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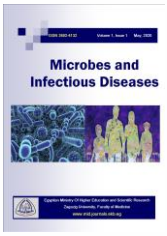

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Original article

Interferon-induced transmembrane protein 3 (IFITM3) gene polymorphisms in COVID-19 patients in Assiut University Hospitals

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ABSTRACT

Background and aims: Interferon-induced transmembrane protein 3 (IFITM3) plays an important antiviral role in the adaptive and innate immune response, it prevents hemifusion of the viral membrane and host cellular membrane in a broad spectrum of enveloped viruses, e.g. influenza A, ebola, marburg or SARS-CoV. This study aimed to evaluate correlation of IFITM3 gene polymorphisms (rs12252) and infection susceptibility, severity and mortality rate of COVID-19 patients. **Patients and methods:** The IFITM3 SNP (rs12252) polymorphism was genotyped by (RT-PCR) in 100 SARS-CoV-2-negative controls and 100 SARS-CoV-2- positive, who admitted to Chest, Assiut University and other quarantine Hospitals respectively in Assiut city, Egypt. **Results:** The minor allele frequency (rs12252 (G)) were significantly more frequent in the patients compared to controls after age and sex matching (p-value = 0.011, OR (95% CI) =2.44 (1.20-4.97). This allele (G) was significantly more common among patients who ICU admitted than non-ICU admitted (p -value = 0.001, OR (95% CI) =3.78 (1.64-8.75)). Also, was significantly more frequent in dead patients than cured patients (p -value = 0.005, OR (95% CI) =3.33 (1.37-8.10**)). Conclusion:** The present study has shown significant association between G (the mutant type) allele variant of IFITM3 rs12252 and COVID-19 infection susceptibility and disease severity and mortality.

Introduction

Coronavirus 2019 (COVID-19) is a respiratory tract infection due to a novel coronavirus, SARS-CoV-2, and it is considered a zoonotic virus. On March 11, 2020, the World Health Organization (WHO) reported the COVID-19 as a pandemic [1]. The clinical picture of COVID-19 ranges from asymptomatic to severe form. The disease can progress over a week or more from mild to severe deterioration, which can be sudden and catastrophic [2]. Septic shock and acute respiratory distress syndrome are the major symptoms. Generally, the mortality rate among diagnosed cases is about 2-3%, with variations among countries [3].

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Still, there is controversial information on its possible complications, pathogenesis, prognostic factors, therapeutic options, and/or available prevention. Many rapidly expanding research projects are being conducted to identify prognostic criteria that can predict disease severity and possible outcomes, and thus plan of management, either at home or in the hospital [4].

Because the host and the pathogen require iron in the evolutionary process, the innate immune response carefully regulates control over iron metabolism to restrict its existence during infection [5]. The oxidative stress that causes physiopathology in the lungs is exacerbated by iron, which can increase virulence and pro-oxidant reactions. In normal, healthy lungs, extracellular excess iron makes them especially susceptible to oxidative damage and infection during viral infections [6].

The abnormalities in plasma iron chemistry (elevated levels of iron saturation of transferrin) and impaired antioxidant protection have been described in viral pneumonia.

Rationale

We aimed to assess total iron binding capacity (TIBC), serum level of iron and transferrin saturation in COVID-19 patients and to detect its relation to disease severity and outcome.

Patient and methodology

This observational cross-sectional analytical study was conducted over 100 confirmed COVID-19 patients by PCR, and they were admitted to Kasr Al-Ainy Hospitals between June and December 2020.The Institutional Review Board and ethics committee of the medical school at Cairo University approved this study. All procedures were carried out per the Helsinki Declaration of 1975, as amended in 2008, and the national and institutional responsible committees on human experimentation's ethical standards.

We included 100 patients of both genders,18 years old or more, and all patients were admitted to Kasr Al-Ainy Hospitals. The COVID-19 severity was assessed according to the WHO classification [7]. Exclusion criteria included those who refused to participate, patients who did not need hospital admission and/or previous history of hematological disease that may affect the results were all excluded.

All included patients who were diagnosed with positive COVID-19 by nasopharyngeal swab signed an informed consent, and were subjected to the following: demographic data including BMI, where obesity will be considered if body mass index (BMI) above 30kg/m2, full medical history, clinical examination, laboratory investigations including (complete blood count(CBC),c-reactive protein(CRP), liver biochemical profile, creatinine, D-dimer,serum iron, TIBC, and transferrin saturation) and chest computed tomography imaging. Based on all the previous data, triage of patients is performed with the categorization of the patient into mild, moderate, or severe and critically ill according to the management protocol of the Ministry of Health and Population (MOHP) for COVID-19 patients in Egypt in May 2020. Blood sampling for serum levels of iron, TIBC, and transferrin saturation was obtained under complete aseptic precautions.

The severity of COVID-19 assessed by using the modified National Early Warning Score (NEWS) which include the following points : age, respiratory rate, O2 saturation, systolic blood pressure, heart rate, any O2 supplementation, conscious level and temperature.

-Each item took score from 0 to 3

-Severity of covid-19 classified according to the score from 0 to ≥ 7 :

0 score = no risk.

1-4 score = low risk .

5-6 score = moderate risk .

≥ 7 score = high risk .

Statistical analysis

Data were tabulated and analyzed through the Statistical Package of Social Science Software program, version 25 (IBM SPSS Statistics for Windows, Version 25. 0. Armonk, NY: IBM Corp.).Classification variables were expressed as frequency and percentage. Continuous variables were defined as mean±SD. Comparison of qualitative variables between groups were performed through the Chi-square test of independence. In contrast, the quantitative variables were compared through an independent sample t-test or Mann-Whitney test (according to the normality of data).

A significance level (alpha error) of 5% were used to reject or accept the null hypothesis (H0).

Results

This observational cross-sectional analytical study was conducted on 100 patients with

COVID-19. The description of the studied group is demonstrated in **table (1)**. Fifty-one percent were males with a mean age=51±14.9. Concerning the patients' disease severity, 53% were moderate, 34% were mild, and 13% were severe. Serum iron parameters were calculated for 96 patients only due to missing data or hemolyzed samples.

In the studied population, the mean ±SD values of iron, TIBC and transferrin saturation were 163.1±105 mcg/dL, 366 ± 162.6 mcg/dL, 44.4 ± 20.2 %, respectively.

Serum iron level was low in 6 (6.3%) patients, normal in 52 (54.2%) patients, and high in 38 (39.6%) patients. Mortality was detected in 31.7 % (19 out of 60 patients with COVID-19) (only data concerning mortality from 60 patients were available). **Table 2** shows factors affecting COVID-19 severity among the studied population. Severe COVID-19 illness had a statistically significantly higher rate in obese patients (p=0.037). There was a statistically significant higher mortality rate in patients with severe COVID-19 compared to patients with mild COVID-19 (p=0.000).

Factors associated with mortality among the studied group of patients (n=60) are presented in **table (3)**. Higher mortality was observed significantly in patients with diabetes mellitus (p=0.041).Iron levels, TIBC levels, and transferrin saturation did not show a significant correlation regarding either COVID-19 severity or mortality(p-values are shown in **tables (2)** and **(3)**.

Table 1. Description of demographic features, co morbidities, iron parameters and mortality among patients with COVID-19.

Variables	**Number=100 (%)**
Gender	
Male	51 (51)
Female	49 (49)
Age	
Range	14 – 80
Mean ± SD	51 ± 14.9
Median (IQR)	52 (39 - 63)
Case severity	
Mild	34 (34)
Moderate	53 (53)
Severe	13 (13)
DM	74 (74)
HTN	82 (82)
Obesity	25 (25)
Iron (n=96) normal (M: 65-175 mcg/dL) (F: 40-150 mcg/dL)	
Range	12 - 532.1
Mean ± SD	163.1 ± 105
Median (IQR)	131.5 (89 - 217.5)
Iron (n=96)	
Low	6 (6.3)
Normal	52 (54.2)
High	38 (39.6)
TIBC (n=96) Normal (200-400 mcg/ dL)	
Range	93 – 846
Mean ± SD	366 ± 162.6
Median (IQR)	322.5 (244.5 - 460.5)
TIBC (n=96)	
Low	11 (11.5)
Normal	51 (53.1)
High	34 (35.4)
Transferrin saturation (%) (n=96)	
Range	3.6 – 109
Mean ± SD	44.4 ± 20.2
Median (IQR)	41.2 (33 - 54.8)
Transferrin saturation (%) (n=96)	
Low	4 (4.2)
Normal	59 (61.5)
High	33 (34.4)
Mortality (n=60)	
Yes	19 (31.7)
No	41 (68.3)

DM: diabetes mellitus, HTN: hypertension, TIBC: total iron binding capacity

Table 2. Factors affecting COVID-19 severity among the studied population.

	Case severity				***p* values of Pairwise comparisons**		
	Mild	**Moderate**	**Severe**	***p* value**	**Mild*Mod**	**Mild*Severe**	**Mod*Severe**
Gender							
Male	18 (52.9)	25 (47.2)	8 (61.5)	0.625*	0.599*	0.596*	0.353*
Female	16 (47.1)	28 (52.8)	5 (38.5)				
Age							
Range	14 - 80	25 - 75	18 - 80				
Mean ± SD	47.1 ± 16.8	53.2 ± 12.9	52.2 ± 16.7				
Median (IQR)	52 (34 - 59)	53 (45 - 64)	48 (46 - 63)	0.283#	0.109@	0.483@	0.771@
DM	24 (70.6)	40 (75.5)	10 (76.9)	0.851*	0.614*	1.000*	1.000*
HTN	28 (82.4)	43 (81.1)	11 (84.6)	0.956*	0.886*	1.000*	1.000*
Obesity	4 (11.8)	15 (28.3)	6 (46.2)	**0.037***	0.069*	**0.017***	0.319*
iron							
Range	12 - 532.1	43 - 510	13 - 300				
Mean ± SD	166 ± 113.3	170 ± 103.6	129.2 ± 87.7				
Median (IQR)	124.9 (98 - 207)	151 (91 - 218.3)	108 (70 - 164)	0.462#	0.820@	0.323@	0.212@
iron							
Low	3 (9.1)	1 (2)	2 (15.4)	0.295*	0.333*	0.542*	0.077*
Normal	17 (51.5)	27 (54)	8 (61.5)				
High	13 (39.4)	22 (44)	3 (23.1)				
TIBC							
Range	93 - 846	165 - 800	110 - 558				
Mean ± SD	377.5 ± 188.1	376.8 ± 150.6	294.8 ± 126				
Median (IQR)	318 (252 - 456)	357 (261 - 480)	270 (200 - 360)	0.235#	0.849@	0.184@	0.080@
TIBC							
Low	4 (12.1)	4 (8)	3 (23.1)	0.349*	0.665*	0.391*	0.112*
Normal	18 (54.5)	25 (50)	8 (61.5)				
High	11 (33.3)	21 (42)	2 (15.4)				
Transferrin saturation (%)							
Range	7.4 - 99.2	16.5 - 109	3.6 - 65.9				
Mean ± SD	44.3 ± 20.5	45.4 ± 20.3	40.8 ± 20				
Median (IQR)	44.9 (33.6 - 53.4)	40.6 (33.7 - 55.6)	46.8 (32.3 - 55.2)	0.976#	0.948@	0.913@	0.786@
Transferrin saturation (%)							
Low	2 (6.1)	0 (0)	2 (15.4)	0.084*	0.117*	0.589*	0.081*
Normal	18 (54.5)	35 (70)	6 (46.2)				
High	13 (39.4)	15 (30)	5 (38.5)				
Mortality	0 (0)	10 (34.5)	9 (69.2)	0.000*	0.008*	0.000*	0.036*

*$p \leq 0.05$= statistically significant, DM: diabetes mellitus, HTN: hypertension, TIBC: total iron binding capacity.

*Chi Square test, #Kruskal Wallis test, @Mann Whitney test . IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

Table 3. Factors related to mortality among patients with COVID-19.

	Mortality		
	Yes (n=19)	**No (n=41)**	***p*-value**
Gender			
Male	11 (57.9)	20 (48.8)	0.511
Female	8 (42.1)	21 (51.2)	
Age			
Range	18 - 80	23 - 72	
Mean ± SD	50.8 ± 16	50.5 ± 13.7	
Median (IQR)	48 (44 - 63)	52 (38 - 62)	0.918
Case severity			
Mild	0 (0)	18 (43.9)	0.000
Moderate	10 (52.6)	19 (46.3)	
Severe	9 (47.4)	4 (9.8)	
DM	15 (78.9)	21 (51.2)	0.041
HTN	15 (78.9)	29 (70.7)	0.503
Obesity	9 (47.4)	14 (34.1)	0.327
Iron (n=96) normal (M: 65-175 mcg/dL) (F: 40-150 mcg/dL)			
Range	13 - 470	12 -363	
Mean ± SD	125.7 ± 103	132 ± 88.2	
Median (IQR)	98 (69 - 140)	110 (69 - 160)	0.691
Iron (n=96)			
Low	3 (15.8)	3 (7.3)	0.552
Normal	13 (68.4)	29 (70.7)	
High	3 (15.8)	9 (22)	
TIBC (n=96) Normal (200-400 mcg/ dL)			
Range	110 - 555	93 - 693	
Mean ± SD	278.5 ± 123.3	318.3 ± 128.2	
Median (IQR)	228 (200 - 360)	288 (240 - 390)	0.127
TIBC (n=96)			
Low	4 (21.1)	6 (14.6)	0.792
Normal	11 (57.9)	27 (65.9)	
High	4 (21.1)	8 (19.5)	
Transferrin saturation (%) (n=96)			
Range	3.6 - 109	7.4 - 98.8	
Mean ± SD	43 ± 23.3	42.2 ± 22.5	
Median (IQR)	43.3 (25.4 - 55.2)	38.8 (21.9 - 55.6)	0.733
Transferrin saturation (%) (n=96)			
Low	2 (10.5)	2 (4.9)	0.699
Normal	11 (57.9)	24 (58.5)	
High	6 (31.6)	15 (36.6)	

*$p \leq 0.05$= statistically significant, DM: diabetes mellitus, HTN: hypertension, TIBC: total iron binding capacity

Discussion

Iron is an important trace element to all organisms. There is a balance between the host defense system and viral proliferation, where high and low serum iron levels may increase the risk of infection [8,9].

This study showed no association between iron indices and COVID-19 severity or outcome. However, it was observed a non-significant higher level of serum iron than lower levels concerning disease severity in the study population. This is as per the study by **Hu *et al.*** that stated an association between higher iron levels and sepsis [10].

The study results differ from **Moreira *et al.*** who found that serum levels of iron and transferrin levels at admission were lower in COVID-19-positive than in COVID-19-negative patients. Moreover, another study by **Yadav *et al.*** reported significantly decreased levels of iron, transferrin, and TIBC, and significantly increased levels of transferrin and ferritin saturation in COVID-19 cases compared to controls [11,12].

A study by **Kang *et al.*** found different results where COVID-19 severity and mortality were closely related to serum iron levels [13]. The discrepancy may be due to the study's lower percentage of severe cases.

It was observed that mortality reached 31.7% among the studied population and was statistically significantly related to severe COVID-19 disease and diabetes mellitus (DM). This is in accordance with a meta-analysis by **Corona *et al.*** who found that DM was the predictor for mortality in patients with COVID-19, and with another meta-analysis by **Singh *et al.*** that found higher mortality among patients with COVID-19 was detected in patients with DM [14,15]. Several mechanisms may be the underlying causes, especially modulation in immune response, higher susceptibility for severe infections, and the use of agents which may modulate the angiotensin-converting enzyme 2 (ACE2) expression [16].

In the study, severe COVID-19 disease was significantly related to obesity. This goes with the meta-analysis by **Singh *et al.*** who stated that obesity was linked to an increased risk of severe COVID-19 [15]. This may be due to the association of obesity with the over expression of the ACE2 receptor, which may increase infection. It acts as a viral reservoir, and ectopic fat may cause upregulation of the proinflammatory cytokines, thus expanding the infection severity [16].

The study concluded that mortality is related to severe COVID-19 infection and diabetes mellitus, and severe infection is associated with obesity. There is no association between iron indices and COVID-19 severity or mortality. Further studies on larger sample sizes are recommended in the future.

Limitations of the study

The limitations were:

a- We could not include critical ill patients (whose need mechanical ventilation) as we could not take a written consent from them.

b- Limited sample collection to mild, moderate, and severe cases only.

Competing interests

Non declared.

Funding

Non declared.

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