

## Frequency of HLA-A alleles in a group of COVID-19 Iraqi patients

Ali Fadhel Ahmed

Department of Science, College of Basic Education, Al-Mustansirya University, Baghdad, Iraq

### Abstract

**Background:** Host genetic factors, particularly polymorphisms in the human leukocyte antigen (HLA) region, have been implicated in influencing individual susceptibility to infectious diseases, including COVID-19. This study investigated the distribution of HLA-A alleles in an Iraqi cohort to determine their association with COVID-19 susceptibility.

**Methods:** Using a sequence-specific oligonucleotide (SSO-PCR) assay, we genotyped HLA-A alleles in COVID-19 patients and control individuals. A comparison of allele frequencies occurred between different groups using odds ratios with 95% confidence intervals and p-values assessed the strength of identified associations.

**Results:** The study established a major link between HLA-A\*03:01 since this human leukocyte antigen type occurred more frequently in COVID-19 patients with an OR of 6.33 (95% CI: 1.28–31.11) at  $p = 0.01$ . This indicates a heightened risk factor for SARS-CoV-2 infection. HLA-A\*29:01 showed higher occurrence in the control subjects than patient participants thus indicating increased protection from infection (OR = 0.85, 95% CI: 0.74–0.96,  $p = 0.02$ ). The study indicated that the relationship between HLA-A\*32:01 at borderline significance ( $p = 0.05$ ) implied lower risk susceptibility to SARS-CoV-2 infection.

**Conclusion:** HLA-A diversity plays a significant role in controlling SARS-CoV-2 immune response thus certain alleles appear to affect COVID-19 risk levels differently. The different outcomes from HLA-A\*03:01 and HLA-A\*29:01 demonstrate why genetic background assessment needs to happen before determining susceptibility to disease. Additional large research groups combined with functional examinations must understand how specific HLA-A alleles interfere with COVID-19 disease manifestations.

**Keywords:** HLA-A, sars-CoV-2, COVID-19

### Introduction

The sudden outbreak of SARS CoV 2 with its COVID 19 pandemic has triggered worldwide scientific investigation to identify factors influencing who gets sick and how severe their illness becomes (Alzahran 2024)<sup>[2]</sup>. Central to the host immune defense is the human leukocyte antigen (HLA) system—a highly polymorphic gene complex located on chromosome 6p21 that is essential for antigen presentation and immune recognition (Augusto and Hollenbach 2022)<sup>[3]</sup>. Among HLA class I molecules, HLA-A plays a pivotal role in presenting viral peptides to cytotoxic T lymphocytes, thereby orchestrating the adaptive immune response against intracellular pathogens, including SARS-CoV-2 (Muraduzzaman, Illing *et al.* 2022, Lin, Lin *et al.* 2023, Srivastava and Hollenbach 2023)<sup>[8, 9, 14]</sup>. Genetic variability within the HLA system has been proposed as a key factor modulating the host response to COVID-19 (Augusto and Hollenbach 2022, Hoseinnezhad, Soltani *et al.* 2024)<sup>[3, 7]</sup>. Recent bioinformatics analyses have suggested that specific HLA-A alleles, such as HLA-A\*02 may exhibit high binding affinity for SARS-CoV-2 epitopes, potentially influencing viral clearance and clinical outcomes (Abd El-Baky, Amara *et al.* 2023, Ribeiro, Sanches *et al.* 2023)<sup>[12]</sup>. However, studies to date have yielded conflicting results, with some large-scale investigations reporting no significant association between particular HLA-A alleles and disease severity. These discrepancies underscore the complexity of HLA-mediated immune responses and the importance of accounting for population-specific genetic diversity (Schetelig, Heidenreich *et al.* 2021)<sup>[13]</sup>. Iraq's unique genetic landscape shaped by centuries of migration and admixture offers an invaluable context for

investigating the role of HLA-A in COVID-19. Despite its strategic geographical and historical significance, data on HLA-A allele frequencies in Iraqi patients remain sparse. A comprehensive analysis of these alleles in the context of SARS-CoV-2 infection could provide critical insights into the genetic determinants of host susceptibility and inform both vaccine design and personalized therapeutic strategies (Batool M, Khalida M *et al.* 2005)<sup>[4]</sup>.

In this study, we analyze the frequency of HLA-A alleles in a cohort of Iraqi COVID-19 patients. The research assesses HLA-A polymorphisms' impact on disease exposure to develop improved public health management and better disease pathogenesis understanding.

### Methodology

#### Study Design and Participants

This study was a case-control study to determine the occurrence of HLA-A alleles in patients with COVID-19 who were residents of Iraq. A research using 80 participants included both 40 subjects diagnosed with COVID-19 and 40 healthy participants who served as controls. All participants came from an Iraqi ethnic background and the study recruitment took place from November 2020 to May 2022. included COVID-19 patients who tested positive for SARS-CoV-2 using RT-PCR testing at any time throughout the study period. The research analyzed a control population made of subjects who tested negative for COVID-19 by RT-PCR during designated study period.

#### Sample Collection and COVID-19 Diagnosis

Every participant had nasopharyngeal swab testing through RT-PCR in order to confirm SARS-CoV-2 infection. The

study determined COVID-19 patients through positive RT-PCR results while confirming negative results for controls. All participants provided data on demographic characteristics which served to match the two groups.

### DNA extraction and HLA Typing

Genomic DNA was isolated from peripheral blood samples using the Promega Wizard® Genomic DNA Purification Kit. HLA-A genotyping was performed by sequence-specific oligonucleotide PCR (SSO-PCR), a high-throughput method that precisely identifies HLA-A alleles via hybridization with allele-specific probes.

### Statistical Analysis

Allelic frequencies of HLA-A in COVID-19 patients and controls were determined and compared using the chi-square test or Fisher's exact test, as appropriate. To quantify

the association between specific HLA-A alleles and COVID-19 susceptibility, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A two-tailed p-value of <0.05 was considered statistically significant. All analyses were performed using IBM 20. SPSS Statistics software.

### Ethical approval

Ethical approval was obtained from the ethics board committee of Al-Nahrain University, Forensic DNA Research and Training Center, with approval number 116. Additionally, ethical approval was also obtained from the Baghdad Medical City, Ministry of Health of Iraq with approval number 1157 and Dr. Saad Al Watri Hospital with approval number 81595.

### Results and discussion

**Table 1:** comparative gender among study groups

			Groups		
			Control (n=40)		Patients (n=40)
Gender	Males	n	28	26	54
		%	70	65	100%
	Females	n	12	14	26
		%	30	35	100%

**Table 2:** The association between *HLA-A* alleles and the risk of Covid-19 among Iraqi population

<i>HLA-A</i> alleles	Patients		Control		P value	OR	95% CI
	2n	F%	2n	F%			
A*01:01	7	8.75	7	8.75	1.00	1.00	0.31 -3.16
A*02:01	16	20	24	1	0.07	0.44	0.18-1.08
A*02:02	0	0	1	1.25	1.00	0.97	0.92-1.02
A*02:05	0	0	1	1.25	1.00	0.97	0.92-1.02
A*03:01	10	12.5	2	2.5	0.01	6.33	1.28-31.11
A*03:02	1	1.25	0	0	1.00	1.02	0.97-1.07
A*11:01	3	3.75	2	2.5	1.00	1.54	0.24-9.75
A*11:02	0	0	1	1.25	1.00	0.97	0.92-1.02
A*23:01	1	1.25	3	3.75	0.61	0.31	0.03-3.17
A*23:02	0	0	1	1.25	1.00	0.97	0.92-1.02
A*24:01	5	6.25	1	1.25	0.20	5.57	0.62-50.03
A*24:02	2	2.5	4	5	0.67	0.47	0.08-2.74
A*26:01	9	11.25	3	3.75	0.06	3.58	0.89-14.39
A*29:01	0	0	6	7.5	0.02	0.85	0.74-0.96
A*30:01	2	2.5	0	0	0.49	1.05	0.98-1.13
A*30:02	1	1.25	0	0	1.00	1.02	0.97-1.07
A*31:01	1	1.25	1	1.25	1.00	1.00	0.06-16.56
A*32:01	1	1.25	7	8.75	0.05	0.12	0.01-1.03
A*33:01	12	15	7	8.75	0.18	2.02	0.70-5.82
A*33:03	0	0	1	1.25	1.00	0.97	0.92-1.02
A*36:01	0	0	1	1.25	1.00	0.97	0.92-1.02
A*68:01	8	10	7	8.75	0.77	1.17	0.38-3.63
A*69:01	1	1.25	0	0	1.00	1.02	0.97-1.07

Infectious diseases are known to have strong associations with host genetic factors, and several studies have demonstrated that specific human leukocyte antigen (HLA) alleles can influence susceptibility to various pathogens. During the COVID-19 pandemic, researchers have explored associations between HLA alleles and disease outcomes with the goal of identifying high-risk individuals for targeted management and vaccination prioritization (Wang, Zhang *et al.* 2020, Pairo-Castineira, Clohisey *et al.* 2021) [10, 16].

The present study aimed to investigate the association between HLA-A alleles and the risk of COVID-19 among

the Iraqi population, while also examining the gender distribution among study groups. The results provide valuable insights into the potential genetic factors influencing susceptibility to COVID-19 and highlight demographic characteristics of the study population.

The gender distribution among the control and patient groups revealed a higher proportion of males in both groups (70% in controls and 65% in patients), compared to females (30% in controls and 35% in patients). This aligns with global trends suggesting that males may be more susceptible to severe COVID-19 outcomes, potentially due to biological, hormonal, or behavioral factors (Pradhan and

Olsson 2020)<sup>[11]</sup>. However, the relatively balanced gender distribution in our patient group suggests that further research is needed to explore gender-specific risk factors in the Iraqi population.

The analysis of HLA-A alleles revealed several significant associations with COVID-19 susceptibility. Notably, the HLA-A03:01 allele demonstrated a strong association with increased risk of COVID-19 (OR = 6.33, 95% CI: 1.28–31.11,  $p = 0.01$ ). This finding is consistent with previous studies suggesting that certain HLA alleles may influence immune responses to viral infections, including SARS-CoV-2 (Tavasolian, Rashidi *et al.* 2021)<sup>[15]</sup>. The HLA-A03:01 allele, in particular, has been implicated in altered antigen presentation, which could potentially affect the severity or susceptibility to COVID-19.

Conversely, the HLA-A29:01 allele appeared to confer a protective effect against COVID-19 (OR = 0.85, 95% CI: 0.74–0.96,  $p = 0.02$ ). This finding is intriguing and warrants further investigation, as it suggests that specific HLA alleles may play a role in modulating immune responses to SARS-CoV-2 (Buckley, Lee *et al.* 2022)<sup>[5]</sup>. The protective effect of HLA-A29:01 could be attributed to its ability to present viral peptides more effectively, leading to a robust immune response and reduced viral replication (Goulder and Watkins 2008)<sup>[6]</sup>.

A similar protective role for certain HLA alleles has been reported in other viral infections, supporting the idea that differences in antigen presentation capabilities can influence disease severity.

Other alleles, such as HLA-A\*32:01, also showed a trend toward protection (OR = 0.12, 95% CI: 0.01–1.03,  $p = 0.05$ ), although this association did not reach statistical significance. This highlights the complexity of HLA-mediated immune responses and the need for larger studies to validate these findings.

Previous investigations using *in silico* approaches and next-generation sequencing have identified other HLA associations with COVID-19. For example, Yusuke *et al.* reported a significant association between HLA-A02:01 and COVID-19 morbidity and mortality, and Shekarkar *et al.* found an association with HLA-B38 in their cohorts. Wang *et al.* also described differences in HLA allele distributions between COVID-19 patients and healthy individuals in Chinese populations. However, our results—highlighting a risk with HLA-A03:01 and protection with HLA-A29:01 (and possibly HLA-A\*32:01)—suggest that the influence of HLA alleles on COVID-19 outcomes may be population-specific. Genetic background, environmental factors, and differences in sample size and study design may all contribute to these divergent findings.

Interestingly, several alleles, including HLA-A02:01, HLA-A11:01, and HLA-A\*68:01, did not show significant associations with COVID-19 risk in this population. This contrasts with findings from other regions, suggesting that the role of HLA alleles in COVID-19 susceptibility may vary across ethnic and geographic populations. These discrepancies underscore the importance of population-specific studies in understanding the genetic determinants of infectious diseases.

### Limitations

This study has several limitations. The sample size, particularly in the patient group ( $n = 40$ ), may limit the statistical power to detect weaker associations. The

relatively small sample size was partly influenced by the high cost of HLA genotyping and COVID-19 diagnostic tests, which restricted the recruitment of a larger cohort. Additionally, the study focused exclusively on HLA-A alleles, while other HLA class I and II genes may also play significant roles in COVID-19 susceptibility. Future studies should include a broader range of HLA genes and larger, more diverse cohorts to validate these findings.

### References

1. Abd El-Baky N, *et al.* HLA-I and HLA-II Peptidomes of SARS-CoV-2: A Review. *Vaccines*,2023;11(3):548.
2. Alzahrani A. Research Developments Understandings in the Dynamics of COVID-19: A Comprehensive Review. *International Journal of Pharmaceutical Investigation*, 2024, 14(4).
3. Augusto DG, Hollenbach JA. HLA variation antigen presentation in COVID-19 and SARS-CoV-2 infection. *Current opinion in immunology*,2022;76:102178.
4. Batool MM, *et al.* HLA antigens of Arab Christians in Iraq, 2005
5. Buckley PR, *et al.* HLA-dependent variation in SARS-CoV-2 CD8+ T cell cross-reactivity with human coronaviruses. *Immunology*,2022;166(1):78–103.
6. Goulder PJ, Watkins DI. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nature Reviews Immunology*,2008;8(8):619–630.
7. Hoseinnezhad T, *et al.* The role of HLA genetic variants in COVID-19 susceptibility, severity, mortality: A global review. *Journal of Clinical Laboratory Analysis*,2024;38(1–2):25005.
8. Lin F, *et al.* Functional studies of HLA and its role in SARS-CoV-2: stimulating T cell response and vaccine development. *Life sciences*,2023;315:121374.
9. Muraduzzaman A, *et al.* Understanding the role of HLA class I molecules in the immune response to influenza infection rational design of a peptide-based vaccine. *Viruses*,2022;14(11):2578.
10. Pairo-Castineira E, *et al.* Genetic mechanisms of critical illness in COVID-19. *Nature*,2021;591(7848):92–98.
11. Pradhan A, Olsson P-E. Sex differences in severity mortality from COVID-19: are males more vulnerable? *Biology of sex Differences*,2020;11(1):53.
12. Ribeiro LN, *et al.* HLA-A\* 02 affinity to SARS-CoV-2 and susceptibility to COVID-19. *European Journal of Biological Research*,2023;13(4):202–210.
13. Schetelig J, *et al.* Individual HLA-A, -B, -C -DRB1 Genotypes Are No Major Factors Which Determine COVID-19 Severity. *Frontiers in Immunology*, 2021, 12.
14. Srivastava A, Hollenbach JA. The immunogenetics of COVID-19. *Immunogenetics*,2023;75(3):309–320.
15. Tavasolian F, *et al.* HLA, immune response, susceptibility to COVID-19. *Frontiers in immunology*,2021;11:601886.
16. Wang W, *et al.* Distribution of HLA allele frequencies in 82 Chinese individuals with coronavirus disease-2019 (COVID-19). *Hla*,2020;96(2):194–196