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Short Communication

Evaluation of serum soluble HLA-G levels post-recovery from COVID-19 and post-vaccination (Sinopharm and Pfizer-BioNTech)

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ABSTRACT

Serum soluble HLA-G (sHLA-G) levels have been shown to be upregulated in COVID-19 patients. In this study, sHLA-G levels were examined in COVID-19 patients 14–21 days post-recovery (100 patients) and 80 uninfected controls. In addition, individuals vaccinated with Sinopharm or Pfizer-BioNTech (50 individuals each) were followed 21 days post-first dose and 21 days post-second dose. Serum sHLA-G levels were significantly higher in recovered patients than in controls. The first and second doses of Sinopharm and Pfizer-BioNTech were associated with significantly elevated levels of sHLA-G compared to controls or recovered patients, except for the first dose of Pfizer-BioNTech where sHLA-G levels did not show significant differences compared to recovered patients. In conclusion, recovery from COVID-19, as well as vaccination with two doses of Sinopharm or Pfizer-BioNTech, were associated with up-regulated levels of sHLA-G molecules, but the first dose of Sinopharm had the greatest effect in raising sHLA-G levels.

1. Introduction

Coronavirus disease 2019 (COVID-19) is one of the most important life-threatening infectious respiratory diseases of the twenty-second century, with significant morbidity and mortality. It is due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly pathogenic and transmissible coronavirus that infects humans and animals [1]. Infection can lead to severe pneumonia and patients may develop acute respiratory distress syndrome (ARDS). Besides, failure of multi-organs may be associated with advanced disease. Multiple lines of evidence suggest that ARDS and multi-organ failure are due to excessive inflammatory responses [2]. In this context, the term cytokine storm has been introduced to describe up-regulated systemic levels of pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-17 and tumor necrosis factor-alpha (TNF-α), key immune mediators implicated in lung damage in patients with ARDS [3]. In addition, dysregulated anti-inflammatory responses have been associated with COVID-19 severity, as well as ARDS and in-hospital mortality [4–6].

Immunity to viruses is also controlled by genes involved in mediating the immune response, in particular those mapped to the human leukocyte antigen (HLA) system. In fact, HLA molecules, by their highly polymorphic nature, serve as key regulators of the host's immune response to invading pathogens, including SARS-CoV-2 [7]. The HLA system is organized into three classes on the short arm of chromosome 6 (I, II and III), and class I molecules are functionally linked to the presentation of viral antigens at the cell surface, a process necessary for cytotoxic T cell to recognize and destroy virus-infected cell [8]. HLA-class I comprises classical (A, B, C) and non-classical (E, F, and G) molecules, and HLA-G molecules have received considerable attention due to their involvement in controlling inflammatory reactions, as well as immune response to viral infections [9]. HLA-G molecules are expressed in two forms, membrane-bound antigens (HLA-G1, -G2, -G3, and -G4) and soluble proteins (sHLA-G5, -G6 and -G7). Functionally, they are potent immunomodulators and dysregulated expression of HLA-G molecules has been implicated in many pathological conditions including viral infections [10]. In COVID-19, HLA-G molecules are suggested to have immune-regulating effects and up-regulated expression of sHLA-G has been shown in patients with severe disease [11]. Besides, HLA-G 14-bp insertion/deletion polymorphism has been associated with susceptibility to COVID-19 [12].

Since there is no effective treatment for COVID-19, vaccination is one of the most effective strategies that may limit the spread of the disease in humans. More than ten candidate vaccines worldwide have received approval for emergency use, including Pfizer-BioNTech (a messenger RNA [mRNA] vaccine) and Sinopharm (an inactivated whole-virus vaccine) [13]. Both vaccines are approved for use in Iraq...
and are given in two doses, 21 days apart. Most COVID-19 vaccines are
designed to induce the immune system to produce antibodies that neu-
tralize SARS-CoV-2 spike (S) protein. However, it has been suggested
that other innate and adaptive immune mechanisms may be elicited
by vaccines and contribute to protection against COVID-19 [14]. There
are no data linking the HLA-G vaccines to COVID-19, but in malaria,
vaccine immunogenicity and the development of parasite infection
may change depending on sHLA-G levels [15].

In this study, sHLA-G levels were analyzed in patients who had
recovered from COVID-19. In addition, the analysis was expanded to
include individuals vaccinated with Sinopharm or Pfizer-BioNTech.
In both vaccines, sHLA-G levels were determined 21 days after the first
and second doses and compared with recovered patients and healthy,
uninfected individuals. To the researchers’ knowledge, this issue has
not been addressed and the current study may contribute to under-
standing the role of sHLA-G in recovery from COVID-19, as well as
the effects of vaccines on sHLA-G levels.

2. Materials and methods

2.1. Populations studied

During the period from January to June 2021, a cross-sectional
study was performed on 280 individuals who were classified into four
groups. The first group (PR) included 100 patients (mean age ± standard deviation = 58.4 ± 15.1 years [16.0% < 40 years
and 84.0% ≥ 40 years]; 49.0% male) who recovered from COVID-19
and were enrolled in the study 14–21 days post-recovery. The second
group was a control group (CTRL) and included 80 uninfected blood
donors and health service workers (39.1 ± 7.5 years [58.8% < 40 years
and 41.2% ≥ 40 years]; 46.3% male). The third group included 50 individuals (38.3 ± 9.8 year [56.0% < 40 years
and 44.0% ≥ 40 years]; 64.0% male) who received Sinopharm vaccine
(Vero Cell; Beijing Institute of Biological Products Co., Ltd). They
were enrolled in the study 21 days post-first dose of vaccination (PS1D)
and 21 days post-second dose of vaccination (PS2D). The fourth group
included 50 individuals (39.1 ± 8.7 year [56.0% < 40 years and 44.
0 ≥ 40 years]; 40.0% male) who received Pfizer-BioNTech vaccine
(mRNA COMINARTY vaccine; Mainz, Germany/New York, United
States) and were grouped into PP1D and PP2D as in the third group.
Participants were vaccinated in primary health care units in Baghdad
(Al-Imamian Al-Kadhimiyaq Medical City and Al-Kindi General
Teaching Hospital). A nasopharyngeal swab was taken from each par-
ticipant at the time of blood collection, and tested for SARS-CoV-2
using the RealLine SARS-CoV-2 kit (Bioron Diagnostics GmbH).
Included participants were those who showed negative molecular test
and were over 18 years of age. Pregnant women were excluded. The
approval of the Ethics Committee of the Iraqi Ministry of Health was
obtained, and all participants provided written consent.

2.2. Immunoassay of sHLA-G

An enzyme-linked immunosorbent assay (ELISA) kit (Catalogue
No.; MBS267094; MyBioSource, California, USA) was used to measure
serum sHLA-G levels according to the manufacturer’s instructions.
This kit uses the double antibody sandwich ELISA technique, which
is based on the characteristics of a target antigen with more than
two potential epitopes that can be simultaneously recognized by both
pre-coated capture antibody and detection antibody. The standard
range of the kit was 0.312–40 ng/L.

2.3. Statistical analysis

Serum sHLA-G levels did not follow a normal distribution as indi-
cated by the Kolmogorov-Smirnov and Shapiro-Wilk normality tests.
Therefore, sHLA-G levels were given as median and interquartile range
(IQR). Significant differences between medians were assessed using
Mann-Whitney U test. A probability (p) ≤ 0.05 was considered statisti-
cally significant. The p-value was adjusted using Dunn’s test. GraphPad
Prism version 8.0.0 (San Diego, CA, USA) was used to perform statis-
tical analysis.

3. Results

Median sHLA-G serum levels were significantly higher in PR than
in CTRL [11.6 [IQR: 10.9–12.8] vs. 10.8 [IQR: 10.7–11.1] ng/mL; p < 0.001]. The first dose of Sinopharm was associated with non-
significantly elevated levels of sHLA-G compared to the second dose
(13.8 [IQR: 12.8–14.9] vs. 13.0 [IQR: 11.7–15.2] ng/mL; p = 0.426), but at both doses, sHLA-G levels were significantly higher
than in CTRL (10.8 [10.7–11.1] ng/mL; p < 0.001). In contrast, the first
dose of Pfizer-BioNTech was associated with significantly lower
levels of sHLA-G compared to the second dose (13.5 [IQR: 12.0–14.2]
vs. 11.6 [IQR: 10.5–11.9] ng/mL; p < 0.001), but again at both doses, sHLA-G levels were significantly higher than in CTRL
(10.8 [10.7–11.1] ng/mL; p = 0.008 and < 0.001, respectively). Com-
pared with PR, both doses of Sinopharm were associated with significa-
cantly elevated levels of sHLA-G (p < 0.001). With respect to Pfizer-
BioNTech, sHLA-G levels did not show significant differences between
the first dose and PR (p = 0.307), while significantly elevated levels of
sHLA-G were encountered at the second dose compared to PR
(p < 0.001). The first dose of Sinopharm was associated with signifi-
cantly elevated levels of sHLA-G compared to the first dose of Pfizer-
BioNTech (p < 0.001), while no significant differences were observed
between the second doses (p > 0.05) (Fig. 1). When sHLA-G levels
were examined in the six study groups (PR, CTRL, PS1D, PS2D,
PP1D and PP2D) after stratification by age group (< 45 years
and ≥ 45 years) and gender, no significant difference was found in
each stratum. However, there was a trend for increased sHLA-G levels
in PS2D females compared to males (14.3 [IQR: 12.6–16.4] vs. 12.3
[IQR: 11.5–14.6] ng/mL; p = 0.059) (Table 1).

4. Discussion

The study demonstrated that sHLA-G levels were significantly ele-
vated in PR compared to CTRL. In a previous study by our group,
sHLA-G levels were also significantly higher in COVID-19 patients with
severe disease than in CTRL [11]. Further, significantly higher plasma
levels of sHLA-G have been reported in COVID-19 patients with respi-
atory failure compared to CTRL, but clinical improvement was also
observed in parallel with higher plasma levels of sHLA-G [16]. In addi-
tion, it has been shown that recovery from critical pneumonia in a
patient with COVID-19 was associated with a fluctuation in HLA-G cel-
ular expression. The percentage of HLA-G-positive peripheral immune
cells (T cells, B cells and monocytes) followed the pattern high (sus-
pected SARS-CoV-2 infection)–low (SARS-CoV-2 RNA positive)–high
(SARS-CoV-2 RNA returned to negative) [17]. An interesting study
was done on a patient who recovered from COVID-19, but after two
weeks, the patient was admitted to the emergency department com-
plaining of abdominal pain followed by intestinal bleeding. Four
weeks after COVID-19-free, examination of the ulcerated intestinal
mucosa revealed co-expression of SARS-CoV-2 nucleocapsid protein
and HLA-G in epithelial cells and lymphocytes [18]. Although these
observations were based on only one patient, they suggest evidence
of an association between persistence of SARS-CoV-2, virus-induced
inflammation and HLA-G expression. HLA-G induction in host cells
infected with SARS-CoV-2 may represent a molecular mechanism for
the development of immune evasion as proposed in several human
pathogenic viruses [10]. During infection with SARS-CoV-2, the
observed decrease in immune-competent cells and the up-regulated
expression of pro-inflammatory cytokines is a prominent feature of immunopathology, especially in patients with severe COVID-19 [19]. Therefore, the observed change in sHLA-G levels in the current study during the recovery phase may reflect a dynamic interplay between virus, cytokines and HLA-G.

To understand the relationship between recovery from COVID-19 and sHLA-G, serum levels of sHLA-G were followed in individuals vaccinated with two doses of Sinopharm or Pfizer-BioNTech. As a general observation, sHLA-G levels exceeded those in PR and CTRL after the first and second doses, but the sHLA-G response pattern was different in Sinopharm and Pfizer-BioNTech. In Sinopharm, the first dose was associated with a non-significant increase in sHLA-G levels compared to the second dose, while in Pfizer-BioNTech, the opposite trend was observed (i.e., the first dose was associated with significantly lower levels of sHLA-G compared to the second dose). Besides, the first dose of Sinopharm induced higher levels of sHLA-G compared to the first dose of Pfizer-BioNTech, while there were no significant differences between the second doses. These differences are probably related to the vaccine technology used, inactivated whole-virus in Sinopharm and mRNA in Pfizer-BioNTech. Although both vaccines rely on the native S protein of SARS-CoV-2 to effectively stimulate neutralizing antibodies, differences between Sinopharm and Pfizer-BioNTech have been proposed in terms of mode of action, presentation of S antigen and promotion of innate immune response [20].

The immunological mechanism of action of Sinopharm and Pfizer-BioNTech in inducing virus-neutralizing antibodies is similar to that of SARS-CoV-2 infection. Since sHLA-G levels have been shown to be up-regulated during SARS-CoV-2 infection [11], sHLA-G is also expected to follow a similar trend during vaccination. Consistent with this view, sHLA-G showed significantly elevated levels after vaccination, particularly after the second dose of both vaccines. Unfortunately, data linking sHLA-G and COVID-19 vaccines are not available, but in adults who received GM22 malaria vaccine, significantly elevated levels of sHLA-G were observed after vaccination [21]. In addition, an increased risk of failure to respond to hepatitis B vaccination has been associated with HLA-G 14-bp insertion allele [22]. Accordingly, it has been suggested that serum sHLA-G levels are essential for the development of appropriate immune responses to vaccination due to immunomodulatory functions. In this context, sHLA-G molecules can be used as a potential target for vaccination in order to reach desirable outcomes [15].

The study faced the limitation of not following COVID-19 patients from clinical disease until recovery. Besides, the sample size of the vaccinated individuals was relatively small. In addition, simultaneous evaluation of pro-inflammatory and anti-inflammatory cytokines has not been performed, and this will certainly contribute to a better understanding of the relationship between sHLA-G molecules and COVID-19 vaccines.

In conclusion, recovery from COVID-19, as well as vaccination with two doses of Sinopharm or Pfizer-BioNTech, were associated with up-regulated levels of sHLA-G molecules, but the first dose of Sinopharm had the greatest effect in raising sHLA-G levels.

Table 1

| Group | Age group, year | Gender | p-value |
|-------|----------------|--------|---------|
|       |     | PR     | CTRL   | PS1D   | PS2D   | PP1D   | PP2D   |
| < 45  |     | 11.8 (10.7-13.0) | 10.8 (10.6-11.7) | 13.8 (12.8-14.7) | 13.4 (12.0-16.0) | 11.5 (10.4-13.9) | 12.5 (12.8-14.0) | 16.4 (12.9-16.4) | 0.059 |
| ≥ 45  |     | 11.6 (10.9-12.7) | 10.8 (10.7-11.0) | 13.4 (12.9-15.3) | 12.5 (11.4-15.0) | 11.7 (11.0-11.9) | 13.6 (12.0-14.3) | 13.5 (12.0-14.2) | 0.579 |
|       |     | 11.4 (10.7-12.6) | 10.9 (10.7-11.2) | 13.7 (12.9-14.7) | 12.3 (11.5-14.6) | 11.7 (10.7-11.9) | 13.3 (12.4-13.9) | 15.0 (12.6-15.1) | 0.659 |
|       |     | 11.9 (10.9-12.9) | 10.8 (10.6-11.0) | 13.8 (12.8-15.1) | 14.3 (12.6-16.4) | 11.6 (10.4-11.9) | 13.5 (12.0-14.2) | 0.859 |
|       |     | 0.318 | 0.876 | 0.803 | 0.235 | 0.395 | 0.675 | 0.059 |
|       |     | 0.154 | 0.121 | 0.059 | 0.059 | 0.859 | 0.579 |
| PR: 14–21 days post-recovery (n = 100); CTRL: Controls (n = 80); PS1D: 21 days post-first dose of Sinopharm (n = 50); PS2D: 21 days post-second dose of Sinopharm (n = 50); PP1D: 21 days post-first dose of Pfizer-BioNTech (n = 50); PP2D: 21 days post-second dose of Pfizer-BioNTech (n = 50); p: Two-tailed probability (Mann-Whitney U test).
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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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References

[1] B. Hu, H. Guo, P. Zhou, Z.L. Shi, Characteristics of SARS-CoV-2 and COVID-19, Nat. Rev. Microbiol. 19 (2021) 141–154. https://doi.org/10.1038/s41579-020-00459-7.

[2] G. Widjaja, A. Turki Jalil, H. Sulaiman Rahman, D.O. Bokov, W. Sukastan, M. Ghaedi, F. Marrof, J. Gholideh Navashenag, F. Jaddidi-Niazhagh, M. Ahmadi, Humoral immune mechanisms involved in protective and pathological immunity during COVID-19, Hum. Immunol. 82 (2021) 733–745, https://doi.org/10.1016/j.humimm.2021.06.011.

[3] S. Montazeranbeh, S.M. Hoseiniyan Khatibi, M.S. Hejazi, V. Tarhriz, A. Farjami, F. Ghasemian Sorbeni, R. Farahzadi, T. Ghasemnejad, COVID-19 infection: an overview on cytokine storm and related interventions, Virol. J. 19 (2022) 92, https://doi.org/10.1186/s12985-022-01814-1.

[4] A.A Ahmed, A.H. Adhiyaw, Interleukin-37 is down-regulated in serum of patients with severe coronavirus disease 2019 (COVID-19), Cytokine. 148 (2021) 155702. https://doi.org/10.1016/j.jcyto.2021.155702.

[5] Q.Q. Liu, A. Cheng, Y. Wang, H. Li, X. Zhao, T. Wang, F. He, Cytokines and their relationship with the severity and prognosis of coronavirus disease 2019 (COVID-19): A retrospective cohort study, BMJ Open. 10 (2020) e041471. https://doi.org/10.1136/bmjopen-2020-041471.

[6] Q. Noz, M. Schmalzing, F. Wedekink, T. Schlesinger, M. Gernert, J. Herrmann, L. Sorgor, D. Weisnman, B. Schmid, M. Sitter, N. Schlegel, P. Kranke, J. Wischhusen, P. Meybohm, C. Lotz, Pro- and Anti-Inflammatory Responses in Severe COVID-19-Induced Acute Respiratory Distress Syndrome-An Observational Pilot Study, Front. Immunol. 11 (2020). https://doi.org/10.3389/fimmu.2020.581338.

[7] I. Saulle, C. Vicentini, M. Clerici, M. Basin, Antigen presentation in SARS-CoV-2 infection: the role of class I HLA and ERAP polymorphisms, Hum. Immunol. 82 (2021) 551–560. https://doi.org/10.1016/j.humimm.2021.05.003.

[8] M. Wiesnork, E.T. Absalouris, J. Sticht, M. Álvaro-Benito, S. Stolenberg, F. Noé, C. Freund, Major histo compatibility complex (MHC) class I and MHC class II proteins: Conformational plasticity in antigen presentation, Front. Immunol. 8 (2017) 292, https://doi.org/10.3389/fimmu.2017.00292.

[9] H.-H. Xu, A. Lin, W.-h., Yan, HLA-G-mediated immunological tolerance and autoimmunity, in: Transl. Autoimmun, Academic Press, 2022, pp. 265–295, https://doi.org/10.1016/B978-0-12-822564-6.00021-5.

[10] S. Jasinski-Bergner, D. Schmiedel, O. Mandelboim, B. Seliger, Role of HLA-G in Viral Infections, Front. Immunol. 13 (2022), https://doi.org/10.3389/fimmu.2022.826074.

[11] N.T. Al-Bayatee, A.H. Adhiyaw, Soluble HLA-G is upregulated in serum of patients with severe COVID-19, Hum. Immunol. 82 (2021) 726–732. https://doi.org/10.1016/j.humimm.2021.07.007.

[12] A.H. Adhiyaw, N.T. Al-Bayatee, (COVID-19) among Iraqi patients, Hum. Immunol. 83 (6) (2022) 521–527.

[13] A. Jamkhande, M.R. Khairnar, N. Gavali, Y. Patil, S.S. Kapare, K.P. Bhosal, A review of approved COVID-19 vaccines, Rocz. Panstw. Zakl. Hig. 72 (2021) 245–252, https://doi.org/10.32394/rzwh.2021.0177.

[14] M. Sadarangani, A. Marchant, T.R. Kollmann, Immunological mechanisms of vaccine-induced protection against COVID-19 in humans, Nat. Rev. Immunol. 21 (2021) 475–484, https://doi.org/10.1038/s41577-021-00579-z.

[15] S. Rashidi, C. Vieira, R. Tuteja, R. Mansouri, M. Ali-Hassanzadeh, A. Muro, P. Nguewa, R. Manzano-Román, Immunomodulatory Potential of Non-Classical HLA-G in Infections including COVID-19 and Parasitic Diseases, Biomolecules. 12 (2022) 257, https://doi.org/10.3390/biom12020257.

[16] D. Bortolotti, V. Gentili, S. Rizzo, G. Schiuma, S. Beltrami, S. Spadaro, G. Strazzabosco, G. Campo, E.D. Carosella, A. Papid, R. Rizzo, M. Contoli, Increased sHLA-G is associated with improved COVID-19 outcome and reduced neutrophil adhesion, Viruses. 13 (2021) 1855, https://doi.org/10.3390/v13091855.

[17] S. Zhang, J. Gan, B.G. Chen, D. Zheng, J.G. Zhang, R.H. Lin, Y.P. Zhou, W.Y. Yang, A. Lin, W.H. Yan, Dynamics of peripheral immune cells and their HLA-G and receptor expressions in a patient suffering from critical COVID-19 pneumonia to convalescence, Clin. Transl. Immunol. 9 (2020) e1128.

[18] R. Rizzo, L.M. Nerì, C. Simioni, D. Bortolotti, S. Occhionorelli, G. Zauli, P. Secchiero, C.M. Semprini, I. Laface, J.M. Sanz, G. Lanza, R. Gañà, A. Passaro, SARS-CoV-2 nucleocapsid protein and ultrastructural modifications in small bowel of a 4-week-negative COVID-19 patient, Clin. Microbiol. Infect. 27 (2021) 936–937, https://doi.org/10.1016/j.cmi.2021.01.012.

[19] A. Lin, W.H. Yan, Perspective of HLA-G Induced Immunosuppression in SARS-CoV-2 Infection, Front. Immunol. 12 (2021), https://doi.org/10.3389/fimmu.2021.788769.

[20] F.X. Heinz, K. Stiasny, Distinguishing features of current COVID-19 vaccines: knowns and unknowns of antigen presentation and modes of action, NPJ Vaccines. 6 (2021) 1–13, https://doi.org/10.1038/s41551-021-00369-6.

[21] O. Nouatin, U. Ateba Ngoa, J. Ibáñez, J.C. Dejon-Agobe, B. Mordmüller, J.R. Edoa, M. Moutairou, S.L. Hoffman, S. Issifou, A.J.F. Luty, M.M. Loembe, S.T. Agnandji, B. Lelli, P.G. Kremsner, A.A. Adegnika, Effect of immune regulatory pathways after immunization with GMZ2 malaria vaccine candidate in healthy lifelong malaria-exposed adults, Vaccine. 38 (2020) 4263–4272, https://doi.org/10.1016/j.vaccine.2020.04.046.

[22] A.A. Shahawy, A.A. Ahmed, M.A. Arafah, M.M. Malek, Influence of HLA-G polymorphism in antibody response to hepatitis B vaccination during the first year of life, Vaccines. 21 (2020) 76–81, https://doi.org/10.2478/vacc-2020-0005. 