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Association of Polymorphism rs1264459 in the HLA-E Gene Promoter with Incidence of (RSA) and its Consequences to Steroid Hormones in Iraqi Women

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Abstract: Recurrent spontaneous abortion (RSA) is one of the common complications that occur after gestation, its multi-factorial issue. This study explored whether the rs1264459 polymorphism in the promoter of HLA-E gene and the concentration of soluble HLA-E in serum is correlated with the risk of RSA. Fifty women with idiopathic RSA and fifty apparently healthy women as control were consulted Al Elwiya teaching hospital, Baghdad, Iraq between January 2019 and April 2019. The concentration of soluble HLA-E measured by Human leukocyte antigen E ELISA Kit. The level of the serum HLA-E in female with RSA was 43.19±0.9 U/ml while in apparently healthy women was 41.35±1.31 U/ml. The genotypes were determined by TaqMan genotyping assay using RT-PCR. The results showed that there were no significant differences in the GG, GA and AA genotypes frequency percentage distribution and alleles frequencies, between the control and female with RSA, the frequency of G allele was 0.26 in female with RSA and 0.20 in control, while the frequency of A allele was 0.74 in female with RSA and 0.8 in control. Results show certain alleles at different loci in HLA-E gene are associated with low concentrations in the levels of some steroidal hormones in females with RSA.

Keywords: HLA-E gene promoter, single nucleotide polymorphism, recurrent spontaneous abortion, steroid hormones.

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Introduction

Recurrent spontaneous abortion is consecutive ending more than 2 of pregnancy before foetus reached 20 weeks after gestation or if the weight of the lost foetus less than 500 grams (1). RSA is multi-factorial disorder including chromosomal abnormalities, maternal infections, immune disorder, endocrine dysfunction, life style issues and abnormalities of reproductive tract, however 50% of cases are idiopathic (2). Most cases of RSA are caused by the elimination of baby by the immune system of the mother (3). To guarantee that immune cells of the mother will not attack and destruction the foetus particular mechanisms must exist (4). Down-regulation to the expression of the polymorphic classic major histo-compatibility complex (MHC) antigens on foetus trophoblast cells is one predictable mechanism (5). The human major histo-compatibility complex (MHC) gene located on chromosome 6, MHC gene has several loci most of which have a directly related in

immunology (6). According to their pattern in expression theses loci classified in to classes MHC class I molecules which express on all nucleated cells and MHC II which express on immune cells such as macrophage and B-lymphocytes (7). MHC or HLA class I sub divided in to two subclasses: HLA class Ia and HLA class Ib, HLA-E Ia contain (HLA- A,B and C) which initiate the adaptive immunity through present antigen-derived peptides to cytotoxic T – lymphocytes (8). HLA Ib consists of (HLA- G, E and F) which play role in immune tolerization (9). HLA-E peptides serve as a mediator to activate or inhibit the natural killer cells by either activating CD94/NKG2C or inhibit CD94/NKG2A which are NKs receptors (10). HLA-E has few polymorphic alleles, despite of it has 13 alleles but only two variants alleles are knowing their functional effects HLA-E*0101 and HLA-E*0103 (11). The study investigates a SNP in non –coding region in the promoter of HLA-E gene **rs1264459** and its association with the concentrations of some steroidal hormones in Iraqi females suffering recurrent spontaneous abortion. Steroidal hormones play important role in maintenance of pregnancy, they are essential for endometrial proliferation and maintain feto-placental functions and maturation as well as uterus - placental circulation and prevents myometrium contractility by decrease the uptake of extracellular calcium that is needed for contraction also progesterone inhibits lymphocyte cytotoxicity, natural killer cell degranulation and release of pro inflammatory cytokines (12).

Materials and methods

Subjects

This study included 100 cases of Iraqi females, fifty females (n=50) with idiopathic RSA (mean age 35.06 ±0.9) and consulted Al Elwiya teaching hospital, Baghdad, Iraq from January 2019 to April 2019 and fifty normal fertile females with at least 2 live births and without history of abortion. Patients with anatomical, infection, endocrine. metabolic disorder and autoimmune diseases were excluded from the study. Ethical approval for the study was obtained from Al Elwiya teaching hospital. Blood collected from patients and control, 3ml for serum collection and 2ml for DNA extraction.

Serum HLA-E concentration

The technique that used was Double Antibody Sandwich ELISA. The principle of it is based on features of the tested antigen with more than two valances which can recognize coated antibody and detection it (13).

Hormonal assays

Hormonal analysis for progesterone, testosterone and estradiol was performed by using Automated Immune Assay (AIA) by the VIDAS auto analyzer, (BioMérieux Company, France). VIDAS hormonal assay is an automated quantitative test for use on the VIDAS instrument for the quantitative measurement of human serum using the ELFA (enzyme linked fluorescent assay) in Hormonal Laboratory at El-Elwiya and Al-Yarmouk teaching hospitals.

DNA extraction and Genotyping

The DNA was extracted by using kit (Quick-gDNA ™ Blood MiniPrep, Zymo research /USA), Nanodrop was used to estimate the purity and the concentration for DNA samples. Genotyping analysis was performed using Real Time PCR, custom TaqMan fluorescent oligonucleotide probes and primers for examined SNP rs1264459 in gene promoter (G>A) were prepared and stored lyophilized at -20 C (ordered from integrated DNA technologies /USA). Taq man SNP genotyping assay using real time thermocycler according to the protocol recommended by the manufacturer. Calculating the total volume of each component needed for assay, using the following table (1). Table (2) show the thermal cycling conditions.

Table (1): The final volume for assay

Components	Volume (µL)
2X TaqMan® Master	5
20X Assay Working	0.5
Nuclease-free	-
DNA Sample Volume	4.5
Total volume	10

Table (2): Thermal cycling conditions.

Steps	Temp.	Duration	Cycles
Enzyme activation	95°C	10 minutes	HOLD
Denaturation	95°C	15 seconds	40
Annealing/Extension	60 C◦	1 minutes	40

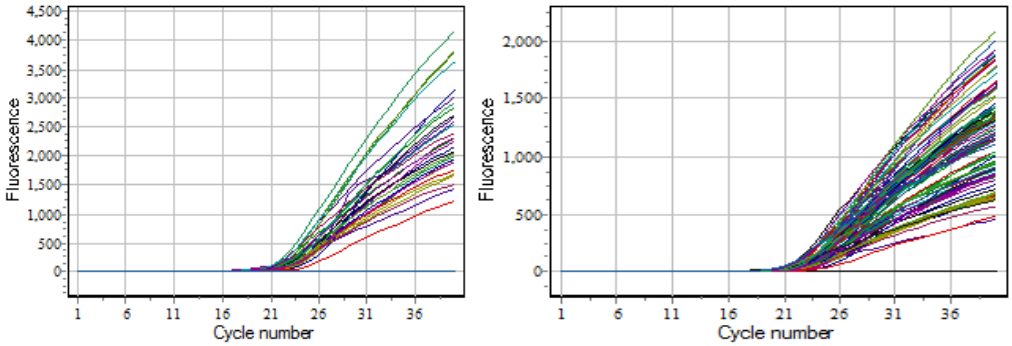

Figure (1): (A) FAM (curves of G allele); (B) HEX (curves of A allele).

Statistical Analysis

The Statistical Analysis System-SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study (14).

Results and discussion

The results show no significant increase in the serum concentration of HLA-E in women with RSA (43.19 ± 0.9) U/ml *versus* control (41.35 ± 1.31) U/ml as shown in the table (3).

Table (3): The concentration of s HLA-E(U/ml) in the serum.

Trait	Control	Female with RSA
HLA-E concentration (U/ml)	41.35 ±1.31	43.19±0.9
p- value	0.4003 NS	
LSD	4.339	

NS means Non- significant

Expression and the level of HLA-E protein at the fetomaternal interface are important to effective gestation by suppressing the response of maternal immune (15). The HLA –E gene expression is controlled by the binding of peptides derived from MHC class I leader sequences, the accuracy of any peptides is very critical to allowing the expression of HLA-E (16). This may be the reason of the non-significant increase; another explanation is that the trophoblast of foetus also expresses the HLA-E protein and may compensates for the depression of HLA-E level from maternal.

The present study investigates SNP rs1264459 located in the promoter of HLA –E gene on chromosome 6 (G>A). The distribution of genotype alleles frequency presented in table (4).

Table (4): Genotype and allele frequencies of rs1264459 at HLA-E gene promoter in Iraqi women with RSA.

Group	Control		Female with RSA		χ^2	OR	CI
	No.	Percentage	No.	Percentage			
GG	2	4%	6	12%	2.029 NS	0.087	0.77-1.60
GA	16	32%	14	28%	1.04 NS	0.085	0.78-1.57
AA	32	64%	30	60%	1.04 NS	0.085	0.78-1.57
Allele frequency							
G	0.20		0.26		--	--	--
A	0.80		0.74		--	--	--

NS means Non- significant

The GG, GA and AA genotypes frequency percentages have non-significant difference noted between the control and women with RSA, the frequency of G allele was 0.26 in women with RSA and 0.20 in control, while the frequency of A allele was 0.74 in women with RSA and 0.8 in control.

The HLA is related with many diseases than any part of the human genome, the HLA-E belongs to non –classical HLA type Ib that considered as immune tolerization molecules through interaction with NK cells and T lymphocytes (17). This study showed that the promoter SNP rs1264459 was not associated with RSA. In spite of the global frequency of G allele is 0.16 and A allele frequency is 0.83 there was one article that study the association of this SNP with non-segmental vitiligo patients in Korean population (18). This may be correlated to that studies that interest with polymorphism focus mainly on the single nucleotide polymorphisms of coding regions of the genes while SNPs on DNA binding motifs of the promoter have been less discovered (19). This study also involved the measurement of HLA-E concentration under the effect of studied SNP as in table (5).

Table (5). HLA-E concentrations as affected by rs1264459 polymorphism at HLA-E gene promoter in Iraqi women with RSA.

Genotypes	HLA- E concentration (U/ml)		p- value
	Control	Female with RSA	
GG	35.52±3.74	44.49±4.38	0.144 NS
GA	45.28±2.98	41.54±1.59	0.609 NS
AA	39.98±1.37	43.86±2.58	0.438 NS
p- value	0.135 NS	0.875 NS	-

(**Mean ± standard error). NS means Non- significant**.

The concentration of serum HLA-E didn't affect neither in women with RSA nor in apparently healthy females by the genotypes of the SNP that examined in this study. To investigate the influence of HLA-E on some steroidal hormones, three hormones which are progesterone, testosterone and estradiol were their concentration measured under the effect of the SNP in the promoter of HLA-E gene.

The heterozygous GA and mutant AA types in rs1264459 in the promoter of HLA-E gene significantly ($p<0.01$) decreased the serum progesterone hormone in women with RSA compared with apparently healthy subjects (9.52 ± 0.49ng/ml *versus* 20.41 ± 1.09 ng/ml and 10.77 ± 0.51ng/ml *versus* 21.77 ± 0.42 ng/ml, respectively). The wild type GG hadn't effect on the concentration of progesterone hormone between women with RSA and apparently healthy subjects, but significantly decrease the progesterone hormone in control compared with the other genotypes in the same group as shown in table (6).

Table (6): Serum progesterone hormone concentrations as affected by rs1264459 polymorphism at HLA-E gene promoter in Iraqi females with RSA.

Genotypes	Progesterone concentration (ng/ml)		P -value
	Control	Female with RSA	
GG	13.76 ± 6.14 b	11.33 ± 0.87	0.455 NS
GA	20.41 ± 1.09 a	9.52 ± 0.49	0.0038 **
AA	21.77 ± 0.42 a	10.77 ± 0.51	0.0051 **
p- value	0.0013 **	0.114 NS	

- (Mean ± standard error). ** means a significant difference at 0.01 level.

Only the wild type GG of rs1264459 has related with the decrease of the testosterone hormone significantly ($p <0.05$) in women with RSA than control (2.566 ± 0.32ng/ml *versus* 4.450 ± 2.55ng/ml a, respectively), also the wild type GG decrease the testosterone hormone concentration significantly ($p <0.05$) in the control group compared with other genotypes in the same group as in table (7).

Table (7): Serum Testosterone hormone concentrations as affected by rs1264459 polymorphism at in Iraqi women with RSA.

Genotypes	Testosterone concentration (ng/ml)		P -value
	Control	Female with RSA	
GG	4.450 ± 2.55 a	2.566 ± 0.32	0.041 *
GA	2.514 ± 0.19 b	2.437 ± 0.37	0.802 NS
AA	2.153 ± 0.15 b	2.409 ± 0.22	0.563 NS
p- value	0.021 *	0.847 NS	

*** Means a significant difference at 0.05 level.**

The heterozygous GA genotype of rs1264459 had significantly ($p<0.05$) decrease the concentration of estradiol hormone in women with RSA compared with apparently healthy subjects (17.92 ± 1.07pg/ml *versus* 30.65 ± 1.69pg/ml, respectively), the mutant AA genotype decrease significantly ($p<0.01$) the hormone concentration in women with RSA compared with apparently healthy subjects (19.67 ± 1.85 pg/ml *versus* 34.11 ± 1.05pg/ml, respectively) notice the table (8).

Table (8). Serum estradiol hormone concentrations as affected by rs1264459 polymorphism at HLA-E gene promoter in Iraqi women with RSA. (Mean ± standard error).

Genotypes	Estradiol concentration (pg/ml)		P -value
	Control	Female with RSA	
GG	24.75 ± 15.25	20.56 ± 1.24	0.468 NS
GA	30.65 ± 1.69	17.92 ± 1.07	0.0366 *
AA	34.11 ± 1.05	19.67 ± 1.85	0.0072 **
p- value	0.122 NS	0.717 NS	

Many studies indicate that the steroidal hormones have positive or negative influence on the HLA-E expression, progesterone and estradiol for example upregulated the HLA-E expression while testosterone downregulate the expression but there is no study indicate the influence of the polymorphism of HLA-E gene on the concentration of these hormones (20). Our results suggest that certain alleles at different loci in HLA-E gene are associated with low concentration in the levels of the hormones in females with RSA, the explanation of this relation is not clear, as there are not enough research on this topic to determine whether this reduce applies to diseases or its present in individual before diseases. Single study suppose that low expression of an enzyme called 21-hydroxylase coded by gene located in HLA gene which is convert 17α-hydroxyprogesterone to deoxycortiosol and progesterone is related with low hormones concentration (21).

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