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COMPARATIVE STUDY BETWEEN VIDAS SYSTEM AND PCR TECHNIQUE IN DIAGNOSIS THE CMV IN ABORTED WOMEN

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Abstract:

Cytomegalovirus, also known as CMV, is one of the microorganisms responsible for the highest number of miscarriages and stillbirths among pregnant women around the world. It is also one of the most major causes of fetal mortality. In order to evaluate the effectiveness of the Vidas system and traditional PCR in the identification of cytomegalovirus in pregnant women experiencing complications related to abortion, this research project was designed. This study was successful in collecting sixty serum samples from female participants from the Babylon province, which were then categorized. In the first group there were forty women who were experiencing recurrent miscarriages. In the second group there were twenty women who were healthy and served as a control. According to the findings, forty of the serum samples that were analyzed using the Vidas system were positive, but only thirty of the samples were positive when tested using the PCR approach. The PCR method has a higher sensitivity than the Vidas system when it comes to the detection of cytomegalovirus in pregnant women who have undergone an abortion.

Keywords: *PCR Technique, Vidas System, CMV, Abortion.*

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Introduction:

Cytomegalovirus is a common infectious agent that affects between fifty percent and eighty five percent of the world's population. Although the initial HCMV infection in healthy persons is frequently asymptomatic, the virus can cause serious and even deadly disease in immune-compromised individuals as well as neonates (Britt, 2008). According to Rawlinson et al. (2017), HCMV is one of the most common causes of birth abnormalities originating from an infectious agent. Twenty percent of congenitally infected infants have lifelong neurological squeals, some of which include blindness, hearing, and/or mental incapacity. According to Navarro (2016), HCMV is capable of causing serious illnesses in organ transplant recipients as well as AIDS patients following either primary infection or reactivation of a dormant infection that had been present. According to Vanarsdall et al.'s research from 2019, HCMV is capable of establishing a protracted infection, during which it remains dormant in the host and goes through productive reactivation cycles that contribute to its effective transmission.

According to Noriega et al. (2012), over the course of evolution, HCMV has picked up a variety of various techniques to modify and escape the human immune response. As a result, the virus has been able to achieve high infection efficiency and widespread diffusion within the body of its host. The following objectives were established for the purpose of determining whether or not human cytomegalovirus is involved in the infection that causes abortions: Evaluation of anti-HCMV antibodies using the Vidas system. Utilization of the PCR method for the detection of HCMV receptors.

Material and Methods:

A. Samples collection

For the purpose of this study, forty patients (females suffering from abortion who were admitted to an outside clinic) ranging in age from 20 to 40 years were used, while twenty healthy women served as the control group. They were given a diagnosis by doctors who specialize in that field (explain the methods that doctors use to diagnose infection with CMV).

B. Blood sampling:

Each participant in the trial had around five milliliters of venous blood drawn from them for the study. After centrifuging a gel tube that contained blood at 3000 rpm for 15 minutes, serum was extracted; after that, the serum was collected and stored at -20 degrees Celsius.

C. **Detection Cytomegalovirus (CMV) by Vidas system**

Forty samples were put through the CMV detection process using the Vidas system that was supplied by Biomerieux France.

D. **Extraction viral DNA** and **PCR technique**

Extraction viral DNA according to (Kleines *et al.,* 2003) Lyophilized primer was dissolved in water that was free of DNase and RNase to produce a final concentration of 100 pmol/ l. This solution was stored at a temperature of -20 degrees Celsius as a stock to prepare a 10 mM concentration of work primer, which was then resuspended at a concentration of 10 pmol/ 1 in 90 ml of free DNase and RNase to produce a final concentration of 10 picmole.

E. Detection of CMV virus by Conventional PCR technique

Primer sequences used in this study for amplification receptor of Cytomegalovirus (ILT2) bp 285 described by Taylor-Wiedeman et al (1991).

Sense primer sequence (3'- TGAGAGTGGTGGGAATGCAC -5) and antisense primer (5-AACCCAACGCAATTTCCAGC-3).

Table 1 outlines the PCR conditions that were used. Following amplification, 8 µl of each product was electrophoresed at 70 V in an agarose gel using TBE buffer and agarose at a concentration of 2%. After being stained with green star 5l, the sample was photographed under ultraviolet light.

Results and Discussion

There were thirty positive samples out of forty serum samples collected from patients who came to our clinic suffering from abortion. These results are recorded in table (1) below, which compares the detection of CMV using the Vidas system with the PCR approach.

Table (1) Comparison of Cytomegalovirus Detection by Vidas system and PCR technique.

Type of method	Number of samples	Number of Positive	Number of negative	Frequency
Vidas system	40	40	0	100%
PCR technique	40	30	10	75%

According to table 2, the findings of the current study revealed that the age range of 20 to 30 years was associated with the highest rates of Cytomegalovirus infection.

Table (2) distribution of CMV infection according to Age

Age	No. positive	No. negative	Frequency
20-30 year	30	10	75%
30-40 year	10	30	25%

Conventional polymerase chain reaction amplification for receptor CMV gene (ILT2) bp 285 was found to be sensitive and specific for CMV detection. The results of the PCR technique performed on the same samples revealed that thirty of the forty serum samples belonging to the same patients' women included the CMV gene's representative 285-bp fragment. These samples were positive for the virus. Gel electrophoresis revealed a positive CMV detection and carried a 285-bp fragment, as demonstrated by these findings, which were reported in Figure (1).

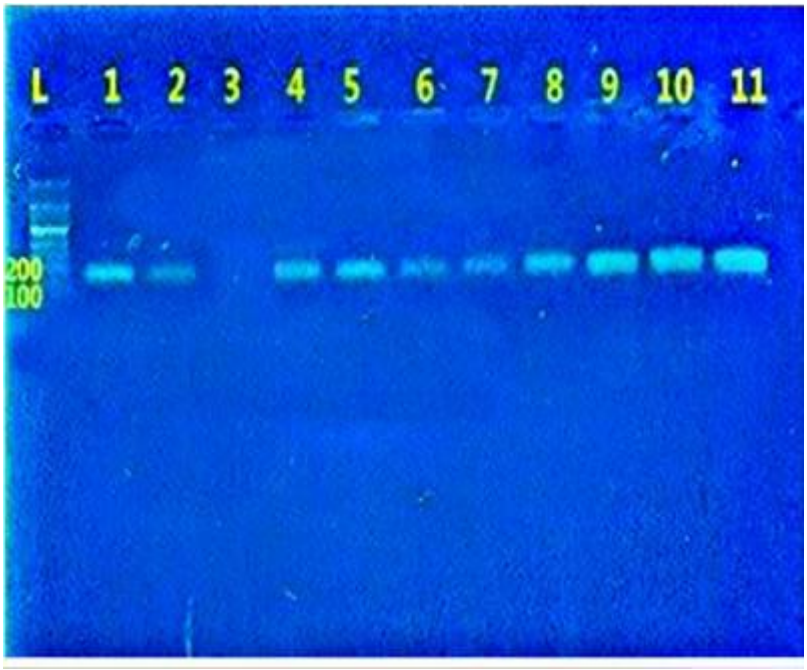

Figure (1) Electrophoresis of PCR products for Cytomegalovirus Receptor gene (ILT2-285bp). Lanes 1-11 revealed to the positive gene (285bp). L 1000, bp DNA ladder . and condition of PCR technique recorded in table (3)

Table 3. PCR condition for CMV diagnosis.

Step	Temperature C°	Time/min.	Cycles
Initial denaturation	94	5	1
Denaturation	94	2	
Annealing	52	1	40
Extension	72	2	
Final extension	72	7	1
Storage	4	∞	

According to the findings of the current research, the investigation of CMV using the Vidas method and the traditional polymerase chain reaction are not the same. In a study that was carried out some time ago, immunoassays such as the VIDAS system were put up against the tried-and-true culture methods, and it was discovered that the VIDAS system had a degree of sensitivity that was marginally lower than that of the other methods.

According to Valiente et al. (2007), this was the very first occasion that the VIDAS system has been put to use with the intention of detecting infections. The Vidas system satisfies the requirements of both first-line laboratories that do regular testing on small series and specialist laboratories that conduct more in-depth analyses of larger samples. Oleksiuk et al. (2014) state that the VIDASTM UP Salmonella (SPT) Assay is noticeably slower in comparison to the PCR Assay. The traditional polymerase chain reaction (PCR), multiplex polymerase chain reaction (PCR), and real time reverse transcription-polymerase chain reaction (RT-qPCR) assays were all part of the molecular methods. Data on the efficiency of techniques based on PCR have also been uncovered by other lines of investigation (Gardênia et al., 2012).

The use of RT-qPCR tests is currently the method of choice in laboratories for the detection of cytomegalovirus. These tests have a high level of sensitivity, allowing them to identify as little as 10 to 100 copies in a single reaction. In addition to being quantitative, RT-qPCR techniques are also able to offer estimations of viral load. The PCR methods have been recommended by numerous studies, both older ones that were conducted many years ago and more contemporary ones that were conducted more recently (Marshall and Bruggink,2006; CDC, 2012). The tests can be used to detect the viruses in various fluids of the body.

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