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Original Research Article

Assessment on serum CRP and serum ferritin level in COVID-19 patients of Dehradun Uttarakhand, India

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ABSTRACT

Background: Since being declared a global pandemic, COVID-19 has ravaged many countries worldwide and has overwhelmed many healthcare systems. The pandemic has also resulted in the loss of livelihoods due to prolonged shutdowns, which have had a rippling effect on the global economy. Even though substantial progress in clinical research has led to a better understanding of SARS-CoV-2 and the management of COVID-19, limiting the continuing spread of this virus and its variants has become an issue of increasing concern, as SARS-CoV-2 continues to wreak havoc across the world, with many countries enduring a second or third wave of out-breaks of this viral illness attributed mainly due to the emergence of mutant variants of the virus. Our study tried to evaluate the fact regarding CRP and feritin parameters concentration efficacy in the detection and evaluation of inflammatory disorders, tissue injury and infections.
Methods: CRP concentration measurements are useful in the detection and evaluation of inflammatory disorders, tissue injury and infections. The VITROS CRP slide method is performed using the VITROS 5600 integrated system. The VITROS CRP slide is a multi-layered, analytical element coated on a polyester support. The immune-rate format for CRP is based on enzymatic heterogeneous, sandwich immunoassay format. In this format a derivative of phosphorylcholine (PC) is covalently bound to polystyrene polymer beads and in the presence of calcium serves as a capture agent, monoclonal anti-CRP antibody conjugated to horseradish peroxidase (HRP) serves as a signal generator.
Results: A total of 175 patients were selected for the study for estimate levels of C-reactive protein and serum ferritin in COVID-19 patients. The data obtained was coded and entered into Microsoft Excel Worksheet. Data was analysed and results were tabulated. In our study, serum CRP and serum ferritin values were found to be elevated in patients infected with COVID-19.
Conclusions: CRP levels were positively correlated with lung lesions. CRP levels could reflect disease severity and should be used as a key indicator for disease monitoring. Serum ferritin was found to be elevated among the COVID-19 patients who could not survive the treatment as compared to the recovered patients. Therefore, serum concentrations of ferritin could be used as a prognostic marker in the management of COVID-19 patients which is easily available and cost effective too.

Keywords: Coronavirus, Ferritin, Pandemic, Respiratory, Serum, SARS, Tissue

INTRODUCTION

The highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has a catastrophic effect on the world's demographics resulting in more than 3.8 million deaths worldwide. After the first cases of this predominantly respiratory viral illness were first reported in Wuhan, Hubei Province, China, in late December 2019, SARS-CoV-2 rapidly disseminated across the world in a short span of time, compelling the World Health Organization (WHO) to declare it as a global pandemic on March 11, 2020. Like other RNA viruses, SARS-CoV-2, while adapting to their new human hosts, is prone to genetic evolution with the development of mutations over time, resulting in mutant variants that may have different characteristics than its ancestral strains. Based on the recent epidemiological update by the WHO, as of June 22, 2021, Coronaviruses (CoVs) are positive-stranded RNA (+ssRNA) viruses with a crown-like appearance under an electron microscope (corona is the Latin term for crown) due to the presence of spike glycoproteins on the envelope. Beta CoV genus is further divided into five sub-genera or lineages. Genomic characterization has shown that bats and rodents are the probable gene sources of alphaCoVs and betaCoVs. In general, estimates suggest that 2% of the population are healthy carriers of a CoVs and that these viruses are responsible for about 5% to 10% of acute respiratory infections.[1]

Common human CoVs: HCoV-OC43, and HCoV-HKU1 (betaCoVs of the A lineage); HCoV-229E, and HCoV-NL63 (alphaCoVs). These viruses can cause common colds and self-limiting upper respiratory tract infections in immunocompetent individuals. However, in immunocompromised subjects and the elderly, lower respiratory tract infections can occur due to these viruses. Other human CoVs: SARS-CoV and MERS-CoV (betaCoVs of the B and C linessage, respectively). These viruses are considered to be more virulent and capable of causing epidemics manifesting with respiratory and extra-respiratory manifestations of variable clinical severity. SARS-CoV-2 is a novel betaCoV belonging to the same subgenus as the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which have been previously implicated in SARS-CoV and MERS-CoV epidemics with mortality rates up to 10% and 35%, respectively.

The main objectives of our study were to know how CRP and ferritin get changes in COVID-19 patients. What is the correlation between these parameters. Why these parameters get affected in COVID-19 affected population.

Role of CRP and serum ferritin in COVID-19 patients

Acute phase reactants are known to be elevated in infectious and inflammatory disease and correlate with the severity of the disease. Acute phase reactants such as CRP and serum ferritin are elevated in various inflammatory conditions and acts a negative acute phase reactant. Coronavirus disease (COVID-19) infected patients may have asymptomatic to symptomatic severe disease and now it is well known that complication such as pneumonia, thrombosis, disseminated intravascular coagulation, and cytokine release syndrome can occur.

Few studies have also shown that CRP and ferritin levels are increased in COVID-19 patients and correlate with severity and therefore help in modifying treatment protocol. Therefore, this study was done to assess the role of CRP and ferritin in moderate-to-severe hospitalized COVID-19 patients.[6]

METHODS

The present study was conducted in the Department of Biochemistry, SGRRIM and HS Dehradun on COVID-19 positive patients.

Source of data

It was a hospital-based study carried on patients attending the Shri Mahant Indresh Hospital (OPD and IPD patients).

Study period and duration

The present one-year study was conducted during the period of (April-2020- April-2021).

Sample size

The sample size was determined the number of patients according to the inclusion criteria for cases during the sample period in 2020. Study population consists of 100 positive patients attending OPD and IPD in SMI Hospital. The total number of patients were 175 included in the study.

Sampling method

The COVID-19 patients who were attending to Shri Mahant Indresh Hospital, Dehradun with a confirmed case of RT-PCR were selected as subjects for study. A blood sample was taken to measure the CRP and ferritin level in the body.

Eligibility

Age group eligible for study were 15-90 years, both male and female.

Method of collection of data

A total of 175 patients with mild to moderate symptoms of COVID-19 were selected for the study and method of collection of data.

Methods

CRP concentration measurements are useful in the detection and evaluation of inflammatory disorders, tissue injury and infections.

Principles of the procedure

The VITROS CRP slide method is performed using the VITROS 5600 integrated system. The VITROS CRP slide is a multilayered, analytical element coated on a polyester support. The immune-rate format for CRP is based on enzymatic heterogeneous, sandwich immunoassay format. In this format a derivative of phosphorylcholine (PC) is covalently bound to polystyrene polymer beads and in the presence of calcium serves as a capture agent, monoclonal anti-CRP antibody conjugated to horseradish peroxidase (HRP) serves as a signal generator. A drop of patient sample was deposited on the slide and was evenly distributed by the spreading layer to the underlying layers.

CRP in the sample binds to PC-linked capture beads and anti-CRP antibody labelled with horseradish peroxidase to form an insoluble sandwich complex in incubation 1. The subsequent addition of 12 µl of VITROS immune-wash fluid to the slide removes unbound materials from the read area, while also providing the hydrogen peroxide required for the enzyme-mediated oxidation of leuco dye. The reflection density of the dye is measured after addition of VITROS immuno-wash fluid at the end of incubation 2. This reflection density proportional to the concentration of CRP in the sample. To determine if an adequate wash has occurred, the wash detection dye is read at 540 nm immediately after incubation 2.

Table 1: Test type and conditions.

Test type	VITROS system	Approximate incubation time	Temperature	Wavelength	Reaction sample volume
Fixed-point immuno-rate	5600,5,1FS,950, 250/350	Incubation 1:5 minutes Incubation 2:2.5 minutes	37°C	670 nm	11 µl

Reaction scheme

CRP + PC* +Ab•HRP $\overset{Ca^{2+}}{\rightleftharpoons}$ PC*-CRP-Ab•HRP + Ab•HRP

Immune-wash + PC*-CRP- Ab•HRP + Ab•HRP wash ⟶ PC*-CRP-Ab•HRP

H_20_2 + leuco dye + PC*-CRP-Ab•HRP ⟶ dye + 2 H_2O

PC*= phosphorylcholine beads

Ab•HRP = anti CRP monoclonal antibody labelled with horseradish peroxidase

Reagents

Slide ingredients

Reactive ingredients per cm^2

Immobilized phosphorylcholine 0.07mg; mouse anti-CRP antibody labelled with horseradish 0.0006 U ; calcium cholrine 0.08mg and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis(4-dimethylaminophenyl) imidazole (leuco dye) 0.04 mg.

Other ingredients

Blinders, buffer, surfactants, cross-linking agent, polymer beads, proteins, stabilizers and wash detection dye.

Methods for serum ferritin

For the quantitative measurement of ferritin in human serum and plasma (heparin) using the VITROS 3600 immunodiagnostic systems and the VITROS 5600 integrated system. Measurements of ferritin aid in the diagnosis of diseases affecting iron metabolism such as hemochromatosis (iron overload) and iron deficiency anemia.

Principles of the procedure

The VITROS ferritin test was performed using the VITROS 3600 immunodiagnostic systems and the VITROS 5600 integrated system using intellicheck technology. A two-step immunometric technique was used, which involves the reaction of ferritin present in the sample with a biotinylated antibody (sheep polyclonal anti-ferritin) in the first step. The antigen-antibody complex was captured by streptavidin coated on the well. Unbound materials were removed by washing. The second step involved the reaction of antigen-antibody complex with a horseradish peroxidase labelled antibody conjugate (mouse monoclonal anti-ferritin). Unbound materials were removed by washing.

The bound HRP conjugate was measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyses the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted

acetanilide) increases the level of light produced and prolongs its emission. The light signals were read by the system, The amount of HRP conjugate bound was directly proportional to the concentration of ferritin present.

Table 2: Test type and condition.

Test type	System	Incubation time	Time to first result	Test temperature	Reaction sample volume
Immunometric	ECi /ECiQ 3600,5600	32 minutes	42 minutes	37°C	15 µl

Statistical analysis

The data obtained was coded and entered into Microsoft Excel worksheet. The categorial data was expressed as rates, ratio and comparison was done. Statistically analysis of serum CRP and serum ferritin in COVID-19 positive and negative patients was done.

RESULTS

The level of serum ferritin was high in the age group of 76-90 years as compared to other age groups. We can come to the conclusion that the level of serum ferritin in COVID positive male is higher than COVID positive females the level of serum ferritin is high in the age group of 76-90 years as compared to other age groups.

Table 3: Age wise distribution in serum ferritin of COVID positive patients.

Age group	Serum ferritin level
15-30	166.62
31-45	520.22
46-60	385
61-75	512.03
76-90	1825.79

Table 4: Age wise distribution in serum ferritin of COVID negative patients.

Age group	Serum ferritin level
15-30	106.66
31-45	123.64
46-60	141.98
61-75	152.08
76-90	146.37

Table 5: Comparison of level of ferritin in male and female in covid positive and covid negative patients in the 15-30 years of age group.

Sex	Sr. ferritin level in COVID +ve patient	Sr. Ferritin level in COVID -ve patient
Male	192.38	125.19
Female	217.27	124.33

Table 6: Comparison of level of serum ferritin in male and female in COVID positive and COVID negative patients in the 76-90 years of age group.

Sex	Sr. ferritin level in COVID +ve patient	Sr. Ferritin level in COVID -ve patient
Male	686	183
Female	430	140

The level of serum ferritin was high in the age group of 61-75 years as compared to other age groups among COVID-19 patients. We can come to the conclusion that the level of serum ferritin is high in the age group of 61-75 years as compared to other age groups among COVID-19 patients.

The level of serum ferritin in male and female (15-30 years) COVID positive female patients were higher than COVID negative patients. We can come to the conclusion that the level of serum ferritin in male and female (15-30 years) COVID positive female patients are higher than COVID negative patients.

The level of serum ferritin in male and female (76-90 years) COVID positive male patients were higher than Covid negative female patients. We can come to the conclusion that the level of serum ferritin in male and female (76-90 years) COVID positive male patients are higher than COVID negative female patients.

DISCUSSION

The purpose of our study is to observe severity between Serum CRP and Serum ferritin in COVID-19 patients.

Serum CRP in COVID-19 patients

In our study we observe the mean value of Serum CRP were 45.50 mg/l.

According to Liu et al, reported that more severe cases infected with COVID-19 expressed significantly higher CRP levels than non-severe patients.[7]

According to Qin et al observed higher CRP levels in severe COVID-19 patients than in non-severe cases,

suggesting that this biomarker can be monitored to evaluate disease progression.[8]

According to Sahu et al performed a meta-analysis to assess CRP levels as a potential biomarker of the COVID-19 prognosis. Their results indicated that CRP concentrations remain high in expired patients and could be a promising biomarker for assessing mortality.[9]

In the present retrospective study, the clinical characteristics of severe COVID-19 patients were compared with those of non-severe patients and analyzed the possible factors associated with disease progression and severity. Furthermore, the prognostic value of the CRP in the progression of COVID-19 cases has been revealed current study evaluated the association between CRP and COVID-19 infection, and the findings indicated that a patient with a CRP level >64.75 mg/l was more likely to develop the severe form of the disease.

In response to infections, the liver synthesizes significant quantities of acute-phase proteins (APPs), such as CRP is acute inflammatory protein is a highly sensitive biomarker for inflammation, tissue damage, and infection. It has been shown that CRP levels are correlated with levels of inflammation.[10]

The current study revealed significantly higher CRP levels in severe cases than in non-severe patients suggesting that the CRP level may be a biomarker of disease severity and progression in patients with COVID-19.

CRP is an exquisitely sensitive systemic marker of acute-phase response in inflammation, infection, and tissue damage, which could be used as indicator of inflammation in the study by Chen et al, although no statistically significant difference was found in the level of CRP between the non-severe and the severe group, the mean level of CRP was higher in the severe group than in the non-severe group.

Serum ferritin in COVID-19 patients

In our study we observe the mean value of serum ferritin were 434.11 ng/ml.

According to Wu et al investigated cases of COVID-19 to study the clinical characteristics and outcomes in patients; their findings showed that higher serum ferritin was an independent risk factor associated with ARDS development.[11]

According to Connelly et al investigated serum ferritin levels in patients at risk for and with ARDS and found serum ferritin to be a predictor of ARDS.[12]

According to Pastora et al in a systematic review on the utility of ferritin in COVID-19 has revealed that ferritin concentrations of COVID-19 patients were generally within the normal range of less than 400 ng/ml in patients with the non-severe disease.[13]

According to Zhang et al have also evaluated the clinical characteristics of 82 deaths cases, laboratory confirmed as SARS-CoV-2 infection. The researchers have reported fever (78.0%), cough (64.6%), and shortness of breath (63.4%) as the prominent symptoms reported in the succumbed victims.[14]

The study by Reddy et al conducted in a tertiary care center in India, showed the serum activities of ferritin were markedly increased in COVID-19 patients who could not survive as compared to the patients who finally recovered from the infection.[15]

Jonathan et al, based on a retrospective review of 942 adult COVID-19 cases from New York city health system database, have also reported almost similar AUC for mortality and severity in COVID-19, being 0.63 and 0.68 respectively.[16]

Recent literature advocates hyperferritinemic syndrome as one of the main modifications in COVID infection suggesting evaluation of ferritin levels as a parameter of infection.

Presently, excluding reviews and metanalysis, only ten papers have been published with the contemporary topic of “ferritin” and COVID.[16]

Ferritin is an iron-storing protein; its serum level reflects the normal iron level and helps the diagnosis of iron deficiency anemia. Circulation ferritin level increases during viral infections and can be a marker of viral replication. Increased levels of ferritin due to cytokine storm and sHLH have also been reported in severe COVID-19 patients. During the cytokine storm in COVID-19, many inflammatory cytokines are rapidly produced, including IL-6, TNF-α, IL-1β, IL-12, and IFN-γ, which stimulate hepatocytes, Kupffer cells, and macrophages to secrete ferritin. The uncontrolled and dysfunctional immune response associated with macrophage activation, hyperferritinemic syndrome, and thrombotic storm finally leads to multiple organ damage. Notably, ferritin is not only the result of excessive inflammation, but also plays a pathogenic role in the inflammation process through its bind with the T-cell immunoglobulin and mucin domain 2 (TIM-2) by promoting the expression of multiple pro-inflammatory mediators. Besides, some studies showed that H chain of the ferritin activates macrophages to secrete inflammatory cytokines.[17]

This study has few limitations. In our study we observed and concluded that the level of serum CRP and serum ferritin was high in the age group of 76-90 years as compared to other age groups among COVID-19 patients. This was explained by the study done my Chen et al and Jonathan et al respectively. CRP is an exquisitely sensitive systemic marker of acute-phase response in inflammation,

infection, and tissue damage, which could be used as indicator of inflammation in the study by Chen et al, although no statistically significant difference was found in the level of CRP between the non-severe and the severe group, the mean level of CRP was higher in the severe group than in the non-severe group. Increased levels of ferritin due to cytokine storm and sHLH have also been reported in severe COVID-19 patients. During the cytokine storm in COVID-19, many inflammatory cytokines are rapidly produced, including IL-6, TNF-α, IL-1β, IL-12, and IFN-γ, which stimulate hepatocytes, Kupffer cells, and macrophages to secrete ferritin.

CONCLUSION

To conclude with this study, we took 175 patients of COVID-19. In which the level of serum CRP and serum ferritin is high in the age group of 76-90 years as compared to other age groups among COVID-19 patients.

CRP levels were positively correlated with lung lesions. CRP levels could reflect disease severity and should be used as a key indicator for disease monitoring.

Serum ferritin was found to be elevated among the COVID-19 patients who could not survive the treatment as compared to the recovered patients. Therefore, serum concentrations of ferritin could be used as a prognostic marker in the management of COVID-19 patients which is easily available and cost effective too.

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REFERENCES

1. Lei J, Kusov Y, Hilgenfeld R. Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. Antiviral Res. 2018;149:58-74.
2. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell. 2020;182(4):812-27.
3. Wang P, Casner RG, Nair MS, Wang M, Yu J, Cerutti G, et al. Increased resistance of SARS-CoV-2 variant P. 1 to antibody neutralization. Cell Host Microbe. 2021;29(5):747-51.
4. Riddell S, Goldie S, Hill A, Eagles D, Drew TW. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol J. 2020;17(1):145.
5. Stokes EK, Zambrano LD, Anderson KN, Marder EP, Raz KM, Felix SE, et al. Coronavirus disease 2019 case surveillance- United States, January 22–may 30, 2020. Morbid Mortal Week Rep. 2020;69(24):759.
6. Aloisio E, Chibireva M, Serafini L, Pasqualetti S, Falvella FS, Dolci A, et al. A comprehensive appraisal of laboratory biochemistry tests as major predictors of COVID-19 severity. Arch Pathol Lab Med. 2020;144(12):1457-64.
7. Davies NG, Barnard RC, Jarvis CI, Russell TW, Association of tiered restrictions and a second lockdown with COVID-19 deaths and hospital admissions in England: a modelling study. Lancet Infect Dis. 2021;21(4):482-92.
8. Challen R, Brooks-Pollock E. Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: matched cohort study. BMJ. 2021;372:n579.
9. Davies NG, Jarvis CI, Edmunds WJ, Jewell NP, Diaz-Ordaz K, Keogh RH. Increased mortality in community-tested cases of SARS-CoV-2 lineage B. 1.1. 7. Nature. 2021;593(7858):270-4.
10. Jackson DK, Gaythorpe K, Groves N, Sillitoe J. Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. Nature. 2021;593(7858):266-9.
11. Lei J, Kusov Y, Hilgenfeld R. Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. Antiviral Res. 2018;149:58-74.
12. Wood S, Ferris A, Miller D, Weaver W. SARS-CoV-2 is rapidly inactivated at high temperature. Environ Chem Lett. 2021;1-5
13. Oreshkova N, Molenaar RJ, Vreman S, Harders F, SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro Surveill. 2020;25(23).
14. Perez LG, Tang H, Moon-Walker A. Evidence that D614G increases infectivity of the COVID-19 virus. Cell. 2020;182(4):812-827.e19.
15. Wenseleers T, Gimma A, Waites W. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science. 2021;372(6538).
16. Ahmad FB, Cisewski JA, Miniño A, Anderson RN. Provisional mortality data- United States, 2020. Morbid Mortal Week Rep. 2021;70(14):519-22.
17. Zarjou A, Black LM, McCullough KR. Ferritin light chain confers protection against sepsis-induced inflammation and organ injury. Front Immunol. 2019;10:131.

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