JBAAR

Journal of Bioscience and Applied Research

https://jbaar.journals.ekb.eg

SPBH-IC: Biomedical pISSN: 2636-4077, eISSN: 2636-4085

SPBH

Testing for Cytomegalovirus (CMV) Infection in Patients with Previous Miscarriage: A Prospective Observational Study

Mohey Eldin Hassan Shikhoun [1]*, Omar Ali Mohmed Farghaly [2] Hatem Aboelftooh Awaga3

[1] Analysis and Laboratories Department, Higher Technological Institute of Applied Health Sciences in Sohag, Ministry of Higher Education, Cairo, Egypt

[2] Biochemistry and Molecular Biology, Faculty of Pharmacy, Al-Azhar University, Asyut Branch, Egypt

3 Obstetrics and Gynecology Department, Faculty of Medicine, Sohag University, Egypt

Corresponding author:** ***M.H. Shikhoun, Analysis and Laboratories Department, Higher Technological Institute of Applied Health Sciences in Sohag, Ministry of Higher Education, Cairo, Egypt, Tel: 01119025409.

Email: moheyshikhoun@gmail.com

DOI:10.21608/jbaar.2024.398061

ABSTRACT

Background: One of the opportunistic viruses is cytomegalovirus (CMV), which is widely distributed and can infect individuals at any stage of life. It can result in significant pregnancy complications and is a primary cause of both perinatal and prenatal infections. **Objective:** This study suggested the necessity of conducting a CMV-IgG test and confirming the results with reverse transcriptase polymerase chain reaction (RT-PCR) technology for pregnant women to avoid miscarriage. **Methodology:** Eighty women who had miscarried were chosen. In addition to the chemical tests, including blood count tests and lupus anticoagulant level assessment, the CMV-IgG test was conducted, and the infection was confirmed by RT-PCR test. **Result:** Our study revealed that 52 (65%) of the 80 women who had miscarried did not have CMV infection. The remaining 28 (35%) cases of miscarried women were found to be infected with CMV using the ELIZA technique to detect CMV-IgG antibodies. The infection was confirmed by RT-PCR for CMV DNA. The results showed a high statistically significant relationship between the levels of white blood cells and the Lupus anticoagulant, as the percentage of white blood cells was lower in aborted women infected with CMV than in aborted women who were not infected with the virus. Also, the level of Lupus anticoagulant increased in aborted women who were not infected with CMV compared to those infected with the virus.

Keywords: Cytomegalovirus, Abortion, Lupus anticoagulant

Introduction

An extremely common herpes virus is the human cytomegalovirus (HCMV). The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) report that HCMV affects individuals of all ages, with about 33% of American kids infected by the time they are five years old **[1].**

Viral infection can spread both vertically (from mother to fetus) and horizontally (via sexual contact or contact with fluids like saliva, breast milk, mother's vaginal secretions, or blood) **[2]**. The prevalence rate of HCMV varies geographically, ranging from 30% to 100% **[3]**.

Blood transfusions, organ transplants, and sexual interaction can all result in the transmission of HCMV infections to an individual during pregnancy, infancy, or maturity **[4]**. Symptomatic, asymptomatic, lytic (productive), and latent (non-

Received: September 1, 2024. Accepted: October 25, 2024. Published: December 19, 2024

productive) are the four likely HCMV infection stages [5].

Cytomegalovirus, commonly referred to as the human herpes virus type 5, is one of the numerous causes of prenatal damage that leads to miscarriage [6]. A spontaneous abortion, sometimes referred to as a pregnancy loss, occurs when a fetus or embryo passes away naturally before it can survive on its own. About 80% of spontaneous pregnancies end in miscarriage during the first trimester, and the rate decreases with each further week of gestation [7].

The search for an alternative laboratory test that might be utilized to detect primary CMV infection with high specificity and sensitivity was prompted by the unsatisfactory results for CMV IgM. Low CMV IgG avidity serves as a specific and sensitive indicator of primary CMV infection, according to an assessment of CMV IgG avidity [8]. It is becoming more widely accepted that CMV IgG avidity is the "gold standard" for differentiating between main and non-primary CMV infection [9].

Materials and Method

Eighty women who had miscarried in the first months of pregnancy were tested to study the extent of the spread of CMV and examine the effect of the virus on blood cells and the Lupus anticoagulant test. Samples were collected from the Obstetrics and Gynecology Department at the Royal Polyclinic in Sohag Governorate from January 2021 to February 2023.

Blood samples: Collection and processing

The infection control precautions were taken while taking samples from all cases. The samples were collected in tubes containing the anticoagulant EDTA to study the level of blood cells and sodium citrate 4% to examine the level of Lupus anticoagulant. The study included three varied groups of patients:

The first group: was 30 normal women (non-pregnant and non-aborted) who were not infected with CMV.

The second group: was 52 women who had miscarriages in the first months of pregnancy and were not infected with CMV.

The third group: was 28 women who had a miscarriage in the first months of pregnancy and were infected with CMV.

Serological assay:

Detection of anti-CMV-IgG: The cases of the three groups in the present study were screened for anti-CMV antibodies IgG using the CMV IgG ELISA Test Kit, which is an enzyme immunoassay designed for the qualitative detection of IgG antibodies to CMV in human serum or plasma. This test is designed to help screen and diagnose the potential infection of CMV (ATLASE MEDICAL CMV ELIZA TEST KIT).

The microwell plate was pre-coated with CMV antigens. In the testing process, the specimens and diluents were introduced to the antigen-coated microwell plate. After that, they were incubated. Should the specimens possess IgG antibodies to CMV, they tend to bend to the pre-coated antigens on the plate, resulting in the formation of immobilized antigen-CMV IgG antibody complexes. In the absence of IgG antibodies against CMV in the specimens, the complexes would fail to form. After the initial incubation, the microwell plate underwent washing to eliminate unbound substances. Enzyme-conjugated anti-human IgG antibodies were introduced to the microwell plate and subsequently incubated. The enzyme-conjugated anti-human IgG antibodies would attach to the immobilized antigen-CMV IgG antibody complexes present. After the second incubation, the microwell plate was rinsed to eliminate unattached substances. Substrate A and substrate B were combined and subsequently incubated to yield a blue tint, signifying the concentration of CMV IgG antibodies in the sample. A sulfuric acid solution was introduced to the microwell plate to terminate the reaction, resulting in a color transition from yellow to blue. The color intensity, indicative of the

quantity of CMV IgG antibodies in the specimens, was quantified using a microplate reader at 450/630-700 nm or 450 nm.

Lupus anticoagulant estimation

Lupus anticoagulant (LA) was estimated following the guidelines of the International Society on Thrombosis and Haemostasis, (ISTH) [10]. Then, LA was screened using the Kaolin Cephalin Clotting Time (KCCT) testing in addition to the dilute Russell's viper venom time (DRVVT) test.

Hematological assessment:

This was done by determining the complete blood count (CBC) with the help of the automated hematology analyzer (DIRUI BCC-3000B).

Molecular assay

RNA isolation and RT-PCR: The reverse transcriptase polymerase chain reaction (RT-PCR) was utilized to test the existing viral genome in the serum by making use of the Amplicon method (Basel, Switzerland). First, the extraction of the viral DNA was carried out by the viral DNA minutes kit according to the manufacturing instructions following the spin column protocol (Qiagen, Hilden, and Germany). After that, it was initially denatured at 95°C for five minutes. Then, the polymerase chain reaction was amplified at 94°C for one minute, 56°C (annealing temperature) for one minute, and 72°C for one minute, totaling 40 cycles with the final extension at 72°C for seven minutes. We used these primer sequences:

forward primer was 5`-ACTTTGCCGATGTAACGTTTCTTG-3`

and reverse primer was 5`-CGGGTCATCTACGGGGACAC-3`.

Electrophoresis agarose gel detection: Detecting the products of the PCR-CMV was carried out by 1.5% agarose gel electrophoresis dyed with ethidium bromide and spotted under the reactions of UV light. Two systems of treatment were used.

Statistical analysis

SPSS version 21 was used to code and enter the data, which were then summarized in the form of means, standard deviations, and medians, maximum and minimum concerning quantitative variables, as well as frequencies (number of cases) and relative frequencies (percentages) regarding categorical variables. The nonparametric Mann-Whitney U test was employed to make group comparisons when drawing comparisons between two groups. In addition, chi-square or Fisher's exact test was utilized to make group comparisons when needed. The calculation of the odds ratios and their 95% confidence intervals was carried out, and the p-value ≤ 0.05 was set as a level of statistical significance.

Results

Eighty women who had miscarried in the first months of pregnancy were tested to study the extent of the spread of CMV and to examine the effect of the virus on blood cells and its effect on the Lupus anticoagulant test. Our study showed that 28 (35%) out of 80 cases of miscarried women were infected with CMV by detecting CMV-IgG antibodies by the ELIZA technique. The infection was established by RT-PCR for CMV DNA. It was found that 52 (65%) of the 80 aborted women were not infected with CMV, as confirmed by RT-PCR for CMV DNA. **(Table: 1)** (**Figure: 1)**

Thirty normal women (non-pregnant, non-aborted) were selected and confirmed to be free of infection with CMV by detecting CMV-IgG antibodies using the ELIZA technique. This result was demonstrated by RT-PCR for CMV DNA.

The study included three groups:

The first group: was 30 normal women (non-pregnant and non-aborted) who were not infected with cytomegalovirus.

The second group: was 52 women who had miscarriages in the first months of pregnancy and were not infected with CMV.

The third group: was 28 women who had a miscarriage in the first months of pregnancy and were infected with CMV.

Table 1: showing the rate of infection with the CMV virus among aborted women

No. of patients	Percent (%)	ELISA results CMV Ab. IgG	PCR results CMV RNA
52	65%	Negative	Negative
28	35%	Positive	Positive

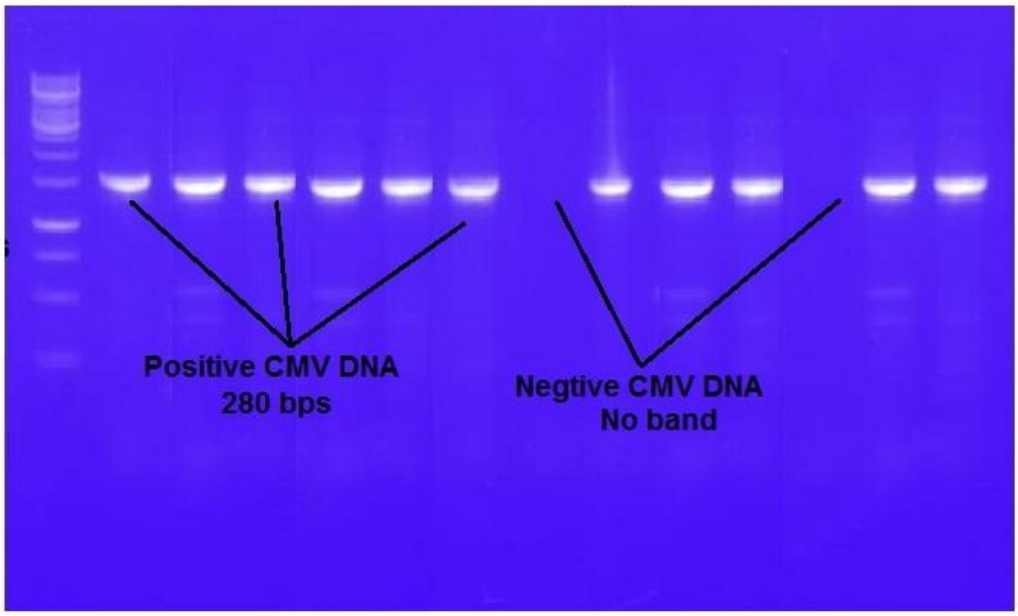

Figure 1: Electrophoresis pattern of some samples, performed for qualitative analysis. (a) Lanes M contain markers, 1 - 6 contain bands that are CMV positive, while lanes 7 and 11 are negative control.

According to our research, there are statistically significant differences (P. Value >= 0.005) in lupus anticoagulant levels and white blood cells (WBCs). Specifically, women who have experienced a miscarriage and are free from CMV had higher levels of lupus anticoagulant than normal women. In contrast, no statistically significant differences (P. Value < 0.005) were noted in hemoglobin (Hgb.) levels, red blood cells (RBCs), and platelets (PLTs) among women who have had a miscarriage and were free from CMV and normal women **(Table: 2 & Figure 2)**

Our results showed that there were high statistical significances (P. Value <= 0.005) in the level of white blood cells (WBCs) (the percentage of white blood cells decreases (leukopenia) in aborted women infected with CMV. In contrast, there were no statistical significances (P. Value > 0.005) at

hemoglobin (Hgb.), RBCs, PLTs, and the Lupus anticoagulant level. **(Table: 3 & Figure 3)**

Our results showed a high statistical significance (P. Value <= 0.005) in the level of white blood cells and the level of Lupus anticoagulant as the percentage of white blood cells was lower in aborted women infected with CMV than in aborted women who were not infected with CMV. Moreover, the level of Lupus anticoagulant increased in aborted women who were not infected with CMV compared to those infected with CMV. The results revealed the lack of statistical significance (P. Value < 0.005) in the levels of Hgb, RBCs, and PLTs among both aborted women with and without CMV. **(Table: 4 & Figure 4)**

Table 2: Compares the results of biochemical testing between normal women and abortion women not infected with CMV.

Biochemical test		**Normal Women**	**Abortion Women Free CMV**	**T. Value**	**P. Value**	**Sig.**
Hgb (g/dl)	Mean ±SD	11.96±0.73	11.51±0.66	2.75	0.010	**N.S**
RBCS (10^6/UL)	Mean ±SD	4.78 ± 0.44	4.66 ± 0.43	0.97	0.342	**N.S**
WBCS (10^3/UL)	Mean ±SD	8.04 ±1.47	10.62±0.89	7.89	0.000	**H.S**
PLTS (10^3/UL)	Mean ±SD	286.83±74.37	243.10±65.10	2.078	0.011	**N.S**
Lupus anticoagulant (Sec./Sec.)	Mean ±SD	37.95 ± 1.94	42.40 ± 5.28	4.240	0.000	**H.S**
N.S: Non-Significant **S:** Significant **H.S:** Highly Significant						

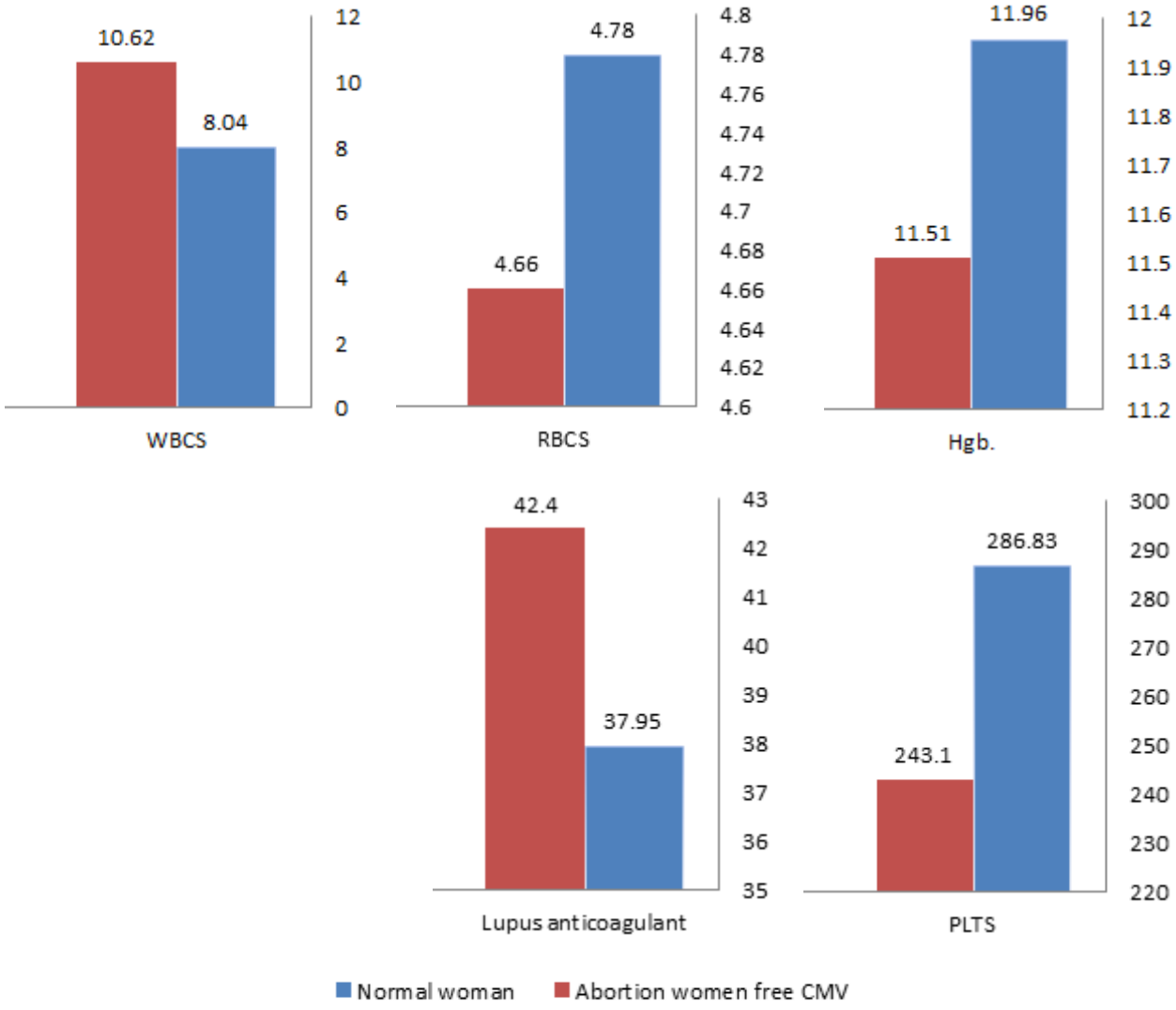

Figure (2): Explains the difference in biochemical testing between normal women and abortion women not infected with CMV.

Table 3: Compares the results of biochemical testing between normal women and abortion women infected with CMV.

Biochemical test		**Normal Women**	**Abortion Women with CMV**	**T. Value**	**P. Value**	**Sig.**
Hgb (g/dl)	Mean ±SD	11.96 ± 0.73	11.71 ± 0.53	1.404	0.172	**N.S**
RBCS (10^6/UL)	Mean ±SD	4.78 ± 0.44	4.76 ± 0.34	0.473	0.640	**N.S**
WBCS (10^3/UL)	Mean ±SD	8.04 ± 1.47	4.93 ± 0.82	9.58	0.000	**H.S**
PLTS (10^3/UL)	Mean ±SD	286.83 ± 74.37	238.81 ± 65.48	2.49	0.019	**N.S**
Lupus anticoagulant (Sec./Sec.)	Mean ±SD	37.95 ± 1.94	37.68 ± 2.45	0.614	0.544	**N.S**
N.S: Non-Significant **S:** Significant **H.S:** Highly Significant						

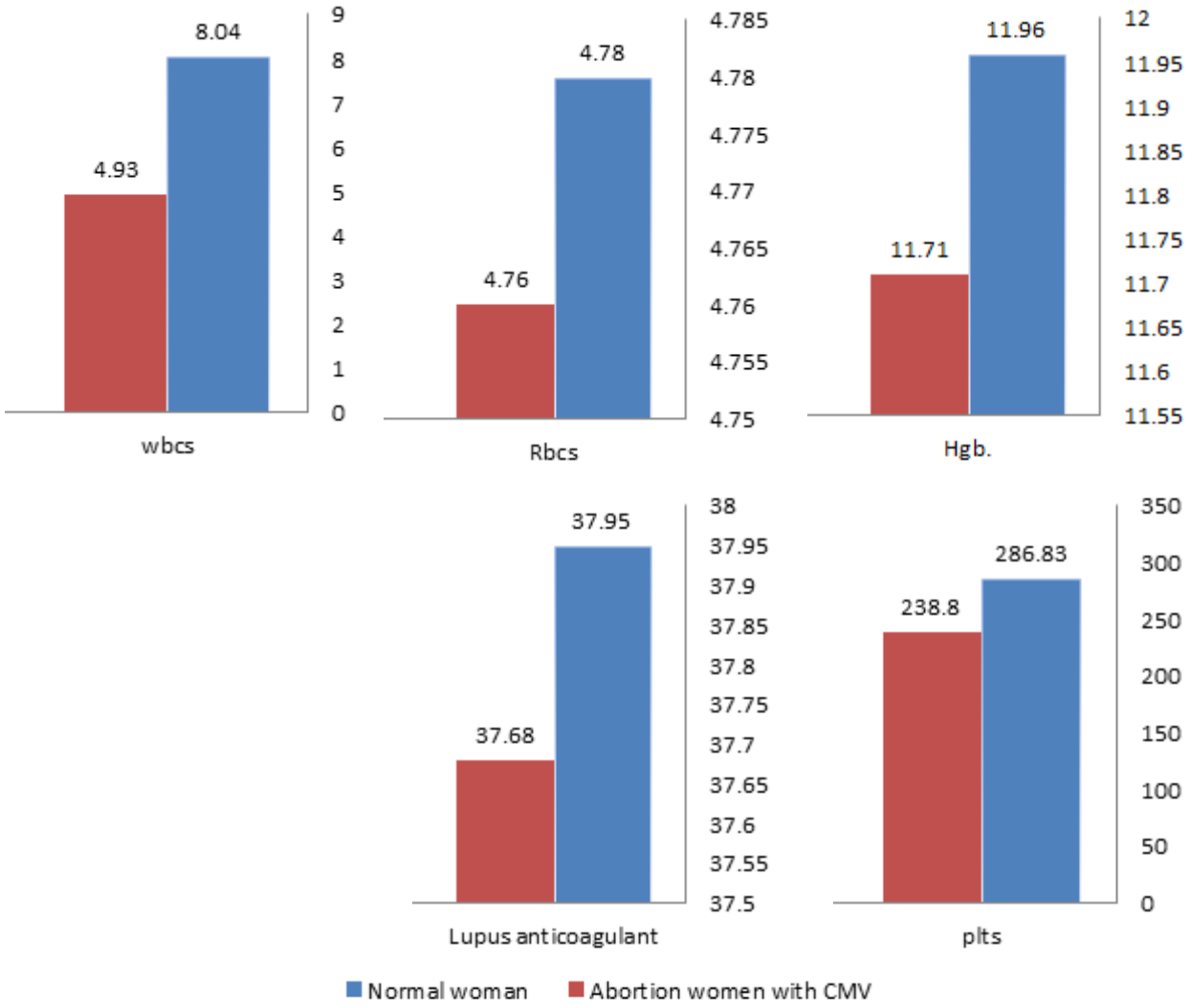

Figure (3): Explains a difference in biochemical testing between normal women and abortion women infected with CMV.

Table 4: Compares the results of biochemical testing between abortion women not infected with CMV and abortion women infected with CMV.

Biochemical test		**Abortion Women Free CMV**	**Abortion Women with CMV**	**T. Value**	**P. Value**	**Sig.**
HGB (g/dl)	Mean ±SD	11.51±0.66	11.71 ± 0.53	-0.74	0.467	**N.S**
RBCS (10^6/UL)	Mean ±SD	4.66 ± 0.43	4.76 ± 0.34	-1.39	0.177	**N.S**
WBCS (10^3/UL)	Mean ±SD	10.62±0.89	4.93 ± 0.82	23.94	0.000	**H.S**
PLTS (10^3/UL)	Mean ±SD	243.10±65.10	238.81 ± 65.48	0.454	0.654	**N.S**
Lupus anticoagulant (Sec./Sec.)	Mean ±SD	42.40 ± 5.28	37.68 ± 2.45	4.424	0.000	**H.S**
N.S: Non-Significant **S:** Significant **H.S:** Highly Significant						

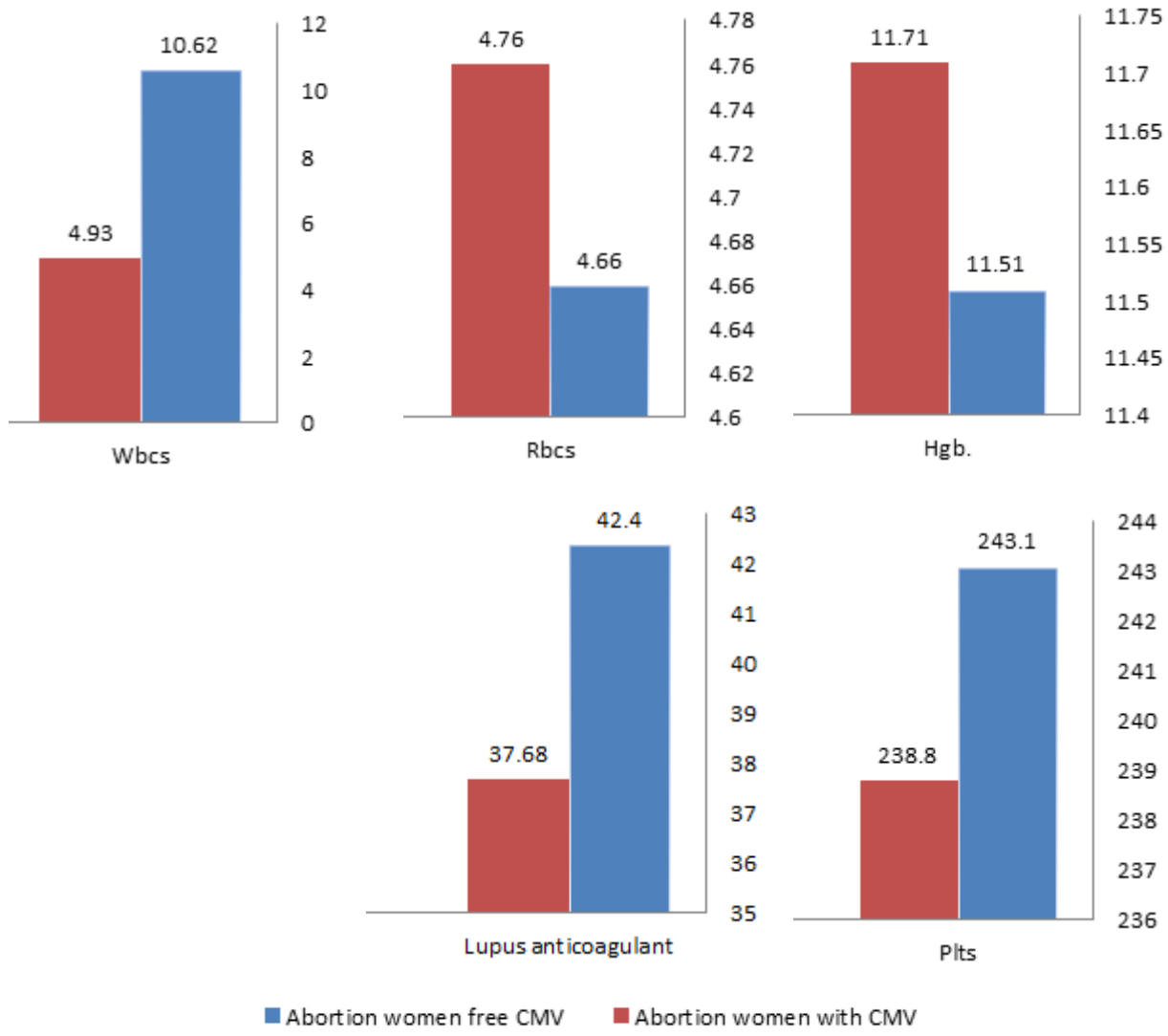

Figure (4): Explains the difference in biochemical testing between normal women and abortion women not infected with CMV.

Discussion

In general, variations in the sample, study design, and geographic location may be connected to variations in the incidence of HCMV infections in the aforementioned research. Age distribution of the women in each piece of research may have contributed to the infection. For instance, Jerman et al. [11] examined the traits of American women who had abortions between 2008 and 2014. Our study showed that 28 (35%) out of 80 cases of miscarried women were infected with CMV by detecting CMV-IgG antibodies using the ELIZA technique. Confirming the infection depended on RT-PCR for CMV DNA, and 52 (65%) of the 80 aborted women were not infected with CMV-IgG.

The incidence of miscarriages was markedly higher in the thirty-six to forty-five-year-old age group compared to those aged twenty to thirty. The IgG titer was elevated in individuals over thirty, indicating that CMV infection was typically latent with the potential for reactivation, consistent with the literature [12].

According to our research, there were statistically significant differences in hemoglobin and lupus

anticoagulant levels. Specifically, women who have experienced a miscarriage and are free of the CMV IgG had higher levels of lupus anticoagulant than did pregnant women in the first few months of their pregnancy. There were no statistically significant differences in the level of RBCs, WBCs, and platelets among women who have had a miscarriage and were free of the CMV IgG, and among pregnant women in the first months.

Human CMV is serious because it results in miscarriage and, in more cases, congenital damage, fetal death, and mental retardation. Congenital CMV is the most frequent intrauterine infection **[12].**

The majority of researchers agreed that LA and anticardiolipin (aCL), the two best-characterized antiphospholipid antibodies, were closely related to recurrent miscarriage **[13].** Our results showed that there was a high statistical significance in the level of white blood cells, as the percentage of white blood cells was lower in aborted women infected with CMV-IgG than in aborted women who were not infected with CMV-IgG. In contrast, there were statistical significances in Lupus anticoagulant, as it increased in aborted women who were not infected with CMV-IgG compared to those infected with CMV-IgG. Our study showed that there was no statistical significance in the levels of HGB, RBCs, and PLTs among both aborted women with and without CMV-IgG.

Congenital HCMV transmission rates reached 50.0% in cases infected with HCMV in pregnancy but were below 2.0% in those without a primary HCMV infection **[14]**. Although several causes result in miscarriage, more than 50.0% of cases are still idiopathic. Most cases of miscarriage happen in the first trimester of pregnancy, accounting for roughly 80.0% of unplanned fetal deaths, resulting from manifestations like bleeding and discomfort that increase maternal worry **[15].**

Leukopenia and anemia are brought on by CMV because the virus suppresses immune system function, particularly in pregnant women **[16]**. Our findings demonstrated the lack of statistically significant differences in the level of lupus anticoagulant among pregnant women and aborted women infected with CMV-IgG. Still, there were significant differences in the percentages of HGB, RBCs, and WBCs among these groups. The percentages of HGB and RBCs increased, while the percentage of WBCs decreased (leukopenia) in aborted women infected with CMV-IgG.

Conclusions:

Thirty-five percent of miscarriages are caused by cytomegalovirus (CMV), which can inhibit the immune system and produce leukopenia.

Ethics approval and consent to participate

Approval was obtained from the Ethics Board of the Faculty of Medicine, Sohag University for the study. All patients signed an informed consent before participating in the study.

Approval number of ethics committee: OHRP#(IRB00013006)

Conflict of interest; NIL

Funding: NIL

References

1- **Al Mana H, Yassine HM, Younes NN, Al-Mohannadi A, Al-Sadeq DW, Alhababi D, et al. (2019):** The Current Status of Cytomegalovirus (CMV) Prevalence in the MENA region: a systematic review. Pathogens., 8 (4):213-236.

2- **Crough T, Khanna R (2009):** Immunobiology of human cytomegalovirus: from bench to bedside. Clin. Microbiol. Rev., 22: 76- 98.

3- **Parsons K, Osterholm E, Hernandez-Alvarado N *et al.* (2021):** Investigating CMV Pathogenesis and Breast Milk Transmission In Premature Infants Who Acquire Symptomatic CMV Viremia. Journal of the Pediatric Infectious Diseases Society, 10: S10.

4- **Badami K (2014):** CMV and transfusions, an old story that's not quite over yet. International

Journal of Clinical Transfusion Medicine, 2: 7–19.

5- **Powers C, De Filippis V, Malouli D *et al.* (2008):** Cytomegalovirus immune evasion. Current Topics in Microbiology and Immunology, 325: 333– 359.

6- **Manicklal S, Emery V, Lazzarotto T *et al.* (2013):** The "Silent" global burden of congenital cytomegalovirus. Clin. Microbiol. Rev., 26: 86-102.

7- **Pinar M, Gibbins K, He M *et al* (2018):** Early pregnancy losses: review of nomenclature, histopathology, and possible etiologies. Fetal and pediatric pathology, 37 (3): 191-209

8- **Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. (2013):** The "Silent" Global Burden of Congenital Cytomegalovirus. ASM journals; 26(1):86-102.

9- **BaAlawi F, Robertson PW, Lahra M, Rawlinson WD. (2012):** Comparison of five CMV IgM immunoassays with CMV IgG avidity for diagnosis of primary CMV infection. Pathology; 44(4):381–3.

10- **Bertolaccini M, Gomez S, Pareja JF, Theodoridou A, Sanna G, Hughes GR, Khamashta MA. (2005):** Antiphospholipid antibody tests: spreading the net. Ann Rheum Dis, 64: 1639-43.

11- **Jerman J, Jones RK, Onda T. (2016):** Characteristics of US abortion patients in 2014 and changes since 2008, a report. 1-26.

12- **Ablfors K, Ivarrson S, Harris (2001):** Secondary Maternal Cytomegalovirus Infection-A Significant Cause of Congenital Disease. Pediatrics 107: 1227- 1228.

13- **Petri M, Rheinschmidt M, Whiting-O'keefe Q, Hellman D, Corash L. (1987):** The frequency of lupus anticoagulant systemic lupus erythematosus. A study of 60 consecutive patients by activated partial thromboplastin time, Russell viper venom time and anticardiolipin antibody level. Arthritis Rheum, 30: 601-6.

14- **Kagan KO, Hamprecht K. (2017):** Cytomegalovirus infection in pregnancy. Arch Gynecol Obstet. 2017;296(1):15–26. https://doi.org/10.1002/bdra.23601.

15- **Atik RB, Hepworth-Jones BE DP. (2010):** Early pregnancy, chapter 2, Risk factors for miscarriage. Cambridge University Press,2010: 9-18.

16- **Zhong XY, Ma TY (1993):** Aclinical study of cytomegalovirus infections during prograte. J Tongji Med Univ 13: 60-64.