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Biochemical and Biophysical Research Communications 631 (2022) 138–145

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

SARS-CoV-2-induced hypomethylation of the ferritin heavy chain (*FTH1*) gene underlies serum hyperferritinemia in severe COVID-19 patients

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ARTICLE INFO

Article history:
Received 30 August 2022
Received in revised form
2 September 2022
Accepted 21 September 2022
Available online 25 September 2022

Keywords:
SASR-CoV-2
Hyperferritinemia
FTH1
TET3
Spike glycoprotein

ABSTRACT

High serum ferritin (hyperferritinemia), a reliable hallmark of severe COVID-19 often associates with a moderate decrease in serum iron (hypoferremia) and a moderate increase in serum hepcidin. This suggests that hyperferritinemia in severe COVID-19 is reflective of inflammation rather than iron overload. To test this possibility, the expression status of ferritin heavy chain (*FTH1*), transferrin receptor 1 (*TFRC*), hepcidin (*HAMP*), and ferroportin (*SLC40A1*) genes and promoter methylation status of *FTH1* and *TFRC* genes were examined in blood samples obtained from COVID-19 patients showing no, mild or severe symptoms and in healthy-donor monocytes stimulated with SARS-CoV-2-derived peptides. Severe COVID-19 samples showed a significant increase in *FTH1* expression and hypomethylation relative to mild or asymptomatic COVID-19 samples. S-peptide treated monocytes also showed a significant increase in *FTH1* expression and hypomethylation relative to that in controls; treatment with ECD or NP did not change FTH1 expression nor its methylation status. *In silico* and *in vitro* analysis showed a significant increase in the expression of the TET3 demethylase in S peptide-treated monocytes. Findings presented here suggest that S peptide-driven hypomethylation of the *FTH1* gene promoter underlies hyperferritinemia in severe COVID-19 disease.

1. Background

Severe COVID-19 disease associates with significant hyperinflammation resulting from increased production of proinflammatory cytokines [1] and marked hyperferritinemia [2–5]. Ferritin, which consists of multiple heavy (FTH1) and light (FTL) chain subunits [2,6], serves as the primary cellular iron storing protein [2] and functions as an acute phase reactant in inflammation [7,8]. Expression of FTH1 and FTL and the subsequent assembly of the ferritin 24-subunit cage-like structure is regulated by complex transcriptional and post-transcriptional mechanisms triggered by changes in iron status or by proinflammatory cytokines [2,9–13].

The serum ferritin level is often evaluated upon COVID-19 patient hospital admission [3] and during hospitalization as a reliable prognostic marker [4,5,14,15]. Several meta-analysis-based studies have established that serum ferritin is significantly higher in non-survivors versus survivors and in severe COVID-19 cases relative to milder ones [5,14,15]. High serum ferritin levels (>2000 μg/L) along with low platelet counts and high triglyceride levels are strong predictors of multi-organ failure and death in critically-ill COVID-19 patients [16]. Drawing on previous work which has dealt with the regulation of ferritin expression [2,17,18], hyperferritinemia in severe COVID-19 has been attributed to perturbations in iron homeostasis and increased proinflammatory cytokine (e.g., TNF-α and IL-1β) production [16]. Consistent with the latter, serum ferritin content was reported to be significantly reduced in patients on tocilizumab, a monoclonal antibody that blocks the IL-6 receptor [19–21].

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https://doi.org/10.1016/j.bbrc.2022.09.083
0006-291X/

Serum levels of the liver-derived peptide hormone hepcidin, which regulates cellular iron release by degrading the cellular iron exporter ferroportin [22], is moderately increased in COVID-19 patients [23,24]. Macrophages and hepatocytes also secrete hepcidin during inflammation as an antimicrobial peptide to sequester iron and limit pathogen growth [25]. Upregulation of hepcidin expression in COVID-19 patients, which strongly correlates with the levels of proinflammatory cytokines, is inflammation-dependent [25]. Moderate hypoferremia (serum iron concentration <6 μMol/L) and low transferrin saturation are additional consistent findings in severe COVID-19 patients [26–29]. However, the moderate increase in serum hepcidin, which is subject to proinflammatory cytokine regulation, and the moderate hypoferremia that is associated with severe disease do not match the rapid and exorbitant increase in serum ferritin levels (>2000 μg/L) [19–21]. Moreover, the observation that hypoferremia in COVID-19 patients poorly correlates with serum hepcidin levels [16] is inconsistent with the well-established negative correlation between the two parameters as they relate to iron homeostasis [2,22]. This suggests that hyperferritinemia does not necessarily reflect a change in iron status robust enough to trigger such excessive hyperferritinemia. Instead, a more direct infection-related and possibly inflammation-dependent mechanism may better explain the excessive hyperferritinemia observed in severe COVID-19 patients [16].

We previously showed that *FTH1* is subject to epigenetic regulation through CpG island demethylation events at its promoter region [30]. Also, we showed that SARS-CoV-2 infection could induce epigenetic mediated upregulation of heat shock proteins via hypomethylation of its promoter region [31]. Herein, we investigated the possibility that SARS-CoV-2 infections could reprogram the epigenetic profile of iron metabolism-related genes like *FTH1* as means of sequestering more iron in severe COVID-19.

2. Methods

2.1. Sample collection and processing

In a prospective clinical study, blood samples were collected from donors who tested positive for COVID-19 and presented with no, mild or severe symptoms between March 20 until July 17, 2020. Patients were diagnosed with COVID-19 using a nasal swab PCR test and later divided into three groups (asymptomatic, mild, and severe) based on their clinical presentation. Each donor gave a 10 ml blood sample, one half of which was collected in a plain tube and the other half in an EDTA vacutainer. A total of 34 samples were collected (12 COVID-19-positive asymptomatic, 10 COVID-19-positive with mild symptoms, and 12 COVID-19-positive with severe symptoms) for the purpose of this study. COVID-19-positive asymptomatic individuals were identified as a result of the national screening campaigns. Asymptomatic individuals included subjects who tested positive for SARS-CoV-2 using a nasopharyngeal swab PCR test but have no symptoms that are consistent with COVID-19. Symptomatic COVID-19 patients were classified as mild or severe based on guidelines from Abu Dhabi Department of Health (circular number 33, April 19, 2020). Patients with mild disease presented with upper respiratory tract infection and symptoms like fever, dry cough, sore throat, runny nose, loss of taste and smell, and muscle and joint pains without shortness of breath, dyspnea, or abnormal chest imaging. Those with severe disease presented with severe pneumonia and symptoms like fever, cough, dyspnea and fast breathing (>30 per minute), in addition to oxygen saturation <90% or lung infiltrates >50%. Average age of study participants was 42.15 ± 7.56 years; there were 67.6% males, which was similar in all three groups; 43.08 ± 8.92 years for asymptomatics, 41.80 ± 7.82 years for those with mild COVID-19 and 41.50 ± 5.48 years for those with severe COVID-19 (Table 1). Immediately upon sample collection, the hospital lab staff separated and tested the serum for CRP, D-dimer, ferritin, IL-6 and LDH; a complete blood count was also performed on each sample. Whole blood samples were aliquoted and frozen at −80 °C for subsequent processing and analysis.

2.2. Serum levels of hepcidin and soluble transferrin receptor

Upon receipt of frozen samples at RIMHS, UOS labs, whole blood samples were thawed and centrifuged; serum was separated and levels of hepcidin (Cat No.733228; MyBiosource, San Diego, California, United States) and soluble transferrin receptor (sTfR) (Cat No. 750294; MyBiosource) were measured using commercially-available colorimetric assay kits; absorbance was read at 450 nm on a microplate reader.

2.3. RNA extraction, cDNA synthesis and real-time (RT) polymerase chain reaction (PCR)

Total mRNA was extracted using RNA Mini Kit (Qiagen) from whole blood samples obtained from the COVID-19 patients. According to the manufacturer's protocol, the cDNA was synthesized from 1 μg of RNA using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). RT-PCR was performed using 1 μl of complementary DNA (cDNA), specific primers (Suppl Table 1), SYBR® Green I, and an iCycler Thermal Cycler. The expression level of target genes was normalized to GAPDH gene expression.

2.4. DNA extraction, bisulfite DNA conversion and methylation-specific PCR (qMSP)

Total genomic (g)DNA was extracted from whole blood samples from the COVID-19 patients using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). An aliquot gDNA (2 μg) was treated with EpiTect Bisulfite Kit (Qiagen, Hilden, Germany). qMSP was conducted using 1 μl of the sodium bisulfite-treated DNA, primers designed explicitly for methylated and unmethylated DNA sequences of the promoter region of *FTH1* and *TFRC* genes (Supplementary Table S1), Promega GoTaq® qPCR Master Mix (Promega GoTaq master mix A6002; Madison, Wisconsin, USA); and results were analyzed using Qiagen Rotor-gene qPCR machine (Qiagen, Hilden, Germany). Fully methylated and fully unmethylated control DNAs were used as positive and negative controls, respectively. Relative methylation levels were analyzed as described previously [32].

2.5. SARS-CoV-2 peptide treatment in freshly isolated peripheral blood monocytes

Blood samples were collected from 5 healthy adults who never tested positive for COVID-19 and received their last vaccine shot at least three months prior to donating the sample; blood samples were then pooled and processed. Freshly-isolated peripheral blood monocytes (PBMCs) were enriched following a previously published protocol [33]. Blood samples were collected in EDTA tubes and pooled in 50 ml falcon tubes. To separate the peripheral blood mononuclear cells (PBMCs), 12.5 ml pooled blood was over-layered onto 10 ml Histopaque-1077 (Sigma-Aldrich, St Louis, MO) followed by centrifugation at 2000 RPM for 20 min at room temperature with break turned off. PBMCs in the interface were aspirated and washed once with warm PBS. Pellets were suspended in 1 ml RPMI-1640 media and viable cells were counted using trypan blue vital dye. Cells were seeded into 60 × 15 mm cell culture dishes at a density of 4.5 × 10^6 cells per 3 ml of RPMI-1640 media

Table 1
Demographics of the COVID-19 patients included in this study.

	Total	Asymptomatic	Mild symptoms	Severe symptoms
	(n = 34)	(n = 12)	(n = 10)	(n = 12)
Age(mean ± SD)	42.15 ± 7.56	43.08 ± 8.92	41.80 ± 7.82	41.50 ± 5.48
Gender				
Male	23	11	10	9
Female	4	1	0	3

supplemented with 10% fetal bovine serum (Sigma) and 1% penicillin/streptomycin (Sigma-Aldrich, St Louis, MO). Flasks were incubated for 24 h at 37 °C and 5% CO_2. Twenty-four hours after culture, floating non-monocytes were removed by gently aspirating the supernatants followed by a gentle wash with pre-warmed PBS. RPMI-1640 alone (employed as control) or containing the peptides was then added to the attached monocytes. SARS-CoV-2 Spike Protein is composed of S1 and S2 domains. S1 contains a receptor-binding domain (RBD) that can specifically bind to angiotensin-converting enzyme 2 (ACE2), the receptor on target cells. ECD is the extra cellular domain of the spike protein. Cells seeded in wells were separately treated with 100 ng/ml extracellular domain (ECD; cat.#Z03481), 100 ng/ml Spike Protein S1 domain (SP; cat.#Z03501) or 1000 ng/ml nucleocapsid protein (NP; cat.#Z03488), or left untreated as negative control; a separate well received 1000 ng/ml the soluble angiotensin-converting enzyme 2 (ACE2) as an internal control (all from GenScript Inc, Piscataway, NJ, USA). On the eighth day following treatment, DNA and RNA constituents were extracted as described above. Blood collection was approved by University of Sharjah ethics committee and donors were asked to sign a consent form authorizing the use of their samples on the understanding that personal information will be kept confidential.

2.6. Western blotting

Cultured PBMCs were washed twice with cold 1X PBS prior to lysis with RIPA lysis buffer containing 150 mM sodium chloride, 1.0% NP-40, 0.1% SDS, 50 mM Tris (pH 8.0), 0.5% sodium deoxycholate and supplemented with protease inhibitors cocktail. Cell lysates were kept on ice for 20 min and then centrifuged at 4 °C for 10 min. The supernatant was collected and protein concentration was quantified; samples were then boiled in 5X loading buffer at 95 °C for 5 min. After separation and transfer, the nitrocellulose membrane was washed with 1X TBST and blocked in 5% non-fat dry milk dissolved in 1X TBST for 1 h at room temperature. The membrane was washed 3 times with 1X TBST and incubated with a primary antibody against FTH (LS-B11085-200; LS Bio, Seattle, WA, USA; at 1:1000), DNMT1 (5032S; Cell Signaling Technology, Danvers, Massachusetts, United States), DNMT3A (ab232391; Abcam, Waltham, MA, United States), DNMT2B (ab239893; Abcam), G9a (D5R4R; Cell Singling Technology, Danvers, MA, USA; at 1:1000), TET1 (A1506, Abclonal), TET2 (A5682, abclonal), TET3 (A18319, abclonal) or GAPDH (8884; Cell Signaling Technology) at 4 °C overnight. The primary antibody solution was discarded and membranes were washed 3 times with 1X TBST and incubated with a secondary antibody (anti-mouse [Cat. No. 7076; Cell Signaling Technology; at 1:1000] or anti-rabbit antibody [ac97040; Abcam; at 1:5000] in 3% BSA in 1X TBST for 2 h at room temperature. Secondary antibody was discarded and the membrane was washed 3 times with 1X TBST prior to incubation with ECL solution; the signal was captured using the Bio-Rad Gel Doc system (Biorad).

2.7. Computational analysis

The National Center for Biotechnology Information Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/), a free public functional genomics database including array- and sequence-based data, was searched for COVID-19-related transcriptomic datasets. Datasets GSE173488, which includes expression profiling by high throughput sequencing of COVID-19 patient-derived macrophages stimulated with SARS-CoV-2 spike protein, lipopolysaccharide or without stimulation (n = 8 for each treatment, and GSE150316, which includes expression profiling by high throughput sequencing from the samples taken from the autopsies of SARS-CoV-2 infected lungs, were selected. Differentially expressed genes (DEGs) were analyzed in COVID-19 versus healthy individuals, and expressions of iron-metabolism-related genes were identified.

2.8. Statistical analysis

The gene expression profiles were downloaded from the GEO database. Raw data from each dataset were processed using R statistical software (version 3.5.1). According to the expression profiling data, DEGs were presented as relative mRNA expression in infected/treated samples compared to normal controls (Limma package; http://www.bioconductor.org/packages/release/bioc/html/limma.html). Bioconductor package version 1.0.2. A log2 fold-change (log2FC) was calculated to present DEGs; an adjusted p-value <0.05 using classical *t*-test was applied. For qMSP analysis, the significant difference was estimated by the student's t-test considering unequal variance; $p < 0.05$ was considered significant.

3. Results

3.1. Severe COVID-19 disease is associated with serum hyperferritinemia

Consistent with previous studies [5,14–16], serum ferritin and sTfR levels were significantly higher in patients with severe COVID-19 compared to those with no or mild symptoms (Fig. 1A). No difference in sTfR levels in mild COVID-19 versus severe COVID-19 patients was observed (Fig. 1B). Serum hepcidin levels showed no significant difference amongst the three groups (Fig. 1C).

3.2. Hypomethylation-dependent upregulation of FTH1 gene expression explains hyperferritinemia in severe COVID-19

To investigate the cause of increased serum ferritin and sTfR levels in patients with severe COVID-19, we sought to analyze the expression status of key iron regulatory genes. RNA extracted from all 34 blood samples was subjected to PCR using specific primers for *FTH1* (ferritin heavy chain), *TFRC* (TfR1), *TFR2* (TfR2), *SLC40A1* (ferroportin) and *HAMP* (hepcidin) genes. Our results showed that *FTH1* expression was significantly higher in samples collected from patients with mild or severe disease as compared with that in asymptomatic counterparts; severe COVID-associated with the highest levels of FTH1 expression. The expression of *TFRC* and *TFR2* was higher in mild COVID-19 samples relative to asymptomatic samples; no significant difference was observed in the mean expression of *TFRC* and *TFR2* in severe COVID-19 versus asymptomatic individuals. Furthermore, no statistically significant differences were observed regarding the expression of *SLC40A1C* or *HAMP* genes among the three groups (Fig. 2A). RNAseq data from lung biopsies of severe COVID-19 patients and healthy individuals, that was generated using a publicly available database showed no significant difference in the mean expression of the tested iron-

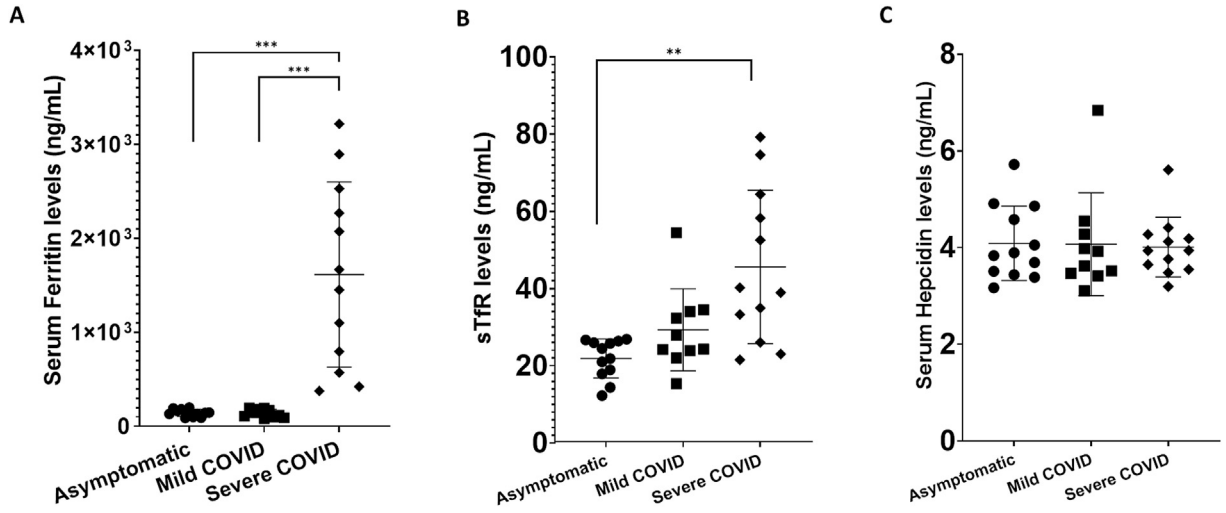

Fig. 1. Analysis of serum iron biomarkers in asymptomatic, mild and severe COVID-19 patients (n = 34). (A) Serum ferittin levels, (B) serum transferrin receptor (sTfR) levels, and (C) serum hepcidin levels were measured and reported as ng/mL for individual study participants. (Error bars represent mean ± SD; **p < 0.001, ***p < 0.0001).

metabolism-related genes (Suppl. Fig 1).

We previously showed that the promoter regions of *FTH1* and *TFRC* genes, which contain CpG islands, is subject to epigenetic (methylation/demethylation) regulation [30]. Using specific primers designed to target CpG islands in the promoter regions of *FTH1* and *TFRC* genes, we performed MSP to investigate SP-induced methylation changes in these genes. MSP analysis showed a very significant hypomethylation in the *FTH1* gene promoter in mild and severe COVID-19 patients compared with asymptomatic counterparts. The methylation changes observed in the *TFRC* gene promoter were not significant amongst the three patient groups (Fig. 2B).

3.3. SARS-CoV-2 spike protein-induced hypomethylation and overexpression FTH1 gene in immune cells

The next question we asked was what triggers this form of epigenetic modification in PBMCs. Previous studies have documented the presence of SARS-CoV-2 RNA in the blood of COVID-19 patients [34,35] but whether SARS-CoV-2 related proteins may trigger epigenetic changes in PBMCs in not well understood. To answer this question, PBMCs extracted from healthy individuals were stimulated with SARS-CoV-2 peptides and the expression and promoter methylation status of *FTH1* and *TFRC* were assessed. As shown in Fig. 3A. Only SP-treated cells showed significant levels of *FTH1* overexpression and promoter hypomethylation. Other peptides showed minimal effect on *FTH1* or *TFRC* promoter methylation (Fig. 3A and B).

3.4. SARS-CoV-2 spike protein-induced regulation of DNA methyltransferases and demethylases

To confirm these results, we analyzed a publicly available RNAseq dataset obtained from patient-derived macrophages stimulated with SP and LPS. Consistently, we found that SP induces *FTH1,* but not *TFRC,* overexpression (Fig. 4A). Given that the DNA

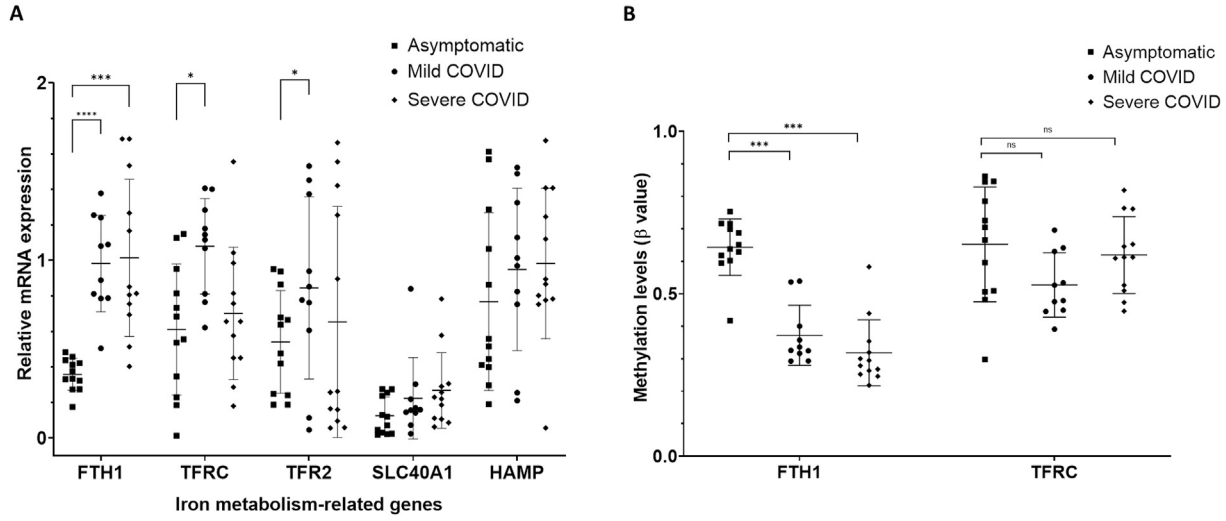

Fig. 2. Expression and promoter methylation levels of key iron metabolism related genes in asymptomatic, mild and severe COVID-19 patients (n = 34). Relative mRNA expression of *FTH1*, *TFRC*, *TFR2*, *SLC40A1*, and *HAMP*. (B) DNA methylation levels of the promoter region of *FTH1* and *TFRC*. (Error bars represent mean ± SD, ns = not significant, *p < 0.01, **p < 0.001, ***p < 0.0001, ****p < 0.00001).

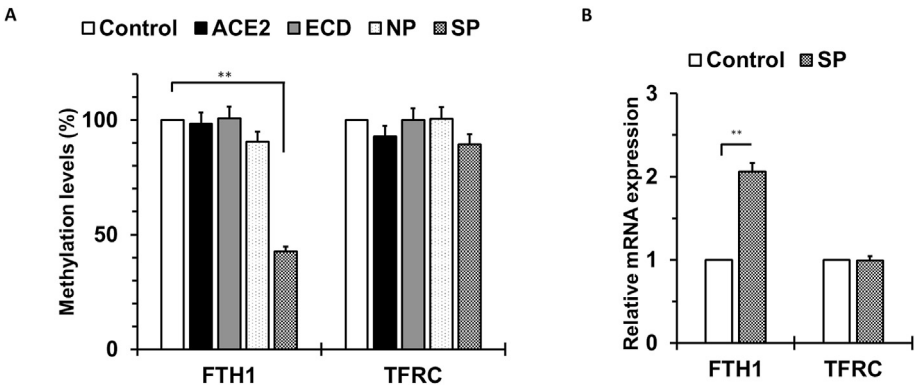

Fig. 3. Effects of SARS-CoV-2 derived peptides on human blood derived monocytes (n = 3 per treatment). Extracellular domain (ECD), Spike 1 (SP) and l nucleocapsid protein (NP); a separate well received 1000 ng/ml of angiotensin-converting enzyme 2 (ACE2) as a positive control. (A) DNA methylation levels of the promoter region of *FTH1* and *TFRC*. (B) Relative mRNA expression of *FTH1* and *TFRC*. (Error bars represent mean ± SD, **p < 0.001).

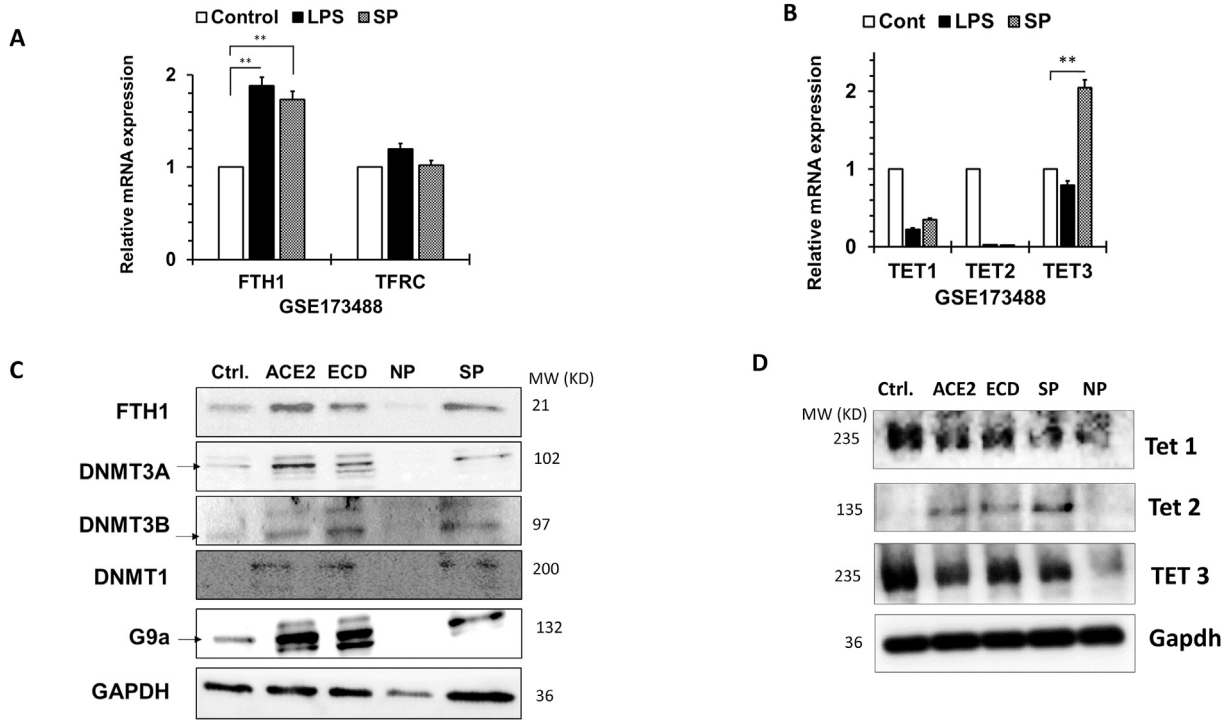

Fig. 4. *In silico* and *in vitro* analysis of patient-derived macrophages stimulated with SARS-CoV-2 derived peptides (n = 8 per treatment). Spike 1 (SP) and Lipopolysaccharide (LPS). (A) Relative mRNA expression of *FTH1* and *TFRC* derived from publicly available RNAseq dataset (GSE173488) (B) Relative mRNA expression of *TET1, TET2* and *TET3*, derived from publicly available RNAseq dataset (GSE173488). (Error bars represent mean ± SD, **p < 0.01). (C and D) In vitro investigation and Western blot analysis of protein expression of FTH1 and key DNA methyltransferases (DNMT1, DNMT3A and DNMT3B) and demethylases (G9a, TET1, TET2, TET3) in blood derived monocytes stimulated with SARS-CoV-2 derived peptides. GAPDH expression was measured as internal control, and data is representative of two independent experiments.

methylation-induced epigenetic regulation might be involved, we searched the same dataset for expression levels of DNA methylases (data not shown) and DNA demethylases (Fig. 4B). We found that ten-eleven translocation methylcytosine dioxygenase (*TET3*) gene expression was significantly higher in SP-treated macrophages relative control or LPS-treated cells. Consistent with previous work, which showed that COVID-19 infection associates with significant hypomethylation [36], the expression of key DNA methyltransferase enzymes including DNMT1, DNMT3A and 3B and G9a (EHMT2) were all upregulated in freshly isolated ACE2-or ECD-treated PBMCs but significantly downregulated in SP-treated cells (Fig. 4C). The expression of DNA demethylases was investigated and the ACE2, ECD and SO treatment showed significant upregulation of TET2 compared to control. The other two TETs (TET1 and TET3) did not show any change compared to control (Fig. 4D). This further confirms that SARS-CoV-2-derived SP upregulates DNA demethylases and downregulates methyltransferases thus leading to increased FTH1 expression.

4. Discussion

It is well-accepted now that COVID-19 causes severe structural lung injury, hyper-inflammation and disruption of host immunity. Several serum markers are used to follow disease progression including lactate dehydrogenase, procalcitonin, CRP, proinflammatory cytokines and ferritin. Elevated serum ferritin levels

were reported to associate with increased morbidity and mortality in COVID-19 patients [37]. However, a direct link between SARS-CoV-2 infection and the exorbitant hyperferritinemia seen in patients with severe COVI-19 is poorly understood. This is especially significant given that the majority of patients with severe COVID-19 often exhibit anemia as indicated by increased serum hepcidin [24,25] and decreased serum iron levels [26–29] rather than iron overload as predicted by hyperferritinemia.

In this study, blood samples from a cohort of 34 COVID-19-positive individuals with variable symptoms (asymptomatic, mild or severe) were interrogated in terms of serum and cellular markers of iron metabolism. Our data showed that patients with severe COVID-19 experience a very significant increase in serum ferritin levels and a moderate increase in serum levels of sTfR. Our data also showed that serum hepcidin levels did not significantly differ in patients with mild or severe COVID-19 patients from that in asymptomatic counterparts. This is in line with previous work which has shown that serum ferritin levels in patients with severe COVID-19 [3,5] far exceed the level expected by the slight/moderate increase in serum hepcidin levels [24,25]. It is also consistent with the observation that hyperferritinemia is a predictor of increased morbidity and mortality in COVID-19 patients [14–16]. Also in agreement with these findings was our observation that the *FTH1* gene is highly expressed in blood leukocytes in patients with mild or severe COVID-19 patients as compared with asymptomatic counterparts (Fig. 2A). This suggests that changes in the expression pattern of iron regulatory genes such as *FTH1*, *TFRC* and others in COVID-19 patients may occur independently of patients' actual cellular or systemic iron status. In this context, previous work has shown that macrophages, upon inflammatory cytokine stimulation or infection, undergo a phenotype switch in terms of their iron handling function by increasing FTH1 expression and reducing that of ferroportin [38,39]. This points to the possibility that viral proteins, perhaps alomg with inflammation-mediated changes in iron status, is responsible for hyperferritinemia in severe COVID-19.

Epigenetic modifications include histone modification, DNA methylation and non-coding RNA-mediated regulation. Previous studies have suggested that DNA methylation, rather than histone acetylation, is the main driver of virus-induced epigenetic changes [40]. Also, acute COVID-19 associated illness was correlated with gene promoter hypomethylation in ~75% of the differentially methylated regions (DMRs) [36]. Based on that, we thought to investigate whether SARS-CoV-2 derived proteins to lead to epigenetic changes specifically targeting expression of *FTH1* gene causing hyperferritinemia. Previously we showed that SARS-CoV-2 infection induces hypomethylation and upregulation of heat-shock protein (HSPA1L) in lung epithelial cells, which could aid in viral protein synthesis and replication [31]. *FTH1* gene contains CpG islands in its promoter region, making it prone to be regulated by hyper or hypomethylation. Previously we showed that DNA methylation mediated the epigenetic regulation of *FTH1* in cancer cells [30]. Analysis of the methylation status of the CpG islands in the *FTH1* and *TFRC* gene promoters showed a very significant level of hypomethylation in the *FTH1* gene promoter in patients with mild or severe COVID-19 and a moderate hypomethylation of the *TFRC* gene promoter in patients with mild disease. This is consistent with our previous work which showed that estrogen (17-β estradiol) signaling triggers hypomethylation and overexpression of *FTH1* and *TFRC* genes in liver cancer cells [30], suggesting that these two genes are subject to epigenetic reprogramming. These findings were further validated *in vitro* and *in silico* where treatment of freshly-isolated PBMCs with different SARS-CoV-2-derived peptides showed that exposure of PBMCs to SARS-CoV-2 SP protein-derived peptides, but not other SARS-CoV-2-derived peptides, induces *FTH1* gene promoter hypomethylation and overexpression. To the best of our knowledge, this is the first study to investigate the molecular mechanism underlying hyperferritinemia in COVID-19 patients and to point to a link between exposure to the SARS-CoV-2 and the induction of hyperferritinemia.

Among the hundreds of enzymes involved in epigenetic regulation the DNMTs, TETs, and Thymine DNA glycosylase (TDG), are involved in the active DNA methylation/demethylation process [41]. These enzymes are responsible for establishing specific methylation patterns on the promoter regions of specific genes, thus resulting in transcriptionally active or silent genes [31,42]. Moreover, genome-wide methylations studies using blood samples revealed a higher incidence of hypomethylated DMRs in severe COVID-19 patients [36,43]. The SARS-CoV-2 virus-mediated changes in methylation levels were previously attributed to inhibition of DNMTs [31]. Another key observation is that SP-derived peptides upregulate the expression of TETs. *In silico* analysis showed upregulation of TET3 but *in vitro* investigation showed upregulation of TET2. This difference could be due to the difference in host infection status and perhaps suggest a broad (non-specific) effect of SARS-CoV-2 SP on the DNA demethylases. Previous studies have established that the demethylase TET3 upregulates in response to inflammation [44,45] and hypoxia [46]. Numerous studies have also documented that COVID-19 patients experience both hyperinflammtion [13,47] and hypoxia [48,49] in a manner that corresponds with disease severity. That said, whether the link between exposure to SARS-CoV-2 and the epigenetic reprogramming that ensues in macrophages in COVID-19 patients is dependent on or independent of inflammation and/or hypoxia is not known.

5. Conclusions

Ferritin, a biomarker that can be quickly and cheaply measured, can be reliably used to predict COVID-19 progression and mortality. The *FTH1* gene promoter hypomethylation level can also be added to the growing list of diagnostic and prognostic biomarkers of COVID-19. Lastly, exposure to SARS-CoV-2-derived spike protein seems capable of reprogramming macrophages' epigenetic profile. Whether such changes are transient or long-lasting, or whether they are somatically heritable and lead to serious health complications is not known.

Data availability statement

The data that support the findings of this study are either openly available in National Center for Biotechnology Information Gene Expression Omnibus (GEO) at https://www.ncbi.nlm.nih.gov/geo/, reference number GSE173488, and GSE150316. In addition, the remaining data that support the findings of this study are available within the article.

Ethical approval and consent of participants

The study was jointly approved by the Ministry of Health, Abu Dhabi and Dubai Health Authority (DOH/CVDC/2020/1949) on the understanding that samples will be number-coded to hide patient identity, that no personal information will be shared with a third party and that no sample analysis can be performed by entities other than the Research Institute of Medical and Health Sciences (RIMHS), the University of Sharjah (UOS) without prior written approval.

Funding

This work was supported by research grant CoV19-0305 (MH),

University of Sharjah, UAE.

Authorship

MH, GEG and JSM were responsible for the conception of the idea, data analysis, and manuscript preparation; JSM, JS, AAQ and MGM performed the experimental work; MH, GEG and JSM edited and finalized the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by research grant CoV19-0305 (MH), University of Sharjah, UAE. The authors wish to acknowledge the generous support of the Research Institute for Medical and Health Sciences, University of Sharjah UAE.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2022.09.083.

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