Absolute Lymphocyte Count and its Correlation with Serum Ferritin Level: An early indicator of disease severity in COVID-19

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Abstract

Introduction: COVID-19 has been a formidable challenge with an already under-resourced health care system. Current literature has already identified several risk factors. Both lymphopenia and hyperferritinemia in SARS-Cov-2 patients had been reported in the literature but so far no study has been done to find the correlation between these two variables.

Objective: To correlate absolute lymphocyte count with serum ferritin levels in patients with COVID-19 admitted in the intensive care unit of a tertiary care hospital.

Material and Methods: This cross-sectional study was carried out at Fauji Foundation Hospital/Foundation University Islamabad from July 2020-December 2020. Data were analyzed on Microsoft excel. The correlation between serum ferritin and absolute lymphocyte count was done using the Pearson correlation coefficient and P-value was calculated. Mean and SD was calculated for age. Frequency and percentage were calculated for quantitative variables

Results: The mean age of the patients was 61 +/- 9 years. The age range was (28-75) years. Patients with lymphopenia are shown in the following table I. The mean lymphocyte count was 1.1 +/- 0.2 SD. The mean Ferritin value was 1148.5 +/- 60 SD. The Pearson correlation coefficient showed an r value of -0.60 which shows a moderate negative correlation. The p-value was < 0.05 which was statistically significant.

Conclusion: There is a significant negative correlation between lymphocyte count and serum ferritin levels which will assist clinicians in the early identification of patients at risk.

Keywords: Serum Ferritin, Absolute Lymphocyte Count, COVID-19.

Introduction

The first case of the novel coronavirus SARS-CoV-2 was identified in Wuhan China in December 2019. Fast forward to April 2021, 133 million cases of COVID 19 have been reported globally with 4.31 million deaths.[1] The symptoms of COVID-19 ranged from asymptomatic to mild-to-severe illness leading to death.[1] All over the world COVID-19 has led to an unprecedented human catastrophe.[2] The COVID-19 infection started to spread across Pakistan in February 2020. In countries like Pakistan COVID-19 has been a formidable challenge with an already under-resourced health care system.[2]

The development of a cytokine storm is a potentially fatal immune condition characterized by hyperactivation of T cells, macrophages, natural killer cells, and the overproduction of inflammatory cytokines and chemical mediators.[3,4] In viral infections, the aberrant release of pro-inflammatory factors leads to apoptosis of epithelial cells which damages the lung microvascular and alveolar epithelial cell barrier, leading to vascular leakage, alveolar oedema, and hypoxia.[5]

Hyperactivated T cells, macrophages, and excess production of chemical mediators, inflammatory cytokines, and macrophages is the hallmark of a potentially life-threatening cytokine storm.[3,4] Apoptosis of epithelial cells occurs due to an aberrant release of pro-inflammatory cytokines which leads to a cascade of lung damage that includes damage to the alveolar as well as microvascular epithelial cell barrier leading to vascular leakage and consequently lung damage and hypoxia.[5]

The massive number of cases with SARS-Cov-2 has put tremendous pressure on healthcare systems all over the world. Most of the countries have declared a health emergency. In these highly challenging times and also to reduce the risk of morbidity early warning signs may be helpful.[6] The early detection of patients at risk who develop severe illness may assist in delivering proper care and modify treatment strategies.

Current literature has already identified several risk factors. Both lymphopenia and hyperferritinemia in SARS-Cov-2 patients had been reported in the literature but so far no study has been done to find the correlation between these two variables. Both hyperferritinemia and lymphopenia have been linked to diseases severity and increased morbidity and mortality among SARS-Cov-2 infected patients. Absolute lymphocyte count is a cheap, cost-effective, quick test that can assist in risk stratifying these patients as early management in this disease is crucial to save a patient.[7,8,9,10]

The present study aimed to correlate absolute lymphocyte count with serum ferritin level an inflammatory marker that heralds the risk for disease complication or cytokine storm to assist in early identification of the disease by picking up these early warning signs so that treatment could be curtailed in due time and risk of morbidity and mortality due to COVID 19 Disease is minimized by early intervention.

Materials and Methods

This cross-sectional study was carried out at --- removed for blind review---from July 2020-December 2020. The study was approved by the Ethical Review Committee of Fauji Foundation Hospital, Rawalpindi. Patients with confirmed COVID-19 infection admitted to the intensive care unit of the hospital from 1st July-to 31st December 2020 were included in the study. A positive COVID-19 patient was the one who had a positive real-time polymerase chain reaction (PCR) for SARS-CoV-2 by sampling through nasopharyngeal swabs. 3 ml of blood is taken for Blood CP in ethylene diamine tetraacetic acid-containing vacutainers and was run on Sysmex-XN series. For serum ferritin 4 ml clotted blood was collected in the plain test tube. Serum ferritin was performed on the AXSYM system by immunoassay using the AXSYM Ferritin reagent kit pack. COVID-19 disease severity classification proposed by WHO was used to categorize the patients. (WHO COVID-19 interim guidelines).[11]

Data were analyzed on Microsoft excel. The correlation between parameters was assessed using the Pearson correlation coefficient and P-value was calculated. Mean and SD was calculated for age. Frequency and percentage were calculated for quantitative variables. Descriptive statistics were computed for all study variables. Categorical variables were expressed as count (percentage) and continuous variables as a median. $P < 0.05$ was counted as statistically significant.

Positive cases were stratified into asymptomatic, mild, moderate, severe, according to the National Institute of Health, Pakistan guidelines. The range for normal lymphocyte count is $1.5-4.5 \times 10^9/L$,[12] the cut-off for lymphopenia was $< 1 \times 10^9/L$. Cut off for mild lymphopenia $< 1 \times 10^9/L$. Moderate lymphopenia was less than $0.8 \times 10^9/L$ and severe lymphopenia was less than $0.5 \times 10^9/L$.[12] The normal reference range of serum ferritin for men was 18-270 ng/ml and for

females, 18-160 ng/ml the cut-off for high serum ferritin for males is >270 ng/ml and for females, it is > 160 ng/ml. Generally, levels between 30-300 μg/L for men and 15-200 μg/L for women are considered normal,[13] which reflects the significant variation in serum ferritin levels. Mild hyperferritinemia was ferritin 300-600ug/L, moderate 600-1000 ug/L, and severe >1000 ug/L.

Results

This cross-sectional study was conducted at Fauji Foundation Hospital from 1st July till 31st December 2020. A total of 91 patients diagnosed with COVID-19 by RT-PCR testing admitted in intensive care units with severe and critical diseases were included in the study. Out of these 91 patients; 41 were males and 50 patients were females. The mean age of the patients was 61+/- 9 years. The age range was (28-75) years. Patients with lymphopenia are shown in the following table I. The median nadir lymphocyte count was 0.2 X 10 9/L. The mean lymphocyte count was 1.1 +/- 0.2 SD. The mean Ferritin value was 1148.5 +/- 60 SD. Figure 1 showing the correlation between lymphocytes and ferritin.

Table 1: Frequency and Percentage of Patients with Lymphopenia

Serial no	Grades of Lymphopenia	Frequency/ Percentage of Patients
1	Normal/Mild	27 (29%)
2	Moderate	23 (25%)
3	Severe	41 (45%)

Table 2: Frequency and Percentage of Patients with Hyperferritinemia

Serial no	Grades of Hyperferritinemia	Frequency/ Percentage of Patients
1	Mild /Normal	25 (27%)
2	Moderate	15 (16%)
3	Severe	51 (56%)

The Pearson correlation coefficient showed an r value of -0.60 which shows a moderate negative correlation. The inverse relationship shows higher the ferritin lower the lymphocyte count. The p-value was < 0.05 which was statistically significant.
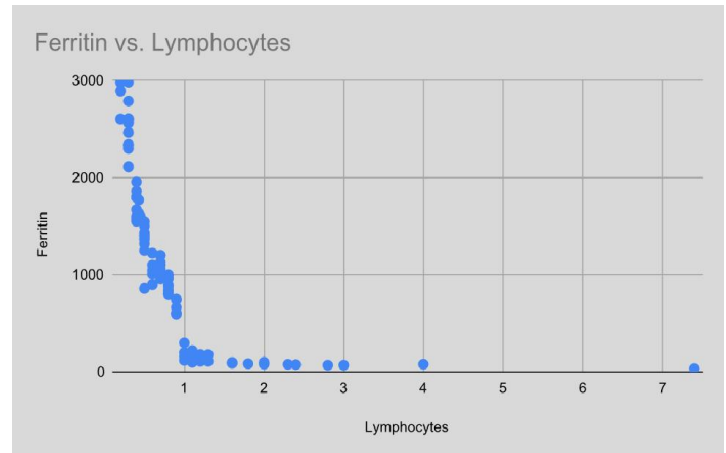

Figure 1: showing the correlation of serum ferritin with Lymphocytes where the absolute number of lymphocytes are shown on the X-axis and Ferritin values are shown on Y-axis

Discussion

The SARS-CoV2 disease has led to unprecedented demands for health care services, especially in the intensive care unit.[14] Ferritin is one of the major proteins which regulates iron homeostasis and it is a clinical biomarker for the status of iron in the body. [15] Serum ferritin is an acute-phase protein and is raised in inflammatory conditions as well as in conditions where iron stores are increased.[16] Serum iron is the main modulator of serum ferritin, its synthesis is also regulated by numerous inflammatory cytokines i.e. IL-6 and IL-1β.[17]

In a study done in high-risk patients with COVID 19 serum ferritin levels strongly correlated with disease severity. In this study, all patients who had severe disease had a serum ferritin level higher than 500 ug/L. Our data does have a high serum ferritin level in patients with serious disease but not all patients had serum ferritin levels higher than 500 ug/L.[18]

It was found that serum ferritin levels in patients who were having critical COVID-19 disease were 2.6 times higher compared to the relatively milder categories. [18] Its crucial to control cytokine storms in the early phase of the disease to reduce morbidity and mortality from the disease by giving immunomodulators and cytokine antagonists (Gandini). In this regard, a blood CP could be an early, readily available and quick, and cost-effective screening tool to assess the disease severity and for early identification for high-risk patients with COVID-19.[18]

A study was done to assess the relationship of C-reactive protein, serum ferritin, and lactate

dehydrogenase at admission with mortality in SARS-CoV2 infection. Out of all the inflammatory markers studies, serum ferritin was found to be one of the most significant inflammatory markers to correlates with disease severity in COVID-19 patients. A serum ferritin level of ≥723 ng/ml had a sensitivity of 95.45% and a specificity of 86.57% for predicting mortality.[19]

A study did even emphasized the need for risk stratification of COVID-19 patients based on serum ferritin levels as high or low risk. The high-risk patients with high serum ferritin levels in these patients had a higher ratio of admission to intensive care units.[20,21]

A high level of serum ferritin is a constant feature of HLH and predicts a poor recovery from COVID-19. 23 Hyperferritinimea in COVID19 and its association with disease severity have been reported in the literature. Hyperferritinemia can assist in recognizing high-risk patients and guide physicians to timely modify their management plan to control inflammation.[22,23]   Hemophagocytic Lymphohistiocytosis is a known complication of many viral infections

In another study done serum ferritin levels remained persistently high in patients who died of COVID-19.[24] Hyperferritinemia persisted in almost 38% of the especially in those having the critical disease. [24]The patients who did not survive had higher serum ferritin levels compared to those who did not survive with COVID-19.[25]

Lymphopenia is also reported in the literature. There is a definite role of lymphocytes in immune homeostasis along with an inflammatory response. Four potential mechanisms of lymphopenia in COVID-19 have been proposed. (1) directly infecting lymphocytes leading to lymphocyte death. The ACE2 receptors are present on Lymphocytes which may act as a receptor for coronavirus.[26] (2) The fact that lymphocytic dysfunction causes direct insult by SARS-CoV-2 on the thymus and spleen needs to be confirmed through further research. (3) Lymphocyte apoptosis can occur due to the dysregulation of inflammatory cytokines.[27] Hyperlacticedemia could lead to reduced lymphocytes as patients who are critically ill with COVID-19 are found to have raised lactic acid levels[28] so the mechanism of lymphopenia can be multifactorial.

A direct correlation between low lymphocyte counts and severity of disease and inflammatory markers was found (P=0.0014). It has been established that those having severe lymphopenia had a prolonged duration of illness. (17.0 days vs. 14.0 days, P = 0.002).[29] This is in agreement with our study as our patients who had severe lymphopenia had extended stays in the hospital on an average of 18 days.[29]

A meta-analysis also showed a worse outcome in those patients who had lymphopenia at the time of admission. This study also evaluated the association of severity of disease and lymphopenia according to age groups and was found to be higher in the elderly as compared to middle age and younger populations. Lymphopenia can be used as a tool to assess the severity of disease and stratify high-risk patients. [30]

In patients with COVID-19, one CBC parameter that has shown to be consistently associated with disease progression is the absolute lymphocyte count. It was also shown that within the first 2 weeks of disease onset lymphocytes were reduced to lower than 5%. In our study, the degree of lymphopenia was irrespective of age or gender as both groups showed lymphocytes less than 5%.[31]

Lymphocyte count is the most commonly deranged CBC parameter inpatient with COVID-19, lymphopenia showed in (approximately 35%-83% of patients).[32,33]

Lymphopenia is found to be persistently low in the severe phase of COVID-19.[32]

The median nadir of the ALC in hospitalized patients with COVID 19 was found to be $(0.4 \times 10^9/L$ vs $1.2 \times 10^9/L)$[33] which was significantly lower. However, the median nadir in our patient was lower than in this study which was found to be $0.2 \times 10^9/L$.

A meta-analysis has shown lymphopenia as a poor prognostic marker in young patients with COVID-19. 34 Lymphopenia defined as a lymphocyte count ≤ of 1100 cells/μL is associated with almost a threefold risk of a poor outcome. As compared to an older patient the association was found to be stronger in younger patients.[34]

The correlation between lymphocytes and ferritin is shown in figure I and it shows a very moderate negative correlation with a correlation coefficient r= -0.6. The correlation will certainly help in the clinical judgment of these patients.

The strong correlation between these variables also established the fact that the pathophysiology between the low lymphocyte counts and serum ferritin seems to have a common origin. The inflammatory cytokine storm plays a primary role in causing lymphopenia. Pro-inflammatory cytokines i.e. IL-6 and TNF-α are closely correlated with lymphopenia. The patients who recover from COVID-19 show almost normal levels of these cytokines.[35] An autopsy performed on lymphoid organs showed massive lymphocyte death which was

primarily due to the high levels of IL-6 as well as Fas-FasL interactions.[35]

The strong negative correlation between these 2 variables (coefficient of -0.60 w) will not just help in assessing the condition and risks of the patient with COVID 19 but will also help to add any intervention and modify the treatment as CBC is a quick cost-effective test and a low lymphocyte count can help in predicting an impending cytokine storm in those patients.

The limitation of the study is the small sample size as we only included patients who were admitted to the ICU. The other limitation is our hospital primarily caters to main women which could not be the true representative of gender distribution the males are mostly those who were our non-registered patients.

Conclusion

There is a significant negative correlation between lymphocyte count and serum ferritin levels which will assist in identifying patients at risk and in their early identification and prompt management.

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