Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 1-7

Evaluating of Genetic Polymorphism and Effect of the (*HLA-G*) Gene on Rheumatoid Arthritis in a Group of Iraqi Patients

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Received: February 20, 2025 / Accepted: March 12, 2025 / Published: November 16, 2025

Abstract: This study aimed to evaluate the genetic variants of the *HLA-G* gene and its effect on the severity of rheumatoid arthritis (RA). One hundred samples were collected, including seventy infected people and thirty free of the disease. The patient samples collected starting from December 2022 to February 2023 in Rheumatology department of Baghdad Teaching Hospital. The level of *HLA-G* was measured in the serum of both infected and healthy people using the ELISA technique, which showed significant differences with statistical significance, where the value of p-value was equal to 0.001 while the value of mean ± SD in healthy people was greater than in patients (84.38 ± 35.99), (45.03 ± 30.03) respectively. The genotypes were determined in the two groups of patients and healthy people using the RT-PCR technique to determine (rs 1063320) SNP for the antigen (*HLA-G*) and it showed that the genotype (heterozygous) gave the largest value among the other types between patients and healthy people mean ± SD (77.69 ± 53.6) for healthy people and (64.65 ± 95.37) for patients and there were no significant differences as the value was p-value (0.6). In the study of genotypes and allele frequency, the study showed that in Co-dominant the (CG) pattern was the most frequent, as it was in patients (55.7%) while in healthy people (40%) and in Dominant the (CG+GG) pattern was the most frequent (68.6%) in patients and (60%) in healthy people. The Recessive (CC+CG) pattern was also more frequent with a ratio of (87.1%) for patients and (80%) for healthy people. The frequency of the allele (C) was higher than the allele (G) as it was in patients (83%) and healthy people (36%) while the proportions of (57%) and (24%) were for patients and healthy people, respectively. The study showed that the analysis of the balance Hardy-Weinberg Equation showed a balance between the observed values and the expected values. We did not notice any statistically significant differences, as the values of p-values were (0.2) and (0.3) for patients and healthy people, respectively.

Keywords: Rheumatoid Arthritis (RA), genetic polymorphism, *HLA-G*, SNP (rs1063320).

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease that primarily affects the elderly and is more common in women than men, it affects roughly 5 people out of every 1000 (1). The disease had been described by Landre Beauvais in 1800 and Garrod first introduced the terminology of RA in 1859 (2) RA mostly affects the synovial joint lining and can lead to progressive disability, early mortality, and financial hardships(3).

Clinical signs of symmetric joint involvement might include redness, edema, and possibly limited range of motion in addition to arthralgia. (4) The

most desirable outcomes (i.e., less joint destruction, less radiologic progression, no functional disability, and disease-modifying antirheumatic drug (DMARD) free remission) and cost-effectiveness are thought to be best achieved with early diagnosis, as the first 12 weeks following the onset of early symptoms are thought to be the most effective therapeutic window. (5) RA symptoms, however, do not just affect the joints; they also affect other organs such as the blood vessels, eyes, skin, lungs, heart, and kidneys. Additionally, amyloidosis, a condition in which amyloid proteins accumulate in several organs, may accompany them. We refer to these symptoms as extra-articular manifestations. (6) It is still difficult to make an early diagnosis as so much clinical data is derived from the patient's medical history, physical examination, blood tests, and imaging studies. The causes of a delayed diagnosis range significantly throughout nations with different healthcare systems. (7) Although genetic and environmental variables have been linked to the development of RA, the precise origin of the illness is still unknown. (8) The secretion of numerous proinflammatory cytokines, chemokines, proteases, and other matrix lysing enzymes that may upset the immune balance, involves both immune (T cells, B cells, dendritic cells, macrophages, and neutrophils) and nonimmune (fibroblasts and chondrocytes) cells. The early onset of bone erosion and the presence of autoantibodies before the clinical onset of arthritis strongly indicate that the autoimmune phase of arthritis development begins extremely early in terms of the initiating events that damage bone and cartilage. (9)

Inducible non-classical major histocompatibility complex class Ib molecules known as human leukocyte antigen (*HLA-G*) antigens are crucial for controlling inflammatory processes and immunomodulation in autoimmune disorders. Numerous research studies have examined the relationship between soluble *HLA-G* (*sHLA-G*) levels and the influence of +3142G>C (rs1063320) polymorphisms on rheumatoid arthritis (RA) to better understand RA susceptibility (10).

Materials and methods

This study involved a total of 100 blood samples that were collected from December 2022 to February 2023. The blood samples included seventy Iraqi patients (14 males and 56 females) with rheumatoid arthritis disease who most regularly visit the chronic arthritis diseases department of Baghdad Teaching Hospital and thirty blood samples of apparently healthy volunteers (15 males and 15 females) were collected.

A questionnaire was taken from the patients, and the case sheet included age, sex, residence, height, weight, occupational status, and previous history of the disease and if the patient suffered from any chronic diseases such as diabetes mellitus, other autoimmune disorders, and other conditions.

Five milliliters (5 ml) of blood samples were obtained from each patient as well as healthy control through vein puncture by using disposable plastic syringes. Each blood sample was divided into 2 ml placed in an EDTA tube for the genetic part of the current study and 3 ml placed in a gel tube for immunological tests

The EDTA tubes were mixed gently for 2 minutes to prevent blood from coagulation and then stored at -20˚C to be used later, whereas the blood samples in the gel tubes were allowed to clot for 30 minutes at room temperature and then centrifuged for 10 minutes at

4000 rpm, then the obtained serum was preserved in 1.5 ml Eppendorf tubes and stored in -20˚C until analysis.

Measurement of *HLA-G* concentrations

The concentration level of (s *HLA-G*) was measured in the serum of infected and healthy samples that were collected and kept at a temperature (-20cº) using an ELISA kit (Sunlog Biotech China) and it was used according to the instructions of the company that manufactured it.

DNA extraction

DNA was extracted from 200 microliters of samples of infected and healthy people that had previously been collected and stored in anticoagulant tubes and stored in (-20Cº) according to the gDNA was extracted as demonstrated in the following protocol of the EasyPure® Genomic DNA Kit using EasyPure® Genomic DNA Transgenbiotech Chain Kit according to the manufacturer's instructions. After that, the concentration and purity of the DNA were measured using a nanodrop device, then the desired DNA was stored at (-20Cº) until used. once again.

***HLA-G* (rs1063320) SNP**

The single nucleotide polymorphism (SNP) *HLA-G*(rs1063320) was genotyped in the current investigation. Data analysis and comparison between samples of a particular gene were done using software.

Using TaqMan PCR SNP Genotyping Assays, the *HLA-G* gene polymorphism rs1063320 was genotyped.

Primers

The precise primers were created for the *HLA-G* gene's rs1063320 region. The manufacturer provided these primers in lyophilized form. To achieve a final concentration of 100 pmol/l, lyophilized primers were dissolved in water devoid of nuclease. Table (1) provides information on these primers, such as their sequence and the size of the PCR result.

Table (1): A set of *HLA-G* +3142 C>G rs 1063320 Genotyping.

Primer and Probe	Sequence (5′→3′ direction)	product size	Primer size (bp)	TM
F.	GGGATGTGTCTCCGTCTCTGTC	121	20	64
R.	CCCATCAATCTCTCTTGGAAA		21	60
Fam-	GGTCCACTGAGCTATAACTTACTTCTG			78
Vic-	GGTGCACTGAGCTATAACTTACTTCTG			78

Table (2): The thermal cycling program was as follows.

Step	Temperature (ºC)	Time (sec.)	Cycles
Enzyme activation	94	30	1
Denaturation	94	5	40
Annealing	58	15	
Extension	72	20	
Dissociation	55 ºC-95 ºC		1

Statistical analysis

Analyses Statistical the IBM SPSS Statistics 26 application was used to determine how various factors affected the study's key performance indicators. T-test and one-way ANOVA were employed to compare means substantially. The chi-square test was used to assess the variation in genotype between the patient groups and the control group, and this test was used to compare percentages (0.05 and 0.01 probability) substantially. This investigation estimated odds ratios using a 95% confidence interval (CI).

Results and discussion

Distribution of RA patient and control groups according to (*HLA-G*) and the relationship between *HLA-G* and the susceptibility to RA

The study shows, as shown in Table (3) that there are significant differences when comparing the group of patients and healthy subjects subject to the examination (*HLA-G*) that was conducted by the ELISA device, where the value of the difference was (p-value 0.001) there is an increase in the proportion of healthy people to patients, according to the following ratios mean SD (84.38 35.99) (45.03 30.42) for healthy and patients respectively. The decreased s*HLA-G* concentrations may lead to chronic activation of inflammatory cells and contribute to the development of the disease.

Table (3): Comparison between Patients and control group in *HLA-G*.

Group	Mean/.ng	Std. Deviation	Std. Error of Mean	p-value
Patients	45.0330	30.42421	5.54875	0.001**
Control	84.3846	35.99050	8.04772	

The study did not agree with another study conducted by (11) from the University of Baghdad / Iraq, where the study confirmed that Serum levels of *HLA-G* showed higher significant differences ($p<0.05$) in patients compared with healthy control**.** On the other hand, this study agrees with a study conducted by (12), which showed that Serum s*HLA-G* protein concentration is significantly lower in RA patients than in controls.

HLA-G SNP in the studied groups

As shown in Table (4) the genotype and allele frequencies of *HLA-G* polymorphism in RA patients and controls. *HLA-G* rs1063320 (+3142G>C) variant increased the risk of RA in codominant for CG 55.7% to patients and 40% to control (OR = 1.7, 95% CI = 0.27–6.68, p = 0.2 for versus GG 12.8% to patients and 20% to control (OR = 0.8, 95%CI = 0.09–5.63, p = 0.7, and dominant showed increased the risk in CG+GG (OR = 1.4, 95% CI = 0.26–4.99, p = 0.4, GC + CC versus GG). While the recessive (OR=0.5,59% CI =0.25-10.57, p =0.3, GG versus CC+CG). tested inheritance models. *HLA-G* rs1063320 G allele significantly reduced the risk of RA (OR = 1.0, 95% CI = 0.33–2.62, p = 0.9) compared to the C allele. Overall, the chi-square comparison analyses calculated in each inheritance model did not reveal an association between *HLA-G* rs1063320 polymorphism and RA risk (Table 4).

Table (4): Association of *HLA-G* polymorphisms and the risk of RA.

SNP rs1063320	Frequencies (%)		P value	X2	Odd ratio (95% CI)
	Patients (n= 70)	Control (n=30)			
Co-dominant					
CC	22 (31.4%)	12 (40%)	---	--	1.00 (Reference)
CG	39 (55.7%)	12 (40%)	0.2	0.146	1.7 (0.2783 to 6.6828)
GG	9 (12.8%)	6 (20%)	0.7	0.093	0.8 (0.0939 to 5.6333)
Dominant					
CC	22 (31.4%)	12 (40%)	---	--	1.00 (Reference)
CG+ GG	48 (68.6%)	18 (60%)	0.4	0.687	1.4 (0.2656 to 4.9932)
Recessive					
CC +CG	61 (87.1%)	24 (80%)	---	--	1.00 (Reference)
GG	9 (12.8%)	6 (20%)	0.3	0.840	0.5 (0.2496 to 10.5781)

Examination of the genotype (*HLA-G*) and allele frequencies in patients and healthy people revealed that there is no significant difference between these frequencies, despite the increase in the frequency of the allele C (80%) and the increase in the frequency of G (57%) observed in patients versus healthy people. As shown in Table (5).

Table (5): Statistical analysis of the association between genotypes and alleles of the *HLA-G gene* (rs1063320 SNP) and rheumatoid arthritis.

Allele	% Patients	% Control	p-value	X2	odd ratio(95%CI)
C	(83) %	(36) %	---		1.00 (Reference)
G	(57) %	(24) %	0.9	0.008	1.0 (0.3312 to 2.6248)

(rs1063320) was presented with three genotypes (CC, CG, GG) and two alleles (G, C) Hardy-Weinberg equilibrium analysis (HWE) in rheumatoid arthritis patients and healthy controls revealed that the genotypes were consistent with the balance. No statistically significant differences were observed between the frequencies of the observed and expected genotypes, as they were the value of (pv =0.2,0.3) for patients and healthy people, respectively, as in (Table 6).

Table (6): Association of *HLA-G* polymorphisms and the risk of RA according to Hardy-Weinberg equilibrium analysis

Groups SNP rs1063320		CC	CG	GG	P-value
Patients	**Observed no.**	22	39	9	0.2
	Expected no.	24.604	33.793	11.604	
Control	**Observed no.**	12	12	6	0.3
	Expected no.	10.800	14.400	4.800	

This study is consistent with an Iranian study conducted by Mohammad Hashemi and his group in 2016. (13).

Serum *HLA-G* level and its association with the *HLA-G* of rs1063320 G>C genotypes

The study of genotypes showed statistical differences in the level of (*HLA-G*) between the patients themselves and the healthy people themselves. There is also a difference when comparing healthy people and patients, As shown in (table 7) the genotype heterozygous CG gave the highest percentage among healthy people, which was (mean±SD =77.69 ±53.16) followed by in patients, where it was (64.65±95.37) while the genotype wild CC gave the patients. (44.11±11.14) and for healthy people (51.07±19.56) while the genotype mutant GG gave different percentages, as it was high in healthy people (69.23±15.22) while its percentage in patients was low (25.23±6.75) while the value of (p-value) was close between patients and healthy people, its value was for patients (0.5) and healthy people (0.6).

Table (7): Serum *HLA-G* level and its association with the *HLA-G* of rs1063320 G>C genotypes.

Group	rs 1063320		*HLA-G*
Patients	Wild CC	Mean	44.1119
		Std. Deviation	11.14872
		Std. Error of Mean	3.36147
	Heterozygous CG	Mean	64.6564
		Std. Deviation	95.37624
		Std. Error of Mean	24.62604
	Mutant GG	Mean	25.2308
		Std. Deviation	6.75523
		Std. Error of Mean	3.37762
	p-value		0.5
Control	Wild CC	Mean	51.0769
		Std. Deviation	19.56852
		Std. Error of Mean	9.78426
	Heterozygous CG	Mean	77.6923
		Std. Deviation	53.16255
		Std. Error of Mean	26.58127
	Mutant GG	Mean	69.2308
		Std. Deviation	15.22999
		Std. Error of Mean	10.76923
	p-value		0.6

Conclusion

The study showed an increased level of *HLA-G* increased in healthy people compared to patients and the study of the genotype and allele frequencies for *HLA-G* rs1063320 (+3142 G>C) showed that the codominant GC genotype increases the risk and development of RA.

The genetic examination of the alleles showed that the allele (G) significantly reduced the risk of developing RA compared with allele (C). The chi-square comparison analyses calculated in each inheritance model did not reveal an association between *HLA-G* rs1063320 polymorphism and RA risk.

Hardy-Weinberg equilibrium analysis revealed that the three genotypes (CC, CG, GG) for (SNP rs1063320) are consistent with equilibrium, and no significant differences were observed between the frequencies of the observed and expected genotypes, was for patients and healthy people.

References

1. Jebur, M. M.; Al-qaisi, A. H. J. and Harbi, N. S. (2022). Evaluation Serum Chemerin and Visfatin Levels with Rheumatoid Arthritis: Possible Diagnostic Biomarkers. International Journal Current Research Review, 14(02): 42.
2. Taha, M. A. and Hussain, D. A. (2022). Role Investigation of Interleukin-IL-17 rs763780 T/C Gene Polymorphism with Rheumatoid Arthritis Patients. Iraqi Journal of Biotechnology, 21(2): 103-114.
3. National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2015.

4. Mahdi, A.H and Hussin D.A. (2022). Role Investigation of Interleukin-IL-21 rs 763780 T/C Gene Polymorphism with Iraqi Rheumatoid Arthritis Patients. Iraqi Journal of Biotechnology, 21(2): 115-123.
5. Cho, S. K.; Kim, D.; Won, S.; Lee, J.; Choi, C. B.; Choe, J. Y., *et al*. (2019). Factors associated with time to diagnosis from symptom onset in patients with early rheumatoid arthritis. The Korean Journal of Internal Medicine, 34(4): 910-916.
6. Matsushita, M.; Yamaji, K. and Tamura, N. (2020). Extra-articular Manifestations of Rheumatoid Arthritis. Juntendo Medical Journal, 66 (1): 21-26.
7. Raza, K.; Stack, R.; Kumar, K.; Filer, A.; Detert, J.; Bastian, H., *et al*. (2011). Delays in assessment of patients with rheumatoid arthritis: variations across Europe. Annals of the rheumatic disease, 70: 1822-1825.
8. Mohammed, M.B and Al-Saffar, J.M. (2022). Impact of Interleukin -21 Gene Polymorphism on IL-21 Serum Level in Iraqi Rheumatoid Arthritis Patients. Iraqi Journal of Biotechnology, 21(2): 326-330.
9. Fang, Q.; Zhou, C. and Nandakumar, K.S. (2020). Molecular and Cellular Pathways Contributing to Joint Damage in Rheumatoid Arthritis. Mediators Inflammation. 17: 3830212.
10. Gautam, S.; Kumar, U.; Kumar, M.; Kanga, U. and Dada, R. (2020). Association of *HLA-G* 3'UTR Polymorphisms with Soluble *HLA-G* Levels and Disease Activity in Patients with Rheumatoid Arthritis: A Case-Control Study. Immunological investigation, 49(1-2): 88-105.
11. Bortolotti, D.; Gentili, V.; Rotola, A.; Cassai, E.; Rizzo, R. and Di Luca, D. (2014). Impact of *HLA-G* analysis in prevention, diagnosis, and treatment of pathological conditions. World Journal of Methodology, 4(1): 11-25.
12. Tabarek A.A. and Reema M. A. (2023). Genetic polymorphisms of *HLA-G* gene in Rheumatoid Arthritis. Iraqi Journal of Agricultural Sciences, 54(2): 378-387.
13. Hashemi, M.; Sandoughi, M.; Fazeli, S.A.; Bahari, G.; Rezaei, M. and Zakeri, Z. (2016) Evaluation of *HLA-G* 14 bp ins/Del and +3142G>C Polymorphism with Susceptibility and Early Disease Activity in Rheumatoid Arthritis. Advances in medicine, 4985745.