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Association of iron-related biomarkers with severity and mortality in COVID-19 patients

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ABSTRACT

Background: Nutritional deficiency is associated with weaken immune system and increased susceptibility to infection. Among other nutrients, several trace elements have been shown to regulate immune responses. Iron is one of the most abundant trace elements present in our body, which is required in various biological processes. Iron has an immunomodulatory function and thus influence the susceptibility to the course and outcome of a variety of viral infections. So, this present study was aimed to study relations of different iron-related biomarkers in association to severity and mortality in SARS-CoV-2 patients.

Materials and methods: A total of 150 individuals infected with COVID-19 and 50 healthy individuals were recruited. Cases were divided based on severity (mild, moderate, and severe) and outcome (discharged or deceased). Serum iron, TIBC, ferritin, transferrin, transferrin saturation levels were analyzed by the direct colourimetric method.

Results: In cases the median levels of serum iron, TIBC, transferrin, transferrin saturation and ferritin are 29 μg/dL, 132.53 μg/dL, 106.3 mg/dL, 17.74 % and 702.9 ng/dL respectively. Similarly, in controls the median levels of serum iron, TIBC, transferrin, transferrin saturation and ferritin are 53 μg/dL, 391.88 μg/dL, 313.51 mg/dL, 12.81 % and 13.52 ng/dL respectively. On comparing the cases with the controls, a significant lower level of iron, TIBC, and transferrin were found in the cases along with the significant higher levels of ferritin and transferrin saturation. On comparing the Receiver operating characteristic (ROC) curves of Iron, Ferritin, Transferrin, Transferrin sat % and TIBC in relation to survival in COVID-19 patients it was found that iron, followed by transferrin and ferritin has the highest area under the curve (AUC) with 74 %, 63 % and 61 % respectively. Further, in pairwise analysis of ROC curve, a significant difference was found between the Iron-transferrin ($p < 0.01$), iron-TIBC ($p < 0.001$) and transferrin-ferritin ($P < 0.01$). The multiple regression model based on Iron and transferrin outperformed any other combination of variables via stepwise AIC selection with an AUC of 98.2 %. The cutoff point according to Youden's J index is characterized with a sensitivity of 98 % and a specificity of 96.8 %, indicating that iron along with transferrin can be a useful marker that may contribute to a better assessment of survival chances in COVID-19.

Conclusion: Our study demonstrated a significantly decreased levels of iron, TIBC, & transferrin and a significantly increased levels of ferritin and transferrin saturation in COVID-19 patients when compared with controls. Further, Iron and transferrin were observed to be a good predictor of mortality in patients with COVID-19. From the above analysis we confirm that iron-related biomarkers play an important role in the development of oxidative stress and further lead to activation of the cytokine storm. So, continuous monitoring of these

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parameters could be helpful in the early detection of individuals developing the severe disease and can be used to decrease mortality in upcoming new waves of COVID-19.

1. Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by the severe acute respiratory distress syndrome-associated coronavirus-2 (SARS-CoV-2) [1]. The deterioration of lung function has been attributed to a maladaptive immune response rather than increased viral loads [2–4]. SARS-CoV-2 infection can activate innate and adaptive immune responses. However, uncontrolled inflammatory innate responses and impaired adaptive immune responses may lead to harmful tissue damage, both locally and systemically in the reason known reasons for the highest mortality [5,6].

Many recent studies have shown that trace elements play a major role in the pathogenesis of the disease [7,8]. Iron is one of the most abundant trace elements present in our body, which is required in various biological processes [9]. Similar to humans, viruses also depend on iron for various fundamental processes. SARS-CoV-2 use angiotensin-converting enzyme 2 (ACE2) receptor to enter the host cell and causes damages to tissue and organs [10]. Iron homeostasis plays a crucial role in the human immune defence system, as its content in macrophages regulates the generation of many pro-inflammatory cytokines [11]. Iron was also found to be promoting the differentiation of macrophages and lymphocytes and immune cell metabolism [12].

Various indices are used to assess the adequacy of iron status. Serum iron, serum ferritin, serum transferrin, transferrin saturation and total iron-binding capacity are some of the available indicators of iron status. Among the listed parameters, serum ferritin is the most commonly used method to assess the iron status in clinical and public health settings [13]. Ferritin, being an iron storage protein, will be dependent on the overall levels of iron present in the body [14]. Apart from ferritin, serum transferrin concentrations are also a sensitive indicator of body iron stores [15].

The association of iron status with COVID-19 prognosis has been preliminarily explored. Haematological abnormalities in COVID-19 is a constitutive component in critically ill patients [16]. Dysregulation of heme iron balance has been observed in COVID-19, mostly including Anaemia, low serum iron, hyperferritinemia, increased variation of red blood cell distribution width (RDW), and hypoxemia [17]. A previous study in SARS found that serum iron levels decreased in confirmed patients [18], and similar reports were also reported in SARS CoV-2 showing serum iron level was one of the independent risk factors for the severity and death in COVID-19 patients[19]. A high concentration of serum ferritin was also found to be associated with poor outcomes in COVID-19 patients[20,21]. However, recent studies were descriptive case series or had a relatively small sample size. An in silico analysis pointed to a relevant role of SARS-CoV-2 proteins in viral physiopathology. In recent in silico studies, it was found that SARS CoV − 2 has a spike protein that mimics the activity of hepcidin, called covidin [22]. So, increasing the levels of both hepcidin and covidin will lead to toxic accumulation of intracellular iron and end-organ damage in COVID-19 [23–25]. Further, rapid onset of hypoxia might be seen due to low erythropoiesis caused due to low serum iron, rapidly deteriorating a patient's condition and leading to high morbidity. Although iron metabolism plays a central role in many viral infections, its role in the COVID 19 patients is still not fully studied. So, this present study was planned to investigate the role of different iron-related markers and their use as early indicators in detecting the severity and mortality in COVID 19 patients.

2. Materials and methods

This study includes150 patients hospitalised in All India Institute of medical sciences (AIIMS), Jodhpur India and 50 healthy controls. All these patients were confirmed with SARS CoV-2 infection by reverse transcription-polymerase chain reaction using the WHO guidelines[26]. All the patients were followed up to the death or discharged from the hospital. The patients were classified into 3 categories: mild, moderate and severe based on the type of ward admission (Stable, HDU and ICU) in the hospital. A total of 50 controls were also included in the study was approved by the institutional ethics committee of AIIMS Jodhpur, India.

All the demographic information and clinical outcomes were taken from the hospital information system of the institute. The inflammatory markers like Interleukin-6 (IL-6), c Reactive protein (CRP), Lactate dehydrogenase (LDH) and iron markers like iron, transferrin, Transferrin saturation %, total iron binding capacity (TIBC) and ferritin were measured. A follow-up sample at the discharge was taken for the discharged patient and deceased patients, a sample near to the time of death was taken.

Serum Iron and UIBC were measured by immunoturbidometric method using the 2,4,6-tripyridyls-triazine (TPTZ) without deproteinization method and 2-Nitroso-5-(N-n-propyl-N-(3-sulfopropyl) amino) phenol method (Nitroso- PSAP) respectively on the on fully automated chemistry analyser Beckman Coulter AU680 [27]. TIBC was calculated from adding the both UIBC and iron. Serum transferrin was measured using the immunoturbidometric method by the Diasys kit (DiaSys Diagnostic Systems, Germany) [28]. Transferrin saturation was measured using formula (serum iron/TIBC)* 100). Serum ferritin was measured using the sandwich chemiluminescent immunoassay using a solid phase monoclonal antibody coated in to a magnetic particle and the other monoclonal antibody (mouse) is linked with the isoluminol derivative (isoluminol-antibody conjugate [29]. For serum Iron and UIBC the quality control tested reference material was taken from Lyphochek assayed Chemistry Control Level 1 (range: Iron 47.80−77.20 μg/dL, UIBC 55–104 μg/dL) and Level 2 (range: Iron 222–283 μg/dL, UIBC 114–168 μg/dL) (Bio-Rad Laboratories). For transferrin the TruLab Protein, a liquid-stable control based on human blood material (plasma) Level 1(range: 105–158 mg/dL) and Level 2 (284–426 mg/dL) was used. For IL-6 For serum ferritin the quality control material was Lyphochek immunoassay plus controls Level 1 (range: 42.5–70.8 ng/mL), Level 2 (range:103–171 ng/mL) and Level 3 (range: 256–444 ng/mL) (Bio-Rad Laboratories). The quality controls were run before analysing the samples and the values below the ± 2 standard deviation were taken as cut off for quality passing.

2.1. Total antioxidant status (TAS)

Further to know the oxidative stress, we have measured the serum total antioxidant status (TAS) levels. It was measured using a spectrophotometric method by Rel Assay Diagnostics kit (Rel assay, Turkey). TAS was measured by using the 2, 2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS+). Depending on the antioxidant concentration present in the sample the ABTS+ (dark blue colour) gets reduced to ABTS form (colourless). The change in the colour is measured at 660 nm, which is directly proportional to the total antioxidant levels of the sample.

2.2. Statistical analysis

The data collected were analysed using Microsoft Excel and SPSS version 21.0 (IBM SPSS Statistics, USA) for windows. The normality of the parameters was checked using the Shapiro-Wilks test and found to be non-parametric. Data were expressed as median (IQR) for continuous variables, whereas categorical variables were described as their

Table 1
Demographic and biochemical parameters of the study population.

Variables		Mild (50)		Moderate (50)		Severe (50)		Controls (50)	
Demographic Data									
		Median	Range	Median	Range	Median	Range	Median	Range
Age (In years)		64	32–96	60.5	18–73	60	18–81	30	19–52
Outcome	Discharged	42		31		26		–	
	Deceased	8		19		24			
Gender	Males	32		36		33		23	
	Females	18		14		17		27	
Biochemical Profile									
Urea (mg/dL)		54	16–154	44	18–155	55	9–297	21	11–37
Creatinine (mg/dL)		0.99	0.48–4.21	0.98	0.64–5.74	1.15	0.08–10.53	0.795	0.57–1.12
Aspartate aminotransferase (U/L)		34.7	11.4–255.2	31.5	12.7–90.3	31	7.5–218.3	7.8	3.1–41.8
alanine aminotransferase (U/L)		47.5	17.4–306	38.15	19.6–142	40.8	12.2–333.5	21.5	13.3–69.6
Total bilirubin (mg/dL)		0.61	0.29–1.99	0.64	0.32–1.83	0.71	0.26–1.88	0.215	0.15–0.84
Direct bilirubin (mg/dL)		0.18	0.05–1.04	0.18	0.06–0.64	0.23	0.05–0.69	0.04	0–0.16
Total protein (g/dL)		6.42	4.51–8	6.285	4.89–7.41	6.01	4.55–7.44	7.615	6.57–8.65
Albumin (g/dL)		3.22	2.13–4.48	2.93	1.96–3.83	2.93	1.71–3.87	4.335	3.54–5.27
ALP (U/L)		104	59–374	117.5	64–256	122	44–742	75.5	31–190
Cholesterol (mg/dL)		116	61–251	166	101–231	117	53–223	155	90–284
Triglycerides (mg/dL)		125.5	61–240	118	62–487	129	55–341	101.5	49–411
High-density lipoprotein (HDL) (mg/dL)		31	12–61	43	24–58	33	15–44	42.5	20–96
Low-density lipoprotein (LDL) (mg/dL)		82.5	31–193	126	52–177	74.5	33–169	100.5	45–185
Sodium (mmol/L)		133	119–144	136	124–144	136	121–149	142	135–154
Pottasium (mmol/L)		4.525	3.18–7.37	4.42	3.44–6.47	4.24	1.9–7.37	4.415	3.66–5.29
Chlorides (mmol/L)		98	81–108	98	88–110	100	74–113	107	101–117
Procalcitonin (ng/mL)		4.205	2.5–15.5	7.304	5.04–23.62	10.322	0.0248–91.6	0.05	0.02–1.1
Interleukin-6 (pg/mL)		26	2.3–122.1	32	2.5–131.8	1554	3.1–5500	2	0–3.9
C-Reactive protein (CRP), (mg/L)		134.7	0.5–190	133.305	29.1–188	76.7	0.9–191.1	1	0.1–12.5
Lactate dehydrogenase (LDH) (mg/dL)		413	112–1166	393	301–623	459	203–1338	109.5	74–198
partial thromboplastin time (APTT) (s)		26.5	18–78	30	18–59	29	18–84	24	21–35
Prothrombin time (PT) (s)		13.65	11.49–20.5	15.75	11.4–29	15.4	11–29	11	11–13.5
international normalized ratio (INR)		1.105	0.98–1.49	1.225	0.89–2.3	1.18	0.9–2.3	0.7	0.50–1.1
D.dimer (ug/Ml)		1.69	0.27–39.9	1.98	0.27–39.9	1.55	0.27–20	0.3	0.1–0.5

Table 2
Comparison of different iron markers between the controls and cases.

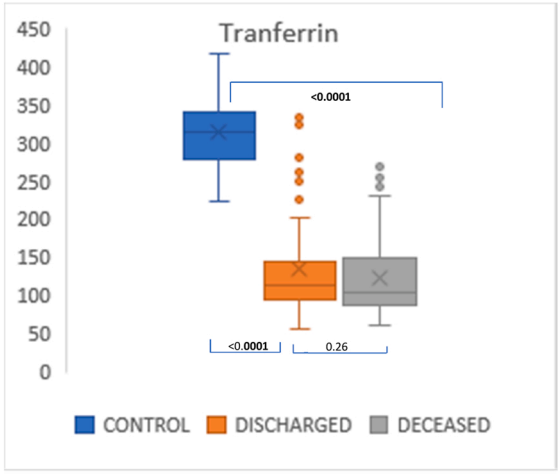
		Iron (μg/dL)	TIBC (μg/dL)	transferrin (mg/dL)	transferrin sat (%)	Ferritin (ng/dL)	TAS (mmol/L)
CONTROL	N	50	50	50	50	50	50
	Median	53	391.88	313.51	12.81	13.52	1.35
	25th	49	353.63	282.91	11.87	5.43	1.24
	75th	63	423.71	338.97	13.05	51.15	1.67
COVID-19	N	150	150	150	150	150	150
	Median	29	132.53	106.03	17.74	702.90	0.73
	25th	17.50	113.51	90.81	9.01	432.70	0.65
	75th	48.50	183.75	147	33.08	1175	1.01
p-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.01

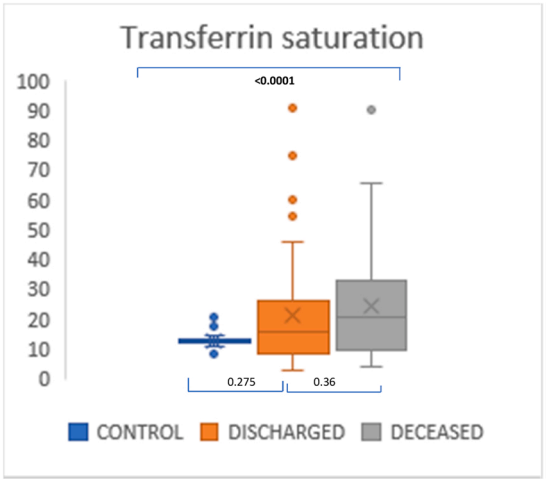
All bold values indicate significant p value ($p < 0.05$)

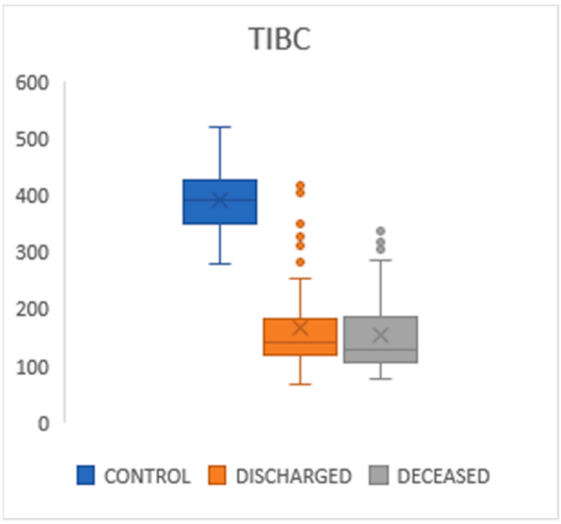
Table 3
Comparison of baseline different iron markers between different grades of severity of COVID and controls.

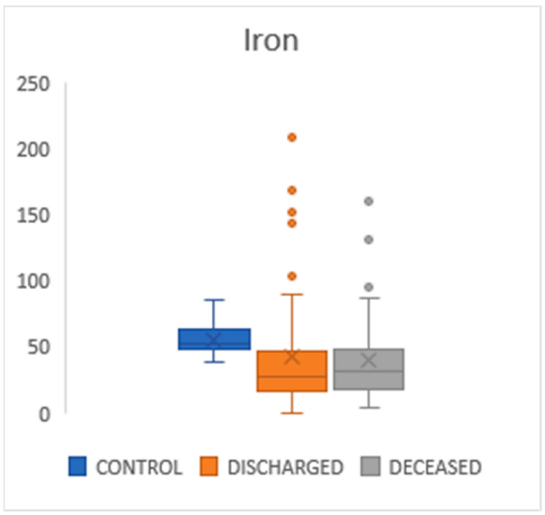
		Iron (μg/dL)	TIBC (μg/dL)	transferrin (mg/dL)	transferrin sat (%)	Ferritin (ng/dL)
Control	N	50	50	50	50	50
	Median	53	391.88	313.51	12.81	13.52
	25th	49	353.63	282.91	11.87	5.43
	75th	63	423.71	338.97	13.05	51.15
Mild	N	50	50	50	50	50
	Median	28.50	165.28	132.23	17.20	674.2
	25th	14	117.34	93.80	8.45	418.8
	75th	87.5	253.53	202.83	36.20	1350.5
Moderate	N	50	50	50	50	50
	Median	30	134.8	107.8	16.85	550.35
	25th	17.72	114.4	91.5	8.91	351.70
	75th	58.75	171.2	137	33.98	926.12
Severe	N	50	50	50	50	50
	Median	26	129.58	103.66	21.3	680.85
	25th	16	105.87	84.70	9.1	169.35
	75th	44	188.88	103.66	28.4	1068.03
p-value		< 0.0001	< 0.0001	< 0.0001	0.062	< 0.0001

All bold values indicate significant p value ($p < 0.05$)









	p-value			
	Di vs De	De vs C	Di vs C	De vs Di vs C
TIBC	0.78	**<0.0001**	**<0.0001**	**<0.0001**
Tranferrin	0.269	**<0.0001**	**<0.0001**	**<0.0001**
Transf sat.	0.369	**0.006**	0.275	<0.**0001**
Iron	0.61	**<0.0001**	**<0.0001**	**<0.0001**

De-Deceased; Di-Discharged patients; C-Controls; All bold values indicate significant p value ($p<0.05$)

Fig. 1. Comparison of different iron parameters between discharged patients, deceased patients and controls:.

respective percentages. The difference in serum trace elements levels between subgroups was analysed using the Kruskal Wallis test and Mann-Whitney U test. Correlation analysis between the parameters was analysed by Spearman correlation. Receiver operating characteristic (ROC) curve analysis was carried out to evaluate the usefulness of different parameters in measuring the survival odds in COVID-19 patients. A p-value < 0.05 was considered statistically significant.

3. Results

A total of 200 individuals were recruited in this study, of this 150 were hospitalised patients who were tested positive for COVID 19 and 50 were healthy controls. The median age of cases was 61.0 years old (IQR: 55–66.0). Among them, 57 (63 %) were male and 33 (37 %) were females. All the patients were classified on admission according to the severity as mild (50), moderate (50) and severe cases (50) (Table 1). All the patients had determined outcomes whether discharged (66 %) or deceased (34 %). Demographic and laboratory parameters including the Inflammatory markers and iron markers were included in Table 1.

The results of the comparison of different iron markers between the controls and cases are summarised in Table 2. The median levels of serum iron, TIBC, transferrin, transferrin saturation and ferritin of the total cases were 29 μg/dL, 132.53 μg/dL, 106.3 mg/dL, 17.74 % and 702.9 ng/dL respectively. Similarly, the median levels of serum iron, TIBC, transferrin, transferrin saturation and ferritin of the controls are 53 μg/dL, 391.88 μg/dL, 313.51 mg/dL, 12.81 % and 13.52 ng/dL respectively. The difference between all the parameters was found to be statistically significant in total cases with lower levels of iron, TIBC, transferrin, and higher levels of ferritin and transferrin saturation.

Furthermore, on comparing the different groups of the cases with the

Table 4
Comparison of baseline and follow up sample of discharged patients.

		Iron (μg/dL)	TIBC (μg/dL)	transferrin (mg/dL)	transferrin sat (%)	Ferritin (ng/dL)
Discharged Patients						
Baseline	N	87	87	87	87	87
	Median	27	142.55	114.07	16.74	597.55
	25th	16.50	113.51	95.105	9.01	276.92
	75th	47.50	183.75	144.735	33.08	1009.32
Follow up	N	87	87	87	87	87
	Median	29	142.5	104.96	15.61	605.90
	25th	19	118.60	84.34	8.30	320.70
	75th	50	181.80	147.12	32.33	1047
p-value		0.48	0.22	0.042	0.52	< 0.001
Deceased Patients						
Baseline	N	63	63	63	63	63
	Median	26.52	128.71	102.97	21.20	702.90
	25th	18	107.80	86.28	9.63	432.7
	75th	47.75	186.07	148.86	33.69	1175
Follow up*	N	63	63	63	63	63
	Median	26	127.20	96.11	22.31	1002.5
	25th	16.30	107.50	84.98	10.61	525.46
	75th	45.50	184.30	125.98	35.32	1258.20
p-value		0.03	0.52	0.26	0.65	< 0.02

* the last sample received to the clinical biochemistry lab before the death of the patient; All bold values indicate significant p value ($p < 0.05$)

controls a significant difference was found in the levels of all the iron markers except for transferrin saturation. All the parameters were found to be low in the case groups when compared to the controls. The median levels of serum iron, TIBC and transferrin were found to be lowest in the severe group. Although the difference was not significant the transferrin saturation was found to be highest in the severe group. The levels of TIBC (mild: 165 μg/dL, moderate: 134 μg/dL & severe: 129 μg/dL) and transferrin (mild: 132 μg/dL, moderate: 107 μg/dL & severe: 103 μg/dL) was found to be decreasing with increasing severity, whereas the transferrin saturation (mild: 17.2 %, moderate: 16.5 % & severe: 21.3 %) and ferritin (mild: 674 ng/dL, moderate: 550 ng/dL & severe: 680 ng/dL was increasing with increasing severity (Table 3). While the total antioxidant status levels were characterized by a significant decrease in patients with mild, moderate, and severe COVID-19, being 13 %, 46 % and 64 % respectively, lower than the healthy controls.

On comparing these cases based on the outcome (i.e., Deceased vs Discharged), 40 % of the total cases had a fatal outcome, of which 15 % were from mild cases, 36 % were from moderate cases and 49 % were from severe cases. On comparing the levels of these iron parameters based on the outcome, we found that no significant difference was found between the deceased and discharged patients (Fig. 1). We also found a significant difference in the levels of Iron ($p < 0.0001$), TIBC ($p < 0.00001$), transferrin ($p < 0.0001$), transferrin saturation ($p < 0.0001$) and Ferritin ($p < 0.0001$) when compared between the controls, discharged and deceased individuals. On comparing the levels of TAS based on the outcome, we found that no significant difference was found between the deceased and discharged patients. Similarly, we have also compared the iron marker levels between the baseline and follow-up samples of discharged patients, the serum levels of Iron, TIBC and transferrin has been increased approximately 3–6 % each in follow-up samples, with a significant difference only in transferrin levels ($p = 0.042$). A similar significant and non-significant decrease in ferritin and transferrin saturation was found respectively in the follow-up samples when compared to the baseline samples (Table 4). Likewise, we have measured these parameters in baseline and follow up samples (the last sample received to the clinical biochemistry lab before the death) for deceased patients. On comparison of baseline and follow up samples between deceased and discharged patients we found a significant increase in the ferritin levels in follow up samples of deceased patients and a significant decrease in the follow up samples of discharged patients. Although iron levels are decreased in follow up samples of deceased patients the change in the levels were not significant (Table 5).

Correlation analysis On doing the correlation analysis between the inflammatory and haematological markers with the Iron markers (Table 6), we find that age has a significant positive correlation with the IL-6 (0.15, $p < 0.05$). Similarly, age also has a significant positive correlation with the LDH and D-Dimer in the COVID 19 cases. Further, the iron has a significant negative correlation with the inflammatory markers like hsCRP (-0.31, $p < 0.01$), LDH (-0.14, $p < 0.05$), Ferritin (-0.26, $p < 0.05$) and IL-6(-0.22, $p < 0.05$). transferrin was found to have a significant positive correlation with the TIBC (0.74, $p < 0.01$) and a significant negative correlation with the IL-6 (-0.23, $p < 0.05$) and ferritin (-0.38, $p < 0.01$).

Finally, receiver operating characteristic (ROC) curve analyses were conducted to assess the potential value of Iron, transferrin, transferrin saturation, TIBC and ferritin for improving the survival odds in COVID-19 (Fig. 2A). Here we have tested all the parameters individually and in combination also using regression analysis. In individual analysis Iron, followed by transferrin and ferritin has the highest area under the curve

Table 5
Comparison of Baseline and follow up samples between deceased and discharged patients.

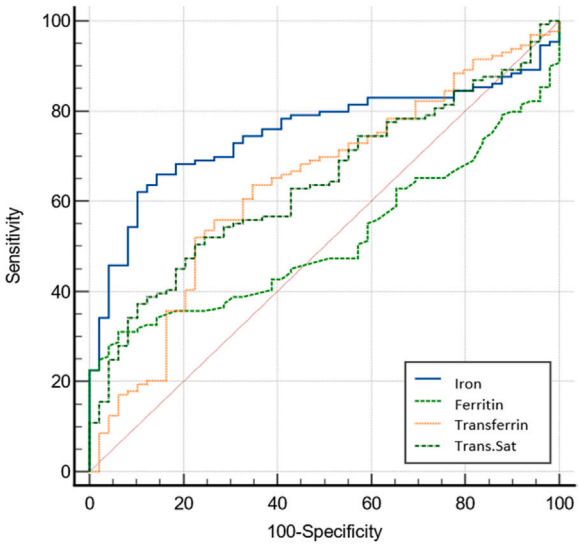
		Iron (μg/dL)	TIBC (μg/dL)	transferrin (mg/dL)	transferrin sat (%)	Ferritin (ng/dL)
Baseline Sample						
Discharged Patients	N	87	87	87	87	87
	Median	27	142.55	114.07	16.74	597.55
	25th	16.50	113.51	95.10	9.01	276.92
	75th	47.50	183.75	144.73	33.08	1009.32
Deceased Patients	N	63	63	63	63	63
	Median	26.52	128.71	102.97	21.20	702.90
	25th	18	107.80	86.28	9.63	432.7
	75th	47.75	186.07	148.86	33.69	1175
p-value		0.46	< 0.001	< 0.0001	0.052	< 0.01
Follow-up sample*						
Discharged Patients	N	87	87	87	87	87
	Median	29	142.50	104.96	15.61	605.90
	25th	19	118.60	84.34	8.30	320.70
	75th	50	181.80	147.12	32.33	1047
Deceased Patients*	N	63	63	63	63	63
	Median	26	127.20	96.115	22.31	1002.50
	25th	16.30	107.50	84.98	10.61	525.46
	75th	45.50	184.30	125.98	35.32	1258.20
p-value		0.23	< 0.01	0.12	0.13	< 0.001

* the last sample received to the clinical biochemistry lab before the death of the patient; All bold values indicate significant p value ($p < 0.05$)

Table 6
Correlation analysis between the inflammatory and haematological markers with the iron markers.

	Age	Trans	T-SAT	TIBC	Ferritin	PCT	IL-6	APTT	PT	INR	MNPT	d.dimer	Fib.	iron	LDH	TAS
Age	1															
Trans	-0.16	1														
T-SAT	0.08	-0.32 **	1													
TIBC	-0.16	0.74 **	-0.32 **	1												
Ferritin	0.34	-0.38 **	0.11	-0.38 **	1											
PCT	-0.12	-0.11	0.12	-0.21	0.04	1										
IL-6	0.15 *	-0.23 *	-0.09	-0.21	0.14 *	0.66 **	1									
APTT	0.01	-0.20	-0.12	-0.20	0.23	0.23	0.25	1								
PT	-0.18 *	-0.07	-0.20	-0.37	-0.17	-0.12	0.21	0.46 **	1							
INR	-0.03	-0.18	-0.09	-0.18	0.15	0.18	0.11	0.56 **	0.85 **	1						
MNPT	-0.11	-0.33	-0.14	-0.33	-0.25	-0.16	0.24	0.14	0.89 **	0.16	1					
d.dimer	0.27	-0.07	-0.23	-0.25	0.09	0.13	0.31	0.19	0.26 **	0.23 *	0.19 *	1				
Fibrinogen	-0.19	0.14	-0.12	0.24	-0.21	-0.43	-0.24	-0.13	0.15	-0.17	0.15	0.38	1			
Iron	0.32	-0.09	0.41 * *	-0.09	-0.26	0.22	-0.22 *	-0.26	-0.16	-0.13	-0.28	-0.13	-0.14	1		
LDH	0.21 *	-0.28	0.22	-0.23	0.17 *	-0.09	-0.33	0.36	0.15	0.28 *	0.08	0.29	-0.20	-0.14 *	1	
TAS	-0.36 *	-0.24	0.12	-0.15	-0.39 *	0.013	-0.45 *	-0.08	0.02	-1.88 *	0.54 *	-0.33 *	-0.21	0.26	-0.45 *	1

*p < 0.05; ** p < 0.001



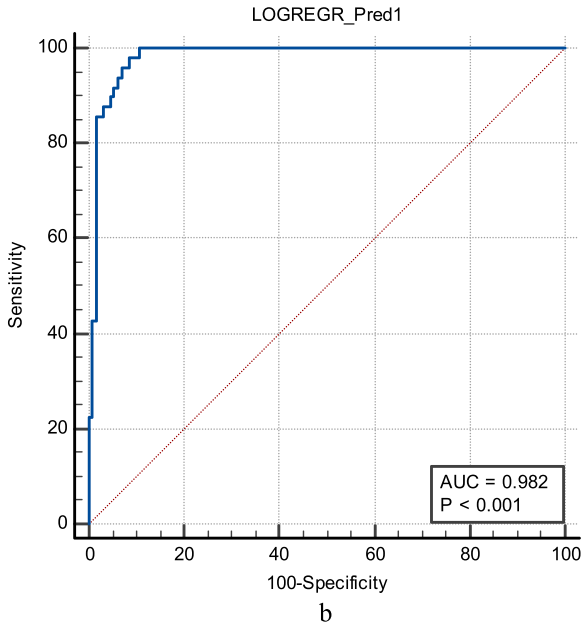


Fig. 2. a Receiver operating characteristic (ROC) curves of Iron, Ferritin, Transferrin, Transferrin sat % and TIBC in relation to survival in COVID-19 patients. b The multiple regression model based on Iron and transferrin outperformed any other combination of variables via stepwise AIC selection. The final model, based on these two parameters, yielded the highest AUC of 98.2 %.

(AUC) with 74 %, 63 % and 61 % respectively. In pairwise analysis, a significant difference was found between the Iron and transferrin ($p < 0.01$), similar results were also seen in Iron-transferrin saturation ($P < 0.01$), Iron-TIBC ($p < 0.01$) and TIBC-transferrin saturation ($p < 0.01$). The results indicate that Iron along with transferrin provides a piece of reliable information on COVID-19 course and survival odds, and outperformed other variables as well as combinations thereof, yielding an area under the curve (AUC) 98.2 % (Fig. 2B). The cut point according to Youden's J statistics is characterized by a sensitivity of 98 % and a specificity of 96.8 %, indicating a useful marker that may contribute to a better assessment of survival chances in COVID-19.

4. Discussion

Iron is an essential trace element, which plays an important role in

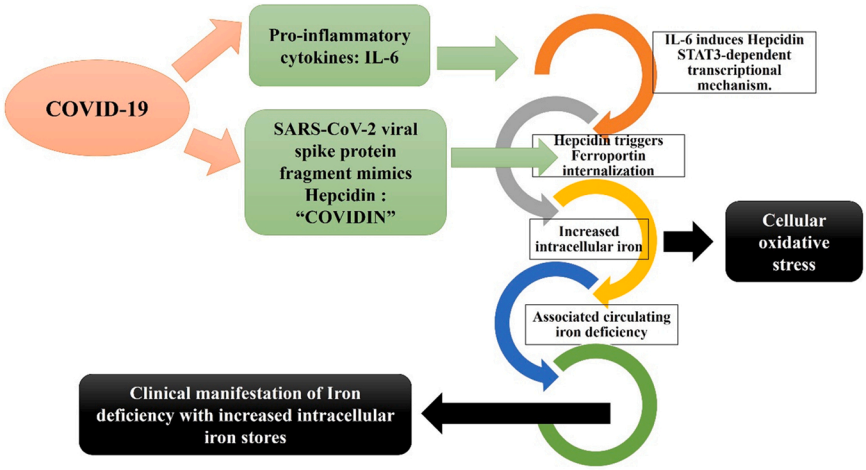


Fig. 3. Mechanism of alteration of iron metabolism in COVID 19. The release of IL-6 increases the production of C reactive protein a marker of systemic inflammation from the liver, similarly, this also leads to an increase in the synthesis of an iron regulating peptide called Hepcidin (covidin in COVID) and decrease the production of transferrin and internalisation of ferroportin. Hepcidin is also released by the macrophages due to the action of IL-6. Hepcidin is a major intracellular regulator of iron by triggering the ferroportin leading to increased intracellular iron. So, this Iron-hepcidin/covidin-transferrin axis is a major pathway responsible for the decreased iron in inflammatory.

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redox reactions. Other than redox reactions it also acts as a cofactor for many essential cell processes like ATP production and cell cycle regulation [30]. Due to this many viruses are known to depend on iron for their replication in the host cell [31]. Some viruses like HCV and HBV are known to selectively affect the iron acquiring cells through the transferrin receptor. Although iron metabolism plays a central role in many viral infections, its role in the COVID 19 patients is still not fully studied. So, this present study was planned to investigate the role of different iron-related markers and their use as early indicators in detecting the severity and mortality in COVID 19 patients.

In this study, we found a significant decrease in serum iron levels in the COVID 19 patients when compared to the controls, however, the iron levels were having an inverted U shaped association with the severity of the cases with the lowest levels in severe patients. Many recent studies had reported similar reports, with a significant decrease of iron levels in severe patients [19,20,32,33]. To contrary, a study by Tojo et al. has reported a U shape association of iron levels with the COVID 19 severity [34]. Similarly, there are reports, that suggest no significant relation in iron levels in COVID patients [35]. However, ICU admission was reported by them to be associated with serum ferritin and transferrin. Although our study iron levels are not in line with the above study, the ferritin and transferrin levels are significantly associated with the severity of the disease. A recent case series from UK and China have also reported low serum iron levels in severe patients [19,20].

Serum ferritin, an acute-phase protein is recognised as a marker of acute & chronic inflammation [36]. It is widely elevated in many inflammatory conditions like acute infections, Autoimmune diseases etc., This increased ferritin reflects the increased total body iron storage, which is recognised as anaemia of inflammation [37]. This type of iron deficiency in inflammation is presumed as a defence mechanism to restrict serum iron from utilization by pathogens [37–39]. In our study, along with the ferritin, transferrin was also found to be decreased in COVID 19 patients, when compared to the controls and also found to be inversely associated with the severity of COVID 19. This is in line with the many previous studies, which reported increased ferritin levels are associated with the severity [40–42]. This high expression of ferritin in COVID 19 is due to the elicitation of IL-6. Transferrin is also an acute-phase protein that tends to decrease in infections. Our results were found to be in line with the previous studies, which reported a decrease in transferrin levels in SARS associated diseases [43]. Similarly, TIBC is also seen to be decreased in COVID 19, because it imitates the transferrin levels.

Various factors regulate the iron metabolism both at cellular and systemic levels. The uptake of iron into the cell is made possible by transferrin receptors that bind to transferrin and endocytose the complex through the clathrin-dependent pathway [44]. The iron regulatory proteins controls the expression of genes of transferrin receptors and ferritin and facilitate a dynamic regulation as per the requirement of the cell [45]. At systemic level, hepcidin/ferroportin axis dominates the iron homeostasis [46]. Ferroportin allowes the release of iron from the iron stores in the body [47]. As a ferroportin regulates the release of iron, its expression is tightly regulated by circulating hepcidin [48]. Aberrant expression of hepcidin culminates in dysregulation of iron metabolism [49].

A major hallmark of the COVID 19 disease is a hyperinflammatory condition caused due to the elevated cytokines called cytokine storm [25]. The major cytokine which was found to be associated with this cytokine storm is IL-6 [6]. The release of IL-6 increases the production of C reactive protein a marker of systemic inflammation from the liver, similarly, this also leads to an increase in the synthesis of Hepcidin and decrease the production of transferrin [50]. Hepcidin is also released by the macrophages due to the action of IL-6. So, this Iron-hepcidin/covidin-transferrin axis is a major pathway responsible for the decreased iron in inflammatory conditions (Fig. 3). Along with the hepcidin- transferrin pathway, it was also found that the hepcidin-ferroportin pathway also plays a major role in the iron homeostasis in the COVID 19 disease [22]. Ferroportin, also known as iron-regulated transporter 1, which transports iron from the cell to the bloodstream [51]. When hepcidin is low, the flow of iron to the peripheral bloodstream is high. Similarly, when the levels of hepcidin increase, the ferroportin gets internalised and iron gets accumulated in the tissues and cells [52]. This intracellular accumulation of iron leads to oxidative stress by activating the Fenton reactions, which releases powerful oxidizing agents leading to oxidative stress. Although an indirect measure of oxidative stress, to confirm these findings, we have measured the TAS levels, which was found to be decreased significantly in the cases. Although measuring TAS is an indirect way of measuring oxidative stress, it gives an overall idea about the oxidative status of the body. This

oxidative stress will further activate ferroptosis (apoptosis), which was also confirmed in the recent biopsies conducted in the COVID 19 patients [53,54].

In recent insilico studies, it was found that SARS CoV − 2 has a spike protein that mimics the activity of hepcidin, called covidin [22]. So, increasing the levels of both hepcidin and covidin will lead to toxic accumulation of intracellular iron and end-organ damage in COVID 19 [23–25]. Further, rapid onset of hypoxia might be seen due to low erythropoiesis caused due to low serum iron, rapidly deteriorating a patient's condition and leading to high morbidity.

5. Conclusion

Given the results from the above analysis, we confirm that iron-related biomarkers reflect the oxidative stress situation which activates the cytokine storm. So, continuous monitoring of these parameters could be helpful in the early detection of individuals developing the severe disease and can be used to decrease mortality in upcoming new waves of COVID-19.

6. Limitations and future prospects

Estimation of hepcidin levels could help us in better understanding the role of iron metabolism in the COVID-19 disease. An estimation of quantitative ratio of Fe2 + vs. Fe3 + to assess the shifting of iron equilibrium toward Fenton reaction and oxidative stress will help in further understanding the role of oxidative stress.

CRediT authorship contribution statement

Conceptualization: PP, DY, SS; Material preparation, data collection and analysis: KKP, PP,SS, DY. Manuscript first draft preparation: KKP, PP, SS, DY; Writing – review & editing: KKP, PP, ST, DY, SS, JC; Sample testing: KKP, ST, AP, VLN; Patient recruiting: KKP, ND, PKB; Supervision: KS, MKG, SM and PS, All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Purvi Purohit, Dharmveer Yadav, Shrimanjunath Sankanagoudar reports financial support was provided by All India Institute of Medical Sciences - Jodhpur.

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