ORIGINAL ARTICLE

Seropositivity of cytomegalovirus in patients with recurrent pregnancy loss

Roya Sherkat, Mohsen Meidani[1], Hossein Zarabian[2], Abbas Rezaei[3], Ali Gholamrezaei[4]

Acquired Immunodeficiency Research Center, [1]Assistant Professor, Infectious Diseases and Tropical Medicine Research Center, [2]Infectious Diseases Specialist, [3]Immunology, [4]Poursina Hakim Research Institution, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Some evidence has shown a relationship between human cytomegalovirus (CMV) infection and pregnancy loss. However, whether recurrent or latent CMV infection or altered immune response to CMV is related to recurrent pregnancy loss (RPL) is unclear. We evaluated CMV infection and avidity of antibodies to CMV in women with RPL. **Materials and Methods:** This case-control study was conducted on 43 women with RPL referred to a clinical immunology out-patient clinic in Isfahan (Iran), and 43 age-matched multiparous women without history of abortion as control subjects. Patients and controls were evaluated for anti-CMV IgG and IgM antibodies and IgG avidity index (AI) using the enzyme linked immunosorbent assay method. Student's *t*-test and Chi-square test were used to analyze the data. **Results:** One case (2.3%) of positive anti-CMV IgM was detected in each group. Anti-CMV IgG positivity was more frequent in patients than in controls (90.6% vs. 69.8%, $P = 0.014$), but there was no difference between the two groups in anti-CMV IgG AI (79.4 ± 11.4 vs. 80.1 ± 10.2, $P = 0.781$). IgG titer was significantly higher in seropositive cases with RPL than seropositive controls (5.18 ± 1.99 vs. 2.00 ± 0.81, $P < 0.001$). **Conclusion:** We found that previous exposure to CMV was significantly higher in patients with RPL than the control group. However, no association was found between IgG AI and RPL. Further investigations are needed to find whether latent CMV infection starts an indirect process of autoimmune etiology in RPL or women with RPL have recurrent or reactivation of CMV infection.

Key words: Avidity index, cytomegalovirus, infection, recurrent pregnancy loss

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INTRODUCTION

Infection with human cytomegalovirus (CMV), a member of the herpesvirida family, is very common world-wide with seropositivity rates ranging from 40% in developed countries up to 100% in developing countries.[1,2] Clinical manifestations are various, but symptomatic disease is rare among immunocompetent hosts. Severe scenarios occur when the immunocompetent host is critically ill[3] and also in immunocompromised hosts.[4-8] The epidemiology and pathogenesis of infection with CMV in pregnancy have been studied over the past decades. Primary infection or reactivation of the previously acquired CMV can occur during pregnancy and can result in congenital CMV; the most important cause of congenital viral infections. If the child survives, up to 90% of the cases will have complications such as hearing loss, vision impairment and varying degrees of mental retardation.[9-13]

Whether primary infection or reactivation of CMV in a pregnant woman can cause pregnancy loss is still under discussion, results have been controversial, and underlying mechanisms are unclear.[13-20] Some studies found high presence of CMV antigens in tissues from abortion[15] and others reported higher seropositivity. [21] One prospective study also found a higher risk of pregnancy loss with CMV infection,[22] though results of other prospective studies did not approved it.[23] Despite these reports on the role of CMV infection in spontaneous pregnancy loss, the role of CMV infection in recurrent losses is less clear. Recurrent pregnancy loss (RPL), usually referred to as three or more consecutive abortions prior to 22nd gestational weeks, is one of the most frustrating and difficult areas in reproductive medicine. While about 15% of pregnant women experience sporadic pregnancy loss, about 2% and only 0.4-1% of them experience two and three consecutive abortions, respectively. The etiology of RPL is still unclear and few evidence-based diagnostic and treatment approaches are available. Etiologic factors associated with RPL are suggested as anatomical, immunological, genetic, endocrine, infectious, thrombophilic, and environmental factors.[24,25] Available reports on the role of CMV infection in RPL showed controversial results. While some authors reported higher prevalence and higher antibody titers to CMV in RPL cases,[26] other studies showed comparable

Address for correspondence: Dr. Mohsen Meidani, Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: meidani@med.mui.ac.ir

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and even less prevalence of antibodies to CMV among women with RPL than normal pregnant women.[27,28] Accordingly, some investigators suggested that RPL cases might have some selective immunological unresponsiveness to CMV.[28,29]

Previous studies on the association between CMV infection and RPL were mainly based on simple serological tests. Also, sample sizes were small among these studies. Considering the lack of data on the etiology of RPL, and also regarding the suggested role of altered immune response in this regard, we evaluated and compared humoral immunological response to CMV in women with and without RPL using IgG and IgM CMV antibody plus IgG avidity index (AI) test which provide complementary data for diagnosis of and immune response to CMV infection.[30,31]

MATERIALS AND METHODS

Participants and settings

This case-control study was conducted on 43 women with RPL referred by gynecologists to a clinical immunology outpatient clinic in Isfahan (Iran). Inclusion criteria were age between 18 and 45 years, at least three recurrent spontaneous abortions before the 22nd gestational week, and no evidence for organic or autoimmune diseases as the etiology for RPL confirmed by laboratory results. Sampling for case group was done with simple non-random consecutive sampling. Control subjects were randomly selected from healthy age-matched multiparous women without history of abortion referring to the gynecology clinic of the hospital. The study was approved by the Ethics Committee of Isfahan University of Medical Sciences (research project number: 389048) and informed consent was obtained from patients and controls.

Assessments

After evaluation of the inclusion criteria and interviewing with participants and gathering demographic data, blood samples were obtained from patients and controls. Samples were centrifuged and stored in −70°C and then were transferred to the Infectious Disease Research Center of Isfahan, Central Laboratory. To evaluate CMV IgG and IgM antibodies the enzyme linked immunosorbant assay method (ELISA) was applied using a commercial kit (EUROIMMUN, Lübeck, Germany). AI of anti-CMV IgG was also studied by ELISA method as proposed by the manufacturer (Focus Diagnostics, California, USA). An AI of <40% was considered as low, higher than 60% as high and between the 40% and 60% as moderate.[30,31] According to serological tests, women were classified as follows:

- CMV seronegative: both CMV-IgM and -IgG negative.
- CMV seropositive: CMV-IgM negative and CMV-IgG positive.
- Primary CMV infection: CMV-IgM positive and CMV-IgG negative.

Either a primary or a recurrent infection: both CMV-IgM and CMV-IgG positive. Differentiation of primary and recurrent CMV infection in women with both CMV-IgM and CMV-IgG positive antibody was determined by the assessment of the anti-CMV IgG AI at the same time. Pregnant women with low AI were considered as having primary maternal infection, while those with high AI were considered as having recurrent maternal infection. Those with moderate AI were conserved as undefined group.[30,31]

Statistical analyses

Data were analyzed with the SPSS software for windows version 16.0 (SPSS Inc., Chicago IL., USA). Quantitative data are expressed by mean ± standard deviation (SD), and categorical variables are described by relative frequencies. For comparisons of the quantitative variables, we used the Student's *t*-test, and for comparisons between the categorical variables, we used the Chi-square test with Pearson modification. The statistical significance criterion was set at $P < 0.05$.

RESULTS

Forty three women with RPL (mean ± SD age = 30.22 ± 4.73 years, mean abortion number = 3.84 ± 1.98) and 43 controls (mean age = 29.87 ± 5.32 years, $P = 0.64$) were included into the study. IgM and IgG titers and final results of the patients and controls are presented in Table 1. There was one (2.3%) case of positive IgM in each group of the RPL and controls. These patients had also positive IgG and high AI and thus were considered as having recurrent or reactivated maternal infection. There were 39 (90.6%) and 30 (69.8%) cases of positive IgG in the RPL and control groups, respectively ($P = 0.014$). No difference was found between the two groups in IgG AI; $P = 0.781$ [Table 1].

Separate analysis of the IgG titer below and above the cut-off (according to the kit instruction: Ratio >0.8 or 16 RU/ml

Table 1: IgM and IgG titers and avidity index in patients and controls

Group	Patients (*n* = 43) (%)	Controls (*n* = 43) (%)	*P*
IgM titer	0.44±0.32	0.37±0.21	0.223
IgM positive	1 (2.3)	1 (2.3)	0.753
IgG titer	4.78±2.2	3.46±1.13	0.024
IgG positive	39 (90.6)	30 (69.8)	0.014
IgG avidity index	79.4±11.4	80.1±10.2	0.781
IgG avidity			
<40%	0	0	0.500
40-60%	2 (4.6)	3 (6.9)	
>60%	41 (95.3)	40 (93.0)	

as positive) in cases showed that IgG titer was significantly higher in seropositive cases with RPL than seropositive controls (5.18 ± 1.99 vs. 2.00 ± 0.81, $P < 0.001$), and also in seronegative RPL cases than seronegative controls (0.82 ± 0.28 vs. 0.53 ± 0.20, $P = 0.015$) [Table 2].

DISCUSSION

The role of infection in RPL has been intensely investigated during the past decades and viruses, in particular CMV, have been under more attention since they can produce chronic/recurrent intrauterine infections. Possible mechanisms are suggested as production of toxic metabolites, fetal, placental, or chronic endometrial infection, and chorio-amnionitis.[12,14] The aim of this study was to evaluate if exposure/infection to CMV and/or an altered immunological response to CMV is related to RPL in women of our population. For this aim, we assessed anti-CMV IgG and IgM specific antibodies along with IgG AI, which is a good and reliable method to differentiate a recurrent/reactivated infection from primary infection.[30,31] As the results showed, there was only one case with a recurrent or reactivated infection in each group of patients and controls. Patients with RPL were significantly more seropositive than controls suggesting that previous exposure to CMV might be a risk factor for RPL. These patients had also higher IgG titer which shows either hyper-response or more frequent exposure to CMV. Because patients and controls were similar in IgG AI, two possibilities are exist both indicating an altered immune response in RPL cases: That recurrent exposure to CMV is a risk factor for RPL, but patients with RPL have altered immune response to CMV exposure which does not lead to higher AI; and that patients with RPL are hyper-responsive to CMV.

Few reports are available on the association between CMV infection and RPL and results have been controversial.[26-28] The study by Odland *et al*. on a large sample of RPL cases and controls found similar prevalence of seropositivity (78% vs. 81.1%).[27] Johnson *et al*.[29] studied women with RPL of unknown etiology for immune responsiveness and found only 35% seropositive cases (compared to 65% in controls). Other investigators also found lower seropositivity in RPL women compared with age-matched female controls and an impaired lymphocyte proliferative response to CMV in seropositive RPL cases compared with seropositive controls.[28] These studies indicated that women with RPL have difficulty in responding to CMV. In contrast to these studies, and similar to our results, Szkaradkiewicz *et al*.[26] found more frequent seropositivity and higher levels of antibodies in women with RPL than controls and suggested that abortion might have resulted from fetal infection due to reactivation of chronic CMV infection in the course of pregnancy. However, other studies with using polymerase chain reaction method did not find CMV in gestational tissue of women with RPL[32-34] suggesting that CMV infection of the gestational tissue is not a direct cause of abortions in RPL cases and highlights the role of immune response in RPL. Differences between the studies' results can be due to differences in the studied population and it is well-known that epidemiology of CMV infection is different among different populations. Also, patients' characteristics have been different among studies. For example, Odland *et al*.[27] found that seropositivity of CMV in RPL cases raised with increasing age (from 76.5% in younger than 20 years to 91.4% in older than 34-year-old women), and previous studies were different regarding patients' age distribution. Future larger and using multi-socioeconomic cases of RSA, also evaluation of different components of their immune response to CMV need to be done to get more clear results.

Table 2: IgG titers and avidity index in seropositive and seronegative patients and controls

IgG titer	Patients	Controls	*P*
Seropositive	5.18±1.99	2.00±0.81	<0.001
Seronegative	0.82±0.28	0.53±0.20	0.015
IgG avidity index			
Seropositive	80.1±10.5	82.5±10.8	0.477
Seronegative	73.2±18.8	79.0±9.9	0.333

CONCLUSIONS

The results of the present study showed that previous exposure to CMV detected by a positive IgG antibody is significantly higher in RPL. However, we found no relationship between IgG AI and RPL. We may conclude: (1) Recurrent exposure to CMV is a risk factor for RPL, (2) other immunological processes like hyper-response (higher IgG titers) to the similar number of exposure with CMV may be another risk factor, (3) recurrent exposure to CMV, but the inability to detect it by higher AI, mostly because of altered immune function might be other state, and (4) autoimmune etiologies and hypergammaglobulinemia in RPL cases may have caused false positivity in CMV antibody checking. Further investigations evaluating these theories and also other mechanisms such as cell-mediated immunological response in RPL patients, with adequate sampling and sample size are required.

AUTHORS' CONTRIBUTION

All authors have contributed in designing and conducting the study. AG, HZ and MM collected the data and RS, MM, and AR did the analysis. All authors have assisted in preparation of the first draft of the manuscript or revising it critically for important intellectual content. All authors have read and approved the content of the manuscript and are accountable for all aspects of the work.

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