Immunogenetic study of herpes simplex virus; GM-CSF in female suffering from recurrent miscarriage

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Abstract

Background: Granulo cyte –macrophage- colony-as stimulating -factor (GM-C.S.F) gene may be related with repeated- pregnancy- loss (RPL); among women with reproductive age.
Objective: Investigate the association between GM-CSF- rs1042658 Gene - Polymorphism and risk of RPL among Iraqi women.
Materials and Methods: Polymerase chain reaction was performed to detect HSV1 and determine; rs1042658 geno-types by (Tetra-primer- amplification -refractory –mutation, system for all specimen (one hundred-fifty) from healthy and aborted woman.
Results: Significant variation detected in distribution frequencies of both heterozygote CT, and carriage of T allele of the rs1042658 between cases and healthy groups.
Conclusion: As results of current study, GM-CSF rs1042658 gene polymorphism may be considered as a risk factor for recurrent abortion among Iraqi women.

Keywords: Granulocyte- macrophage-colony-stimulating- factor, recurrent abortion, Polymorphism, HSV1

1. Introduction

Spontaneous abortion is one of most common complications of pregnancy with loss to fetus naturally before twenty weeks of gestation occurring most often in the first trimester and ranging from mild to severe symptom [1, 2].
Recurrent pregnancy loss (RPL) defined as three or more consecutive pregnancy miscarriages [3].
GM-CSFs show a local effect through regulating cytotoxicity of natural killer cells and uterine cell cytotoxicity with reducing the interleukin production therefore GM-CSF plays the main role in embryo implantation and early pregnancy progression while, neutralizing, GM-CSF increases of spontaneous micarrage so, the chance for a successful pregnancy increases with GM-CSF supplementation due to having an angiogenic effect moreover as therapeutic roles of abortion [4].
Viral intrauterine infections like herpes simplex viruses as etiological, causes of first trimester pregnancy loss of pregnant women at aged 20 -30 years [5, 6].

2. Subjects and Methods

Polymerase chain reaction was performed to detect HSV1 and detection SNP of GM-CSF rs1042658 gene polymorphism by amplification –refractory mutation system (ARMS) for all specimen (one hundred-fifty) from healthy and aborted woman from hospitals of Middle Euphrates -Iraq. The collection of sample from February 2020 to Sep-tember 2021.

Herpes simplex virus -1 detection by PCR

By using Patho Gene-spinTM DNA Extraction Kit (Intron/Korea); genome was purifying and migrated using gel (agarose) from the endometrium; swab from cervix;fetal fluid for amplify the Herpes virus DNA.
Program of PCR: Denaturation 95°C- 3 mi; Denaturation 95°C - 30 sec;Annealing 58.3 °C -30 sec; Extension 72°C - 20 sec; Final extension 72 °C for 5 min: for 35 cycles
HSV1-gpD migrating on agarose gel at (75V) for 1h and visualized by special dye; gel was photographed with (Cleaver- Scientific - UK).

Detection of GM-CSF rs1042658 Gene Polymorphism by ARMS

Total DNA for SNP of GM-CSF rs1042658 polymorphism was extracted with G-spin™ Total DNA Extraction Mini Kit; DNA quantity and purity was determined using a spectrophotomete (Nano drop) at the absorbance at 260nm and 280nm respectively from peripheral blood and swabs of female patients to amplification of a target region from a DNA template with specific oligonucleotides by using ARMS technique.
PCR amplification was done using conventional thermal cycler (Biometra - Germany) as follows: Template DNA

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(about 2 µl) was added into PCR master mix tubes. Forward and reverse primers were added 1 µl into PCR master mix tubes (for each one). Distilled water was added to PCR Premix tubes to a total volume of 25 µl.

PCR products of target regions GM-CSF rs1042658 polymorphism was electrophoresed on 1.5% agarose at 75 V for 1h and visualized by bromophenol blue dye that reached at the end edge of the gel then gel was photographed using gel documentation system (Cleaver Scientific - UK).

3. Results

The polymerase chain reaction study done for the 40 cases Herpes simplex virus -1, shows that about 8 cases (20 %) of the samples were infected with herpes simplex virus -1 while 80% (32 out of 40 cases) as negative, as shown in Table 1 as well as Figures 1.

(Table 1) Percentage of HSV-1 Positive Signals in Women Patients with RPL by Using PCR Technique.

Total Viral genome	No.	%	Chi-Square (P-value)
Positive	8	20	P=0.03
Negative	32	80	Sign.
Total	40	100	(P>0.05)

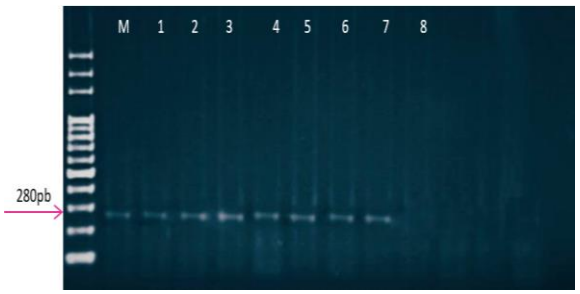

Figure 1: Detection of HSV-1 by PCR.

According to the result of amplified of GM-CSF rs1042658 target sequences of studied groups were by ARMS technique was appeared the presence of two bands (T Allele= 335 bp and C Allele= 456 bp (due to the presence of the T>C mutation. Whereas the wild type was identified by a single 738 bp fragment. It can be seen that the frequency of CC genotypes in women patients with RPL and AHC groups which reached 13% and 1%, respectively it was significantly increased in women patients than control. While, the frequency of CT genotypes in women patients with RPL and AHC groups which reached 9% and 4%, respectively. It was non- significantly in women patients compared with control group. On the other hand, the frequency of TT genotype in women patients with RPL and AHC groups was 10% and 4%, respectively, that increased in women patients compared with control group Table (2). Finally, was found which CT genotype decreased as rate OR=1.6 compared with CC genotype and equal to TT genotype among studied groups. According to the results, both of TT and CC were statistically higher than those of the control group according to the gene expression levels (P<0.05) as shown table 2 and figure 2

Table 2: Comparison between women with and without clinical spontaneous abortion based on percentages of GM-CSF rs1042658 expressed gene polymorphism.

Genotype	Study group		P value	OR [Control]	95% C.I for OR [Control]		OR [Patients]	95% C.I for OR [Patients]	
GM-CSF rs1042658	Control N=50	Patients N=100			Lower	Upper		Lower	Upper
TT	4%	10%	0.07	1.64	1.8	2.3	1.6	1.7	1.8
CC	1%	13%	0.001	1.7	1.7	2.0	1.7	1.8	2.1
CT	4%	9%	**0.01**	1.6	1.6	**1.8**	**1.5**	**1.55**	**1.85**

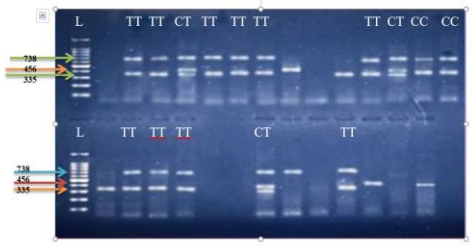

Figure 2: Allelo typing patterens of GM-CSF rs1042658 gene using PCR-ARMS; Showed a heterozygous allele (TC) had a two band (335 and 456 pb) molecular size in women with RPL. While, homozygous allele had a single band with 738 bp molecular size. M: DNA ladder 100-1100 bp.The amplified products using PCR-ARMS migrated into 3% agarose, 75V, 20 mA for 120 min; 15 µl in each well; stained with ethidium bromide.

The concentration of serum GM-CSF were detected by ELISA technique. The mean of serum GM-CSF concentration in women patients with RPL and apparently healthy control (AHC) groups were 9.3± 1.3 pg/ml and 14.88 ± 2.6 respectively. Significant difference (p=.03) was found by comparing the mean of serum GM-CSF concentration among AHC and women with RPL groups

Table 2: Results of serum GM-CSF concentration by ELISA for AHC and women patients with RPL

Immune Variables	AHC (Normal pregnancy) (pg/ml)	Women with RPL (pg/ml)
GM-CSF	14.88 ± 2.6	9.3± 1.3
P value	P<0.05	

According to the Spearman's rho statistical testing to evaluate studied molecular marker in relation with HSV-1 infections in women with clinical spontaneous abortion was appeared there is no significant correlations among HSV-1 and GM-CSF {r = 0. 267, P = 0.182 (p <0.05 Table (3).

Table 3: Spearman's rho statistical testing to evaluate studied molecular marker in relation with HSV-1 infections in women with clinical spontaneous abortion

Spearman's rho		GM-CSF
HSV-1	r.	.267
	P-value	-.182

4. Discussion

Among samples taken from endometrium; fetal fluids and

Blood specimens of spontaneous aborted female patients were found to have 20 % (8 out of 40 cases) with HSV-1, while 80% (32 out of 40 cases) as negative of control healthy group

Current study is supported other survey which reported that infections with HSV1 led to miscarriage [7].

A controversy was noticed regarding the frequency of HSV-1 infection in aborted women since previous studies showed low relation between HSV-1 infection and recurrent miscarriage [8].

Study done by Chow et al. [8] who using multiplex PCR for examined 105 pregnant women, They found 2 cases of spontaneous abortion, but they did not detect any HSV infection, which could be associated in their study with low number of abortion cases enrolled

However, the outcome of occurrence of primary HSV-1 infections in pregnancy are thought to be more severe than those of recurrent HSV-1 infections and as stated by Maitra et al. [9].

More recently in a study done by AHMAD et al. [10].They found only 1.4% are due to HSV infection of aborted woman.

The reasons that to explain the herpes simplex virus-1 cause recurrent miscarriage due to mixed infection of HSV-1 with cytomegalovirus (CMV), chlamydia with and opportunistic flora among women of reproductive age also, immune suppuration with pregnancy can increase to viral infections [11].

Current result detected an association between RPL and variant in GM-C-SF, substitution of the C allele with the T allele as well as significant difference was found by comparing the mean of serum GM-CSF and CD56 concentration in women patients with RPL 9.3± 1.3; 15.78 ±7.02 pg/ml and healthy groups were 14.88 ± 2.6 & 10.74± 4.5 pg/ml, respectively. This indicated a probable risk factor for UN RPL with GM-CSF rs1042658 gene polymorphism

Our finding are inconsistent with Nasiri et al. [12] that showed

GM-CSF rs1042658 gene polymorphism may be risk factor of recurrent miscarriage.

5. Conclusions

In woman with recurrent pregnancy loss and pregnant women (HSV—1) in noticeable level. The presence HSV--1 in women suffering repeated abortion was higher than woman with pregnant, this indicate of this infectious virus as cause recurrent abortion.

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