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Research Article

Evaluation of HLA-G 14 base-pair Insertion /Deletion Polymorphism and Soluble HLA-G Level in Patients with Rheumatoid Arthritis in Mosul City: A Case-Control Study

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Abstract

Background: HLA-G antigens are unconventional “class Ib” entities that are found on human leukocytes; they are essential in immunomodulation and regulation of inflammatory processes. Studies on the effects of the gene variations of HLA-G on rheumatoid arthritis have inconsistent results. ***Objective***: To estimate the potential relationship between soluble HLA-G and the rs66554220 14 base-pair insertion/deletion polymorphism of HLA-G with disease activity parameters of rheumatoid patients in Iraqi patients. ***Methods***: Using a DNA extraction kit, the genomic DNA was retrieved from 190 rheumatoid cases and 190 control individuals. Using primers for the HLA-G gene, the polymerase chain reaction was utilized for the genotyping of HLA-G 14 base-pair insertion/deletion variants. The levels of soluble HLA-G were assessed by means of an ELISA test. ***Results***: The results did not show a link between polymorphic HLA-G 14 base-pair insertion/deletion alleles and the possibility of getting rheumatoid disease. Serum levels of soluble HLA-G were substantially lower than those of controls (median=1.548 *vs.* median=7.391 U/mL), respectively. sHLA-G has a statistically substantial adverse link with ESR (r= -0.245, p<0.05). Furthermore, there is a statistically substantial differences between the DAS-28 activity score and the sHLA-G level. ***Conclusion***: There is no link between the risk of developing RA and polymorphic HLA-G 14 base-pair insertion or deletion in Iraqi patients. However, sHLA-G might serve as a diagnostic predictor of disease activity.

Keywords: Autoimmune diseases, HLA-G 14 polymorphism, Human leukocyte antigen, Rheumatoid arthritis, Variants.

تقييم تعدد الأشكال ادخال/حذف زوج القاعدة HLA-G 14 ومستوى HLA-G القابل للذوبان في مرضى التهاب المفاصل الرثوي في مدينة الموصل: دراسة حالة وشواهد

الخلاصة

الخلفية: مستضدات HLA-G هي كيانات "فئة "Ib غير تقليدية توجد في كريات الدم البيضاء البشرية. وهي ضرورية في التعديل المناعي وتنظيم العمليات الالتهابية. الدراسات حول آثار الاختلافات الجينية ل HLA-G على التهاب المفاصل الرثوي لها نتائج غير متسقة. **الهدف**: تقدير العلاقة المحتملة بين HLA-G القابل للذوبان و 14 rs66554220تعدد أشكال إدخال/حذف زوج القاعدة ل HLA-G مع معلمات نشاط المرض لمرضى الروماتيزم العراقيين. **الطرق**: باستخدام مجموعة استخراج الحمض النووي، تم استرداد الحمض النووي الجينومي من 190 حالة رماتيزم و 190 فردا صحيحا. باستخدام البادئات لجين HLA-G ، تم استخدام تفاعل البلمرة المتسلسل للتنميط الجيني لمتغيرات إدخال/حذف زوج القاعدة14 HLA-G . تم تقييم مستويات HLA-G القابلة للذوبان عن طريق اختبار ELISA. **النتائج**: لم تظهر النتائج وجود صلة بين أليلات إدخال/حذف زوج القاعدة HLA-G 14 متعددة الأشكال وإمكانية الإصابة بمرض الروماتيزم. كانت مستويات مصل HLA-G القابلة للذوبان أقل بكثير من مستويات الضوابط (الوسيط = 1.548 مقابل الوسيط = 7.391 وحدة/مل) ، على التوالي. sHLA-G له ارتباط سلبي كبير إحصائيا مع ESR ، علاوة على ذلك، هناك فروق ذات دلالة إحصائية بين درجة نشاط DAS-28 ومستوىsHLA-G. **الأستنتاج**: لا توجد صلة بين خطر الإصابة بالتهاب المفاصل الرثوي وإدخال أو حذف زوج القاعدة HLA-G 14 متعدد الأشكال في المرضى العراقيين. ومع ذلك، قد يعمل sHLA-G كمؤشر تشخيصي لنشاط المرض.

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune illness, afflicting nearly 0.5–1% of adults globally. It is distinguished by immune cell entrapment in the synovium, which promotes progressive joint damage, functional impairment, and early death. The disease is 2–3 times more likely to affect women than men [1]. RA has a considerable genetic basis [2]. The HLA system contributes to the emergence of RA, particularly HLA-DRB1 alleles. However, these alleles are only found in about 60–70% of arthritis cases [3]. Further inherited influences, including the non-classic HLA genes HLA-E, DOA, and G, were also examined for their association with RA; they were found on the sixth chromosome and play a role in immunological regulation [4]. The HLA-G locus, positioned on the 6p21.31 chromosome, is a non-typical Ib particle [5]. Since HLA-G is a tolerance-inducing particle, its disruption in autoimmune illnesses has harmful effects. HLA-G was categorized by the production of seven distinct forms through alternate splicing: HLA-G 5 to -7 proteins, which are soluble, while the HLA-G1-4 proteins are cell-linked [6]. Mostly through post-transcriptional mechanisms, the 3'UTR appears essential for controlling HLA-G transcription. HLA-G protein concentration is influenced by mutations in the 3'UTR, such as the 14 base-pair insertion/deletion, 3142G>C, and 3187G>A variants [7,8]. We are unaware of any research on the link between RA and the HLA-G gene variations in Mosul, Iraq. The current study is therefore focused on examining the relationship between sHLA-G levels and the 14 base-pair insertion/deletion (rs66554220) HLA-G 3'UTR variants and its associations with RA disease probability, susceptibility and activity in a cohort of Iraqi RA cases.

METHODS

Study design and patient selection

In this case-control study, 380 individuals participated in total, 190 of whom were RA cases diagnosed in accordance with the 2010 ACIR/EULAR criteria, in addition to the 190 controls. Patients were referred to the rheumatology department at Al-Salam Hospital, located in Mosul, Iraq. The ESR was determined utilizing standard methodologies. The measurement of CRP and RF was conducted utilizing a commercial latex fixation test reagent (Plasmatec, UK).

Genotyping of HLA-G gene's polymorphic 14 base-pair insertion/deletion (rs66554220) variant

Five milliliters of whole blood were drawn from each individual. For the genotype of the HLA-G 14 base-pair insertion-deletion variants, two milliliters of blood were evacuated into EDTA tubes and maintained at -70°C until testing. For the ELISA study, the remaining 3 ml of blood were collected into gel tubes and centrifuged at 2000 g for 15 min. The serum was then separated and kept at -20°C until the assay. Whole blood samples were used to obtain genomic DNA, gDNA Miniprep ReliaPrep® Kit (Catalog number: A5081, Promega, USA), obeying the guidelines of the manufacturer. Utilizing polymerase chain reaction (PCR), genotyping of the rs66554220 HLA-G (14 base pair insertion-deletion) variant's carried out corresponding to procedure outlined by Hashemi and colleges [9], the pertinent area of the HLA-G gene was augmented by the use of sequence-particular primers: forward (5′-TCACCCCTCACTGTGACTGATA-3′) primer and reverse (5`-GCACAAAGAGGAGTCAGGGTT-3`) primer (Macrogen, Korea) via thermal device (BioRad, USA). PCR products were then examined under UV light after being electrophoresed on an agarose gel. DNA size in test tracks was calculated using the DNA ladder (100 bp) in the first track as a reference. The PCR products with sizes of 127 bp were for the deletion allele, and those of 141 bp were for the insertion allele. Products with sizes of 141/127 bp were for the insertion-deletion of HLA-G exon 8 (Figure 1).

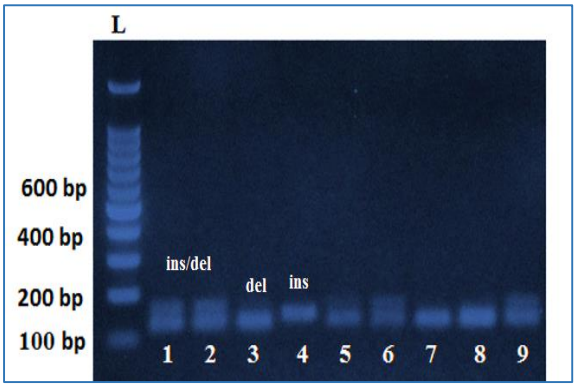

Figure 1: Outcomes of polymerase chain reaction-based 14 base-pair HLA-G insertion-deletion variant. L: DNA Ladder; Lines 1,2,6,9: ins/del; Lines 3, 5, 7 and 8: del /del; Line 4: ins/ins. del, deletion/ ins, insertion.

sHLA-G concentration measurement

The enzyme-linked immunosorbent test (ELISA) (Shanghai YL Biont, China, Catalog No. YLA1602HU) was used to measure soluble HLA-G according to the manufacturer's instructions. Using an ELISA reader, the intensity was measured at 450 nm wavelength. A calibration curve was generated to determine the ELISA detection limits; the sensibility of the ELISA sHLA-G was determined by this process to be 0.05 U/ml.

Ethical approval

The investigation was conducted in accordance with the ethical principles delineated in the Helsinki Declaration. The local Ethics Committee of the College of Medicine, Mosul University, assessed and

granted approval for the research protocol and subject consent form (reference number 20 dated December 27, 2021).

Statistical analysis

Categorical data were represented as percent (%), and continuous data were described in terms of mean±SD. sHLA-G levels for each group are shown as medians. The chi-square test is utilized for estimating genotype and allele rates. The receiver operation characteristic was employed to compute the area underneath the curve. Utilizing the MedCalc® Statistical Software, the data were examined (version 19.1). A *p*-value less than 0.05 was considered a significant difference.

RESULTS

Table 1 summarizes the demographic facts related to both RA and healthy individuals. In this study, 190 RA cases, 165 females and 25 males (mean age 42.8±10.9 years) and 190 controls (mean age 40.5±12.1 years), were recruited.

Table 1: Information on the study cohort's demographics

Variables	RA patients (*n*=190)	Control (*n*=190)	*p*-value
Age groups n(%)			
< 30 years	48(25.3)	52(27.3)	
31-49 years	120(63.2)	122(64.2)	0.135
≥ 50 years	22(11.5)	16 (8.5)	
DAS-28 Mean±SD = (7.42±1.13)			
High activity	106(55.8)		
Moderate activity	41(21.6)		
Low activity	34(17.9)		
Remission	9(4.7)		
ESR of RA patients (Mean±SD) = 40±20.31 mm/hr.			
CRP of RA patients *n*(%)	Positive: 159 (83.7) Negative: 31 (16.3)		
Rheumatoid factor (RF) *n*(%)	Positive: 168 (88.4) Negative: 22 (11.6)		

RA, Rheumatoid Arthritis; SD, standard deviation.

The 14 base-pair variations analysis shows that there are no variances in the allele frequencies and genotypes between RA cases and healthy persons. The allele occurrence for the wild allele type "del" in RA and control subjects was 78.4% (*n*=149) and 68.4% (*n*=130) (*p*=0.326), respectively; the mutant "ins" type allele frequency was 76.3% (*n*=145) and 69.5% (*n*=132) in RA and control subjects, respectively (*p*=0.411) (Table 2).

Table 2: HLA-G 14 base-pair insertion/deletion alleles in the studied groups

	RA patients (*n*=190)	Control (*n*=190)	*p*
HLA-G Allele frequency	n(%)	n(%)	
HLA-G insertion allele	145(76.3)	132(69.5)	0.411
HLA-G deletion allele	149(78.4)	130(68.4)	0.326

The occurrence of the homozygous mutant genotype (insertion/insertion) of the HLA-G 14 base-pair insertion/deletion variants was 33 (17.4%) versus 29 (15.3%) in RA and healthy individuals (*p*>0.05), the prevalence of the heterozygosity insertion-deletion genotype was found to be 121 (63.7%) versus 111 (58.4%) (*p*>0.05) in RA and healthy subjects, while the deletion-deletion genotype that is wild-type was observed as 36 (18.9%) versus 50 (26.3%) in RA and healthy subjects, correspondingly (*p*>0.05) as demonstrated in Table 3. Soluble HLA-G levels in RA cases were considerably lower than those of healthy individuals (median=1.548 U/mL versus median=7.391 U/mL), correspondingly (*p*< 0.05), according to Figure 2.

Table 3: Genotypes occurrences of 14 bp HLA-G insertion-deletion (rs66554220) variants in cases with RA and healthy controls

14-base-pair insertion/deletion (rs66554220) alleles	Patients *n*(%)	Control *n*(%)	*p*
ins/ ins	33(17.4)	29(15.3)	0.378
ins/del	121(63.7)	111(58.4)	0.154
del/del	36(18.9)	50(26.3)	0.420

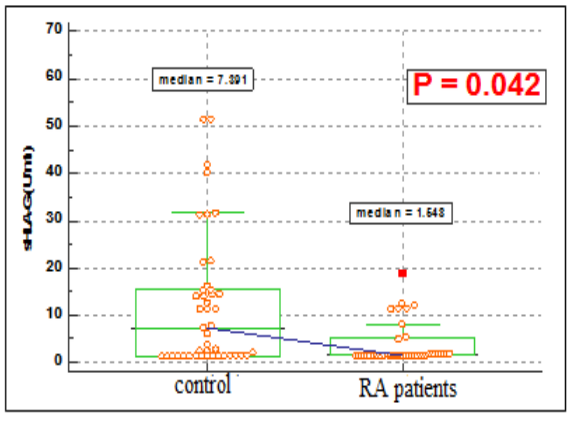

Figure 2: Value of sHLA-G in patients with RA (median=1.548) and healthy individuals (median=7.391) (*p*=0.042).

The validity properties of sHLA-G when used as a biomarker to distinguish RA patients from controls and predict the disease diagnosis are shown in Table 4.

Table 4: Validity parameters of sHLA-G when employed as biomarker to distinguish RA patients from controls (* represent the optimal cut-off value)

Criterion	Sensitivity%	Specificity%	+PV	-PV	Youden index (accuracy rate)
>1.267 (Highest sensitivity)	97.87	19.99	50.6	50.1	0.241 (24)
*>1.871	64.4	97.8	95.7	65.7	0.400 (40)
>31.254 (Highest specificity)	13.33	97.78	6.00	0.89	0.163 (16)

ROC curve analysis of the sHLA-G showed that the specificity and sensitivity were 75.6% and 64.4%, respectively, at a threshold of < 1.871 U/mL, where sHLA-G was used to distinguish between RA and healthy individuals. The *AUC* was 0.643, and the *p-value* was 0.0193, as shown in Figure 3A, while Figure 3B demonstrates the *ROC* curve when using the sHLA-G in differentiating between active and inactive RA.

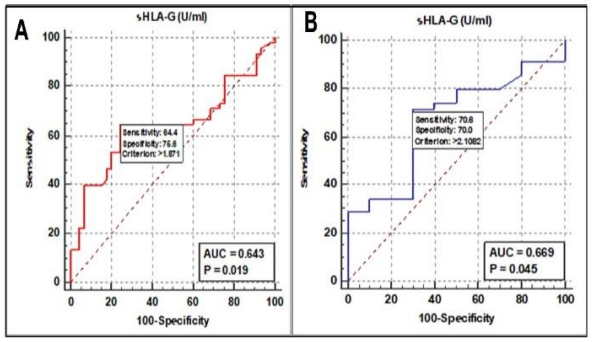

Figure 3: sHLA-G ROC curve [Sensitivity = 64.4%; Specificity = 75.6%; Cut-point value > 1.871 U/mL; *AUC* = 0.643; 95% CI=0.535 - 0.741, *p*=0.0193] (A). B. sHLA-G *ROC* curve for differentiating active from inactive RA [Sensitivity= 70.6%; Specificity= 70.0%; Cut-point value > 2.1082 U/mL; 95% CI=0.512- 0.802, *AUC* = 0.669, accuracy 41.4%, *p*=0.045] (B).

Correspondingly, the study shows a substantial adverse link between sHLA-G concentration and ESR (*r*=-0.245, *p* = 0.012); Figure 4A). As well, there is a statistical difference ($p<0.05$) between the sHLA-G level and the DAS-28 activity score. The sHLA-G level is lower in patients who have high disease activity (median=4.1 U/mL), while it is higher in patients who are in remission (median=11.8 U/mL), as shown in Figure 4B. Figure 4C shows a statistically significant relationship between sHLA-G level and RF factor ($p>0.05$).

DISCUSSION

HLA-G is an immunomodulatory particle; it keeps the adaptive and innate responses of cells like macrophages and T and B lymphocytes under control [10]. Variants in the HLA-coding and noncoding regions may have an impact on the molecule's biological properties; variants in the promotor area and 3′UTR affect the HLA-G gene expression degree, besides how much sHLA-G is present [11]. The HLA-G genome variations in 3'UTR in addition to 5'URR control its expression and are related to an augmented hazard of autoimmune disorders [12]. The mRNA stabilization and protein production rates are influenced by 14 base-pair insertion-deletion in the 3'UTR, which may be a hazard factor for RA development [13,14]. In the current research, we evaluated the genotype and allele occurrences of the 14 base-pair variants and the likelihood of RA development in a cohort of Iraqi inhabitants. However, we found no statistically substantial discrepancy in the allele or genotype frequency ($p>0.05$) between the RA and control groups. The 14 base-pair insertion allele was the principal allele in our RA cases, in contrast to controls. Previous studies suggested that the insertion allele was linked to decreased levels of bound and sHLA-G [15].

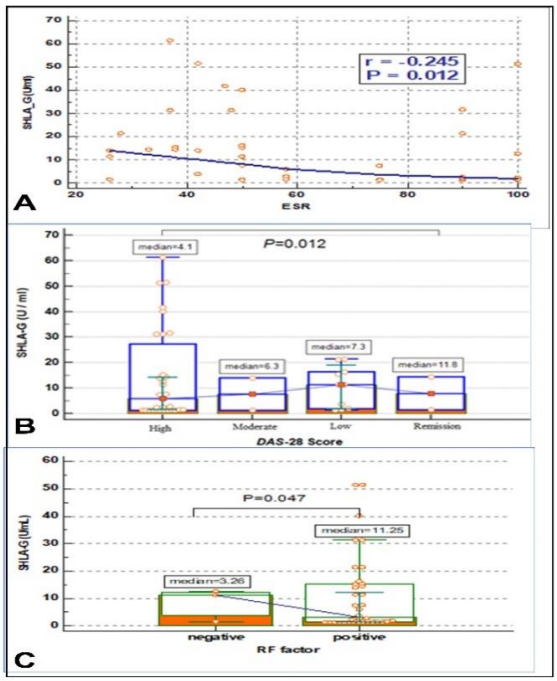

Figure 4: The association between the sHLA-G score and ESR in RA patients (r= -0.245, *p*=0.012) (A). The correlation between DAS 28-Score and sHLA-G concentration in RA patients (B). The association between RF factor and sHLA-G concentrations in RA patients (C).

These locations are connected to the HLA-G gene's post-transcriptional modulation; changes there have an impact on mRNA synthesis [16]. The mRNA instability is more associated with the 14 base-pair insertion allele [17]. Ablation of the 14 nucleotide bases leads to more stable messenger RNA than the addition of the 14 bases does [9]. However, no statistically significant correlation was found between the 14 base-pair polymorphism and the incidence of RA, according to a meta-analysis established by Lee and his colleges [18]. Mariaselvam *et al*. research on south Indian people [7], Catamo *et al*. study on Brazilians [19], and Hashemi *et al*. study on the Iranian population [9] all produced similar negative

results. The 14-base pair polymorphism has a clear consequence for protein expression levels and might be a hazardous issue for RA development. Low sHLA-G concentrations were connected with the 14 bp del/del genotype ($p<0.01$) in this study. Our results show that a lower sHLA-G value was shown in cases in contrast to controls ($p>0.05$), these lower levels may be the reason why the inflammatory state characteristic of RA disease developed. Our findings are consistent with other reports that have been published. According to Verbruggen *et al.*, RA patients had considerably lower sHLA-G levels than controls [20]. Veit *and* colleges also found the same results [21]. According to the ROC curve study, sHLA-G may act as an immunological biomarker for the development and severity of RA. According to studies, low sHLA-G values cannot repress the immune system's self-reactive cells, which results in tolerance loss, the likelihood of acquiring autoimmunity, and the emergence of autoimmune disorders that result in rheumatoid arthritis [7,17]. Additionally, the lower sHLA-G values in cases with RA suggest that HLA-G may be engaged in RA pathophysiology; decreased sHLA-G shows that T lymphocytes and natural killer cell actions really aren't effectively inhibited by sHLA-G in RA [22]. When we examined any potential relationships between the sHLA-G level and the disease's clinical indicators (*DAS*-28 and *ESR*, *RF*), there was a substantial link between the disease activity metrics and the sHLA-G level ($p<0.05$). Similar findings were found by Dreaj *et al*. in Iraq [23], besides Rizzo and colleges [24]. This indicates that sHLA-G is linked to illness physiopathology, suggesting HLA-G as a potential indicator to evaluate disease severity in addition to the prognosis in RA patients.

Study limitations

The study is limited by the tiny sample size and the fact that it was conducted exclusively on the 3 UTR of the HLA-G gene. It is crucial that the identified associations be validated through research involving larger patient and control populations that are representative of various populations.

Conclusion

The findings revealed a strong association between sHLA-G and RA susceptibility, in addition to a correlation between sHLA-G level and disease activity and severity.

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Conflict of interests

No conflict of interest was declared by the author.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Res*. 2018;6:15. doi: 10.1038/s41413-018-0016-9.
2. Dedmon LE. The genetics of rheumatoid arthritis. *Rheumatology (Oxford)*. 2020;59(10):2661-2670. doi: 10.1093/rheumatology/keaa232.
3. Silva GL, Junta CM, Sakamoto-Hojo ET, Donadi EA, Louzada-Junior P, et al. Genetic susceptibility loci in rheumatoid arthritis establish transcriptional regulatory networks with other genes. *Ann N Y Acad Sci*. 2009;1173:521-537. doi: 10.1111/j.1749-6632.2009.04629.x.
4. Weyand CM, Goronzy JJ. Association of MHC and rheumatoid arthritis. HLA polymorphisms in phenotypic variants of rheumatoid arthritis. *Arthritis Res*. 2000;2(3):212-216. doi: 10.1186/ar90.
5. Castelli EC, Mendes-Junior CT, Veiga-Castelli LC, Roger M, Moreau P, Donadi EA. A comprehensive study of polymorphic sites along the HLA-G gene: implication for gene regulation and evolution. *Mol Biol Evol*. 2011;28(11):3069-3086. doi: 10.1093/molbev/msr138.
6. Paul P, Cabestre FA, Ibrahim EC, Lefebvre S, Khalil-Daher I, Vazeux G, et al. Identification of HLA-G7 as a new splice variant of the HLA-G mRNA and expression of soluble HLA-G5, -G6, and -G7 transcripts in human transfected cells. *Hum Immunol*. 2000;61(11):1138-1149. doi: 10.1016/s0198-8859(00)00197-x.
7. Mariaselvam CM, Chaaben AB, Salah S, Charron D, Krishnamoorthy R, Tamouza R, et al. Human leukocyte antigen-G polymorphism influences the age of onset and autoantibody status in rheumatoid arthritis. *Tissue Antigens*. 2015;85(3):182-189. doi: 10.1111/tan.12521.
8. Al-hasso IKQ, Al-Derzi AR, Abbas AA, Gorial FI, Alnuimi AS. The impact of the HLA-G 14BP insertion/deletion polymorphism and soluble HLA-G serum level in systemic lupus erythematosus. *Ann Trop Med Public Health*. 2020;23(S12):SP231227.
9. Hashemi M, Sandoughi M, Fazeli SA, Bahari G, Rezaei M, Zakeri Z. Evaluation of HLA-G 14 bp Ins/Del and +3142G>C polymorphism with susceptibility and early disease activity in rheumatoid arthritis. *Adv Med*. 2016;2016:4985745. doi: 10.1155/2016/4985745.
10. Contini P, Murdaca G, Puppo F, Negrini S. HLA-G expressing immune cells in immune mediated diseases. *Front Immunol*. 2020;11:1613. doi: 10.3389/fimmu.2020.01613.
11. Amodio G, Gregori S. HLA-G genotype/expression/disease association studies: Success, hurdles, and perspectives. *Front Immunol*. 2020;11:1178. doi: 10.3389/fimmu.2020.01178.
12. Agnihotri V, Gupta A, Kumar R, Upadhyay AD, Dwivedi S, Kumar L, et al. Promising link of HLA-G polymorphism, tobacco consumption and risk of Head and Neck Squamous Cell Carcinoma (HNSCC) in North Indian population. *Hum Immunol*. 2017;78(2):172-178. doi: 10.1016/j.humimm.2016.12.007.
13. Rebmann V, König L, Nardi Fda S, Wagner B, Manvailer LF, Horn PA. The potential of HLA-G-bearing extracellular vesicles as a future element in HLA-G immune biology. *Front Immunol*. 2016;7:173. doi: 10.3389/fimmu.2016.00173.
14. Ali DM, El Shahaway AA, Ismail NA. Human leukocyte antigen G 14 base pair Insertion/Deletion Polymorphism and COVID-19 Susceptibility and Severity in Egyptian patients: A case control study. *Egypt J Med Microbiol*. 2023;32(1):151-156. doi: 10.21608/EJMM.2023.277795.
15. Rousseau P, Le Discorde M, Mouillot G, Marcou C, Carosella ED, Moreau P. The 14 bp deletion-insertion polymorphism in the 3' UT region of the HLA-G gene influences HLA-G

mRNA stability. *Hum Immunol*. 2003;64(11):1005-1010. doi: 10.1016/j.humimm.2003.08.347.
16. Donadi EA, Castelli EC, Arnaiz-Villena A, Roger M, Rey D, Moreau P. Implications of the polymorphism of HLA-G on its function, regulation, evolution and disease association. *Cell Mol Life Sci*. 2011;68(3):369-395. doi: 10.1007/s00018-010-0580-7.
17. Hviid TV, Christiansen OB. Linkage disequilibrium between human leukocyte antigen (HLA) class II and HLA-G--possible implications for human reproduction and autoimmune disease. *Hum Immunol*. 2005;66(6):688-699. doi: 10.1016/j.humimm.2005.03.003.
18. Lee YH, Bae SC, Song GG. Meta-analysis of associations between functional HLA-G polymorphisms and susceptibility to systemic lupus erythematosus and rheumatoid arthritis. *Rheumatol Int*. 2015;35(6):953-961. doi: 10.1007/s00296-014-3155-3.
19. Catamo E, Addobbati C, Segat L, Sotero Fragoso T, Domingues Barbosa A, Tavares Dantas A, et al. HLA-G gene polymorphisms associated with susceptibility to rheumatoid arthritis disease and its severity in Brazilian patients. *Tissue Antigens*. 2014;84(3):308-315. doi: 10.1111/tan.12396.
20. Verbruggen LA, Rebmann V, Demanet C, De Cock S, Grosse-Wilde H. Soluble HLA-G in rheumatoid arthritis. *Hum Immunol*. 2006;67(8):561-567. doi: 10.1016/j.humimm.2006.03.023.
21. Veit TD, Vianna P, Scheibel I, Brenol CV, Brenol JC, Xavier RM, et al. Association of the HLA-G 14-bp insertion/deletion polymorphism with juvenile idiopathic arthritis and rheumatoid arthritis. *Tissue Antigens*. 2008;71(5):440-446. doi: 10.1111/j.1399-0039.2008.01019.x.
22. Carosella ED, Rouas-Freiss N, Paul P, Dausset J. HLA-G: a tolerance molecule from the major histocompatibility complex. *Immunol Today*. 1999;20(2):60-62. doi: 10.1016/s0167-5699(98)01387-5.
23 Dreaj HA, Abbas AAH, Hathal HD, Alosami MH, Jafar AM. HLA-G gene polymorphism and soluble HLA-G serum level in rheumatoid arthritis patients under treatment with TNF-γ antagonists. *HIV Nursing*. 2022;22:3487-3492.
24. Rizzo R, Farina I, Bortolotti D, Galuppi E, Rotola A, Melchiorri L, et al. HLA-G may predict the disease course in patients with early rheumatoid arthritis. *Hum Immunol*. 2013;74(4):425-432. doi: 10.1016/j.humimm.2012.11.024.