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Research article

Soluble HLA-G is upregulated in serum of patients with severe COVID-19

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ABSTRACT

Soluble HLA-G (sHLA-G) molecules are considered potent immunomodulators, and their dysregulated expression has been implicated in several pathological conditions, including coronavirus disease 19 (COVID-19). Therefore, a case-control study (103 COVID-19 patients and 105 controls) was performed to determine sHLA-G role in severity of COVID-19. Results revealed that median levels of sHLA-G were significantly increased in serum of patients compared to controls (19.3 vs. 12.7 ng/mL; $p < 0.001$). When patients and controls were stratified by age group, gender, body mass index, chronic disease, or ABO and Rh blood groups, the sHLA-G level did not show a significant difference in each stratum. Logistic regression analysis demonstrated that the up-regulated expression of sHLA-G was associated with an elevated risk of developing COVID-19. Receiver operating characteristic curve analysis showed that sHLA-G was a very good predictor of COVID-19, and at a cut-off value of 15.4 ng/mL, the sensitivity and specificity of sHLA-G were 79.6 and 79.0%, respectively. Spearman rank correlation analysis revealed that sHLA-G was positively correlated with age, erythrocyte sedimentation rate, white blood cell count, and random blood glucose, while a negative correlation was recorded with vitamin D. In conclusion, up-regulated expression of sHLA-G was indicated in patients with severe COVID-19.

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1. Introduction

During the past two decades, the world's populations have experienced three outbreaks of severe acute respiratory syndrome (SARS); SARS in Asia in 2002 [1], Middle East coronavirus (MERS – CoV) in Saudi Arabia in 2012 [2], and SARS-CoV-2 in China in late 2019 [3]. The latter is perhaps the most progressive SARS and has spread to 222 countries affecting millions of people worldwide, and as of June 23, 2021, there were 180,142,412 confirmed cases with 3,902,294 deaths [4]. On 11 February 2020, the World Health Organization (WHO) declared SARS-CoV-2 infection to be a pandemic public health threat with the official name coronavirus disease 19 (COVID-19) [5]. The COVID-19 can either be controlled or it can progress to a more severe disease with worsening pulmonary symptoms leading to pneumonia and acute respiratory distress syndrome with increased morbidity and mortality [6]. There are a large number of risk factors that have been identified to predispose people to COVID-19 and the severity of disease; for instance, age, gender, blood groups, obesity, chronic diseases and vitamin D

status [7]. In addition, COVID-19 patients are presented with dys-regulated immune responses manifested in hyper-inflammation and a cytokine storm, which are proposed to mediate the immunopathogenesis of COVID-19 and associated morbidity and mortality [8]. Therefore, understanding the immune function is fundamental to identifying the mediators that cause dysregulated immune responses and hyper-inflammation in patients with COVID-19.

The immune response against pathogens is controlled by some genetic factors associated with susceptibility or resistance to viral, bacterial, fungal and parasitic infections, in particular, those encoded by genes of the human leukocyte antigen (HLA) system, which are mapped to the short arm of human chromosome 6 [9]. The HLA genes are organized into three classes (I, II and III), and their products perform integral functions in innate and adaptive immune responses. The classical class I (A, B and C) and class II (DR, DQ and DP) HLA molecules are involved in mediating antigen presentation of intracellular and extracellular peptides, respectively [10]. Non-classical class I HLA molecules (E, F, and G) are other important products encoded by genes in the HLA region, and constitute the core molecules involved in controlling the immune response to infectious agents, as well as inflammatory reactions [11].

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HLA-G antigens are among the non-classical class I HLA glycoproteins that were first distinguished by their expression at the maternal-fetal interface to protect the fetus from the maternal immune system [12]. Seven isoforms of HLA-G have been recognized, including four membrane-bound antigens (HLA-G1, -G2, -G3 and -G4) and three soluble molecules (sHLA-G5, -G6 and -G7) [13]. These molecules are considered potent immunomodulators, and their dysregulated expression has been implicated in several pathological conditions [14]. In viral infection, two hypotheses have been proposed to explain the role of HLA-G in virus immunopathogenesis. HLA-G may enhance immune escape of the virus, and this is supported by HLA-G's immunosuppressive properties. Conversely, HLA-G expression and/or secretion may reflect a robust response to inflammatory reactions that occur during viral infections [15]. In the context of COVID-19, the role of HLA-G in immunopathology of disease has not been well investigated. However, there have been some suggestions that HLA-G molecules may have immune-regulating effects in COVID-19 [16–19]. Therefore, this study sought to explore the role of sHLA-G in immunopathogenesis of COVID-19 in severely-ill patients.

2. Materials and methods

2.1. Patients and controls

A total sample of 208 subjects was included in a case-control study (103 COVID-19 patients and 105 healthy controls). The patients were admitted to three hospitals in Baghdad (Dar Al-Salam Field Hospital, Al-Karkh General Hospital and Al-Furat General Hospital) during the period from September to December 2020, and they showed signs and symptoms of COVID-19. Laboratory diagnosis of COVID-19 was performed on nasopharyngeal swabs using the RealLine SARS-CoV-2 kit (Bioron Diagnostics GmbH). In addition, a chest tomography (CT) scan was performed to confirm the diagnosis. Patients were studied 4–7 days after admission, and only those with a positive molecular test and CT scan indicating COVID-19 were included. In the initial plan of our study, we considered identifying all patient groups in the hospital, but we found that the wards mostly contained severe cases, and mild cases tended to leave the hospital. Therefore, only severe cases were included for initial evaluation. The severity was determined according to the world health organization (WHO) Interim Guidance [20]. According to this guidance, the infection was considered severe if the patient met one of the following conditions: severe respiratory distress, respiratory rate ≥ 30 breaths/minute and pulse oxygen saturation (SpO_2) $\leq 93\%$ on resting state. Patients were characterized for age, gender, body mass index (BMI), ABO and Rh blood group systems, chronic diseases (cardiovascular and diabetes), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), hemoglobin (Hb), platelet count, white blood cell count (WBC), random blood glucose (RBG) and vitamin D status (Table 1). The healthy controls were blood donors and health service personnel. Their serum was negative for antibodies to viruses (human hepatitis viruses, HIV and SARS-CoV-2) and CRP. Besides, their ESR was <20 mm/h, they had no respiratory infection in the past 12 months, and they did not suffer from chronic disease such as respiratory allergy, diabetes or cardiovascular disease. Written consent was obtained from participants after approval of the Ethics Committee at the Iraqi Ministry of Health and Environment.

2.2. Determination of sHLA-G

Serum level of HLA-G was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit designed for quantitative detection of total HLA-G in serum, plasma, cell culture

supernatant, cell lysates and tissue homogenates. Instructions of manufacturer were followed to determine HLA-G level (Cat. No E0443Hu, Bioassay Technology Laboratory, China). Briefly, the provided plate was pre-coated with anti-human HLA-G antibody. Standards and serum samples were added to pre-assigned wells in the plate, and after an incubation period, biotinylated anti-human HLA-G antibody was added. This was followed by the addition of streptavidin-horseradish peroxidase. After incubation, the wells were washed and then a substrate solution was added. A color proportional to the HLA-G concentration was developed. The reaction was terminated with an acidic stop solution and absorbance was measured at 450 nm. Concentrations of standards were plotted against their respective absorbances, and then a curve-fitting equation was obtained and used to calculate the HLA-G level of unknown samples. The standard curve range of the kit was 0.3 – 90 ng/mL.

2.3. Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) and GraphPad Prism version 8.0.0 (San Diego, California USA). Categorical variables were given as number and percent, and significant differences were assessed using two-tailed Fisher exact or Chi-square test. Continuous variables were tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Parametric variables (i.e. normally distributed) were expressed as mean \pm standard deviation (SD), and significant differences were tested using least significant difference test. Non-parametric variables were given as median and interquartile range (IQR: 25 – 75%), and significant differences were determined using Mann-Whitney *U* (to compare two groups) or Kruskal-Wallis (to compare more than two groups) test. Multinomial logistic regression analysis was performed to estimate odds ratio (OR) and 95% confidence interval (CI) after classifying sHLA-G levels into four quartiles, which were 1 ($<25\%$), 2 (25–50%), 3 ($>50\text{--}75\%$) and 4 ($>75\%$). Four models were followed to perform the analysis; I (unadjusted), II (adjusted for age), III (adjusted for age and gender) and IV (adjusted for age, gender and BMI). Receiver operating characteristic (ROC) curve analysis was performed to determine area under the curve (AUC), 95% CI, cut-off value, sensitivity and specificity. The Youden index was used to select the best cut-off value. Spearman rank correlation analysis was applied to estimate correlation coefficient between study variables. A probability (*p*) value ≤ 0.05 was taken statistically significant. The *p*-value was corrected (*pc*) due to multiple comparisons using Bonferroni correction.

3. Results

3.1. Baseline characteristics

A significant increase in median age was observed in COVID-19 patients compared to controls (57 vs. 32; $p < 0.001$). Further, the patients and controls were divided into two age groups (<45 and ≥ 45 years). It was found that most patients were classified under the age group ≥ 45 years (80.6%), while most of controls were classified under the age group <45 years (87.6%). This difference was significant ($p < 0.001$). Stratification by gender, BMI, and blood groups ABO and Rh did not reveal significant differences between patients and controls. Patients were characterized by increased incidence of cardiovascular disease and diabetes (57.2%), and this was accompanied by a significant increase in the median level of RBG compared to controls (185 vs. 93 mg/dL; $p < 0.001$). ESR and WBC were also significantly increased in patients (57 vs. 9 mm/h and 12.3 vs. $9.4 \times 10^9/L$, respectively;

Table 1
Baseline characteristics of COVID-19 cases and controls.

Characteristic	Cases; n = 103	Controls; n = 105	p-value
Age; years	57.0 (47.0–66.0)	32.0 (25.0–39.0)	<0.001
Age groups; years			<0.001
	<45	20 (19.4)	
	≥ 45	83 (80.6)	
Gender	Male	76 (73.8)	0.495
	Female	27 (26.2)	
Body mass index; kg/m ²	Normal weight	28 (27.2)	0.124
	Overweight/obese	75 (72.8)	
ABO system	A	34 (33.0)	0.713
	B	17 (16.5)	
	AB	14 (13.6)	
	O	38 (36.9)	
Rh system	Positive	84 (81.6)	0.658
	Negative	19 (18.4)	
Chronic diseases	Cardiovascular	30 (29.1)	0 (0.0)
	Diabetes	9 (8.7)	0 (0.0)
	Cardiovascular/diabetes	20 (19.4)	0 (0.0)
	No disease	44 (42.7)	100 (100.0)
C-reactive protein	Negative	0 (0.0)	105 (100.0)
	Positive	103 (100.0)	0 (0.0)
Erythrocyte sedimentation rate; mm/hour	57.0 (39.0–80.0)	9.0 (6.0–13.0)	<0.001
Hemoglobin, g/dL	14.3 ± 9.8	13.4 ± 1.8	0.356
Platelets × 10 ⁹ /L	282.9 ± 135.9	273.2 ± 95.5	0.554
White blood cell count × 10 ⁹ /L	12.3 ± 5.2	9.4 ± 1.9	<0.001
Random blood glucose; mg/dL	185.0 (144.0–290.0)	93.0 (87.0–106.0)	<0.001
Vitamin D (25OHD3); ng/mL	41.2 (35.6–47.9)	49.2 (46.4–52.3)	<0.001

Values were given as median followed by interquartile range (25 – 75%) in parentheses (non-parametric variable), mean ± standard deviation (parametric variable) or number followed by percentage in parentheses (categorical variable). p: Probability of Mann-Whitney U test (to compare non-parametric variables), least significant difference test (to compare parametric variables) or Pearson Chi-square test (to compare categorical variables). Significant p-value is indicated in bold.

p < 0.001), while median levels of vitamin D were significantly decreased (41.2 vs. 49.2 ng/mL; p < 0.001) (Table 1).

3.2. Serum level of sHLA-G

The median levels of sHLA-G were significantly increased in serum of COVID-19 patients compared to controls (19.3 vs. 12.7 ng/mL; p < 0.001) (Fig. 1). When patients and controls were

stratified by gender, BMI, chronic disease, or ABO and Rh blood groups, the sHLA-G level did not show a significant difference in each stratum (Table 2). Regarding age groups (<45 and ≥ 45 years), although their frequencies showed a significant difference between patients and controls, neither the patients nor the controls showed a significant difference in sHLA-G level between these age groups (Fig. 2). Logistic regression analysis revealed that patients in the quartiles >50–75% and >75% of sHLA-G level were more likely to have COVID-19 even after adjusting the analysis for age, gender and BMI (Model IV); the estimated OR under this model was 11.56 (95% CI = 3.15–42.37; p < 0.001) and 43.34 (95% CI = 9.63–195.12; p < 0.001), respectively (Table 3).

3.3. ROC curve analysis

ROC curve analysis revealed that up-regulated level of sHLA-G was a very good predictor of COVID-19. The AUC was 0.871 (95% CI = 0.822 – 0.919; p < 0.001). At a cut-off value of 15.4 ng/mL (optimized by the Youden index), the sensitivity and specificity of sHLA-G were 79.6 and 79.0%, respectively (Fig. 3).

3.4. Spearman rank correlation

Spearman rank correlation analysis revealed that sHLA-G was positively correlated with age, ESR, WBC and RBG (correlation coefficient = 0.495, 0.558, 0.204 and 0.501, respectively; p < 0.01), while a negative correlation was recorded with vitamin D (correlation coefficient = -0.155; p < 0.01) (Table 4).

4. Discussion

The median age of the patients recruited was 57 years, and thus age is indicated as a critical risk factor for the development of COVID-19 and progression to severe disease. Previous studies have also documented that severity and outcome of COVID-19 depends largely on age of patients, with adults in their sixth decade of life accounting for majority of hospitalizations and having

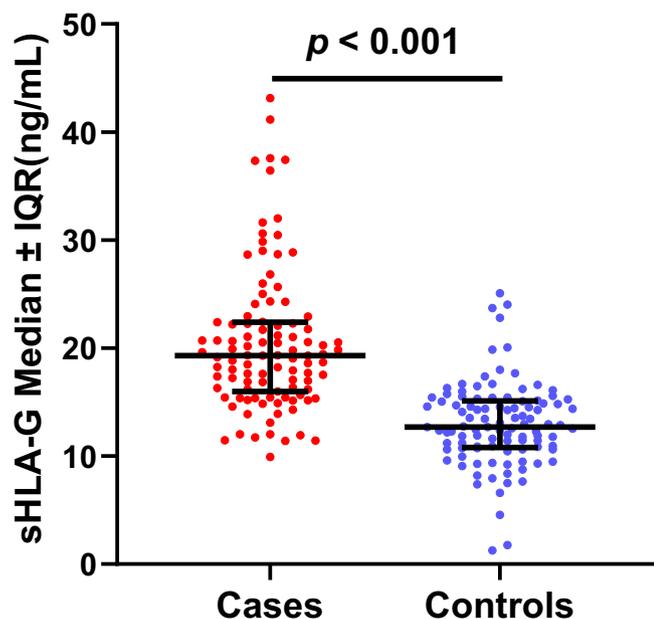


Fig. 1. Scatter dot plot of soluble HLA-G (sHLA-G) levels in serum of COVID-19 cases and healthy control subjects. The horizontal lines indicate the median concentration for each group, while vertical lines denote interquartile range (IQR). The difference between COVID-19 cases and controls was assessed by the non-parametric Mann-Whitney U test.

Table 2
Median levels of soluble HLA-G in serum of COVID-19 cases and controls stratified by some characteristics.

Characteristic		HLA-G median (IQR: 25% – 75%); ng/mL	
		Cases; n = 103	Controls; n = 105
Gender	Male	18.8 (15.5–22.1)	12.7 (10.8–15.1)
	Female	20.5 (16.9–30.5)	13.3 (10.8–15.3)
	<i>p</i> -value	0.141	0.551
Body mass index; kg/m ²	Normal weight	20.8 (17.5–26.4)	14.1 (11.9–15.5)
	Overweight/obese	19.0 (15.4–21.7)	12.4 (10.4–14.9)
	<i>p</i> -value (<i>pc</i>)	0.102	0.129
ABO system	A	19.0 (16.2–24.1)	14.1 (11.9–15.8)
	B	19.3 (16.3–20.0)	13.2 (10.0–16.0)
	AB	19.8 (14.6–22.4)	12.4 (11.0–14.6)
	O	19.6(16.9–22.4)	11.9 (10.3–14.4)
	<i>p</i> -value (<i>pc</i>)	0.991	0.106
Rh system	Positive	19.0 (15.8–22.2)	12.9 (10.8–15.3)
	Negative	20.2 (17.7–28.9)	11.8 (9.5–14.3)
	<i>p</i> -value (<i>pc</i>)	0.366	0.358
Chronic diseases	Cardiovascular	19.3 (15.7–24.3)	NA
	Diabetes	18.7 (15.4–25.0)	NA
	Cardiovascular/diabetes	19.7 (17.7–23.1)	NA
	No disease	19.0 (10.4–21.1)	NA
	<i>p</i> -value	0.545	

IQR: Interquartile range; *p*: Probability of Mann-Whitney *U* test (to compare two groups) or Kruskal-Wallis test (to compare more than two groups); *pc*: The probability value is adjusted by the Bonferroni correction for multiple tests. Significant *p*-value is indicated in bold.

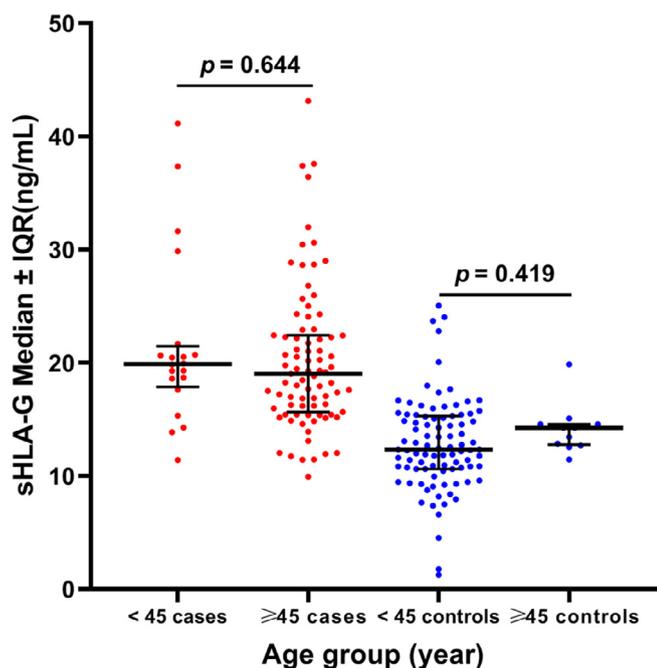


Fig. 2. Scatter dot plot of soluble HLA-G (sHLA-G) levels in serum of COVID-19 cases and healthy control subjects stratified by age groups. The horizontal lines indicate the median concentration for each group, while vertical lines denote interquartile range (IQR). The difference between age groups was assessed by the non-parametric Mann-Whitney *U* test.

a higher risk of death than younger adults [21]. This may be related to two immune phenomena linked to aging, immunosenescence and cellular senescence, which can cause impaired immunological responses to SARS-CoV-2 and increased systemic inflammation due to irreversible cell cycle arrest [22]. Besides, age-related morbidities, such as cardiovascular disease and diabetes, may also increase susceptibility to COVID-19 and are likely to be associated with a more severe illness [23]. These findings are reinforced in the current study and >50% of COVID-19 patients had chronic diseases (cardiovascular disease and diabetes). Further, their RBC was

185 mg/dL, which is indicative of pre-diabetes according the American Diabetes Association Criteria [24]. Consistent with our observation, it has been found that hyperglycemia occurred in 33.2% of COVID-19 patients during hospitalization, and the proportion was higher in severe cases compared to non-severe cases (45.4 vs. 21.5%) [25]. Accordingly, it has been hypothesized that the SARS-CoV-2 may reduce insulin secretion through its effects on pancreatic β -cell function [26]. The COVID-19 receptor (ACE2: angiotensin-converting enzyme 2) has been shown to be expressed by pancreatic cells, as well as other metabolic organs and tissues (for instance, adipose tissue, intestine and kidneys). Therefore, the virus may cause multidirectional changes in glucose metabolism that could complicate pre-existing diabetes or lead to the development of hyperglycemia in non-diabetic patients [27]. It has also been demonstrated that COVID-19 patients are markedly characterized by elevated serum levels of pro-inflammatory cytokines; for instance, interleukin-1 (IL-1), IL-6, IL-12, interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α) [28]. Inflammation due to high production of cytokines may lead to insulin resistance. Furthermore, these cytokines may impair insulin production by pancreatic β -cell [29].

This study also revealed that patients with severe COVID-19 had a significantly lower serum level of vitamin D compared to controls. Thus, it can be indicated that this vitamin represents an additional risk factor for disease progression and severity. Vitamin D is an essential food supplement that contributes to maintaining good health and preventing diseases, especially respiratory diseases. Low levels of vitamin D have been associated with an elevated risk of developing pneumonia and viral upper respiratory infections [30]. In the case of COVID-19, vitamin D can be considered as a protective factor due to its role in regulating the production of pro-inflammatory cytokines from pulmonary epithelial cells and macrophages, as well as in suppressing the cytokine storm, which is associated with immunopathogenesis of SARS-Cov-2 infection [31]. These beneficial effects in improving COVID-19 outcomes have been experimentally demonstrated, and patients who received adjunctive Pulse D therapy (vitamin D) showed a significant reduction in inflammatory markers associated with the clinical course of disease [32].

In addition to age, cardiovascular disease, diabetes and vitamin D status, sHLA-G can be considered an additional risk factor linked

Table 3
Multinomial logistic regression analysis of sHLA-G between COVID-19 patients and controls.

Model	Analysis	Serum level of sHLA-G; ng/mL			
		Quartile 1 (<25%)	Quartile 2 (25–50%)	Quartile 3 (>50–75%)	Quartile 4 (>75%)
I	OR (95% CI)	Reference	1.75 (0.66–4.66)	12.09 (4.64–31.50)	41.21 (13.22–128.49)
	p-value		0.265	<0.001	<0.001
II	OR (95% CI)	Reference	1.46 (0.40–5.30)	8.88 (2.51–31.40)	26.78 (6.53–109.79)
	p-value		0.562	0.001	<0.001
III	OR (95% CI)	Reference	1.47 (0.41–5.29)	9.04 (2.58–31.72)	29.39 (7.05–122.58)
	p-value		0.552	0.001	<0.001
IV	OR (95% CI)	Reference	1.73 (0.47–6.34)	11.56 (3.15–42.37)	43.34 (9.63–195.12)
	p-value		0.401	<0.001	<0.001

Model I: Unadjusted analysis; Model II: Adjusted for age; Model III: Adjusted for age and gender; Model IV: Adjusted for age, gender and body mass index; OR: Odds ratio; CI: Confidence interval; p: Probability (significant p-value is indicated in bold).

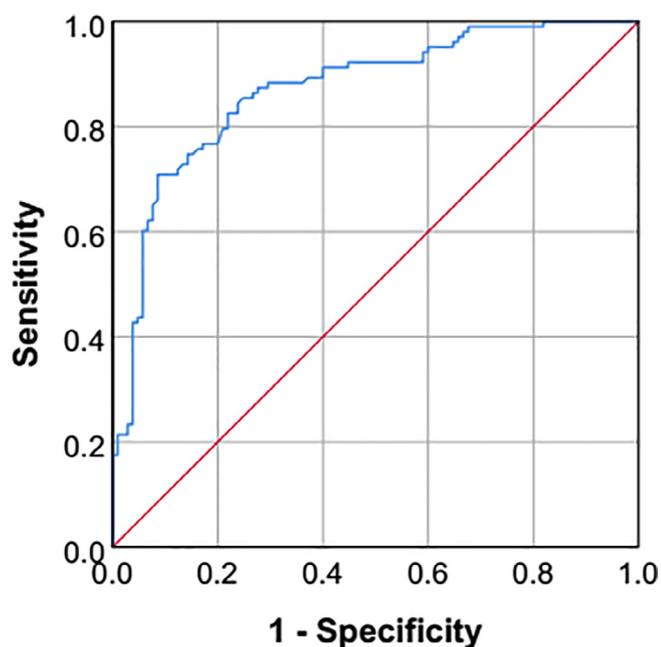


Fig. 3. Receiver operating characteristic (ROC) curve plot of soluble HLA-G for predicting COVID-19 (Area under curve = 0.871; 95% confidence interval = 0.822 – 0.919; p-value <0.001; Youden index = 0.586; Cut-off value = 15.4 ng/mL; Sensitivity = 79.6%; Specificity = 79.0%).

to COVID-19 immunopathogenesis. Levels of sHLA-G were significantly up-regulated in serum of patients with severe COVID-19 compared to controls. Patients who were high producers of sHLA-G were 14.73-folds more likely to develop COVID-19 compared to the low producers. Moreover, the ROC curve analysis demonstrated the predictive significance of sHLA-G in differentiating between COVID-19 patients and controls. (AUC = 0.871). However, there is a point of concern regarding age mismatch between

patients and controls; the median age of patients was 57 years, while it was 32 years in controls. To test the hypothesis that sHLA-G might be affected by age, patients and controls were divided into two age groups (<45 and 45 years), and sHLA-G serum levels were examined in these groups. It was found that both age groups of patients showed a significant increase in sHLA-G levels compared to corresponding groups in controls. Further, there was no significant difference between the age groups of patients or controls (Fig. 2). This may indicate that age had no effect on sHLA-G, and that the observed up-regulated level of these molecules was associated with the risk of COVID-19 infection regardless of age. In addition, sHLA-G level was also not affected by gender, BMI, or ABO and Rh blood group systems, and this may confirm the significance of sHLA-G in immunopathogenesis of COVID-19.

There is no direct evidence to support or refute these findings, but several studies have indicated that HLA-G plays a prominent role in controlling the pathogenesis of a number of autoimmune and inflammatory diseases, such as inflammatory bowel disease, celiac disease, psoriasis, asthma, rheumatoid arthritis, juvenile idiopathic arthritis and systemic lupus erythematosus [14]. The main function of sHLA-G is to inhibit innate and adaptive immune responses by interacting with its ligands on target cells to transmit inhibitory signals. Natural killer cells, CD4 + and CD8 + T cells, B cells, macrophages, dendritic cells and neutrophils are among the target cells functionally influenced by sHLA-G. Thus, most features of the immune response, including differentiation, proliferation, cell lysis, cytokine production, and immunoglobulin release, can be inhibited by sHLA-G [15,33]. This is apparent in viral infections and patients infected with human immunodeficiency virus [34], human cytomegalovirus [35], herpes simplex type I virus [36], H1N1 influenza virus [37] or hepatitis B virus [38] have shown up-regulated expression of cell-bound HLA-G or sHLA-G molecules, which were associated with persistent viral infection. On the contrary, a down-regulated expression of the HLA-G5 isoform was found in cervical cancer cases infected with human papillomavirus [39]. Therefore, increased expression of sHLA-G may enhance the virus ability to escape from immune mechanisms;

Table 4
Spearman rank correlation analysis between study variables.

Variable	sHLA-G	Age	ESR	WBC	RBG	Vitamin D
sHLA-G	1.000	0.495**	0.558**	0.204**	0.501**	–0.155*
Age		1.000	0.657**	0.302**	0.656**	–0.294**
ESR			1.000	0.370**	0.665**	–0.362**
WBC				1.000	0.180**	–0.181**
RBG					1.000	–0.346**
Vitamin D						1.000

ESR: Erythrocyte sedimentation rate; WBC: White blood cell count; RBG: Random blood glucose.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

otherwise, sHLA-G secretion may represent an exaggerated immune response to counteract the inflammatory response during viral infection [15]. In COVID-19, the first indication of HLA-G involvement in COVID-19 was documented by Zhang and colleagues. The authors followed the dynamics of peripheral immune cells (WBC, neutrophils, monocytes, B cells and CD4 + and CD8 + T cells), cytokines (IL-4, IL-6, IFN- γ and TNF- α) and HLA-G and its inhibitory receptors immunoglobulin-like transcript (ILT) 2, ILT4 and KIR2DL4 (killer cell immunoglobulin-like receptor, two immunoglobulin domains and long cytoplasmic tail four) in a patient with severe COVID-19 pneumonia up to the recovery period. Their data indicated that frequency of HLA-G + T cells, B cells and monocytes followed the pattern high-low-high, while frequency of cells expressing the receptors ILT2, ILT4 and KIR2DL4 remained relatively stable [17]. Next, Zidi proposed that HLA-G molecules could be considered as potent determinants in inhibiting immune cell functions during SARS-CoV2 infection, thereby promoting virus subversion [18]. Fraga-Silva and colleagues also suggested that HLA-G expression induced by SARS-CoV2 may be associated with increased morbidity and mortality, and increased expression of HLA-G in COVID-19 patients may predict a worse outcome [19]. In a man infected with SARS-CoV-2, HLA-G expression was found in epithelial cells of the intestinal mucosa and in some local lymphocytes. The authors concluded that HLA-G is involved in the viral immune escape mechanism, as well as in neoangiogenesis, and this may cause COVID-19-dependent hemorrhage [16]. Besides, a genome-wide association study in critically ill patients with COVID-19 from the UK identified that the HLA-G variant rs9380142 was associated with susceptibility to disease [40]. These observations may suggest that the expression pattern and genetic variants of HLA-G in COVID-19 is warranted to guide research to understand the mechanisms involved in disease control. However, it has been commented that HLA-G expression in COVID-19 is a complex process influenced by various factors, including infection stage, HLA-G variants, administered therapy and cytokine profile, which may create an immunological environment involved in determining the outcome of COVID-19 [19].

The up-regulated level of sHLA-G was positively correlated with age, ESR, WBC and RBG. The relationship between sHLA-G and age has not been well presented and conflicting results were found. In healthy Malian individuals, a negative correlation was found between sHLA-G and age, and those aged 3–25 years showed significantly higher levels of sHLA-G compared to individuals over 26 years of age [41]. Further, no correlation was found between age and sHLA-G in bone marrow plasma samples of healthy Italian donors [42]. The median age of the current COVID-19 patients was 57 years and this may explain the positive correlation between sHLA-G and age, especially if we take into account the observations that SARS-CoV-2 can induce the expression of sHLA-G and also disproportionately affect the elderly [19,21]. With regard to WBC and ESR, the results may be justified because both are biomarkers of inflammation [43], and sHLA-G molecules are involved in modulating inflammatory responses [13]. In the case of RBG, it has been demonstrated that sHLA-G was more frequent in patients with type 2 diabetes mellitus or impaired glucose tolerance than in normal glucose tolerance. Moreover, plasma levels of sHLA-G were gradually elevated across glucose tolerance classes [44]. In contrast to these positive correlations, sHLA-G showed a negative correlation with vitamin D. This may indicate that up-regulation of sHLA-G and down-regulation of vitamin D could contribute to viral persistence in COVID-19 patients, because it is suggested that the outcomes of both changes are linked to immunosuppressive effects, and these effects are associated with an increased risk of developing COVID-19 [13,19,31,45]. The presented correlations have not been well defined in COVID-19 and further studies are warranted to understand their mechanisms.

The study suffered from a number of limitations. First, the patients were not monitored for the level of sHLA-G from admission until recovery. Second, the profile of pro- and anti-inflammatory cytokines was not determined. Third, the viral load was not taken into account in the analysis. Fourth, patients with asymptomatic, moderate and critical COVID-19 were not investigated. Fifth, the low sample size of patients (particularly the age group <45 years) and controls (particularly the age group ≥ 45 years) was an important limitation of the study.

In conclusion, sHLA-G showed up-regulated expression in the serum of patients with severe COVID-19. This up-regulation was positively correlated with age, ESR, WBC and RBG, while it was negatively correlated with vitamin D status.

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.

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