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RESEARCH ARTICLE

RELATIONSHIP BETWEEN HUMAN CYTOMEGALOVIRUS INFECTION AND RECURRENT PREGNANCY LOSS

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Abstract

Background and aims: Pregnancy loss and human cytomegalovirus (CMV) infection have been linked in several studies. It is unclear, therefore, if recurrent pregnancy loss (RPL) is linked to latent or recurrent CMV infection or a changed immunological response to CMV. We compared women with RPL to healthy controls to assess CMV infection and the level (titer) of CMV antibodies.
Materials and Methods: In this comparative study, 149 women with recurrent miscarriage, referred to gynecology clinics in Sana'a, Yemen, and 149 multiparous women of the same age who had never had a miscarriage served as controls. Enzyme-linked immunosorbent assay (ELISA) was used to assess IgG and IgM antibodies to cytomegalovirus (CMV), as well as IgG levels in patients and controls. Data were analyzed using chi-square and Student's t-tests.
Results: The study found that the majority of patients with recurrent miscarriage were in the 20-24 age group (34.9%), followed by the 15-19 age group (25.5%) and the 30+ age group (22.1%). The prevalence of CMV IgM indicating current CMV infection was 5.3% in RPL cases, with the highest prevalence in cases aged ≥30 years (6.1%), followed by 20-24 years (5.8%) and 15-19 years (5.2%). The control group had a 3.4% positive IgM rate. No significant difference in IgG-CMV antibody prevalence between patients and controls, with a crude prevalence of 97.98% in the patient group and 97.3% in healthy controls. Patients aged ≥ 30 had a lower prevalence of IgG (93.9%). The study found significant differences in IgM antibody levels between patients and controls. Patients had higher IgM antibodies than controls; also, CMV-IgM-positive cases had higher IgM antibodies.
Conclusions: According to the current study's findings, RPL had a somewhat greater level of prior CMV exposure than controls, as shown by positive IgG antibodies. Nonetheless, we discovered a statistically significant correlation between elevated IgG levels and RPL, with patients exhibiting higher antibody levels than healthy controls. We can therefore conclude that hyper-response (greater IgG titers) to a comparable number of CMV exposures may be another risk factor, and that repeated exposure to CMV is a risk factor for RPL.
Keywords: Cytomegalovirus, infection, recurrent pregnancy loss (RPL), Sana'a, Yemen.

INTRODUCTION

A member of the *Herpesvirida* family, human cytomegalovirus (CMV) is highly prevalent world-wide, with rate of seropositivity ranging from 40% in industrialized nations to 100% in impoverished nations[1-4]. Although there are many different clinical presentations, immunocompetent hosts rarely experience symptomatic illness. Severe situations can arise in immunocompromised hosts as well as in critically ill immunocompetent hosts[5-7]. Over the past few decades, research has been conducted on the pathophysiology and epidemiology of CMV infection during pregnancy. Congenital CMV is the most common cause of congenital viral infections and can be caused by primary infection or reactivation of previously acquired infection during pregnancy. Up to 90% of children who survive will experience consequences include hearing loss, vision impairment, and various levels of mental retardation[4,8]. Results have been conflicting, the underlying mechanisms are unknown, and it is still up for debate whether a

pregnant woman's initial infection or reactivation of CMV might result in pregnancy loss[9,10]. Abortion tissues were shown to have substantial levels of CMV antigens in some investigations[11], whereas other studies indicated higher seropositivity[1,2,12]. Although other prospective studies' findings did not support it, one study also indicated a greater risk of pregnancy loss with CMV infection[13]. It is unclear how CMV infection contributes to repeated losses, despite recent evidence on its involvement in spontaneous pregnancy loss. One of the most distressing and challenging areas of reproductive medicine is recurrent pregnancy loss (RPL), which is typically defined as three or more consecutive abortions before the 22nd gestational week. While spontaneous pregnancy loss affects 15% of pregnant women, two and three consecutive abortions occur in 2% and 0.4–1% of pregnant women, respectively. There are currently few evidence-based diagnoses and therapy methods for RPL, and its pathogenesis is still unknown. Anatomical, immunological, genetic, endocrine, infectious, thrombophilia, and environmental variables are proposed as etiologic factors linked to RPL[4,14-17]. Reports that are currently available on the connection between CMV infection and RPL have produced conflicting findings. Other investigations revealed that the prevalence of CMV antibodies in RPL women was similar to or even lower than that of normal pregnant women, despite some authors reporting higher prevalence and higher antibody titers to CMV in RPL cases[18]. Accordingly, several researchers hypothesized that RPL patients would have some degree of CMV-specific immune unresponsiveness[18].

Prior studies on the relationship between CMV infection and RPL have relied on basic serological tests. These studies likewise had small sample sizes. Using anti-CMV IgG and IgM antibodies and IgG and IgM levels, which offer further information for the diagnosis of CMV infection and the immunological response to it, we assessed and contrasted the humoral immune response to CMV in Yemeni women with and without RPL. Given the paucity of data regarding the aetiology of RPL and the suggested involvement of a modified immune response in this context, this action was taken[4,19].

In Yemen, there have been recent studies discussing viral infections and their association with cancers and birth defects, for example, a study of CMV infection among children with leukemia[1] and the prevalence of potential CMV risk factors and congenital CMV among female physicians[2]. In addition, Epstein-Barr virus has been linked to breast cancer[20,21] and systemic lupus erythematosus[22]. Hepatitis C virus (HCV) infection, leukemia, oncology and chronic liver disease[23-29], poliovirus and non-nutritional diseases[30]. However, no study has addressed whether recurrent or latent CMV infection or altered immune response to it is associated with recurrent pregnancy loss (RPL) in Yemen. Therefore, our objectives were to evaluate CMV infection and the level (titer) of CMV antibodies in women with RPL compared to healthy controls.

MATERIALS AND METHODS

Participants and settings:

In this study, 149 women with RPL who were referred by gynaecologists to a clinical outpatient clinic at the Family Planning Centre in Sana'a, Yemen, were the subjects of this case-control research. Age between 18 and 45 years, at least three consecutive spontaneous abortions prior to the 22nd week of pregnancy, and the absence of laboratory data supporting organic or autoimmune disorders as the cause of RPL were the requirements for inclusion.

Simple non-random consecutive sampling was used to sample the case group. Healthy age-matched multiparous women without a history of abortion who were sent to the Family Planning Centre's gynaecology clinic were chosen at random to serve as control subjects.

Ethic consideration: The Ethics Committee of the Faculty of Medicine and Health Sciences, Sana'a University, approved the study with document number: 2024-15 dated January 25, 2025, and both patients and study controls gave their informed consent.

Assessments: After the inclusion criteria were evaluated, participant interviews were conducted, and demographic information was gathered, blood samples were drawn from both patients and controls. The samples were centrifuged and stored at -20°C before being sent to the Central Laboratory in Sana'a City. CMV IgG and IgM antibodies were evaluated using the enzyme linked immunosorbant assay method (ELISA) with a commercial kit (EUROIMMUN, Lübeck, Germany). Based on serological testing, women were classified as 3-primary CMV infection (CMV-IgM positive and CMV-IgG negative), 4-primary or recurrent infection (both CMV-IgM and CMV-IgG positive), 2-CMV seropositive (CMV-IgM negative and CMV-IgG positive), and 1-CMV seronegative (both CMV-IgM and -IgG negative).

Statistical analysis

SPSS software for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA), was used to analyse the data. While relative frequencies are used to represent categorical variables, mean±standard deviation (SD) is employed to express quantitative data. The Student's t-test was used to compare the quantitative variables, while the Chi-squared test with Pearson correction was used to analyse the categorical variables. The threshold for statistical significance was set at $p<0.05$.

RESULTS

The age distribution of CMV-tested women in Sana'a City, Yemen, who had a history of miscarriages and healthy controls is displayed in Table 1. The cases ranged in age from 17 to 44 years, with a mean age of 24.7 years±SD 6.3 years. The controls ranged in age from 17 to 44 years old, with a mean age of 27.1 years±SD 5.3 years. Patients aged 20–24 years had the highest rate of recurrent miscarriages (34.9%), followed by those aged 15–19 (25.5%) and 30+ (22.1%). Table 2 displays the prevalence of current CMV infection in Sana'a City, Yemen, among women

who had recurrent miscarriages and healthy controls who underwent CMV-IgM testing. Among RPL cases, the crude prevalence of CMV IgM, a measure of current CMV infection, was 5.3%, whereas among healthy controls, it was 3.4%. When age categories were examined, the cases aged ≥30 years had the highest prevalence (6.1%), followed by those aged 20–24 years (5.8%) and 15–19 years (5.2%), while the 25–29 year age group had the lowest frequency (3.8%). The crude positive IgM rate in the control group was 3.4%; the highest rate was 5.6% in the 20–24 age group and 3.6% in the 25–29 age group. The other age groups had zero rates (0.0%) (Table 2).

Table 1: Age distribution of women with recurrent miscarriage cases and healthy controls tested for CMV.

Age in Years	Cases n=149		Controls n=149	
	N	%	N	%
15- 19 years	38	25.5	12	8.1
20 - 24 years	52	34.9	36	24.2
25 -29 years	26	17.4	56	37.6
≥30 years	33	22.1	45	30.2
Mean	24.7 years		27.1 years	
SD	6.3 years		5.3 years	
Median	23 years		27 years	
Mode	19 years		27 years	
Min to Max	17-44 years		17-44 years	

Table 2: Prevalence of current CMV infections of women with recurrent miscarriage cases and healthy controls tested for CMV-IgM.

Age in Years	Cases PositiveIgM		Controls Positive IgM	
	N	%	N	%
15-19 years	2/38	5.2	0/12	0.0
20-24 years	3/52	5.8	2/36	5.6
25-29 years	1/26	3.8	2/56	3.6
≥30 years	2/33	6.1	0/45	00
Total	8/149	5.3	5	3.4

There was no significant difference in the rate of IgG-CMV antibodies between patients and controls. The crude prevalence among the patient group was 97.98% and among healthy controls was 97.3%. A lower prevalence of IgG-CMV was found in the patient group in the age group ≥ 30 years (93.9%) while a rate of 100% was found in 15–19 years and 20–24 years. Considering the control group, a lower prevalence of IgG-CMV was found in the age group ≥ 30 years (93.3%) while a rate of 100% was found in 15-19 years and 97.2% in the age group 20-24 years (Table 3). The mean±standard deviation of IgM antibodies in patients was 0.44±0.32 IU/ml versus lower the mean±standard deviation of IgM antibodies in controls which was 0.31±0.21 IU/ml with a difference of -0.13 and this difference was statistically significant (p=0.0001). The mean±standard deviation of IgM antibodies in CMV-IgM positive cases was 4.9±0.5 IU/ml versus 2.4±0.2 in IgM positive healthy controls with a difference of -2.5, 95% CI -2.59 to -0.41. The mean±standard deviation of IgG antibodies in patients was 5.78±2.2 IU/ml versus less than the mean±standard deviation of IgG antibodies in controls which was 3.46±1.13 IU/ml with a difference of -2, 395% (Table 4). Considering place of residence, 69.1% of cases were urban residents and only 30.9% were rural residents. Considering education, secondary school was the most common educational status (38.3%).

Table 3: prevalence of CMV infections of women with recurrent miscarriage cases and healthy controls tested for IgG-CMV antibodies.

Age in Years	Cases Positive IgG		Controls Positive IgG	
	N	%	N	%
15-19 years	38/38	100	12	100
20-24 years	52/52	100	35	97.2
25-29 years	25/26	96.2	56	100
≥30 years	31/33	93.9	42	93.3
Total	146	97.98	145	97.3

Table 4: The amount of IgM CMV and IgG –CMV antibodies of women with recurrent miscarriage cases comparing to healthy controls.

Age in Years	Patients (mean±SD) n=149	Controls (mean±SD) n=149	Difference	95% CI	t-statistic	*p*
IgM titer	0.44±0.32 IU/ml	0.31±0.21	-0.130	-0.19 to -0.07	-4.146	0.0001
IgM positive	8 (4.9±0.5)	5 (2.4±0.2)	-2.500	-2.59 to -0.41	-56.668	<0.0001
IgG titer	5.78±2.2	3.46±1.13	-2.320	-2.72 to -1.92	-11.450	< 0.0001
IgG positive	146 (97.98)	145 (97.3)	0.7 %	-3.38% to 4.9%	0.159	0.6905

Table 5: Residence, education level and occupation of cases of women with recurrent miscarriage and healthy controls tested for CMV antibodies.

Characters	Cases		Controls	
	N	%	N	%
Residence				
Urban	103	69.1	60	40.3
Rural	46	30.9	89	59.7
Education status				
Uneducated	14	9.4	27	18.1
Read and write	45	30.2	26	17.4
Primary school	17	11.4	21	14.1
Secondary school	57	38.3	52	34.9
University	15	10.1	23	15.4
Occupations				
Employee	10	6.7	6	4.02
Farmer	3	2	2	1.34
Housewife	131	87.9	137	91.9
Students	5	3.4	4	2.7

Considering occupation, housewife was the most common (87.9%) (Table 5). Table 6 shows the age of first pregnancy, number of children, recurrent miscarriage, history and frequency of women with recurrent miscarriage who were tested for CMV antibodies. Considering maternal age for first pregnancy, 15–25 years was the most common age at 92.6% in cases and controls. For cases, the first pregnancy did not occur after 35 years (0.0%). Considering the number of children for mothers with recurrent miscarriage (cases), one child was 38.9%, followed by two children (38.3%), while three or more children was less frequent.

Table 6: Age of first pregnancy, number of children, recurrent, history and frequency of cases of women with recurrent miscarriage and healthy controls tested for CMV antibodies.

Characters	Cases		Controls	
	N	%	N	%
Age of first pregnancy				
15-25 years	138	92.6	138	92.6
26-35 years	11	7.4	7	4.7
36-45 years	0	0	4	2.7
Children own				
One	58	38.9	31	20.8
Two	57	38.3	52	34.9
Three	19	12.8	41	27.5
More than three	15	10.1	25	16.8
Previous recurrent abortion				
Zero	0	0	149	
One	0	0	0	0
Two	125	83.9	0	0
Three	22	14.8	0	0
More than three	2	1.3	0	0
Time of the abortion				
First trimester	120	80.5	0	0
Second trimester	25	16.8	0	0
Third trimester	4	2.7	0	0
Causes of abortion				
Stress	5	3.4	0	0
Infections	27	18.1	0	0
Uterine surgery	4	2.7	0	0
Unknown	113	75.8	0	0

For the control group, having three or more children was more common. Considering previous recurrent miscarriage, having two miscarriages was the predominant event in the case group at 83.9%, while having three miscarriages occurred in only 14.8% and having more than three miscarriages occurred in 1.3% of cases. Considering the time of miscarriage, most miscarriages occurred in the first trimester (80.5%), while only 16.8% and 2.7% occurred in the second and third trimesters, respectively.

DISCUSSION

In the present study, the crude prevalence of IgG antibodies against CMV among the patient group was 97.98%, and among healthy controls, it was 97.3%. The high incidence of CMV in Yemeni society is

summed up by this finding. The socioeconomic relationship between CMV and this high prevalence is supported by numerous studies that demonstrate that, at any given age, the prevalence of CMV is higher among people from lower socioeconomic classes, as is the situation in Yemen[31]. It has also been noted that after relocating to their adopted nation, children of people born in impoverished nations with high CMV prevalence have decreased prevalence[32]. Accordingly, it appears likely that non-specific behavioural and cultural factors will also interact with socioeconomic groups to affect the incidence of CMV.Additionally, as previously noted, the higher occurrence among women of childbearing age is probably caused by their exposure to children[31,33]. Since viruses, especially CMV, can cause chronic/recurrent intrauterine infections, there has been a lot of research done in recent decades on the role of infection in recurrent miscarriages, also known as recurrent pregnancy loss (RPL). The generation of toxic metabolites, chorioamnionitis[4], and foetal, placental, or chronic endometrial infections are proposed as potential causes. The purpose of this study was to determine whether RPL in Yemeni women is associated with exposure to or infection with CMV and/or a changed immune response to CMV. Anti-CMV IgG and IgM specific antibodies were evaluated for this purpose, which is a good and trustworthy way to distinguish between a primary infection and a recurring or reactivated infection[4].

According to the findings, 5 cases (3.4%) in the healthy control group and 8 (5.3%) in the sick group had a recurring or reactivated infection. Prior exposure to CMV may be a risk factor for RPL in our study, as patients with RPL had considerably higher seropositive rates than controls. In addition, these patients' IgG titers were greater (5.78±2.2) than controls' (3.46± 1.13; $p<0.0001$), indicating either hyper-response or more frequent exposure to CMV. There are two possible explanations for the same IgG positive rates between patients and controls (97.98% for patients versus 97.3% for controls, $p=0.69$), both of which point to a changed immune response in RPL cases: Recurrent CMV infection is a risk factor for RPL; nevertheless, patients with RPL are hyperresponsive to CMV and have altered immunological responses to CMV exposure. There aren't many reports on the connection between RPL and CMV infection, and the findings have been debatable[19]. In a large sample of RPL cases and controls, Odland *et al.*[34], showed a decreased prevalence of seropositivity (78% vs. 81.1%)[35]. They also looked at immunological response in women with RPL of uncertain aetiology and found only 35% of them to be seropositive (compared to 65% in controls).

Other researchers also discovered that RPL women had lower seropositivity than age-matched female controls, and that seropositive RPL individuals had a worse lymphocyte proliferative response to CMV than seropositive controls. According to these studies, women with RPL have trouble responding to MV, however the ladies in our study respond well to CMV. Contrary to these studies and consistent with our findings, Szkaradkiewicz *et al.*[35], discovered that women with RPL had higher levels of antibodies and more frequent seropositivity than controls. They also hypothesised that abortion may have been caused by foetal infection as a result of chronic CMV infection reactivating during pregnancy.

Nevertheless, other research employing the polymerase chain reaction technique failed to detect CMV in the foetal tissue of women with RPL[36], indicating that the immune response plays a part in RPL and implying that CMV infection of the prenatal tissue is not a direct cause of abortions in RPL instances. It is widely known that the epidemiology of CMV infection varies among populations, and variations in the examined population may account for discrepancies in the study's findings.

The age group ≥30 years had the lowest prevalence rate of IgG-CMV in the patient group in the current study (93.9%), whereas the younger age groups (15–19 years and 20–24 years) had a 100% rate. This contrasts with a report by Odland *et al.*[34], that indicated that the seropositivity of CMV in RPL cases increased with age (from 76.5% in women under 20 to 91.4% in women over 34), and that the age distribution of patients varied in earlier investigations. To obtain more precise results, larger research involving multi-socioeconomic instances with RSA and assessing many aspects of their immune response to CMV must be conducted in the future.

There are multiple reasons why these findings are significant. They will first aid in estimating the illness burden resulting from congenital infections, which have a complicated relationship with the sero-status of the mother. Even though it is thought that pregnant women who contract primary infections are most likely to give birth to infants who are seriously harmed by intrauterine infections, these women are uncommon in many nations across the world[36]. Instead, due to their prevalence in the community, the majority of kids are born to mothers who were seropositive before becoming pregnant.

The majority of new-borns may contract CMV due to the mother's reactivation or reinfection, even in nations with comparatively low seroprevalence[37]. The findings reported here should help us better understand the diseases caused by CMV around the world and help vaccine manufacturers decide where to find participants for clinical trials. Seronegative may receive vaccinations to avoid initial infection, or seropositive may receive vaccinations to increase immunity[38].

Limitations of the study

There were several restrictions on this work. Few prevalence studies were conducted in Yemen. We did not report prevalence by age or sex because our analysis was limited to adult women. Given that CMV prevalence is known to rise with age, the lack of age stratification in our study may be the cause of the disparity with other research.Although some variance within the nation is to be expected, the prevalence figures gathered were exclusive to Sana'a city. As a result, our study's seroprevalence values could not be nationally representative. Using data from a small number of studies runs the danger of producing seroprevalence estimations that are not accurate

representations of Yemen's current epidemiological state.

CONCLUSIONS

According to the current study's findings, RPL had a somewhat greater level of prior CMV exposure than controls, as shown by positive IgG antibodies. Nonetheless, we discovered a statistically significant correlation between elevated IgG levels and RPL, with patients exhibiting higher antibody levels than healthy controls. In summary, repeated exposure to CMV is a risk factor for RPL. Other immunological processes, such as hyper-response (higher IgG titers) to comparable numbers of CMV exposures, may also be risk factors. Recurrent exposure to CMV can be identified by higher IgG-CMV antibody titer, primarily due to altered immune function in other states. False positive results in CMV antibody testing may have been generated by autoimmune etiologies and hypergammaglobulinemia in RPL patients. More research is needed to evaluate these theories and additional mechanisms, like the cell-mediated immune response in RPL patients, using a bigger sample size and proper sampling. Future vaccine development and national health care models of disease burden will benefit from knowledge of CMV distribution.

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AUTHOR'S CONTRIBUTION

Al-Huraibi BS: writing original draft, methodology. **El-moflehi AM:** methodology, investigation. **Al-Moyed KA:** methodology, investigation. **Hassan SMA:** formal analysis, data curation. **Al-Madhaji AG:** conceptualization. **Al-Jaufy AY:** data curation, conceptualization. **Al-Shamahy HA:** formal analysis, data curation, conceptualization. **Aljabri AAS:** writing, review. **Al-Haidary NM:** editing, methodology. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The empirical data used to support the study's results can be obtained upon request from the corresponding author.

CONFLICT OF INTEREST

None to declare.

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