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## **Molecular detection of human herpes virus 7 in women patients with recurrent pregnancy loss**

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**Abstract**--Viral infections during pregnancy have been associated with adverse pregnancy outcomes and birth defects in the offspring. Viruses rarely cross the placental barrier, but when the virus does reach the fetus, it can result in severe birth defects such as microcephaly or even fetal death. Human herpes virus 7 (HHV-7) is thought to cause severe complications is spontaneous abortion. These experiments were conducted to study the effect of human herpes virus -7 on the pregnant women and cause abortion. This case control study was done for 100 patients including different ages that range from 18-47 age that sever recurrent miscarriage. Also the study includes 50 apparently healthy control (AHC) this age were similar with the patients age. The specimens were collected during winter from August 2021 to February 2022. Endometrium; Cervical swabs; fetal fluids swabs as well as Blood specimens were collected and processed to extract viral genome. The mean age of the patients with RPL was (32.70±12.41 years) was less than the mean age of the AHC (30.67 ± 11.17 years). There are non-significant statistical differences (p=0.47) between RPL and AHC. A strong positive relationship (with highly significant correlation) was found between number of participants; number abortion; week of abortion and Maternal age (P < 0.001). However, there are no significant correlation between number of participants with control maternal age. Out of 100 patients, 55 (55%) were found to have a viral infection where as 45 % as negative with RPL. The positive result according to PCR shows 34.5 % (19 out of 55 cases) as positive while 65.5% (36 out of 55 cases) as negative. In women with RPL, the most commonly affected age stratum infected with DNA -HHV-7 was (30-39 years) which constituted 48% (12 out of 25 cases), while the age stratum (17-29 years) was constituted 32% (8 out of 25 cases), followed by 20% (5 out of 25) in age stratum (40 – 49 years). Conclusion: HHV-7 might be one of the most recently identified uterus, cervical viruses in Iraqi female patients suffering from

recurrent miscarriage in the Iraqi population. The positive results of HHV-7\_DNA PCR in endometrium; cervical & fetal fluids swabs of female patients group, these findings lead to the proposal that HHV-7 acts as cofactor in the process of pregnancy loss as well as in recurrent miscarriage in female patients suffering from abortion.

**Keywords**---HHV-7, Abortion, Recurrent Pregnancy Loss (RPL).

## 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 (Latt et al., 2019). In addition, recurrent pregnancy loss (RPL), defined as the spontaneous loss of two or more pregnancies or the loss of two or more pregnancies before the 24th week of gestation, presents several still incompletely defined aspects. Among these is the outcome of the successive pregnancy in women with a history of RPL (Carlo et al., 2020).

Indeed, there is considerable discrepancy between the reported birth rates and the rates of gestational complications of the successive pregnancy in women with RPL. The likelihood of a live birth in the successive pregnancy in untreated women with RPL has been reported to range 42–86% after three miscarriages and decreases with increasing the number of pregnancy losses, reaching only 23–51% after ≥5 losses (Christiansen et al., 2020). This observation suggests that the number of miscarriages—a likely indicator of the gravity of the condition—is a major determinant of the reproductive success of women with RPL; in fact, it has been reported that the live birth rates in the successive pregnancy in women with two consecutive losses is around 75% (Green, and O'Donoghue, 2019; Christiansen et al., 2020).

In fact, in only around 50% of RPL cases can defined causes/risk factors can be found, including advanced maternal age, genetic abnormalities, selected maternal autoantibodies, endocrine dysfunctions, and uterine abnormalities. The remaining RPL cases currently are unexplained (uRPL) (Ticconi et al., 2019). Recent studies have shown that the etiology of RSA is complex and varied, including advanced age, genetic abnormalities, infections, immune disorders, coagulation abnormalities, and endocrine dysfunction (Guo et al., 2020).

Human betaherpesvirus 7 (HHV-7) is one of nine known members of the Herpesviridae family that infects humans. HHV-7 is a member of Betaherpesvirinae, a subfamily of the Herpesviridae that also includes HHV-6 and Cytomegalovirus (HHV-5 or HCMV). HHV-7 often acts together with HHV-6, and the viruses together are sometimes referred to by their genus, Roseolovirus (Guo et al., 2020).

The complete HHV-6 and HHV-7 genome is integrated into every nucleated cell of an individual with HHV-6. Extremely high copy numbers of HHV-6 and HHV-7 DNA can be detected in clinical specimens containing nucleated cells, which can

lead to a misdiagnosis of active viral infection. Theoretically, a parent with HHV-7 has a 50% chance of transmitting the integrated HHV-6 and HHV-7 genome to the next generation (Gaccioli et al.,2020). HHV-6 and HHV-7 can integrate into human chromosomes, resulting in genetic transmission from parent to child. Individuals of either sex with inherited chromosomally integrated human herpesvirus 7 (HHV-7) harbor the virus in every cell. Viral reactivation from the integrated HHV-6 and HHV-6 genome can occur in pregnancy (Miura et al.,2020). We designed this study to determine the percentage of Human Herpes Virus 7(HHV-7) in Women Suffering from Abortion as well as Recurrent Miscarriage.

## Materials & Methods

### Patients population

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 Middle Euphrates -Iraq. The age range of the study population was 18 years to 47 years. The specimens were collected during period from August 2021 to February 2022. Endometrium; Cervical swabs; fetal fluids swabs as well as Blood from each study group of female patients suffering from recurrent pregnancy loss should be enrolled, that classify into:

- 1) 1.One –hundred endometria; cervical swabs; fetal fluids swab as well as Blood specimens from women suffering from abortion as well as recurrent Miscarriage.
- 2) Fifty blood and cervical swabs specimens of apparently healthy persons as control group.

All these specimens were submitted for genetic part for screening human Herpes virus-7 (HHV-7) in patients and apparently healthy person control groups by polymerase chain reaction (PCR).

## Results

### 1. Study population according to their age:

Table 1 shows the mean age groups of the study population. The mean age of the patients with Recurrent Pregnancy Loss (RPL) was (32.70±12.41 years) was more than the mean age of the apparently healthy control (AHC) (30.67 ± 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

| Study groups | No. | Mean of age (years) | S. D  | S. E  | Range(years) |         |
|--------------|-----|---------------------|-------|-------|--------------|---------|
|              |     |                     |       |       |              |         |
|              |     |                     |       |       | Minimum      | Maximum |
| AHC          | 50  | 30.67               | 11.17 | 2.403 | 20           | 45      |

|       |     |                                  |       |       |    |    |
|-------|-----|----------------------------------|-------|-------|----|----|
| RPL   | 100 | 32.70                            | 12.41 | 1.979 | 18 | 47 |
| Total | 150 | P-value = 0.3 No. sign. (P<0.05) |       |       |    |    |
| Total | 150 | P-value = 0.3 No. sign. (P<0.05) |       |       |    |    |

## 2. A comparison of the Pattern of Miscarriage Between Women with and without Clinical Spontaneous Abortion

A strong positive relationship (with highly significant correlation) was found between number of participants; number abortion; week of abortion and Maternal age ( $P < 0.001$ ). However, there are no significant correlation between number of participants with control maternal age and as illustrated in Table 2.

Table 2: A comparison of the pattern of miscarriage between women with and without clinical spontaneous abortion

| Variables                             | Study groups (cases) |                   |                  | Study groups (control) |                  |                  |
|---------------------------------------|----------------------|-------------------|------------------|------------------------|------------------|------------------|
| Maternal age (Y*)                     | 18-29<br>No.(42)     | 30-39<br>No. (38) | 40-47<br>No.(20) | 20-29<br>No.(20)       | 30-39<br>No.(20) | 40-45<br>No.(10) |
| Number of participants<br>Means (SEM) | 23(±0.7)             | 33(±0.3)          | 42(±0.6)         | 25(±0.7)               | 32(±0.5)         | 42(±0.8)         |
| Number abortion<br>Means (SEM)        | 1.6(±0.4)**<br>*     | 2.5(±0.1)**       | 4.5(±0.9)**      | NF                     | NF               | NF               |
| Week of abortion<br>Means (SEM)       | 12(±0.9)             | 13(±0.6)          | 14(±0.7)         | NF                     | NF               | NF               |

NF: Not found: \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$

## 3. Detection of Human Herpes Virus -7 (HHV-7) by Real-Time Polymerase Chain Reaction Technique (RT.PCR)

### 3.1 Extraction nucleic acid by specific Viral DNA/RNA extraction kit:

Out of 100 Endometrium; Cervical swabs; fetal fluids swab as well as Blood specimens involved in this study 55 (55%) were found to have a viral infection with RPL as shown in Table (3) and Figure (1) While, no viral nucleic acid was detected among all the examined apparently healthy specimens (50) as control group. There were statistically highly significant differences ( $p = 0.01$ ) between women patients with Recurrent Pregnancy Loss with viral genome and those without the Viral Genome Table (3).

Table 3: Percentage of Viral Genome Extraction of Women Patients with RPL and AHC Groups

| Viral Genome |   | Study Groups  |               | Pearson Chi-Square (P-value)                              |
|--------------|---|---------------|---------------|-----------------------------------------------------------|
|              |   | RPL No. (100) | AHC No. (100) |                                                           |
| Positive     | N | 55            | 0             | <i>P=0.01</i><br><i>Hig.sign.</i><br>( <i>P&gt;0.05</i> ) |
|              | % | 55%           | 0.00%         |                                                           |
| Negative     | N | 45            | 50            |                                                           |
|              | % | 45%           | 100%          |                                                           |
| Total        | N | 100           | 50            |                                                           |
|              | % | 100%          |               |                                                           |

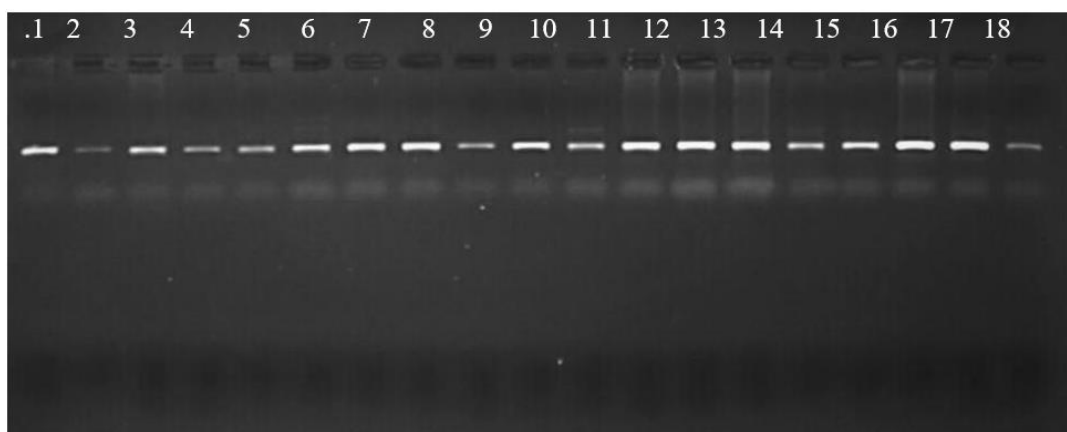


Figure 1: Extraction of Viral Genome from women Patients with Recurrent Pregnancy Loss, 1 % Agarose Gel Electrophoresis, TBE 1X , at Voltage 75 Volt for 45 min, Lanes (1-19) were Positive.

### 3.2 Detection of HHV-7 –DNA by PCR:

The positive result according to PCR shows 34.5 % (19 out of 55 cases) as positive while 65.5% (36 out of 55 cases) as negative, as shown in Table (4) as well as Figure (2). Statistically significant differences ( $p = 0.04$ ) among patients group.

Table 4: Percentage of HHV-7 Positive Signals in Women Patients with RPL by Using qRT. PCR Technique

| Total Viral genome | No. | %    | Chi-Square (P-value)                                  |
|--------------------|-----|------|-------------------------------------------------------|
| Positive           | 19  | 34.5 | <i>P=0.04</i><br><i>sign.</i><br>( <i>P&gt;0.05</i> ) |
| Negative           | 36  | 65.5 |                                                       |
| Total              | 55  | 100  |                                                       |
|                    |     |      |                                                       |

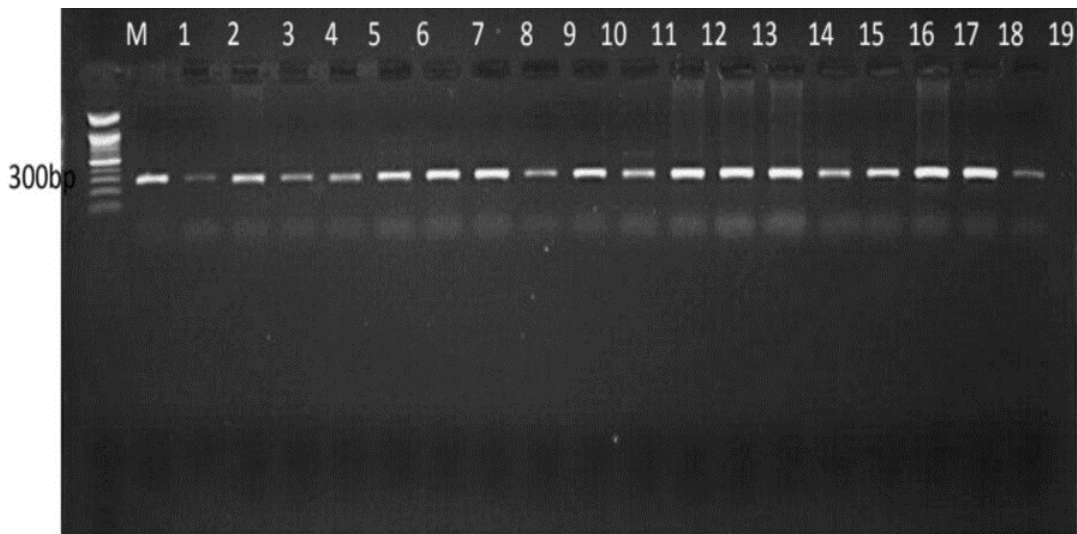


Figure 2: The electrophoresis pattern of HHV-7 DNA (300bp) detection in Endometrium; Cervical swabs; fetal fluids swabs of RPL patients and healthy control groups. Lane 1 - lane 19 refers to HHV-7 DNA samples; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (5  $\mu$ l in each well), stained with red safe solution.

### 3.3 The results of HHV-7 in the Patients with Women with RPL according to the age stratum

In women with RPL, the most commonly affected age stratum infected with DNA - HHV-7 was (30-39 years) which constituted 48% (12 out of 25 cases), while the age stratum (17-29 years) was constituted 32% (8 out of 25 cases), followed by 20% (5 out of 25) in age stratum (40 – 49 years). Statistical comparison of these HHV-7 in the Patients with Women with RPL according to age stratum revealed significant differences ( $p < 0.05$ ) Table (5).

Table 5: Frequency of HHV-7 PCR Signal Among the Patients with Women with RPL According to the Age Stratum

| Age Stratum | Years | HHV-7 |          |          | P value                                |
|-------------|-------|-------|----------|----------|----------------------------------------|
|             |       | No.   | Positive | Negative |                                        |
|             | 17-29 | 18    | 6        | 12       | Anova test<br>P=0.04<br>S.<br>(P<0.05) |
|             |       | 32.7% | 10.9%    | 21.8%    |                                        |
|             | 30-39 | 25    | 10       | 15       |                                        |
|             |       | 45.5% | 18.2%    | 27.3%    |                                        |
|             | 40-49 | 12    | 3        | 9        |                                        |
|             |       | 21.8% | 5.4%     | 16.4%    |                                        |
| Total       |       | 55    | 19       | 36       |                                        |
|             |       | %100  | 34.5%    | 65.5%    |                                        |

## Discussion

Miscarriage is a common outcome of pregnancy, but the rate is challenging to estimate because of inconsistent registration and documentation. The provision of abortion services has changed in the past decade. Medication abortion accounts for at least half of all abortions in the majority of countries. Also in the majority of countries, over 90% of all abortions were completed before 13 weeks, and more than two-thirds of abortions occurred before the first 9 weeks of gestation. Over the past 10 years there has been an increase in both the proportion of abortions that were medication abortions, and the proportion that were obtained before 9 weeks' gestation (Popinchalk and Sedgh, 2019). The most of the spontaneous abortions occur in the early weeks of pregnancy, and therefore, it can be confused with menstrual bleeding. Generally, it is very difficult to determine the rate of spontaneous and unwanted abortions because in countries where legal abortion is prohibited, there is a possibility of false report. Besides, the study of spontaneous abortion in low- and middle-income countries is also very challenging because most abortions have not been reported to and recorded in their official health system (Delicious et al., 2016). Accordingly, the risk for abortion in women with the first marriage and pregnancy at age greater than 40 years old more than the other age groups; This finding was also consistent with previous researches by (Cohain et al., 2017; Kebede et al., 2018).

Although causal relationships between abortion and infections are difficult to establish, the detection rate of several viruses such as HHV-6, HHV-7; B19V, CMV, and HSV during pregnancy is an important way to analyze their relationship with first-trimester spontaneous abortion. It is generally thought that the optimal specimen type for detecting HHV-7 is fetal swab; cervical swab as well as blood specimens, although this may not be true for all HHV-6 & HHV-7 and detection techniques. Given the complexity and invasive nature of the procedure used to acquire swabs specimens have emerged as an alternative specimen type. Fetal swab; cervical swab as well as blood specimens were shown to be superior or equivalent to specimens for RT-PCR in many studies (Francesca et al., 2020).

Out of 100 Endometrium; Cervical swabs; fetal fluids swab as well as Blood specimens involved in this study 55 (55%) were found to have a viral infection with RPL of them were discovered to have viral infection by using Real Time PCR technique table (3). A study done by (Sayyadi-Dehno et al., 2019; Miura et al., 2021) and for aborted women, which revealed (38.2%) and (34.7%) positively viral infected women, respectively that support current study in the total of viral infection.

Furthermore, our results are consistent with previously published studies that investigated the etiology of RPL, especially those focused on viral infection associated with recurrent miscarriage (van der Eijk et al., 2016; Bhatnagar et al., 2017).

It may be possible that the quantity or the physical status of viral DNA in the gestational tissue of pregnancy loss does not permit its easy detection and requires the use of the more sensitive and specific nested PCR technique. In addition, it remains unclear whether Endometrium; Cervical swabs; fetal fluids

swabs as well as blood specimens are optimal for detection of most aborted viral by molecular technique such as multiplex PCR or RT-PCR. HHV-7 is an etiological agent or a risk factor in a portion of pregnancy loss. A previous report has tied HHV-7 infection to spontaneous abortion and neonatal hypotonia. Accordingly, to these investigators have attempted to clarify the relationship between HHV-7 infections during pregnancy (Ohashi et al.,2002; Caserta et al.,2007; Suzuki. et al.,2022).

In the current study, the positive result according to PCR shows 34.5 % (19 out of 55 cases) as positive while 65.5% (36 out of 55 cases) as negative, as shown in Table (4). A follow up study revealed similar results for women who developed PR in the first 15 weeks of pregnancy, with 39% of pregnancies ending in miscarriage (Giulia et al ., 2017).

In addition, the present result of HHV7 is compatible with Al-Buhtori et al.,2011 and Luis et al., 2011 who found HHV-7 in 34% and 66.9% in spontaneous aborted women, respectively. Furthermore, a recent study found HHV-7, only one pregnant woman did not shed viral DNA at all, and most subjects (46/54, 85.2%) shed viral DNA in all five samples (Suzuki. et al.,2022).

The concept of the relationship between HHV-7 and spontaneous abortion is based on the identification of HHV-7 genome sequences in fetal tissues, umbilical cord blood, and villous tissue and antigen has also been found in villous tissue (Aoki et al.,2016).

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### **Conflicts of Interest**

The Authors declare no conflicts of interest.

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### **Author's Contribution**

Ghufran Salam Hadi designed the study and analyzed and interpreted the data. Shakir H. Mohammed Al-Alwany. Both authors read and approved the final version of the manuscript.

### **Data Availability**

All datasets generated or analyzed during this study are included in the manuscript.

### **Ethics Statements**

This project has formal ethics approval by the college of science \ University of Babylon.



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