Short Communication

Diagnosis of Cytomegalovirus by Real Time Polymerase Chain Reaction in Pregnant Women in Kirkuk City

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Abstract

Background: Cytomegalovirus (CMV) is the most prevalent viral infection in humans, particularly in pregnant women due to decreased immunity. Most accurate laboratory method for the diagnosis of this virus is via RT-PCR to detect vial DNA. **Objectives:** This study attempted to identify the presence of the cytomegalovirus among pregnant women in Kirkuk City. **Materials and Methods:** For the real time polymerase chain reaction (RT-PCR) test, viral DNA was extracted from blood samples of pregnant women in different trimesters and residency from Azadi Teaching Hospital in Kirkuk city. **Results:** Enzyme-linked immuno-sorbent assay testing revealed 128 pregnant women who had the infection, (4.68%) were positive in RT-PCR and majority of them (57.81%) were at their trimester and most of them (78.13%) were from city center. **Conclusions:** RT-PCR is most accurate, specific, and rapid method for diagnosis of viral infection such as cytomegalovirus.

Keywords: Cytomegalovirus, DNA, pregnant women, RT-PCR

Introduction

Cytomegalovirus (CMV) is a double-stranded DNA virus that is a member of the Herpesviridae family (HHV-5). Similar to other herpes viruses, the CMV creates a latent infection that persists for the duration of the person's life after the initial infection.[1] The effects of infection are predominantly seen in larger cells, and the presence of unique intracellular inclusions surrounded by a halo of low reflection gives the appearance of "Owl-eyes."[2] The complicated natural history of CMV infection consists of three different types of infections. When a person contracts this virus for the first time and lacks immunity, they experience primary infection. After that, the virus enters a state of latency from which it may reactivate cause a second infection. The third type of infection is reinfection, which occurs when a person who has once been infected becomes superinfected after coming into contact with an infectious person despite having natural immunity. Any of these three infection subtypes can complicate pregnancy, making CMV the most common congenital infection source.[3] The CMV infection is asymptomatic; the most prevalent clinical sickness among immune-competent persons is infectious mononucleosis, which is characterized by malaise, headache, sore throat, and tiredness. Fever is another common symptom of CMV mononucleosis and can persist for weeks.[4] Fever especially in children must be managed as soon as possible because this may lead to serious complication.[5] The virus has the ability to induce infection in the entire body and has been found in various tissues like, the kidneys, lungs, liver, esophagus, colon, monocytes, T and B lymphocytes.[6]

CMV has the ability to evade the immune system and it continues to be dormant in the body. When the host encounters an immunosuppressive circumstances (such as pregnancy, chemotherapy or immune deficiency condition), the virus can reactivate from latency and

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induce a new proliferation cycle which result in releasing more particles in the system to invade new cells.[7] CMV transmission needs contact with body fluids that contain the virus such as urine, saliva; tears, cervical secretions, semen, and breast milk are all examples of bodily fluids where CMV is shed. The highest rates of CMV transmission occur right after direct contact with bodily fluids.[8] Additionally, blood transfusions and organ and tissue transplants are effective methods of transmission.[9] The most common method of horizontal transmission of a virus from child to child or from child to adult, particularly in group childcare settings, is by saliva on hands and toys.[10]

Infection with CMV happens more commonly in human during pregnancy.[11] Pregnant women encounter insufficient immunity at various stages of pregnancy, which can make it easier for them to contract infections and transmit them to developing fetuses, potentially leading to abortion. CMV and, more recently, covid-19 are the most common causes of this.[12] The humoral immunity plays an important role in limiting viral spread and provides significant protection against CMV transmission, thereby reducing the disease's clinical symptoms; the cellular immune response to CMV is critically dependent on several subsets of T cells.[13] Additionally, women with initial CMV infections had a higher rate of vertical transmission than women with reactivated or recurrent CMV infections. Pregnant women who experience primary CMV infections in the third trimester of their pregnancy are at a larger risk of congenital transmission than those who get infections in the first trimester.[14] In the general population, diagnosis is rare, often because there are no or a few clinical signs.[15]

Laboratory confirmation can be achieved using molecular techniques. Real-time PCR, which has excellent specificity and sensitivity, has become a better method for identifying the molecular responses to diverse infectious illnesses.[16] The primary benefits of RT-PCR are its quick and advanced detection and quantification of target DNA sequences therefore RT-PCR is crucial for the identification, measurement, and characterization of viral pathogens.[17] The aim of this study is to detect CMV infection among pregnant women by RT-PCR that considered more accurate and specific than other technique.

Materials and Methods

Clinical samples collection

Blood samples have been obtained from 180 pregnant women (128 patients—52 pregnant women as a control group) in different trimesters and from viable residents during the period from October 2022 to March 2023 at Azadi teaching hospital. Blood samples (5 mL) were collected in ethylenediaminetetraacetic acid (EDTA) tubes from pregnant women.

Statistical analysis

Statistical analysis was performed using GraphPad Prism statistical software.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 48206 (including the number and the date in November 1, 2022) to get this approval.

Results

The current study involves detection of Cytomegalovirus in pregnant women using RT-PCR. Table 1 revealed that the 180 participants (128 infected women) were spread out throughout different regions (78.13% were located in urban areas, whereas 21.88% were in rural areas), and the majority of them (57.81%) were in the third trimester, whereas 11.72% and 30.47% were in the first and second trimesters, respectively as shown in Table 2. Furthermore, Table 3 reveals that (4.68%) was tested positive by RT-PCR.

Discussion

CMV infection in most case is asymptomatic in pregnant women, which makes it detection difficult clinically.

Table 1: Residency of patients and control group

Residency	Patient %	Control %
Urban	78.13	71.15
Rural	21.88	28.85

Table 2: Pregnancy trimester percentage in patients and control group

Gestational stage	Patient %	Control %
1st trimester	11.72	11.54
2nd trimester	30.47	34.62
3rd trimester	57.81	53.85

Table 3: RT-PCR result

PCR data	Patient %	Control %	*P* value
PCR+	4.68	0	0.1953
PCR -	-	95.31	100.00

Whenever they do appear, the symptoms are typically nonspecific and mimic a mononucleosis- or flu-like syndrome with fever, cervical lymphadenopathy, sore throat, lethargy, and myalgia. Lymphocytosis and increased liver enzyme levels may be detected in the laboratory. Compared to women with recurring infections or reactivations, pregnant women with initial infections are more likely to experience clinical symptoms.[18] Higher rates of symptomatic congenital infection and fetal loss are associated with primary CMV infection during pregnancy. Primary infection is indicated by the presence of IgM antibodies.[19] Because infections during the first trimester disrupt organogenesis and infections during the second or third trimesters may also result in neurologic disease, CMV infection is frequently dangerous to the fetus.[20] Women at any stage of pregnancy are at risk of intrauterine transmission.[21] However different investigation indicated that women who were in their third trimester of pregnancy had a significant prevalence of CMV infection.[22] Our findings agree with previous research that found higher CMV infection in pregnant women throughout the third trimester of pregnancy.[23,24] In contrast to our data, other researchers reported that the highest prevalence rate of CMV were noticed in pregnant woman during second trimester and lowest prevalence was recorded during the third trimester.[4,25]

On the other hand, some researchers observed that the number of persons infected with CMV in urban area is greater than in rural areas which agrees with our study.[26,27] In on the contrary, other study found that CMV prevalence is great in rural areas.[1,28]

The conflictions in the data observed could reflect the population size included in the study as well as to the biased selection of patients and inclusion as well as exclusion criterion. Infection with CMV is more common in pregnant women, and the risk increases with age.[20]

Regarding CMV diagnosis via RT-PCR, Dina *et al.* recorded CMV positive DNA in (6.7%) of women assessed with RT-PCR which is close to our result.[29] On the contrary, Ali *et al*. reported CMV DNA in about (12.9%) which is higher than our data.[16] Real-time PCR allowed for the detection of the amplified product using fluorescent dyes. Usually, oligonucleotide probes that accurately bind to the amplified product during thermal cycling are connected to these colors. Without having to reopen the reaction tubes after the PCR cycle, accumulating products can be detected using real-time monitoring of fluorescence intensities during real-time PCR.[30] PCR technique mainly realized on amplification of specific target of DNA these provide a sensitive and precise detection of the amplified region with minimum error or contamination in addition RT-PCR advantage on conventional technique is the lack of post amplification analysis which is a necessity in conventional PCR. In addition the time for complete run with RT-PCR is minimum compared to conventional PCR. The major advantages of these technologies include the reduction of contamination and the ability to perform quantitative applications.[31] The simultaneous detection and identification of several samples are possible using real-time PCR.[16]

Nucleic acid amplification has become a frequently used CMV diagnostic technique. One of the most effective techniques for diagnosing CMV is PCR because it is highly accurate and precise in detecting the presence of CMV DNA. When compared to individuals with asymptomatic and/or reactivated CMV infection, patients with initial infection have significantly higher CMV DNA levels during infection.[32-35]

Conclusion

Diagnosis of viral infection such as cytomegalovirus must performed by technique such as RT-PCR because it is most accurate, specific, and rapid than other tests.

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Conflicts of interest

There are no conflicts of interest

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