Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2256
DOI URL: http://dx.doi.org/10.21474/IJAR01/2256
ISSN NO. 2320-5407

ORGINAL ARTICLE

HLA-G 14 bp INSERTION/ DELETION POLYMORPHISM IN RECURRENT PREGNANCY LOSS AMONG A SAMPLE OF IRAQI WOMEN.

Reema M.abed, Hasan F. Al-azzawie and Kareem A-Kazzaz.
Dept.of biotechnology, College of science, Baghdad University.

Manuscript Info

Manuscript History

Received: 29 September 2016
Final Accepted: 30 October 2016
Published: November 2016

Key words:-
Single nucleotide polymorphism, Recurrent spontaneous abortion,HLA-G,Insetion/deletion

Abstract

HLA-G displays immunotolerant properties and hence plays important roles in the maintenance of a successful pregnancy and maternal tolerance of the semiallogeneic fetus. HLA-G gene Polymorphism may potentially affect the biological properties of the protein, and a 14-bp insertion/deletion polymorphism in exon 8 of the 3 untranslated region (3 UTR) of the HLA-G gene is thought to influence HLA-G expression. To study the association of the 14-bp insertion/deletion (INDEL) polymorphism with the risk of recurrent pregnancy loss (RPL), polymerase chain reaction (PCR) amplification and genotyping were enrolled on 100 women in the case group (women who have had three or more unexplained RPL) and 100 women in the control group (women who have had at least two normal pregnancy). Our results showed that the frequencies of the+14 bp/+14 bp genotypes were not observed in women with RPL, while that of the +14 bp/−14 and -14 bp/-14 bp genotype was significantly increased in RPL compared with the control group of normal fertile women, in addition a significant differences in the allele frequencies of the HLA-G 14-bp polymorphism were observed. These results suggest a possible significance of the HLA-G 14-bp INDEL polymorphism in the outcome of pregnancy. However, further studies on other polymorphic sites in the 3 UTR and 5 UTR regions, as well as monitoring the serum HLA-G concentration are necessary in order to determine the potential implications of this marker in our population

Copy Right, IJAR, 2016,. All rights reserved.

Introduction:-

The human leukocyte antigen-G gene (*HLA-G*) is located on the short arm of chromosome 6 within the *HLA* region . It consists of 7 intorns and 8 exons that code for the heavy chain of the HLA-G molecule. Exons 7 and 8 are always absent in the mature mRNA because of the presence of a stop codon in exon 6 **(1).** Seven expressed isoforms have been described, 4 of which (HLA-G1-G4) are membranous and 3 (HLA-G5-G7) of which are soluble molecules (2). The proteolytic cleavage of the HLA-G1 isoform generates the soluble HLA-G1 form (3). Compared to the classical HLA molecules, the *HLA-G* gene contains a modest 46 polymorphisms that map to either the coding or non-coding regions. At the protein expression level, only 15 variants have been reported (4). The polymorphisms mapped to the non-coding regions, particularly those in the 5' upstream regulator region (5' UTR) and 3' untranslated regions (3' UTR), reportedly influence the function of HLA-G molecules and have been implicated in some pathologies such as

Corresponding Author: Hasan F.Al-azzawie.
Address:- Dept.of biotechnology, College of science, Baghdad University.

infertility, preeclampsia, failure in *in vitro* fertilization, and RPL **(1,5).** Several studies have demonstrated the importance of the 3' UTR in the *HLA-G* expression profile **(6,7)**. This region contains several regulatory elements, including a poly-A signal and AU-rich motifs involved in maintaining mRNA stability and isoform alternative splicing patterns, which may influence the function of HLA-G, particularly during pregnancy (8)gly, the 14-base pair (bp) (5'-ATTTGTTCATGCCT-3') insertion/deletion (indel) polymorphism mapped to position 3741 in the 3' UTR of exon 8 has gained interest (6). Thus, several reports have indicated that this indel polymorphism is related to *HLA-G* mRNA stability and splicing patterns involved in generating HLA-G isoforms (6,9,10). Moreover, the 14-bp insertion allele was reported to be associated with low levels of both *HLA-G* mRNA and circulating soluble HLA-G (sHLA-G) isoforms (10,11). It was also reported that plasma levels of sHLA-G were dramatically lower with the genotype +14-bp/+14-bp than with +14-bp/-14-bp and -14-bp/-14-bp genotypes (7,10). Thus, based on the results of several studies, the HLA-G molecule is considered to be a key player during early and mid-term pregnancy by contributing to the maintenance of gestation throughout pregnancy (8,12,13).

RPL was initially defined as death of the fetus before 24 weeks of gestation at least 2 consecutive times (14). It occurs in 2-4% of reproduce tive-aged women, of which 40-55% cases remain unexplained (15,16). RSA is one of the most common complications associated with early pregnancy and remains a challenge in gynecology (17). Different potential etiologic factors have been implicated in this condition, such as endocrine regulation, autoimmune reaction, thrombophilia, environmental, psychological, and genetic background, and viral infections (15,18,19).

The interface between the fetus and mother may contribute to the development and maintenance of the pregnant uterus as an immune-privileged site. In fact, the immunologic relationship between the mother and the fetus is determined by fetal antigens and the maternal immune system. Inadequate recognition of fetal antigens may result in failed pregnancy (20,21). Since its discovery, the crucial role of the non-classical HLA-G molecule in pregnancy success has been demonstrated (22,23). Various studies have reported that HLA-G molecules are responsible for maintaining the immune-regulated and tolerogenic environment during pregnancy (8,24,25). Indeed, these molecules are predominately expressed on extra villous cytotrophoblasts at the fetal-maternal interface during pregnancy (26). Currently, there is convincing experimental support for expression of HLA-G molecules conferring protection against cytolysis mediated by different maternal cytotoxic subpopulations, such as those of the natural killer cells, T lymphocytes, and dendritic cells. Another study indicated that altered expression of HLA-G molecules is associated with RSA (27).

In this study, we examined the association between successful and unsuccessful pregnancies and the *HLA-G* 14-bp insertion/deletion polymorphism using 2 groups of women: an RPL group and a normal, fertile control group of unrelated women in a Iraqi population.

Material and Methods:-

This study included 100 cases of Iraqi women (mean age 34.18 ± 6.22 years) who had RPL and had consulted the Al-yarmouk teaching . Hospital, Baghdad, Iraq, between April 2014 and June 2015. The control group included 100(mean age 34.67 ± 7.75 years) unrelated, normal fertile Iraqi women with 2 or more uncomplicated pregnancies, without a history of RPL, and with at least 2 live births. Patients with anatomical, endocrine, or metabolic disorders or immunodeficiency and autoimmune diseases were excluded from the study. Ethical approval for the study was obtained from the medical ethics committee of Al-Mustansiryia medicine College /Al-Yarmouk Hospital. All patients and controls provided informed consent and agreed to give blood samples for this case-control study.

Genomic DNA extraction and 14-bp polymorphism genotyping:-

Genomic DNA was extracted from peripheral blood using the Pure gene purification kit (Qiagen; Hilden, Germany) according to the manufacturer protocol. Exon 8 of the *HLA-G* gene was amplified by polymerase chain reaction (PCR) using the primers GE14HLAG (5'-GTGATGGGCTGTTTAAAGTGTCACC-3') and RHG4 (5'-GGAAGGAATGCAGTTCA GCATGA-3') (Hviid et al., 1999).The PCR protocol consisted of an initial step of denaturation at 94°C, followed by 35 cycles for 20 s at 94°C, 30 s at 64°C, and 60 s at 72°C, as well as a final extension for 10 min at 72°C. The fragment sizes of the PCR products were analyzed (210/224 bp) based on the presence or absence of a specific band on a 3% agarose gel stained with ethidium bromide and visualized on an ultraviolet transilluminator using a gel documentation system .

Statistical analysis:-

Frequencies of the *HLA-G* 14-bp indel polymorphism were calculated using the direct counting method. Differences between populations were assessed using a c2 test and Fisher exact test with the SPSS software (ver. 17.0; SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium was evaluated for these polymorphisms within each group using the X^2 test. Statistical significance was defined at the 5% level. We applied Yates correction for continuity and Fisher's exact test (2-tail) when the sample was small

Results:-

In this case-control study, we examined the distribution of alleles and genotypes of the most commonly studied *HLA-G* 14-bp indel polymorphism among women with at least 3 unexplained RSAs and healthy women with at least 2 live births and without a history of RSA. Clinical and demographic characteristics of the RSA and control subjects are reported in Table 1. We observed that among RSA subjects, the number of spontaneous abortions varied from 3 to 8. The percentage of women with 3, 4, 5, 6, 7, and 8 RSAs were 37.5, 26.56, 17.2, 4.7, 6.25, and 8%, respectively. Within the RSA group, 36% of women had no children (100% abortions), while for the remaining women, the loss rate was greater than 50%.

Table 1:- Clinical and demographic characteristics of the cases and control subjects.

Characteristic	Cases	Controls	P value
Mean age (years)	29.23±4.76	29.89±5.22	p>0.05
body mass index BMI(Kg/m^2)	22.37±4.24	22.78±3.88	p>0.05
Menarche (years)	13.46±1.62	13.35±1.57	p>0.05
Irregular menstrual history %	65	15	p<0.01
Number of pregnancies	4.37±1.22	2.6±1.38	p<0.01
Abortion	3.5±0.72	0	p<0.001

The role of 14bp INDEL polymorphism in RSA:-

Genotyping for the 14-bp deletion polymorphism was done by electrophoresis. The amplified PCR products were either of 224 or 210 bp depending on the deletion of the 14 bp from exon-8. The PCR product was visualized and scored under Gel documentation - UV trans-illuminator after being stained the gel with ethidium bromide stain. (Figure 1). Differences between the two groups were seen by using X^2 analysis (Hardy –Weinberg equilibrium).

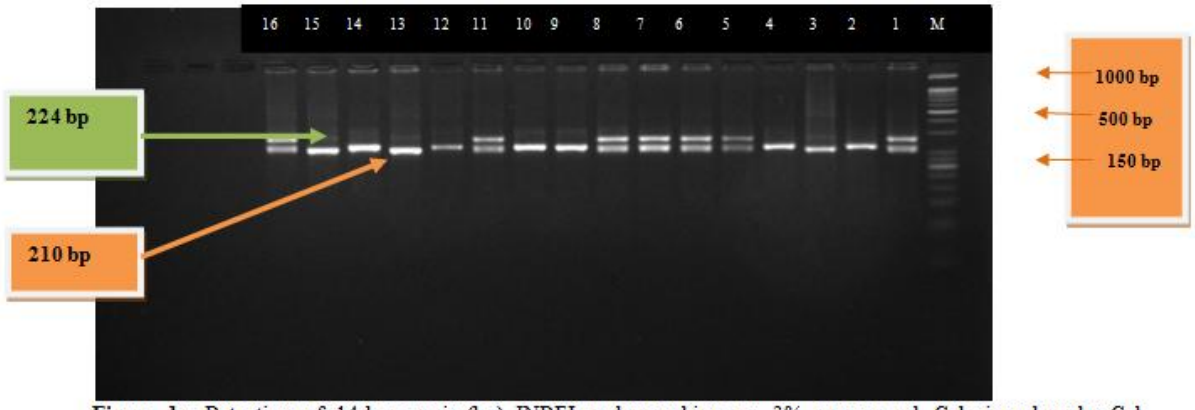

Figure 1:- Detection of 14-base pair (bp) INDEL polymorphism on 3% agarose gel. Gel viewed under Gel documentation - UV trans-illuminator after being stained with ethidium bromide stain. Lane M: 25/100 bp mixed DNA marker, Lane 1, 5-8,11,16: Heterozygote for deletion, Lane 2-4,9,10,12-15:Homozygote for deletion.

In this case-control study, we examined the distribution of alleles and genotypes of the most commonly studied *HLA-G* 14-bp ins/del polymorphism located in exon 8 of the HLA-G gene among Iraqi women with at least 3 unexplained RSAs and healthy women with at least 2 live births and without a history of RSA.The genotype distribution of the 14bp polymorphism in URSA and controls were not in Hardy-Weinberg equilibrium (p <0.05); more heterozygote's were observed compared to expectation. This indicates that 14bp HLA-G polymorphism may have some significance in pregnancy outcome and fetal survival.

There are many homozygotes for the -14bp/-14bp sequence (del/del) in RSA compared to fertile control. While homozygote for the +14bp/+14bp sequence (ins/ins) in this study was absent from RSA women and control groups. The distributions of allele and genotype frequencies of *HLA-G* 14-bp ins/del polymorphisms are reported in table (3-6 A and B). The frequency of 14bp (-14bp/-14bp genotype) in URSA was (52.0%), while the frequency of 14bp (-14bp/-14bp genotype) in control group was (27.0%), and the odd ratio was 2.93(95%CI=1.63-5.27) for both groups.

The estimated frequencies of 14bp (+14bp/-14bp) genotype in URSA was (48.0%), while the frequency of 14bp (+14bp/-14bp) genotype in control group was (73.0%), and the odd ratio was 0.34(95%CI=0.19 - 0.61). The significance analysis was similar in both 14bp (-14bp/-14bp and +14bp/-14bp) genotypes which was (5×10^{-4}).The significance of such association was assessed Fisher`s Exact Probability. Such assessment is more preferred, because it allows for correction of probability and it is not affected by small numbers (less than 5). It seems that the chance of successful pregnancy is greater when the mother is heterozygotes (odd ratio of (+14bp/-14bp) was 0.34) rather than homozygotes (odd ratio of (-14bp/-14bp) was 2.93). Maybe this is reflection of the association between the (–14 bp) sequence and altered balance in HLA-G mRNA (thereby probably protein) isoforms and isoform concentrations. Survival of fetus from heterozygotes mothers, rather than from homozygotes, may be an advantage for the species because of greater variety of MHC haplotype. It may also contribute to avoiding inbreeding.

Table 2 A:- Allele and genotype frequencies and Hardy-Weinberg equilibrium of 14-bp insertion/deletion polymorphism in unexplained recurrent spontaneous abortion patients and controls

Groups			14-bp Insertion/Deletion Polymorphism					
			Genotypes			HWE	Alleles	
			del/del	*ins/del*	*ins/ins*	$p \leq$	*del*	*ins*
URSA patients (No. = 100)	Observed	No.	52	48	0	0.01	152	48
		%	52.0	48.0	0.0		76.0	24.0
	Expected	No.	57.8	36.5	5.7		Not estimated	
		%	57.8	36.5	5.7			
Controls (No. = 100)	Observed	No.	27	73	0	0.001	127	73
		%	27.0	73.0	0.0		63.5	36.5
	Expected	No.	40.3	46.4	13.3		Not estimated	
		%	40.3	46.4	13.3			

URSA: Unexplained Recurrent Spontaneous Abortion; HWE: Hardy-Weinberg Equilibrium

Table 2B:- Statistical analysis of 14-bp insertion/deletion polymorphism in unexplained recurrent spontaneous abortion patients and controls**.**

Groups	HLA-G 14-bp Insertion/Deletion Polymorphism				
	Genotypes			Alleles	
	del/del	*ins/del*	*ins/ins*	*del*	*ins*
Odd Ratio	2.93	0.34	-	1.82	0.55
Etiological or Preventive Fraction	0.34	0.48	-	0.34	0.16
Fisher's Exact Probability	5×10^{-4}	5×10^{-4}	-	0.009	0.009
95% Confidence interval	1.63 -5.27	0.19 -0.61	-	1.18 -2.81	0.36 - 0.85

Discussion:-

The genotype distribution of the 14bp polymorphism in URSA and controls were not in Hardy-Weinberg equilibrium (p <0.05). To achieve the equilibrium five conditions must be met: Population must be very large, population must be isolated from other populations (no immigration or emigration), no alleles mutations (deletion or insertion), random mating (no inbreeding) and no natural selection (i.e. every individual has an equal chance of survival). If the five conditions are not met then evolution occurs and there is a change in allele frequency in the population and Hardy–Weinberg equilibrium is not present(28). Because the mutation occurred in allele by deletion 14bp in this study and inbreeding (increased recessive alleles) may occurred that led to deviate in Hardy - Weinberg equilibrium and the genotypes distribution were not in this equilibrium.

This result was agreed with (6) that the genotype distributions of the 14bp polymorphism in fertile controls were not in Hardy-Weinberg equilibrium. This 14-bp polymorphism has been associated with HLA-G mRNA isoforms which lack 92 base sequences in the first part of exon-8 (3' UTR) (29). The 14-bp sequence at the beginning of

exon-8 may be responsible for the alternative splicing of the HLA-G transcript. At the time of mRNA processing, this sequence may function as a cryptic branch point sequence for mRNA splicing. The presence of 14 b in the transcript induces alternative splicing of HLA-G mRNA as a result of which surrounding 92 bases are removed from the mature transcript. This mature transcript without 92 bases is stable in nature. However, when 14b is deleted there is retention of 92 bases in the mature transcript and these results into unstable transcript. This way 14-bp deletion/insertion polymorphism may influence both, the HLA-G isoform's splicing patterns (9) and HLA-G mRNA stability (13) therefore may be associated with miscarriages. The mRNA stability may be influenced by AU-rich element of 3' UTR. These AU-rich elements consist of one or many copies of AUUUA pentamer and facilitate the degradation of mRNA. It is hypothesized that because the pentameric initial AUUUG of 14b sequence has AU pentamer-like effect (9), it may be involved in deadenylation and subsequent decay of mRNA. The absence of such a motif in the 92b deleted transcript may interfere with the deadenylation and thus may provide better resistance to mRNA degradation.

Since its discovery by Ellis (30), the non-classical HLA-G class I molecule has been considered to be a key effector in the immune tolerance of the fetus in pregnant women (12,13,14). Based on its specific tissue distribution and level of expression in the body, this molecule received attention for its potential role in pregnancy disorders. Compared to classical *HLA* genes, *HLA-G* is characterized by a low level of polymorphism (5,9). Correlations between these polymorphisms, particularly in the 5' UTR and 3' UTR and some pregnancy disorders, have been well-documented. Numerous polymorphic sites have been reported in the 3' UTR (2). The most thoroughly examined is the 14-bp INDEL polymorphism that is important in HLA-G expression, mRNA stability, and alternative splicing and is associated with a wide spectrum of diseases, including pregnancy disorders (31). Thus, this polymorphic site is an important candidate for studying pregnancy disorders, particularly those with unidentified common factors such as RSA.

Our results regarding the association study between the 14-bp INDEL polymorphism and RSA in the Iraqi population agree with those reported in other populations such as the Amerindian populations from the Brazilian Amazon, Finnish, Hungarian, Polish, Japanese, Danish, Chinese, and some Indian populations (2,27,32.33). However, in other studies, the association between the *HLA-G* 14-bp polymorphism and RSA were confirmed in some Indian and Chinese populations (17). However in a previous study in an Indian population, the *HLA-G* 14-bp polymorphism was not associated with RSA disorder (20). To assess the strength of the association between the *HLA-G* 14-bp polymorphism and RSA, a meta-analysis based on 14 studies examining the association in different populations were controversial. This analysis confirmed a strong association between the del (-14bp) allele and RSA risk. However, the association between the heterozygote (+14bp/-14bp) and RSA remains controversial (28.)

References:-

1. **Donadi** EA, Castelli EC, Arnaiz-Villena A, Roger M, et al. (2011). Implications of the polymorphism of HLA-G on its function, regulation, evolution and disease association. *Cell. Mol. Life Sci*. 68: 369-395.
2. **Rull** K, Nagirnaja L, Laan M. Genetics of recurrent miscarriage: challenges, current knowledge, future directions. Front Genet 2011; 3:34
3. **O'Brien** M, McCarthy T, Jenkins D, Paul P, Dausset J, Carosella ED and Moreau P (2001) Altered HLA-G transcription in pre-eclampsia is associated with allele specific inheritance: possible role of the HLA-G gene in susceptibility to the disease. Cell Mol Life Sci 58,1943–1949
4. **Hviid** TV, Hylenius S, Hoegh AM, Kruse C, et al. (2002). HLA-G polymorphisms in couples with recurrent spontaneous abortions. *Tissue Antigens* 60: 122-132.
5. **Menie**r C, Rouas-Freiss N, Favier B, LeMaoult J, et al. (2010). Recent advances on the non-classical I HLA-G molecule. *Tissue Antigens* 75: 201-206 .
6. **Pfeiffer** KA, Fimmers R, Engels G, van der Ven H, van der Ven K. The HLA-G genotype is potentially associated with idiopatic recurrent spontaneous abortion. Mol Hum Reprod 2001; 7(4):373-8
7. **Martelli-Palomino** G, Pancotto JA, Muniz YC, Mendes-Junior CT, et al. (2013). Polymorphic sites at the 3' untranslated region of the HLA-G gene are associated with differential hla-g soluble levels in the Brazilian and French population. *PLoS One* 8: e71742.
8. **Fuzzi** B, Rizzo R, Criscuoli L, Noci I, et al. (2002). HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *Eur. J. Immunol*. 32: 311-315.
9. **Hviid** TV, Hylenius S, Rorbye C and Nielsen LG (2003). HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. *Immunogenetics* 55: 63-79.

10. **Chen** XY, Yan WH, Lin A, Xu HH, et al. (2008). The 14-bp deletion polymorphisms in HLA-G gene play an important role in the expression of soluble HLA-G in plasma. *Tissue Antigens* 72: 335-341
11. **Boukouaci** W, Busson M, Fortier C, Amokrane K, et al. (2011). Association of HLA-G low expressor genotype with severe acute graft-versus-host disease after sibling bone marrow transplantation. *Front. Immunol.* 2: 74
12. **Kovats** S, Main EK, Librach C, Stubblebine M, et al. (1990). A class I antigen, HLA-G, expressed in human trophoblasts. *Science* 248: 220-223.
13. **Rouas-Freiss** N, Marchal RE, Kirszenbaum M, Dausset J and Carosella ED (1997) The alpha1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors? Proc Natl Acad Sci USA 94,5249–5254
14. **Rico**-Rosillo MG, Vega-Robledo GB. Immunological mechanisms involved in pregnancy. Ginecol Obstet Mex 2012; 80(5);332-40
15. **Li** TC, Makris M, Tomsu M, Tuckerman E, et al. (2002). Recurrent miscarriage: aetiology, management and prognosis. *Hum. Reprod. Update*. 8: 463-481.
16. **van der** Meer A, Lukassen HG, van Lierop MJ, Wijnands F, Braat DD and Joosten I (2004) Membrane-bound HLA-G activates proliferation and interferon-gamma production by uterine natural killer cells. Mol Hum Reprod 10,189–195.
17. **Shankarkumar** U, Shankarkumar A, Chedda Z, Ghosh K.Role of 14-bp deletion /insertion polymorphism in exon 8 of the HLA-G gene in recurrent spontaneous abortion patients. J Hum Reprod Sci 2011; 4(3):143-6.
18. **Hunt** JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. FASEB J 2005;
19. **Shiroishi** M, Tsumoto K, Amano K, Shirakihara Y,Colonna M, Braud VM, et al. Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially to HLAG.Proc Natl Acad Sci U S A 2003; 100(15):8856-61.
20. **Tripathi1** P, Naik S, Agrawal1 S. Role of HLA-G, HLAE and KIR2DL4 in Pregnancy. Int J Hum Gene 2007;7(3):219-33
21. Haematology AoGaCiOa. HLA-G (major histocompatibility complex, class I, G). 2009.
22. **Pistoia** VM, F.Ferrone, S. Soluble HLA-G: Are they clinically relevant? Semin Cancer Biol 2007; 17(6):469-79.
23. Hunt JS, Pace JL, Morales PJ, Ober C. Immunogenicity of the soluble isoforms of HLA-G. Mol Hum Reprod 2003; 9(11)729-35.
24. **Martelli-Palomino** G, Pancotto JA, Muniz YC, Mendes-Junior CT, Castelli EC, Massaro JD, et al. Polymorphic Sites at the 3' Untranslated Region of the HLA-G Gene Are Associated with Differential hla-g Soluble Levels in the Brazilian and French Population. PLoS One 2013;8(10):e71742.
25. **Azarpira** N, Aghdaie MH, Kazemi K, Geramizadeh B,Darai M. HLA-G Polymorphism (rs16375) and Acute Rejection in Liver Transplant Recipients. Disease Markers 2014 .
26. **Harrison** GA, Humphrey KE, Jakobsen IB, Cooper DW.A 14 bp deletion polymorphism in the HLA-G gene. Hum Mol Genet 1993; 2(12):2200.
27. **Yan** WH, Lin A, Chen XJ, Dai MZ, Gan LH, Zhou MY,et al. Association of the maternal 14-bp insertion polymorphism in the HLA-G gene in women with recurrent spontaneous abortions. Tissue Antigens 2006;68(6):521-3.
28. **Edwards,** A., W. (2008). G. H. Hardy (1908) and Hardy-Weinberg equilibrium,Genetics.179 (3):1143–1150.
29. Hiby S, King A, Sharkey A, Loke YW: Molecular studies of trophoblast HLA-G: polymorphism, isoforms, imprinting and expression in preimplantation embryo.Tissue Antigens 53:1, 1999
30. **Ellis** SA, Sargent IL, Redman CW and McMichael AJ (1986). Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. *Immunology* 59: 595-601.
31. **Wang** X, Jiang W, Zhang D. Association of 14-bp insertion/deletion polymorphism of HLA-G gene with unexplained recurrent spontaneous abortion: a metaanalysis.Tissue Antigens 2013; 81(2):108-15
32. **Xue** S, Yang J, Yao F, Xu L, Fan L. Recurrent spontaneous abortions patients have more -14 bp/+14 bp heterozygotes in the 3'UT region of the HLA-G gene in a Chinese Han population. Tissue Antigens 2007; 69 (Suppl 1):153-5.
33. **Aruna** M, Sudheer PS, Andal S, Tarakeswari S, et al. (2010). HLA-G polymorphism patterns show lack of detectable association with recurrent spontaneous abortion. *Tissue Antigens* 76: 216-222.