IMMUNOGENETIC STUDY OF HUMAN HERPES VIRUS-6 AND IL-10GENE POLYMORPHISM IN WOMEN SUFFERING FROM RECURRENT PREGNANCY LOSS

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ABSTRACT

Background: HHV-6A includes several adult-derived strains and its disease spectrum is not well defined. Interleukin 10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. Several single nucleotide polymorphisms (SNPs) have been identified in the IL-10 gene promoter region which regulates the expression of IL-10. Among these SNPs located in the promoter region of the IL-10 gene, three single nucleotide polymorphisms (G/A at -1082, C/T at -819, and C/A at -592 positions) in the proximal region, are associated with an increased frequency of spontaneous abortions.

Patients and methods: This case control study was done for a one hundred-fifty specimens collected from female patients subjected to recurrent pregnancy loss and apparently healthy persons as control group. All these specimens were submitted for genetic part for screening human Herpes virus-6 (HHV-6) in patients and apparently healthy person control groups by real time polymerase chain reaction (RT-PCR).

Results: The positive result of HHV-6 DNA according to qRT-PCR shows 43.1% (25 out of 58 cases) as positive while 56.9% (33 out of 58 cases) was negative. According to the results of IL-10, both of TT and CC were statistically higher than those of the control group according to the gene expression levels (P<0.05).

Conclusions: HHV-6 one of the most recently identified vaginal viruses in Iraqi women patients suffering from recurrent miscarriage. In addition, highly serum concentration of IL-10 level in women patients with RPL could point for its possible roles in protective against pathogenesis of women patients suffering from recurrent miscarriage.

Keywords: RPL, HHV-6, RT-PCR, IL-10; ARMS

1. INTRODUCTION

Abortion is the ending of a pregnancy by removal or expulsion of an embryo or fetus. An abortion that occurs without intervention is known as a miscarriage or "spontaneous abortion" and occurs in approximately 30% to 40% of pregnancies (Lattet et al., 2019).

Human herpesvirus 6 (HHV-6) was the sixth human herpesvirus discovered. It belongs to the β-Herpesvirinae subfamily. Although horizontal transmission is considered to be the main route of HHV-6 infection, it can be genetically transmitted from parent to child as inherited chromosomally integrated HHV-6 (ichiHHV-6) (Miura et al., 2018).

HHV-6 can integrate into human chromosomes, resulting in genetic transmission from parent to child. Individuals of either sex with inherited chromosomally integrated human herpesvirus 6 (ichiHHV-6) harbor the virus in every cell. Viral reactivation from the integrated HHV-6 genome can occur in pregnancy (Miura et al., 2020). HHV-6A includes several adult-derived strains and its disease spectrum is not well defined, although it is thought by some to be more neurovirulent (Kofman et al., 2011).
Transmission is believed to occur most frequently through the shedding of viral particles into saliva. Both HHV-6B and HHV-7 are found in human saliva, the former being at a lower frequency. Studies report varying rates of prevalence of HHV-6 in saliva (between 3–90%), and have also described the salivary glands as an in vivo reservoir for HHV-6. The virus infects the salivary glands, establishes latency, and periodically reactivates to spread infection to other hosts (Arbuckle et al., 2011; Araujo et al., 2011).

In normal pregnancy, a shift to Th2 cytokine production with abundant interleukin-10 (IL-10) is considered important. During pregnancy, IL-10 is produced locally in the fetoplacental unit by cytotrophoblasts and decidual T cells, and it up-regulates the human leukocyte antigen (HLA)-G expression of cytotrophoblasts at the fetomaternal barrier (Camil and Viorica, 2014).

In humans, interleukin 10 is encoded by the IL10 gene. IL-10 signals through a receptor complex consisting of two IL-10 receptor-1 and two IL-10 receptor-2 proteins. Consequently, the functional receptor consists of four IL-10 receptor molecules. IL-10 binding induces STAT3 signalling via the phosphorylation of the cytoplasmic tails of IL-10 receptor 1 + IL-10 receptor 2 by JAK1 and Tyk2 respectively (Mosser et al., 2008).

IL-10 is a cytokine with multiple, pleiotropic, effects in immunoregulation and inflammation. It downregulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. IL-10 can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway (Iyer et al., 2012).

II. MATERIALS AND METHODS

This case control study was done for a one hundred-fifty specimens collected from female patients subjected to recurrent pregnancy loss and apparently healthy persons as control group from general hospitals as well as many private clinical in Diyala province and Middle Euphrates—Iraq.

The age range of the study population was 18 years to 42 years. The specimens were collected during period from February 2020 to September 2020.

Endometrium; Cervical swabs; fetal fluids swabs as well as Blood samples were collected and processed for screened for human Herpes Virus 6 (HHV-6) and IL-10 gene polymorphism. A special kit (Human Herpes Virus-6 Real-Time PCR kit; GPS / Spain) was used by the RT PCR device to detect the presence of the HHV-6 in the 58 samples that gave a positive result in gel electrophoresis.

Detection of SNPs IL10 (rs1518111) polymorphism were extracted from peripheral blood and swabs of female patients using ARMS technique. Is based on two major processes: isolation of total DNA from specimens and Polymerase chain reaction (ARMS-PCR) is allows the amplification of target region from a DNA template by using specific oligonucleotides.

SPSS program (Version– 23) & P value was considered Sig. when p <0.05 was used to explain the statistical variable between study populations.

III. RESULTS

1. Age Frequency of Patients with RTI and Healthy Control Groups

The mean age groups of the study population. The mean age of the patients with Recurrent Pregnancy Loss (RPL) was (30.70±12.41 years) was less than the mean age of the apparently healthy control (AHC) (28.67 ± 11.17 years). There are non-significant statistical differences (p=0.47) between RPL and AHC.

Table 1: Distribution of Women Patients with RLP and AHC according to Their Age.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>No.</th>
<th>Mean of age (years)</th>
<th>S. D</th>
<th>S. E</th>
<th>Range(years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minimum</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maximum</td>
</tr>
</tbody>
</table>
IV. DETECTION OF HUMAN HERPES VIRUS-6 (HHV-6) BY MOLECULAR TECHNIQUE.

Out of 150 Endometrium; Cervical swabs; fetal fluids swabs as well as Blood specimens involved in this study 58 (58%) were found to have a viral infection where as 42 % (42 out of 100 cases) as negative with RPL as shown in Figures (1). While, no viral nucleic acid was detected among all the examined apparently healthy specimens (50) as control group. There were statistically significant differences (p = 0.03) between women patients with recurrent pregnancy loss with viral genome and those without the Viral genome.

Table 2: Percentage of Viral Genome Extraction of Women Patients with RPL And AHC Groups.

<table>
<thead>
<tr>
<th>Viral Genome</th>
<th>Study Groups</th>
<th>Pearson Chi-Square (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPL No. (100)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>N 58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 58%</td>
<td>P = 0.03 sign. (P &gt; 0.05)</td>
</tr>
<tr>
<td>Negative</td>
<td>N 42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 42%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>N 100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 100%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Extraction of Viral Genome from women Patients with Recurrent Pregnancy Loss , 1% Agarose Gel Electrophoresis, TBE 1X, at Voltage 75 Volt for 45 min, Lanes were Positive.

The quantity HHV-6 system is a quantitative test that allows the DNA amplification and quantification, by means of Real-Time PCR, of HHV-6 genome. The analysis of results was made by using a Real-Time PCR analyzer (Rotor-gene Q MXQ/thermal cycler integrated with a system for fluorescence detection and a dedicated software). The positive result of HHV-6 –DNA according to qRT-PCR shows 43.1% (25 out of 58 cases) as positive while 56.9% (33 out of 58 cases) was negative, as shown in Table (3) as well as Figures (2 A, & B). Statistically significant differences (p = 0.04) among patients group.
Table 3: Percentage of HHV-6 Positive Signals in Women Patients with RPL by Using qRT-PCR Technique

<table>
<thead>
<tr>
<th>Total Viral genome</th>
<th>No.</th>
<th>%</th>
<th>Chi-Square (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25</td>
<td>43.1</td>
<td>P=0.04 sig. (P&gt;0.05)</td>
</tr>
<tr>
<td>Negative</td>
<td>33</td>
<td>56.9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Detection of HHV-6 by qRT-PCR.

V. GENOTYPING OF IL-10(RS1518111) GENE POLYMORPHISMS

The amplified of IL-10(rs1518111) target sequences of studied groups were by ARMS technique are summarized in table (4-8) and figure (4-5). The result of amplified was appeared the presence of two bands (T Allele= 183 bp and C Allele= 133bp) due to the presence of the T>C mutation. Whereas the wild type was identified by a single 316 bp fragment. It can be seen that the frequency of CC genotypes in women patients with RPL and AHC groups which reached 11% and 0%, respectively it was significantly increased in women patients than control. While, the frequency of TC genotypes in women patients with RPL and AHC groups which reached 17% and 16%, 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 9% and 4%, respectively, that increased in women patients compared with control group. Finally, was found which TC genotype decreased as rate OR=1.7 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) Table (4).

Table 4: Comparison between women with and without clinical spontaneous abortion based on percentages of IL-10 expressed gene polymorphism.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Study group</th>
<th>p value</th>
<th>OR [Control]</th>
<th>95% C.I for OR [Control]</th>
<th>OR [Patients]</th>
<th>95% C.I for OR [Patients]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control N=50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients N=100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>4%</td>
<td>9%</td>
<td>0.08</td>
<td>1.94</td>
<td>1.9</td>
<td>2</td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td>11%</td>
<td>0.001</td>
<td>1.9</td>
<td>1.8</td>
<td>2.04</td>
</tr>
<tr>
<td>TC</td>
<td>16%</td>
<td>17%</td>
<td>0.01</td>
<td>1.8</td>
<td>1.7</td>
<td>1.9</td>
</tr>
</tbody>
</table>

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VI. DISCUSSION

In our study, there was a correlation between number of abortion and age that determined by p value for number of miscarriage and age group. Interestingly, previous observations support our results on the possible adverse effect of HHV-6A on pregnancy: Gervasi et al., 2012 observed the presence of HHV-6 infection in amniotic fluid of a patient that developed gestational hypertension at term and of a patient who delivered at 33 weeks for premature rupture of membranes and give Loss of subclinical pregnancy is even higher, and is reported to be approximately 60% based on the measurement of human chorionic gonadotrophin levels. Gibson et al., 2008 observed the association of HHV-6 (and HHV-7, Varicella zoster) newborn infection with preterm pregnancies and pregnancy-induced hypertensive disorders. Spontaneous abortion is a common problem in early pregnancy. Spontaneous miscarriages occur in approximately 14% to 16% of naturally conceived pregnancies and approximately 15% of clinically recognized first-trimester pregnancies undergo miscarriage (Aren et al., 1999). Although causal relationships between abortion and infections are difficult to establish, the detection rate of B19V, CMV, and HSV during pregnancy is an important way to analyze their relationship with first-trimester spontaneous abortion.

Michou et al., (2012) identified nine HHV-6-positive samples among 109 whole semen specimens tested using a sensitive real-time PCR method, but found no positive samples in the sperm fraction after purification using a Pure Sperm gradient. In contrast, overestimation due to nested PCR techniques may explain anomalously high HHV-6 prevalence in semen, between 66.3% and 70%, reported by other authors (Neofyto et al., 2009).

IL10, which shows dual (inhibitory and stimulatory) immunologic functions and thus could not be classified as either Th1 or Th2 (Conti et al., 2003).

In these results was found the frequency of TC genotypes in women patients with RPL and AHC groups which reached 17% and 16%, 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 9% and 4%, respectively, that increased in women patients compared with control group.

One report from Brazil agreed entirely with our finding that there was no significant association observed between the polymorphisms and risk of recurrent miscarriage (Daher et al., 2003), while another work from India showed that the mutant alleles of the three polymorphisms increased the risk of recurrent miscarriage (Parveen et al., 2013). Besides these two previous reports, the IL-10 -1082A > G polymorphism had also been investigated in the South Korean population (Lee et al., 2013), and no statistically significant finding was observed. These discrepancies clearly showed that the polymorphisms influence the risk of recurrent miscarriage in a population-specific manner.

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