Relationship of 14-bp insertion/deletion gene polymorphism of HLA-G with Consanguinity in recurrent abortion in Baghdad, Iraq

Abdulkhaliq Roua Jamal[1], Mohammed Sabaa Taher[2], Abbas Ahmed Abdul-Hassan[3] and Muhaidi M.J.[4*]
1. College of Veterinary Medicine, University of Fallujah, IRAQ
2. College of Science, AL-Mustansiriyah University, IRAQ
3. College of Medicine, Al-Nahrain University, IRAQ
4. College of Science, University of Fallujah, IRAQ
*mjm20002014@uofallujah.edu.iq

Abstract

The 14 bp insertion/deletion gene polymorphism of insertion /deletion (+14 bp -14 bp) in the exon 8 of (HLA-G) human leucocyte antigen-G gene has been usually recognized to associate with frequent abortion (RA). This study was designed to determine the possible association between 14bp of HLA-G deletion/ insertion gene polymorphism with frequent abortion in husband's relatives and husbands strange. Peripheral blood was collected from ninety (90) women with periodic abortion, three abortions or more with unknown causes (husband's relatives was 58 and husband's strange was 32). DNA from the peripheral blood was extracted, the concentration and purity of DNA were measured then, DNA primer was designed by using genious software program. HLA-G gene of the both groups was amplified by using polymerase chain reaction (PCR) and analyzed by gel electrophoresis. The result revealed that homozygous insertion (+14bp/+14bp) was in husband's relatives (13), and husband's strange (11), and homozygous deletion (-14bp/-14bp) results were in husband's relatives (11), and husband's strange (5) while heterozygous (+14bp/-14bp) results were in husband's relatives (34), and husband's strange (16).

In the present study any significant variation was not found between heterozygous and homozygous amongst husband's relatives and husbands strange in Iraqi women. We also did not found any implication between husband's relatives and husbands strange in the field of alleles (+14bp (insertion) or -14bp (deletion) in recurrent abortion).

Keywords: HLA-G**,** frequent abortion**,** polymorphism**,** husband's relatives**,** husband's strange.

Introduction

Frequent abortion (RA) is the majority communal complicated of early pregnancy, showing one of the most common reproductive disease. It is well-defined as (at least three or more than three) frequent pregnancy losses; earlier the fetus has not reached a viable gestational age. The most common causes of miscarriage include genetics, immunologic factors, endocrine disorder, infection disease and maternal disease such as thyroid disease and diabetes mellitus[15]. It is persisting normally in the uterus of mother during the complete gestational period in the event of normal successful pregnancy[11].

(HLA-G) is non-classical class I major histocompatibility complex[10] and can perform a climacteric role in organizing ($CD8^+$ T) cells through pregnancy by removing all reactive anti-paternal T cell[7]. HLA-G protein possibly exists as seven isoforms inclusive of four membrane-bound HLA-G4, HLA-G3, HLA-G2 and HLA-G1 in addition to three secreted soluble HLA-G7, HLA-G6 and HLA-G5 proteins[3]. HLA-G gene is existing on the short arm of chromosome 6. It is (4170) bp long and consists of 8 exons and 7 introns coding for the heavy chain of the HLA-G[6]. HLA-G gene has a modest 46 polymorphisms which maps to either the non-coding or coding regions[3].

The aim of study was to inspect if there is any association of 14bp deletion/insertion polymorphism of HLA-G gene with recurrent abortion in husband's relatives and husbands strange and to investigate whether gene polymorphisms and alleles for the HLA-G could be applied as markers of predisposition to recurrent abortion.

Material and Methods

Patient and control: The recent study included ninety (90) women with recurrent abortion during the first-trimester for three abortions or more. The ages of these women ranged between 20-30 years. Blood was collected from different Hospitals in Baghdad (AL-Yarmook Teaching Hospital and Baghdad Teaching Hospital) during the period from March to December in 2016. The venous blood was taken at the time of miscarriage by using sterile disposable syringes. 2ml of blood samples were transferred to K_2EDTA (di-potassium ethylene diamine tetra acetic acid) tubes and incubated in the refrigerator for extraction of DNA.

Genetic variation study

DNA extraction: The peripheral blood samples was used to extracted the Genomic DNA using the salting-out procedure[12].

Determination of DNA Concentration and purity: The concentration and purity of DNA were measured by Quantus Fluorometer.

Primer design: PCR reaction was performed by using specific primer pairs designed for HLA-G gene. Based on the NCBI database, all gene information, and sequence Single-nucleotide polymorphism (SNPs) details were collected. By using genious software, the sequences of this primer were explained in the table 1.

Amplification of DNA by PCR: For analyzing the HLA-G of (14bp) Insertion/ Deletion Polymorphism among patient and control, PCR amplification of the HLA-G gene on exon 8 was achieved for all cases by using a specific primer pair planned for this project. Sanger sequencing was done for all PCR products.

PCR program: In this reaction, specific temperature for HLA-G gene, the reaction was executed as in the table 2.

Agarose Gel Electrophoresis: After extraction of DNA and PCR amplification, it was prepared according to Sambrook et al[14] method.

Standard Sequencing: PCR product was sent for Sanger sequencing utilizing automated DNA sequences, ABI3730XL by Macrogen Corporation- in Korea, and received the results by email and then analyzed by the genius software.

Statistical analysis: Chi-square test was used to significant by compare genotype for each sample. Odd ratio and confidence intervals were used to assess the risk or beneficial effect of the studied factor between groups [5].

Results and Discussion

In the result of the gene HLA-G polymorphism in husband's relatives and husbands strange of HLA-G gene polymorphism, the frequencies of homozygous (insertion) +14bp/+14bp were in husband's relatives (13), and husband's strange(11), and homozygous (deletion) -14bp/-14bp results were in husband's relatives(11), and husband's strange (5). Heterozygous+14bp/-14bp results were in husband's relatives (34), and husband's strange 16. revealing statistically no significant differences of HLA-G (14bp) insertion/deletion genotype between a patient with husband's relatives and husband's strange as shown in the table 3.

Distribution of 14 bp alleles (Insertion or Deletion) in the two study groups: The result of the 14 bp alleles in husband's relatives and husband's strange showed insertion +14bp allele in husband's relatives (60) and husband's strange (38), while deletion -14bp allele were in husband's relatives (56), and husband's strange (26). The frequencies of the insertion 14bp alleles in husband's relatives were more than in husband's strange also, deletion 14bp alleles in husband's relatives were more than in husband's strange but did not reach significant level at $p < 0.05$ as shown in the table 4.

The current study did not found any correlation between 14 bp HLA-G genotype and consanguinity marriage. Many researchers showed that the average of unprompted abortion in non-consanguineous and consanguineous mating is the same[4,13]. Other studies show that the rates of spontaneous abortion in non-consanguineous marriage were higher than it in consanguineous marriage and this is related to the genetic compatibility of the parents in consanguineous marriage and the differences in genes between mother and her fetus which may be foreign to the maternal immune system because of the genetic difference in parents of non-consanguineous marriage[9]. Fewer studies noticed a higher rate of prenatal losses among consanguineous couples[2,8] but not in the field of HLA-G and consanguineous marriage.

Alomar et al[1] observed that the distribution of allele frequencies of 14bp insertion or deletion was not significant difference in the recurrent abortion and healthy controls. In this study no correlation was found between the distribution of HLA-G 14-bp (insertion/deletion) polymorphism with recurrent abortion in husband's relatives and husbands strange. Also, study did not show any significant differences in HLA-G for 14bp deletion or insertion allele between groups of recurrent abortion in husband's relatives and husband's strange which indicated that these alleles do not play a role in the success of pregnancy.

Conclusion

In the current study, any significant alteration between heterozygous and homozygous of the HLA-G 14bp (deletion/ insertion) polymorphism in exon 8 amongst husband's relatives and husbands strange was not found along with implication between husband's relatives and husbands strange in the field of alleles (+14bp insertion or -14bp deletion).

Table 1
Primer Sequence used for gene

Gene	Primer Sequence
14(ins/del) F Primer with M13 Tail	5'-TGATGTGTGTGGGTTGTTGAGGG-3' 5'-TGTAAAACGACGGCCAGTTGATGTGTGTGGGTTGTTGAGGG-3'
14(ins/del) R Primer with M13 Tail	3'-ACAAGAAACACGTGTACTGTGGAAA-5' 3'-CAGGAAACAGCTATGACCACAAGAAACACGTGTACTGTGGAAA-5'

Table 2
PCR program for PCR amplification

Steps	C°	min: sec	Cycles
Initial Denaturation	95 C°	5 min	1
Denaturation	95 C°	30 sec	35
Annealing	60 C°	30 sec	35
Extension	72 C°	30 sec	35
Final Extension 2	72 C°	7 min	1
Holding	4 C°	--	1

Table 3
Frequencies of the 14 bp (deletion/insertion) of HLA-G gene polymorphism in husband's relatives and husband's strange.

Study groups	+14bp / +14bp N (%)	-14bp / -14bp N (%)	+14bp / -14bp N (%)	Total N (%)
husband's relatives	13 (14.4%)	11 (12.2%)	34 (37.8%)	58(64.4%)
husband's strange	11 (12.2%)	5 (5.5%)	16 (17.8%)	32(35.5%)
Total N (%)	24 (26.6%)	16 (17.8%)	50 (55.6%)	90(100%)

The chi-square statistic is 1.51. The P-value is 0.46. The result is not significant at $p < 0.05$.

Table 4
Frequencies of the 14bp alleles among two groups.

Study groups	+14bp	-14bp	Total	Odds ratio	confidence interval (95% CI)	P-value
husband's relatives	60	56	116	0.7331	0.39 to 1.35	0.32
husband's strange	38	26	64	------	------	------
Total	98	82	180	-------	------	------

Between husband's relatives and husband's strange: The chi-square statistic is 0.97. The p-value is 0.32. This result is not significant at $p < 0.05$.

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