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Evaluation of Serum Ferritin in Hospitalized Patients with COVID-19 as A Potential Biomarker for Assessing COVID-19 Severity

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Abstract:

Introduction: *Corona virus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), has become global pandemic. Pro-inûammatory mediator, serum ferritin is reported to be elevated significantly by different studies in COVID-19. Our study was aimed to find whether serum ferritin level can be employed as a biomarker to assess the disease severity in COVID-19 cases.*

Methods: *This cross sectional observational study was carried out at Armed Forces Institute of Pathology (AFIP), a tertiary referral laboratory between 15 March 2020 and 15 June 2020. Total 2418 hospitalized RT-PCR confirmed COVID-19 patients from Combined Military Hospital (CMH) were included in our study. Serum ferritin was measured by electrochemiluminescence immunoassay and was compared between the severe and non-severe groups. P-value < 0.05 was considered statistically significant.*

Results: *Total patients were 2418, among them 337 (13.9%) from intensive care unit (ICU) and 2081 (86.1%) from non-ICU. Median age and IQR were 60.5 (51.5–68.0) years in ICU patients versus 38.0 (28-46.1) years in non-ICU patients (p <0.0001). Most (86.8%) patients were males; 82.8% in ICU and 87.5% in non-ICU. Serum ferritin was significantly higher (p<0.0001) in ICU patients; median and IQR was 952.8 (529.9 - 1520.5) ng/mL versus 254.2 (156.1 - 441.9) ng/mL (p <0.0001) in non-ICU patients. Serum ferritin, at cut off value (COV) <550 ng/mL had sensitivity 82.36% and specificity 73.59% for categorization of COVID-19 cases as non-severe. Comparison of proportions of ICU and non-ICU patients was found highly significant (p <0.0001 at 95% confidence interval) with this cut off value.*

Conclusions: *Serum ferritin level was significantly high among COVID-19 patients requiring ICU admission than non-ICU cases. Serum ferritin may be used for categorizing COVID-19 patients. Cut off value 550 ng/mL can be meaningfully used for this categorization, above which should be considered severe and need more careful monitoring.*

Key Words: *COVID-19, SARS-CoV-2, Ferritin, Disease Severity, ICU*

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Introduction:

Corona virus disease 2019 (COVID-19), first identified in Wuhan City, Hubei Province, China is caused by severe acute respiratory syndrome corona virus type 2 (SARS-CoV-2).[1] This COVID-19 was officially stated pandemic on the 11th March 2020 by the World Health Organization (WHO).[1] WHO marked six-month anniversary of the COVID-19 outbreak on 29 June 2020 coincides with reaching 10 million cases and 500,000 deaths.[2] As of 27 July 2020, 06:00 GMT, COVID-19 update revealed worldwide cases confirmed 16,114,449 and deaths confirmed 646,641 over the 216 countries or territories.[3]

This COVID-19 is characterized by highly variable clinical presentation; fever, cough, malaise; viral pneumonia; respiratory failure, heart failure, multi-organ dysfunction and death etc.[1] Unremitting fever, hyperferritinemia and hyper-inûammatory process with massive release of pro-inûammatory cytokines are evident in severe COVID-19 has similarity to hyperferritinemic syndrome.[4] This syndrome embraces

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adult onset still's disease (AOSD), systemic juvenile idiopathic arthritis (SJIA), secondary hemophagocytic lymphohistiocytosis (sHLH), catastrophic anti phospholipid syndrome (cAPS), and septic shock.[4] Hyperferritinemia is a common feature in all of these conditions.[6]

Ferritin, an iron storage protein has critical role in iron homeostasis. Apoferritin, the iron-free form of this protein forms a roughly spherical shell of 24 subunits within which ferric iron remains stored. The subunits are of two types, termed H and L. The ratio of these subunits varies widely depending on tissue type and can be modified under inflammatory and infectious conditions.[7]

The heavy H-subunit is primarily responsible for the ferroxidase activity required for the conversion of ferrous to ferric form for internalization and sequestration of iron in the ferritin complex, whereas the light L-subunit facilitates the storage of iron into the ferritin core.[7,8] Majority of serum ferritin is immunologically related to ferritin L-subunit.[7]

Serum ferritin is a surrogate marker of iron status; is also elevated in oxidative stress and inflammation irrespective of iron status, and also in liver disease, decreased lung function and malignancy.[7,9,10] This inflammatory marker has been reported to be significantly associated with the high risk of the development of severe COVID-19.[9]

Inflammatory responses triggered by rapid viral replication of SARS-CoV-2 and cellular destruction recruit macrophages and monocytes that induce the release of cytokines and chemokines.[11] These cytokines and chemokines then attract immune cells and activate immune responses, lead to cytokine storms.[4, 9, 12] Cytokines and chemokines increased in plasma in COVID-19 are IL-1², IL-2, IL-4, IL-7, IL-10, IL-12, IL-13, IL-17, GCSF,IFN-³, TNF, pro-inflammatory IL-6, IP-10, macrophage inflammatory protein 1± (MIP1±), MIP1² and MCP.[4,11] Defective immune response leads to further accumulation of immune cells in the lungs, cause overproduction of pro-inflammatory cytokines. It eventually damages the lung infrastructure along with the resultant cytokine storm circulates to other organs and leads to multi-organ damage. In addition, non-neutralizing antibodies produced by B cells may enhance SARS-CoV-2 infection through antibody-dependent enhancement (ADE), further exacerbates organ damage.[11] Speciûc genetic susceptibility also causes exaggeration of such cytokines release in severe COVID-19. Pro-inûammatory mediators including serum ferritin are found elevated significantly in blood in these COVID-19 patients.[4]

The aim of the study was to compare the serum ferritin status in between severe and non-severe COVID-19 patients and to find its usefulness with a cut off value for categorization of COVID-19 cases.

Methods:

This observational cross sectional study was carried out at Armed Forces Institute of Pathology (AFIP), a tertiary referral laboratory between 15 March 2020 and 15 June 2020. Total 2418 patients with COVID-19 hospitalized patients in CMH (Combined Military Hospital) were enrolled in our study irrespective of clinical presentation, age and sex. Patients were confirmed for COVID-19 by detecting SARS-CoV2 RNA by real-time polymerase chain reaction (RT-PCR) from their nasopharyngeal and oropharyngeal swabs at AFIP. Patients requiring ICU support were considered severe and those admitted in wards/cabins (non-ICU) were considered non-severe cases.

Serum Ferritin was measured in both the groups by using electrochemiluminescence immunoassay by Cobas 6000 analyzer (Roche, USA) with measuring range 0.5 – 2000 ng/mL or up to 100000 ng/mL with 50 fold dilution. Statistical analyses were performed by SPSS software version 20. Data were presented as number and percentages for categorical variables. Continuous variables were expressed as mean ± SD as well as median ± IQR (Inter Quartile Range). Serum ferritin levels were compared between the ICU and non-ICU groups by employing independent t-test; p-value < 0.05 was considered statistically significant.

Results:

Among the total 2418 in-hospital patients, 337 (13.9%) patients were from ICU care and 2081 (86.1%) belonged to non-ICU care.

Mean ± SD of age was 58.9 ± 15.4 years in ICU patients versus 37.7 ±13.0 years in non-ICU patients. Median and inter quartile range was 60.5 years and 51.5 – 68.0 years in ICU patients versus 36.0 years and 28.0- 46.1 years in non-ICU patients (p<0.0001). In this study, among the COVID-19 patients, 86.8% were male and 13.2% were female. Among the ICU patients 82.8% and among the non-ICU patients 87.5% were male versus 17.2% of ICU care and 12.5% of non-ICU care were female (Fig 1).

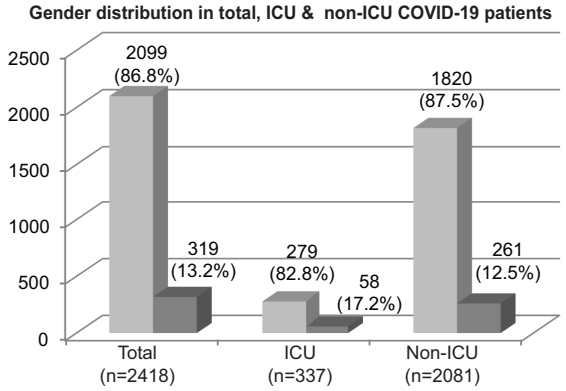

Fig.-1: *Gender Distribution in Total, ICU & non-ICU COVID-19 Patients*

Serum Ferritin level in ICU versus non-ICU patients, mean was 1729.45 ng/mL versus 382.19 ng/mL ; median & IQR was 952.8 (529.9 - 1520.5) ng/mL versus 254.2 (156.1 - 441.9) ng/mL (p <0.0001) (Table I).

Considering sensitivity and specificity for different cut off values (COV) of serum ferritin, COV <550 ng/mL seems to be preferable for non-severe cases with sensitivity 82.36% and specificity 73.59% for categorization of COVID-19. (Table II). p-value for comparison of proportions of ICU and non-ICU patients was found highly significant (p <0.0001) at 95% confidence interval with this COV.

Table-I

Serum Ferritin in ICU & non-ICU COVID-19 Patients

Serum Ferritin	Total (n=2418)	ICU Patients (n=337)	Non-ICU Patients (n=2081)	p-value
Mean (ng/mL)	569.96	1729.45	382.19	<0.0001
Median (IQR) (ng/mL)	289.4 (169.5 – 560.4)	952.8 (529.9 - 1520.5)	254.2 (156.1 -441.9)	

Table-II

Different Cut off Values of Serum Ferritin with Sensitivity and Specificity for the Differentiation of ICU & non-ICU Patients

Serum Ferritin COV	Frequency among ICU patients (n=337)	Frequency among Non-ICU patients (n=2081)	ICU (%) Vs non-ICU (%) patients (p-value)	Sensitivity for non-ICU cases	Specificity for non-ICU cases
<250 ng/mL	30/337	1025/2081	8.9% vs 49.2% (p<0.0001)	49.26%	91.10%
<350 ng/mL	45/337	1370/2081	13.35% vs 65.8% (p<0.0001)	65.83%	86.65%
<450 ng/mL	71/337	1572/2081	21% vs 75.5% (p<0.0001)	75.54%	78.93%
<550 ng/mL	89/337	1714/2081	26.4% vs 82.4% (p<0.0001)	82.36%	73.59%
<650 ng/mL	109/337	1801/2081	32.3% vs 86.5% (p<0.0001)	89.65%	67.66%
<750 ng/mL	125/337	1873/2081	37.1% vs 90% (p<0.0001)	90.00%	62.91%

* COV: Cut off value

Discussion:

We found serum ferritin increased significantly in severe patients with median (IQR), 952.8 (529.9 - 1520.5) ng/mL in compare to non-severe patients, 254.2 (156.1 - 441.9) ng/mL ($p < 0.0001$) (Table I). It indicates serum ferritin can be used as a very effective and independent marker for assessment of severity of COVID-19. Increase in serum ferritin in bacterial and/or viral infection results from the release of iron from the reticulo-endothelial system, decreased ability of transporting ferritin in liver and spleen and increased synthesis and release of intracellular ferritin. Elevated serum ferritin can predict a poor outcome in hospitalized patients with influenza infection.[13] Chen et al. reported that most patients (63%) of COVID-19 had their serum ferritin beyond the upper reference limit.[14] Bo Zhou et al in their study found, serum ferritin level was increased in both severe and very severe COVID-19 cases but was significantly elevated in very severe patients compared with that of severe patients; median 1006.16 ng/ml (IQR: 408.265-1988.25) vs 291.13 ng/ml (IQR: 102.1-648.42), respectively.[13] In agreement with this, Fei Zhou et al study revealed serum ferritin was elevated in non-survivors compared with survivors throughout the clinical course, and increased with deterioration of illness. They found median (IQR) of serum ferritin 1435·3 (728·9–2000·0) in non-survivors versus 503·2 (264·0–921·5) in survivors ($p < 0·0001$).[15] A retrospective, multicenter study on 150 confirmed COVID-19 cases in Wuhan, China, observed elevated ferritin, mean 1297·6 ng/ml in non-survivors vs 614·0 ng/ml in survivors ($p<0·001$), with elevated IL-6 ($p<0·0001$) suggested that mortality might be due to virally driven hyperinflammation.[16] Mehta et al[16] recommended all patients with severe COVID-19 should be screened for hyperinflammation using laboratory trends including elevated ferritin for whom immunosuppression might improve critical and life-threatening illness. A total of 16 studies comprising 3962 patients with COVID-19 were included in a meta-analysis by Furong Zenga et al showed the non-severe group had lower levels for serum ferritin (Weighted Mean Difference = -398.80 mg/l, 95% CI = [-625.89, -171.71], $P < 0.001$), compared with the severe group. They recommended further research to find the positive correlation between serum ferritin level and the severity of COVID-19.[9]

In our study, median and inter quartile range of age was 60.5 (51.5 – 68.0) years In ICU patients and 36.0 (28-46.1) years in non-ICU patients (p value <0.0001), was statistically highly significant. We observed older age was related to severity of the disease, is also supported by Zhou et al study.[15]

Most of our patients were males, in our study 2099 (86.8%) were male, among them 1820 (87.5%) were in non-ICU care and 279 (82.8%) were in ICU care. Exact mechanism behind this is yet to establish. But, it can be assumed that immune-related genes of the whole genome are largely on X chromosome and female because of their two copies of X chromosomes have more pronounced innate and adaptive immunity for better response than male against COVID-19.[17,18]

Clinical features, other infection biomarkers and variable timing for ferritin estimations were not considered in this study were the limitations of our study.

Conclusion:

Severe COVID-19 (ICU cases) had significantly high ferritin levels in comparison with non-severe group (non-ICU cases). Serum ferritin level was related to the severity of COVID-19. Hence, serum ferritin might be used as a relatively available and prognostic decisive marker for categorization of COVID-19 effectively. Measurement of serum ferritin can contribute clinicians in management of such patients. Further research should go on to evaluate, either this high ferritin is an active pathogenic initiator or is a consequence of inûammation in COVID-19.

Conflict of interest:

The authors have no conflict of interest.

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