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BIOCHEMICAL AND INFLAMMATORY BIOMARKERS ABNORMALITY ASSOCIATED WITH MORTALITY IN COVID-19- A RETROSPECTIVE OBSERVATIONAL STUDY IN A TERTIARY CARE CENTRE IN INDIA

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ABSTRACT

Background and objectives: COVID 19 caused by SARS-CoV-2 virus was declared a pandemic by WHO on March 11, 2020. With primary involvement of the respiratory system, it causes ARDS, sepsis, septic shock, metabolic acidosis, coagulopathy, and multi-organ failure in case of severe infection leading to death. Thus comes the role of laboratory analysis of biochemical profile and immune biomarkers in disease management of COVID 19. Method It was a retrospective observational study which was conducted on data of 730 patients who were admitted with COVID 19 in Jayaarogya Hospital Gwalior. Baseline biomarkers were collected which included routine biochemical markers (RFT, LFT and electrolytes) and inflammatory parameters (IL-6, ferritin, Troponin I and procalcitonin). The data was collected and compared between deceased (n=97) and survivors (n=633). **Result** There was a significant increase in urea, creatinine and uric acid and AST in the deceased group along with significant hypoalbuminemia. All four inflammatory markers were significantly increased in the deceased group. Kaplan-Meier survival curves also showed that patients with IL-6, Troponin-I, or procalcitonin higher than the normal values had a significantly higher probability of mortality (log-rank, $P < 0.05$). The multivariate Cox model showed that Troponin-I ($P = 0.028$) could be used as independent factors to predict mortality. **Conclusion** Laboratory analysis of biochemical profile and immune biomarkers in patients infected with SARS-CoV-2 not only plays a role in monitoring treatment for better patient management in COVID 19 but also in prediction of disease severity and mortality.

KEYWORDS: Biochemical profile, COVID 19, inflammatory markers, Mortality.

INTRODUCTION

From December 2019, when a pneumonia-like syndrome was first observed in Wuhan, China,[1] it was attributed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease it caused was named coronavirus disease (COVID-19) by The International Committee on Taxonomy of Viruses.[2] It was declared a pandemic by WHO on March 11, 2020. Since its origin, COVID 19 has rendered the world helpless with current global cases running at 15,83,34,639 causing 32,93,120 deaths. India is facing the second wave of COVID 19, with 2,26,73,240 cases and 2,46,178 deaths till May 2021.[3]

SARS-CoV-2 primarily affects the respiratory system leading to the rapid development of pneumonia.[4] Acute respiratory distress syndrome (ARDS) and multi-organ failure are sequelae in case of severe infection leading to death. Progression to ARDS is seen in 10–15% cases of the COVID-19.[5] And it has been attributed to rapid replication of virus in endothelial and epithelial cells, resulting in the immune system developing significant numbers of proinflammatory cytokines and chemokines termed as cytokine storm.[6] The risk of severity and mortality in covid 19 is directly proportional to age and underlying comorbidities, such as hypertension, diabetes, cardiac disease, chronic lung disease, cancer, and immune-compromised individuals.[7] Severe COVID-19 cases show quick progression to ARDS, sepsis, shock, metabolic acidosis, coagulopathy, and multi-organ failure.[8] A study that analysed the clinical characteristics of deceased coronavirus patients identified sepsis, ARDS, respiratory failure, and heart failure as the most critical complications of the disease.[9] Role of laboratory medicine in COVID 19 pandemic ranges not only in etiological diagnosis and monitoring of disease progression but also in assessing the disease severity and prediction of mortality.[10] Routine biochemical tests like liver function tests (LFT) renal function tests (RFT) and electrolytes assist in disease

monitoring and therapeutic intervention along with emergence of role of immune and inflammatory biomarkers in COVID 19.[11] IL-6, procalcitonin, troponin-I and ferritin are various inflammatory markers which are increased in Covid-19. Interleukin 6 (IL 6), a pro-inflammatory marker, plays an important role in patients with lung damage that is caused by SARS CoV-2.Baseline IL-6 level at the time of admission was found to be a good prognostic indicator for negative outcome of combined endpoint progression to severe disease and/or mortality.[12] Increased Procalcitonin (PCT) levels have been associated with a 5-fold higher risk of evolution towards severe disease.[13] Studies have shown that higher baseline procalcitonin is associated with higher risk of death/ICU admission.[14] Cardiac injury in COVID 19 mandates evaluation of cardiac biomarkers (Troponin I) upon admission in hospital and monitoring for the early detection of cardiac injury in COVID-19 patients.[15]

Thus despite battling COVID 19 on a war like front, there is a considerable dearth of Indian studies elaborating biochemical profile associated with mortality and role of inflammatory biomarkers in predicting mortality. With this in mind, this study was an attempt to analyse biochemical and immune biomarkers in a subset of Indian population in central India.

MATERIAL AND METHOD

It was a retrospective observational study which was conducted on data of 730 patients who were admitted in a tertiary care hospital in Gwalior with COVID 19 during the period of August 2020- December 2020. All patients were confirmed positive for SARS-CoV-2 by nucleic acid RT-PCR (Ct value ≤ 30.0, BGI, Shenzhen, China) using specimens derived from nasopharyngeal swabs or sputum, prior to or during the hospitalization. Baseline biomarkers of the day of admission of the patients were collected which included routine biochemical markers (RFT, LFT and electrolytes) and inflammatory parameters (IL-6, ferritin , Troponin I and procalcitonin). The outcome of the disease ((deceased/survived) along with duration of their stay in hospital was followed. Then the comparative analysis was done between the survivors (n=633) and deceased (n=97).

Biochemical data which was collected included- Renal function tests (Urea - 20-40 mg/dl; Creatinine – 0.5-1.2 mg/dl, Uric acid – 2- 7 mg/dl), Liver function tests (Bilirubin - <1 mg/dl; AST – 6-40 IU/L ; ALT – 6-40 IU/L ; Alkaline phosphatase – 28-111 IU/l ; Total protein – 6.4-8 g/l; Albumin -3.5-5.2 g/l), and Serum electrolytes (Sodium – 135-145 meq/l; Potassium – 3.5-5.5 meq/l). Markers of inflammation included IL 6 (0-7 pg/ml), Ferritin (25-291 ng/ml), Procalcitonin (0-0.05 ng/ml), and Troponin I (0- 0.1 ng/ml).

The data was analysed using SPSS version 25. The tests of significance included T test, Mann Whitney U test, Chi square test. P value of less than 0.05 was taken as significant. Kaplan-Meier curves were constructed for analyzing survival data. A multivariate Cox proportional risk model was used to determine predictive factors for disease risk.

RESULTS

Baseline biochemical profile and inflammatory markers of 730 patients were included which were admitted with COVID 19 during the period of August 2020 –December 2020 in a tertiary care hospital Gwalior. Out of the total cohort of admitted patients in whom laboratory data was available on admission, 97 patients died during hospital stay. Data was categorised in two groups, the deceased group (n=97) and survivor group (n= 633). There was no significant gender difference in 2 groups. Median age in the survivor group was 61.15 (56.52–69.80) years and deceased were 65.50 (59.18–74.53) years, p <0.001. On comparing the biochemical parameters between the two groups it was found that there was significant increase of urea, creatinine and uric acid and AST in the deceased group but there was significant fall of serum albumin in the deceased group. Among the inflammatory markers all the four inflammatory markers (IL-6, Ferritin, Procalcitonin and Troponin I) were significantly increased in the deceased group. (Table 1)

Table I: Outcome status wise description of biochemical markers in patients with COVID 19.

Biochemical profile	Outcome Status	N	Mean	Std. Deviation	P
Urea (mg/dl)	Deceased	97	61.37	42.17	**0.000***
	Survivor	633	44.07	30.87	
Creatinine (mg/dl)	Deceased	97	1.58	1.85	**0.000****
	Survivor	633	0.93	0.81	
Uric acid (mg/dl)	Deceased	97	6.13	3.50	**0.000***
	Survivor	633	4.70	2.26	
Bilirubin (mg/dl)	Deceased	97	0.93	0.86	
	Survivor	633	0.79	0.78	0.150*
AST (IU/L)	Deceased	97	122.56	636.06	**0.036****
	Survivor	633	55.55	47.95	
ALT (IU/L)	Deceased	97	88.22	368.37	0.575**
	Survivor	633	61.33	62.28	
ALP	Deceased	97	138.70	108.22	

(IU/L)	Survivor	633	132.16	79.24	0.569*
Total Protein (g/dl)	Deceased	97	6.312	1.09	
	Survivor	633	6.50	2.24	0.170*
Albumin (g/dl)	Deceased	97	3.36	0.80	
	Survivor	633	3.73	2.12	**0.002***
Sodium (mEq/l)	Deceased	97	137.45	15.44	
	Survivor	633	139.28	11.47	0.264*
Potassium (mEq/l)	Deceased	97	4.30	2.48	0.058*
	Survivor	633	4.04	0.89	
IL-6 (pg/ml)	Deceased	97	325.12	926.90	**0.001****
	Survivor	633	296.26	928.81	
Ferritin (ng/ml)	Deceased	97	1221.18	1002.76	**0.000***
	Survivor	633	857.44	845.97	
Troponin I	Deceased	97	0.78	1.65	**0.000****
	Survivor	633	0.32	0.94	
Procalcitonin (ng/ml)	Deceased	97	2.38	9.25	**0.001****
	Survivor	633	1.23	6.50	

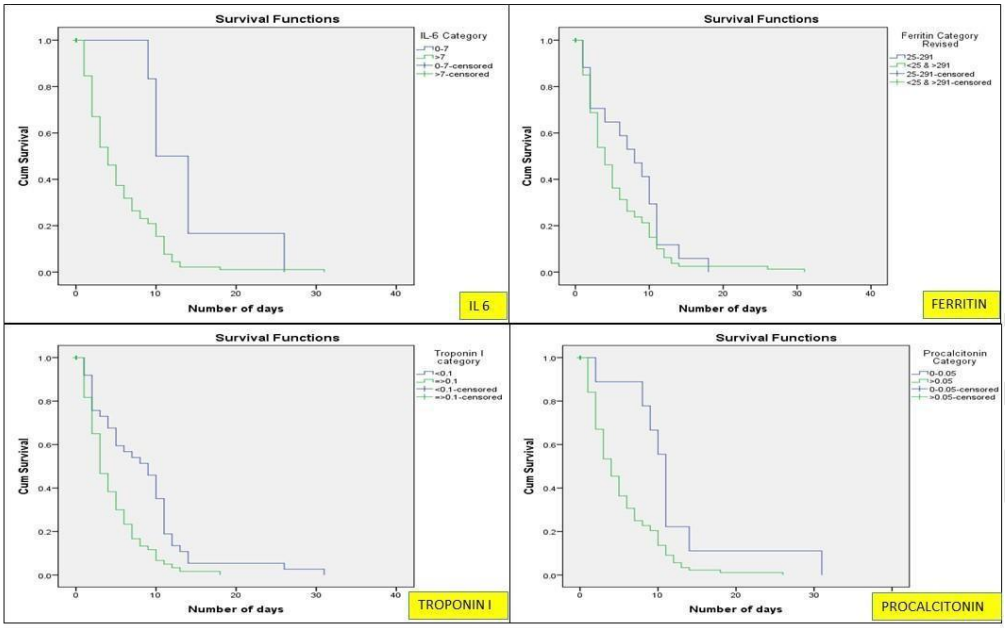
*P values in bold are Significant. *t-test; **Mann-Whitney U test. ALT= alanine tranaminases, AST aspartate tranaminases, ALP alkaline phosphatase IL-6 interleukin-6*

Estimated time until death was 5 days for males and 3 days for females following admission due to COVID19 (log-rank, p= 0.270). On comparing the number of days survived in deceased patients with respect to inflammatory biomarkers, it was found that number of days survived was significantly less if the patients had higher value of Il-6, troponin and procalcitonin at the time of admission. (Table 2) Kaplan-Meier survival curves also showed that patients with IL-6, Troponin-I, or PCT higher than the normal values had a significantly higher probability of mortality (log-rank, P < 0.05). (Figure 1)

Table II: Comparison of number of days (medians) survived according to variables among Deceased Patients.

Variables	Total N=97	Number of days survived		Log Rank	p
		Median	S.E.		
Gender					
Male	66	5.000	0.669	1.218	0.270
Female	31	3.000	1.192		
IL-6					
0-7	06	10.000	1.531	7.433	**0.006***
>7	91	4.000	0.528		
Ferritin					
25-291	17	8.000	2.058	1.725	0.189
>291	80	4.000	0.515		
Troponin					
<0.1	37	9.000	1.516	13.152	**0.000***
=>0.1	60	3.000	0.483		
Procalcitonin					
0-0.05	09	11.000	0.416	7.580	**0.006***
>0.05	88	4.000	0.519		

***Significant**



The multivariate Cox model showed that Troponin-I (P = 0.028) could be used as independent factors to predict the mortality in COVID19 whereas IL-6 (P =0.171), Ferritin (P = 0.379), and PCT (P=0.179) were not found to be significant predictors. (Table 3)

Table III: Multivariate Cox model analysis of inflammatory biomarkers.

Variables	Exp(B)	Sig.	95.0% CI for Exp(B)	
			Lower	Upper
IL-6	**1.900**	**.171**	.758	4.760
Ferritin	**1.286**	**.379**	.734	2.253
Troponin-I	**1.708**	**.028***	1.061	2.749
Procalcitonin	1.719	**.179**	.789	3.785

**Significant*

DISCUSSION

The second wave of covid -19 is already going on in India with a looming fear of an impending third wave in the later part of the year. Although there are a large number of studies on laboratory data, combined studies on biochemical and inflammatory markers predicting mortality in a large population of India are not there. Therefore, a need has arisen to make informed decisions in COVID 19 management. This study was aimed to compile a comprehensive biochemical and inflammatory profile in a substantial cohort of COVID 19 patients from Indian population.

In our study (which is based on Indian population) it was found that the median age of the deceased was 65.50 years (59.18–74.53 years) which was lower than Danish (77.5 years) British (81 years) and Chinese studies,[14,16,17] but was similar to study of Asghar et al (63 years).[18] In an Indian study by Bairwa M et al, the deceased group had a median age of 55 years, which was significantly higher than the survivors' group (P < 0.05), similar to our results.[19] The reason for this may be that the older population have more comorbidities, limited organ function, reduced lung capacity, impaired immune system and biological aging.[20]

Our results indicated significantly deranged baseline renal function markers i.e. urea, creatinine and uric acid in deceased patients as compared to survivors, similarly reported for urea in the Danish cohort study by Hodges G et al.[14] with an additional data of creatinine in the study of Asghar MS et al.[18] We found significant hyperuricemia in deceased patients as reported by Zheng T et al thus supporting their theory that in view of increased biomarkers of tissue damage such as LDH, AST, ALT, and ferritin in severely ill or deceased COVID-19 patients, hyperuricemia can be attributed to tissue damage and cell death and uric acid lowering therapy may be beneficial in COVID-19 patients with hyperuricemia.[21]

Amongst the liver function tests, at the time of admission, AST was found to be significantly raised in deceased groups as compared to survivors. Previous studies have noted the same.[14,22] In a Chinese cohort, Feng lei et al noted a dramatic elevation of AST on admissions followed by an increase in ALT. and a positive correlation of raised AST with markers of disease severity (Neutrophil: Lymphocyte ratio), thus theorizing that liver impairment in severe COVID 19 can be attributed to immune mediated inflammation.[22] This

sets SARS-CoV-2 apart from other viruses, such as hepatitis B virus, in which ALT elevation is the primary manifestation of liver injury.[23]

Similar to our results, Hypoalbuminemia has previously been reported in severe COVID 19,[24] and has come out as an independent predictor of mortality.[25] It can be attributed to decreased hepatic synthesis.[26] and/or albumin extravasation due to increased capillary permeability in immune mediated inflammation by SARS-CoV-2.[27]

Amongst the immune markers that we tested, all of them i.e. IL 6, Ferritin, PCT andTroponin I were significantly raised in deceased group on admission as compared to survivors, supported by results of other studies as well.[4,14] In a multivariate analysis by Gadhiya KP et al raised Ferritin, PCT and Troponin I were independent risk factors for in-hospital mortality.[28] Our results showed significance of Troponin I in prediction of mortality, however, the role of Ferritin and PCT was not found to be significant, probably due to a smaller deceased cohort in our study. Although, the role of raised IL 6 in cytokine storm in pathophysiology of COVID 19 has already been established and it has come out an adequate predictor of severity in COVID-19.[29,30] Isolated increased IL-6 levels failed to predict mortality in a meta analysis by Liu X et al,[30] corroborating with our results. This can be attributed to fluctuating IL 6 Ievels throughout the day, variable intensity of Il 6 response to infection in different individuals and obesity.[30]

Our study has some limitations pertaining to availability of only baseline laboratory data of hospitalised patients of COVID 19 and non availability of comprehensive clinical confounding factors in analysis of biochemical and inflammatory markers. Further studies are required assessing serial and timely estimation of biochemical and inflammatory markers during hospital stay of COVID 19 patients with the endpoint (discharge/death) taken into consideration.

CONCLUSION

Multiple waves of COVID 19 pandemic raging through the world for more than a year, mandates a comprehensive study of multiorgan involvement by the disease. Laboratory analysis of biochemical profile and immune biomarkers in patients infected with SARS-CoV-2 not only plays a role in monitoring treatment for better patient management in COVID 19 but also in prediction of disease severity and mortality.

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