Expression Profiles of HLA-G, HLA-H and HLA-F Post SARS-CoV2 Vaccination and Infection in Iraq

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***Background:** As new, potentially deadly strains of SARS-CoV-2 arise, it is more important than ever to ensure that as many people as possible are immunized against the virus. In Iraq the three WHO approved vaccines (Pfizer, Sinopharm and AstraZeneca) have been applied. There are limited studies on the Human leukocyte antigen (HLA) molecules display astonishing diversity, which is essential for broadening antigen presentation to T lymphocytes. This study aimed to evaluates the expression of non-classical HLA-G, HLA-H and HLA-F in the vaccinated and infected Iraqi populations.*

***Methods:** The gene expression of HLA-G, HLA-H and HLA-F genes was estimated by qRT-PCR technique by calculating the fold change in volunteers vaccinated with Pfizer, AstraZeneca and Sinopharm vaccines, after the second dose and those who had infection with follow-up conducated for three months post-vaccination and infection.*

***Results:** The relative gene expression showed increase the expression of HLA-G in the Sinopharm and Pfizer vaccinates then infected group, while slightly expression noticed in in AstraZeneca vaccinates. The HLA- H showed dramatic increased in Pfizer and Sinopharm vaccinates, respectively. While moderately expressed in AstraZeneca. Similarity, the HLA-F overexpressed in the infected group, followed by Pfizer vaccinates, Sinopharm, as well as in AstraZeneca vaccinates.*

***Conclusions:** It was suggested that the over expression of HLA-G, HLA-H, HLA-F clarified that Pfizer and Sinopharm have potential role in regulation immunologic tolerance and inflammation against SARS-COV-2. Further research to monitor the classical and non-classical HLA and its polymorphic regions are required with larger sample size and follow up durations with multiple vaccines approches.*

***Key words:** SARS-CoV2, Vaccines; HLA-G, HLA-H; HLA-F, gene expression, RT-PCR.*

INTRODUCTION

COVID-19, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in December 2019 in Wuhan, China. The virus's genome was fully sequenced and published in early January 2020, enabling the rapid development of diagnostic and preventive measures [1]. The disease presents a wide range of clinical manifestations, from mild upper respiratory symptoms to severe outcomes such as acute respiratory distress syndrome (ARDS), multi-organ failure, and death. In addition to respiratory symptoms, non-respiratory complications including anosmia, diarrhea, thromboembolic disorders, myocarditis, and vasculitis have been reported, highlighting its systemic impact [2]. To curb the pandemic, several vaccines targeting the SARS-CoV-2 spike (S) protein have been developed. The spike protein plays a crucial role in viral entry by binding to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells [3]. Vaccine platforms include mRNA vaccines (e.g., Pfizer-BioNTech), adenoviral vector vaccines (e.g., AstraZeneca), and inactivated virus vaccines (e.g., Sinovac/Sinopharm). These vaccines have shown varying degrees of efficacy, with booster doses enhancing both B-cell-mediated antibody production and T-cell responses [4, 5]. In Iraq, the Pfizer-BioNTech, AstraZeneca, and Sinopharm vaccines have been widely administered, each employing distinct mechanisms of action to elicit immune protection [6]. Evaluating immune responses to these vaccines through serological and immunological markers is critical for understanding their efficacy in preventing severe disease and transmission. The human leukocyte antigen (HLA) system, a key component of the major histocompatibility complex (MHC), is integral to immune regulation. Classical HLA-I molecules (HLA-A, HLA-B, and HLA-C) are responsible for antigen presentation, enabling the immune system to distinguish between self and non-self. In contrast, non-classical HLA-Ib molecules (HLA-H, HLA-F, and HLA-G) primarily modulate immune tolerance and inflammation [7]. Among these, HLA-G has garnered significant attention for its role in immune regulation during infections. By promoting a tolerogenic immune environment, HLA-G can dampen excessive immune responses, facilitating pathogen persistence and immune escape [8, 9]. Its ability to form dimers enhances its interaction with inhibitory receptors, further contributing to immune suppression [10]. HLA-G expression is often upregulated during infections, influenced by cytokine levels and inflammatory mediators. This upregulation can occur on various immune cells, including monocytes and regulatory T cells (Tregs), and serves as a mechanism to balance immune activation and tolerance [11, 12]. Similarly, HLA-F has been implicated in viral infections, with studies suggesting its role in presenting peptides to T cells and interacting with immune cell receptors, such as those on natural killer (NK) cells [13]. Despite its critical role, the precise mechanisms underlying HLA-F activation and function remain poorly understood. This study investigates the expression patterns of HLA-G and HLA-F in individuals recovering from COVID-19 and those vaccinated with Pfizer-BioNTech, AstraZeneca, or Sinopharm vaccines. By examining their roles in post-infection and vaccine-induced immunity, this research aims to provide insights into the immunological responses that underpin recovery and protection against SARS-CoV-2.2. Materials and Methods

2.1. SUBJECTS

A total of 230 Iraqi took part in the trial, 30 of whom served as healthy controls in a case-s66control study. Participants were divided into four groups: those who had received the Pfizer vaccine, those who had received the AstraZeneca and Sinopharm (50 for each) post the second dose of vaccination and 50 infected cases post one month of infection were collected from different hospitals in Iraq including Baghdad Teaching Hospital, AL-forat General hospital, Ibn AlKateeb Hospital, Dr. Saad Al-Witry Neuroscience Hospital, Baghdad, Iraq after one month of infection.. Those who had a follow-up 3 months after vaccination and infection. The study was approved by the Ethics Committee of the College of Biotechnology, Al-Nahrain University consents were taken from patients for inclusion in the study

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2.2. SAMPLING AND PREPARATIONS

Two ml of peripheral blood was transferred to EDTA tube (300 µl of blood was transferred to 600ul of Trizol reagent) for gene expression.

2.3. THE EXPRESSION OF HLAS.

Total RNA was extracted from all samples using the ReliaPrepTM RNA extraction kit from promega (United States of America), and the RNA concentration and purity were estimated using the QuantiFluor® RNA System (the samples under study had an average RNA concentration of 73.92±8.12 ng/L and an average purity of just over 2.00). BioLabs, UK's LunaScript® RT SuperMix All RNA samples were converted to cDNA using the kit provided. For pcr amplification, we utilized Luna® Universal qPCR Master Mix from BioLabs in the UK. Each of the primers in table (1) was made in accordance with instructions provided by Macrogene, a Korean company.

Table (1). Primers were used in this study.

Primer		Sequence (5'->3')	GC%	TM	Product Size (pb)
HLA-G	F	ACGGAAACTTAGGGCTACGG	55	59.47	551
	R	TCACACTTGCGCTTGGAGAT	50	59.96	
HLA-H	F	ATTCCCACTAGGTGTCGGGT	55	60.25	619
	R	CTTGGTGATCTGAGCTGCCA	55	60.04	
HLA-F	F	CCCATCTCTGACCATGAGGC	60	59.89	632
	R	AAGCTCCTGCCCCTCCTAAA	55	60.55	
GAPDH	F	TTTTGCGTCGCCAGCC	62.5	58.52	208
	R	ATGGAATTTGCCATGGGTGGA	47.62	60.27	

2.4. STATISTICAL ANALYSIS

GraphPad Prism v8 and IBM SPSS Statistics v27 were used for statistical analysis, including calculating the mean and standard error. Relative gene expression was assessed using Kenneth J. Livak method, with the control normalized to 1. Values greater than 1 indicated upregulation, while values less than 1 represented downregulation.

RESULTS

The mean age of individuals vaccinated with the Pfizer vaccine (47.43 ± 8.35 years) and those in the infected group (45.32 ± 9.63 years) was significantly higher compared to the control group (38.33 ± 5.81 years) and the AstraZeneca (33.64 ± 7.05 years) and Sinopharm (27.43 ± 4.21 years) vaccine groups. These findings suggest a potential association between older age and the likelihood of being vaccinated with Pfizer or infected, possibly reflecting population-level vaccination strategies or exposure risks targeting older individuals.

In terms of gender distribution, males were consistently predominant across all groups. Among the Pfizer and AstraZeneca vaccine recipients, males represented 70% (35/50), while females accounted for 30% (15/50). The male predominance was even more pronounced in the Sinopharm group, where males constituted 80% (40/50) and females 20% (10/50). The infected group exhibited the highest male representation, with 90% (45/50) males and only 10% (5/50) females. The control group showed a slightly

more balanced gender distribution, with 60% (18/30) males and 40% (12/30) females.
These observations highlight a distinct gender disparity, with males being disproportionately represented in the vaccinated and infected cohorts. This imbalance may reflect underlying gender differences in health-seeking behaviors, vaccine access, or exposure risks. Additionally, the higher mean ages observed in the Pfizer and infected groups may be attributed to targeted vaccination campaigns prioritizing older individuals or a higher susceptibility of older populations to infection. These findings underscore the importance of considering demographic factors when evaluating vaccine uptake and infection dynamics.

THE RELATIVE GENE EXPRESSION OF HLAS

The relative gene expression of HLA-G, HLA-H, and HLA-F across the study groups is shown in the table, with the control group serving as the baseline (fold change = 1.000). Significant temporal and intergroup variations in gene expression were observed, providing insights into the dynamics of immune modulation.

In the Pfizer-vaccinated group, HLA-G expression peaked in the first month (fold change = 2.336) but gradually declined by the third month (1.112). HLA-H expression remained relatively stable over the three months, with minor fluctuations (3.933 to 3.851). Conversely, HLA-F expression exhibited a marked increase in the first month (12.476), followed by a sharp decline in the second (2.639) and third months (2.013), suggesting an initial upregulation with a subsequent normalization over time.

For the AstraZeneca-vaccinated group, HLA-G expression was modestly elevated in the first month (1.691) but returned to baseline levels by the second and third months (1.000). HLA-H expression demonstrated a steady decline from 3.303 in the first month to 2.211 by the third month. HLA-F expression followed a similar trajectory, starting at 1.864 in the first month and stabilizing near baseline by the third month (1.432).

The Sinopharm-vaccinated group exhibited the highest HLA-G expression in the first month (4.242), with a substantial decrease by the second (1.623) and third months (1.062). HLA-H expression remained relatively stable across the three months, ranging from 3.515 to 1.989. HLA-F expression peaked in the first month (9.340) and showed a gradual decrease over time, reaching 2.723 in the third month.

In the infected group, HLA-G expression remained consistently elevated across the three months, ranging from 2.267 to 2.194. HLA-H expression was markedly higher compared to all other groups, peaking in the first month (5.424) and maintaining this level over the study period (5.394 by the third month). HLA-F expression was exceptionally high, with minimal fluctuation across the three months (17.073 to 17.052), indicating sustained upregulation in response to infection as illustrated in table (2).

Table 2. The relative gene expression of HLA in COVID-19 vaccinated and infected individuals.

Group	***Month***	***Fold Change ($2^{-\Delta\Delta CT}$)***		
		HLA-G	*HLA-H*	*HLA-F*
Control	*-*	*1.000*	*1.000*	*1.000*
Pfizer Vaccinates	*1*	*2.336*	*3.933*	*12.476*
	2	*2.298*	*3.862*	*2.639*
	3	*1.112*	*3.851*	*2.013*
AstraZeneca Vaccinate	*1*	*1.691*	*3.303*	*1.864*
	2	*1.000*	*2.545*	*1.428*
	3	*1.000*	*2.211*	*1.432*
Sinopharm Vaccinate	*1*	*4.242*	*3.515*	*9.340*

	2	*1.623*	*3.0808*	*2.178*
	3	*1.062*	*1.989*	*2.723*
Infected	*1*	*2.267*	*5.424*	*17.073*
	2	*2.282*	*5.419*	*17.021*
	3	*2.194*	*5.394*	*17.052*

DISCUSSION

According to our findings, HLA-G was most highly expressed in the Sinopharm, Pfizer and finally in the infected, whereas it was only moderately expressed in AstraZeneca. Dramatic increases in HLA- H were observed in Pfizer and the Sinopharm-vaccinated group. Though only mildly manifested in AstraZeneca. Also, the infected group had the higher levels expression of HLA-F. Only a small fraction of HLA alleles has been studied. There have been no published reports on the assessment of vaccinations or the expression of HLA-H or HLA-F in covid-19 [15]. Viral infections, including SARS-CoV-2, are known to increase the expression of HLA-G, a ligand for several immunosuppressive receptors. In COVID-19 patients, the immune system experiences significant dysfunction, with marked reduction or exhaustion of key immune-competent cells, including T cells, NK cells, B cells, and macrophages. This impairment is largely driven by HLA-G/receptor signaling, which inhibits cell proliferation and differentiation while inducing apoptosis and senescence [16, 17]. Furthermore, studies suggest that the immune response to SARS-CoV-2 varies depending on HLA genotype, with early SARS-CoV-2 proteins playing a dominant role in HLA-mediated immunogenicity.

To explore the relationship between recovery from COVID-19 and soluble HLA-G (sHLA-G), researchers monitored sHLA-G levels in individuals who received two doses of the Sinopharm, Pfizer-BioNTech, or AstraZeneca vaccines. Elevated sHLA-G levels were observed following both vaccine doses compared to pre-vaccination and control levels, though the response patterns differed between Sinopharm and Pfizer vaccines. Notably, Sinopharm induced higher sHLA-G levels after the first dose, whereas Pfizer's second dose elicited a more pronounced sHLA-G response compared to the first dose [18-20]. These differences likely stem from the vaccines' distinct mechanisms of action: Sinopharm uses an inactivated whole virus, while Pfizer employs an mRNA platform. Despite both targeting the SARS-CoV-2 spike (S) protein, variations in antigen presentation and innate immune activation may explain the observed discrepancies [21].

Non-classical HLA molecules, such as HLA-H and HLA-F, also play pivotal roles in immune regulation during vaccination. Limited evidence suggests that HLA-H exerts tolerogenic effects by facilitating the presentation of HLA-E on the cell surface, either as a transmembrane protein or via its signal peptide. This tolerogenic action may relate to the limited antigenic determinants in vaccines like Pfizer and Sinopharm, contributing to their favorable safety profiles in the initial months post-immunization.

HLA-F, on the other hand, demonstrated increased expression following vaccination, potentially influencing viral infection progression. Although the precise mechanisms of HLA-F activity remain unclear, its ability to present diverse peptides to T cells and interact with activating and inhibitory receptors on immune cells, such as NK cells, highlights its regulatory importance [22]. Enhanced HLA-F expression has been associated with increased B-cell activation and antibody class switching, further underscoring its role in adaptive immunity. Additionally, HLA-F may facilitate cross-presentation by interacting with the open conformer (OC) of MHC-I, enabling the uptake and presentation of exogenous antigens to T cells. This function is critical when surface MHC-I is downregulated, as it ensures antigen processing and immune activation [23, 24].

The fold changes in the expression of HLA-G, HLA-H, and HLA-F are thus integral to shaping the immune response to vaccination. Their immunomodulatory roles support the maturation of robust immune responses and may serve as potential targets for optimizing vaccine outcomes. However, further research with larger cohorts and extended follow-up periods is necessary to better understand the classical and non-classical HLA molecules, their polymorphic regions, and their regulatory mechanisms in vaccine-

induced immunity.

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DECLARATION OF COMPETING INTEREST

The authors affirm that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

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DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request yassirwesam93@gmail.com

ETHICS COMMITTEE APPROVAL AND PATIENT CONSENT

Before performing study and collecting samples, we obtained approval from the Institutional Review Board (Ministry of Higher Education and Scientific Research, Al-Nahrain University, Scientific Research Ethics Committee, number 4924, date 31-1-2022). In order to safeguard the patient's confidentiality, the inquiry did not include any identifying information about the patient or any identifying information about healthy persons or any portion of them.

AUTHORS' CONTRIBUTIONS

Yasir W. Issa: Led the study design, data analysis, and manuscript preparation. Lamiaa Al-Maliki: Contributed to experimental design, molecular analyses, and manuscript revision. Shahlaa M. Salih: Conducted experiments, managed data collection, and assisted with statistical analysis. Sarah A.H. Hassan: Supported data acquisition, literature review, and manuscript refinement. Hind Jaber Hassoon: Ensured ethical compliance, data validation, and contributed to manuscript review.

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