ARTICLE / INVESTIGACIÓN

Serological detection of Cytomegalovirus in blood samples from infected women in Misan province, Iraq

Hasan S. H. Alrashedi[1]*, Hind J. Hassoon[2]

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[1] Department of Biology, College of Sciences, University of Misan, Misan, Iraq.
[2] Department of Medical Laboratory Technology, College of Health and Medical Technology, Middle Technical University, Baghdad, Iraq.
Corresponding author: hasan.salam@uomisan.edu.iq

Abstract: Cytomegalovirus is a species of Herpesviridae can that infect humans and cause latent and severe infections. Severe human infection caused by Human Cytomegalovirus (HCMV) usually occurs in humans who suffer from immunodeficiency. This research aims to detect antibodies IgM as a primary infection and IgG as a secondary infection in women suffering from repeated abortion and determines the more ages susceptible to HCMV infection. Blood sera were taken from females of different ages, from 15-45 years, based on clinical manifestation and abortion. The sample sizes in this study were taken from 150 patients, of which 100 cases were positive for HCMV. We recorded a high percentage of CMV infection (25%) and a high abortion rate (27.5%) in patients at age 20-24 years with a high rate of IgG, and we noticed a low rate of CMV infection (10%) in the age group 35-39 years with a low rate of IgG, we also reported a high abortion rate (27.27%) related with present IgM. The percentage of abortion was (26.66%) reported in patients ages 15-19 and 40-45 years, with (a 33.33%) rate of IgM and IgG.

Key words: Human Cytomegalovirus HCMV, abortion, IgM, IgG.

Introduction

Human Cytomegalovirus (HCMV) refers to the Herpesviridae family, subfamily Betaherpesvirinae. HCMV can cause severe human diseases, especially in immune-compromised patients and children. The CMV infections in adult women cause abortion, while in children can lead to hearing loss[1]. The virus can be transmitted horizontally and vertically, by direct contact, from the mother to her fetus during pregnancy, or through breastfeeding[2–4].

Furthermore, it was reported that the virus transmits sexually three and via blood transfusion four from one infected person to another. HCMV virion is round in shape with 150 to 200 nm diameter and surrounded by a lipid bilayer envelope[5]. The viral genome composes of double-strand DNA linear with a size reaching 236 KB[6]. The genome consists of two segments (units), long unit (UL) and short unit (US), containing around 150 open reading frames (ORFs) that encode 158 proteins, 47 essential and 117 nonessential proteins[7].

The virus infects the human body by attaching to the target cell via viral glycoprotein that is prolonged from the viral envelope[5]. HCMV uses some tegument proteins to invade the immune system of humans, including intrinsic, innate, and adaptive immune systems[8,9]. The virus sheds in body fluids such as saliva, blood, milk, tears and urine and causes asymptomatic infection[8,10]. On another side, the virus causes symptomatic infections in people suffering from weak immunity or immunodeficiency[4].

Detection of IgM and IgG are used for virus identification widely. Testing antibodies in blood samples is highly sensitive and straightforward for viral diagnosis. It was reported that the IgM antibody is the first line of defense, and IgG has a significant role in long-term immunity[11]. IgM can be detected in the case of SARS coronavirus infection after 3-6 days of infection; IgG can also be seen after eight days[12,13]. Therefore, detecting antibodies is one of the effective methods for testing samples from patients suspected of virus infection.

Materials and methods

Collection of blood sera

One hundred samples were obtained from women attending to Obstetrics gynecology Out-patients department in Al-Sadder Hospital and Central Health laboratory in Misan city. Blood sera were taken from women suffering from CMV infection and abortion, fifty women were chosen as a healthy control group, and their ages ranged from 15-45 years. The sera were separated and stored at -20 °C for HCMV antibodies estimation using the Minividas technique.

Detection of Cytomegalovirus

The sera samples were tested using VIDAS (CMVG), and BioMerieux and the procedure were carried out according to the manufacturer's instructions. The serum samples were placed in a ten-pits strip. The pits were: 1: Sample well (serum) contains antigen, Empty well: 2, 3, 4, 5, Conjugate wells: 6, Wash buffer well: 7, 8, 9: Substrate wells (SPR): 10 contains anti-antibody. SPR sucks out the serum in well 1, and then the antigen interacts with the antibody in well 6. During this process, SPR sucks the washing buffer from one of the 7, 8, and 9 pits and return it to empty wells for

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antigen-antibody conjugation, as this process repeats several time. Next, the unreacted cognates aspirate and return to the empty well. Then, SPR interacts with fluorinated material in the last well, where the enzyme is attached to the substrate to produce a flash (phosphorescent light). Finally, the Mini VIDAS (BioMerieux) device records and analyses the results according to the phosphorescent light produced.

Statistical analysis

The collected data were analyzed using SPSS 26 software.

Results

Effect of CMV infection on fetal cases

Infection with CMV significantly impacts fetus status during and at the end of the pregnancy period in women. In this study, we noticed that the high percentage of fetal death was 70%, while the low rate was 2.4% for abnormal birth. It was recorded that 27.5% of fetuses were normal, as shown in Figure 1. This variation in fetus status is due to many factors, such as congenital CMV that affect fetus growth, high CMV viral load due to low immunity or changes in normal flora count in the uterus and vagina. Many women use contraceptive drugs. These findings agree with the study carried out by (14) as they recorded a high percentage of abortion at 21 weeks and premature fetus cases in women infected with CMV. Therefore, from the figure shown below, it is clear that the infection of CMV does not entirely affect the fetus, and some other factors may be correlated with the normality or abnormality of fetus delivery.

Relation between age groups and CMV infections

This study was based on variables to identify the relationship between age groups and women infected with CMV. The sample size was taken from 125 patients, and 100 cases were positive for CMV. We found that the high percentage of CMV infection was at age 20-24 years (25%), and the low rate of CMV infection was at age 35-39 years (10%), as shown in Figure 2. This might be because the account of normal flora in the uterus and vagina of infected women with HCMV is changed due to the estrogen hormone that affects the immune system and the uterus environment. These results are similar to the finding done in the United States by (15). The authors reported a high rate of CMV infections in women aged between 20 and 24. Therefore the age of the women has a significant role in resistance to CMV infection.

Relation between education levels and CMV infections

Infection with CMV in humans can also depend on the patient's education level factor. In this study, it was found that the high rate of CMV infection, 45%, was in primarily educated women, while the low rate of CMV infection, 15%, was in university-educated women, as shown in Figure 3. This could be because of a lack of health awareness compared to women with higher education levels who care for their health, including preventing pathogens and biological vectors. However, these findings disagreed with a report showing that CMV infection was high in secondary educated women level and low rate of CMV infection in women with primarily educated women[16].

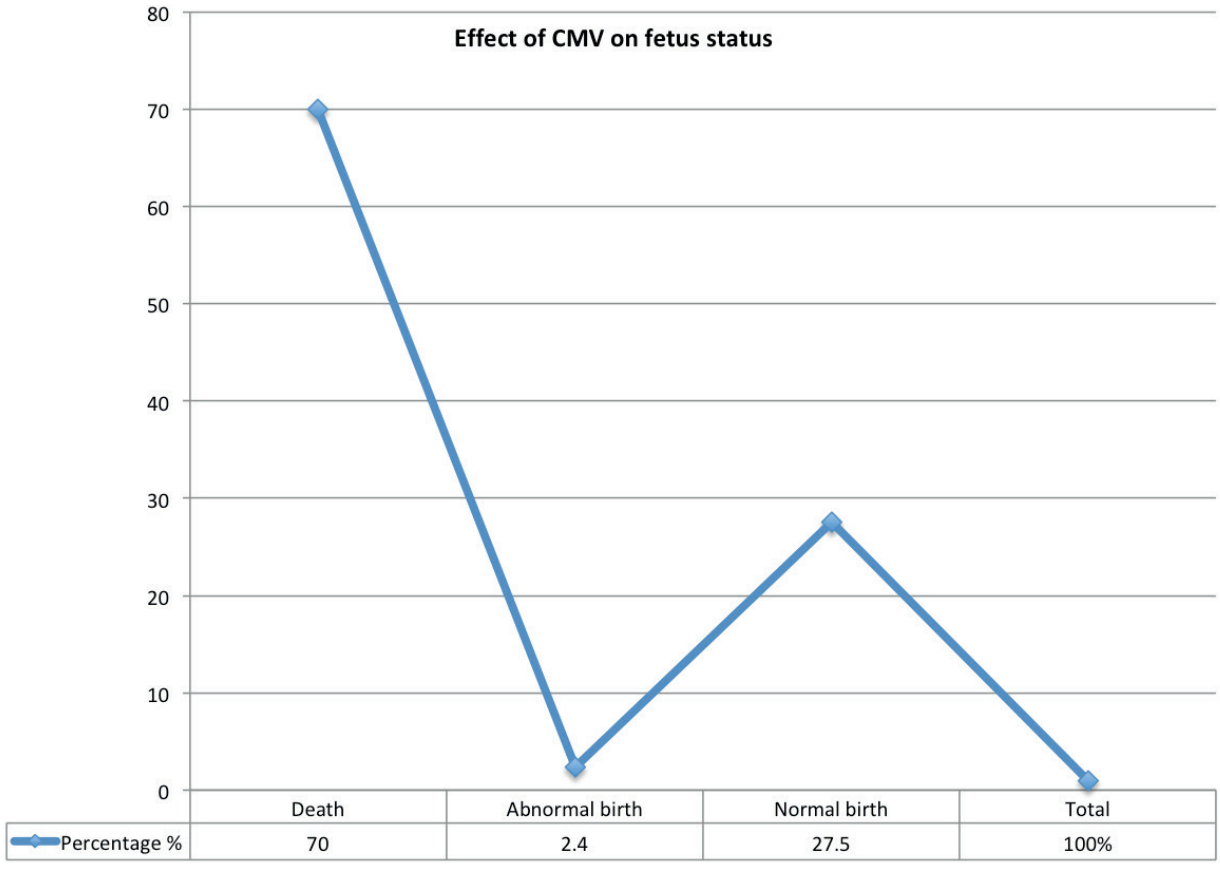
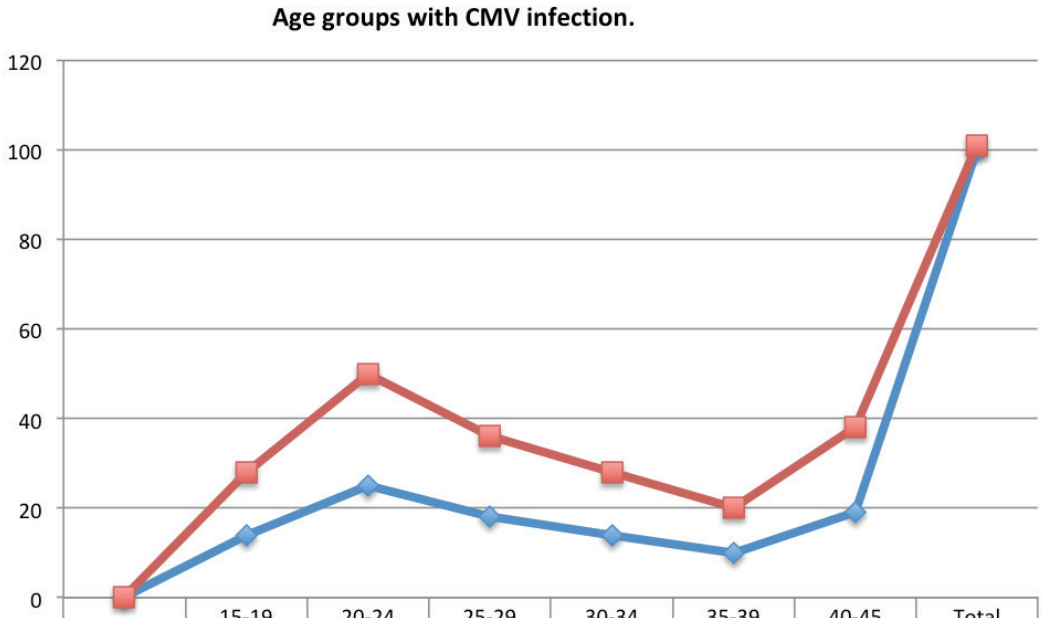
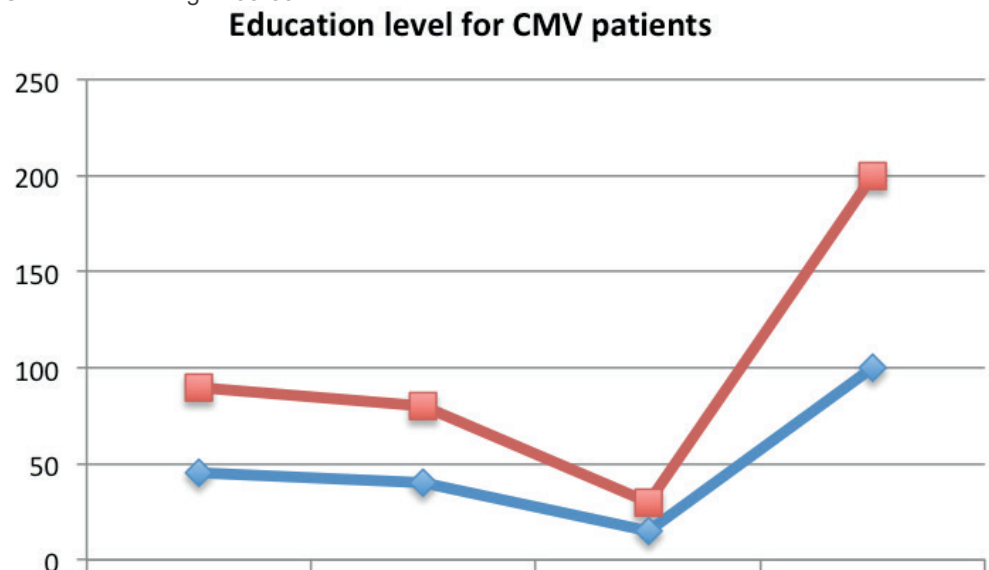


Figure 1. A percentage of cases of birth. The figure shows a high rate of fetal death cases, around a quarter percentage of normal birth, and a low rate of abnormal birth.



Age group	15-19	20-24	25-29	30-34	35-39	40-45	Total
Percentage %	14	25	18	14	10	19	100%
Number of cases	14	25	18	14	10	19	100

Figure 2. Age groups of women infected with CMV. There is a high rate of CMV infection in women aged between 20-24 years and a low rate of CMV in women aged 35-39.



	Primary	Secondary	Higher	Total
Percentage %	45	40	15	100
Number of cases	45	40	15	100

Figure 3. The effect of education level in CMV patients. There is a high percentage of CMV infection in women with a low education level compared to infected women at the university level.

Relation between Immunoglobulin (IgM, IgG) and age of infected women

The levels of immunoglobulin IgM and IgG vary during HCMV infections. In this present study, we detected Immunoglobulin IgM in women that suffer from acute infection. While IgG in women with reactivation or past infection, we also detected IgM and IgG in reactivation or recent infection. In addition, we found that the high rate of IgG was 26 (26.96 %), IgM was 1(20)%, and both IgG and IgM were 2(33.33%), as shown in Table 1. These IgG and IgM rate variations could happen because of many factors, such as changing hormones and infection in reproductive age groups. These findings agreed with other results carried out by (17), in which they reported a high level of IgG. Contrary to these results, a low IgG rate was revealed in women infected with CMV in the Kurdistan region of Iraq[18].

Immunoglobulin and age-related abortion in women

In this study, as shown in Table 2, we found that the high rate of abortion was 27.5 % in the age group 20-40 years associated with IgG 24 (26.9%); it was noticed that the low rate of abortion was 11.1% at age group 35-39 with

Age groups	IgG	Percentage %	IgM	Percentage %	IgM & IgG	Percentage%
15-19	11	12.35	1	20	2	33.33
20-24	24	26.96	1	20	0	0
25-29	16	17.97	1	20	1	16.66
30-34	13	14.60	0	0	1	16.66
35-39	9	10.11	1	20	0	0
40-45	16	17.97	1	20	2	33.33
Total	89	100	5	100	6	100

Table 1. Relation between immunoglobulin and the age group.

a lower rate of IgG 9 (10.1%). In the case of the level of IgM, it was recorded a high rate of abortion in the age group 25-29 and 35-39 years old with 1 (20%)IgM level, while there was no abortion occurred in the case of 0 IgM level at age 15-19. Moreover, the results show that the high rate of abortion was 26.66% in the age group 15-19 and 40-45 years old, corresponding to 2 (33.3%) levels of IgM and IgG that similar to results carried by (19), whereas no abortion in case of 0 IgG and IgM level at age 20-24 and 35-39 years. There is a positive correlation between IgG, IgM and abortion in women infected with CMV.

Relation between IgG, IgM and miscarriage

Miscarriage can cause by HCMV infection. In this study, we recorded a high rate of IgG 89(89%) and 90% miscarriage, while the rate of IgG and IgM were 6(6%) and miscarriage was 5.76%, as shown in Figure.4. Therefore, it is clear that there is a high rate of a miscarriage occurring in women associated with a high level of IgG. These findings are similar to the result revealed by (20). Therefore, there is a high level of IgG measured in women who suffered from miscarriage caused by HCMV.

Conclusions

Human Cytomegalovirus (HCMV) causes abortion in women. In this study, we found that the infections with CMV were different according to age groups, and level of the education of the infected women. We found 25% of CMV infection that is the highest rate in an age group ranging from 20-24 years, this is could be because of the women were in reproductive age, changing hormones, contraceptive and more susceptible to CMV. The relation between IgM is equal in all age groups as found that it was 1(20%) as a primary immune response and IgG as a secondary immune response was 24(26.9%), with age group 20-24 years and 40-45 years. Finally, the total IgM and IgG 2(33.33)% at age 15-19 and 40-45 years respectively, In addition, the percentage of abortion was 4(26.66)% at the same age groups. Therefore, CMV infection in women can lead to abortion and changing fetus status, and further investigation about HCMV in women are recommended.

Age	IgG	Abortion	IgM	Abortion	IgG & IgM	Abortion
15-19	11(12.3 %)	9.8 %	1(20 %)	9.09 %	2(33.3 %)	26.66 %
20-24	24(26.9 %)	27.5 %	1(20%)	18.18%	0	0
25-29	16(17.9%)	20.9%	1(20%)	27.27%	1(16.6%)	20%
30-34	13(14.6%)	16.2%	0	0	1(16.6%)	13.33%
35-39	9(10.1%)	11.1%	1(20%)	27.27%	0	0
40-45	16(17.9%)	14.5%	1(20%)	18,18%	2(33.3%)	26.66%
Total	100%	100%	100%	100%	100%	100%

Table 2. Immunoglobulin and age-related with abortion in women.

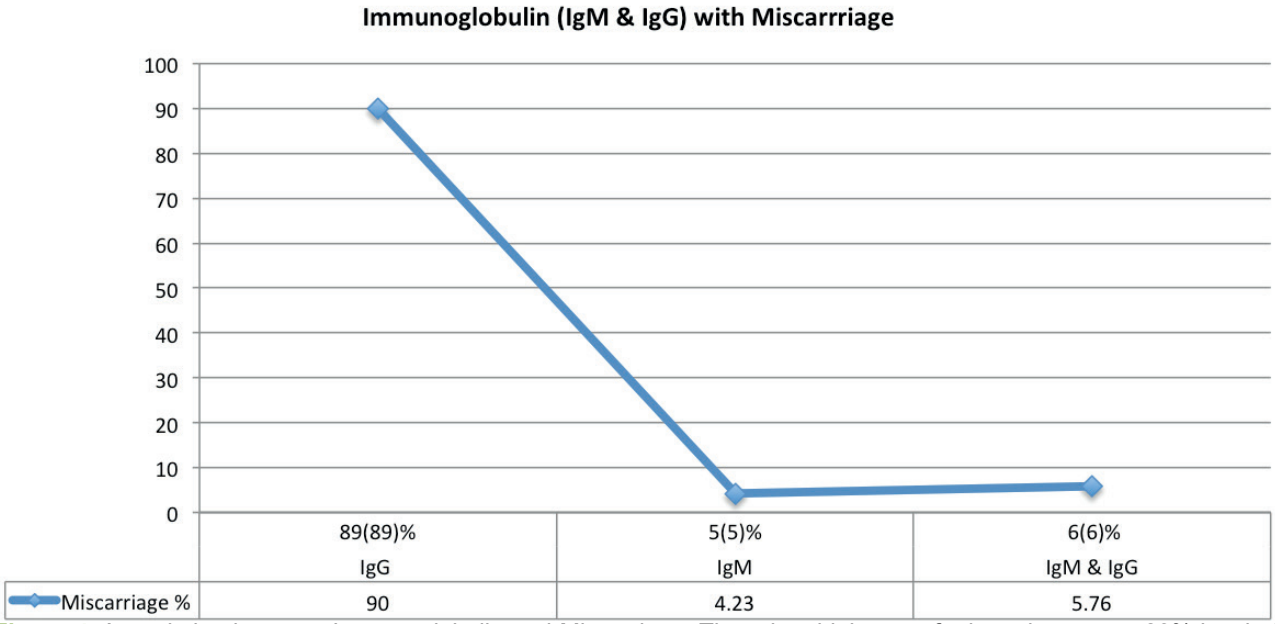


Figure 4. Association between Immunoglobulin and Miscarriage. There is a high rate of miscarriage at an 89% level of IgG, while a low rate of miscarriage at a 5% level of IgM.

Author Contributions

HSHA writing—original draft preparation, editing and project administration. HJH review and validation.

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Conflicts of Interest

The authors declare no conflict of interest.

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