Evaluation of Some Immunological Factors Associated with SARS-Cov-2 Infection

Hadeel Salah Mahde AL-Masudi[1], Abdalnabi J Abid[2], Raheem Tuama Al Mammori[3]

[1, 2]Department of Biology, College of Science for Women, University of Babylon, Babylon Iraq

[3]Babylon GIT and Liver Center, Merjan Medical City, Babylon, Iraq

Email: ahmedAlwazni93@gmail.com

Abstract

Coronavirus (CoV) is a family of RNA viruses that cause disease in humans and other animals by entering the body via ACE2 receptors found in various organs such as the heart, lungs, kidneys, and digestive system. Coronaviruses are large particle viruses that are enveloped, spherical, have surface protrusion-forming spikes, and have a large positive- sense single-stranded RNA genome. A total 90 samples were collected from people suspected of being infected with SARSCoV-2, 50 blood samples were collected from patients, and 40 samples were collected from healthy individuals as a control group, who aged 20-80 years and carried out during September (2020) to January (2022) at Al-Hussein Teaching Hospital. Some immunological parameters were studied on serum of 50 patients and 40 control by using enzyme linked immunoassay sorbent assay (ELISA). The result found the gender ratio and HLA typing (HLA-G, HLA-DR). There is no noticeable difference between males and females in the values of HLA-DR, while there is a clear increase in the values of HLA-G in male patients than in females, and there is an increase in values between patients and healthy subjects. Reduced amounts of HLA-DR can also place COVID-19 patients at high risk of secondary and severe bacterial nosocomial infections. The results of the age groups were the HLA types (HLA-DR, HLA-G) show that The young age groups have lower level of HLA-DR in comparison with adult age groups, the Mean ± SD, of young age groups (30 -39y and 40 -49y) was revealed that 2.89 ± 0.65 and 2.59 ± 0.64) respectively, at LSD value (1,07) . While the result of HLA-G show that the higher level were spotted at adult age groups (40 -49 y, at 606.66 ± 376.33) in comparison with other age groups as well as control groups, with noted that higher level in control groups at adult age group (50 - 59 year, at 989.07 ± 488.84), at LSD value 54.8., The newly infected patients with Sars cov2 virus have reduced in HLA –DR expression in comparison with cured patients as well as in control (3.31 ± 1.07, 5.33 ± 2.99, 4.08 ± 2.09) at P. Vale < 0.05 . While the cured patients have higher level of HLA-G than newly infected patients and healthy control at mean ± SD (792.95 ± 179.71, 597.99 ± 317.51 and 534.40 ± 360.84) respectively at P Value <0.05). The smoker patients have lower level of Both HLA –DR and HLA-G in comparison with nonsmoker of patients and control, at mean ± SD (2.70 ± 0.42, 456.15 ± 221.23), respectively of HLA-DR and HLA-G.

1. Introduction

Coronavirus have a single-stranded positive-sense RNA (+ssRNA) genome that is larger (27–32 kb) than any other RNA virus. Outside the nucleus, the nucleocapsid protein (N) forms the capsid, and the genome is further packed by an envelope that is made up of three structural proteins: membrane protein (M), spike protein (S), and envelope protein (E) (Brian et al.; 2005). The genome size of SARS-CoV-2, which was recently sequenced as a member of the coronavirus family, is approximately (29.9 kb), (Li et al.; 2020). SARS-CoV-2 has sixteen non-structural proteins (nsp1-16) and four structural proteins (S, E, M, and N).

The human leukocyte antigen (HLA) system or complex is a complex of genes on chromosome 6 in humans which encode cell-surface proteins responsible for the regulation of the immune system.(Choo, 2007) The HLA system is also known as the human version of the major histocompatibility complex (MHC) found in many animals.(MS Trust .,2020). 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 (Wieczorek etal. 2017). 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.(Halenius etal.,2015).

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 (Djurisic and Hviid, 2014). 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) (Alegre etal. 2014). These molecules are considered potent immunomodulators, and their dysregulated expression has been implicated in several pathological conditions (Morandi etal. 2016). In viral infection, two hypotheses have been proposed to explain the role of HLA-G in virus immunopathogenesis. HLA-DR is a class II human leukocyte antigen (HLA) expressed on the cell surface of antigen-presenting cells, including monocytes,

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differentiated macrophages and dendritic cells, as well as B cells. Since the first description of role of HLA-DR in immunosuppression, (Volketal. 1991).

2. Materials and Methods

Totally, 90 samples were collected from people that probably patients and healthy with SARS-CoV-2 and 50 blood samples were collected from patients, and 40 samples from healthy as control group. Aged (20-80 years) during 1/September/2020 to 20/January/2022, in AL-Hussein Teaching hospital. The serum samples were placed in a gel tube and use in the study, enzyme linked immunoassay sorbent assay (ELISA). We prepared all the reagents and made the standard dilution

3. Results and Discussion

Relationship of gender and HLA types (HLA-DR and HLA-G)

There is no noticeable difference between males and females in the values of HLA-DR, while there is a clear increase in the values of HLA-G in male patients than in females, and there is an increase in values between patients and healthy subjects. This result was mentioned in table (1 – 1).

Table (1 - 1) Relationship of Gender and HLA-Types.

Parameters		Gender	N	Mean	Std. D.	P. Value
HLA –DR	Patients	Male	26	3.27	1.113	0.000
		Female	24	3.35	1.045	
	Control	Male	23	3.82	1.98	
		Female	17	5.53	2.76	
HLA –G	Patients	Male	26	628.961	304.680	0.034
		Female	24	564.455	334.188	
	Control	Male	23	601.204	417.669	
		Female	17	599 .62	169 .78	

Reduced amounts of HLA-DR can also place COVID-19 patients at high risk of secondary and severe bacterial nosocomial infections. This observation is consistent with a clinical report of secondary bacterial infections and end-organ injury among COVID-19 patients requiring ICU care. (Elliott teal, 2021)

Relationship of Age groups and HLA types (HLA-DR and HLA-G).

In relation to age groups distribution of HLA studied types (HLA-DR and HLA- G) .The result of table (1 - 2) show that The young age groups have lower level of HLA-DR in comparison with adult age groups, the Mean ± SD, of young age groups (30 -39 and 40 - 49 was revealed that 2.89 ± 0.65 and 2.59 ± 0.64) respectively, the control age groups were have higher level that patients especially at adult age groups or in patient at more than 50 years old, the LSD value (1,07) . While the result of HLA-G show that the higher level were spotted at adult age groups (40 -49 yeas, at 606.66 ± 376.33) in comparison with other age groups as well as control groups, with noted that higher level in control groups at adult age group (50 - 59 year, at 989.07 ± 488.84), at LSD value 54.8.

Table (1 - 2) Age groups distribution and HLA types.

Age Groups and HLA		N	Mean	S.D	LSD Value
HLA –DR	20 -29 Pat	1	3.46	.	1.07
	30 -39 Pat	10	2.89	0.65	
	40 --49 Pat	9	2.59	0.64	
	50 -59 Pat	11	3.82	1.68	
	> 60 Pat	19	3.58	.74	
	20 -29 Con	17	4.15	2.36	
	30 -39 Con	8	4.53	2.70	
	40 -49 Con	3	4.81	3.36	
	50 -59 Con	5	5.93	3.27	
	> 60 Con	6	4.53	1.95	
HLA –G	20 -29 Pat	1	416.15	.	54.8
	30 -39 Pat	10	573.52	248.93	
	40 --49 Pat	9	606.66	376.32	
	50 -59 Pat	11	578.60	264.14	
	> 60 Pat	19	574.93	32 .55	
	20 -29 Con	17	469.23	40.80	
	30 -39 Con	8	749.90	271.17	
	40 -49 Con	3	469.23	76.37	
	50 -59 Con	5	989.07	299.86	
	> 60 Con	6	606.92	174.82	

In comparison with other study, (Venet etal. 2007) coronavirusdisease2019 (COVID-19) patients requiring hospitalization in an intensive care unit (ICU). A significant number of patients appeared to develop immune dysfunction, as evidenced by reduced amounts of monocytic human leukocyte antigen-DR (mHLA-DR). A similar observation has been reported in patients who survive severe sepsis or trauma, but later develop life-threatening immunosuppression. (Venet teal. 2018)

The infected groups and HLA types (HLA-DR and HLA-G).

The newly infected patients with Sars cov2 virus have reduced in HLA –DR expression in comparison with cured patients as well as in control (3.31 ± 1.07, 5.33 ± 2.99, 4.08 ± 2.09) at P. Vale < 0.05. While the cured patients have higher level of HLA-G than newly infected patients and healthy control at mean ± SD (792.95 ± 179.71, 597.99 ± 317.51 and 534.40 ± 360. 84) respectively at P Value <0.05). The results were listed in table (1 - 3).

Table (1 - 3) the infected patients and HLA Types.

Infected groups and HLA		N	Mean	Std. D.	P. Value
HLA –DR	Newly Patients	50	3.31	1.07	.001
	Cured Patients (+ve)	15	5.33	2.90	
	Healthy (-ve Control)	25	4.08	2.09	
HLA –G	Newly Patients	50	597.99	217.51	.006
	Cured Patients	15	792.95	179.71	
	Healthy Control)	25	534.40	260.84	

In comparison with other study, (Nguyen etal. 2020, Giamarellos etal. 2020). Subsequent studies have recently confirmed that immune dysregulation in COVID-19 patients with respiratory failure is associated with a significant downregulation of

monocyte HLA-DR.

Leukocyte antigen-G (HLA-G) is a ligand for multiple immune inhibitory receptors, whose expression can be upregulated by viral infections. HLA-G/receptor signalling, such as engagement with immunoglobulin-like transcript 2 (ILT-2) or ILT-4, not only inhibit T and natural killer (NK) cell immune responses, dendritic cell (DC) maturation, and B cell antibody production. (Aifen and Wei, 2021).

HLA-G can induce profound immune suppression, which favours the escape of SARS-CoV-2 from immune attack. Although detailed knowledge on the clinical relevance of HLA-G in SARS-CoV-2 infection is limited, we herein review the immunopathological aspects of HLA-G/receptor signalling in SARS-CoV-2 infection (Wilder et al., 2020), which could provide a better understanding of COVID-19 disease progression and identify potential immunointerventions to counteract SARS-CoV-2 infection (Aifen and Wei, 2021). Consequently, differential alteration in HLA antigen expression by viral infection makes the host antiviral immune system vulnerable (Garcia, 2020).

Other diseases associated with HLA types (HLA-DR and HLA-G).

Some patients were choosing to the present study, have other chronic diseases rather than Sars-Cov 2 infection, mostly as hypertension, Diabetes, pulmonary thrombosis and certain groups have both diabetes and hypertensive at the same time. The level of HLA –DR was reduced in patients having pulmonary thrombosis than other conditions (HT, DM, and in patients have both HT and DM) at mean ± SD (2.55 ± 0.89) at LSD value 1.12. While The HLA –G level were more elevated in patients with pulmonary thrombosis than other conditions at mean ± SD (953 ± 224. 43) as shown in table (1 - 4).

Table (1 - 4) Other diseases and HLA Types.

Other diseases and HLA types		N	Mean	Std. De	LSD Value
HLA –DR	Hypertension	9	3.69	1.10	1.12
	Diabetes	9	3.78	1.53	
	Pulmonary thrombosis	3	2.55	0.89	
	HT and DM	5	3.16	0.58	
	None	24	3.12	0.90	
	Control	40	4.55	2.47	
HLA –G	Hypertension	9	614.52	126.84	52.4
	Diabetes	9	615.28	104.34	
	Pulmonary thrombosis	3	953.58	224.43	
	HT and DM	5	496.30	105.31	
	None	24	562.05	69.44	
	Control	40	580.73	121.61	

In comparison with other study, The most distinctive comorbidities of 32 non-survivors from a group of 52 intensive care unit patients with novel coronavirus disease 2019 (COVID-19) in the study by Xiaobo Yang and colleagues (Yang etal.;2020) were cerebrovascular diseases (22%) and diabetes (22%). Another study (Guan etal. 2020) included 1099 patients with confirmed COVID-19, of whom 173 had severe disease with comorbidities of hypertension (23·7%), diabetes mellitus (16·2%), coronary heart diseases (5·8%), and cerebrovascular disease (2·3%). In a third study, (Zhang etal.; 2020) of 140 patients who were admitted to hospital with COVID-19, 30% had hypertension and 12% had diabetes.

The effect of Smoking on HLA types (HLA-DR and HLA-G).

The smoker patients have lower level of Both HLA – DR, and HLA-G in comparison with nonsmoker of patients and control, at mean ± SD (2.70 ± 0.42, 456.15 ± 221.23) respectively of HLA-DR and HLA-G .as listed in table (1 – 5) .

Table (1 - 5) The effect of smoking on HLA types

Smoking and HLA types		N	Mean	Std. D.	LSD Value
HLA –DR	Smoker (Pat)	7	2.70	.42	1.17
	Non-Smoker (Pat)	43	3.41	1.11	
	Smoker (Con)	15	5.33	2.90	
	Non-Smoker (Con)	25	4.08	2.09	
HLA –G	Smoker (Pat)	7	456.15	221.23	38.7
	Non-Smoker (Pat)	43	621.08	126.68	
	Smoker (Con)	15	679.95	177.71	
	Non Smoker (Con)	25	534.40	160.84	

This result might be show that the smoking have that ability to reduced expression of certain genes and immunological element because the high toxicity of smoking materials after metabolize in the human body. Evidence on the role of smoking in COVID-19 has been inconsistent. (Guo FR., 2020. Elliott etal ,2021) Several studies conducted early in the pandemic reported a lower prevalence of active smokers among COVID-19 patients relative to the general population, and a large population-based study conducted in the UK found that smoking was associated with lower risks of COVID-19 mortality (Hippisley etal ,2020) on adjustment for multiple prognostic factors. In contrast, current smoking was associated with higher risks of COVID-related death, adjusted for age and sex, in another large population-based study (OpenSAFELY), (Williamson etal,2020) higher risks of self-reported SARS-CoV-2 infection in online surveys, (Jackson etal ,2020) an increased burden of COVID-19 symptoms in the ZOE COVID-19 symptom study (Hopkinson etal ,2021) and increased risk of severe COVID-19 with respiratory failure in a Mendelian randomisation study of lifetime smoking.(Ponsford etal ,2020).

Correlation between HLA-DR and HLA-G.

The result of figure (1 - 6) show that direct relationship or positive correlation between HLA-Class II (HLA- DR) and HLA- Class I (HLA-G), this result might be refer to that increased in HLA-DR associated with increased in HLA-G, both of them are most important in regulation of immune response at

both humeral and cellular activity against viral infections especially at Sars –Cov2 infection

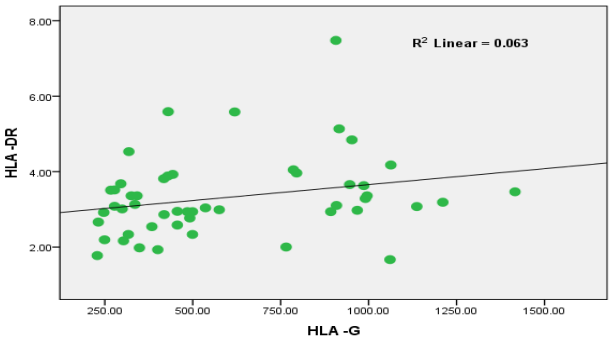

Figure (1 - 6) Correlation between HLA-DR and HLA-G.

More evidence can be accumulated to solidify the basic and clinical aspects of HLA-G in COVID-19 progression andoutcome. Aspects expected to be explored include (a) HLA-G expression is reported to be correlated with the progression of various infectious diseases (Amiot etal. 2014, Li C etal. 2013). We hypothesise that cell surface HLA-G and circulating soluble HLA-G levels are related to the severity, outcome, or viral load in patients with COVID -19 b,The upregulation of HLA-G expression by cytokines, such as IFN-g, IL-6, and IL-10, is dramatically increased in patients with severe COVID-19 .(Persson etal.;2020, Ragab etal.; 2020).

4. Conclusions

Through the present study, the results of patients with corona virus for some immune factors, HLA HLA- DR ,HLA-G) We found- Increased of HLA-Class II and Class I infeacted covid-19 pateint have the most regulation of immune system at both humeral and celluler immunty . HLA-Classe II (DR), more predicted work in female rather than male at the infected population increase of HLA-G Class I.

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