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Original paper

Genotyping and evaluation of interleukin-10 and soluble HLA-G in abortion due to toxoplasmosis and HSV-2 infections

Abdulla L. JIAD[1], May K. ISMAEL[2], Taher A. SALIH[3], Salma N. MALIK[3], Bahaa Abdullah Laftaah AL-RUBAII[2]

[1]Department of Anesthesia Techniques, Institute of Medical Technology, Middle Technical University, Baghdad, Iraq
[2]Department of Biology, College of Science, University of Baghdad, Iraq
[3]Branch of Radiotherapy, Institute of Medical Technology, Middle Technical University, Baghdad, Iraq

Corresponding Author: Bahaa Abdullah Laftaah Al-Rubaii; email: bahaa.abdullah@sc.uobaghdad.edu.iq

ABSTRACT. Abortion is a condition that occurs due to one of the pathological injuries, often one of the members of the TORCH is the real cause. The current study aimed to investigate the impact of toxoplasmosis, HSV-2 infections with abortion, and also, the identification of immunogenetics marker (interleukin-10) that may be associated with abortion. Anti-*Toxoplasma* IgG, IgM, Herpes simplex virus-2 IgM, human soluble leukocyte antigen class I–G and interleukin-10 were estimated by ELISA technique, while the expression of IL-10 gene was investigated by using the real-time PCR. The results showed that among aborted women the rate of anti-*Toxoplasma* and HSV-2 IgM antibodies occurred within the age groups (21–30) years and (31–40) years 32(100.0%) and 36(100.0%), respectively. A significant relationship was found between IL-10 and cases with a P=0.005. The pattern of distribution of HLA-G in the studied groups showed that there was a significant relationship between HLA-G and cases with a P=0.005. Regarding the *IL-10* rs gene, the results revealed an amplified product of 377 bp and there was a high Ct value for patients and controls with a high Ct value of templates, preoperational to the gene concentration. We concluded that there was a significant relationship between human leukocyte antigen-G and the cases. It was found that there was a high Ct value for patients and controls with a high Ct value for templates.

Keywords: genotyping, toxoplasmosis, Herpes simplex virus-2, soluble HLA-G, abortion

Introduction

Abortion is one of the extreme problem among women that may happen and is defined as termination of pregnancy [1,2]. Genetic and uterine abnormalities, endocrine and immunological dysfunctions, environmental agents including infectious agents are extreme essential reasons of spontaneous abortion [3]. There are different microorganisms infecting the mothers such as TORCH agents [4]. Toxoplasmosis is one of human infections that may cause abortion [5]. Human Herpes simplex virus can infect the placenta and cause disorders to fetal growth and spontaneous abortion [6]. HSV-2 is a member of Herpesviridae family; is a DNA virus that causes primary infection and has the ability to establish lifelong latency in sensory neural ganglia [7], and is able to cross the placenta and extends to the fetus; as a result, it may affect the gestation via prompting the miscarriage of fetal growth disorders [8]. Anti-inflammatory cytokines as interleukin-10 is a critical cytokine for protection of a normal pregnancy [9], and maintaining immune tolerance, thus the dysregulation of IL-10 is associated with adverse pregnancy complications such as miscarriage and fetal growth restriction [10]. Another molecule that acts as an immune-modulatory molecule is the human leukocyte antigen (HLA-G) due to its role in maintaining immune tolerance at the feto-maternal interface, enhancing graft tolerance and decreasing immune responses [11]. At the maternal-fetal

Table 1. Distribution of toxoplasmosis HSV-2 IgM in studied groups according to age

Age	Parameters		Abortion		Normal	
	Toxoplasmosis	Herpes simplex virus-2 HSV-2 IgM	Count	%	Count	%
<=20	-ve	-ve	0	0.0	16	100.0
	+ve	+ve	6	100.0	0	0.0
21–30	-ve	-ve	0	0.0	24	100.0
	+ve	+ve	32	100.0	0	0.0
31–40	-ve	-ve	0	0.0	40	100.0
	+ve	+ve	36	100.0	0	0.0

interface, the HLA-G molecules are specifically expressed by fetus-derived cells [12] so any deficiency in HLA-G expression is associated with miscarriage [13].

The current study aimed to investigate the impact of toxoplasmosis, HSV-2 infections with abortion, and also, the identification of immunogenetics marker (interleukin-10) that may be associated with abortion.

Materials and Methods

The ELISA kit was used for the detection of anti-*Toxoplasma* IgM and IgG antibodies and Herpes simplex virus-2 IgM, human soluble leukocyte antigen class I–G and for the detection of interleukin-10 depending on the manufacturer s instructions of the kit (MyBiosource, USA). The polymerase chain reaction (PCR) was used to assess the *IL10* (−1082) polymorphism amplification, then digested by a specific restriction enzyme. The sequences of PCR primers were 5′-CCAAGACA ACACTACTAAGGCTCCTTT-3′ and 5′-GCTTCT T TATGCTAGTCAGGTA-3′ with expected PCR product size of 377 bp. Four µl of extracted DNA was added in 0.2 ml PCR tube and then added to it 1 µl of RT primer. The total volume must be 5 µl**.** Thermal cycler program for first reaction is: annealing 70°C, m: s at 1 cycle. Hold 4°C, m: s at 1 cycle.

Statistical analysis

The statistical analysis was done by the SPSS-20 software program (Inc, Chicago, Illinois, USA). The (P<0.05) value was regarded as a statistically significant. Data are presented as mean ± SD or number and percentage as applicable. Comparison between abortion and normal groups was done using Student's t-test. Relation between qualitative data was studied using Chi-square test.

Results

Table 1 shows the incidence of toxoplasmosis and Herpes simplex virus-2 IgM antibodies rates in the studied groups according to the age groups. It was shown that the highest *Toxoplasma* and HSV-2 IgM antibody rates occurred within the age groups (21–30) years and (31–40) years 32(100.0%) and 36(100.0%) respectively. There was a significant relationship between IgM and cases in all age groups (P=0.005). Table 2 demonstrated the prevalence of interleukin-10 in aborted women and control groups. There was a significant relationship between IL-10 and cases with P=0.005.

The pattern of distribution of human leukocyte

Table 2. Evaluation of interleukin-10 in the studied groups

Interleukin-10	Parameters				*P*-value
	Miscarriage		Control		
	Count	%	Count	%	
-ve	26	21.7	40	33.3	0.005
+ve	94	78.3	80	66.7	

Table 3. Distribution of human leukocyte antigen-G in the studied groups

Human leukocyte antigen-G	Cases			
	Aborted women		Normal patients	
	Count	%	Count	%
Decreased	110	91.6	0	0.0
Normal	8	6.7	72	60.0
Increased	2	1.7	48	40.0

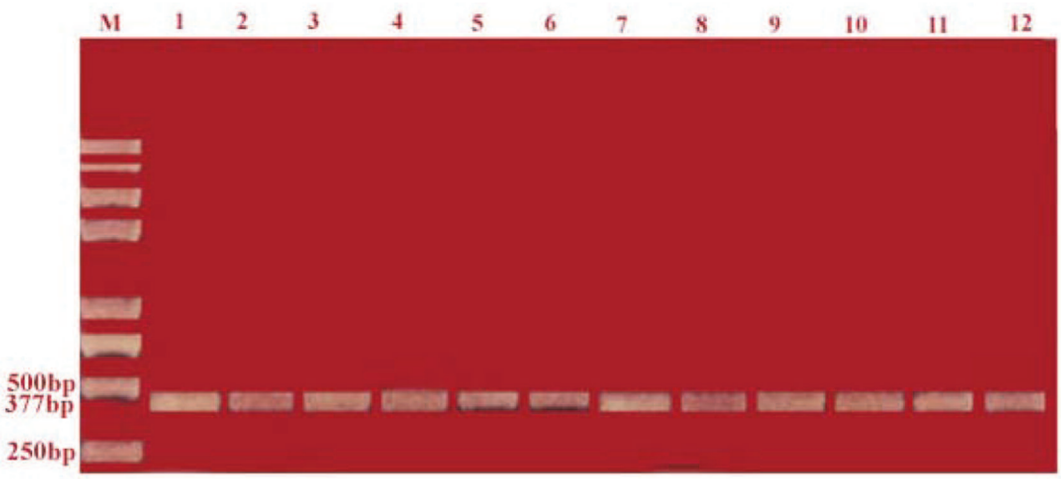

Figure 1. Amplified products of *IL-10* (−1082) polymorphism gene after electrophoresis on 1% agarose gel

antigen-G in studied groups is noticeable in table 3. There was a significant relationship between human leukocyte antigen-G and the cases with P=0.005.

A total of 50 aborted women and 50 normal individuals were genotyped to detect the *IL-10* rs gene. PCR was used for amplification of IL-10 (−1082) polymorphism gene in aborted women by using specific primers. The results in figure 1

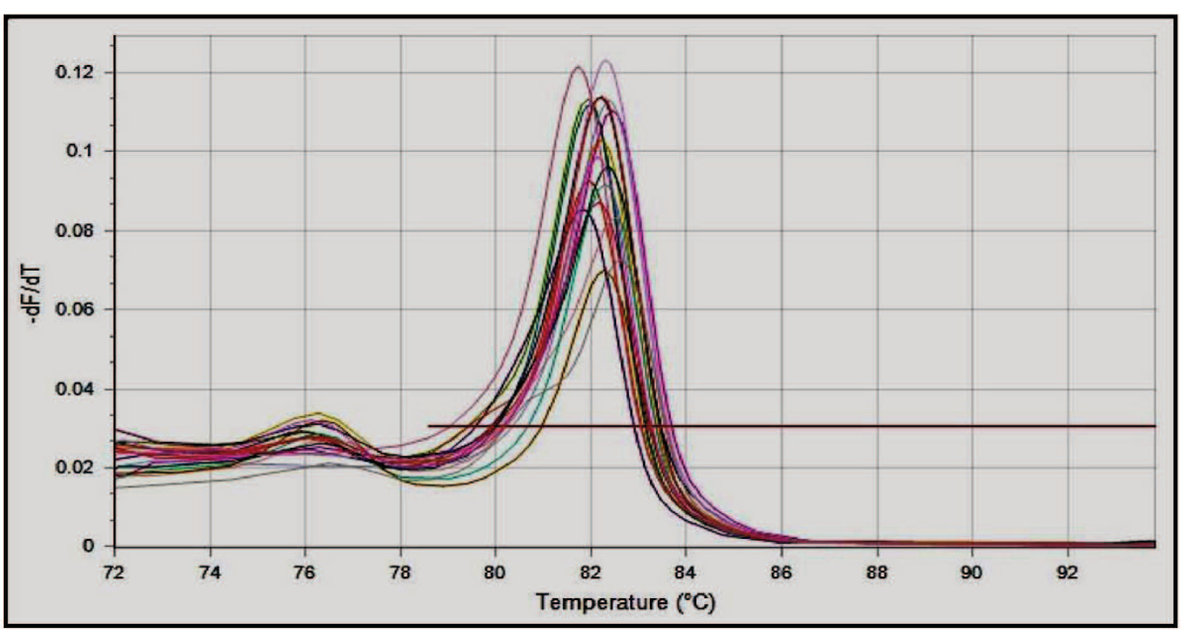

Figure 2. Melting curve analysis of the real-time PCR for *IL-10* expression

showed that there was an amplified product of 377 bp.

Expression of *IL-10* gene was investigated in aborted women and healthy controls by using real-time PCR. It was found that there was a high Ct value for patients and controls with a high Ct value for templates, preoperational to the gene concentration (Fig. 2).

Discussion

Toxoplasmosis association with Herpes-2 virus infection poses a serious threat to the reproductive health of the population. Primary infection or relapse during pregnancy is so serious for the fetus and also can lead to spontaneous miscarriage or developmental malformations [14]. The trophoblast cells express entry mediators of Herpes simplex virus and causes failure in placental invasion and spontaneous abortion [6]. In the current study, the prevalence of HSV-2 IgM in aborted women was 60(100.0%) with (P=0.005).

According to various studies conducted in India, they were shown to be nearly compatible with the current results which showed that the prevalence of toxoplasmosis and Herpes-2 antibodies varies from 3.6% to 61.3% [15], and another study which reported that the seropositivity rate of Herpes simplex virus-IgM was 31.06% [16]. Another result was also almost similar to the results of the current study which reported that the toxoplasmosis and Herpes-2 IgM rate was 73.9% in the patients group [17].

However, another study showed that the elevated toxoplasmosis and Herpes-2 positivity rate was seen in the age group (30–39) years 7(18.9 %) with a highly significant difference (P<0.001) [18]. IL-10 actively suppresses the maternal immune system to avoid rejection of the fetal allograft [10]. In the current study, there was an elevated level of IL-10 among aborted women carrier with toxoplasmosis and Herpes-2 IgM with a significant relation between IL-10 and cases, and these results were nearly compatible with the results obtained by [19] who showed that the increasing levels of IL-10 was observed in aborted women than pregnant controls with highly significant difference. Nevertheless, a study reported that normal pregnant women were characterized by transiently decreased pro- and raised anti-inflammatory expressions [20]. The results of a study conducted by [21] showed a moderate increase in the anti-inflammatory cytokines such as IL-10 in the pregnant women with threats of miscarriage than the apparently healthy pregnant women, and these results agreed with a study which demonstrated that the estimated levels of serum IL-10 for recurrent pregnancy loss women was significantly lower [22]. The current study revealed that the aborted women carriers with toxoplasmosis and Herpes-2 had decreased HLA-G. These results were almost compatible with a study which found that the sHLA-G level increased in normal pregnancy. The HLA-G expression in maternal-fetal tolerance is important in protection of fetal semi-allograft against lysis by maternal NK cells [23], and decreased levels of HLA-G is seen in women with history of two or more abortions [24]. The PCR was used for amplification of IL-10 (−1082) polymorphism gene in aborted women by using specific primers. The results showed that there was an amplified product of 377 bp. Camil et al. [24] stated that the genotype of IL-10 (−1082) gene in aborted women was detected by using conventional PCR with product 377 bp. Expression of *IL-10* gene was investigated in abortions and healthy controls by using the real-time PCR. The results revealed that there was a high Ct value for patients and controls with a high Ct value of templates, preoperational to the gene concentration. These results agreed with Qaddourah [25] who reported the IL-10 expression by using (RT-PCR) to detect the inflammatory cases in aborted women due to TORCH diseases.

We concluded that there was a significant relationship between human leukocyte antigen-G and the cases, it was found that there was a high Ct value for patients and controls with a high Ct value for templates.

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