Assessment of liver function enzymes, MDA and GSH in the Sera of COVID-19 patients

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Abstract---A cluster of unexplained pneumonia cases was reported in Wuhan, China, in late December 2019. A new coronavirus was identified as the causative cause of this mysterious pneumonia a few days later. The World Health Organization has temporarily given this causal virus the designation of severe acute respiratory syndrome coronavirus 2 and the related infected disease as coronavirus disease 2019 (COVID-19). The primary objective of this study is to examine the liver efficiency and assess the oxidative stress status of COVID-19 patients by measuring liver function enzymes, glutathione and MDA. The study included 120 cases ranging from 20 to 60 years, divided into three groups, COVID-19 patients, recovered, and control groups. Group. The results obtained indicated a significant increase in ALT, AST, and ALP activity (p< 0.01) in patients compared to those recovering and control. The results also indicated a significant increase in MDA in contrast to a significant decrease in the GSH activity of COVID-19 patients compared to recovered and healthy subjects. These results reflect the strong relationship between COVID-19 and impaired liver function as well as impaired oxidative stress / antioxidant balance.

Keywords---COVID-19, liver enzymes, Glutathione, MDA.

1. Introduction

Coronavirus disease (COVID-19) was initially detected when cases of viral pneumonia were reported in December 2019 in Wuhan, Hubei Province, China [1]Since then, the disease has spread globally and has been declared a global

pandemic by the World Health Organization (WHO). According to this pandemic, it resulted in a large number of hospitalizations and deaths, which in turn put pressure on health care resources. Over time, knowledge of the condition has grown, and it is now clear that it affects not only the lungs, but also the digestive system, heart ,and liver [2].In fact, liver involvement has been clearly documented in two recent pathogenic coronaviruses, SARS-COV and MERS-COV.These two viruses had remarkable genetic similarities (particularly SARS-COV) to the new coronavirus, SARS-CoV-2, and thus the hepatic involvement is not surprising [3]. In fact, several studies have indicated that SARS-CoV-2 the infection causes an increase in liver transaminases. The suggested mechanisms include the direct effect of the virus on hepatocytes or the biliary epithelium, liver injury caused by a severe immune response (cytokine storm) and immune damage, drug toxicity (such as acetaminophen, antivirals, and hydroxychloroquine), and ischemic hepatitis, which can occur in patients with multi-organ dysfunction and hemodynamic instability. This study aims to verify liver efficiency and assess oxidative stress status by measuring MDA and glutathione levels of COVID-19 patients compared to a recovered and healthy individual.

2. Materials And Methods

2.1. Subjects and study design
The study included three groups: patients, recovered ,and control groups with an age range between 20 and 60 years.The first group consist of 40 patients infected with Covid 19 who were diagnosed by physicians after the PCR analysis showed a positive result. The second group consists of 40 cases recovered from the Covid 19, and the third group was the control group which consisted of 40 healthy individuals. Subjects were collected from patients who attended the consulting clinic/Baghdad Teaching Hospital/Medical City, Isolated Shifa Center Baghdad, Iraq between October and December 2020. Informed consent was obtained from all study subjects prior to their participation. None of the patients was a smoker, alcoholic or pregnant. Patients with other diseases such as diabetes, hypertension, hyperthyroidism, psoriasis were excluded.

2.2. Samples collection

Collecting samples
Ten ml of blood was withdrawn from each case by vein puncture using disposable syringes, 2 ml were collected in an EDTA tube, and 8 ml was collected in a gel tube. Whole blood samples were stored in a refrigerator at 2–4 °C after collection. Samples in gel tubes were centrifuged for 10 min at 2000 and the serum was then kept at −20 °C.

2.3. Sample Analysis

The levels of AST and ALT in serum were determined using a colorimetric method (Reitman and Frankel) using the protocol of commercially available Randox kits from Randox Laboratories Ltd. (United Kingdom). ALP level was determined calorimetrically according to the protocol of commercially available Diasys kit was supplied by Diasys Diagnostic System GmbH, Holzheim, Germany. values were

determined. The lipid peroxidation was determined in serum by measuring malondialdehyde concentration, depending on the reaction between malondialdehyde and thiobarbituric acid (TBA) in an acidic medium to give a pink color supernatant that is read at a wavelength λ= 535 nm, following the method, described previously by Buege and Aust [4]. GSH concentration was measured according to the Ellman method by measuring the serum thiol concentration at λ= 420nm, following the procedure described by Nasif [4].

2.4. Statistical analysis

Data were analyzed using SPSS statistical software, version 23. Student analysis ANOVA test was performed for independent samples between patients, recovery ,and control groups, and the resulting values were expressed as mean and standard deviation (SD). Statistical tests were significant at $p < 0.05$ and highly significant at $p < 0.01$ with 95% confidence interval. ROC CURVE was carried out for the studied parameters and the area under the curve (AUC) was determined. The sensitivity and specificity of the above-mentioned analyzes were also determined, and the cut-off value was determined.

3. Results and Discussion

Liver Function Tests

The data analysis of liver enzymes (AST, ALT, and ALP) is summarized in Table 1. The mean activity of three enzymes for patients with Covid 19 showed a significant increase in activity compared to the recovered and control groups respectively, while no significant differences were recorded between recovered and control groups, as represented in Figures 1-3.

Table 1: The activity of Liver function enzymes AST, ALT and ALP for patient with Covid 19, Recovered and control group

| Parameters | Patient | | Recovered | | Control | | *p*-value |
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
AST (IU/L)	49.13	21.77	25.91	7.83	25.04	3.96	0.001*
ALT (IU/L)	43.64	19.13	25.94	6.85	26.71	6.18	0.009*
ALP (IU/L)	105.72	42.66	85.77	21.50	90.90	18.28	0.001*

* Significant at p<0.01.

Several studies agreed with the results obtained during this study [5][6] in which AST and ALT showed a high value, indicating liver impairment that can progress to cirrhosis[7] An increased AST level can also be used to diagnose a heart injury. In fact, if this is the case, it could be one of the causes of death, as elevated levels of both enzymes indicate liver dysfunction in its early stages, and liver function should be checked frequently for Covid-19 patients.

Figure 1: AST activity for patients, recovered and control groups.
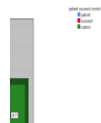

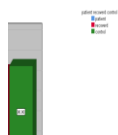Figure 2: ALT activity for patients, recovered andcontrol groups.

Figure 3: ALP activity for patients, recovered and control groups.

The data analysis for GSH and MDA of Covid 19 patients, recovered and the control groups are shown in Table 2. The mean ± SD estimated for GSH for the three study groups were 448.5 ±153.3µmol/l, 1012.6 ± 153.8µmol/land 1189.1 ±139.3 µmol/l respectively. Statistically, the GSH level in the patients appeared to be substantially lower (p<0.001) than in the recovered and control groups, as represented in Figure 4. In contrast, the mean ± SD values of MDA for the three study groups were 0.369 ± 0.127 µmol/l, 0.157 ± 0.027 µmol/l and 0.171 ± 0.029 µmol/l respectively. The results revealed that MDA values were significantly higher (p<0.01) in covid 19 patients in comparison to the recovered and control groups, as shown in Table 2 and represented in Figure 5.

Table 2: Serum concentrations of GSH and MDA for patient with Covid 19, Recovered and control groups

Parameters	Patient		Recovered		Control		
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	p-value
GSH (µmol/l)	448.5	153.3	1012.6	153.8	1189.1	139.3	0.001*
MDA (µmol/l)	0.369	0.127	0.157	0.027	0.171	0.029	0.001*

* Significant at p<0.01.

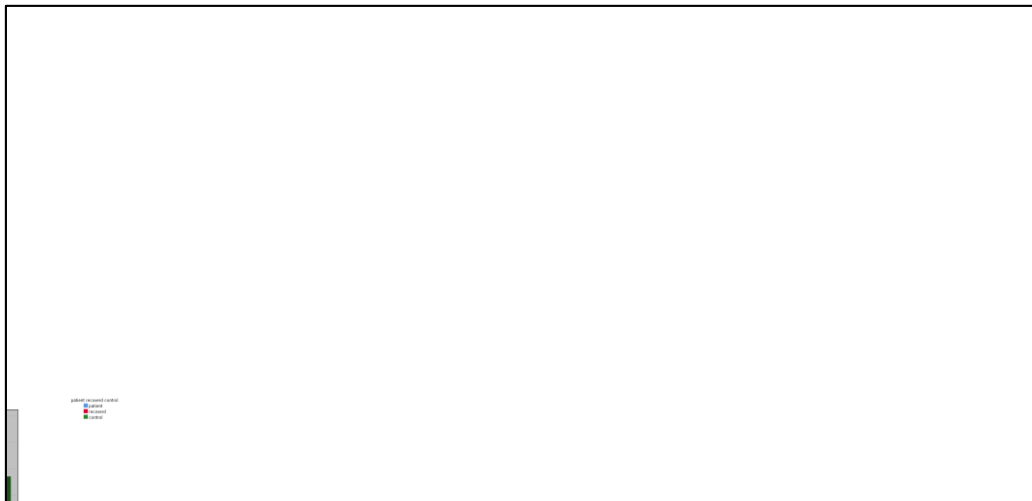Figure 4: GSHvalues for patients, recovered and control groups.

Figure 5: MDA values for patients, recovered and control groups.

According to these results, there was a decrease in the concentration of glutathione in Covid-19 patients when compared with the concentration of glutathione in the recovering and control groups. These findings strongly similar and support the findings recorded in other studies [8][9]Previously, endogenous glutathione insufficiency, characterized by decreased synthesis and/or increased GSH depletion has been linked to the pathophysiology of a number of diseases via oxidative stress and inflammatory pathways [8].Glutathione is an essential antioxidant that has been shown to have an important function in cellular resistance to oxidative damage [10].In COVID-19, neutrophil phagocytosis of immune complexes releases reactive oxygen species (ROS), which may subsequently, interact with immune complexes in addition to the plasma component to produce the chemotactic factor that attracts more neutrophils to the inflamed area [11].It has been shown that excessive production of ROS accelerates the process of cell destruction.

Several studies have shown that increased levels of glutathione increase an individual's response to viral infections. Glutathione, in particular, is known for its ability to protect host immune cells through its antioxidant effect and is also responsible for the proper function of a range of immune system cells. It should be noted that glutathione has been shown to inhibit viral replication at different stages of the virus life cycle and that this antiviral property of GSH appears to prevent viral loads and the subsequent a massive release of inflammatory cells in the lungs (cytokine storm) [8]. Serum GSH concentrations revealed that Covid 19 patients showed oxidatively stress.These findings could be related to a variety of clinical conditions, including length of disease, disease activity, treatment, reduced immunity, and even the condition of the patient at the time of sample collection and the drugs used as treatment.

The MDA findings of this research are consistent with those of another study, which found a fundamental difference and increased levels of MDA in COVID-19 patients compared to recovered and control subjects[12][13][14]. One of the most dangerous a consequences of oxidative stress is lipid peroxidation. MDA is a product of polyunsaturated fatty acid peroxidation that is used as a measure of oxidative damage [15]because free radicals play a key role in the cellular immune and inflammatory response in COVID-19, they are indirectly involved in cell damage. Increased MDA and decreased antioxidant status markers, such as GSH, support the idea that oxidative stress plays a role in the progression of disease severity [16][17]. Lipid peroxidation markers can be used as surrogate markers of disease activity. The MDA findings in this study could indicate increased lipid peroxidation and oxidative stress in COVID-19 patients, resulting in decreased the concentration of the antioxidant GSH.

The ROC Curve study was carried out to explore if liver functions enzymes could be utilized to diagnose COVID-19 and the results are represented in Figure 4. The area under the curve (AUC) for AST was found 0.971=excellent, with Specificity: 90, sensitivity: 100 and the cutoff value26.3 U/L. In light of this, all results higher than 26.3 U/L within the patient population, and results in less than 26.3 U/L are classified within the healthy population.

The results for ALT showed that AUC was 0.878 = good. The specificity and sensitivity were calculated and found to be 49 and 97 respectively, while the cutoff value is 27.03 U/L. ROC curve analysis of ALP revealed that the AUC value for both was weak and useless. It was0.598, as shown in Figure 4. It can be suggested from these results that the ASTvalue can be used as a reliable test for diagnosing and monitoringCOVID-19.
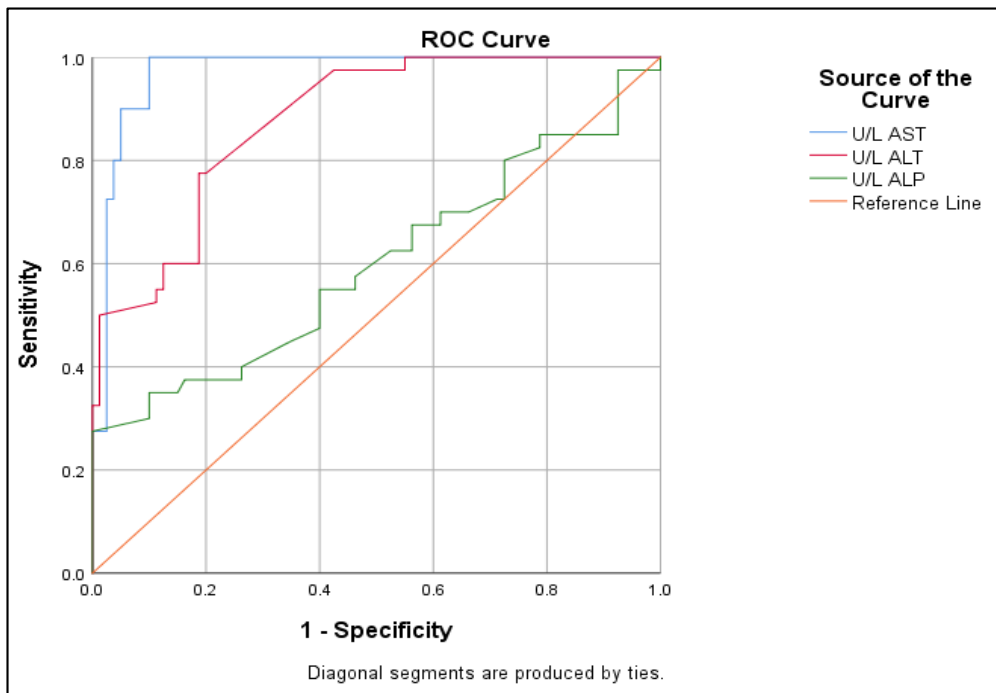
Figure 6: ROC curve for AST, ALT and ALP of Covid 19 patients.

The ROC curve for GSH showed 0.988=excellentAUC value. Specificity: 93, sensitivity: 97 and the cutoff value870 µmol/l, Figure 7. In light of this, all results higher than 870 µmol/l within the patient population, and results in less than 870 µmol/l are classified within the healthy population.For LDH, the results revealed that the AUC was found 0.953 = excellent. Specificity: 54, sensitivity: 95, and the cutoff value0.1750 µmol U/I, Figure 8. This result suggested that the LDH test can be used as a reliable test for monitoring Covid 19 disease.From these results, it can be suggested that the GSH level can be used as a reliable test for monitoring the disease.
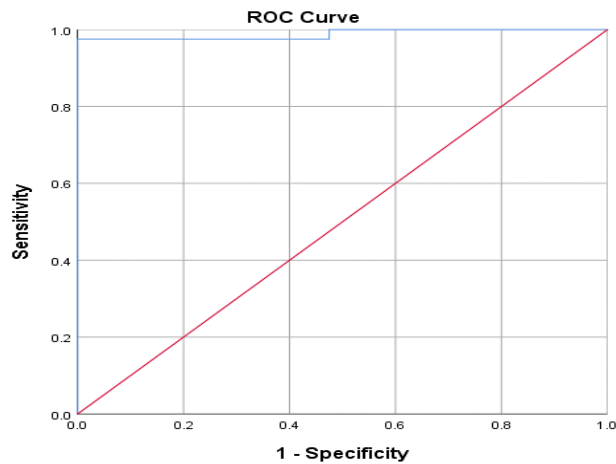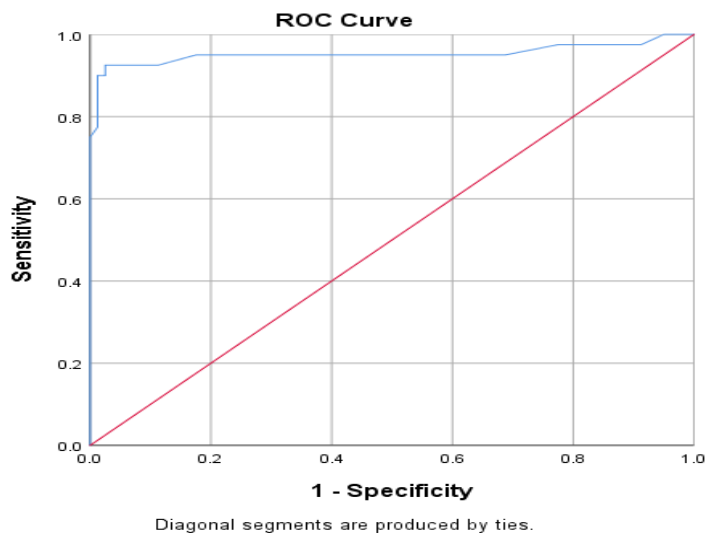
Figure 7: ROC curve for GSH of Covid 19 patients.

Figure 8: ROC curve for MDA of Covid 19 patients.

4. Conclusion

The COVID-19 infection has a similar onset to other pneumonia. ACT scan may be a reliable test for screening for cases of COVID-19.Liver function damage is more common with COVID-19 than it is with other. This case was revealed in this study from the high level of liver enzymes for Covid 19 patients. GSH and MDA may be important markers for assessing COVID-19, as the study revealed a strong relationship between COVID-19 and a high level of MDA in addition to a decreased level of GSH in the serum of patients infected with coronavirus, which affects oxidative stress / antioxidant balance in patients. This indicates that

COVID-19 is a serious disease that can increase oxidative stress in humans. High levels of MDA suggest the involvement of adipose tissue in elevated reactive oxygen and low glutathione not only locally but at the systemic level. Therefore, excessive free radical generation, rather than decreased antioxidant activity, is the major cause of oxidative stress in covid 19.

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