

A retrospective cross sectional study to find out the role of biochemical parameters in assessing the severity of SARS Covid 19

ABSTRACT

Background and objective: SARS Covid viruses are a group of viruses which are responsible for the present pandemic. The clinical presentation of this pandemic has been varied from simple flu like symptoms to severe pneumonia. It is also found to affect multiple organ systems in our body. This study was conducted to find out the variations in the biochemical parameters among SARS Covid patients admitted in our hospital. **Materials & methods:** This study was done among 258 SARS Covid patients admitted in Chettinad hospital. Among them 183 were males, 75 were females. Their liver profile, renal profile, cardiac profile, ferritin levels and LDH levels were studied. **Results:** In this study we found a significant difference in the levels of ferritin, LDH, serum albumin, troponin I, CK-MB and BNP levels between patients admitted in ICU and ward. The patients were divided into three groups based on their age as 20-50, 50-80 and more than 80 years. We found out a significant difference in the levels of total protein, serum albumin, ALT, BUN, troponin I and CK –MB values. Regression analysis showed ferritin, LDH and serum albumin to be independent markers in predicting disease severity. **Conclusion:** This study has shown that ferritin, LDH and serum albumin are promising biochemical markers in the diagnosis and prognosis of SARS Covid 19 infection.

Key words: Cardiac profile; Ferritin; Liver profile; Renal profile; SARS Covid; Lactate dehydrogenase¹

INTRODUCTION

SARS COVID viruses are a family of viruses that can cause respiratory and intestinal diseases in animals and humans.¹ These viruses tend to attack the cells of the upper respiratory tract. It can cause moderate to severe illnesses from cold to pneumonia. While most of the Covid-19 cases are found to be mild, some patients also present with respiratory failure, septic shock and /or multi organ failure and death. Corona virus has been declared as pandemic by the World Health Organization (WHO) having led to deaths and hospitalization of millions of people worldwide. The number of SARS Covid-19 cases in India has risen to -more than 269789 cases by July 9th 2020 with more than 21000 deaths according to the data given by the Ministry of Health and Family Welfare, India.

Laboratory diagnostic tests play an important role in the diagnosis, assessment of prognosis, severity and therapeutic monitoring of different diseases among which the role of biochemical blood tests in assessing the severity and prognosis of the present pandemic is valuable.²

According to the centre of disease control and prevention, elderly patients with comorbidities such as hypertension, diabetes and cardiac problems had a higher chance of contracting SARS Covid infection.³ Some studies have shown that SARS Covid infected patients with cardiovascular morbidity had a greater risk of progressing to severe stages.⁴

The target organ for corona virus is lungs and respiratory pathways. But it has been found to affect multiple organs in our body like gastrointestinal tract, cardiac system and can even present atypically in the form of meningitis. The patho physiology behind multi organ involvement may be due to the immune response specific to each organ towards corona virus which in turn point towards the involvement of immunological system in the development of multi organ failure⁵. Recent studies have shown the involvement of liver and that Angiotensin Converting Enzyme - 2 (ACE-2) is the cell entry receptor for SARS Covid-19 virus and this enzyme is expressed in a

higher concentration in the type II alveolar cells of lungs and also in the bile duct cells.^{6,7} These findings suggest that SARS Covid-19 virus can affect the bile ductules and cause abnormal liver function.

Early detection of patients with chances of deterioration should be done at the earliest to prevent mortality from SARS Covid-19 cases. This study will help to give a better understanding into the effects of SARS Covid -19 on the alterations in liver, renal and cardiac functions in our body. If we find out a link between the biochemical parameters and severity of SARS Covid-19 cases, we can help in the better management of SARS Covid -19 patients in the near future. Aim of this study was to analyze the variations in the liver profile, cardiac profile, renal profile, ferritin and LDH among SARS Covid patients.

MATERIALS AND METHODS

The clinical criterion for diagnosis was as per the ICMR guidelines. All previously healthy individuals who were tested positive for SARS Covid-19 virus after examination of SARS-Covid RNA in the nasal swab of the patients by reverse transcriptase polymerase chain reaction by analyzing the E gene and the S gene, in the Microbiology Department of Chettinad Hospital and Research Institute, Kelambakkam will be included in the study.

Study design: This is a single centre retrospective cross sectional study done over a period of three months from July to September 2020

Inclusion criteria: Details of 500 patients between the age group of 20-80 years of both sex who attended Chettinad Hospital and Research Institute from July 2020-September 2020 diagnosed as SARS Covid- 19, were included in the study. Among them 242 subjects were excluded from the study due to incomplete data and presence of other comorbidities.

Exclusion criteria: Patients admitted to our hospital due to diseases other than SARS Covid- 19.

SARS Covid- 19 patients with previous H/O liver diseases, renal diseases, cardiac problems, hematological disorders, HIV infection, pregnant women, patients less than 18 years of age, patients with cancer, were excluded from this study. Institute ethical committee approval was obtained before commencement of the study. The medical records of all patients were collected and examined. The details of biochemical investigations, clinical, drug history and demographic details of the patients were collected. The patients in our hospital were grouped based on their admission in ICU or ward. The allocation into ICU/ward was based on the ICMR treatment guidelines for SARS Covid -19 patients.

Details regarding patients age, gender, previous comorbidities, previous drug history, present clinical profile were collected. Data regarding biochemical investigations like liver profile, lactate dehydrogenase, serum creatinine, blood urea, troponin I, CK-MB and serum ferritin were retrieved from the Hospital Information system. Any value above the reference interval of our laboratory was considered as abnormal. The normal values of total protein was 6.2-8.4g/dl, serum albumin levels were 2.5-3.5g/dl, total bilirubin levels were 0.2-1.2mg/dl and direct bilirubin levels were 0-0.2 mg/dl, AST-0-40 IU/L ALT-0-40 IU/L, ALP-0-85IU/L, GGT-15-35mg/dl, ferritin levels- (Males -23.9-336, females-2,11-306.8ng/ml) ,LDH-230-460U/L, troponin I – (Males- upto19.8pg/ml, females-upto11.6pg/ml) CK-MB-0.6-6.3ng/ml, BNP-0-100pg/ml, creatinine<1.3 , blood urea nitrogen levels were between 7-22mg/dl. Categorical variables were expressed as percentages and continuous variables as mean and standard deviation. Mean +/- SD to be done for all parameters for 258 participants (total protein, albumin, Total bilirubin, direct bilirubin, AST, ALT, alp, GGT, cretinine, BUN, Troponin I, BNP,CK-MB, electrolytes (sodium, potassium). The whole 258 was divided into two groups based on whether they were admitted in ward or ICU. All the parameters were compared between the two groups using student's t test. The 258 subjects were divided again into three groups based on age (20-50)(51-80)(>80). Compare of all the parameters between the three groups were done using ANOVA test. Regression analysis was done to find out the role of ferritin, albumin and LDH individually in assessing the severity of the disease.

RESULTS:

The study group included 258 cases of SARS Covid patients admitted to Chettinad Hospital and research institute. The group included 187 males and 77 females. In this study we found a significant difference in the levels of ferritin, LDH, serum albumin, troponin I, CK-MB and BNP levels among patients admitted in ICU and ward. The patients were divided into three groups based on their age as 20-50,50-80 and more than 80 years. We found out a significant difference in the levels of total protein, serum albumin, ALT, BUN, troponin I and CK –MB values. Regression analysis showed ferritin, LDH and serum albumin to be independent markers in predicting disease severity.

DISCUSSION:

This study was done among 258 SARS Covid patients admitted in Chettinad hospitals. Among them 183 were males and 75 were females. This number shows that males are more affected than their female counter parts. A study done by Dr Kyle Sue shows the effect of hormones on the immunologic responses of males leading to a less robust immune response among males⁸. X chromosomes carry the largest number of immune related genes causing superior immune response in females.⁹ Animal models have shown that the ACE 2receptor which has been proposed to be the entry point of SARS Covid to be more expressed among males.¹⁰ The variables did not show much statistical difference between males and females, except for albumin, BUN creatinine and troponin I. Multiple organ involvement has been found to be increasing the morbidity of SARS Covid 19 patients. Covid 19 pandemic has given way for understanding various biochemical alterations in the liver and cardiac profile of patients suffering from this viral disease. Various studies are being done to understand the correlation between these parameters and the severity of the disease. Ferritin has been considered as a marker for finding out the severity of SARS Covid 19 patients. Ferritin is the storage form of iron in tissues. It has two subunits H & L. The levels of these two subunits vary from tissue to tissue. Among these subunits, H subunit is found to have an inflammatory and immunomodulatory function. Ferritin has been found to increase the levels of inflammatory markers in the blood like interleukins. The increase in the levels of serum ferritin during inflammation may be due to its active secretion from the hepatocytes or by macrophages through a non-classical pathway.^{11,12,13} In our study we found out that the levels of ferritin among SARS Covid patients were higher than the normal reference interval and when compared between the patients admitted in ICU(454.12+/-396.95) and ward(323+/-362.4), the difference between the levels were statistically significant. (pvalue =0.006).(Table 3)Free iron released from ferritin is a pro inflammatory mediator, responsible for alteration in the coagulation profile leading to vascular thrombosis in various organs especially in lungs.Hence iron chelation therapy may be helpful in decreasing the iron induced coagulopathy. This may form the therapeutic basis for iron chelating agents in the treatment of severe SARS Covid infection as evidenced by the increased levels of ferritin in the biochemical profile.[14,15,16]A recent study done among 39SARS Covid 19 patients showed that serum ferritin levels correlated with the severity of the disease.¹⁷ This study shows a significant difference in LDH levels between patients admitted in ICU(402.6+/-199.3) and ward(328.16+/-250.62)(p value 0.008).(Table 3)LDH is an inflammatory marker which is found to be elevated in obstructive pulmonary diseases and interstitial lung diseases. Our study is on par with another study done in Wuhan by Wang D, Hu B, Hu C, et al.¹⁸

Ferritin and LDH levels showed no significant differences among the different age groups of SARS covid patients as shown in (Table2) Ferritin and LDH were independently associated with the disease as shown by the regression analysis. (Figure 2). SARS Covid 19 has been found to affect multiple organs in our body especially liver. Studies have shown Liver profile derangements were found among 50% of patients infected with SARS Covid 19.¹⁹ Studies have found out that SARS Covid 19 virus infect the Angiotensin converting enzyme (ACE) receptor on cholangiocytes leading to its dysfunction and inturn causing widespread systemic inflammatory response.²⁰ We have analyzed the liver profile of all the SARS Covid 19 patients which included Total protein, albumin, Aspartate transaminase(AST),Alanine amino transferase(ALT),Alkaline phosphatase(ALP) and GGT(Gamma glutamyltransferase). In this study, the various liver parameters were found to be mildly elevated than the normal values. Total protein levels and albumin levels were found to have significant difference between different age groups. (Table:3)

Albumin levels were found to have a significant difference between patients admitted in ICU(3.24+/-0.5) than those admitted in ward(3.46+/-0.5)(p value=0.009). Albumin is a negative acute phase protein. Cytokines released during inflammation caused by SARS Covid 19 affect the liver which is the main organ producing all the proteins in our body and result in pre translational modification of gene expression of all acute phase proteins released from liver thereby decreasing the levels of negative acute phase proteins and increasing the levels of positive acute phase proteins like ferritin and CRP.²¹ Liver is the sole source of albumin. Albumin synthesis in liver depends on the amino acids reaching the intestine from the various food intakes, but during infections, the amount of food intake by the patients will be less which in turn leads to a decrease in the synthesis of albumin.²² Albumin levels were found to be independently associated with serum ferritin levels and as the ferritin levels increased, the albumin levels were found to decrease.(Figure 1). This again emphasizes the use of albumin as an independent marker for the prediction of disease severity. ALT levels were found to have a significant difference among patients of different age groups 20-50 years-58.04 ± 63.86, 50-80 years-43 ± 30.51 and >80 years -24.5 ± 6.149(p value=0.02). There was no significant difference in the levels of other liver enzymes among the different age groups and also between those admitted in ICU and ward as was shown by some recent studies^{23,24}. Cardiac parameters like Troponin I, BNP and CK-MB were found to have significant difference between the patients admitted in ICU and ward pointing to the risk of developing cardiac abnormalities among SARS Covid patients. A study done by Lippi G, Pebani M has shown that 8-12% of the SARS Covid 19 patients had severe cardiac abnormalities.²⁵ Another study done by Huang C, Wang Y, Li X, et al has shown that myocardial injury among SARS Covid patients presented as marginal increase in the troponin I levels.²⁶

In this study we assessed the variations in the levels of renal parameters like Blood urea nitrogen and creatinine levels. It was found out that the mean levels of creatinine was 1.84 ± 5.04 and the mean value of BUN was 18.38 ± 17.88 respectively. ANOVA test done among the different age groups showed a significant difference in the levels of BUN among the different age groups (p value =0.0007), but creatinine values did not show much difference between the different age groups. There was no significant difference in the values of BUN and creatinine levels among patients admitted in ward and ICU. Although the exact mechanism of the involvement of kidney in SARS Covid remain unclear, a number of causes like hyperferritinemia, hypercoagulability, hypercomplementemia, damage to the kidney by direct injury to the tubular cells has been proposed²⁷. ACE2 receptors, the source of entry of SARS Covid virus has been found in kidney cells.[28,29] Studies also report the isolation of SARS Covid virus in the blood and urine of patients affected with the disease.³⁰ This study has thrown light on the importance of biochemical parameters in the treatment of SARS Covid patients. Ferritin, serum albumin and LDH levels seem to be promising markers in assessing the severity of SARS Covid patients. Proper monitoring of these parameters can help treating physicians in finding out the progress of the disease condition.

ETHICAL CLEARANCE: Ethical Clearance for this study was got approved from the Institutional Human Ethical Committee (IHEC).

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Tables

Table 1 Shows the descriptive statistics of variables among Covid 19 patients

Variable	N	N Miss	Mean + STD
Total Protein	258	0	6.84 ± 0.63
Albumin	258	0	3.34 ± 0.54
Total_bilirubin	258	0	0.6 ± 0.8
Direct_bilirubin	258	0	0.18 ± 0.17
AST	258	0	48.97 ± 50.76
ALT	258	0	48.37 ± 46.37
ALP	258	0	76.71 ± 31.54
GGT	258	0	63.44 ± 85.48
FERRITIN	258	0	394.25 ± 386.55
LDH	258	0	367.98 ± 227.26
Creatinine	258	0	1.84 ± 5.04
BUN	258	0	18.38 ± 17.88
Trop_I	257	1	289.8 ± 184.54
CK_MB	254	4	4.65 ± 8.7
BNP	258	0	259.29 ± 807.34

*AST-Aspartate transaminase, ALI-Alanine amino transferase, ALP-Alkaline phosphatase, GGT-Gammaglutamyltransferase, BUN-Blood urea nitrogen, CK-MB-creatine kinase, BNP-Brain natriuretic peptide (p value < 0.05 is considered significant)

Table:2 ANOVA analysis to compare the variables between the three groups divided based on age 20-50,51-80 and >80 years.

Total N = 258 Variables	Age_Group (20-50) N = 97		Age_Group (51-80) N = 157		Age_Group (> 80) N = 4		Pval
	N	Mean	N	Mean	N	Mean	
Protein (g/dl)	97	7.02 ± 0.64	157	6.72 ± 0.59	4	6.9 ± 0.5	0.0008
Albumin (g/dl)	97	3.57 ± 0.5	157	3.21 ± 0.52	4	3.25 ± 0.24	<.0001
Total_bilirubin (mg/dl)	97	0.59 ± 0.82	157	0.61 ± 0.8	4	0.48 ± 0.23	0.9457
Direct_bilirubin (mg/dl)	97	0.17 ± 0.19	157	0.19 ± 0.15	4	0.12 ± 0.09	0.5614
AST(IU/L)	97	44.66 ± 33.78	157	51.68 ± 59.3	4	47.5 ± 20.14	0.5652
ALT(IU/L)	97	58.04 ± 63.86	157	43 ± 30.51	4	24.5 ± 6.14	0.0243
ALP(IU/L)	97	73.3 ± 19.63	157	78.92 ± 37.2	4	72.75 ± 16.17	0.3755
GGT(mg/dl)	97	65.02 ± 63.57	157	63.51 ± 97.49	4	22.25 ± 10.14	0.6199
FERRITIN (ng/ml)	96	368.72 ± 426.33	157	411.03 ± 362.25	4	348.5 ± 364.6	0.682
LDH (U/L)	97	345.04 ± 280.04	157	379.14 ± 187.87	4	486.5 ± 193.32	0.2941
Creatinine (mg/dl)	97	1.08 ± 1.52	157	2.3 ± 6.3	4	2.23 ± 2.08	0.1701
BUN(mg/dl)	97	12.79 ± 10.49	157	21.4 ± 20.18	4	35.5 ± 28.34	0.0001
Trop_I(pg/ml)	96	55.36 ± 271.36	157	386.94 ± 2267.66	4	2103.9 ± 3941.93	0.0538
CK_MB (ng/dl)	93	2.46 ± 4.63	157	5.61 ± 9.24	4	17.7 ± 29.42	0.0002
BNP(pg/ml)	97	215.25 ± 790.27	157	282.43 ± 827.43	4	419.25 ± 356.46	0.7517

*AST-Aspartate transaminase,ALT-Alanine amino transferase,ALP-Alkaline phosphatase,GGT-Gammaglutamyltransferase,BUN-Blood urea

nitrogen,CK-MB-creatin kinase,BNP-Brain natriuretic peptide(p value<0.05 is significant)

Table:3 Independent sample t test shows the comparison between variables among patients admitted in ICU and ward

Variable	Mean+/-SD	Mean+/-SD	Pvalue
	Cases admitted in ICU	Cases admitted in ward	
Total protein(g/dl)	6.8+/-0.67	6.8+/-0.6	0.19
Albumin(g/dl)	3.24+/-0.5	3.46+/-0.5	0.009*
Total bilirubin(mg/dl)	0.54+/-0.3	0.67+/-1.13	0.17
Direct bilirubin(mg/dl)	0.19+/-0.13	0.17+/-0.19	0.41
AST(IU/L)	52.6+/-44.6	44.7+/-56.8	0.22
ALT(IU/L)	49.9+/-54.35	46.6+/-35.18	0.57
ALP(mg/dl)	77.26+/-31.9	76.08+/-31.2	0.76
GGT(mg/dl)	64.8+/-54.8	61.8+/-111	0.78
Ferritin(ng/ml)	454.12+/-396.95	323+/-362.4	0.006*
LDH(u/l)	402.6+/-199.3	328.16+/-250.62	0.0084*
Creatinine(mg/dl)	1.63+/-2.7	2.08+/-6.8	0.48
BUN(mg/dl)	20.097+/-19.7	16.4+/-15.3	0.09
Troponin I(pg/ml)	155.84+/-528.2	44.916+/-140.9	0.03*
CK-MB(ng/ml)	5.8+/-10.4	3.3+/-5.9	0.02*
BNP(pg/ml)	357.4+/-996	146.5+/-490	0.036*

*AST-Aspartate transaminase,ALT-Alanine amino transferase,ALP-Alkaline phosphatase,GGT-Gammaglutamyltransferase,BUN-Blood urea nitrogen,CK-MB-creatin kinase,BNP-Brain natriuretic peptide(p value<0.05 is significant)

Table :4 Independent sample T test showing the difference in variables among male and female SARS Covid patients

	SEX	Mean	Std. Deviation	Std. Error Mean	P value
ALB(g/dl)	F	3.342	.4626	.0538	0.019*
	M	3.355	.5679	.0436	
Total bilirubin(mg/dl)	F	.547	.9004	.1047	0.711
	M	.638	.7847	.0602	
Direct bilirubin(mg/dl)	F	.146	.1398	.0162	0.271
	M	.202	.1804	.0138	
AST(IU/L)	F	48.88	45.959	5.343	0.835
	M	49.11	54.363	4.169	
ALT(IU/L)	F	45.36	61.777	7.181	0.698
	M	49.78	38.406	2.946	
ALP(U/L)	F	74.54	23.334	2.713	0.131
	M	78.78	34.794	2.669	
GGT(mg/dl)	F	51.20	56.383	6.554	0.17
	M	69.75	97.434	7.473	
FERRITIN(ng/ml)	F	363.28	405.064	47.088	0.89
	M	396.19	385.631	29.577	
LDH(U/L)	F	389.81	177.561	20.641	0.88
	M	354.71	250.018	19.175	
CREAT(mg/dl)	F	1.224	1.5393	.1789	0.05*
	M	2.123	6.0938	.4674	
BUN (mg/dl)	F	15.21	13.828	1.607	0.018*
	M	19.35	19.133	1.467	
Troponin I(pg/ml)	F	576.651	3232.1630	375.7313	0.04*
	M	183.101	784.7633	60.1886	
CK-MB(ng/ml))	F	4.908	10.6705	1.2404	0.94
	M	4.739	7.9537	.6100	
BNP(pg/ml)	F	207.31	603.943	70.207	0.28
	M	262.52	843.434	64.688	

*AST-Aspartate transaminase,ALT-Alanine amino transferase,ALP-Alkaline phosphatase,GGT-Gammaglutamyltransferase,BUN-Blood urea nitrogen,CK-MB-creatinekinase,BNP-Brain natriuretic peptide(p value<0.05 is significant)

Figure :1 Shows the regression analysis of ferritin and LDH

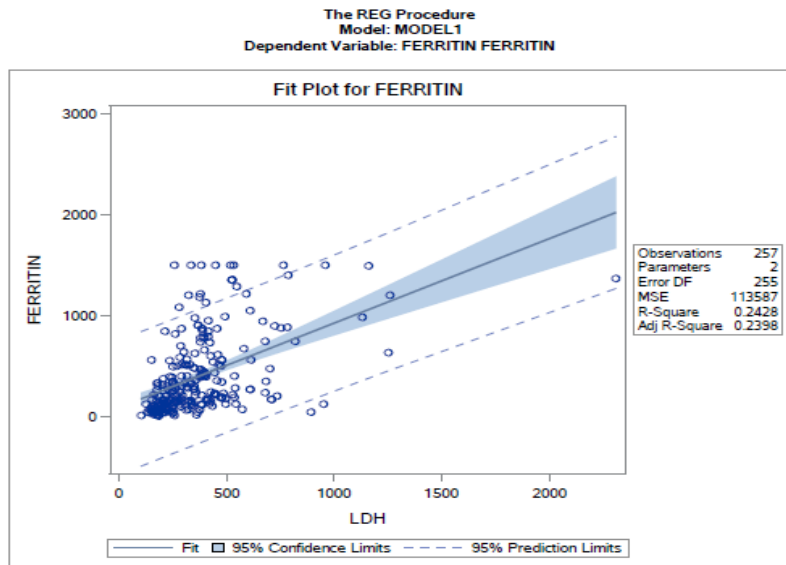
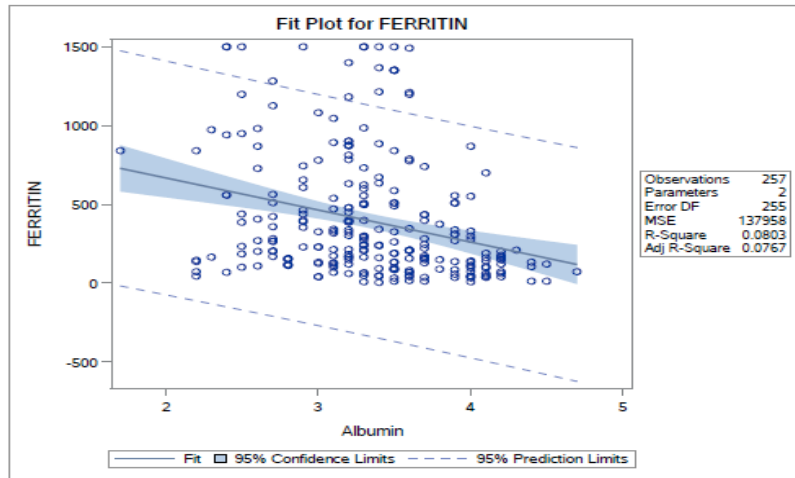


Figure :2 Shows the regression analysis of ferritin and albumin

The REG Procedure
Model: MODEL1
Dependent Variable: FERRITIN FERRITIN



UNDER PEER REVIEW