four study groups (p<0.001). Serum ALP levels were not significantly changed within any of the four study groups. However, both ALT and AST levels were significantly changed within all the four study groups (p<0.001). The levels of TSB changes significantly within the renal group (Group 3), (p=0.017). The levels of S. Creatinine showed significant changes in all the study groups except the renal group (Group 3). The levels change significantly within all the study groups except the control group (Group 1), while fasting blood glucose levels changes significantly in the control group only (Group 1), (p=0.004).

**Conclusions:** Following COVID-19 infection, there were significant changes in the levels of ALT, AST, S. Creatinine and B.Urea after 14 days of the disease progression. While in patients with existing renal disease, there were significant changes in the levels of TSB, AST, ALT and B. Urea following COVID-19 infection. In diabetic patients, there were significant increase in the level of fasting blood glucose after 14 days of COVID-19 infection. there were no significant changes in serum levels of ALP and FBG in patients with chronic illnesses (liver disease, renal disease, and diabetes) when compared to control group.

| Keywords: Coronavirus; liver disease; kidney disease; diabetes mellitus; cytokine storm. |

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by coronavirus-2 (SARS-CoV-2). This virus identified first in Wuhan, China in December 2019 [1,2]. By October 2020, more than 34.4 million people were infection with this virus across the globe [2]. COVID-19 is characterized by cough, malaise, shortness of breath, fever, and loss of sense of taste and smell. While most people have mild symptoms, others may develop respiratory complications due to a strong immune response associated with cytokine influx, failure to multiple organs in the body, septic shock, and coagulation abnormalities. The incubation period range between 2-14 days [2].

The primary mode for viral transmission for this virus is via small aerosol droplets from coughing, sneezing, and talking in the infected patients [1,2]. In addition to touch the contaminated surfaces and then touching their faces [1,2]. The standard method of diagnosis of COVID-19 is by Real Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) from a nasopharyngeal swab. Computer tomography scan (CT scan) of the chest may assist in diagnosis in patients with high risk factors, however, it is not recommended for routine diagnosis of the disease [3].

The disease can be prevented through regular personal hygiene, such as frequent hand washing and sanitizing, physical distancing, as well as covering the face while sneezing and avoid touching the face with hands [4]. COVID-19 can cause serious inflammatory response via the production of large number of cytokines via the immune system. A drastic increase in the levels of various catecholamines, which may aggravate the clinical condition. This exaggerated and abnormal immune response can precipitate a generalized inflammatory response, acute respiratory disorder, multi-organ failure, shock, and eventual death [5].

In addition, COVID-19 may infect the endothelial cells, causing vasoconstriction in several organs. This will be associated with hypercoagulability, and edema. This response may be attenuated in patients with existing immune disorders, and who are taking anti-cytokine and immunomodulatory therapies [9].
COVID-19 viral infection may exacerbate the management of chronic illnesses, such as liver disease, renal disease and diabetes mellitus. This study aims to measure and follow-up a series of relevant biochemical markers (fasting blood glucose, blood urea, serum creatinine, total serum bilirubin, as well as the liver enzymes AST, ALT, ALP) in healthy participants and in patients with liver disease, renal disease and diabetes mellitus following COVID-19 infection.

2. MATERIALS AND METHODS

2.1 Participants

This is a case-control study which included forty-six subjects (29 males & 17 females) who were infected with coronavirus; however, they did not have any pre-existing medical conditions. In addition, the study included 145 patients with pre-existing medical conditions (30 patients with liver disease, 28 patients with kidney disease and 30 patients with diabetes) who were also infected with COVID-19. The study was conducted at the three hospitals in Iraq (Sheikh Zayed Hospital, Ibn Zuhur Hospital, and Ibn-Alkhateeb Hospital). Participants were excluded from the study if they were taking hormone replacement therapy or lipid lowering medications.

2.2 Sample Collection

Twelve milliliters of venous blood samples were collected after an overnight fasting in plain and EDTA tubes to measure various biochemical markers of the underlying conditions. The measured biomarkers included Fasting blood glucose, blood urea, serum creatinine, total serum bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), on three time periods (Day 1, Day 7 and Day 14) following COVID-19 infection. These parameters were measured colorimetric using ELISA kits from Abcam, USA.

2.3 Statistical Analysis

Data were presented using mean and standard deviation. The statistical analysis was done using Statistical Package for Social Sciences (SPSS) software version 18, IBM, USA. Independent sample student's t-test was performed to look for the significant differences between two variables, while ANOVA was used to check for significance between more than two means. Pearson's correlation coefficient was used for the determination of the correlation between two quantitative data in different groups. Significant differences were considered at P < 0.05.

3. RESULTS

3.1 Clinical Characteristic of Study Subjects

The clinical characteristic of study subjects is shown in the (Table 1). The study subjects included 46 patients with Control and 30 patients with liver disease and 30 patients with kidney disease and 40 patients with diabetes mellitus. The mean age of control group was (33.93±13.57) years, whereas the mean age of patients with liver disease was (47±11.9) and of patients with kidney disease was (58.79±15.02) and of patients with DM disease was (50.08±14.43), there was a highly significant difference in the mean age between the group of Control and the groups of (liver, kidney, DM) disease (p < 0.001).

Apparently, the healthy group included (29 males and 17 females), while liver disease group included (15 males and 15 females), kidney disease group included (17 males and 17 females), DM disease group included (17 males and 23 females), there was no statistically significant difference in gender distribution between these groups, that show in the Table 1.

| Table 1. Comparison of age and sex among four study groups by ANOVA |
|-------------------|------------|------------|------------|------------|----------|
|                   | Control N=46 Mean±SD | Liver Disease N=30 Mean±SD | Kidney Disease N=28 Mean±SD | Diabetes N=40 Mean±SD | P value |
| Age (yr)          | 33.93 ±13.57 | 47 ±11.99  | 58.79 ±15.02 | 50.08 ±14.34 | <0.001   |
| Male / Female     | 29/17       | 15/15      | 13/15       | 17/23       | 2.26     |
3.2 Evaluation of Different Biochemical Parameter

3.2.1 Liver function tests

Table 2 shows the difference of Alkaline phosphatase within the disease duration. There was no significant difference in all study group (p = 0.307) in Control and (p = 0.539) in Liver disease and (p = 0.601) in kidney disease and (p = 0.594) in DM, but there were significant differences among all the study groups across the three time points of the study (p< 0.001).

There were highly significant differences in the levels of alanine aminotransferase during the three sampling days across all the four study groups, Table 3, Fig. 1.

Table 2. Comparison of alkaline phosphatase (ALP) according to the days in each group and among four study groups by ANOVA

| Group       | ALP Mean±SD          | P value |
|-------------|----------------------|---------|
|             | Day 1                | Day 7   | Day 14   |
| Control     | 195.8±54.41          | 205.53±3.03 | 213.46±57.06 | 0.307 |
| Liver dis.  | 353.87±153.01        | 361.47±161.55 | 397.27±168 | 0.539 |
| Kidney dis. | 221.54±93.63         | 234.29±95.68 | 248.29±107.11 | 0.601 |
| DM          | 262.18±77.67         | 272.2±77.27 | 279.83±77.36 | 0.594 |
| P value     | <0.001               | <0.001   | <0.001   |

Fig. 1. Alanine aminotransferase at day 1, 7, and 14 in all study groups

Table 3. Comparison of Alanine aminotransferase according to the days in each group and among four study groups by ANOVA

| Group       | ALT Mean±SD          | P value |
|-------------|----------------------|---------|
|             | Day 1                | Day 7   | Day 14   |
| Control     | 30.37±10.44          | 35.54±11.87 | 40.67±15.77 | 0.001 |
| Liver dis.  | 86.3±34.14           | 94.33±40.47 | 111.27±42.27 | 0.046 |
| Kidney dis. | 34.29±10.56          | 40.96±13.52 | 51.46±20.06 | <0.001 |
| DM          | 33.9±11.89           | 41.58±15.65 | 53.48±21.51 | <0.001 |
| P value     | <0.001               | <0.001   | <0.001   |
The levels of aspartate aminotransferase were significantly increased following COVID-19 infection among all the four study groups Control and DM (\(p < 0.001\)), and (\(p = 0.025\)) in Liver disease and (\(p = 0.001\)) in kidney disease. In addition, there were significant differences between the four study groups among the three timepoints of the study, \((p< 0.001)\). Table 4.

Table 5 shows the changes in total serum bilirubin that occur during the days in the disease groups. Only the kidney disease group shows a significant difference (\(p = 0.017\)), Table 5.

### 3.2.2 Renal function tests

Table 6 shows the differences in serum creatinine within the four study groups. There were significant differences in the control group (\(p = 0.043\)), Liver disease group (\(p = 0.028\)) and DM group (\(p=0.005\)). No similar changes noticed in the Kidney group (\(p = 0.744\)), Table 6.

### Table 4. Comparison of aspartate amino transferase according to the days in each group and among four study groups by ANOVA

| Group       | AST Mean±SD Day 1 | AST Mean±SD Day 7 | AST Mean±SD Day 14 | P Value |
|-------------|-------------------|-------------------|-------------------|---------|
| Control     | 29.8±7.42         | 34.67±11.45       | 40.37±14.47       | <0.001  |
| Liver dis.  | 81.97±31.97       | 89.49±40.82       | 107.57±36.88      | 0.025   |
| Kidney dis. | 32.79±8.35        | 41.32±12.74       | 51.96±22.03       | 0.001   |
| DM          | 34.8±14.62        | 43.93±17.38       | 58.1±22.86        | <0.001  |
| P value     | <0.001            | <0.001            | <0.001            |         |

### Table 5. Comparison of total serum bilirubin according to the days in each group and among four study groups by ANOVA

| Group       | TSB Mean±SD Day 1 | TSB Mean±SD Day 7 | TSB Mean±SD Day 14 | P value |
|-------------|-------------------|-------------------|-------------------|---------|
| Control     | 0.79±0.22         | 0.88±0.26         | 0.89±0.22         | 0.080   |
| Liver dis.  | 2.36±1.33         | 2.7±1.81          | 3.2±2.26          | 0.208   |
| Kidney dis. | 0.93±0.16         | 1.01±0.17         | 1.07±0.18         | 0.017   |
| DM          | 0.9±0.24          | 0.95±0.27         | 0.98±0.25         | 0.438   |
| P value     | <0.001            | <0.001            | <0.001            |         |

### Table 6. Comparison of serum Creatinine according to the days in each group and among four study groups by ANOVA

| Group       | S. Cr Mean±SD Day 1 | S. Cr Mean±SD Day 7 | S. Cr Mean±SD Day 14 | P-value |
|-------------|---------------------|---------------------|---------------------|---------|
| Control     | 0.67±0.18           | 0.72±0.13           | 0.74±0.11           | 0.043   |
| Liver dis.  | 0.81±0.12           | 0.86±0.22           | 0.95±0.23           | 0.028   |
| Kidney dis. | 2.26±3.55           | 1.88±0.78           | 2.27±0.93           | 0.744   |
| DM          | 0.9±0.23            | 1.09±0.4            | 1.17±0.46           | 0.005   |
| P value     | <0.001              | <0.001              | <0.001              |         |

### Table 7. Comparison of blood urea according to the days in each group and among four study groups by ANOVA

| Group       | Blood Urea Mean±SD Day 1 | Blood Urea Mean±SD Day 7 | Blood Urea Mean±SD Day 14 | P-value |
|-------------|---------------------------|---------------------------|---------------------------|---------|
| Control     | 26.89±5.67                | 28.41±7.41                | 32.63±20.22               | 0.089   |
| Liver dis.  | 30.53±4.99                | 38±16.59                  | 39.2±10.33                | 0.010   |
| Kidney dis. | 60.75±20.78               | 74.04±22.69               | 90.54±27.18               | <0.001  |
| DM          | 35.03±10.77               | 46±17.26                  | 50.6±19.87                | <0.001  |
| P value     | <0.001                    | <0.001                    | <0.001                    |         |
Table 8. Comparison of fasting blood glucose according to the days in each group and among four study groups by ANOVA

| Group      | FBS Mean±SD   | P-value |
|------------|---------------|---------|
|            | Day 1         | Day 7   | Day 14  |          |
| Control    | 96.22±17.66   | 104.17±25.26 | 110.96±19.25 | 0.004    |
| Liver dis. | 112.67±60.57  | 123.07±49  | 131.73±47.28 | 0.377    |
| Kidney dis.| 146.68±101.71 | 141.07±75.04 | 173±101.79  | 0.400    |
| DM         | 232.63±104.74 | 229.43±79.19 | 260.68±86.59 | 0.243    |
| P value    | <0.001        | <0.001   | <0.001   |          |

Although there was no significant difference in the levels of blood urea in the Control group, the changes in the levels of this marker were significant in the three disease groups (p<0.01), Table 7.

3.2.3 Fasting blood glucose levels

Fasting blood glucose levels were significantly increased in the Control group (p = 0.004). No significant changes were noticed in the three study groups, Table 8.

4. DISCUSSION

4.1 Demographic and Clinical Characteristic of the Study Participants

The study showed that the mean age of patients infected with coronavirus was (47 – 58) years. The increase in MERS-CoV infection in older people can be explained due to the presence of multiple risk factors in elderly, including chronic inflammatory condition, low immune response associated with cytokine storm and coagulopathy [6]. In young healthy adults, the SARS-CoV-2 virus is associated with angiotensin converting 2 (ACE2) enzymes. These are located on the inner wall of the upper respiratory tract. From there, viruses can spread to the alveoli and activate the alveoli macrophages to generate cytokine and activate other inflammatory cells [6,7].

Gender variation was not taken into consideration because the models were grouped in the hospital regardless of the patient's gender. Having said, the immune system and body immune response may cause a gender variation in the severity of infection between males and females [8]. Females produces more antibody response as compared males and last for a longer duration. This difference may be linked to estrogen and chromosome X [8]. The generated cytokine storm is responsible for the exaggerated inflammatory response following COVID 19 infection. Interleukin-6 plays a key part in this process and its level was significantly elevated in males as compared to females. This can be related to worse outcomes when compared to females [9]. Kumar et al, 2020 indicated an increased level of neutralizing antibodies in females when compared to males following COVID 19 infection [10].

4.2 Liver Function Test Parameters

In this study, it was found that there was an increased liver enzyme (AST, ALT) in patients with MERS-CoV and have existing liver, kidney and/or diabetes, as well as those who do not have any diseases. This can be due to several reasons, including: the used medications for the related liver disease following COVID 19 infection. This includes the use of combined medications, like antibiotics, antivirals, antibodies, and sedatives, may cause further liver damage (add references). However, it is still unknown if hepatic dysfunction is entirely due to the drug use or due to other associated conditions following COVID 19 infection [11]. Studies have found that significant increase in the levels of liver enzymes in patients who died following serious COVID 19 infection [11]. This could be related to the management or due to liver complications following the viral infection, the use of antiviral medication, and the development of its complications [11,12].

4.3 Renal Function Test Parameters

There was significant increase in the levels of urea and creatinine in the kidney disease group who were infected with MERS-CoV. We found that patients with acute kidney injury (AKI) were more likely to have a higher rate of glucocorticoid and a lower percentage of antiviral drugs and renin-angiotensin-aldosterone treatment regimens on admission when compared to
patients with non-AKI. This can be explained due to the use of glucocorticoids in patients with AKI to reduce the degree of inflammation even if there is controversy about the use of glucocorticoids in patients with COVID-19 [13].

Given that ACE2 is a functional future of SARS-CoV-2, the safety and efficacy of renin-angiotensin-aldosterone in patients with COVID-19 should be carefully considered, the kidneys are involved through direct or indirect mechanisms. Kidney participation is mainly manifested as protein and serious kidney injury (AKI). Organ interactions, ventricular weakness, hypercoagulation and sepsis are other possible mechanisms of AKI. Furthermore, reduced oxygen delivery to the kidneys may lead to ischemic infection. Understanding basic molecular pathways and pathological physiology of kidney injuries and AKI in Covid-19 is necessary to develop management strategies and design effective treatment [14,15].

4.4 Fasting Blood Glucose

This study showed that there was a slight increase in the concentration of blood glucose level, and there was no significant difference between the study groups. Samanta et al. 2019, showed that SARS-CoV can causes higher fasting glucose levels and that it causes damage to pancreatic beta cells, result in the development of "acute diabetes". The virus can also affect glucose regulation through the Na+/H+ exchanger (NHE) and lactate pathways. When ACE2 is blocked, increase the levels of angiotensin II that activate NHE. This can lead to cellular hypoxia and the generation of reactive oxygen types leading to blanket damage and insulin resistance. Besides, an oxidic pancreatic oxygen types leading to blanket damage and angiotensin II that when ACE2 is blocked, increase the levels of Na+/H+ exchanger (NHE) and lactate pathways. Also affect glucose regulation through the development of "acute diabetes". The virus can to pancreatic beta cells, result in the increase in the concentration of blood glucose after 14 days of COVID-19 infection. In diabetic patients, there were significant increase in the level of fasting blood glucose after 14 days of COVID-19 infection. There were no significant changes in serum levels of ALP and FBG in patients with chronic illnesses (liver disease, renal disease, and diabetes) when compared to control group.

ETHICAL APPROVAL AND CONSENT

The study was approved by the Human Research Ethics Committee, College of Medicine, Al-Nahrain University, Iraq and all participants consented to participate in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/69056