Sudan University of Science and Technology

Collage of Graduate Studies

Plasma Aminotransferases EnzymesActivity Levels among COVID -19.

نشاط مستويات انزيمات الامانوترانسفيريزس في البلازما لدى الأشخاص المصابين بفيروس كرونا 19.

A dissertation submitted in partial fulfillment for the requirements of M.sc degree in Medical Laboratory Science (Clinical Chemistry).

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الآية

(وينصرك الله نصرا عزيزا)

سورة الفتح الآية 3

Dedication

To who give me life meaning

To my father and mother

To my sisters and brothers

To my family and friends

Acknowledgments

Firstly foremost, thanks to ***ALMIGHTY ALLAH*** *for guiding me and give me the knowledge and strength to complete this work.*

give deep, great thanks to my great role model supervisor ***Dr.***

Nuha ELJaili *for her continued guidance, advice, support and help, my sincere gratitude and appreciation for for her confidence on me and am very grateful for always finding open heart and door.*

Finally, I thank all my family and close friends, who encouraged, believed and helped during this study.

Abstract

Background: Corona virus disease is challenging in everyday lives because it has devastating effects on human health and well-being and negative impact on economy.

Aim: This study was amid to assessment plasma aminotransferases Enzymes activity levels among COVID -19 patients at Khartoum state.

Methods: This was case control study conducted during the period from faraway to June 2022. fiftieth patients with COVID -19 collected as case group compared with fiftieth healthy volunteer as control group. Blood samples were collected from both groups and plasma activities of aminotransferases were estimated using full automated spin 200 instrument. the procedure of biochemical test was performed according to manufactured instruction, statistical analysis was done using SPSS version 25

Results: The mean of plasma AST, ALT were significantly higher in COVID - 19 patients (62.08u/l,47.6 u/l) when compared with control group (28.2,21.3) with p value (0.00) also this study shows no significant different in mean of AST, ALT levels between gender (male, female)in case group (51.3)(39.1) ,(66.2,52.7) *with p* value (0.23,0.195).

This result showed no correlation between AST levels and age with p value (0.113) R (0.159) on the other hand their correlation between ALT levels *and age* *p* value (0.003) R (0.294).

Also showed no correlation between AST, ALT levels and duration of disease with *p* value (0.215, 0.182) R (-0.178, 0. 192).

Conclusion: COVID -19 patients had high levels of AST and ALT, with ALT levels increased due to aging.

المستخلص

المقدمة: يمثل مرض فيروس كرونا تحديا في الحياة اليومية لأنه له آثار مدمرة على صحة الإنسان ورفاهيته وتأثيره السلبي على الاقتصاد.

الهدف: تقييم مستويات انزيمات الأماينوترانسفيريزس في بلازما لدى الأشخاص المصابين بفيروس كرونا 19 في ولاية الخرطوم.

المواد والطرق: أجريت هذه الدراسة في الفترة من فبراير الي يونيو في عام 2022 وتمت هذه الدراسة باستخدام بلازما لعدد 50 من الأشخاص المصابين بالفيروس كما تم اختيار 50 شخص سليم كمجموعة ضابطة. قدرت مستويات الانزيمات باستخدام جهاز اسبين 200 سستم كامل التشغيل الالي. وتم تجهيز وتحليل البيانات باستخدام الحزمة الإحصائية للعلوم الاجتماعية رقم الاصدار 25.

النتائج: بينت النتائج ان هناك ارتفاع ذو دلاله معنويه في مستوي الانزيمات في دم الأشخاص المصابين بفيروس كورونا (62.08, 47.6) وانه ليس هناك فرق ذو دلاله معنويه في متوسط الانزيمات بين النوع (ذكر، انثي) (66.2 ,52.7).

كما أظهرت الدراسة عدم وجود علاقة بين مستويات الانزيمات والعمر الفترة الزمنية للمرض.

الخلاصة: هذه الدراسة بينت ان هناك زيادة في مستويات الاماينوترانسفيريزس في المرضى بفيروس كوفيد 19 كما بينت ان هنالك علاقة إيجابية بين الألنين اماينوترانسفيريز والعمرز.

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List of abbreviation:

AST	Aspartate amino transferase
ALT	Alanin amino transferase
LFT	Liver function test
RNA	Ribo nucleic acid
SARS	Sever acute respiratory syndrome
ARTI	Acute respiratory infection
ACE-2	Angiotensin converting enzyme -2
PCR	Polymerase chain reaction
RT-PCR	Real time polymerase chain reaction

Chapter one

Introduction, rationale and objectives

1.1 Introduction:

SARS-CO2 (COVID -19) affected more than 500 million confirmed cases and over six million deaths which have been reported globally by WHO weekly statistical report (Ertuglu *et al*., 2020).

COVID 19 has wide range of clinical presentation, varying from asymptomatic carrier state to viral pneumonia in addition to various extra -pulmonary manifestations, including liver affection (Rajinik *et al*., 2020) many covid 19 patients especially those who have severe or critical disease experienced some form of liver injury (Li *et al*., 2021).

One study has shown that 14-53% patients displayed abnormal levels of aminotransferases during disease progression, and that liver abnormalities could be used to stratify patients' risk and monitoring illness severity (Guan et *al*., 2020). Abnormal liver blood tests have been found in one -half of patients the ASRS-COV-2 infection course. a large systemic review of 11studies evaluated the liver laboratory parameters of 2541 patient infected with SARSCOV-2, in cases of severe COVID -19, the incidence of liver injury can reach 93% indicating a possible association between COVID -19- related liver disease and mortality. (Cai *et al*., 2020). However, there is little information available related to modifications of liver function test and their role as a marker of severity in patients with severe COVID -19 among Sudanese population. Another study showed elevated levels of aminotransferases (25%) (Kukla *et al*., 2020). Accordingly, this study highlights the changes in the liver enzymes AST and ALT among Sudanese patients with COVID -19

1.2 Rationale

Covid 19 remains common cause of mortality and morbidity in Sudan. The first case was reported on 31 March 2020 and up to 3 July 2020 there are 9894 confirmed cases and 616 death. The incidence of liver injury can reach 93% indicating a possible association between COVID -19- related liver disease and mortality.

AST and ALT enzymes are more enzymes affected in liver by covid -19, there are significant elevation in AST and ALT in patient with COVID -19 So, this result may provide base line data to avoid complications of COVID -19.

1.3. Objectives

1.3.1. General objective

To study plasma aminotransferases activity among patients with COVID -19 at Khartoum state.

1.3.2. Specific objectives:

1-To estimate aminotransferases in study groups.

2-To compare mean concentration of aminotransferases in case and control groups

3o To correlate between biochemical parameters aminotransferases and study variables (age, gender and duration of disease).

Chapter two

2. Literature review

2.1 COVID-19

2.1.1 Definition of COVID-19

Coronavirus is infectious disease caused by a new strain of coronaviruses, are broad subfamily of RNA viruses that often cause respiratory tract and gastrointestinal tract infections. (Walls *et al*., 2020).

2.1.2 History of COVID -19

The latest outbreak of another ARTI (acute respiratory tract infection), COVID-19, has once again brought the attention of the world towards the deadly viruses and tested our capability of dealing with the threat of highly contagious viruses including coronaviruses which are a known health threat (Docea *et al*., 2020). Coronavirus has been known to cause human infections since the 1960s; however, the potential of this virus to cause deadly epidemics came to fore in the last two decades only. COVID-19 is the third major outbreak of respiratory disease in twenty years related to coronavirus, which has significantly disturbed the socioeconomic balance of the entire world. SARS-CoV-2 belongs to the family Coronaviridae, which belongs to the order Nidovirales (King *et al*., 2011).

2.1.3 Transmission and Pathogenesis of COVID 19

Infection is acquired either by inhalation of these droplets or touching surfaces contaminated by them or then touching the nose, mouth and eyes. The virus is also present in the stool and contamination of the water supply and subsequent transmission via aerosolization/ feco oral route is also hypothesized (World Health Organization, 2020). As per current

information, transplacental transmission from pregnant women to their fetus has not been described. However, neonatal disease due to post natal transmission is described . The incubation period varies from 2 to 14 days. Studies have identified angiotensin receptor 2 (ACE2) as the receptor through which the virus enters the respiratory mucosa. The basic case reproduction rate (BCR) is estimated to range from 2 to 6.47 in various modelling studies (Cheng *et al*., 2020).

2.1.4 Sign and symptoms of COVID 19

The most common symptoms are fever, dry cough, shortness of breath, myalgia, fatigue. Potential complications include pneumonia, acute respiratory distress syndrome, multi-organ failure and death (Guan., 2019) Potential gastrointestinal manifestations of SARS- CO -2 have been reported, including nausea, vomiting, diarrhea and abnormal liver function test (LFT) .SARS-CoV-2 infection presents a significant heterogeneity in its clinical course, ranging from asymptomatic presentations to life-threatening disease such as acute respiratory distress syndrome or multiple organ failure (Wu *et al*., 2020). A great number of infected patients mainly those critically ill with SARS-CoV-2, present gastrointestinal manifestations, particularly acute alteration of liver function (lei *et al* 2020).

2.1.5 Diagnosis of COVID- 19

PCR method is considered as the gold standard for the detection of some viruses and is characterized by rapid detection, high sensitivity, and specificity. As such, real-time reverse transcriptase-PCR (RT-PCR) is of great interest today for the detection of SARS-CoV-2 due to its benefits as a specific and simple qualitative assay. Moreover, real-time RT-PCR has adequate sensitivity to help us much for diagnosing early infection.

Therefore, the „criterion-referenced" real-time RT-PCR assay can be considered as a main method to be applied to detect the causative agent of COVID-19, SARS-CoV-2.it is used as a diagnostic tool using nasal swab, tracheal aspirate or bronchoalveolar lavage samples. Several studies have shown that SARS-CoV-2 RNA can also be detected in blood and stool specimens. The primary, and preferred, method for diagnosis is the 12 collection of upper respiratory samples via nasopharyngeal and oropharyngeal swabs (Long *et al*., 2020).

COVID19 conditions involve multiple organ systems; thus, a thorough physical examination should be completed. For patients who report previous infection with SARS-CoV-2, in addition to standard vital signs (i.e., blood pressure, heart rate, respiratory rate, pulse-oximetry, body temperature) and body mass index, healthcare professionals should evaluate ambulatory pulse oximetry for individuals presenting with respiratory symptoms, fatigue, or malaise (Schaller *et al*., 2020).

2.1.6 Prevention of COVID- 19

Since at this time there are no approved treatments for this infection, prevention is crucial. Isolation of confirmed or suspected cases with mild illness at home is recommended. The ventilation at home should be good with sunlight to allow for destruction of virus. Patients should be asked to wear a simple surgical mask and practice cough hygiene. Caregivers should be asked to wear a surgical mask when in the same room as patient and use hand hygiene every 15–20 min.

The greatest risk in COVID-19 is transmission to healthcare workers (Chang *et al*., 2020). The doctor who first warned about the virus has died too. It is important to protect healthcare workers to ensure continuity of care and to prevent transmission of infection to other patients. Patients should be placed in separate rooms or cohorted together. Negative

pressure rooms are not generally needed. The rooms and surfaces and equipment should undergo regular decontamination preferably with sodium hypochlorite. Healthcare workers should be provided with fit tested N95 respirators and protective suits and goggles. Airborne transmission precautions should be taken during aerosol generating procedures such as intubation, suction and tracheostomies. All contacts including healthcare workers should be monitored for development of symptoms of COVID-19. Patients can be discharged from isolation once they are afebrile for at least 3 d and have two consecutive negative molecular tests at 1 d sampling interval. This recommendation is different from pandemic flu where patients were asked to resume work/school once afebrile for 24h or by day 7 of illness. Negative molecular tests were not a prerequisite for discharge. At the community level, people should be asked to avoid crowded areas and postpone non-essential travel to places with on-going transmission. They should be asked to practice cough hygiene by coughing in sleeve/tissue rather than hands and practice hand hygiene frequently every 15–20 min. Patients with respiratory symptoms should be asked to use surgical masks. The use of mask by healthy people in public places has not shown to protect against respiratory viral infections and is currently not recommended by WHO. However, in China, the public has been asked to wear masks in public and especially in crowded places and large scale gatherings are prohibited (entertainment parks etc). China is also considering introducing legislation to prohibit selling and trading of wild animals. The international response has been dramatic. Initially, there were massive travel restrictions to China and people returning from China/ evacuated from China are being evaluated for clinical symptoms, isolated and tested for COVID-19 for 2 weeks even if asymptomatic. However, now with rapid world wide spread of the virus these travel restrictions have

extended to other countries. Whether these efforts will lead to slowing of viral spread is not known. A candidate vaccine is under development (Li *et al*., 2020).

2.1.7 Vaccines of COVID -19

Vaccination is the most effective method for a long- term strategy for prevention and control of COVID-19 in the future. Many different vaccine platforms against SARS- CoV-2 are in development, the strategies of which include recombinant vectors, DNA, mRNA in lipid nanoparticles, inactivated viruses, live attenuated viruses and protein subunits159–161. As of 2 October 2020, ~174 vaccine candidates for COVID-19 had been reported and 51 were in human clinical trials (COVID-19 vaccine and therapeutics tracker). Many of these vaccine candidates are in phase II testing, and some have already advanced to phase III trials. A randomized double- blinded phase II trial of an adenovirus type 5- vectored vaccine expressing the SARS- CoV-2 S protein, developed by CanSino Biologicals and the Academy of Military Medical Sciences of China, was conducted in 603 adult volunteers in Wuhan. The vaccine has proved to be safe and induced considerable humoral and cellular immune response in most recipients after a single immunization. Another vectored vaccine, ChAdOx1, was developed on the basis of chimpanzee adenovirus by the University of Oxford. In a randomized controlled phase I/II trial, it induced neutralizing antibodies against SARS- CoV-2 in all 1,077 participants after a second vaccine dose, while its safety profile was acceptable as well. The NIAID and Moderna co- manufactured mRNA-1273, a lipid nanoparticle- formulated mRNA vaccine candidate that encodes the stabilized prefusion SARS-CoV-2 S protein. Its immunogenicity has been confirmed by a phase I trial in which robust neutralizing antibody responses were induced in a dose- dependent manner and increased after a second dose. Regarding

inactivated vaccines, a successful phase I/II trial involving 320 participants has been reported in China. The whole- virus COVID-19 vaccine had a low rate of adverse reactions and effectively induced neutralizing antibody production. The verified safety and immunogenicity support advancement of these vaccine candidates to phase III clinical trials, which will evaluate their efficacy in protecting healthy populations from SARS- CoV-2 infection (Wang, 2020).

2.2. **Aspartate aminotransferase** (AST)

AST is an enzyme belonging to the class of transferases. It is commonly referred to as a transaminase and is involved in the transfer of an amino group between aspartate and -keto acids. The older terminology, serum glutamic-oxaloacetic transaminase (SGOT, or GOT), may also be used. Pyridoxal phosphate functions as a coenzyme. The transamination reaction is important in intermediary metabolism because of its function in the synthesis and degradation of amino acids. The ketoacids formed by the reaction are ultimately oxidized by the tricarboxylic acid cycle to provide a source of energy. (Michael**,** Bishop., 2005).

2.2.1. Tissue Source of AST

AST is widely distributed in human tissue. The highest concentrations are found in cardiac tissue, liver, and skeletal muscle, with smaller amounts found in the kidney, pancreas, and erythrocytes. (Michael**,** Bishop., 2005).

2.2.2 Diagnostic Significance of AST:

The clinical use of AST is limited mainly to the evaluation of hepatocellular disorders and skeletal muscle involvement. In AMI, AST levels begin to rise within 6 to 8 hours, peak at 24 hours, and generally return to normal within 5 days. However, because of the wide tissue distribution, AST levels are not useful in the diagnosis of AMI. AST

elevations are frequently seen in pulmonary embolism. Following congestive heart failure, AST levels also may be increased, probably reflecting liver involvement as a result of inadequate blood supply to that organ. AST levels are highest in acute hepatocellular disorders. In viral hepatitis, levels may reach 100 times ULN. In cirrhosis, only moderate levels—approximately four times ULN— are detected. Skeletal muscle disorders, such as the muscular dystrophies, and inflammatory conditions also cause increases in AST levels (4–8 ULN). AST exists as two isoenzyme fractions located in the cell cytoplasm and mitochondria. The intracellular concentration of AST may be 7,000 times higher than the extracellular concentration. The cytoplasmic isoenzyme is the predominant form occurring in serum. In disorders producing cellular necrosis, the mitochondrial form may be significantly increased. Isoenzyme analysis of AST is not routinely performed in the clinical laboratory.

(Michael**,** Bishop., 2005).

2.3 Alanine aminotransferase (ALT):

ALT is a transferase with enzymatic activity similar to that of AST. Specifically, it catalyses the transfer of an amino group from alanine to-ketoglutarate with the formation of glutamate and pyruvate. The older terminology was serum glutamic-pyruvic transaminase (SGPT, or GPT). Equation 12-12 indicates the transferase reaction. Pyridoxal phosphate acts as the coenzyme. (Michael**,** Bishop., 2005).

2.3.1 Tissue Source of ALT:

ALT is distributed in many tissues, with comparatively high concentrations in the liver. It is considered the more liver-specific enzyme of the transferases. (Michael**,** Bishop., 2005).

2.3.2 Diagnostic Significance of ALT:

Clinical applications of ALT assays are confined mainly to evaluation of hepatic disorders. Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders. In acute inflammatory conditions of the liver, ALT elevations are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half-life of ALT in serum (16 and 24 hours, respectfully). Cardiac tissue contains a small amount of ALT activity, but the serum level usually remains normal in AMI unless subsequent liver damage has occurred. ALT levels have historically been compared with levels of AST to help determine the source of an elevated AST level and to detect liver involvement concurrent with myocardial injury. (Michael**,** Bishop., 2005).

2.4 Relationship between COVID 19 and aminotransferases

Several mechanisms are proposed to explain injury during covid -19 infection hypoxia and cardiac failure in critically affected covid 19 cases can predispose to hypoxic hepatitis (li, xiao., 2020) the use of high levels of positive end -expiratory pressure may cause hepatic congestion by increase right atrial pressure and hindering venous return .however these mechanism alone cannot explain liver affection in all patient ,as liver laboratory test abnormalities are commonly encountered in stable patients who did not require mechanical ventilation (Huang ., 2020).

ACE2recptors, found on many cells including the lung, heart, liver, kidney, and blood vessels, interact with SARS-cov-2direct cytopathic effects (Simpson *et al*., 2020) recently, the single -cell RNA-seq data in healthy human hepatic samples suggest that ACE2 expression by cholangiocytes (59.7) is considerably higher than its expression by hepatocytes (2.6%) . Despite this major difference in ACE2 expression

(Chau., 2020, Ong., 2020). Abnormal liver blood tests have been found in almost one -half of patients, throughout the SARS -COV-2 infection course. large systematic review of 11 studies evaluating the liver laboratory parameters of 2541patients infected with SARScov-2 showed the following results; elevated aminotransferases (25%) (Kukla., 2020).

2.5 previous studies

Several studies have shown that patients with COVID-19 have evidence of liver damage on admission for hospitalization (ranging from 14% to 53%), expressed mainly by abnormal levels of liver transaminases but also by slightly elevated bilirubin levels (Sun., 2020). In cases of severe COVID-19, the incidence of liver injury can reach 93%, indicating a possible association between COVID-19-related liver disease and mortality (Gai ., 2020). However, there is little information available related to modifications of liver function tests (LFT) during hospitalization and their role as a marker of severity in patients with severe COVID-19 (Gan., 2021). large systematic review of 11 studies evaluating the liver laboratory parameters of 2541patients infected with SARScov-2 showed the following results; elevated aminotransferases (25%) (Kukla., 2020).

Chapter three

Material and Methods

3.1 Materials

3.1.1. Study approach:

A quantitative method was used to measure the level of serum AST and ALT enzymes in COVID 19 Sudanese patients during the period from February to June 2022

3.1.2. Study design:

The study was case control.

3.1.3. study area

This study was carried out at Jabra hospital, Khartoum state.

3.1.4 Ethical consideration

the study was approved by Fatima Mohammed and informed consent was obtained from each participant appendix 1

the data was collected through structured questionnaire appendix 11

3.1.5. study population and sample size

This study was conducted on 50 subjects with COVID -19 as case group and 50 subjects from healthy individuals as control group (ages and gender are matched).

3.1.5.1. inclusion criteria

Patients adult clinically diagnosed with COVID -19 and healthy participants as control group were included in this study.

3.1.5.2 Exclusion criteria

Patients adult with COVD-19 that had hepatitis, cirrhosis, fatty liver, jaundice, pancreatic disease, gallstone was excluded.

3. 1.6 sampling collection and processing

About 3ml of venous blood were collected separated by centrifugation in 3000 RPM for 5 min (-20) until time of an analysis.

Serum level of AST, ALT, were measured using commercial kites of spin and practical was performed by using for AST, ALT, spin full automation.

3.2 Methods

3.2.1. Measurement of AST:

Activity level was evaluated by method in appendix 11

3.2.3. Measurement of ALT

Activity level was evaluated by method in appendix 111

3.3 Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of the test and control samples.

3.4 Statistical analysis:

Data analysis using SPSS software program version 25. the results were expressed as mean ± stander deviation (SD). independent t test was used to compare mean of liver enzymes in COVID 19 patient and control group. person correlation test was used to study the relation between study parameters liver enzymes AST and ALT and study variable (age, gender and duration of disease) *p* value less than 0.05 was considered as statistically significant.

Chapter four

Results

4.1 Results

The results of biochemical determine serum of AST and ALT in COVID 19 patients and control group are given in tables and figures.

Table (1) illustrate age of study groups and duration of disease, age range between (24-82) years and duration of disease between (4-13) days.

Table (2) represent the comparison of mean ± SD of AST and ALT levels in case versus control group. The result showed increase in case compared to control group (61.08 ±14.4 versus 28.2 ±310.) *p* value (

0.00), (47.6 ±12 versus 21.3 ±9.8) p value 0.00) respectively.

Table (3) shows comparison of mean levels of liver enzymes AST, ALT in case group according to gender, there were no different. the mean of AST (51.3 ±8.3 vs 66.2 ± 5.1) *p* value=0.23 and mean of ALT (39.1 ± 3.3 vs 52.7 ± 4.2) *p* value (0.195)

Figure (1) shows correlation between ALT and age, there was significant positive correlation between ALT levels and age in COVID 19 (r 0.297, *p* 0.003).

Figure (2) shows no correlation between AST levels and age in case group (r 0.159, *p* 0.113)

Figure (3) shows no correlation between AST levels and duration of disease with (r 0.178, *p* value 0.215).

Figure (4) shows no correlation between ALT levels and duration of disease. (r 0.192, *p* value 0.182).

Table 1: Descriptive Statistics of Demographic Data

Variables	N	Minimum	Maximum	Mean	Std. Deviation
Age	100	24year	82 year	61.7	11.5
Duration	50	4 day	13day	9.2	2.1

Table 2: Comparison of mean levels of liver enzymes between case and control group.

Parameters	case group (N= 50)		control group (N= 51)		P value
	Mean	SD	Mean	SD	
AST U/l	61.08	14.4	28.2	10.3	0.00
ALT U/l	47.6	12	21.3	9.8	0.00

Table 3: Comparison of mean levels of liver enzymes in case group according to gender

Parameters	Male (N= 31)		Female (N= 25)		P value
	Mean	SD	Mean	SD	
AST U/l	51.3	8.3	66.2	5.1	0.23
ALT U//l	39.1	3.3	52.7	4.2	0.195

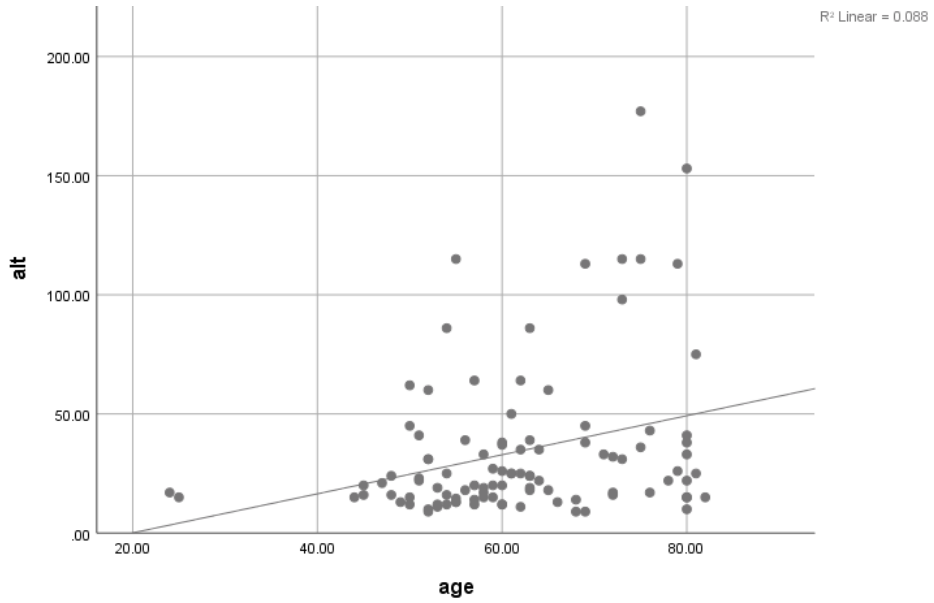

Figure 1: correlation between ALT levels and age of patients (r = 0.297, p value = 0.003)

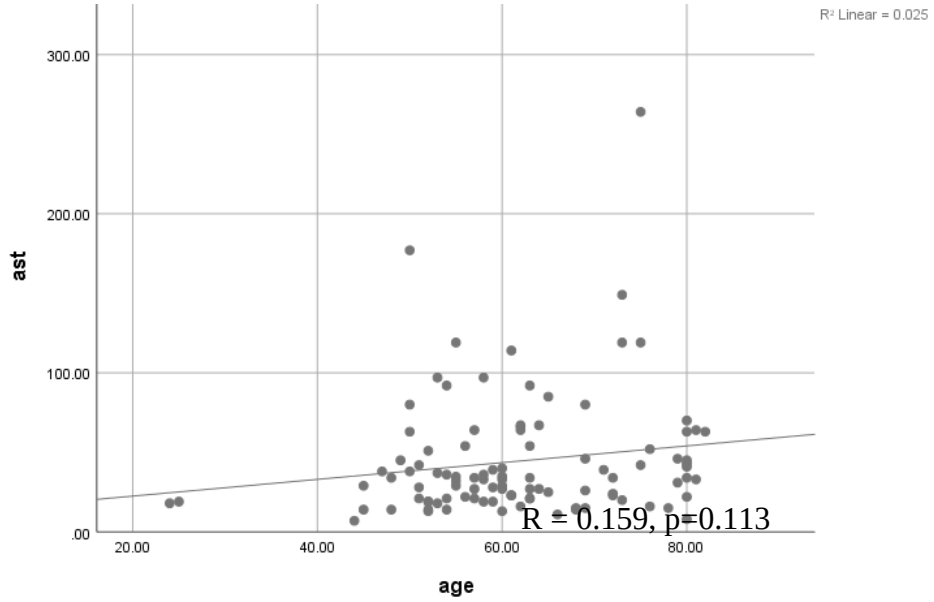

Figure 2: correlation between AST levels and age in case group (r = 0.159, p value = 0.113)

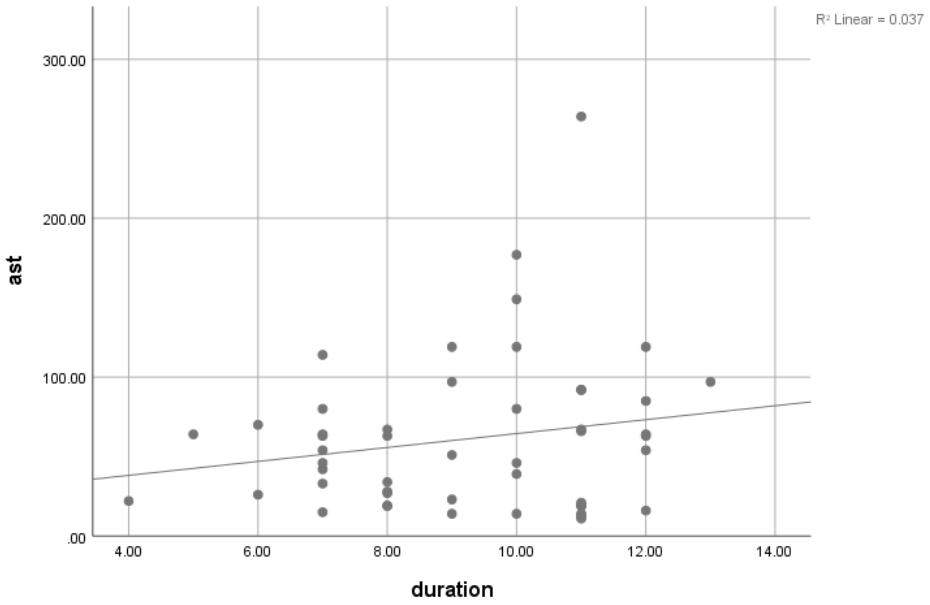

Figure 3: correlation between AST levels and duration of disease in case group
(r = -0.178, p value = 0.215)

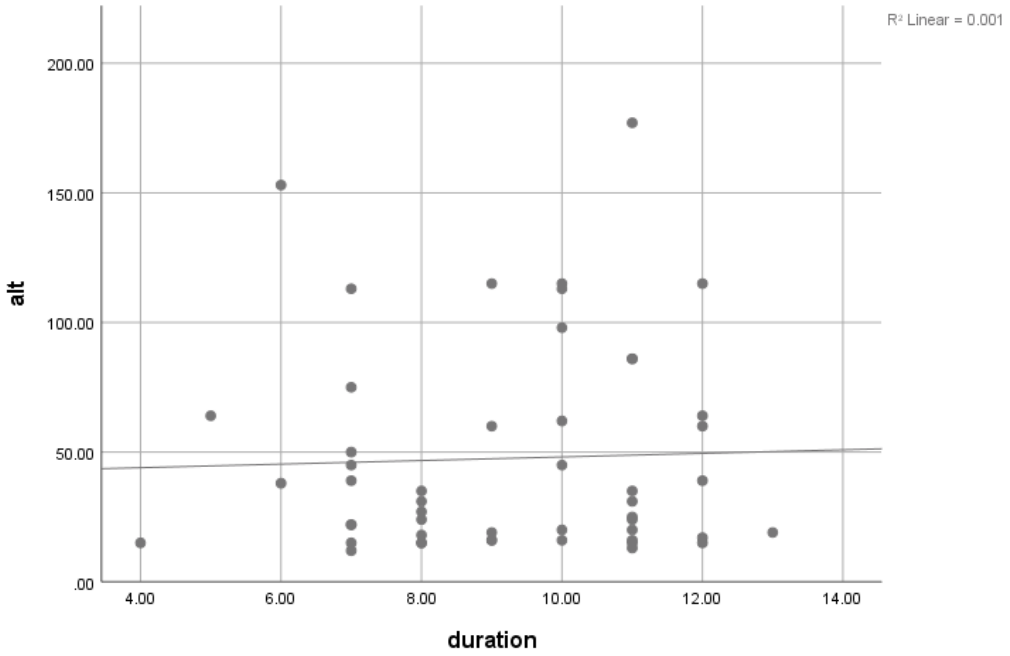

Figure 4: correlation between ALT levels and duration of disease in case group
(r= 0. 192, p value = 0.182)

Chapter five

Discussion, Conclusion and Recommendations

5.1 Discussion

Abnormal liver function tests (LFT) are frequently observed in patients with COVID-19, of which the underlying pathogenesis is incompletely understood (lai *et al.,* 2020).

This study described 50 adult patients who were hospitalized with COVID-19 in jabber hospital in Sudan and 50 healthy volunteers as control group. The study aimed to assess the levels of Aminotransferases in COVID-19 Patients.

Result of this study showed that, there were significant increase in AST and ALT in patient with COVID 19. This result is similar to another result of (Huang *et al.,* 2020; Guan *et al.,* 2020) , which reported that , bile duct cell has high specific expression of coronavirus receptor ACE-2 and low expression of hepatocytes, indicating the capability of COVID-19 to bind to ACE-2 positive bile duct cells directly, leading to the dysfunction of the bile duct, while biliary epithelial cell plays a key role in liver regeneration and immunoreaction. Additionally, most scholars believe that cytokine storm syndrome (CCS) and drug-induced liver injury may be the main mechanism behind hepatic injury.

On the other hand, the study demonstrated positive correlation of ALT with age of patients with COVID-19 (r =0.297, p value 0.003) while there was no correlation of AST with age and duration of disease (r= 0. 159 p, value = 0. 113) (r= 0.178, p value = 0.215) respectively and there was no correlation between ALT and duration of disease (r = 0.192, p value 0.182) This finding is quite similar with study of (Ram *et al*., 2020**)** who reported significantly higher proportion of patients with abnormal liver functions were elderly and duration of admission. The difference can attribute to low sample size and instrumental difference.

5.2 Conclusions

This study concluded increased in activity of aminotransferase in COVID-19 Patients Also, there was positive correlation between ALT and age.

5.3 Recommendations

_Further studies are still required to validate these results and define the role of liver tests in diagnostic algorithms.

_ The follow-up should be done to know whether this hepatic damage would have repercussions after hospital discharge.

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Appendix 1

Sudan University of Science and Technology

Collage of Graduate Studies

Assessment of Plasma Levels of AST and ALT in Sudanese Patient with COVID- 19 at Khartoum State.

Name: ……

Age: ……..……

Gender: Male ☐ Female ☐

Duration of disease: ……

Chronic disease?

Liver cancer……

Cirrhosis……..

Ascites……..

Hepatitis……

Jaundice……

Pancreatic disease……

Gall stone……..

Hypertension……

Biliary disease……

Autoimmune disorder……

Lung disease……

None……

Do you taka any medication?.....

Yes …… No……

Investigation result:

AST ……

ALT……

Appendix II

SPINREACT

CE

GOT (AST)-LQ

GOT (AST)-LQ

NADH. Kinetic UV. IFCC rec.Liquid

Quantitative determination of aspartate aminotransferase GOT (AST)
IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD
Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:

$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Glutamate} + \text{Oxalacetate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+$$

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of AST present in the sample[1].

CLINICAL SIGNIFICANCE
The AST is a cellular enzyme, is found in highest concentration in heart muscle, the cells of the liver, the cells of the skeletal muscle and in smaller amounts in other weaves.
Although an elevated level of AST in the serum is not specific of the hepatic disease, is used mainly to diagnostic and to verify the course of this disease with other enzymes like ALT and ALP.
Also it is used to control the patients after myocardial infarction, in skeletal muscle disease and other[1,4,5].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	TRIS pH 7.8	80 mmol/L
	Lactate dehydrogenase (LDH)	800 U/L
	Malate dehydrogenase (MDH)	600 U/L
	L-Aspartate	200 mmol/L
R 2 Substrate	NADH	0.18 mmol/L
	α-Ketoglutarate	12 mmol/L

PRECAUTIONS
R1: H290-May be corrosive to metals.
Follow the precautionary statements given in MSDS and label of the product.

PREPARATION
All the reagents are ready to use.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.

Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm < 1.00.

ADDITIONAL EQUIPMENT
- SPIN640 / SPIN640Plus Autoanalyzer.
- General laboratory equipment.

SAMPLES
Serum or plasma[1]: Stability 7 days at 2-8°C.

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

BARCODED REAGENTS LOAD MUST BE PRECEDED OF A SPINREACT "DATABASE" COPY INTO THE ANALYZER SOFTWARE. IT IS AVAILABLE UNDER REQUEST TO SPINREACT.

SPIN640 APPLICATION

TEST INFORMATION		**REAGENT VOLUME**	
Nº	**	Vol. R1	240
Test	GOT	Vol. R2	60
Full Name	GOT	Vol. R3	
Standard nº		Vol. R4	
SAMPLE VOLUME		**RESULT SETUP**	
Vol. Sample Stand.	30	Decimal 1	Slope 1
Vol. Sample Increas.		Unit U/L	Inter. 0
Vol. Sample Dec			
REACTION PARAMETERS			
Reac. Type	Kinetic	Direction	Decrease
Pri. Wave.	340	Reagent Blank	0-0
Sec. Wave.		React. Time	50-70

SPIN640Plus APPLICATION

EDIT PARAMETERS			
Test	GOT	No.	**
Full name	GOT	Print name	GOT
Reac. Type	Kinetic	Direction	Derease
Pri. Wave.	340	Sec. Wave.	
Unit	U/L	Decimal	0.1
Reagent Blank	0 - 0	React. Time	57 - 77
Vol. Sample	30 ul	R1	240 ul
Increased		R2	60 ul
Decreased		R3	
Samole blank		R4	

*The Calibration is stable until **35 days.** After this period the Calibration must be performed again in order to obtain good results.*

REFERENCE VALUES[1]

	25°C	30°C	37°C
Men	up to 19 U/L	26 U/L	38 U/L
Women	up to 16 U/L	22 U/L	31 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: From detection limit of 0 U/L to linearity limit of 467 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L and multiply the result by 10.
Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	48,1	159	47,4	156
SD	0,56	0,57	1,42	4,35
CV (%)	1,16	0,36	3,00	2,79

Sensitivity: 1 U/L = 0,00053 ΔA/min.
Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0,99956.
Regression equation: y= 1,042x - 0,342.
The results of the performance characteristics depend on the analyzer used.

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PACKAGING

Ref: MD41264	Cont.	R 1:	4 x 40 mL
		R 2:	2 x 20 mL

MDBEIS46-I 24/04/17

SPINREACT,S.A./S.A.U. Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
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Appendix III

SPINREACT

CE GPT (ALT)

GPT (ALT)

NADH. Cinético UV. IFCC rec.

Determinação quantitativa de alanina aminotransferase GPT (ALT)
IVD
Conservar a 2-8ºC

PRINCÍPIO DO METODO
A alanina aminotransferase (ALT) inicialmente chamada de transaminase glutâmico pirúvica (GPT) cataliza a transferência reversível de um grupo amino da alanina al α-cetoglutarato com formação de glutamato e piruvato. O piruvato produzido é reduzido a lactato na presença de lactato desidrogenase (LDH) e NADH:

$$\text{Alanina} + \alpha\text{-Cetoglutarato} \xrightarrow{\text{ALT}} \text{Glutamato} + \text{Piruvato}$$

$$\text{Piruvato} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactato} + \text{NAD}^+$$

A velocidade de diminuição da concentração de NADH no meio, determinado fotométricamente, é proporcional à concentração catalítica de ALT na amostra testada[1].

SIGNIFICADO CLINICO
A ALT é uma enzima intracelular, que se encontra principalmente nas células do fígado e do rim.
A sua melhor aplicação é no diagnóstico das patologias hepáticas.
São observados niveis elevados em patologias hepáticas como a hepatite, patologias dos músculos e traumatismos.
Quando utilizada em conjunto com a AST ajuda no diagnóstico de enfartes de miocárdio, já que o valor da ALT se mantém dentro dos limites normais e aumenta nos niveis de AST[1,4,5].
O diagnóstico clínico deve ser feito mediante todos os dados clínicos e de laboratório.

REAGENTES

R 1 Tampão	TRIS pH 7,8	100 mmol/L
	L-Alanina	500 mmol/L
R 2 Substrato	NADH	0,18 mmol/L
	Lactato desidrogenase (LDH)	1200 U/L
	α-Cetoglutarato	15 mmol/L

PREPARAÇÃO
Reagente de trabalho (RT):
Ref: 1001170 Dissolver (→) um comprimido de R2 Substrato num frasco de R1 tampão.
Ref: 1001171 Dissolver (→)um comprimido de R2 Substrato em 15 mL de R1.
Ref: 1001172 Dissolver (→)um comprimido de R2 Substrato em 50 mL de R1.
Tapar e misturar suavemente até à dissolução do conteúdo.
Estabilidade: 21 dias a 2-8°C ou 72 horas a temperatura ambiente (15-25ºC).

CONSERVAÇÃO E ESTABILIDADE
Todos os componentes do kit são estáveis, até ao final do prazo de validade indicado no rótulo, quando mantidos nos frascos bem fechados, a 2-8ºC, protegidos da luz e evitando a sua contaminação.
Não usar os comprimidos se eles estiverem fragmentados.
Não usar reagentes após a data indicada.

Indicadores de deterioração dos reagentes:
- Presença de partículas e turvação.
- Absorvância do Branco a 340 nm < 1,00.

MATERIAL ADICIONAL
- Espectrofotómetro ou analisador para leituras a 340 nm.
- Banho termostável a 25ºC, 30ºC ou 37ºC (± 0,1ºC)
- Cuvetes de 1,0 cm de passo de luz.
- Equipamento habitual de laboratorio.

AMOSTRAS
Soro ou plasma[1]. Estabilidade da amostra: 7 dias a 2-8ºC.

PROCEDIMENTO
1. Condições da análise:
   Comprimento de onda: . . . . . .340 nm
   Cuvete:. . . . . .1 cm passo de luz
   Temperatura constante . . . . . 25ºC / 30ºC / 37ºC
2. Ajustar o espectrofotómetro a zero com água destilada ou ar.
3. Pipetar numa cuvete:

RT (mL)	1,0
Amostra (µL)	100

4. Misturar, incubar 1 minuto.
5. Ler a absorvância (A) inicial da amostra, pôr o cronómetro a funcionar e ler a absorvância a cada minuto durante 3 minutos.
6. Calcular a média do aumento da absorvância por minuto (ΔA/min).

CÁLCULOS
ΔA/min x 1750 = U/L de ALT

Unidades: A unidade internacional (UI) é a quantidade de enzima que converte 1 µmol de substrato por minuto, em condições standardizadas. A concentração é expressa em unidades por litro (U/L).

Factores de conversão de temperaturas
Os resultados podem ser transformados a outras temperaturas multiplicando por:

Temperatura de medição	Factor Conversão a		
	25ºC	30ºC	37ºC
25ºC	1,00	1,32	1,82
30ºC	0,76	1,00	1,39
37ºC	0,55	0,72	1,00

CONTROLO DE QUALIDADE
É conveniente analisar juntamente com as amostras, os soros controlo valorizados: SPINTROL H Normal e Patológico (Ref. 1002120 y 1002210).
Se os valores determinados estiverem fora do intervalo de tolerância, verificar o equipamento, os reagentes e o calibrador.
Cada laboratório deve dispor do seu próprio Controlo de Qualidade e estabelecer correcções caso os controles não cumpram com as tolerâncias.

VALORES DE REFERÊNCIA[4,5]

	25ºC	30ºC	37ºC
Homens	Até 22 U/L	29 U/L	40 U/L
Mulheres	Até 18 U/L	22 U/L	32 U/L

Em recém-nascidos normais foram descritos valores de referência até ao dobro do dos adultos, devido à sua imaturidade hepática, valores que normalizam aproximadamente aos três meses.
Estes valores são orientativos. É recomendável que cada laboratório estabeleça os seus próprios valores de referência.

CARACTERÍSTICAS DO METODO
Intervalo de medida: Desde o *limite de detecção* 0 U/L até ao *limite de linearidade* 400 U/L.
Se a concentração da amostra for superior ao limite de linearidade, diluir 1/10 com NaCl 9 g/L e multiplicar o resultado final por 10.
Precisão:

	Intrasérie (n= 20)		Intersérie (n= 20)	
Média (U/L)	42	112	41	111
DP	0,47	0,96	0,79	2,21
CV (%)	1,12	0,85	1,90	1,98

Sensibilidade analítica: 1 U/L = 0,000503 ΔA / min.
Exactidão: Os reagentes de SPINREACT (y) não mostram diferenças sistemáticas significativas quando comparando com outros reagentes comerciais (x).
Os resultados obtidos com 50 amostras foram os seguintes:
Coeficiente de regressão $(r)^2$: 0,9869.
Equação da recta de regressão: y= 1,0589x - 0,6075.
As características do método podem variar segundo o equipamento utilizado.

INTERFERÊNCIAS
Os anticoagulantes de uso corrente como a heparina, EDTA, oxalato ou fluoreto não afectam os resultados. A hemólise interfere com a determinação[1]. Foram descritas várias drogas e outras substâncias que interferem na determinação da ALT[2,3].

NOTAS
SPINREACT dispõe de instruções detalhadas para a aplicação deste reagente em diferentes equipamentos.

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APRESENTAÇÃO

Ref: 1001170	Cont.	R1: 20 x 2 mL ,R2: 20 → 2 mL
Ref: 1001171		R1: 1 x 150 mL, R2: 10 → 15 mL
Ref: 1001172		R1: 10 x 50 mL, R2: 10 → 50 mL

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Appendix IV