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Long-term Clinical and Biochemical Alterations in Mild-to-Moderate COVID-19 Survivors: A Case-Control Study from South India.

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Abstract

Background: COVID-19, caused by SARS-CoV-2, has evolved from a predominantly respiratory illness to a multisystem disease with long-term clinical and biochemical sequelae, now recognized as Post-Acute Sequelae of SARS-CoV-2 infection (PASC) or Long COVID. This study aimed to evaluate the long-term changes in biochemical parameters among individuals who recovered from mild to moderate COVID-19.
Objectives: To evaluate key biochemical parameters in post-COVID-19 patients. To determine the clinical implications across different organ systems.
Methods: A case-control study was conducted from May 2022 to May 2023 at ACSR Government College and Hospital, Nellore. Fifty recovered COVID-19 patients (≥12 weeks post-infection) were compared with 50 age- and gender-matched healthy controls. All participants underwent a comprehensive biochemical assessment including inflammatory, hematological, hepatic, pancreatic, renal, and lipid parameters. Statistical analysis was performed using SPSS v25.0, with significance set at $p \leq 0.05$.
Results: COVID-19 survivors exhibited significantly elevated inflammatory markers (CRP, ESR, D-dimer, PT; $p < 0.001$) and hepatic enzymes (ALT, AST, ALP, GGT; $p < 0.001$). Pancreatic enzymes (amylase, lipase) and renal markers (urea, creatinine, uric acid) were also significantly higher, with reduced eGFR ($p = 0.025$).
The lipid profile showed increased total cholesterol, LDL, VLDL, and triglycerides in survivors compared to controls ($p < 0.001$). Haematological changes included elevated WBC and RBC counts and reduced haematocrit and MCHC.
Conclusion: Recovered COVID-19 patients demonstrated persistent biochemical abnormalities across multiple organ systems even months after infection, despite mild to moderate disease severity. These findings underscore the importance of long-term follow-up and biochemical surveillance to monitor post-COVID complications and guide clinical management strategies.

Keywords: COVID-19, Long COVID, Post-acute sequelae, Biochemical markers, Liver enzymes, Renal function, Dyslipidemia

Introduction

Since its emergence in late 2019, Coronavirus Disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has evolved from an acute respiratory illness to a systemic disease with significant multi-organ involvement. Although most individuals recover from the acute phase within weeks, a considerable proportion experience prolonged symptoms and biochemical abnormalities, a phenomenon now recognized as Post-Acute Sequelae of SARS-CoV-2 infection (PASC) or Long COVID. [1–3] COVID-19 induces a robust inflammatory response, endothelial dysfunction, coagulation abnormalities, and metabolic dysregulation, leading to both acute and chronic organ injury. [4–6]. While initial efforts focused on acute disease management, there is growing recognition of long-term complications affecting the liver, kidneys, endocrine system, and cardiovascular system, often mirrored by persistent abnormalities in clinical biochemistry parameters. [7–9]

Markers such as C-reactive protein (CRP), D-dimer, ferritin, liver transaminases, and renal function indicators have been shown to remain altered for several weeks or months following the resolution of viral infection. Additionally, new-onset

diabetes mellitus, thyroid dysfunction, and sustained hypercoagulability have been reported in several follow-up studies. [10–12]

Understanding the trajectory of these biochemical parameters is critical not only for clinical monitoring but also for anticipating long-term morbidity, guiding follow-up strategies, and informing therapeutic interventions. This study aims to comprehensively analyse the long-term course of key biochemical parameters in post-COVID-19 patients and to highlight their clinical implications across different organ systems.

MATERIALS AND METHODS

Study design: This is a case-control study.
Study setting: Department of Biochemistry at ACSR Government College and Hospital, Nellore, Andhra Pradesh.
Study population: Cases included participants with a history of mild to moderate COVID-19 illness without admission or oxygen therapy were selected to avoid confounding influence of severe illness. Controls included the patient attenders of the same age group and gender who are healthy and without history of COVID-19 or other morbidities.
Study period: May 2022 to May 2023
Sampling technique and sample size calculation: All cases and controls were selected consecutively. A convenient sampling technique was employed for this study. A total of 50 participants who had previously tested positive for SARS-CoV-2 and had completed at least three months post-infection were recruited consecutively from the post-COVID follow-up outpatient clinic as cases.

Inclusion criteria for cases:

1. Age ≥ 18 years.
2. Confirmed diagnosis of COVID-19 based on a positive RT-PCR or antigen test.
3. Mild-moderate disease severity, managed entirely on an outpatient basis (i.e., not requiring hospitalization or oxygen therapy).
4. Recovered from the acute phase of infection and completed at least 12 weeks (3 months) post-diagnosis.
5. Willingness to participate in the study and ability to provide informed consent.
6. Able to undergo routine blood sampling for biochemical analysis during follow-up.

Exclusion criteria for cases:

1. History of severe COVID-19 or any hospitalization for COVID-19.
2. Pre-existing chronic systemic diseases (e.g., chronic liver disease, chronic kidney disease, uncontrolled diabetes, thyroid disorders) that could confound post-COVID biochemical results.
3. Use of systemic corticosteroids, immunosuppressants, or cytotoxic therapy for any indication within 3 months of COVID-19 diagnosis.
4. Pregnancy or lactation at the time of follow-up.
5. Presence of malignancy, autoimmune disease, or other ongoing acute infections.
6. Incomplete clinical records or inability to complete the required biochemical evaluations during the follow-up period.

And 50 controls who gave consent and willing to participate in the study were selected from the patient attenders of same age group and gender. History of COVID-19 and other morbidities were enquired. Only those who were free from it were included in the study.

Method of data collection:
Data was collected through a combination of retrospective chart review and prospective follow-up of patients at the post-COVID outpatient clinic of ACSR Government Hospital. Cases fulfilling the eligibility criteria were contacted via phone or during their routine follow-up visits. And controls, fulfilling the criteria were randomly selected from the patient wards. A structured data collection form was used to record:

- **Demographic details**: age, sex, BMI, comorbidities.
- **COVID-19 illness history**: date of diagnosis, symptom profile, duration of illness, severity (mild/moderate).
- **Treatment history**: medications used during the acute phase.
- **Biochemical Evaluation**

- At least 12 weeks post-COVID diagnosis, all enrolled patients underwent biochemical testing.
- A 5 ml blood sample was collected via venepuncture after an overnight fast of 8–12 hours at the hospital's central laboratory.
- The following biochemical parameters were evaluated:
    - **Inflammatory markers**: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin.
    - **Coagulation markers**: D-dimer, Prothrombin time (PT)
    - **Hematological parameters**: White blood cells (WBCs), Red blood cells (RBCs), Haemoglobin (Hb), Haematocrit (HCT), Mean corpuscular Volume (MCV), Mean corpuscular haemoglobin (MCH), Mean

corpuscular haemoglobin concentration (MCHC), Red blood cell distribution width (RDW), Platelets, Mean platelet volume (MPV).

- **Liver function tests (LFTs)**: Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), Serum bilirubin, Albumin.
- **Pancreatic enzymes**: Amylase, lipase
- **Renal function tests (RFTs)**: Serum creatinine, blood urea, eGFR.
- **Lipid profile**: Total cholesterol, Low-density lipoproteins (LDL), High-density lipoproteins (HDL), Very-low-density lipoproteins (VLDL), Triglycerides (TG)
- **Others**: Vitamin D3, Magnesium, Calcium, Phosphorous

All the data was anonymized and entered into a secure, password-protected electronic database. Laboratory data was cross-checked with hospital records to ensure accuracy. Any abnormal findings were reviewed by a senior biochemist for clinical correlation and interpretation.

Ethics approval:
Approval was obtained from the Institutional Ethics Committee, ACSR Government Medical College, Nellore before commencement of the study. Informed written consent was obtained from all the study participants.

Data analysis:
Data was entered in Microsoft Excel and Analysis was performed using SPSS software (Trial version 25). Descriptive statistical analysis has been carried out in the present study. Categorical variables were represented as proportions/percentages and quantitative variables were represented as mean with standard deviation or median with interquartile range (IQR). Shapiro–Wilk test was performed to determine the normality of distribution of all continuous variables. Mann-Whitney *U* Test was employed for non-normal variables, and independent samples T-Test for normal variables. P values ≤ 0.05 were considered statistically significant.

Results:
A total of 100 participants were included in the study, with 50 COVID-19 survivors and 50 age- and gender-matched healthy controls. The mean age was identical in both groups (33.45 ± 2.37 years), and the sex distribution was also balanced, with males comprising 52% of each group. Among COVID-19 survivors, the period since the negative RT-PCR report ranged from 3 to over 6 months, with 40% presenting at 3–4 months post-recovery, 28% at 4–5 months, 18% at 5–6 months, and 14% beyond 6 months.
Body weight and BMI values were comparable between groups. However, COVID-19 survivors had significantly higher SBP (122.63 ± 3.88 mm Hg) compared to controls (120.55 ± 5.32 mm Hg), with a p-value of 0.027, indicating statistical significance. No significant difference in DBP was observed between the two groups ($p = 0.866$). This suggests that only systolic BP might be transiently affected in the post-COVID state, reflecting persistent cardiovascular or autonomic alterations post-COVID-19, even in mild to moderate outpatient cases.

Regarding the inflammatory markers, ESR and CRP were significantly elevated in COVID-19 survivors ($p < 0.001$), suggesting a persistent low-grade inflammatory response even months after negative RT-PCR. D-dimer levels were also significantly higher ($p = 0.017$), indicating possible subclinical coagulopathy or endothelial dysfunction post-infection. Prothrombin Time (PT) was significantly prolonged in COVID-19 survivors compared to controls ($p = 0.001$), which indicates a persistent alteration in coagulation status, which might reflect subclinical endothelial dysfunction or residual hepatic effects in recovered individuals, even several months post-infection. Ferritin levels were higher but not statistically significant ($p = 0.072$); may still have clinical relevance in long COVID cases.

Haematological Findings showed that WBC and RBC counts were significantly higher in survivors, which could reflect immune system activation or compensatory erythropoiesis. Although Haemoglobin showed no significant difference, haematocrit was lower in cases ($p = 0.008$), possibly reflecting subtle haemodilution or inflammation-associated changes. MCHC was significantly lower ($p = 0.014$), which may point to changes in red cell indices after COVID, even in mild-moderate outpatient cases. No significant difference was noted in platelet count, RDW, or MPV, although platelet counts tended to be higher (approaching significance, $p = 0.062$).

Liver and pancreatic enzymes are significantly higher in COVID-19 survivors, suggesting possible residual inflammation or hepatic/pancreatic injury. Renal function markers (urea, creatinine, uric acid) are also significantly elevated, while eGFR is slightly lower (78.99 ± 20.55 vs 89.71 ± 19.32 ml/min/1.73 m², $p = 0.025$)., indicating mild post-infectious renal impairment. Lipid profile is markedly worse in survivors (↑ TC, LDL, VLDL, TG), which may raise concern for post-COVID metabolic syndrome or increased cardiovascular risk. HDL was lower in survivors, but not significantly (59.32 ± 9.32 vs 64.14 ± 21.05 mg/dL, $p = 0.077$). These findings underscore the need for long-term follow-up in COVID-19 survivors, especially in monitoring hepatic, renal, and metabolic health.

Table 1 Baseline Characteristics of Study Participants with Statistical Comparison

Variable	Cases (n=50)	Controls (n=50)	P value
Age (years)	33.45 ± 2.37	33.45 ± 2.37	1.000
Sex			1.000
Males	26 (52%)	26 (52%)	
Females	24 (48%)	24 (48%)	
Body weight (kgs)	83.51 ± 5.23	80.98 ± 8.26	0.056
Body mass index (BMI)	26.91 ± 3.3	27.65 ± 4.1	0.312
Systolic Blood Pressure (mm Hg)	122.63 ± 3.88	120.55 ± 5.32	**0.027**
Diastolic Blood Pressure (mm Hg)	80.22 ± 6.92	79.94 ± 8.16	0.866
Time Since Negative RT-PCR		-	-
3-4 months	40%		
4-5 months	28%		
5-6 months	18%		
>6 months	14%		

Table 2 Inflammatory and haematological Markers in COVID-19 Survivors and Controls

Variable	Cases (n=50)	Controls (n=50)	P value
ESR (mm/h)	49.32 ± 15.22	13.14 ± 5.65	**< 0.001**
CRP (mg/L)	18.92 (5.4–125.6)	7.5 (4.5–9.3)	**< 0.001**
Ferritin (ng/mL)	199.44 ± 65.38	185.32 ± 22.83	0.072
D-dimer (mg/L)	0.44 ± 0.31	0.31 ± 0.22	**0.017**
Prothrombin Time (seconds)	11.00 ± 0.65	10.00 ± 1.2	**0.001**
WBCs (x 10^9/L)	8.12 ± 0.89	6.53 ± 2.88	**0.001**
RBCs (10^6/µL)	5.93 ± 2.12	4.32 ± 2.64	**0.003**
Haemoglobin (g/dL)	12.55 ± 1.25	12.32 ± 1.95	0.461
Haematocrit (%)	36.22 ± 3.92	38.29 ± 2.31	**0.008**
MCV (fL)	85.29 ± 8.67	83.51 ± 7.45	0.268
MCH (pg)	26.32 ± 4.11	25.29 ± 3.70	0.248
MCHC (g/dL)	33.24 ± 2.02	34.01 ± 1.22	**0.014**
RDW (%)	14.33 ± 3.96	14.22 ± 4.20	0.885
Platelets (10^3/µL)	285.24 ± 163.59	233.15 ± 139.46	0.062
MPV (fL)	8.55 ± 1.65	8.36 ± 1.23	0.456

Table 3: Biochemical Parameters in COVID-19 Survivors vs Controls

Variable	Cases (n=50)	Controls (n=50)	P value
Liver function tests			
ALT (IU/L)	81.65 ± 12.95	34.28 ± 5.04	**<0.001**

AST (IU/L)	66.21 ± 6.98	25.83 ± 8.27	**<0.001**
ALP (IU/L)	116.12 ± 18.74	91.06 ± 9.53	**<0.001**
GGT (IU/L)	66.25 ± 22.93	31.63 ± 13.27	**<0.001**
Bilirubin - Total (mg/dL)	1.2 ± 0.03	0.98 ± 0.11	**0.030**
Albumin (g/dL)	4.15 ± 0.54	3.14 ± 1.09	**<0.001**
Pancreatic enzymes			
Amylase (U/L)	107.32 ± 22.47	82.66 ± 17.29	**< 0.001**
Lipase (U/L)	222.87 ± 97.49	147.51 ± 19.64	**< 0.001**
Kidney function tests			
Blood urea (mg/dL)	21.74 ± 3.87	9.74 ± 5.66	**< 0.001**
Creatinine (mg/dL)	1.35 ± 0.10	1 ± 0.24	**< 0.001**
Uric acid	6.55 ± 1.22	5.08 ± 1.33	**< 0.001**
eGFR (ml/min/1.73 m^2)	78.99 ± 20.55	89.71 ± 19.32	**0.025**
Lipid profile			
Total cholesterol (mg/dL)	212.05 ± 18.69	155.64 ± 31.97	**< 0.001**
LDL (mg/dL)	125.39 ± 25.89	75.87 ± 24.11	**< 0.001**
HDL (mg/dL)	59.32 ± 9.32	64.14 ± 21.05	0.077
VLDL (mg/dL)	31.04 ± 8.46	22.57 ± 2.52	**< 0.001**
Triglycerides (mg/dL)	202.74 ± 56.35	127.84 ± 15.33	**< 0.001**

Discussion

This study demonstrates that even after clinical recovery, individuals who had mild to moderate COVID-19 show significant biochemical disturbances across multiple organ systems—particularly in inflammatory, hepatic, renal, pancreatic, and lipid parameters.

These findings reflect a persistent post-COVID pathophysiological footprint, aligning with the concept of post-acute sequelae of SARS-CoV-2 infection (PASC), or Long COVID. The results align with and build upon previous reports from global and regional cohorts.

COVID-19 survivors in our study had significantly elevated CRP, ESR, D-dimer, and prothrombin time, suggestive of ongoing low-grade inflammation and coagulation activation. This is consistent with Townsend et al., who reported persistently raised D-dimer and CRP in patients several months post-infection [12], and Nalbandian et al., who described systemic inflammation as a key driver of long COVID symptoms. [1] Liver enzymes (ALT, AST, ALP, GGT) were significantly higher in the COVID-19 group, indicating subclinical hepatocellular and cholestatic injury. These results corroborate the findings of Zhou et al. and Yadav et al., who documented liver enzyme abnormalities in recovered COVID-19 patients. [13,14] Importantly, our results are closely aligned with the study by Gameil et al [15], who observed that ESR, CRP, ferritin, D-dimer, ALT, AST, GGT, and ALP were significantly higher in COVID-19 survivors compared to healthy controls, even months after recovery. Like our findings, they also reported persistently elevated pancreatic enzymes (amylase and lipase), and increased renal parameters (blood urea, creatinine, urine ACR), supporting the idea of multisystem residual involvement. A study by Wang et al. found that 17% of COVID-19 patients had pancreatic enzyme abnormalities, attributing this to viral affinity for ACE2 receptors in the pancreas. [16]

Consistent with studies by Bowe et al. [17] and Gameil et al. [15], we found significantly higher urea and creatinine, and lower eGFR in COVID-19 survivors, indicating persistent glomerular stress. The elevation in uric acid may reflect both oxidative stress and renal excretory imbalance, as also noted in the Egyptian cohort studied by Gameil et al. [15] The current study found substantial elevations in total cholesterol, LDL, VLDL, and triglycerides in the post-COVID group. This profile is suggestive of post-infectious dyslipidemia and possible risk of cardiometabolic complications. Our results are

reinforced by findings from Masana et al. [18] and Xie et al. [19], and were similarly reported in Gameil et al.'s [15] study, who identified significant post-COVID lipid disturbances.

Implications

The presence of long-term clinical and biochemical residues in individuals with previously mild or moderate disease, even in outpatient settings, highlights the importance of structured post-COVID surveillance. Routine follow-up of hepatic, renal, and metabolic markers may help in early identification of long COVID-related complications and enable timely intervention.

Strengths and Limitations

This study's strengths include a well-matched control group and comprehensive biochemical profiling. However, limitations include a relatively small sample size, lack of baseline pre-infection labs, and no longitudinal follow-up beyond 6 months. Future studies should include larger cohorts and assess temporal recovery patterns of these abnormalities.

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