



## Antibodies Against Cytomegalovirus Infection in Pregnancy with Recurrent Miscarriage

Nashmel Farhad Mamand\* Ariana Khalis Jawad\*\*

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### Abstract

**Background and objectives:** Recurrent pregnancy loss leads to serious obstetrical and psychosocial consequences specifically if causes are unknown. The aim of this study is to evaluate the role of cytomegalovirus infection in women with recurrent pregnancy loss.

**Methods:** This is a case-control study implemented in outpatient's clinic of Maternity hospital in Erbil city over duration of one year from 1<sup>st</sup> of June, 2022 to 31<sup>st</sup> of May, 2023 included 100 women with miscarriages recurrences and 100 women without history of miscarriage. A sample of 5 ml blood was drawn from selected women and sent for investigation. Serologically, cytomegalovirus infection was confirmed through recording cytomegalovirus immunoglobulin-G and immunoglobulin-M anti-bodies detection in private laboratory.

**Results:** The age, body mass index, educational level, occupation, residence and parity history of study participants were not statistically different between study groups ( $p>0.05$ ). The consanguinity was statistically related to controls (40%) and the positive family history of repeated pregnancy loss was statistically related to women with repeated pregnancy loss (12%) ( $p\leq 0.05$ ). Cytomegalovirus immunoglobulin M seropositivity was not statistically different between study groups (2% versus 2%) ( $p>0.05$ ), however, cytomegalovirus immunoglobulin G seropositivity was prevalent in women with recurrent pregnancy loss (100%) ( $p=0.04$ ).

**Conclusions:** The cytomegalovirus infection is a risk factor for miscarriages recurrence.

**Keywords:** Cytomegalovirus infection, IgG, IgM, Miscarriage recurrence

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\*MBChB., Kurdistan Higher Council of Medical Specialties in Obstetrics & Gynecology; Maternity Teaching Hospital, Erbil City, Kurdistan Region/Iraq.

Email: nashmel\_gardi@yahoo.com. Coresponding author:

\*\*MBChB, FICOG; Professor in Obstetrics &Gynecology; Kurdistan Higher Council of Medical Specialties Kurdistan region/Iraq. Email:aryianadr@yahoo.com



## Introduction

Recurrent pregnancies loss (RPL) is known as 3 or extrasequential spontaneous miscarriages in 1<sup>st</sup> twenty weeks of gestation. Nowadays, different authors and organizations documented that RPL could be known as 2 and extra consecutive sequential spontaneous miscarriages before 20<sup>th</sup> weeks of gestation.<sup>1,2</sup> Incidence of RPL is ranged between 1-2% among women at reproductive age.<sup>3</sup> However, the RPL is regarded as frequent obstetrical disease recorded among 15% to 25% of pregnant women with higher incidence reported with increasing age especially over age of 40 years.<sup>4</sup> The RPL is accompanied with clinical, emotional and social co-morbidities and considered as serious obstetrical disorder.<sup>4</sup> Generally, occurrence of RPL was various in regard to discrepancies in introductions, inclusion characteristics and various communities.<sup>5</sup> Miscarriage risk is increased in subsequent pregnancies by 30% after two consecutive miscarriages, while increased by 33% after three consecutive miscarriages.<sup>6</sup> In Iraq the RPL incidence was (10.9%), while the incidence of RPL in Kurdistan region was 16.3%.<sup>7</sup> The etiology of RPL is unknown in about half of studied cases. However, it was shown that 80% of the unexplained miscarriages might be attributed to immune factors.<sup>8</sup> For women with well-defined etiology, women immunology (auto and allogeneic), thrombotic variables (genetic and acquired), anatomical abnormalities of uterus and endocrine disorders were the main important etiologies of RPL.<sup>9</sup> The embryonic chromosomal abnormalities are the commonest etiology of RPL in addition to infectious diseases and male factors.<sup>10</sup> Human cytomegalovirus (CMV) is a part of Herpesviridae strain and related to beta Herpesviridae sub-strain. The cytomegalovirus is distributed all over the world and has the ability to infect all

humans with various age groups, ethnicities and socioeconomic classes, with lack of season or transmissions effect.<sup>11</sup> Main transmission modes of cytomegalovirus infections are through salivary, urinary and other body fluids. Diagnosis of CMV can be done by several methods: Serologic testing which is done by detecting CMV IgG and IgM, detection of CMV DNA in blood or urine by PCR, Amniocentesis if fetal infection is suspected.<sup>11,12</sup> In healthy non-pregnant women, the CMV infection that is acquired after birth or from blood transfusion, the symptoms of CMV infection is mild and not accompanied with complications.<sup>12</sup> In women who acquired the infection throughout gestation, the risk is high and commonly associated with many adverse outcomes and this infection is mainly caused by longer duration of contiguity with children for lower than 3 years of age and those children were either in the home or child care centers or schools.<sup>13</sup> The seroreactivity and low socioeconomic status are regarded as risk factors for CMV infection.<sup>14</sup> Other risk factors are weak immunity that is related to disorder or pharmacotherapy like transplantation or human immunodeficiency disorder.<sup>15</sup> Epidemiology and etiology of cytomegalovirus infection during pregnancy is studied since last decades. The first contamination or reactivating of previous acquired cytomegalovirus infection may be detected in pregnancy and may leads to congenital cytomegalovirus infection. Unfortunately, in children who survived, 90% of them will have many complications like deafness, blindness and sometimes retarded mental status.<sup>16, 17</sup> Recurrent pregnancy loss is considered as a serious physically and emotionally obstetrical disorder affecting women at reproductive age.<sup>12</sup> The embryo-fetal infestation by CMV has shown to recurrent pregnancy loss.<sup>17</sup> Mechanism of RPL due to cytomegalovirus





might be attributed to either directly, or by indirectly by insufficient placenta and immunosuppression.<sup>17</sup> Other authors found high CMV antibodies prevalence among women with RPL, while others found no effect of cytomegalovirus antibodies on women with recurrent pregnancy loss.<sup>18</sup> Although scarcity of literatures exploring role of cytomegalovirus in miscarriages recurrences among women Kurdistan region/Iraq, some authors found a relationship between the cytomegalovirus and miscarriages/or stillbirth and others reported higher cytomegalovirus distribution in pregnant women living in Kurdistan region.<sup>19,20</sup> This study aimed to evaluate the role of CMV infection in women with RPL.

### Patients and methods

This study was a case-control project implemented in outpatient's clinic of Maternity hospital of Erbil city over duration of one year started as 1<sup>st</sup> of June, 2022 to 31<sup>st</sup> of May, 2023. The inclusion standards were women between the ages between 18-45 with 3 or more sequential pregnancy losses prior to 22 weeks of pregnancy who had tested positive form CMV. Exclusion criteria were women older than 45 and younger than 18 years of age, uterine anatomical or structural disorders, aneuploidy, congenital Mullerian tract anomalies, women with chronic diseases and missing or incomplete data. Ethics of current literature was subjected to guidelines of ethics committee, agreement of hospital administration and consent of study participants. One hundred women with recurrent pregnancy loss were enrolled and another sample of one hundred women without RPL (controls) were selected from women at reproductive age attended the outpatient clinic without history of preceding miscarriage. Information of study participants was gathered in direct way through the researchers or from their recorded information in hospital files and

contented in survey form prepared by the researchers. This form contained: basic properties, clinical and gestational properties and cytomegalovirus seropositivity. The recurrent pregnancy loss was known as 3 or extrasequential spontaneous miscarriages in 1<sup>st</sup>20 weeks of pregnancy.<sup>1, 2</sup> A sample of 5 ml blood was drawn from selected women and sent for investigation. Serologically, cytomegalovirus infection was confirmed through recording cytomegalovirus immunoglobulin-G and immunoglobulin-M anti-bodies detection in private laboratory. Statistical analysis was implemented with help of statistical package of social sciences software version 26. Different statistical tests were used in categorical variables analysis. P value was significant if it was  $\leq 0.05$ . To ensure ethical conduct, this study was approved by the Kurdistan Higher Council of Medical Specialties Research Protocol Ethics Committee on September 18, 2022 (reference number 1618). All women who agreed to participate (provided informed consent) did so in writing during the first interview. They were guaranteed confidentiality, meaning their information would be kept private and used only for research purposes. Throughout the study, we followed the ethical guidelines set forth by the Institutional Research Committee and the Declaration of Helsinki for research involving human subjects

### Results

The age, body mass index, educational level, occupation and residence of study participants were not statistically different between study groups, Table (1). The consanguinity was statistically related to controls and the positive family history of RPL was statistically related to women with RPL ( $p \leq 0.05$ ). Parity history of study participants was not statistically different between study groups ( $p > 0.05$ ), Table (2). Cytomegalovirus immunoglobulin M seropositivity was not statistically different





between study groups, however, cytomegalovirus immunoglobulin G seropositivity was prevalent in women with RPL ( $p \leq 0.05$ ), Table (3).

**Table (1):** Basic properties in regard to study groups.

Variable	Study groups				P
	RPL		Controls		
	No.	%	No.	%	
<b>Age</b>					0.09
<20 years	0	-	4	4.0	
20-29 years	36	36.0	36	36.0	
30-39 years	52	52.0	42	42.0	
≥40 years	12	12.0	18	18.0	
<b>Body mass index</b>					0.2
Normal	42	42.0	30	30.0	
Overweight	36	36.0	44	44.0	
Obese	22	22.0	26	26.0	
<b>Educational level</b>					0.07
Illiterate	14	14.0	28	28.0	
Primary	28	28.0	28	28.0	
Secondary	16	16.0	14	14.0	
College/institute	42	42.0	30	30.0	
<b>Occupation</b>					0.18
Housewife	72	72.0	80	80.0	
Employee	28	28.0	20	20.0	
<b>Residence</b>					0.14
Urban	78	78.0	86	86.0	
Rural	22	22.0	14	14.0	

**Table (2):** Clinical and gestational history in regard to study groups.

Variable	Study groups				P
	RPL		Controls		
	No.	%	No.	%	
<b>Consanguinity</b>					<b>0.002</b>
Positive	20	20.0	40	40.0	
Negative	80	80.0	60	60.0	
<b>Family history of RPL</b>					<b>0.03</b>
Positive	12	12.0	4	4.0	
Negative	88	88.0	96	96.0	
<b>Parity history</b>					0.11
Nulliparous	34	34.0	36	36.0	
Para 1-4	54	54.0	60	60.0	
Para ≥5	12	12.0	4	4.0	

**Table (3):** Cytomegalovirus seropositivity in regard to study groups.

Variable	Study groups				P
	RPL		Controls		
	No.	%	No.	%	
<b>CMV-IgM titer&gt;1.0 AU/ml</b>					1.0
Positive	2	2.0	2	2.0	
Negative	98	98.0	98	98.0	
<b>CMV-IgG titer&gt;2.0 AU/ml</b>					<b>0.04</b>
Positive	100	100.0	96	96.0	
Negative	0	-	4	4.0	

### Discussion

The recurrent pregnancy loss represented a great challenge for Gynecologists and for women and their families especially if the cause was unknown. Studying risk factors of recurrent pregnancy loss is important in reaching its prevention and management.<sup>21</sup> The current study showed no significant differences in age, BMI, educational level, occupation and residency between women with RPL and controls. Similarly, a literature carried out by Gowri et al. reported no significant difference in general characteristics of RPL cases and their control group.<sup>22</sup> This study failed to prove the effect of consanguinity on miscarriage recurrences. We found that a higher incidence of consanguinity between controls (40%) compared to RPL cases (20%). Consistently, a study conducted by Abdulrahman et al. revealed no effect of consanguinity on miscarriages recurrences.<sup>23</sup> Current literatures showed a positive link between RPL and positive family history where we found a 12% rate of positive family history among the RPL cases and 4% positive family history in controls. This finding coincides with the findings of Eleje et al.'s study in which they also found that positive family history significantly affects the occurrence of RPL.<sup>24</sup> In this study, we found that the highest percentage of RPL was among women whose parity were between 1-4; however, the parity had no





statistical effect on miscarriage recurrences. In Islam et al.'s study, it was reported that there was statistical effect of parity history on recurrent pregnancy loss.<sup>25</sup> This discrepancy may be related to the difference in etiology and management protocols of RPL in multiple populations. The present literature found that cytomegalovirus immunoglobulin M seropositivity was not statistically different between study groups. The rate of positive CMV IgM was equal between both groups (2% vs. 2%). Our study result regarding CMV-IgM is parallel to results of Manicklal et al.'s review.<sup>12</sup> However, this finding is inconsistent with results of recent national case control study that found a significant role of CMV-IgM seropositivity in detection of miscarriage.<sup>19</sup> This variance may be related to variances in sample size and methodology between two studies. On other hand, our study showed that the cytomegalovirus immunoglobulin G seropositivity was more prevalent in women with RPL (100%) compared to controls (96%). This finding is consistent with results of different national and international literatures.<sup>20</sup> A case control study by Aljumaili et al. found that Cytomegalovirus IgG seropositivity was accompanied by bad obstetric history.<sup>26</sup>

## Conclusion

the cytomegalovirus infection is a risk factor for miscarriages recurrence. The family history of recurrent pregnancy loss is the common risk factor for recurrent pregnancy loss. This study recommended use of CMV seropositivity test in miscarriage assessment routinely. Supporting further national multi-centers studies in assessing role of CMV infection in development of recurrent pregnancy loss using avidity index.

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## Conflict of interest

Declared none.

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