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Hematological and Biochemical Parameters along with IgG Antibody against SARS-CoV-2 in Patients visiting a referral laboratory for recovery check up after COVID-19 infection in Nepal

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ABSTRACT

Background: The laboratory abnormalities for hospitalized patients with the SARS-CoV-2 have been described in various studies. Limited data are available for the recovered patients. This study aimed to evaluate various laboratory findings in the recovered SARS-CoV-2 patients.

Methods: In this cross sectional study, the laboratory findings of various hematological and biochemical parameters along with antibody against SARS-CoV-2 of 150 patients who visited Samyak Diagnostic Pvt. Ltd for recovery check up after SARS-CoV-2 were studied from October 2020 to March 2021.

Results: Out of total 150 participants, 84% of SARS-CoV-2 recovered patients, who had mild or moderate illness, reported persistence of milder symptoms. Persistence of high serum inflammatory markers such as CRP, Ferritin and LDH along with abnormal cell count and morphology of leukocyte lineage was present in 45.4% of these patients. Similarly, 98.7 % had SARS-CoV-2 IgG antibody after 37 median days of recovery.

Conclusions: Various laboratory abnormalities may persist after SARS-CoV-2 recovery in addition to the presence of SARS-CoV-2 IgG antibody. Follow up study is needed to determine the period up to which these abnormalities are present and the protection from antibody is conferred.

Keywords: SARS-CoV-2; SARS-CoV-2 recovery; SARS-CoV-2 IgG antibody

INTRODUCTION

As the number of patients testing positive for Severe acute respiratory syndrome corona virus- 2 (SARS-Cov-2) continues to rise, a significant numbers have already recovered. There are uncertainties about what laboratory parameters are altered in the recovery stage of SARS-Cov-2 infection. The negative test result for reverse transcriptase- polymerase chain reaction (RT-PCR) is generally considered as the final point of overcoming this novel SARS-CoV-2 infection, though precautionary measures are still advised. Nepalese guideline also confirms the recovery as a negative RT-PCR report. We evaluated the laboratory findings in persons who had recovered from SARS-Cov-2 infection and referred themselves or by the treating physician to our institution for observational research. Follow-up study of SARS-CoV-2 recovered patients will be helpful to evaluate any changes in the other organs in human systems and know about the natural course of disease.

METHODS

A cross sectional observational study was done in 150 cases who visited Samyak Diagnostic Pvt. Ltd from October 2020 to March 2021 for recovery check up after infection with SARS-CoV-2. The initial positive report and subsequent negative report of RT-PCR test was required for confirming recovered cases. All age group individuals were included. The patients who were known case of malignancy or immunological disorders such as Systemic lupus erythematosus, Rheumatoid arthritis and immunosuppressive drugs users were excluded from the study. All patients were interviewed for persistence of any symptoms and received blood investigation.

Only mild and moderate cases were involved in this study however its categorization was not done. In Nepal, the milder cases were also hospitalized and few moderate cases were treated at home. Similarly, laboratory investigation reports or hospital records at the time of

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diagnosis were also not available for each patient that could categorize the severity of illness. However none of the patients who required intensive care unit (ICU) admission came for recovery check up for this study. Ethical approval to conduct this study was taken from the Nepal health research council (Protocol number-737/2020 P)

Whole blood (1 ml) was used for complete blood count test which included hemoglobin, red blood cells, white blood cells, platelets and various red blood cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC). Sysmex automated hematology analyzer XN 330 (Sysmex Corporation, Kobe, Japan) was used for this measurement. Two-level control material was used as internal quality control material which was found within a normal range. Peripheral blood smear (PBS) examination was done for each case using Wright stain.

Measurement of various biochemical parameters such as blood glucose, liver aminotransferase, total protein, albumin, urea, creatinine, total and conjugated bilirubin, alkaline phosphatase and lactate dehydrogenase (LDH) was done using RX Imola auto-analyzer (Randox Laboratories Ltd, UK). Daily maintenance for this auto analyzer was conducted and internal quality control sample from Bio-Rad was run which were found to be within the acceptable range. Serum Ferritin was measured using Siemens Advia Centaur XP immunoassay (Siemens Healthcare Diagnostics Inc. Tarrytown, NY 10591-5097 USA). Serum C- reactive protein (CRP) was measured using the principle of nephelometry in MISPA-i3 specific protein analyzer (Agappe Diagnostics Ltd, India).

IgG antibodies against SARS-CoV-2 was measured using a two-step chemiluminescent microparticle Abbott architect i1000 immunoassay (Abbott Laboratories Diagnostic Division, Abbott Park, IL 60064 USA). In this assay, the patient samples are incubated with SARS-CoV-2 antigen-coated paramagnetic microparticles followed by anti-human IgG acridinium-labeled conjugate to generate a chemiluminescent reaction.[1] This Architect platform requires a minimum of 100 µl of serum or plasma. The result, expressed as an index value, is calculated by comparing relative light units in the sample to the calibrator relative light units. Samples are interpreted as positive or negative according to the manufacturer's instructions with 1.4 as a cutoff index value.[1] Using this manufacturer's recommended index value cutoff of 1.4 for determining positivity, various studies have reported assay specificity above 99% and sensitivity of more than 92%.[2-4] Any result above 1.4 is protective.

The data were entered into the excel sheet and all calculations were done in Microsoft Excel 2010.

RESULTS

In total, 150 recovered SARS-CoV-2 patients, who had mild or moderate illness and did not require ICU admission, were enrolled in this study. Patients had a mean age of 44 ± 16.2 years and 96 (64%) were men. The study was done from October 2020 to March 2021. The median presentation time at our laboratory after recovery (a negative RT-PCR report) was 37 (13- 46) days.

Only 16% of the recovered patients were symptom free, however the remaining 84% reported lasting symptoms out of which 43% had one or two symptoms and 41% had three or more symptoms. None of the patients had fever. Fatigue, muscle weakness, sleep disturbance, anxiety and fear of reinfection were the five commonest symptoms in recovered patients (Figure 1).

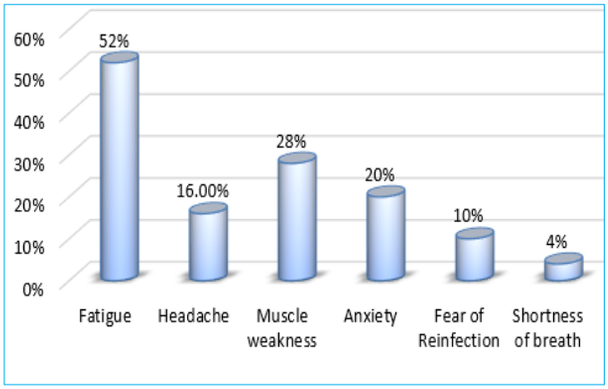

Figure 1. Symptoms present in SARS-CoV-2 recovered patient.

82 (54.6%) recovered patients had no laboratory abnormalities detected during their recovery check up. While 68 (45.4%) recovered patients had hematological or biochemical abnormalities present. Thus, the patients were divided into two categories based on normal or abnormal laboratory findings after recovery which is shown in table 1. The SARS-CoV-2 IgG antibody was present in 98.7 % of the recovered cases.

Table 1.Laboratory abnormalities in SARS-CoV-2 recovered patients.

Abnormal laboratory parameter	Recovered patients with normal laboratory parameter N (Mean ± SD)	Recovered patients with abnormal (↑ or ↓) laboratory parameter N (Mean ± SD)	Percentage of recovered patients with abnormal findings	Normal Reference range with Unit
Alanine Transaminase	95 (23.9 ± 7.8)	55 (76.5 ± 18.6) (↑)	36.7%	10- 45 U/L
C-Reactive Protein	132 (2.9 ± 1.4)	18 (8.6 ± 1.6) (↑)	12.0%	< 6.0 mg/L
Lactate dehydrogenase	141(142.9 ± 32.2)	9 (238.7 ± 21.5) (↑)	6.0%	100-210 U/L
Ferritin	141 (125.5 ± 70.9)	9 (510.6 ± 363.4) (↑)	6.0%	21.81-274.66 ng/mL
Total leukocyte count	138 (6225.9 ± 2195.2)	12 (3685.3 ± 333.7) (↓)	8.0%	4000-11,000 Cells/ cumm
Lymphocyte Count	30.8 ± 7.7	47.6 ± 1.6 (↑)	10.0%	20 -45 %

Symbols: ↑= increase, ↓= decrease

Only the abnormal laboratory parameter is listed in this table. Rest of the parameters that were studied such as serum aspartate aminotransferase, total protein, albumin, urea, creatinine, total and conjugated bilirubin and alkaline phosphatase were in normal range.

Blood smears from all 150 cases were examined and the morphological abnormalities concerning leukocyte lineages were found in (n=18) 12% of the recovered cases. By observing blood smears colored by Wright stain, the characteristics of a neutrophil granulocyte with heavily clumped chromatin with toxic granules and cytoplasmic vacuoles was noted. Ring-shaped nuclei were noted with platelet surface attachment as well as occasional slides revealed C-shaped, fetus-like nuclei with aberrant nuclear projections. Lymphocytes with round to indented nuclei, condensed chromatin, prominent nucleoli, cytoplasmic pod formation and apoptosis were also observed in three cases. The cytoplasmic blebbing of monocytes was also seen in three cases.

DISCUSSION

After SARS-CoV-2 illness, recovered patients may continue to report a wide variety of complaints. Recovery period is likely to be longer for patients who suffered a more severe form of the disease and who had pre-existing illness. Many patients are anxious and present themselves to the healthcare centre for post recovery check up. However, not all patients are clinically indicated for laboratory or radiological investigation post recovery.

Previous studies have shown that clinical features such as fever, cough, a sore throat or fatigue may persist or reoccur in the recovered patients.[5, 6] In our study, the most common symptoms present in recovered patients were fatigue, muscle weakness, sleep disturbance, anxiety and fear of reinfection. One Italian study reported persistence of at least one symptom, particularly fatigue and dyspnea in 87.4% recovered patients.[6] Our study had similar finding where 84 % of participants reported lasting symptoms (Figure 1). Persistence of these symptoms further increases an anxiety of a recovered patient. Reassurance and symptomatic treatment helps most of these patients.

Increased liver alanine transaminase in 36.7% of recovered patients was found in our study. The degree of elevation was only mild. Hepatic involvement is a well recognized feature of SARS-CoV-2 infection and it is related to the direct effect of the virus on hepatocytes or biliary epithelium, liver injury related to accentuated immune response, drug toxicity (because of drugs like acetaminophen, multivitamins, various spices, herbs and Ayurvedic medications), hemodynamic instability and ischemic hepatitis which could occur in SARS-CoV-2 infection.[7- 9] Increase in liver transaminase after SARS-CoV-2 recovery should be regarded a post viral effect and further work up is not required if SARS-CoV-2 antibody is positive. However, the exact duration up to which the enzymes rise, should be studied from the follow up studies.

The serum inflammatory markers such as CRP, ferritin and LDH were found increased in 12%, 6% and 6% of recovered patients respectively. The degree of elevation of these markers was only mild. CRP is an important regulator of inflammatory processes as it modulates the immune response, via its induction of anti-inflammatory cytokines and limitation of free radical damage.[10] Many cytokines are rapidly produced during active viral infection which stimulates hepatocytes and macrophages to secrete ferritin. [11] LDH is an intracellular enzyme found in nearly all organ systems. Its abnormal values can result from multiple organ injury including

lung and decreased oxygenation. These inflammatory laboratory parameters have been associated with worse outcomes in patients with SARS-CoV-2 infection.[12-14] Elevation of these biomarkers in few recovered cases of our study suggests either moderate to severe course of illness in these cases or persistent inflammatory response. Individuals with mild infection are expected to recover relatively quickly (within two weeks) whereas many individuals with moderate to severe disease have a longer time to recovery (two to three months).[15] The median time of presentation for laboratory investigation in our study was 37 (13- 46) days. However, the mild and moderate categorization of recovered patients was not done in our study. In Nepal, the milder cases were also hospitalized and few moderate cases were treated at home, so the actual medical records were obscured. Similarly, laboratory investigation reports at the time of diagnosis were also not available for each patient that could categorize the severity of illness. Nevertheless, elevation of these inflammatory biochemical parameters suggests persistent inflammation and our result is similar to that reported in a study done in recovered patients in London where 9.5% had elevated CRP.[16]

Leucopenia in 8% and lymphocytosis in 10% along with prominent morphological changes in leukocyte lineages in 12% of recovered patients was found in our study. The common hematological abnormalities seen in SARS-CoV-2 infection are anemia, leucopenia mostly lymphopenia, and leukocytosis including neutrophilia and monocytosis.[17, 18] Peripheral blood smear assessment at the time of diagnosis in SARS-CoV-2 patients can provide information about the stage and severity of the disease and the length of hospital stay.[19] Intense monocytes activation and infiltration into the target tissues is the mechanism of lung injury in SARS-CoV-2 infection. Infected monocytes differentiate into macrophages with a greater expression of chemokine receptors, the stimulation of which induces the expression of pro-inflammatory cytokines. A sustained expression of inflammatory monocytes and activated T cells even three weeks after SARS-CoV-2 recovery was reported from a study in India.[20] Presence of persistent hematological abnormalities in our study suggests a moderate to severe course of disease in these individuals.

The SARS-CoV-2 IgG antibody in our study was present in 98.7% of the recovered cases. Similar finding was reported in two larger studies done in Iceland and Spain, where over 90% of PCR-positive persons tested positive with SARS-CoV-2 antibody assays.[21,22] Presence of this antibody helps in identifying potential donors of convalescent plasma, measuring immunogenicity in vaccine development, and determining the degree of herd immunity in the community.

There are various reasons for absence of SARS-CoV-2 antibody in the remaining two recovered cases in our study. These two cases presented at 3 weeks and 4.3 weeks respectively after recovery at our laboratory. Detectable antibodies generally take several days to weeks to develop, and the time to antibody detection varies by test. [23] The rate of positive IgG approaches 100 percent by 16 to 20 days of recovery. [24] Since both cases had presented at a time where antibody was supposed to have developed, so this might not be the cause. A recent study has shown that the SARS-CoV-2 antibody may not be long lasting in persons with mild illness,[25] and this mild illness group comprised the majority of Nepalese patients with Covid-19. However, the mild and moderate categorization of recovered patients was not done in our study. Other possibilities for undetectable IgG antibody are that some patients infected by SARS-CoV-2 may have undetectable levels of antibodies reactive to the spike and nucleocapsid proteins. In our study, the serology for SARS-CoV-2 IgG to nucleocapsid protein was performed using the Abbott Architect i1000 chemiluminescent microparticle which is a qualitative test with manufacturer's recommended index value cutoff of 1.4 for determining positivity. The confirmatory virus neutralization test, which is the gold standard to confirm antibody production, may be required in such case. There is also possibility that some RT-PCR delivered false positive results for these cases.

CONCLUSIONS

Various biochemical and hematological abnormalities may persist after SARS-CoV-2 recovery. Mild elevation of liver enzyme and inflammatory markers along with cell count and morphological changes in leukocyte lineage after SARS-CoV-2 recovery of mild and moderate cases should be regarded a post viral effect. The further diagnostic work up for these deranged parameters in recovered patients is only required if it is in increasing trend or if patient has any chronic illness or the SARS-CoV-2 antibody is not present. This is particularly important for patients who have unknowingly recovered from SARS-CoV-2 infection and has now performed laboratory test for other reason. The exact duration up to which these parameters rise and the protective effect from antibody is conferred, should be studied from the follow up studies.

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