ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Retrospective Cross Sectional Analysis of Biochemical Profile of COVID-19 Confirmed and Suspected Patients in a Tertiary care Hospital in North India.

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ABSTRACT

Biochemical parameters are altered in COVID patients due to the disease pathology and treatment. We conducted this retrospective cross sectional study to estimate the prevalence of abnormalities and analyze the profile of biochemical parameters in COVID confirmed and suspected patients of our tertiary care hospital during the period 1st April 2020 to 31st March 2022. We analyzed the data with IBM-SPSS software version 20.0, using descriptive statistical tools (Mean, frequency, percentage) and Pearson's Chi-square test for any association of age and gender with the test results considering $P < 0.05$ significant at 95% confidence interval. Out of 1445 patients who had undergone the relevant investigations (13928), there were 5720 abnormal reports (41.07%). Of them, 888 (61.45%) were males and 557 (38.55%) females. Mean age of the patients overall was 49.79 years. Maximum patients (40.35%) were in the 41-60 years age group and 46.16% had age between 41-80 years. D-dimer was increased in 69.85% patients, C-reactive protein (CRP) in 86.94%, serum ferritin in 84.54%, blood urea in 39.02%, serum creatinine in 11.29%, serum total and direct bilirubin in 16.67% and 14.71% respectively, alanine aminotransferase (ALT) in 64.34%, aspartate aminotransferase (AST) in 63.84% and alkaline phosphatase (ALP) in 30.53%, whereas total serum protein and serum albumin were decreased in 35.86% and 30.23% respectively. There was a significant association between D-dimer, serum Ferritin (in females only), CRP, serum creatinine, ALT and ALP with age and CRP, ALT, ALP with male and serum Ferritin with female gender. Biochemical markers of coagulation, inflammation, renal and hepatic function were altered in the patients.
Keywords: COVID-19, D-dimer, CRP, Ferritin, Renal function test, Hepatic function test

https://doi.org/10.33887/rjpbcs/2023.14.4.8

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ISSN: 0975-8585

INTRODUCTION

The COVID-19 pandemic has posed many challenges in management of the patients. COVID-19 is a multisystem disease involving a complex interplay of immunological, inflammatory and coagulative cascades. Clinical laboratories have been a source of support for timely diagnosis and hospitalization, risk stratification, effective utilization of intensive care services, selection of appropriate therapies, monitoring and timely discharge of the patients. Laboratory markers or biomarkers can provide additional, objective information which can significantly impact these components of patient care [1].

Inflammatory and coagulation markers, hepatic and renal parameters such as D-dimer, C-reactive protein (CRP), serum ferritin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine have been found to be altered during the course of infection with COVID-19 [2] although none of them are specific for COVID-19 infection only.

Alterations in biochemical parameters in COVID patients are often consistent with severity of infection and levels of these markers are seen more in critical than non-critical patients and in COVID non-survivors rather than in survivors [2-20]. According to Gemicioglu *et al*, high blood urea nitrogen/Albumin ratio (BAR) and neutrophil/albumin ratios may be a better predictor of severity of COVID-19 than other routinely used parameters tested on admission [21]. Ghosh *et al*, Zemlin *et al*, Pitamberwale *et al*, Rostam SRK *et al*, however found no significant difference in most of the biochemical parameters between severe and non-severe patients [22-25].

D-dimers are produced when plasmin cleaves the fibrin to dissolve clots. [26] As such, D-dimer level in blood is measured as an indicator of pulmonary and/or venous thromboembolism, arterial thrombosis and disseminated intravascular coagulation [27].

C-reactive protein (CRP) is an acute inflammatory protein that increases up to 1,000-fold at sites of infection or inflammation [28].

Ferritin is a key acute-phase reactant and hyperferritinemia may be a biomarker of uncontrolled inflammation [29].

The most commonly used endogenous marker for the assessment of glomerular function is creatinine. Serum creatinine is a more accurate assessment of renal function than urea (blood urea nitrogen); however, urea is increased earlier in renal disease. Serum urea levels increase in conditions where renal clearance decreases (in acute and chronic renal failure/impairment) [30].

Serum bilirubin and liver enzymes which are markers of hepatic dysfunction such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) are increased whereas total protein and serum albumin are decreased in hepatic and/or bile duct derangement [31,32].

Hence, we conducted this study to analyze the biochemical profile of confirmed and suspected COVID-19 patients in our tertiary care hospital. Our objectives were:

- To estimate the prevalence of biochemical abnormalities in suspected or confirmed COVID-19 patients
- To analyze the pattern of biochemical abnormalities in suspected or confirmed COVID-19 patients

MATERIALS AND METHODS

Ethical committee permission

We obtained permission of the institutional ethics committee to conduct the study, vide Letter No. MC/IEC/2022-23/06, dated 8.5.2022.

Study design, setting & duration

With the permission of the Medical Superintendent for accessing the data, we conducted a retrospective cross sectional study of the clinical biochemistry laboratory investigation records of our tertiary care hospital from 1st April 2020 to 31st March 2022.

Study sample

We collected the investigation reports of all COVID-19 suspected and confirmed patients which were sent from COVID designated areas of our tertiary care hospital through continuous sampling during the study period.

Operational definitions

Suspected case

A. A person who meets the clinical and epidemiological criteria:

Clinical Criteria

- Acute onset of fever AND cough; OR
- Acute onset of ANY THREE OR MORE of the following signs or symptoms: Fever, cough, general weakness/ fatigue, headache, myalgia, sore throat, coryza, dyspnoea, anorexia/nausea/vomiting, diarrhoea, altered mental status.

Epidemiological Criteria

- Residing or working in an area with high risk of transmission of virus: closed residential settings, humanitarian settings such as camp and camp-like settings for displaced persons; any time within the 14 days prior to symptom onset; or
- Residing or travel to an area with community transmission any time within the 14 days prior to symptom onset; or
- Working in any healthcare setting, including with in health facilities or within the community; any time within the 14 days prior of symptom onset.

B. A patient with severe acute respiratory illness: (SARI: acute respiratory infection with history of fever or measured fever of ≥38 C°; and cough; with onset within the last 10 days; and requires hospitalization)

Thus, a suspected COVID 19 patient was defined by the above mentioned criteria as per Ministry of Health and Family Welfare, Government of India guidelines from time to time but with negative Real Time Reverse transcription - Polymerase chain reaction (RT-PCR) report [33-35].

Probable case

A. A patient who meets clinical criteria above AND is a contact of a probable or confirmed case, or linked to a COVID-19 cluster
B. A suspect case with chest imaging showing findings suggestive of COVID-19 disease
C. A person with recent onset of anosmia (loss of smell) or ageusia (loss of taste) in the absence of any other identified cause.
D. Death, not otherwise explained, in an adult with respiratory distress preceding death AND was a contact of a probable or confirmed case or linked to a COVID-19 cluster [33-35].

Confirmed case

A. A person with a positive Nucleic Acid Amplification Test (NAAT) including RT-PCR or any other similar test approved by ICMR.
B. A person with a positive SARS-CoV-2 Antigen-rapid diagnostic test (RDT) AND meeting either the probable case definition or suspect criteria OR

C. An asymptomatic person with a positive SARS-CoV-2 Antigen-RDT who is a contact of a probable or confirmed case [33-35].

Suspected and probable symptomatic COVID patients were treated in the suspected COVID ward of our hospital as per his/her clinical condition. COVID 19 confirmed patients were treated in the confirmed ward and/or COVID intensive care unit of our hospital.

Study parameters

Apart from estimation of D-dimer, which was done by Neph Plus-4, the investigations such as C-reactive protein (CRP), serum ferritin, blood urea, serum creatinine, total and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), were performed using Randox IMOLA fully automatic clinical Biochemistry analyzer and its test kits with calibrators and controls. Patient samples were analyzed after instrument calibration and acceptable quality control. The biochemical parameters that were investigated along with the reference ranges of our clinical biochemistry laboratory are as shown in Table 1.

Table 1: Investigations performed, methods and their reference ranges

Sl. No	Test	Method	Reference range of our Clinical Biochemistry Laboratory
1	D Dimer, Quantitative	Nephelometry (NEPH PLUS-4)	0.00-0.50 mg/L FEU
2	C-reactive protein (CRP), Quantitative	Immunoturbidimetry	0-5 mg/L
3	Serum Ferritin	Immunoturbidimetry	20-300 ng/ml (Males) 10-120 ng/ml (Females)
4	Blood Urea	Enzymatic kinetic method	10-45 mg/dl
5	Serum Creatinine	Colorimetric method	0.6-1.3 mg/dl
6	Serum bilirubin (total)	Colorimetric method	Up to 1.0 mg/dl
7	Direct bilirubin	Colorimetric method	Up to 0.3 mg/dl
8	ALT (SGPT)	UV method	Up to 37 U/L
9	AST (SGOT)	UV method	Up to 40 U/L
10	Alkaline phosphatase (ALP)	Colorimetric method	30-90 U/L
11	Total serum Protein	Biuret method	6.4-8.3 g/dl
12	Serum Albumin	Bromocresol Green method	3.5 – 5.2 g/dl.

Study procedure

The collected data were entered in a predesigned proforma in MS Excel, including all COVID-19 suspected and confirmed subjects availing patient care services in COVID designated areas of our tertiary care hospital with a continuous sampling strategy during the study period.

Data analysis

Data were recorded and maintained confidentially by investigators and analyzed with IBM-Statistical Package for Social Sciences for Windows (IBM-SPSS software) version 20.0, using descriptive statistical tools (Mean, frequency, percentage) and Pearson's Chi-square test for any association of age and gender with the test results, taking $P < 0.05$ as significant at 95% confidence interval (C.I.)

RESULTS

A total of 1445 patients had undergone one or more of the relevant investigations in the Clinical Biochemistry Laboratory of our tertiary care hospital from 1st April 2020 to 31st March 2022. 5720 out of a total of 13,928 relevant investigations were in the abnormal range (41.07%). Out of 1445 patients, 888 (61.45%) were males and 557 (38.55%) were females. Mean age of the patients overall was 49.79 years, with minimum of 13 and maximum age of 98 years. Maximum patients were in the 41- 60 year age group (40.35%) and 46.16% patients had age between 41-80 years. Age distribution of the patients is shown in Table 2.

ISSN: 0975-8585

Table 2: Age distribution of COVID-19 suspected and confirmed patients

AGE GROUPS (YEARS)				
≤ 18 y	**19-40 y**	**41-60y**	**61-80y**	**≥80y**
11 (0.76%)	435 (30.10%)	583 (40.35%)	84 (5.81%)	40 (2.77%)

Association of abnormal test results of parameters with age and gender is summarized in Table 3. CRP, ALT and ALP abnormalities were significantly ($P < 0.05$) associated with both age and gender, whereas abnormal reports of D-dimer, serum Ferritin (only in females) and serum creatinine were significantly associated with age and those of serum ferritin were significantly associated with female gender.

Table 3: Association of abnormal test results with age and gender using Asymp. Sig. (2-sided) Pearson's Chi-square test. $P < 0.05$ regarded as significant at 95% C.I. (* = Significant and ns = Not significant)

Sl. No	Parameter	**Abnormal test results with Age** Asymp. Sig. (2-sided) Pearson's Chi-square test	**Association of abnormal test results with Gender** Asymp. Sig. (2-sided) Pearson's Chi-square test
1	D-dimer	*0.000	0.386 ns
2	CRP	*0.000	*0.007
3	Serum Ferritin	0.125 ns among males in different age groups *0.008 among females in different age groups	ns *
4	Blood urea	0.170 ns	0.523 ns
5	Serum creatinine	*0.002	0.785 ns
6	Total bilirubin	0.518 ns	0.110 ns
7	Direct bilirubin	0.938 ns	0.677 ns
8	AST	*0.004	*0.042
9	ALT	0.284 ns	0.188 ns
10	ALP	*0.020	*0.024
11	Total protein	0.938 ns	0.956 ns
12	Serum albumin	0.848 ns	0.783 ns

The distribution of patients with abnormal reports of the relevant investigations are given in Table 4.

Table 4: Shows distribution of COVID-19 suspected and confirmed patients with abnormal reports of the relevant investigations

Sl. No	Test	No. of Tests performed	No. of patients with abnormal test results (%)	Minimum and Maximum values of the test results
1	D Dimer, Quantitative	587	410 (69.85%)	0.08, 37.60
2	C-reactive protein (CRP), Quantitative	804	699 (86.94%)	0.39, 580
3	Serum Ferritin	679	574 (84.54%)	12.20, 5000
4	Blood Urea	1330	519 (39.02%)	0.30, 396
5	Serum Creatinine	1329	150 (11.29%)	0.30, 19.50
6	Serum bilirubin (total)	1320	220 (16.67%)	0.00, 108
7	Direct bilirubin	1319	194 (14.71%)	0.10, 600
8	ALT (SGPT)	1318	848 (64.34%)	0.10, 630
9	AST (SGOT)	1319	842 (63.84%)	1.20, 1110
10	Alkaline phosphatase (ALP)	1294	395 (30.53%)	0.00, 622
11	Total serum Protein	1319	473 (35.86%)	0.20, 68
12	Serum Albumin	1310	396 (30.23%)	1.40, 63.60
		13928	5720 (41.07%)	

DISCUSSION

We conducted a retrospective cross sectional analysis of the biochemical profile of COVID-19 confirmed and suspected patients from the clinical biochemistry laboratory records of our tertiary care hospital from 1st April 2020 to 31st March 2022. We estimated the prevalence and profile of biochemical abnormalities (markers of inflammation, coagulation, renal and hepatic function) in the patients.

41.07% of the reports of various biochemical parameters in the studied population were in the abnormal range which is high. There were more males (61.45%) than females (38.55%) among the COVID confirmed and suspected patients, comparable to previous reports. [4,5,8-12,14,15,18,20,22,24] whereas other researchers found female preponderance [12,23] Mean age of the patients overall was 49.79 years. Most patients were in the 41-60 year age group (40.35%) and 46.16% had age between 41-80 years, which is comparable to the results of some researchers. [5,6,8-10,12,14,15,17-20,23,24] but different from results of other researchers who observed most patients in 21-40 year age group. [4,22] Variations were observed in abnormal reports of CRP, ALT, ALP, D-dimer, serum Ferritin according to age and/or gender, which is in contrast to the findings of Sarhan, *et al*, Rostam SRK, *et al* [4,25].

We observed alterations in the levels of all biochemical parameters in the studied population.

In our study, D-dimer levels were increased in the COVID confirmed and suspected patients. Similar observations were reported by other researchers in their COVID patients [2-7, 11,13,14,16,19,20, 22,25,36]. D-dimer elevation indicates the state of hyperfibrinolysis state and increased inflammatory burden induced by SARS-COV-2 infection [26] However, several conditions may cause patients to have raised D-dimer in the absence of pulmonary embolism, deep vein thrombosis or disseminated intravascular coagulation such as pregnancy, cigarette smoking, malignancy, trauma, infection, sepsis, inflammation, chronic liver diseases and vasculitis. Furthermore, elderly patients, immobilized patients, autoimmune disorder patients or those who have had recent surgery may have an elevated D-dimer [26,27]. In contrast to our study, Pitamberwale, *et al*, Shah SSTH, *et al*, Huang D *et al* observed no increase in D-dimer in their studies on COVID-19 patients [17,24,37].

We observed increased CRP levels, which were also reported by other researchers who studied CRP levels in COVID-19 patients like Bairwa, *et al*, Ciaccio *et al*, Sarhan *et al*, Rostam *et al*, Huang CY *et al*, Bilgir *et al*, Qaddoori *et al*, Ghweil *et al,* Zlojutro, *et al,* Pore *et al,* Layla *et al*, Lashmar *et al*, Kesari *et al*, Kantri *et al*, Bats M-L, *et al*, Al Mashhadani, *et al,* Shah SSTH, *et al*, Da Silva DAA, *et al*, Dubey *et al.* Zemlin *et al,* Letelier *et al*, Azar *et al*, Banerjee *et al,* Anani *et al* [2-18,20,23,36,38-40]. CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. Baseline CRP levels can be altered by age, gender, smoking status, weight, lipid levels and blood pressure apart from infections and inflammation. CRP plays important roles in inflammatory processes and host responses to infection including the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, and the production of cytokines, particularly interleukin-6 and tumor necrosis factor-α. As an acute-phase protein, the plasma concentration of CRP deviates by at least 25% during inflammatory disorders [28]. CRP levels are also raised in bacterial and viral infections. [26] CRP can trigger a cellular response storm in COVID-19 pneumonia, which is associated with higher mortality. [39] Ghosh *et al*, Rostam SRK *et al*, Huang D *et al* [22,25,37] however did not observe raised CRP in their study population of COVID patients [20,25,37]. In a study on clinical and biochemical parameters for defining safe criteria for discharge of COVID-19 patients, falling CRP levels from admission to discharge was considered a good prognostic marker [12].

Serum ferritin was increased in our study, which was also observed in COVID patients by Bairwa, *et al*, Rostam SRK *et al*, Huang CY *et al*, Bilgir *et al*, Qaddoori *et al*, Ghweil *et al*, Pore *et al*, Layla *et al*, Kesari *et al*, Kantri *et al*, Bats M-L, *et al*, Al Mashhadani, *et al,* Shah SSTH, *et al*, Dubey *et al,* Ghosh *et al*, Niraula *et al*, Banerjee, *et al,* Letelier *et al*, Anani *et al* [2,5-8,10,11,13-17,20,22,26,36,38,40]. Ferritin is an intracellular protein for iron storage and plays an integral role in iron metabolism [42]. Hepatocytes, Kupffer cells, proximal tubular renal cells and macrophages secrete ferritin. Increased ferritin level is seen in bacterial and/or viral infections, hemochromatosis and long-term transfusion [26]. During infection, increased ferritin levels represent an important host defense mechanism that deprives bacterial growth of iron and protects immune cell function, although some authorities implicate its role as pro-inflammatory [29].

Increased ferritin during infection is probably due to release of iron in the reticuloendothelial system, decrease in ability to transport ferritin in the liver and spleen and increased synthesis and release

of intracellular ferritin [43]. Although elevated ferritin level is observed more in bacterial than viral infection, it might indicate severe secondary bacterial infection in COVID patients. Moreover, higher serum ferritin level in COVID-19 is associated with severe disease manifestations like ARDS, multi-organ failure and death. [26] However, Zemlin *et al,* Pitamberwale, *et al* and Huang D *et al* observed no increase in serum ferritin in their COVID patients in contrast to our study findings [23,24,37].

CRP, ferritin and D-dimer could be elevated in response to the release of proinflammatory cytokine interleukin-6 (IL-6) during SARS-CoV-2 infection. The increase in the serum ferritin levels following bacterial or viral infection could be attributed to iron release into the reticuloendothelial system, and increased intracellular ferritin synthesis and release, with the decreased ability of ferritin transport into spleen and liver [5].

Markers of kidney function like blood urea and serum creatinine (renal function tests) were raised in our study, which indicate renal damage. Bairwa *et al*, Bilgir *et al*, Pitamberwale, *et al*, Pore *et al*, Da Silva DAA, *et al*, [2,6,10,18] reported increase in both blood urea and serum creatinine in COVID patients, whereas Qaddoori *et al*, Dubey *et al,* Gemcioglu *et al* and Anani *et al* [7,20,21,40] reported increase in blood urea in COVID-19 patients and Layla *et al*, Rostam SRK *et al*, reported increase in serum creatinine alone [11,25]. In the kidneys, angiotensin converting enzyme 2 (ACE-2) receptor (where the COVID virus binds) is highly expressed on the brush border of the proximal tubular cells and to a lesser extent in the podocytes. Renal disease in COVID-19 patients can present as acute renal injury, hematuria or proteinuria. It is unclear whether acute renal injury is due largely to hemodynamic changes and cytokine release or the direct toxicity of the virus. Sometimes it may also be due to use of nephrotoxic drugs. Pathological features observed include severe acute tubular necrosis and lymphocyte infiltration [38]. Organ cross-talk (e.g., cardiomyopathy and acute viral myocarditis can contribute to renal vein congestion, hypotension and renal hypoperfusion) has also been proposed as a mechanism of renal damage. Moreover, circulating inflammatory mediators like CRP could result in renal endothelial dysfunction, microcirculatory derangement and tubular injury. Acute kidney injury has been associated with increased mortality risk. [3] However, prevalence of acute kidney injury in COVID patients is low [26]. Ghosh *et al*, Zemlin *et al,* Huang D *et al* and Letelier *et al* found no increase in blood urea and serum creatinine in their patients in contrast to our observations [22,23,37,38].

Markers of liver function like serum bilirubin (total and direct), AST, ALT and ALP were also raised in our study, whereas total serum protein and serum albumin (hepatic function tests) were decreased. Increase in serum total bilirubin was also reported by Bairwa *et al*, Bilgir *et al*, Pore *et al*, Rostam SRK *et al*, Letelier *et al* [2,6,10,25,38] and rise in direct bilirubin by Bairwa *et al*, Zlojutro, *et al*, Pore *et al* [2,9,10]. On the other hand, Bilgir *et al,* Ghosh *et al*, Zemlin *et al,* Pitamberwale, *et al*, Huang D *et al* [6,22-24,37] did not find any increase in total bilirubin and Da Silva DAA, *et al*, Pitamberwale, *et al* and Huang D *et al* [18.24,37] did not find increase in direct bilirubin contrasting with our findings. Both AST and ALT elevation was reported by Bairwa *et al*, Ciaccio *et al*, Sarhan *et al*, Qaddoori *et al*, Ghweil *et al*, Kantri *et al*, Bats M-L, *et al*, Da Silva DAA, *et al*, Niraula *et al*, Anani *et al*, Marc *et al* [2,3,4,7,8,14,15,18,26,40,44] whereas Bilgir *et al*, Pore *et al*, Pitamberwale, *et al*, Huang D *et al* [6,10,24,37] found AST elevation alone and Rostam SRK *et al*, Letelier *et al* [25,38] found ALT elevation alone. Dubey *et al,* Ghosh *et al*, Letelier *et al*, reported no rise in AST [20,22,38] and Layla *et al*, Dubey *et al*, Ghosh *et al*, Zemlin *et al,* Pitamberwale, *et al*, Huang D *et al* [11,20,22-24,37] reported no rise in ALT in their set of COVID patients in contrast with our observations. ALP was found to be raised in reports by Bairwa *et al*, Niraula *et al* [2,26] but no rise was reported by Bats M-L, *et al,* Ghosh *et al*, Pitamberwale, *et al*, [15,22,24] unlike our study results. Bairwa *et al*, Ghweil *et al*, Pore *et al*, Bats M-L, *et al*, Da Silva DAA, *et al* [2,8,10,15,18] reported fall in total serum protein and serum albumin. Ciaccio *et al*, Pitamberwale, *et al*, Anani *et al* [3,24,40] also reported fall in serum albumin in their patients. Layla *et al*, Gemcioglu *et al*, Ghosh *et al*, Huang D *et al* [11,21,22,37] reported no fall in serum albumin in their patients in contrast to our study results.

Although hepatocytes and bile duct epithelial cells express ACE 2 receptor, no significant altered histopathological features have been detected in such cells from COVID-19 patients. Secondary liver damage due to the administration of hepatotoxic drugs, immune mediated damage from systemic inflammatory response, respiratory distress syndrome-induced hypoxia and multi-organ failure in COVID-19 have been postulated as probable causes [3]. Another postulated mechanism for liver damage in COVID-19 is the direct cytotoxicity due to active viral replication in biliary epithelial cells which express ACE 2. Drug induced liver injury may result from administration of many drugs which can potentially cause hepatotoxicity. However, during treatment with antiviral drugs Lopinavir/Ritonavir, Remdesivir and

Favipiravir in COVID-19 patients, some researchers observed that there was no significant increase in AST, ALT and no evidence of drug-induced liver injury [44]. Patients with elevated liver enzymes at admission or during hospitalization have shown significantly higher odds of progressing to severe COVID-19 [26].

Increased levels of liver dysfunction biomarkers have been associated with severe COVID-19 and a worse prognosis. Multi organ failure can also cause secondary liver damage [3,4] Hypoalbuminemia in critically ill patients is multifactorial and is attributed to increased capillary permeability, decreased protein synthesis, increased turnover, decreased serum albumin total mass, increased volume of distribution and increased expression of vascular endothelial growth factor [1].

Comparison of our study with the reports of other researchers is summarized in Table 5.

Table 5: Comparison of COVID-19 suspected and confirmed patients with abnormal reports of the relevant investigations

Sl. No.	Parameters measured	Reports comparable with our study results	Reports contrasting with our study results
1.	D-dimer	Bairwa, *et al*, Ciaccio *et al*, Sarhan *et al*, Rostam *et al*, Huang *et al*, Bilgir *et al*, Qaddoori *et al*, Ghosh *et al*, Layla *et al*, Kesari et al, Kantri *et al*, Al Mashhadani, *et al*, Banerjee, *et al*, Dubey *et al* [2-7, 11,13,14,16,19,20, 22,25,36]	Pitamberwale, *et al*, Shah SSTH, *et al*, Huang D *et al* [17,24,37]
2.	CRP	Bairwa, *et al*, Ciaccio *et al*, Sarhan *et al*, Rostam *et al*, Zlojutro, *et al*, Huang CY *et al*, Bilgir *et al*, Qaddoori *et al*, Ghweil *et al*, Pore *et al*, Layla *et al*, Lashmar *et al*, Kesari *et al*, Kantri *et al*, Bats M-L, *et al*, Letelier *et al*, Azar *et al*, Al Mashhadani, *et al*, Zemlin *et al*, Shah SSTH, *et al*, Da Silva DAA, *et al*, Anani *et al*, Banerjee, *et al*, Dubey *et al* [2-18,20,23,36,38-40]	Ghosh *et al*, Rostam SRK *et al*, Huang D *et al* [22,25,37]
3.	Serum Ferritin	Bairwa, *et al*, Rostam *et al*, Huang CY *et al*, Bilgir *et al*, Qaddoori *et al*, Ghweil *et al*, Pore *et al*, Ghosh *et al*, Layla *et al*, Kesari *et al*, Letelier *et al*, Kantri *et al*, Bats M-L, *et al*, Al Mashhadani, *et al*, Shah SSTH, *et al*, Niraula *et al*, Anani *et al*, Banerjee, *et al*, Dubey *et al* [2,5-8,10,11,13-17,20,22,26,36,38, 40]	Pitamberwale, *et al*, Huang D *et al*, Zemlin *et al* [23,24,37]
4.	Blood urea	Bairwa *et al*, Pitamberwale, *et al*, Bilgir *et al*, Qaddoori *et al*, Pore *et al*, Gemcioglu *et al*, Da Silva DAA, *et al*, Anani *et al*, Dubey *et al* [2,6,7,10,18,20,21,40]	Ghosh *et al*, Letelier *et al*, Zemlin *et al*, Huang D *et al* [22,23,37,38]
5.	Serum creatinine	Bairwa *et al*, Pitamberwale, *et al*, Rostam *et al*, Bilgir *et al*, Pore *et al*, Layla *et al*, Da Silva DAA, *et al* [2,6,10,11,18,25]	Ghosh *et al*, Letelier *et al*, Zemlin *et al*, Huang D *et al* [22,23,37,38]
6.	Total serum bilirubin	Bairwa *et al*, Rostam *et al*, Bilgir *et al*, Pore *et al*, Letelier *et al* [2,6,10,25,38]	Pitamberwale, *et al*, Ghosh *et al*, Zemlin *et al*, Bilgir *et al*, Huang D *et al* [6,22-24,37]
7.	Direct bilirubin	Bairwa *et al*, Zlojutro, *et al*, Pore *et al* [2,9,10]	Pitamberwale, *et al*, Da Silva DAA, *et al*, Huang D *et al* [18,24,37]
8.	AST	Bairwa *et al*, Ciaccio *et al*, Sarhan *et al*, Pitamberwale, *et al*, Bilgir *et al*, Qaddoori *et al*, Ghweil *et al*, Pore *et al*, Kantri *et al*, Bats M-L, *et al*, Da Silva DAA, *et al*, Niraula *et al*, Anani *et al*, Huang D *et al*, Marc *et al* [2,3,4,6,7,8,10,14,15,18,24,26,37,40,44]	Ghosh *et al*, Letelier *et al*, Dubey *et al* [20,22,38]
9.	ALT	Bairwa *et al*, Ciaccio *et al*, Sarhan *et al*, Rostam *et al*, Qaddoori *et al*, Ghweil *et al*, Letelier *et al*, Kantri *et al*, Bats M-L, *et al*, Da Silva DAA, *et al*, Niraula *et al*, Anani *et*	Pitamberwale, *et al*, Ghosh *et al*, Layla *et al*, Zemlin *et al*, Huang D *et al*, Dubey *et al* [11,20,22-24,37]

		al, Marc *et al* [2,3,4,7,8,14,15,18,25,26,38,40,44]	
10.	ALP	Bairwa *et al*, Niraula *et al* [2,26]	Pitamberwale, *et al,* Ghosh *et al,* Bats M-L, *et al* [15,22,24]
11.	Total protein	Bairwa *et al*, Ghweil *et al*, Pore *et al*, Bats M-L, *et al*, Da Silva DAA, *et al* [2,8,10,15,18]	
12.	Serum albumin	Bairwa *et al,* Ciaccio *et al,* Pitamberwale, *et al*, Ghweil *et al,* Pore *et al*, Bats M-L, *et al*, Da Silva DAA, *et al*, Anani *et al* [2,3,8,10,15,18,24,40]	Ghosh *et al*, Layla *et al*, Gemcioglu *et al,* Huang D *et al* [11,21,22,37]

Regarding the correlation or association with disease severity, Oussalah A, *et al* reported a lack of association between the inflammatory markers and the risk of death but there was a significant association between renal dysfunction and the risk of COVID- 19 related acute respiratory failure and death [45]. Wang D, *et al* in their correlation analyses observed that C-reactive protein > 64.79 mg/L, D-dimer > 0.96 µg/mL, albumin < 36 g/L accelerated the progress of COVID-19 to critical stage [46]. Others have also reported the prognostic value of CRP levels in COVID patients [47]. High CRP, D-dimer, serum ferritin, blood urea and low serum albumin were reported as predictors of disease severity by Ranjbar, *et al.* [48] Liver biochemical parameters were strongly correlated with COVID-19 mortality in a meta-analyses by Ye L, *et al* [49].

However, analysis of correlation or association between serum levels of the biomarkers and severity of illness was not done which is a limitation of our study.

Knowledge of alteration in biochemical parameters in COVID 19 patients can be the clinician's guide for timely diagnosis and hospitalization, risk assessment, selection of appropriate treatment options, monitoring the progress of disease, prognosis and response to treatment.

CONCLUSION

A high percentage of biochemical abnormalities were detected in COVID 19 confirmed and suspected patients. Majority of them were middle aged adults and middle age and elderly combined constituted the greatest proportion of patients. There were more males than females in the studied population. Markers of coagulation (D-dimer), inflammation (CRP, serum ferritin), renal (blood urea and serum creatinine) and hepatic function (total and direct bilirubin, AST, ALT, ALP) were raised while other liver function parameters (total protein and serum albumin) were decreased in the study population. There was a significant association between D-dimer, serum Ferritin (in females only), CRP, serum creatinine, ALT and ALP with age and CRP, ALT, ALP with male and serum Ferritin with female gender.

ACKNOWLEDGMENT

We acknowledge the contribution of Dr. Nidhi Nautiyal, Asst. Professor, Statistics for statistical analysis and data entry operator of Medical Research Unit (MRU) of our institute, Mr. Bhanu Pratap for his help in data recording.

REFERENCES

[1] Samprathi M and Jayashree M. Biomarkers in COVID-19: An Up-To-Date Review. Front. Pediatr. 2021;8:607647. doi: 10.3389/fped.2020.607647

[2] Bairwa M, Kumar R, Beniwal K, Kalita D, Bahurupi Y. Haematological profile and biochemical markers of COVID-19 non-survivors: A retrospective analysis. Clin Epi Glob Health 11 (202!) 100770 https://doi.org/10.1016/j.cegh.2021.100770

[3] Ciaccio M, Agnello L. Biochemical biomarkers alterations in Coronavirus Disease 2019 (COVID-19). Diagnosis 2020;7(4):365-72

[4] Sarhan AR, Hussein TA, Flaih MA, Hussein KR. A biochemical analysis of patients with COVID 19 infection. Hindawi Biochemistry Research International Volume 2021, Article ID 1383830, 8 pages https://doi.org/10.1155/2021/1383830

[5] Huang CY, Tsai HW, Liu CY, Liu TH, Huang HL, Chang CC, *et al*. The predictive and prognostic role of hematologic and biochemical parameters in the emergency department among coronavirus disease 2019 patients. Chin J Physiol 2021;64:306-11.

[6] Bilgir F, Çalık S, Demir I, Bilgir O. Roles of certain biochemical and hematological parameters in predicting mortality and ICU admission in COVID-19 patients. Rev Assoc Med Bras 2021;67(Suppl 1):67-73 https://doi.org/10.1590/1806-9282.67.Suppl1.20200788

[7] Qaddoori HT, Hassan A, Hamzah MS, Kalild YS, Mudhafar M. Comparative study of some biochemical and immunological parameters of patients with COVID-19 disease and non-infected people. Egypt. J. Chem. 2022;65(10):217-23

[8] Ghweil AA, Hassan MH, Khodeary A, Mohamed AO, Mohammed AM, Abdelazez1 AA, *et al.* Characteristics, outcomes and indicators of severity for COVID-19 among sample of ESNA Quarantine Hospital's Patients, Egypt: A Retrospective Study. Infection and Drug Resistance 2020;13:2375-83

[9] Zlojutro B, Jandric M, Momcicevic D, Dragic S, Kovacevic T, Vlado Djajic V, *et al*. Dynamic changes in coagulation, hematological and biochemical parameters as predictors of mortality in critically ill COVID–19 patients: A prospective observational study. Clinical Hemorheology and Microcirculation 2023;83:137–48 DOI 10.3233/CH-221583

[10] Pore MM, Padwal MK, Raichurkar AV. Biochemical Markers Associated with COVID-19 Disease Severity in a Tertiary Care Teaching Hospital. Indian J Med Biochem 2021;25(2): 83–7.

[11] Layla KN, Yeasmin S, Khan SA, Chowdhury MU, Azad AB, Mukta FS. Biochemical parameters of mild, moderate & severe COVID-19 patients. World journal of Advanced Research and Reviews 2021;11(2):031-9

[12] Lashmar V, Siddavaram S, D'Cruz LG, Khan AB, Husain SA. Using Clinical and Biochemical Parameters for Safer discharges in COVID-19: A Comparative Study. J Health Care and Research. 2021 Mar 29;2(1):33-45

[13] Kesari M, Patil YV, Agrawal SD, Kesari HV, Gadge PV, Patil LY, Rehman H. Biochemical and haematological parameters predicting severity of Covid 19 infection: Lessons from first wave of pandemic. Indian J Pathol Oncol 2021;8(3):327-33.

[14] Kantri A, Ziati J, Khalis M, Haoudar A, El Aidaoui K, Daoudi Y, et al. (2021) Hematological and biochemical abnormalities associated with severe forms of COVID-19: A retrospective single-center study from Morocco. PLoS ONE 16(2): e0246295. https://doi.org/10.1371/journal.pone.0246295

[15] Bats M-L, Rucheton B, Fleur T, Orieux A, Chemin C, Rubin S, et al. (2021) Covichem: A biochemical severity risk score of COVID-19 upon hospital admission. PLoS ONE 16(5): e0250956. https://doi.org/10.1371/journal.pone.0250956

[16] Al Mashhadani, MA, Al Samarrai, OR. Estimation of Cxcl10 with some biochemical parameters in Covid-19 patients. Journal of Pharmaceutical Negative results 2022;13 (8):2905-11

[17] Shah SSTH, Naeem I, Wahid B. Analyzing correlation of clinical severity of COVID-19 with other biochemical parameters: A retrospective study from Pakistan. Tohoku J. Exp. Med. 2021; 255: 315-23.

[18] Da Silva DAA, e Silva LEDA, Desiderio CS, Farnesi-de-Assunção TS, Oliveira ACDM, Trevisan RO, *et al.* Relation between hematological and biochemical parameters per days of symptoms in hospitalized patients with flu-like syndrome and COVID-19. Research, Society and Development 2022; 11(4):e54411427439 DOI: http://dx.doi.org/10.33448/rsd-v11i4.27439

[19] Yao Y, Cao J, Wang Q, Shi Q, Liu K, Luo Z, *et al*. Journal of Intensive Care 2020;8:49 https://doi.org/10.1186/s40560-020-00466-z

[20] Dubey DB, Mishra S, Reddy HD, Rizvi A, Ali W. Hematological and serum biochemistry parameters as a prognostic indicator of severally ill versus mild Covid-19 patients: A study from tertiary hospital in North India. Clinical Epidemiology and Global Health 2021;12:100806 https://doi.org/10.1016/j.cegh.2021.100806

[21] Gemcioglu E, Davutoglu M, Catalbas R, Karabuga B, Kaptan E, Aypak A, *et al*. Predictive values of biochemical markers as early indicators for severe COVID-19 cases in admission. Future Virol. 2021;16(5):353-67

[22] Ghosh T, Dwivedi T, Agarwal H, Iyer H, Tiwari P, Mittal S, *et al*. Impact of various hematological and biochemical parameters on mortality in coronavirus disease 2019 (COVID-19): A single-center study from North India. Lung India 2022;39:230-3.

[23] Zemlin AE, Allwood B, Erasmus RT, Matsha TE, Chapanduka ZC, Jalavu TP, *et al*. Prognostic value of biochemical parameters among severe COVID-19 patients admitted to an intensive care unit of a tertiary hospital in South Africa. IJID Regions 2022;2:191-7

[24] Pitamberwale A, Mahmood T, Ansari A, Ansari SA, Limgaokar K, Singh L, *et al*. (August 30, 2022) Biochemical Parameters as Prognostic Markers in Severely Ill COVID-19 Patients. Cureus 14(8): e28594. DOI 10.7759/cureus.28594

[25] Rostam SRK, Shekhany KAM, Smail HO. Comparative study of some biochemical parameters among COVID-19 symptoms and non COVID-19 symptoms individuals. Biovalentia: Biological Research Journal 2020;6(2):9-14

[26] Niraula A, Baral N, Lamsal M, Bataju M, Thapa S. Potential role of biochemical markers in the prognosis of COVID-19 patients. SAGE Open Medicine 2022;10:1-10 ps://doi.org/10.1177/20503121221108613

[27] Bounds EJ, Kok SJ. D Dimer. [Updated 2022 Jul 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK431064/

[28] Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front Immunol. 2018 13;9:754. doi: 10.3389/fimmu.2018.00754. PMID: 29706967; PMCID: PMC5908901

[29] Kernan KF, Carcillo JA. Hyperferritinemia and inflammation. Int Immunol. 2017;29(9):401-9. doi: 10.1093/intimm/dxx031. PMID: 28541437; PMCID: PMC5890889.

[30] Gounden V, Bhatt H, Jialal I. Renal Function Tests. [Updated 2022 Jul 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507821/

[31] Kim, WR., Flamm, SL., Di Bisceglie, AM., and Bodenheimer, HC. Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease, Hepatology 2008;47(4):1363-70. doi: 10.1002/hep.22109. PMID: 18366115

[32] Giannini, EG., Testa, R., and Savarino, V. Liver enzyme alteration: a guide for clinicians, Canadian Medical Association Journal 2005;172(3):367-79

[33] Government of India. Ministry of Health and Family Welfare. Clinical management protocol for COVID-19 (in adults) Version 6 24.05.21. Available from https://www.mohfw.gov.in/pdf/UpdatedDetailedClinicalManagementProtocolforCOVID19adults dated24052021.pdf Accessed June 9 2023.

[34] National centre for disease control. DGHS, Ministry of Health and Family Welfare, Govt. of India. Case definition. Available from https://ncdc.mohfw.gov.in/showfile.php?lid=461 Accessed June 10 2023

[35] National centre for disease control. DGHS, Ministry of Health and Family Welfare, Govt. of India. Revised case definition for COVID-19. Available from https://ncdc.mohfw.gov.in/showfile.php?lid=611 Accessed June 10 2023

[36] Banerjee A, Banerjee J, Mazumdar M, Phukan JP. Impact of various hematological and biochemical parameters in severe and nonsevere COVID-19 patients: A retrospective single-center study. Mustansiriya Med J 2022;21:178-83.

[37] Huang D, Yang H, Yu H, Wang T, Chen Z, Yao R. Diagnostic Value of Hematological and Biochemical Parameters Combinations for Predicting Coronavirus Disease 2019 COVID-19) in Suspected Patients. Am J Med Sci 2021;362(4):387–95

[38] Letelier P, Encina N, Morales P, Riffo A, Silva H, Riquelme I, Guzmán N. Role of biochemical markers in the monitoring of covid-19 patients. J Med Biochem 2021;40:115-28 DOI: 10.5937/jomb0-29341

[39] Azar MM, Shin JJ, Kang I, Landry M. Diagnosis of SARS-CoV-2 infection in the setting of the cytokine release syndrome. Expert review of Molecular Diagnostics 2020;20(11): 1087-97 https://doi.org/10.1080/14737159.2020.1830760

[40] Anani M, Amer SAAM, Kishk RM, Hassan A, Hassan S, Attia F. Evaluation of blood and biochemical parameters of COVID-19 patients in Suez Canal University Hospital; A retrospective study. J Infect Dev Ctries 2022; 16(4):592-599. doi:10.3855/jidc.14591

[41] Niraula A, Gelal B, Lamsal M, Karki D, Chhetri R. Biochemical parameters in confirmed Covid-19 patients: A Hospital Based Study from Eastern Nepal. Kathmandu University Med J 2021;76(4):415-9.

[42] Lalueza A, Ayuso B, Arrieta E, et al. Elevation of serum ferritin levels for predicting a poor outcome in hospitalized patients with inflfluenza infection. Clin Microbiol Infect. 2020. doi:10.1016/j. cmi.2020.02.018

[43] Senjo H, Higuchi T, Okada S, Takahashi O. Hyperferritinemia: causes and signifificance in a general hospital. Hematology. 2018;23 (10):817–22. doi:10.1080/10245332.2018.1488569

[44] Marc F, Moldovan C, Hoza A, Restea P, Sachelarie L, Romila, *et al*. Evaluation of Hepatic Biochemical Parameters during Antiviral Treatment in COVID-19 Patients. Biology 2022;11:13. https://doi.org/ 10.3390/biology11010013

[45] Oussalah A, Gleye S, Urmes IC, Laugel E, Barbe F, Orlowski S. The spectrum of biochemical alterations associated with organ dysfunction and inflammatory status and their association with disease outcomes in severe COVID-19: A longitudinal cohort and time-series design study. EClinicalMedicine 2020;27:100554

[46] Wang D, Li R, Wang J, Jiang Q, Gao C, Yao C, *et al*. Correlation analysis between disease severity and clinical and biochemical characteristics of 143 cases of COVID-19 in Wuhan, China: a descriptive study. BMC Infectious Diseases 2020;20:519 https://doi.org/10.1186/s12879-020-05242-w

[47] Ketenci S, Saraçoğlu I , Duranay R, Elgörmüş CS and Aynacıoğlu AS. Retrospective analysis of biochemical markers in COVID-19 intensive care unit patients. The Egyptian Journal of Bronchology 2022;16:27 https://doi.org/10.1186/s43168-022-00129-7

[48] Ranjbar R, Hosseini HM and Dehkordi FS. A Review on Biochemical and Immunological Biomarkers used for Laboratory Diagnosis of SARS-CoV-2 (COVID -19). The Open Microbiology Journal 2020;14:290-6 DOI: 10.2174/1874434602014010290

[49] Ye L, Chen B, Wang Y, Yang Y, Zeng J, Deng G, *et al*. Prognostic value of liver biochemical parameters for COVID-19 mortality. Annals of Hepatology 2021;21:100279 https://doi.org/10.1016/j.aohep.2020.10.007.