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Detection of Human Cytomegalovirus among Women with Miscarriage

A thesis

Submitted to the Council of College of Science/ University of Diyala In
Partial Fulfillment of the Requirements for the Degree of Master of
Science in Biology

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2023 AD

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1. Introduction

The most common member of the herpes virus family is the cytomegalovirus (CMV). In affluent nations, human cytomegalovirus (HCMV) is the more frequent cause of congenital deformity brought on by viral intrauterine infection (Ariani and Chaichi, 2014). In the majority of the world, HCMV is endemic. In various geographic regions, the prevalence rate of HCMV ranges from 30 to 100% (Crough and Khanna, 2009). Seroprevalence is more common in elderly people, women, and those from lower socioeconomic groups (Bate *et al.*, 2010).

Viral infection can spread horizontally (via sexual contact or contact with fluids like saliva, maternal vaginal secretions, breast milk, or blood) as well as vertically (transplacentally from mother to fetus) (Parsons *et al.*, 2021). It is also transmissible through stem cells transplantation or solid organ transplantation (Badami *et al.*, 2014).

The human herpes virus (HSV) with the highest genetic content is cytomegalovirus compared to HSV, it has a genomic DNA that is 240 kbp bigger. Only a few of the many proteins encoded by the virus (~200) have been characterized. One, a cell surface glycoprotein, acts as an Fc receptor that can nonspecifically bind the Fc portion of immunoglobulin. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulin's (McSharry *et al.*, 2012).

Because of its extraordinarily wide tissue tropism, HCMV may infect almost all of the body's organ systems (Sinzger *et al.*, 2000). HCMV infection may be either latent (non-productive), lytic (productive), asymptomatic, or symptomatic (Powers *et al.*, 2008)

When a fetus or embryo naturally dies before it can survive on its own, it is known as a spontaneous abortion or pregnancy loss (Hurt *et al.*, 2012). Twenty weeks is the threshold after which fetal death is referred to as a stillbirth. Numerous elements, including infections and genetic disorders, are linked to spontaneous abortion (Atik *et al.*, 2010). The first trimester accounts for around 80% of spontaneous pregnancies lost, and the incidence decreases with each gestation week (Pinar *et al.*, 2018).

One of the many causes of prenatal harm that results in abortion is the cytomegalovirus, also known as human herpes virus type 5 (Manicklal *et al.*, 2015). Infection with CMV during pregnancy could lead to immunodeficiency causing coinfection of bacteria leading to bacterial vaginitis (Mostafa., 2020). Bacterial infections that rise from the lower genitals through the cervix to the uterus, such as chlamydia, gonorrhea, mycoplasma, and bacterial vaginosis (BV), are frequently to blame for infections connected to abortions. If left untreated, the infection may spread to the fallopian tubes and cause infertility (Hay, 2017).

Primary CMV infection occurs in 0.15%-2.00% of all pregnancies and may be transmitted to the fetus in 40% of cases (Altayeb *et al.*, 2016). The congenital CMV infection rate is 0.87% in a population with high maternal seropositivity (Geris *et al.*, 2022). When diagnosing congenital CMV, saliva samples may give false-positive results and urine should be tested. CMV-DNA could still be detected in urine samples for up to two years after birth. In highly seroimmune populations sequelae may occur during follow-up, comprehensive studies are needed on this subject (Chanswangphuwana *et al.*, 2022).

The molecular approach can be helpful for the goal and even for the low degree of pathogenicity since it is crucial to quickly and accurately

diagnose infections before the onset of their consequences (Zaker Bostanabad *et al.*, 2017). The best approach for detecting CMV was determined to be rt-PCR, followed by ELISA and fast testing. All of these methods were shown to be helpful for both diagnosis and therapy (Sofy *et al.*, 2017).

Antiviral medications including ganciclovir (GCV), cidofovir, and foscarnet are used to treat HCMV infection. GCV, a nucleoside analog, is a prodrug that has to be phosphorylated in order to be active (Chou *et al.*, 2013). The viral kinase (*UL97*) performs this phosphorylation (Roy *et al.*, 2019). Numerous investigations supported the discovery of mutations in the *UL97* gene that are linked to resistance, as did the research by (Anja *et al.*, 2018)

Aim of study

This study was suggested to serological and molecular screening for cytomegalovirus among sample women who suffered miscarriage which is done through the following work steps:-

1. Detection of cytomegalovirus by using ELISA method.
2. Molecular identification of CMV by Real time PCR.
3. Molecular identification of the *UL97* gene by PCR technique.
4. Studying and determining the extent of the spread of the virus and its relationship to some risk factors such as age, place of residence, educational attainment, and the presence of infections and vaginal ulcers

Summary

The human cytomegalovirus (HCMV) is considered one of the opportunistic viruses, with a worldwide distribution, that can infect humans at any stage of life. This study aims to serological and molecular diagnostic of HCMV among women with miscarriage and their correlation with socio-demographic characteristics (e.g., age, living, academic achievement, number of abortion, and fetus age) and detection of unique long 97 (*UL97*) gene. One hundred samples included 80 women suffering from abortion which were referred to Al-Batool Teaching Hospital and 20 pregnant women with no history of abortion in the period between November 2021 and January 2022.

Anti-HCMV antibodies IgG and IgM was estimated by Enzyme Linked Immune Sorbent Assay (ELISA). Results showed that the abortion women shown highest percentage of seropositive to CMV for IgG (96.3%) and (40%) IgM with a significant difference as compared to the control group ($P < 0.05$). Anti-CMV IgG and IgM seropositivity was higher in younger women (20-30 years old). The current study found that the age groups (21-30) years had the highest rate of abortions (53.8%) with significant differences ($P < 0.05$). The findings of this study also show that uneducated women, and the women living in rural areas have a higher rate of abortion (58.8% and 72.5%), respectively with significant difference ($P < 0.05$). Furthermore, the current study found a statistically significant relationship between the number of miscarriages and fetal age, with the highest miscarriage rate for fetuses less than 3 months (98.3%).

Molecular detection of HCMV DNA in whole blood sample using Real Time PCR shows the HCMV DNA was detected in 10.0% (8) out of 80 patients were found to be CMV positive. In order to determine the timing of initial infection, the result confirms the relationships between IgG and IgM and real-time PCR for HCMV in aborted women.