JOURNAL OF TECHNIQUES

Journal homepage: http://journal.mtu.edu.iq

RESEARCH ARTICLE - MEDICAL TECHNIQUES


# The Effects of HLA-G Gene Polymorphism and sHLA-G Level in Women with Threatened Abortion


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**Article Info.**

*Article history:*

Received
13 April 2022

Accepted
23 May 2022

Publishing
30 June 2022



**Abstract**

The most frequent first-trimester problem is a miscarriage, which affects 20% of pregnancies. In approximately 20-25 percent of confirmed pregnancies, threatened miscarriage is indeed a risk factor that may develop or resolve. This study aimed to assess the impact of certain HLA-G gene SNPs and sHLA-G serum levels to determine whether they play a role in threatening abortions. This study included 90 subjects who were separated into three groups: 30 patients (threatened abortion patients), and 60 controls (30 healthy pregnant women and 30 healthy non-pregnant women). All of the study groups were between the ages of 20 and 35 years old. The mean sHLA-G level in pregnant women's sera was found to be 430.38 Pg/ml, compared to 307.98 Pg/ml in the threatened abortion group and 192.11 Pg/ml in non-pregnant women (P= 0.001). The rs2249863 T/C/G was a statistically significant difference between the T allele and the G allele, with an odds ratio of 1.720 (p-value = 0.038). In conclusion, serum sHLA-G levels in threatened abortion patients' sera are significantly lower than in normal pregnant women. Allele frequencies of HLA-G gene snps were non-significant changes between TA patients and controls, except rs2249863 T/C/G.


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Publisher : Middle Technical University

**Keywords**: sHLA-G; HLA-G gene; snps; Threatened Abortion; Pregnancy.


## 1. Introduction

The term "threatened abortion" refers to abnormal bleeding and abdominal pains that occur while the pregnancy is still going on. While vaginal bleeding is typical in early pregnancy, anything other than spotting during the first trimester might indicate a threatened abortion [1].

Pregnancy is an immunological contradiction in which the fetus and placenta, which carry foreign antigens to the mother's immune system, grow without being rejected by it. For a successful pregnancy and to avoid rejection, secreted substances like cytokines, hormones, and also extracellular vesicles facilitate cell-to-cell contact between the fetus and the mother [2].

HLA system is a gene cluster that primarily encodes antigens for MHC proteins existing on human leukocyte cell membranes, hence the name. These cell surface proteins are primarily in charge of human immunological control, at the humoral and cellular levels. Because that's the most important region of the human genome for infections and autoimmune diseases, it's also necessary for both adaptive and innate immunity. HLA gene cluster is situated in a 3-Mbp locus on the short arm of chromosome 6p21 [3]. The Human leukocyte antigens (HLA) are composed of three regions: HLA I, II, and III. however, both MHC-I & MHC-II complexes display particularly short peptides to T-cells. MHC-I binds endogenous synthesis cellular peptides, such as normal protein molecules, genetically altered (mutated), defected, deteriorated, or misfolded proteins, as well as proteins from the viral origin to Tc lymphocyte (CD8+T-cell). Exogenous driven peptides, as proteins from the bacterial origin, are delivered to Th lymphocytes (CD4+T-cell) via Class II MHC [4].

HLA-G is a famous non-classical immunological modulating molecule. In the physiological conditions, surface expressions of HLA-G are limited to the maternal–fetal interface as well as immunologically privileged adults organ and tissue, while soluble HLA-G is distinguished in diverse body fluids. HLA-G can be expressed in pathological situations such as cancers, chronic infections, and allogeneic transplantation. HLA-G influences immunological responses both in positive and negative ways, enhancing tolerance and modulating innate and adaptive immune responses while also producing immune escape mechanisms [5]. Human leukocyte antigen-G (HLA-G) is important for placentation and generating tolerance to the semiallogeneic fetus. HLA-G inhibits immunological cellular activities and may safeguard the fetus from harm caused by the mother's immunological system [6]. A soluble form of HLA-G (sHLA-G) is generated by alternative splicing and proteolytic cleavage and is expressed by all trophoblasts, immune cells, and other tissues. sHLA-G, like its membrane-bound counterpart, can decrease immunological responses by activating receptors on immune cells.

**Nomenclature & Symbols**

| | | | |
|---|---|---|---|
| CD | Cluster of Differentiation | ELISA | Enzyme-linked immunosorbent assay |
| EDTA | Ethylenediaminetetraacetic acid | HLA-G | Human Leukocyte Antigen-G |
| MHC | Major histocompatibility complex | NK | Natural Killer |
| PCR | Polymerase Chain Reaction | sHLA-G | Soluble Human Leukocyte Antigen-G |
| SNPs | Single Nucleotide Polymorphisms | TA | Threatened abortion |
| URR | upstream regulatory region | UTR | untranslated region |

In assisted reproduction research, the relevance of sHLA-G has been established. sHLA-G appears to be important in controlling the maternal immune system response to embryonic tissues in the early stages of pregnancy [7].

Exosomes can also be used to release HLA-G from the placenta and modify NK and T cell activity outside of the placenta. Trogocytosis, in which NK and T cells with HLA-G become immunosuppressive, is a novel method by which HLA-G modifies dNK cell and T cell function [8].

HLA-G surface expression is limited to the maternal-fetal interaction and immunologically-privileged tissues in the human body but solubilized forms of HLA-G may be found in a variety of body fluids. In contrast to the standard HLA class I gene, the non-coding (3′UTR) as well as the 5′ upstream regulatory regions (5′URR) of the HLA-G locus are highly variable. In the instance of the 3′ UTR, variation in these regions affects HLA-G expression by changing mRNA stability or permitting posttranscriptional regulation, whereas, in the case of the HLA-G promoter regions (5′ URR), it senses the microenvironment and responds to particular stimuli. Because of the impact of genetic polymorphisms on HLA-G expression, it's a promising biomarker for tracking disease risk, progression, and treatment response [5]. The objective of this study was to see if certain HLA-G gene SNPs and sHLA-G serum levels play a role in threatening abortions.

## 2. Materials and Methods

### *2.1. Subjects*

This study included a total of 30 patients (30 threatened abortion cases) and 60 healthy controls (30 pregnant and 30 non-pregnant healthy women). The gynecologist specialist physician diagnosed TA in the first 20 weeks of the pregnancy period. The exclusion criteria were as follows: Ages < 20 or > 35ss years old, patients and/or control were positive for TORCH (Toxo/Rubella/CMV/HSV1-2) test, patients and/or control were positive for anti-phospholipid antibodies (APA), other types of abortions, infection, uterine anomalies, and exposure to environmental and occupational dangers such as high amounts of radiation or toxic chemicals. The inclusion criteria were as follows: Single fetus pregnancy with gestational age 6 - 20 weeks, ultrasonography confirmed intrauterine pregnancy with a viable fetus, and vaginal bleeding with closed cervical os. The study was approved by the ethics committee of the Babylon Health Department, and patients' consent was taken to conduct the study.

### *2.2. Specimens*

The blood samples were obtained with a 5 ml for each participant, and a portion of the blood sample (2 ml) was placed in EDTA tubes for HLA-G study by PCR method as well as sequencing, and a portion of the blood sample (3 ml) was placed in gel tubes for each sample will be separated into serum for ELISA testing of sHLA-G levels.

### *2.3. Study protocols*

Quantitative measurement of sHLA-G by ELISA Kit, according to the commercial ELISA kit (SUNLONGBIOTECH, Manufacturer in Hangzhou, China) manufactures protocol. Detecting of HLA-G gene snps was done by PCR method and PCR Sequencing according to Promega, USA. In PCR, the following specific primers (Macrogen, Korea) were being used, see Table 1.

Table 1 Specific designed primers used (the primers were designed)

| Primer Name | Seq. | Annealing Temp. (°C) | Product size (bp) |
|---|---|---|---|
| rs1233334 -F | 5`-TGTAAAACGACGGCCAGTGTCATGGAACAGTGCTAGAG-3` | 60 | 966 |
| rs1233334 -R | 5`-CAGGAAACAGCTATGACCCTGGGATTGTAGGTGTAAAG-3 | | |

### *2.4. Statistical analysis*

Statistical processes and data presentation were performed using SPSS version 24. To examine for differences among study groups, descriptive statistics, and ANOVA. Also, Odds ratios were employed.

## 3. Results

The data in the Table 2 represent the levels of sHLA-G distributed among studied groups. The mean sHLA-G level was significantly elevated (430.4 Pg/ml) in the pregnant women sera in comparison with the threatened abortion group (308.0 Pg/ml) and non-pregnant women (192.1 Pg/ml) (P= 0.001).

The findings indicate that there was a highly significant difference (P≤0.01). The three groups were obtained and concluded that at least one pair of groups were not equal and that the test of comparisons needed to be continued by using the least significant difference (LSD ) test as illustrated in the next Table 3.

Table 3 displayed the findings of multiple comparisons using the LSD method, which expressed a statistically differences with regards to the various groups of concentrations as the following:

Concerning sHLA-G level, the findings demonstrate a highly significant difference (P≤0.01) obtained between the threatened abortion group and the leftover groups.

Association of the HLA-G gene snps with threatened abortion and serum sHLA-G level was examined. The genotypes and allele frequency distributions of HLA-G gene snps ( rs1736936 G/A, rs1736935 A/G/T, rs3823321 G/A/T, rs1736934 A/T, rs17875389 A/G, rs3115630 T/A/C/G, rs1632947 G/A, rs1632946 C/G/T, rs1233334 G/A/C/T, rs2249863 T/C/G, rs2735022 A/G/T, rs35674592 G/T, rs1632944 G/A/C ) were investigated in 30 TA patients , 30 healthy pregnant women and 30 healthy non-pregnant women by polymerase chain reaction then sent for DNA sequencing .

Table 4 showed genotype frequency among threatened abortion, pregnant women, and non-pregnant women, Genotype frequencies of HLA-G gene snps were non-significant differences between TA cases and controls. Hardy-Weinberg equilibrium in patients and controls samples to reveal genotypes and sampling errors, our results were distributed in control groups in Hardy-Weinberg equilibrium as the non-significant deviation of the observed value from the expected value.

Table 5 shows alleles frequency among threatened abortion, pregnant women, and non-pregnant women, alleles frequencies of HLA-G gene snps were non-significant differences between TA cases and controls, except rs2249863 T/C/G was a significant difference between T allele and G allele at OR= 1.720 ( p-value = 0.038).

In our study, we studied whether certain HLA-G polymorphisms and their protein levels in patients' serum may predispose someone to TA and affect pregnancy outcomes. Thirteen SNPs were identified, suggesting that genetic variations might be one of the reasons for threatening abortions.

Table 2 Serum levels of sHLA-G across study groups

| | **Threatened abortion** | | | **Pregnant women** | | | **Non-pregnant women** | | | **p-value** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **Min.** | **Max.** | **Mean** | **Min.** | **Max.** | **Mean** | **Min.** | **Max.** | |
| **sHLA-G level pg/ml** | 308.0 | 148.1 | 631.4 | 430.4 | 281.9 | 723.0 | 192.1 | 126.7 | 269.1 | 0 .001 H.S |

*ANOVA, H.S: highly significance, p-value≤ 0.05

Table 3 Multiple Comparisons of sHLA-G among study groups by LSD Post Hoc

| **Dependent Variable** | **(I) group** | **(J) group** | **Mean Difference (I-J)** | **Std. Error** | **Sig.** |
|---|---|---|---|---|---|
| **sHLA-G level** | **threatened abortion** | **pregnant women** | -122.4-* | 24.7 | 0.001 |
| | | **non-pregnant women** | 115.9* | 24.7 | 0.001 |
| | **pregnant women** | **threatened abortion** | 122.4* | 24.7 | 0.001 |
| | | **non-pregnant women** | 238.3* | 24.7 | 0.001 |
| | **non-pregnant women** | **threatened abortion** | -115.9-* | 24.7 | 0.001 |
| | | **pregnant women** | -238.3-* | 24.7 | 0.001 |

p-value≤ 0.05

Table 4 Genotype frequency among studied groups

| **Genotype** | | **threatened abortion patients No.=30** F (%) | **pregnant women No.=30** F (%) | **Non-pregnant women No.=30** F (%) | **OR** | **95 CI** | **p-value** |
|---|---|---|---|---|---|---|---|
| **rs1736936 G/A** | AA | 6 (20.0) | 8 (26.7) | 5 (16.7) | 0.904 | 0.374 – 2.183 | 0.822 |
| | GA | 11 (36.7) | 14 (46.7) | 12 (40.0) | 0.757 | 0.364 – 1.575 | 0.456 |
| | GG | 13 (43) | 8 (26.7) | 13 (43.3) | 1.420 | 0.680 – 2.966 | 0.351 |
| | H.W | p = 0.219 | p= 0.715 | p= 0.447 | | | |
| **rs1736935 A/G/T** | AA | 8 (26.7) | 2 (6.7) | 12 (40.0) | 1.195 | 0.522 – 2.734 | 0.673 |
| | AG | 13 (43.3) | 18 (60.0) | 11 (36.7) | 0.817 | 0.398 – 1.678 | 0.583 |
| | GG | 9 (30.0) | 10 (33.3) | 7 (23.3) | 1.084 | 0.493 – 2.383 | 0.841 |
| | H.W | p= 0.468 | p= 0.110 | p= 0.178 | | | |
| **rs3823321 G/A/T** | GA | 9 (30.0) | 10 (33.3) | 13 (43.3) | 0.689 | 0.323 – 1.472 | 0.337 |
| | GG | 21 (70.0) | 20 (66.7) | 17 (56.7) | 1.450 | 0.679 – 3.098 | 0.337 |
| | H.W | p= 0.334 | p= 0.273 | p= 0.130 | | | |
| **rs1736934 A/T** | AA | 18 (60.0) | 12 (40.0) | 21 (70.0) | 1.227 | 0.594 – 2.534 | 0.580 |
| | AT | 10 (33.3) | 16 (53.3) | 9 (30.0) | 0.7000 | 0.333 – 1.471 | 0.346 |
| | TT | 2 (6.7) | 2 (6.7) | 0 (0.0) | 2.0714 | 0.365 – 11.762 | 0.411 |
| | H.W | p= 0.708 | p= 0.273 | p= 0.334 | | | |
| **rs17875389 A/G** | AA | 22 (73.3) | 22 (73.3) | 27 (90.0) | 0.617 | 0.259 – 1.472 | 0.277 |
| | AG | 8 (26.7) | 8 (26.7) | 3 (10.0) | 1.620 | 0.679 – 3.862 | 0.277 |
| | H.W | p= 0.399 | p= 0.399 | p= 0.773 | | | |
| **rs3115630 T/A/C/G** | CC | 28 (93.3) | 26 (86.7) | 30 (100.0) | 1.000 | 0.238- 4.198 | 1.000 |
| | TC | 2 (6.7) | 4 (13.3) | 0 (0.0) | 1.000 | 0.238 | 1.000 |
| | H.W | p= 0.850 | p= 0.696 | p= 0.926 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **rs1632947 G/A** | AA | 6 (20.0) | 8 (26.7) | 5 (16.7) | 0.904 | 0.374 – 2.183 | 0.822 |
| | GA | 11 (36.7) | 13 (43.3) | 12 (40.0) | 0.810 | 0.389 – 1.689 | 0.575 |
| | GG | 13 (43.3) | 9 (30.0) | 13 (43.3) | 1.321 | 0.635 – 2.748 | 0.456 |
| | H.W | p= 0.219 | p= 0.468 | p= 0.447 | | | |
| **rs1632946 C/G/T** | CC | 13 (43.3) | 8 (26.7) | 13 (43.3) | 1.420 | 0.680 – 2.966 | 0.350 |
| | CT | 11 (36.7) | 14 (46.7) | 12 (40.0) | 0.757 | 0.364 – 1.575 | 0.456 |
| | TT | 6 (20.0) | 8 (26.7) | 5 (16.7) | 0.899 | 0.363- 2.224 | 0.817 |
| | H.W | p= 0.219 | p=0.715 | p=0.447 | | | |
| **rs1233334 G/A/C/T** | CC | 14 (46.7) | 18 (60.0) | 19 (63.3) | 0.544 | 0.263 – 1.125 | 0.101 |
| | CT | 7 (23.3) | 6 (20.0) | 2 (6.7) | 1.978 | 0.761 – 5.141 | 0.161 |
| | GC | 8 (26.7) | 4 (13.3) | 8 (26.7) | 1.454 | 0.620 – 3.413 | 0.389 |
| | GT | 1 (3.3) | 2 (6.7) | 1 (3.3) | 0.655 | 0.105 – 4.069 | 0.454 |
| | H.W | p= 0.046 | p= 0.171 | p= 0.219 | | | |
| **rs2249863 T/C/G** | GG | 6 (20.0) | 8 (26.7) | 5 (16.7) | 0.904 | 0.374 – 2.183 | 0.822 |
| | TG | 11 (36.7) | 14 (46.7) | 12 (40.0) | 0.757 | 0.364 – 1.575 | 0.456 |
| | TT | 13 (43.3) | 8 (26.7) | 13 (43.3) | 1.420 | 0.680 – 2.966 | 0.351 |
| | H.W | p=0.218 | p=0.715 | p=0.447 | | | |
| **rs2735022 A/G/T** | AA | 13 (43.3) | 8 (26.7) | 12 (40.0) | 1.529 | 0.729 – 3.208 | 0.261 |
| | AG | 11 (36.7) | 13 (43.3) | 13 (43.3) | 0.757 | 0.364 – 1.575 | 0.456 |
| | GG | 6 (20.0) | 9 (30.0) | 5 (16.7) | 0.821 | 0.344 – 1.962 | 0.658 |
| | H.W | p=0.219 | p= 0.468 | p= 0.648 | | | |
| **rs35674592 G/T** | GG | 13 (43.3) | 8 (26.7) | 12 (40.0) | 1.529 | 0.729 – 3.208 | 0.261 |
| | GT | 11 (36.7) | 14 (46.7) | 13 (43.3) | 0.708 | 0.341 – 1.470 | 0.354 |
| | TT | 6 (20.0) | 8 (26.7) | 5 (16.7) | 0.904 | 0.374 – 2.183 | 0.822 |
| | H.W | p= 0.219 | p= 0.715 | p= 0.648 | | | |
| **rs1632944 G/A/C** | AA | 4 (13.3) | 7 (23.3) | 5 (16.7) | 0.6154 | 0.232 – 1.634 | 0.330 |
| | GA | 13 (43.3) | 15 (50.0) | 12 (40.0) | 0.9346 | 0.455 – 1.921 | 0.854 |
| | GG | 13 (43.3) | 8 (26.7) | 13 (43.3) | 1.4202 | 0.680 – 2.966 | 0.350 |
| | H.W | p= 0.794 | p= 0.995 | p= 0.447 | | | |

H.W; Hardy-Weinberg equilibrium ,OR: odds ratio, 95CI: confidence interval is 95% ,p-value≤ 0.05

Table 5 Allele frequency among studied groups

| Alleles | | threatened abortion patients No.=30 F (%) | pregnant women No.=30 F (%) | Non-pregnant women No.=30 F (%) | OR | 95 CI | p-value |
|---|---|---|---|---|---|---|---|
| **rs1736936 G/A** | G | 37 (61.7) | 30 (50) | 38 (63.3) | 1.230 | 0.735 - 2.060 | 0.431 |
| | A | 23 (38.3) | 30 (50) | 22 (36.7) | | | |
| **rs1736935 A/G/T** | A | 29 (48.3) | 22 (36.7) | 35 (58.3) | 1.034 | 0.623 - 1.716 | 0.897 |
| | G | 31 (51.7) | 38 (63.3) | 25 (41.7) | | | |
| **rs3823321 G/A/T** | G | 51 (85.0) | 50 (83.3) | 47 (78.3) | 1.344 | 0.683 - 2.643 | 0.392 |
| | A | 9 (15.0) | 10 (16.7) | 13 (21.7) | | | |
| **rs1736934 A/T** | A | 46 (76.7) | 40 (66.7) | 51 (85.0) | 1.047 | 0.578 - 1.898 | 0.879 |
| | T | 14 (23.3) | 20 (33.3) | 9 (15.0%) | | | |
| **rs17875389 A/G** | A | 52 (86.7) | 52 (86.7) | 57 (95.0) | 0.656 | 0.291 - 1.479 | 0.310 |
| | G | 8 (13.3) | 8 (13.3) | 3 (5.0) | | | |
| **rs3115630 T/A/C/G** | C | 58 (96.7) | 56 (93.3) | 60 (100) | 1.000 | 0.244 - 4.094 | 1.000 |
| | T | 2 (3.3) | 4 (6.7) | 0 (0.0) | | | |
| **rs1632947 G/A** | G | 37 (61.7) | 31 (51.7) | 38 (63.3) | 1.189 | 0.710 - 1.992 | 0.511 |
| | A | 23 (38.3) | 29 (48.3) | 22 (36.7) | | | |
| **rs1632946 C/G/T** | C | 37 (61.7) | 30 (50) | 28 (46.7) | 1.189 | 0.710 - 1.992 | 0.511 |
| | T | 23 (38.3) | 30 (50) | 32 (53.3) | | | |
| **rs1233334 G/A/C/T** | G | 9 (15.0) | 6 (10) | 9 (15.0) | 1.235 | 0.591 - 2.582 | 0.574 |
| | C | 43 (71.7) | 46 (76.7) | 48 (80.0) | | | |
| | T | 8 (13.3) | 8 (13.3) | 3 (5.0) | | | |
| **rs2249863 T/C/G** | T | 37 (61.7) | 30 (50.0) | 28 (46.7) | 1.720 | 1.029 - 2.874 | 0.038 |
| | G | 23 (38.3) | 30 (50.0) | 32 (53.5) | | | |
| **rs2735022 A/G/T** | A | 37 (61.7) | 29 (48.3) | 37 (61.7) | 1.316 | 0.787 - 2.202 | 0.295 |
| | G | 27 (38.3) | 31 (51.7) | 23 (38.3) | | | |
| **rs35674592 G/T** | G | 37 (61.7) | 30 (50) | 37 (61.7) | 1.273 | 0.760 - 2.130 | 0.359 |
| | T | 23 (38.3) | 30 (50) | 23 (38.3) | | | |
| **rs1632944 G/A/C** | G | 39 (65.0) | 31 (51.7) | 38 (63.3) | 1.373 | 0.815 - 2.312 | 0.234 |
| | A | 21 (35.0) | 29 (48.3) | 22 (36.7) | | | |

OR: odds ratio, 95CI: confidence interval is 95%, p-value≤ 0.05

## 4. Discussion

The human leukocyte antigen system (HLA), which is the largest polymorphic complex in the human genome, plays a key role in immunological homeostasis by interacting with both immunological stimulating and suppressive receptors found on various immune cells. By this, more evidence of genetic predisposition and molecular activities of the non-classical HLA class I antigen (HLA-G) in the clinical importance of autoimmune diseases has indeed been identified. HLA-G expression in tissue was originally discovered on extravillous cytotrophoblasts, and its immune suppressive properties have since been thoroughly characterized. HLA-G is including very limited genetic variations, HLA-G1, G2, G3, and G4 membrane isoforms as well as HLA-G5, G6 and G7 soluble isoforms have already been discovered [9].
In this research, we observed that the mean maternal serum sHLA-G levels in women with threatened abortion were lower than those in healthy pregnant controls, while were higher than healthy non-pregnant controls. Tunisian research concluded that Pregnant women had higher levels of sHLA-G than non-pregnant women or women who had abortions [10].
It's unknown how a semiallograft fetus' immunological tolerance develops throughout pregnancy. The human leukocyte antigen G is expressed by extravascular trophoblasts, which is necessary for the maternal immune system's detection of prenatal tissues as self and the establishment of immunological tolerance to them. The presence of the soluble form of the HLA-G molecule (sHLA-G) in a mother's serum has been associated with the prevention of pregnancy rejection [11]. Various studies have suggested that the HLA-G plays an important role in various stages of reproduction, including the time preceding fertilization. The HLA-G is critical in maintaining a healthy pregnancy. sHLA-G levels in female serum during pregnancy are 2-5 times greater than those found in non-pregnant women, according to research [12].
sHLA-G in non-pregnant women was Almost similar to the results of the Malian study showed the sHLA-G mean value in the 219 plasma samples of healthy individuals had been 143 UI/ml (178.7 pg/ml) [13].
The presence of single nucleotide polymorphisms that affect the HLA-G gene has been linked to fetal mortality in a variety of studies [14].
The result of the rs1736936 G/A snp is consistent with the Iranian study, which found no significant difference in alleles and genotypes frequency of rs1736936 (P value = 0.323), OR (95 CI percent) = 1.056 (0.844–1.319), and this outcome indicates that the rs1736936 snp does not predispose to TA in the study population [15].
The influence of the rs1736936 promoter polymorphism of the HLA-G gene on rheumatoid arthritis in the Korean population was examined by Kim, S. K., et al. They discovered no significant changes in genotype and haplotype distributions between RA patients and control persons [16]. The genotype and allele frequency of rs1736935 single nucleotide polymorphisms in the HLA-G 5'-upstream regulatory region did not differ significantly between cases of idiopathic repeated spontaneous abortion and controls in an Indian study (OR = 0,78), (P value = NS). This Indian study supports our findings on this snp. Non-significant differences were also discovered for the following snps: rs1736936 (OR= 0.78), rs3823321 (OR= 1.88) and rs1736934 (OR=1.23) in the same research [17].
Between the TA patient and control groups, There's no significant difference in the rs1632947 genotype. Whereas in other gynecological diseases, a Chinese study indicated that the HLA-G rs1632947: GG genotype was attributed to protection against Endometriosis and Endometriosis severe stages [18].
The rs1233334 SNP results are consistent with the Iranian study, which found that the frequencies of allele C and genotype CC in the rs1233334 polymorphism differed between the case (recurrent spontaneous abortion) and control groups, although the changes were not statistically significant. According to Khasevani, L., et al., rs1233334 polymorphisms are linked to recurrent spontaneous abortion in the examined group and might be used as possible risk factors for the condition. On the other hand, our findings were in agreement with Khasevani, L., et al. Regarding rs2249863 snp, they found the frequencies of alleles and genotypes in the rs2249863 polymorphism were significantly different between the case and control groups ($P<0.05$) [19]. Nowak, I., et al. discovered that the rs1233334 SNP of the HLA-G gene polymorphisms plays no role in spontaneous miscarriage, with no statistically significant change in allele and genotype frequencies [20].
The SNPs rs1632946, rs2249863, rs2735022, rs35674592, and rs1632944 have been linked to autoimmune Rheumatologic diseases, according to Rizzo, et al. [21].
Our findings contradict those of another Iranian study, which found that the rs2735022 polymorphism, Which is placed within the HLA-G promoter, may impact its gene expression. The Human leukocyte antigen G promoter possesses various specificities, and the quantity of protein generated by HLA-G is altered by many polymorphisms in the HLA-G gene, including rs2735022 snp. Analysis of alleles, as well as genotypes frequency of rs2735022 amongst RPL with control participants, showed that allele risk (odds ratio=1.897) and genotype risk (odds ratio=1.932) was significant statistical (P=<0.05) between the two groups, and they exhibited a positive relationship with the recurrent pregnancy miscarriage [22].
Kim, S.K., et al. investigated the influence of HLA-G gene promoter polymorphisms rs1736936 and rs2735022 on Rheumatoid arthritis in the Korean population. They discovered no statistically significant changes in genotype and haplotype frequencies among Rheumatoid arthritis patients and healthy people [16].

## 5. Conclusion

Low levels of serum sHLA-G in TA patients' sera compared to normal pregnant women, resulting in a feto-maternal immunotolerance defect. Nevertheless, note that the level of sHLA-G in TA patients' sera is higher than those of non-pregnant women. Genotype frequencies of HLA-G gene snps were non-significant differences between TA cases and controls. Allele frequencies of HLA-G gene snps were non-significant changes between TA patients and controls, except rs2249863 T/C/G, which was a significant difference at the allele level between T allele and G allele at OR= 1.720 (p-value = 0.038), that means who has T allele more prone to threatened abortion. However, further investigations with a high number of participants are also recommended.

**Acknowledgement**

All thanks and appreciation to those who helped me or contributed something simple to complete this work.

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