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## Estimation of some immunological biomarkers in aborted women infected with human cytomegalovirus (HCMV) in Salah Al-deen province

### ABSTRACT:

**Background** Human Cytomegalovirus (CMV) is the most common cause of congenital malformation resulting from viral intrauterine infection in developed countries. Congenital HCMV infection causes severe morbidity and mortality in newborns and is the infectious cause of deafness, vision loss, and mental retardation and neurodevelopmental abnormalities in children. **The aim** of this study was to estimate of some immunological parameters in aborted women infected with Human Cytomegalovirus (HCMV) in Salah Al-deen province.

**Pateints & Methods:** This study was carried out on 128 pregnant women with abortion at 15-45 years who attended Tikrit teaching hospital in Salah Al-deen province. Five ml of blood were drawn from each pregnant woman for separation of the sera. The ELISA test was used for estimating the levels of CMV-IgM and CMV-IgG antibodies in patient's serum.

**The Results** The present study revealed that 21 out of 128 (16.40%) aborted women had antibodies against CMV (either IgM or IgG), while 83.59% of women were without known cause of abortion. The aborted pregnant women with CMV infection was 21 (16.40%) out of 128 pregnant women with abortion, while, it was 107(83.59%) aborted women had other causes of abortion, meantime the major of pregnant women with abortion were positive for CMV-IgG which reached 71.42%, whereas the percentage of CMV-IgM positive was 28.57%. The most infected age groups with CMV infection was 25 – 34 years old, which represented 12 (57.14%), case. The most infected patients from rural area was 71.42%, while in urban was 28.57%. The most pregnant women with CMV infection were had previous abortions within first trimester (less than 12 weeks of gestational age) was 55.5% case. **The conclusion** of this study was the seropositive of pregnant women with CMV high at 25-34 years age from rural area especially at first trimester.

### Keywords:

HCMV,  
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## **Introduction:**

Human cytomegalovirus (HCMV) is a human Beta herpes virus, which is an enveloped double-stranded linear DNA virus that like other members of the herpes virus family, establishes lifelong latency following primary infection results in a persistent or latent infection that can be found within various tissues, peripheral blood mononuclear cells (PBMCs), and endothelial cells [1, 2, 3]. Furthermore, compared to other human herpes viruses, CMV is the largest, with a genome of ~235 kbp encoding ~165 genes [4]. The CMV is highly species-specific and only human strains are known to produce human disease, the virus is transmitted horizontally, vertically and via infected blood transfusions, saliva, sexual contact, placental transfer, breastfeeding, in addition to organ and hematopoietic stem cell transplantation [5].

Human Cytomegalovirus (CMV) is the most common cause of congenital malformation resulting from viral intrauterine infection in developed countries [6, 7]. Congenital HCMV infection causes severe

morbidity and mortality in newborns and is the infectious cause of deafness, vision loss, and mental retardation and neurodevelopmental abnormalities in children [8, 9]. The frequency of congenital HCMV infection resulting from primary maternal infection contracted during pregnancy or from the reactivation of HCMV in a seropositive mother during pregnancy is about 0.64% of live births; however, the incidence can vary among different study populations [10].

The antigens or antibodies in body fluids against CMV are typically produced in response to an infection and can be detected by using techniques such as direct immune fluorescence assays or enzyme immune assays (ELISA) [19]. The presence of CMV-specific Immunoglobulin M (IgM) is a very sensitive marker for primary infection, can be detected for many months following primary infection, and may be produced following reinfection or reactivation [20]. In addition, Igmx<sub>d</sub> M may not be indicative of primary infection, since it is also produced during reactivation and reinfection

[21]. The use of IgG testing has been shown to be useful for distinguishing primary and non-primary CMV infections [22], since the most seropositive patients show high IgG levels in the first serum sample collected for testing [20].

## **Materials and Methods:**

### **1. Patients:**

The study population was 128 pregnant women with abortion, with aged between 15 - 45 years old. A structured interview using a standard maternal questionnaire were included: age, parity, gynecologic and medical history of abortion and residence. The clinical examination and laboratory investigations were carried out for the study subjects to exclude other causes of fetal loss, such as hypertension, diabetes mellitus, syphilis, Rh (rhesus) incompatibility and physical causes of abortion.

### **2. Immunological Assays:**

The ELISA technique was performed using kits for detection of CMV-IgM and CMV-IgG. The kits were brought from Sigma Diagnostics (USA), the techniques were performed

according to the manufacturer's instructions.

### **Principle of the assay**

Purified CMV antigen is coated on the surface of micro wells. Diluted patient serum is added to the wells, and the CMV IgG specific-antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of CMV IgG-specific antibody in the sample. A micro well reader compared in a parallel manner with calibrators and controls reads the results.

### **Results:**

The current study was performed to detect of HCMV IgG and IgM antibodies in 128 pregnant women with abortion including, 21 out of 128 (16.40%) cases were seropositive, while 107 (83.59%) had negative serotype as shown in Figure 1.

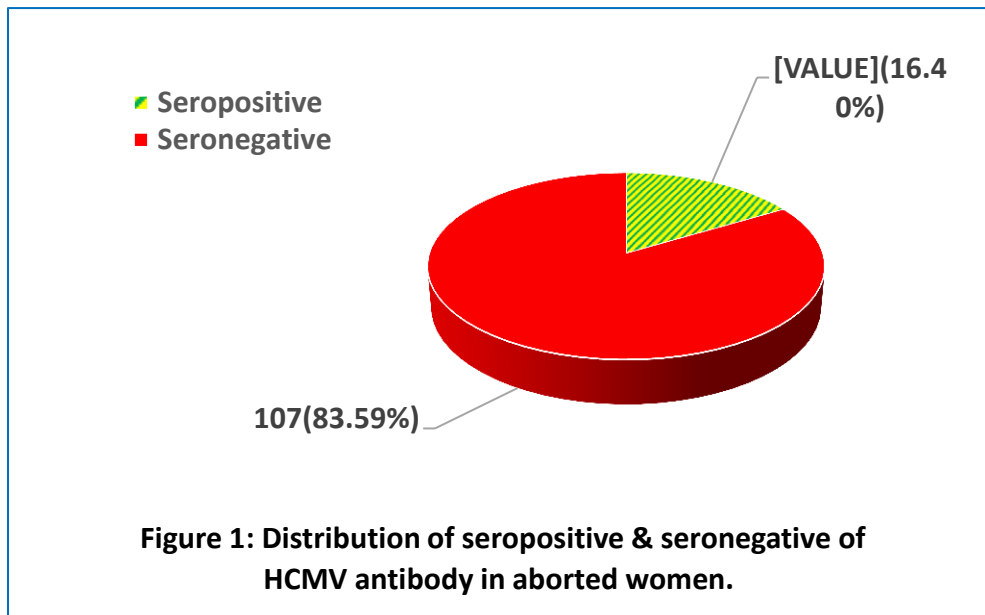


Table (1) shows the serological status of pregnant women including the frequency of IgG Ab 15 (71.42%) case out of 21 of seropositive HCMV and IgM Ab 6 (28.57%) case out of 21 seropositive.

The distribution of pregnant women with abortion caused by CMV according to age and the most affected age group was between 25 – 34 years

which presented 12 (57.14%) cases, then between 15 – 24 years which presented 6 (28.57%) cases and the last group between 35 – 44 years was 3 (14.28%) case as shown in table 2.

The most infected pregnant women with abortion had CMV infection was in rural area presented 15 (71.42%) while in urban was 6 (28.57%) as illustrated in Figure 2.

**Table 1: HCMV serological status in pregnant women with abortion.**

Seropositive	No.	%
IgG Ab	15	71.42
IgM Ab	6	28.57
Seronegative	107	83.59

**Table 2: Distribution of pregnant women with abortion due to CMV infection at different age categories.**

Age group (yr)	No.	%
15 – 24	6	28.57
25 – 34	12	57.14
35 – 44	3	14.28
<b>Total No.</b>	<b>21</b>	<b>100</b>

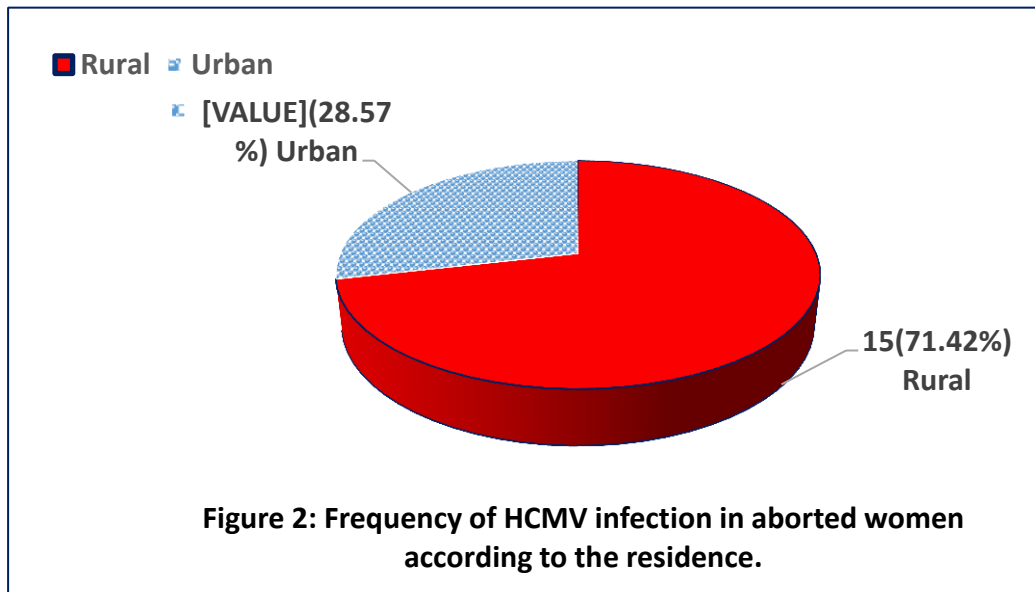
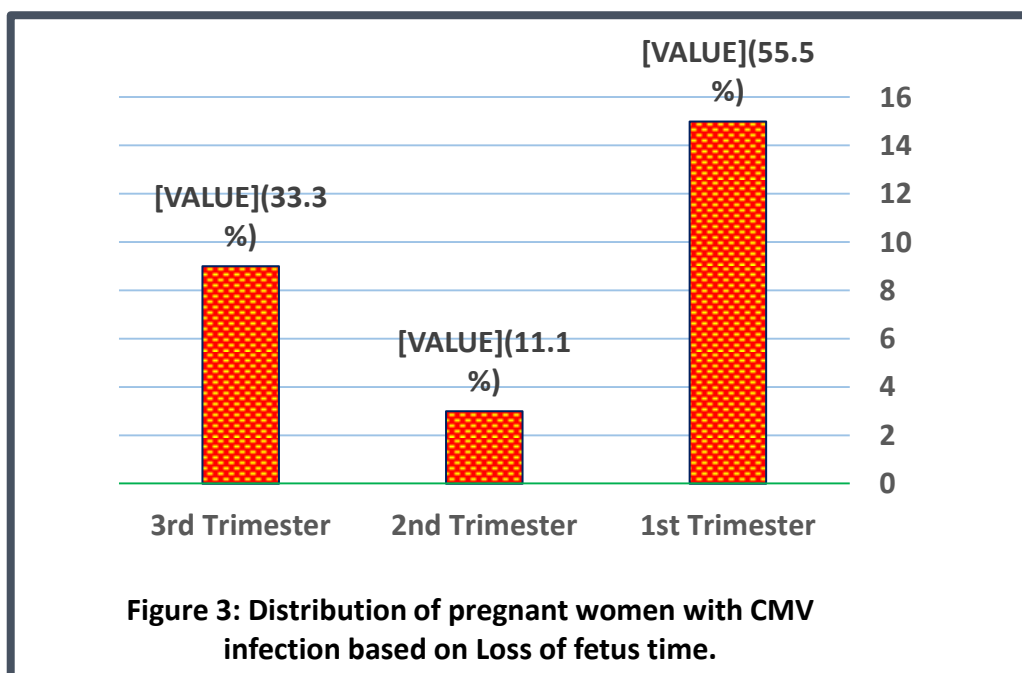


Figure 3 shows frequency of pregnant women with CMV infection according to abortion's time. The highest rate of infection was at first trimester 15 (55.55%), then was in the third trimester 9 (11.11%) but the low rate was in second trimester 3 (33.33%).



## Discussion:

In the present study, we tried to determine the percentage rates of both CMV-IgG and IgM seropositivity among pregnant women who had abortion. This study showed 21 (16.40%) out of 128 cases were seropositive, while 107 (83.59%) had negative serotype or had other causes of abortion.

Results of this study were agreed with Salih [23] in 2013 in Sulaimani who mentioned that the majority of tested women were positive for IgG 90.2%; the percentage of CMV-IgM positive without IgG was 9.18 %. Also, agreed with study was done in Mosul, that noticed that the rates of CMV-IgG and IgM seropositivity among pregnant women were 90% and 2.5%, respectively [24]. In addition to that other study was performed in Baghdad at 2014 which detected the percentage of IgG was higher than IgM presented as 85% and 10% respectively among aborted women [25]. CMV- IgG seroprevalence between pregnant women was 92.8%

and IgM positivity was 5.8% in pregnant women [18].

Also other studies showed the rate of CMV- IgG was higher than CMV- IgM like in Iran in which 93% of the cases were seropositive for HCMV - IgG and 5.4% cases were seropositive for HCMV - IgM. Another study in Iran reported that the percentage for CMV- IgG was 94% and 5.2% women were positive for CMV- IgM [26, 27].

In the present study and other studies show a high seroprevalence of CMV- IgG antibodies, may be attributed to the previous exposure of the pregnant women and now they have immune against CMV, especially when they have IgM negative, these women as mentioned can considered immune and their primary infection with CMV was assumed to have been taken place before the current pregnancy.

However, the preconception immunity against CMV provide incomplete protection against intrauterine transmission, and adverse outcomes can occur in infected children born to women who were

seropositive prior to pregnancy [28, 29]. Previous immunization with CMV is not perfectly protective against either reinfection or vertical transmission of infection from mother to fetus [30, 31]. Approximately one third of the seroimmune women were noted to have CMV reinfection during follow up [32]. Other literature mentioned that the incidence of congenital CMV infection increases with increasing maternal CMV seroprevalence. Transplacental transmission of CMV in women with preexisting seroimmunity may be a secondary to virus reactivation [33] or to infection with a new different CMV strain (reinfection) during pregnancy [32].

Table 2 shows distribution of aborted women according to age the most affected age group was 25 – 34 years presented with 12 (57.14%) cases, while age group between 15 – 24 years was 6 (28.57%) cases. The lowest affected age was between 35 – 44 years presented 3 (14.28%) cases. These results agreed with the results in Al-Kufa [34], which showed higher percentage of positivity at age group

27- 32 years, also other study, showed 94% of positivity at age 25-34 years [35]. As well as Abdolreza [27] who mentioned the average age was  $25.6 \pm 7.6$  years in aborted women. Arabpour [26] reported the age group between 20 – 24 years more affected about 55% and 25 – 26 years was 30%.

In compared with other study showed the CMV- IgG seropositivity was 95.6% in women with age of < 20 years, and then declined in women with age. CMV- IgM seroprevalence was lowest in women with 30-39 years 3.3%, and highest 4.6% in women with age of 14 – 29 years [18]. Also other reported the age 41 - 45 years old may be regarded as the most age class which showed high prevalence of anti-HCMV antibodies which represent 100% IgG for and 11% for IgM, while the youngest age group 16 - 20 years old showed the least prevalence of anti-HCMV- IgG antibodies 75%, but the highest for IgM 17%. This may be referred to many factors such as multiple pregnancies, abortion, physiological and hormonal changes and increase

possibility of virus accessibility with increasing age [25].

The high prevalence of aborted women with high seropositivity of CMV in rural area 71.42% than urban area 28.57%. This result was agreed with study was carried out in Kirkuk [18] which mentioned women living in rural areas demonstrate 92% seropositivity for CMV- IgG, while in urban area the seropositivity was 90.9%. The result of present study was disagreed with observations reported by other researchers in Sulaimani [23] that reported CMV- IgG and IgM seropositivity among tested women according to their residence area. From urban residency, 91% were positive for CMV- IgG antibodies and 8% were positive for CMV- IgM antibodies. From rural residency, 89% were positive for CMV- IgG antibodies and 11% were positive for CMV-IgM antibodies. In addition, the present study agreed with Arabpour [26], which mentioned HCMV seroprevalence rate was higher in women from rural as compared to those of urban areas. The residence showed similar effects, although

hypothesis for the probable role of geographical influence on HCMV seroprevalance might be the route of infection. In rural areas, saliva is probably the main route through which the virus is transmitted postnatally. This is likely to be the route through which the virus is transmitted early in life amongst infants and young children due to poor sanitation [36]. On the other hand, in urban areas sexual transmission seems to be the major route of infection later in life during child-bearing age.

The present study showed the most aborted women with CMV seropositive had bad previous obstetric including spontaneous abortion and missed abortion especially within first trimester was 55.55% of cases, then in the third trimester including preterm labor or stillbirth or congenital abnormalities babies was 11.11% of cases. The lowest miscarriage pregnancy in women with CMV seropositivity was 33.3% of cases.

In other study mentioned in case of recurrent abortion, the rate of IgM seropositive was 20% [25]. As well as other study showed that aborted



women suffered from habitual abortion (more than three times), show a higher percentage of IgM seropositive compared to those had less than three times [37]. In addition, Yasir reported high prevalence of IgM at age 21 – 26 years in recurrent abortions and falls [34].

In study was done in Kirkuk [18] that reported CMV- IgM seroprevalence among women was 4.1% and it was vary significantly between pregnant represent 5.8% and in non-pregnant was 2.2% women. Although, CMV- IgM seroprevalence was lower rate in women with bad obstetric history 3.8% as compared to women with normal pregnancy was 4.5%, but the difference was not significant, in addition, the bad obstetric history in women with CMV infection was 60.2% in Waset, Iraq [38].

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