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# Prevalence of Cytomegalovirus in First Trimesteric Spontaneous Abortion: A Prospective Serological and Molecular Case–control Study

**Radi Alsafi[a,*], Saad Alghamdi[a].**

[a] *Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia.*

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**ABSTRACT**

**Aim:** To measure the prevalence and assess the risk of cytomegalovirus (CMV) infection in women diagnosed with first-trimester spontaneous abortion. Comparison

**Methods:** This was a prospective case-control study; a total of 551 women were recruited from two Maternity and Children Hospitals (Makkah and Jeddah cities) in the Western province of the kingdom between March 2013 and June 2016

Serum and products of conceptions were collected prospectively from 201 women diagnosed with first-trimester spontaneous abortion. Another set of serum samples and placental tissues were collected from 350 healthy pregnant women at the time of normal vaginal delivery. CMV IgM and IgG antibodies were measured serologically by ELISA while viral DNA was detected in tissue specimens by PCR. Socio-economic data were analysed to identify potential risk factors of abortion and/or CMV infection.

**Results:** The overall prevalence was 88.4%, 13.6% and 10.1%, for IgG+, IgM+ and PCR+, respectively. IgG+ frequency was comparable between cases (89%) and controls (88%). Both exclusively IgM+ (7.9% vs. 0.6%) and dual IgM+/IgG+ (22.4% vs. 3.4%) antibodies were significantly more prevalent in abortion ($P < 0.01$). PCR+ was also significantly higher ($P < 0.01$) in abortion (25.4% vs. 1.4%). Women with exclusive IgM+ (OR 17.1; 95%CI [3.38 – 86.68]; $P = 0.001$) and those who were positive for all tests (OR 29.3; 95%CI [6.52–131.71]; $P < 0.001$) had a significantly increased risk for abortion.

**Conclusions:** The majority of the study sample of pregnant women in Saudi Arabia were previously exposed to CMV. First trimester CMV infection appears to be an independent risk for spontaneous abortion and viral spread to the intrauterine cavity was associated with the highest risk for miscarriage. More studies are required to measure the precise prevalence of CMV and its associated sequelae during pregnancy in the kingdom.

## 1. Introduction

Spontaneous miscarriage/abortion is a common form of early pregnancy loss [1, 2] and the World health Organization (WHO) defines it as a natural termination of a pregnancy before 20 weeks gestational age and it is believed that 10-20% of pregnancies are naturally lost during the first trimester [3]. Several risk factors associated with early pregnancy loss have been identified, among which maternal upper genital tract infection has been shown to have a major impact on the incidence of spontaneous abortions [4-6]. Cytomegalovirus (CMV) could be transmitted sexually and several studies have linked the virus with poor reproductive outcomes [7, 8]. Infection with CMV during human pregnancy is also common, usually latent, persistent and the majority of patients are asymptomatic [7, 9]. Additionally, the virus could result in spontaneous abortion as well as severe neonatal neurological manifestations following vertical transmission during pregnancy [10, 11].

Screening for CMV infection during pregnancy is mainly achieved by serological detection of IgM and IgG antibodies against the virus and the differentiation between primary and reactivation/re-infection status is established by performing IgG avidity test [12-15]. However, substantial numbers of those patients positive for both IgM and IgG antibodies will show a moderate IgG avidity and, hence, limiting the dating of infection and mandating further serial serological testing [14, 15]. Nucleic acid amplification tests (e.g. PCR) are more sensitive and specific methods that can complement/confirm the serological results [15-17].

Similar to many other countries [18, 19], screening for CMV infection is not routinely performed during clinical antenatal work-up in Saudi Arabia, except for selected cases, and therefore little is known regarding whether CMV infection could represent a risk factor for spontaneous abortion in the kingdom. The aim of this study was to measure the prevalence of IgM and IgG antibodies against CMV in serum as well as viral DNA by PCR in products of conception specimens obtained from women diagnosed with first trimesteric spontaneous miscarriage and the results were compared with those obtained from normal pregnancy following vaginal delivery. A better understanding about the role of CMV infection in the risk of spontaneous abortion could possibly lead to the development of appropriate policies regarding screening and/or treatment of CMV during pregnancy in the kingdom.

## 2. Subjects and methods

### *2.1. Ethical approval*

Ethical approval was obtained from the Faculty of Applied Medical Sciences Ethics Committee (AMSEC 10-15-9-2013) and all serum and tissue samples were collected following obtaining informed written consent from all the participants.

### *2.2. Study design*

This was a prospective case-control study; a total of 551 women were recruited from two Maternity and Children Hospitals (Makkah and Jeddah cities) in the Western province of the kingdom between March 2013 and June 2016; for whom TORCH screening was not performed during their routine clinical examination.

* **Corresponding Author**
*Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia.*
*E-mail address:* rtsafi@uqu.edu.sa (Radi Alsafi)
1685-4732 / 1685-4740 

Serum samples and fresh products of conception were collected from 201 pregnant women (cases) who attended the Emergency Department with complaints of vaginal bleeding or lower abdominal pain, had positive pregnancy test following natural conception and were diagnosed with spontaneous miscarriage during the first trimester (≤ 13 weeks gestation). None of the cases had a previous bad obstetric history, underwent infertility treatment or had a previous history of sexually transmitted diseases and/or pelvic inflammatory disease. Cases with pregnancy loss following accident or induced abortion were excluded. Treatment and management were performed according to the clinical background. The average estimated gestational age was calculated by subtracting the date of last menstrual period from the date of admission. The mean (SD) gestational age was 10.4 ± 2.3 weeks. The mean (SD) of serum β-human chorionic gonadotropin level was 3,751 ± 1,988 IU/L.

Since it is not ethically possible to collect placental tissue samples from first trimesteric healthy pregnant women to measure CMV by PCR, another set of serum samples and placental specimens were obtained from 350 healthy singleton pregnant women (controls) following normal vaginal delivery of a healthy new-born. All women in the control group were multiparous with livebirths, had no history of chronic diseases (e.g. diabetes, hypertension, etc.), previous abortion, sexually transmitted infections, pelvic inflammatory disease, ectopic pregnancy, uterine diseases (e.g. fibroid), bad obstetrics history or a family history of genetic diseases.

The study measured the frequency of IgM and IgG antibodies against CMV in serum together with viral DNA by TaqMan real-time PCR in all tissue specimens and the results were compared between the case and control groups. Socio-economic characteristics were also collected from all participants to measure and identify any potential associations with spontaneous miscarriage and/or CMV infection.

### *2.3. Enzyme linked immunosorbant assay (ELISA)*

ELISA was used for the qualitative detection of IgG and IgM antibodies against CMV; all the used kits (Human Diagnostics, Germany) in the present study are IVD CE certified and from the same batch. All samples were processed in duplicate using a fully automated ELISA system (Human Diagnostics) according to the manufacturer's instructions including the internal controls provided within each kit for quality control. The sensitivity, specificity, inter and intra-assay coefficient of variation for each kit as reported by the manufacturer were 100%, 99.3%, 3.4% and 3.6% for the CMV IgG kit and, 93.75%, 99.2%, 5.6% and 7.8% for the CMV IgM kit, respectively. The threshold indices were calculated, and the cut-off value was 0.71 and 0.51 for CMV (IgG and IgM) antibodies, respectively. The samples were considered positive if the value of the sample was > (cut-off + 10% cut-off value) as recommended by the manufacturer. The validation criteria set by the manufacturer for each kit was checked and passed prior to the interpretation of the results.

### *2.4. Real-time TaqMan PCR for the detection of CMV in tubal samples*

The extraction of DNA from the collected products of conception and placental tissue specimens was carried-out using MagMAX™ DNA Multi-Sample Kit (Thermo Fisher Scientific, Warrington, UK) according to the manufacturer's instructions and following homogenisation using a tissue raptor and sterile plastic tubes containing beads (Omni International, GA, USA). The quality and quantity of extracted DNA were measured on the BioSpec-nano (Shimadzu Corporation, Tokyo, Japan) and typically had an A260/A280 ratio of 1.7 to 1.9. Extracted DNA samples were stored at -20°C until used. Single plex TaqMan PCR was performed by using the IVD CE certified FTD Cytomegalovirus kit (Fast-track diagnostics, Junglinster, Luxembourg) on ABI® 7500 platform (Thermo Fisher Scientific) and according to the manufacturer's protocol. The detection limit of the kit was $10^3$ copies/ml as reported by the manufacturer.

The internal control provided with the kit consisted of murine CMV, which was added at the lysis buffer stage with each tissue specimen during the DNA extraction process. Confirmation of proper isolation of nucleic acid was confirmed by the successful amplification of the internal control, which also assured the absence of PCR inhibitors. Furthermore, human genomic DNA was also detected by conventional PCR using primers specific for the human β-globin gene (PCO3 and PCO6) to also verify the absence of PCR inhibitors [20]. Extra in-house validation protocol was also performed for all negative samples as previously described [20].

The PCR reaction per 'well' specimens consisted of 12.5 µl mastermix, 1 µl enzyme, 1.5 µl of primers and 10 µl DNA. The amplification was performed as instructed by the manufacturer. The validation of the results was performed according to the manufacturer's guidelines and by using the provided positive and negative controls within the kit as previously described [7, 9].

### *2.5. Statistical analysis*

Statistical analysis of the results was performed with SPSS version 16 (SPSS Inc., Chicago, USA). Categorical variables were compared by Chi square ($\chi^2$) or Fisher's tests following cross-tabulation and crude odds ratio (COR) with 95% confidence interval (CI) were calculated. Binary regression was used to measure odds ratio (OR) and 95% CI of the potential risk factors that could be associated with CMV infection and/or spontaneous abortion. All tests performed were two-sided and significance was considered if $P < 0.05$.

## 3. Results

### *3.1. Subject characteristics*

A total of 551 pregnant women were recruited from two maternity hospitals in the Western province of Saudi Arabia between March 2013 and June 2016. The participants were 201 cases (36.5%) diagnosed with first-trimester spontaneous miscarriage and 350 (63.5%) age-matched healthy ... multiparous women in the immediate period following normal vaginal delivery. For the case group, 57.2% (n = 115) of women were recruited from Makkah city and the remained were from Jeddah. The control subjects were equally distributed between both cities. Details of the numbers of recruited patients from each study centre are summarised in Table 1. Additionally, there were no significant difference (P = 0.6) in age between the case (Range 20-40 years; mean± SD 34.6 ± 5.3 years) and control (Range 20-40 years; mean ± SD 33.9 ± 5.6 years) groups.

**Table 1:** Details of recruitment of the 551 study participants according to study groups and study centres during the 3 years period of the study.

| | Maternity Hospital-Makkah | | | | Maternity Hospital-Jeddah | | | |
|---|---|---|---|---|---|---|---|---|
| | Year 1 | Year 2 | Year 3 | Total | Year 1 | Year 2 | Year 3 | Total |
| Abortion group (n = 201) | 40 | 36 | 39 | 115 | 26 | 33 | 27 | 86 |
| Control group (n = 350) | 58 | 63 | 54 | 175 | 61 | 55 | 59 | 175 |
| Total (n = 135) | 98 | 99 | 93 | 290 | 87 | 88 | 86 | 261 |

The socio-economics characteristics of the study population are summarised in table 2. In general, all women from both groups were multiparous (≥ 1) with livebirths except for 9.9% of the case group (n = 20/201) who were primigravida. Regarding educational levels among the study participants, there was no significant difference between both groups and 6.9% (n = 38) had primary or lower education, 13.6% (n = 74) attended high school and 79.5% (n = 439) with college or higher education (Table 2).

Separating the study population according to profession showed that 22.8% (n = 126) were housewives, 14.7% (n = 81) were working in a day-care/nursery, 11.3% (n = 62) were healthcare professionals and the remaining (51.2%) were in assorted professions. The frequencies of housewives (33.8% vs. 16.6%; P = 0.0001) and working in a child day-care/nursery (22.4% vs. 10.3%; P = 0.0003) were significantly more prevalent in the case compared with control group. Based on the data of household income per month, 5.2% (n = 29) earned < 3000 Saudi riyals (SR), 18.9% (n = 104) were ≥ 3000 and < 5000 SR, 43.4% (n = 239) were ≥ 5000 and < 10000 SR and the remainder (32.5%) were ≥ 10000 SR (Table 2).

Additionally, the rates of having a child enrolled in a day-care/nursery was significantly (P = 0.01) more frequent in the case group (43.3%) compared with control (31.7%) and was associated with a significantly 1.5 times higher risk of having abortion (COR 1.65; 95% CI: 1.13–2.4; P = 0.01). Similarly, 28.4% of the case group were resident in a rural area and this prevalence was significantly higher ($P = 0.01 \times 10^{-5}$) than the control group (13.2%) and was associated with six times greater risk of developing abortion (COR

6.77; 95% CI: 3.73–12.29; P = 0.01 X $10^{-5}$). Not sure about p values some further in text $10^{-17}$?

**Table 2:** Maternal Socio-economic characteristics and their distribution in the study control and case groups.

| *Maternal Characteristics* | | Control Group % (n = 350) | Abortion group % (n = 201) | P Value | Total % (n = 551) |
|---|---|---|---|---|---|
| Age groups (years) | *20 – 25 years* | 16.6% (58) | 16.4% (33) | 0.6 | 16.5 (91) |
| | *25 – 30 years* | 28.3% (99) | 29.8% (60) | 0.8 | 28.8 (159) |
| | *30 – 35 years* | 28.6% (100) | 24.9% (50) | 0.4 | 27.2 (150) |
| | *> 35* | 26.6% (93) | 28.8% (58) | 0.6 | 27.4 (151) |
| Parity | *≤ 1* | 32% (112) | 33.3% (67) | 0.3 | 32.5 (179) |
| | *2* | 29.1% (102) | 34.3% (69) | 0.2 | 31 (171) |
| | *≥ 3* | 38.8% (136) | 32.3% (65) | 0.1 | 36.5 (201) |
| Educational level | *Primary or below* | 6% (21) | 8.4% (17) | 0.4 | 6.9 (38) |
| | *High school* | 8.3% (29) | 22.4% (45) | 0.06 | 13.6 (74) |
| | *College and above* | 81.1% (284) | 69.2% (155) | 0.03 | 79.5 (439) |
| Monthly Household income | *≤ 3000 SR* | 4.8% (17) | 5.8% (12) | 0.2 | 5.2 (29) |
| | *> 3000 & ≤ 5000 SR* | 17.1% (60) | 21.9% (44) | 0.2 | 18.9 (104) |
| | *> 5000 & ≤ 10000 SR* | 44.3% (155) | 41.8% (84) | 0.05 | 43.4 (239) |
| | *> 10000 SR* | 33.7% (118) | 30.3% (61) | 0.001 | 32.5 (179) |
| Profession | *Housewife* | 16.6% (58) | 33.8% (68) | 0.0001 | 22.8 (126) |
| | *Child day-care/nursery* | 10.3% (36) | 22.4% (45) | 0.0003 | 14.7 (81) |
| | *Healthcare* | 10.8% (38) | 11.9% (24) | 0.1 | 11.3 (62) |
| | *Other* | 62.3% (218) | 31.8% (64) | 0.01 X $10^{-12}$ | 51.2 282) |
| Having a child in a day-care/nursery | *No* | 68.3% (239) | 56.7% (114) | 0.01 | 64.1 (353) |
| | *Yes* | 31.7% (111) | 43.3% (87) | | 35.9 (198) |
| Residency | *Urban* | 86.8% (304) | 71.6% (144) | 0.01 X $10^{-5}$ | 81.3 (448) |
| | *Rural* | 13.2% (46) | 28.4% (57) | | 18.7 (103) |

### *3.2. Seroprevalence of CMV IgM and IgG antibodies*

The overall seroprevalence of IgG and IgM antibodies against CMV in the study participants were 88.4% (cases) and 13.6% (controls), respectively. The frequency of exclusively positive cases for IgM antibodies was 3.2% while the rate of concurrently positive cases for both antibodies was 10.3% (Table 3). None of the tested samples showed equivocal reaction against IgG antibodies but 15 women (2.7%) had equivocal results against CMV IgM antibodies.

By further analysis, there was no significant difference (P = 0.7) in the total rates of detecting CMV IgG antibodies between the control (88%) and case (89.1%) groups. However, a significant increase (P = 0.06 X $10^{-18}$) in the overall frequency of IgM against CMV was detected in the case (30.3%) compared with control (4%) and the detection of CMV IgM was associated with a significantly greater risk of having early spontaneous abortion (COR 10.4; 95% CI: 5.66–19.31; P = 0.03 X $10^{-16}$). Additionally, the prevalence of dual seropositivity for both IgM and IgG was significantly higher (P = 0.01 X $10^{-10}$) in the case group (22.4%) compared with controls (3.4%) and was associated with a significantly increased risk of developing miscarriage (COR 8.1; 95% CI: 4.18–15.79; P = 0.06 X $10^{-10}$). Similarly, the sole detection of IgM antibodies was significantly more prevalent (P = 0.02 X $10^{-11}$) in the case (8%) compared with controls (0.6%) and was associated with a significantly higher risk of abortion (COR 15.04; 95% CI: 3.42–66.15; P = 0.0004). The age-adjusted odds ratio with 95% CI for the detection of CMV infection by the different methods are summarised in table 3.

**Table 3:** Distribution of CMV serological and PCR results in the study groups with age adjusted odds ratio (A-OR) and 95% confidence intervals (95% CI) for developing spontaneous abortion.

| *CMV Serology* | | N (%) | Control (%) | Abortion (%) | A-OR [95% CI] | P value |
|---|---|---|---|---|---|---|
| Overall IgG positive | *No* | 64 (11.6) | 42 (12) | 22 (10.9) | 1.09 [0.63 – 1.89] | 0.7 |
| | *Yes* | 487 (88.4) | 308 (88) | 179 (89.1) | | |
| | *Total N* | 551 (100) | 350 | 201 | | |
| Overall IgM positive | *No* | 461 (83.7) | 332 (94.9) | 129 (64.2) | 6.82 [4.12 – 11.28] | 0.7 X $10^{-13}$ |
| | *Equi-vocal* | 15 (2.7) | 4 (1.1) | 11 (5.5) | | |
| | *Yes* | 75 (13.6) | 14 (4) | 61 (30.3)* | | |
| | *Total N* | 551 (100) | 350 (100) | 201 (100) | | |
| Overall PCR positive | *No* | 495 (89.8) | 345 (98.6) | 150 (74.6) | 24.24 [9.45 – 62.16] | 0.3 X $10^{-10}$ |
| | *Yes* | 56 (10.2) | 5 (1.4) | 51 (25.4)* | | |
| | *Total N* | 551 (100) | 350 (100) | 201 (100) | | |
| Sole IgG positive | *No* | 127 (23.1) | 51 (14.6) | 76 (37.8) | 0.27 [0.17 – 0.41] | 0.8 X $10^{-9}$ |
| | *Yes* | 424 (76.9) | 299 (85.4) | 125 (61.2)* | | |
| | *Total N* | 551 (100) | 350 (100) | 201 (100) | | |
| Sole IgM positive | *No* | 533 (96.7) | 348 (99.4) | 185 (92) | 16.03 [3.62 – 70.99] | 0.02 X $10^{-11}$ |
| | *Yes* | 18 (3.3) | 2 (0.6) | 16 (8)* | | |
| | *Total N* | 551 (100) | 350 (100) | 201 (100) | | |
| Dual IgM & IgG positive | *No* | 494 (89.7) | 338 (96.6) | 156 (77.6) | 8.29 [4.25 – 16.17] | 0.5 X $10^{-9}$ |
| | *Yes* | 57 (10.3) | 12 (3.4) | 45 (22.4)* | | |
| | *Total N* | 551 (100) | 350 (100) | 201 (100) | | |
| PCR & sole IgM positive | *No* | 537 (97.5) | 349 (99.7) | 188 (93.5) | 25.42 [3.28 – 197.12] | 0.0002 |
| | *Yes* | 14 (2.5) | 1 (0.3) | 13 (6.5)* | | |
| | *Total N* | 551 (100) | 350 (100) | 201 (100) | | |
| PCR with dual IgM & IgG positive | *No* | 517 (93.8) | 348 (99.4) | 169 (84.1) | 34.1 [8.05 – 144.38] | 0.1 X $10^{-5}$ |
| | *Yes* | 34 (6.2) | 2 (0.6) | 32 (15.9)* | | |
| | *Total N* | 551 (100) | 350 (100) | 201 (100) | | |

* = P < 0.01 compared with control by Chi-square analysis.

### *3.3. Rates of CMV infection by PCR in tissues collected from the study groups*

All standards that are included within the analysis kit as positive control measures showed an overall positive signal with the detection of human *β-globin* gene in all extracted samples (data not shown). Additionally, there was no signal detected in the negative control wells, reassuring the observed results of this study.

In general, CMV DNA was detected in 10.2% of tissue samples obtained from the 551 women of both study groups (Table 3). There were 51 (25.4%) positive samples from the products of conception and the rate was significantly higher (P = 0.03 x$10^{-17}$) than those detected in placental tissues collected from controls (1.4%). The detection of CMV by PCR in the products of conception was also associated with a 24 fold greater risk of developing spontaneous abortion (Table 3).

Among those cases with positive IgM serology, 64% were also positive by PCR. Interestingly, 8 cases (53.3%) of those participants with equivocal IgM results (n = 15) were also positive with PCR. Additionally, 14 subjects (77.8%) from both study groups were exclusively positive for IgM antibodies and also had positive PCR results for CMV in the collected tissue specimens. The overall rate of detecting both CMV antibodies (IgG & IgM) and PCR was 6.2%. None of the women with sole positive IgG antibodies was positive by PCR in both study groups. The distribution of CMV PCR findings according to study groups, serological results and maternal socio-economic characteristics is summarised in Table 4.

**Table 4:** Distribution of PCR CMV positive tissue samples according to study groups, serological results and maternal socio-economic characteristics.

| | | | N | PCR with sole positive CMV IgM | PCR with dual positive CMV IgG & IgM |
|---|---|---|---|---|---|
| | | | | N positive cases (%) | N positive cases (%) |
| Age groups (years) | > 20 & ≤ 25 | C G | 58 | ND (0) | ND (0) |
| | | A G | 33 | 6 (18.2)[b] | 5 (15.1)[b] |
| | > 25 & ≤ 30 | C G | 99 | ND (0) | 1 (1) |
| | | A G | 60 | 3 (5) | 14 (23.3)[b,**] |
| | > 30 & ≤ 35 | C G | 100 | 1 (1) | 1 (1) |
| | | A G | 50 | 2 (4) | 8 (16)[b] |
| | > 35 | C G | 93 | ND (0) | ND (0) |
| | | A G | 58 | 2 (3.4) | 5 (8.6)[b] |
| Parity | ≤ 1 | C G | 112 | ND (0) | 1 (0.9) |
| | | A G | 67 | 5 (7.4)[b] | 5 (7.4)[a] |
| | 2 | C G | 102 | ND (0%) | ND (0) |
| | | A G | 69 | 4 (5.8)[a] | 11 (15.9)[b,**] |
| | ≥ 3 | C G | 136 | 1 (0.75) | 1 (0.75) |
| | | A G | 65 | 4 (6.1)[a] | 16 (24.6)[b,**] |
| Educational level | Primary or below | C G | 21 | 1 (4.7) | 1 (12.5) |
| | | A G | 17 | 4 (23.5) | 10 (42.8) |
| | High school | C G | 45 | ND (0) | 1 (3.8) |
| | | A G | 29 | 9 (31)[b] | 12 (44)[b] |
| | College and above | C G | 284 | ND (0) | ND (0) |
| | | A G | 155 | ND (0) | 10 (10.2)[b,*] |
| Monthly Household income | ≤ 3000 SR | C G | 17 | 1 (5.9) | 1 (5.9) |
| | | A G | 12 | 4 (33.3)[a] | 5 (41.6)[a] |
| | > 3000 & ≤ 5000SR | C G | 60 | ND (0) | 1 (1.6) |
| | | A G | 44 | 8 (18.2)[b] | 13 (29.5)[b,*] |
| | > 5000 & ≤ 10000 SR | C G | 155 | ND (0) | ND (0) |
| | | A G | 84 | 1 (1.2) | 10 (11.9)[b,**] |
| | > 10000 SR | C G | 118 | ND (0) | ND (0) |
| | | A G | 61 | ND (0) | 4 (6.5)[b,*] |
| Profession | Housewife | C G | 58 | ND (0) | 2 (3.4) |
| | | A G | 68 | 5 (7.3)[b] | 17 (25)[b,**] |
| | Child day-care/nursery | C G | 36 | 1 (2.7) | ND (0) |
| | | A G | 45 | 8 (17.8)[b] | 8 (17.8)[b] |
| | Healthcare | C G | 38 | ND (0) | ND (0) |
| | | A G | 24 | ND (0) | 3 (12.5)[a] |
| | Other | C G | 218 | ND (0) | ND (0) |
| | | A G | 64 | ND (0) | 4 (6.2)[b,*] |
| Having a child in a daycare / nursery | No | C G | 239 | ND (0) | ND (0) |
| | | A G | 114 | 3 (2.6)[b] | 9 (7.9)[b,**] |
| | Yes | C G | 111 | 1 (0.9) | 2 (1.8) |
| | | A G | 87 | 10 (11.5)[b] | 23 (26.4)[b,**] |
| Residency | Urban | C G | 304 | ND (0) | ND (0) |
| | | A G | 144 | 7 (4.6)[b] | 13 (9)[b,**] |
| | Rural | C G | 46 | 1 (2.2) | 2 (4.4) |
| | | A G | 57 | 6 (10.5)[b] | 19 (33.3)[b,**] |

C G: Control Group A G: Abortion Group

(N/A = not applicable, ND = not detected, a = P < 0.05 compared with control, b = P < 0.01 compared with control, * = P < 0.05 compared with IgM only positive cases and ** = P < 0.01 compared with IgM only positive cases).

### *3.4. Factors associated with spontaneous abortion and/or CMV genital tract infection among the study population*

Logistic regression model that included the socio-economic characteristics, CMV serology and PCR results showed that the odds ratio of developing spontaneous abortion was significantly higher in housewives, above 35 years of age, ≤ 1 parity, primary or lower education level and living in a rural area (Table 5). Additionally, the exclusive detection of IgM antibodies against CMV with or without positive PCR significantly increased the risk of spontaneous abortion by more than 15 folds (Table 5). The highest significant odds ratio was observed for those women with positive IgM, IgG and PCR results and was associated with a 27 fold increase in the risk of spontaneous abortion. Interestingly, the sole detection of CMV IgG antibodies significantly decreased the risk of abortion by 3 fold (Table 5).

**Table 5:** Maternal socio-economic characteristics, CMV serology and PCR results and odds ratio (OR) with 95% confidence intervals [95% CI] for developing spontaneous abortion.

| | Variable Categories | N | Control (%) | Abortion (%) | Logistic Regression | |
|---|---|---|---|---|---|---|
| | | | | | OR [95% CI] | P value |
| Age groups (years) | 20 - 25 years | 91 | 58 (63.8) | 33 (36.2) | Ref. | - |
| | 26 - 30 years | 159 | 99 (62.7) | 60 (37.3) | 1.23 [0.63 – 2.4] | 0.5 |
| | 31 - 35 years | 150 | 100 (66.7) | 50 (33.3) | 1.17 [0.57 – 2.41] | 0.6 |
| | > 35 years | 151 | 93 (61.6) | 58 (38.4) | 2.64 [1.18 – 5.91] | 0.01 |
| Parity | ≤ 1 | 179 | 112 (62.6) | 67 (37.4) | Ref. | - |
| | 2 | 171 | 102 (61.5) | 69 (38.5) | 1.01 [0.58 – 1.76] | 0.9 |
| | ≥ 3 | 201 | 136 (67.7) | 65 (32.3) | 0.40 [0.20 – 0.78] | 0.007 |
| Educational level | Primary or below | 38 | 21 (56.3) | 17 (44.7) | Ref. | - |
| | High school | 74 | 45 (60.8) | 29 (39.2) | 0.10 [0.039 – 0.28] | 0.0001 |
| | College and above | 439 | 284 (64.7) | 155 (35.3) | 0.046 [0.010 – 0.22] | 0.0001 |
| Monthly Household income | ≤ 3000 SR | 29 | 17 (58.6) | 12 (41.4) | Ref. | - |
| | > 3000 & ≤ 5000SR | 104 | 60 (57.7) | 44 (42.3) | 0.90 [0.56 – 1.45] | 0.7 |
| | > 5000 & ≤ 10000 SR | 239 | 155 (64.9) | 84 (35.1) | 0.31 [0.14 – 1.62] | 0. 1 |
| | > 10000 SR | 179 | 118 (65.9) | 61 (34.1) | 0.43 [0.22 – 0.86] | 0.01 |
| Profession | Housewife | 126 | 58 (46.9) | 68 (53.1) | Ref. | - |
| | Child day-care/nursery | 81 | 36 (44.5) | 45 (55.5) | 0.48 [0.23 – 0.99] | 0.04 |
| | Healthcare | 62 | 38 (62.5) | 24 (37.5) | 0.31 [0.14 – 0.67] | 0.003 |
| | Other | 282 | 218 (77.3) | 64 (22.7) | 0.15 [0.08 – 0.29] | 0.2 X$10^{-8}$ |
| Having a child in a day-care/nursery | No | 353 | 239 (67.7) | 114 (32.3) | Ref. | - |
| | Yes | 198 | 111 (56.1) | 87 (43.9) | 1.29 [0.82 – 2.02] | 0.2 |
| Residency | Urban | 448 | 304 (67.9) | 144 (32.1) | Ref. | - |
| | Rural | 103 | 46 (44.7) | 57 (55.3) | 1.73 [0.92 – 3.26] | 0.08 |
| Sole IgG positive | No | 127 | 51 (40.2) | 76 (59.8) | Ref. | - |
| | Yes | 424 | 299 (70.5) | 125 (29.5) | 0.27 [0.16 – 0.46] | 0.1 X$10^{-4}$ |
| Sole IgM positive | No | 533 | 348 (65.3) | 185 (34.7) | Ref. | - |
| | Yes | 18 | 2 (21.1) | 16 (88.9) | 17.1 [3.38 – 86.68] | 0.001 |
| Dual IgM & IgG positive | No | 494 | 338 (68.4) | 156 (31.6) | Ref. | - |
| | Yes | 57 | 12 (21.6) | 45 (78.4) | 8.39 [3.81 – 18.43] | 0.1 X$10^{-6}$ |
| PCR & sole IgM positive | No | 537 | 349 (65) | 188 (35) | Ref. | - |
| | Yes | 14 | 1 (7.2) | 13 (92.8) | 27.99 [3.17 – 246.52] | 0.003 |
| PCR with IgM & IgG positive | No | 517 | 348 (67.3) | 169 (32.7) | Ref. | - |
| | Yes | 34 | 2 (5.9) | 32 (94.1) | 29.3 [6.52 – 131.71] | 0.1 X$10^{-5}$ |

Ref. = indicates reference group to which the other group(s) were compared

Furthermore, regression analysis also revealed that age < 30 years, multiparous (≥ 2), primary or lower education, resident of rural areas and working or having a child in a nursery significantly increased the

risk of CMV reproductive tract infection that can be detected by PCR (Table 6).

**Table 6:** Maternal socio-economic characteristics and adjusted odds ratio (AOR) with 95% confidence intervals (95% CI) for reproductive tract infection with CMV as indicated by PCR results.

| | Variable Categories | N | Reproductive tract CMV infection | | Logistic Regression | |
|---|---|---|---|---|---|---|
| | | | Yes (%) | No (%) | AOR [95% CI] | P value |
| Age groups (years) | 20 – 25 years | 91 | 12 (13.2) | 79 (86.8) | Ref. | - |
| | 25 – 30 years | 159 | 20 (12.6) | 139 (87.4) | 0.61 [0.18 – 1.99] | 0.4 |
| | 30 – 35 years | 150 | 14 (9.3) | 136 (90.7) | 0.29 [0.075 – 1.2] | 0.08 |
| | > 35 years | 151 | 9 (5.9) | 142 (94.1) | 0.10 [0.02 – 0.47] | 0.004 |
| Parity | ≤ 1 | 179 | 11 (6.1) | 168 (93.9) | Ref. | - |
| | 2 | 171 | 18 (10.5) | 153 (89.5) | 2.76 [1.1 – 8.48] | 0.03 |
| | ≥ 3 | 201 | 26 (12.9) | 175 (87.1) | 8.25 [2.31 – 29.44] | 0.001 |
| Educational level | Primary or below | 38 | 16 (42.1) | 22 (57.9) | Ref. | - |
| | High school | 74 | 22 (29.7) | 52 (70.3) | 0.78 [0.24 – 2.51] | 0.6 |
| | College and above | 437 | 17 (3.9) | 422 (96.1) | 0.27 [0.07 – 0.86] | 0.03 |
| Monthly Household income | ≤ 3000 SR | 29 | 11 (37.9) | 18 (62.1) | Ref. | - |
| | > 3000 & ≤ 5000SR | 104 | 22 (21.1) | 82 (78.9) | 0.69 [0.18 – 2.57] | 0.6 |
| | > 5000 & ≤ 10000 SR | 239 | 15 (6.3) | 224 (93.7) | 0.41 [0.10 – 1.59] | 0.2 |
| | > 10000 SR | 179 | 7 (3.9) | 172 (96.1) | 0.31 [0.08 – 0.78] | 0.02 |
| Profession | Housewife | 126 | 25 (19.8) | 101 (80.2) | Ref. | - |
| | Child day-care/nursery | 81 | 21 (25.9) | 60 (74.1) | 1.7 (0.80 – 4.77) | 0.03 |
| | Healthcare | 62 | 4 (6.4) | 58 (93.6) | 0.61 (0.15 – 2.5) | 0.4 |
| | Other | 282 | 5 (1.8) | 277 (98.2) | 0.14 (0.04 – 0.50) | 0.002 |
| Having a child in a day-care/nursery | No | 353 | 15 (4.2) | 338 (95.8) | Ref. | - |
| | Yes | 198 | 40 (20.2) | 158 (79.8) | 5.13 (2.30 – 11.43) | 0.6 $X10^{-5}$ |
| Residency | Urban | 448 | 21 (4.7) | 427 (95.3) | Ref. | - |
| | Rural | 103 | 34 (32.7) | 69 (67.3) | 5.52 (2.41 – 12.64) | 0.5 $X10^{-5}$ |

Ref. = indicates reference group to which the other group(s) were compared

## 4. Discussion

Herein, the prevalence of CMV was measured by serology and PCR in samples obtained from women diagnosed with first trimesteric spontaneous abortion and the results were compared with those samples collected at the time of normal vaginal delivery from age-matched healthy post-delivery women. The results showed a high seroprevalence rate of IgG antibodies in the study case population with no significant difference in the frequency between both study groups. However, the seroprevalence of CMV IgM antibodies was significantly higher, with or without co-detection of IgG antibodies, in the spontaneous abortion group. Furthermore, the viral DNA was more frequently detected by PCR in the products of conception from the case group compared with those placental specimens obtained from controls. These observations are in parallel with the findings of several previous studies that showed high rates of CMV immunity among their populations and support the notion that this viral infection is more commonly detected in women with early pregnancy loss [21-26].

Additionally, this is the first study to report that, among several factors, CMV infection during early pregnancy independently and significantly increased the risk of developing spontaneous abortion in Saudi Arabia. The present study also showed that the detection of CMV IgM exclusively or together with IgG antibodies significantly increased the risk of abortion by 17 and 8 fold, respectively. These observations suggest that acute CMV infection in early pregnancy, as indicated by sole IgM positivity, could have a worse pregnancy outcome compared with viral re-activation/re-infection in early pregnant women. Interestingly, the detection of CMV in tissues by PCR was associated with 28- and 29-fold increase in spontaneous abortion when simultaneously positive with either sole IgM or both IgM and IgG antibodies, respectively. The current findings, therefore, suggest that intrauterine CMV presence during early pregnancy, either in the form of acute infection or viral re-activation/re-infection/persistence, could be a major risk factor for early pregnancy loss in the kingdom.

CMV has been estimated to infect 0.15–2% of pregnant women and the virus can later be transmitted in utero leading to several pre- and postnatal complications [6, 18]. This viral infection is usually asymptomatic, latent and the rates increase dramatically during pregnancy, mainly due to the associated suppression of maternal immune system [27]. The acquisition of CMV during pregnancy could occur either following primary exposure, viral reactivation or reinfection with a different viral strain [28]. Additionally, the rates of recurrent CMV is more common than primary infection during pregnancy [29], suggesting that a prior exposure to the virus does not provide full immunity against intrauterine transmission [21].

The present findings correlate and provide further support to the aforementioned observations since they demonstrated an overall seroprevalence of 88.4% for IgG antibodies and 13.6% for IgM antibodies. Currently, little is known about the frequency of CMV during pregnancy in Saudi Arabia and only a single study has reported a seroprevalence of 92.1% for CMV IgG antibodies in 926 first trimester healthy pregnant women, which is in agreement with the current findings [30]. Furthermore, similar seroprevalence of CMV has also been reported in pregnant women from several other regional [21, 23] and international countries [24-26]. The current observations advocate that the majority of women of childbearing age in the Western province of Saudi Arabia were previously exposed to CMV infection and have acquired natural immunity against the virus prior to pregnancy. Therefore, these women could, theoretically, be at higher risk of acquiring CMV infection during subsequent pregnancies following viral reactivation and/or re-infection with a different strain [28, 31]. This hypothesis could further be supported by the observed significant higher rate of simultaneously positive IgM and IgG antibodies (10.3%) than the sole detection of IgM against the virus (3.2%) in our study population.

Nevertheless, the results of IgM should be cautiously interpreted since the majority of available IgM serological assays are associated with high false-positive rate [12, 13]. Additionally, IgM antibodies could persist for months following a primary exposure to the virus and/or could be produced against other viruses such Epstein-Barr virus [15]. Hence, serological results cannot accurately differentiate between primary and reinfection/reactivation of CMV infection [12-15]. The IgG avidity test is an alternate method that is currently applied for more precise diagnosis of primary infection serologically in clinical settings. Nevertheless, the performance of this diagnostic assay is limited due to the high rate of intermediate avidity reaction and lack of appropriate cut-off for low avidity [14, 15]. Therefore, the present study used an IVD CE singleplex PCR kit to confirm the serological findings since the detection of CMV DNA by PCR is another feasible approach for the diagnosis of current infection and is associated with a much higher specificity ranging between 97-100% and positive predictive value of almost 100% [15, 17].

The results showed an overall prevalence of intrauterine CMV infection by PCR in 10.2% of the study participants and the frequency was significantly higher in the spontaneous abortion group. The present findings also revealed that, 77.8% of tissue specimens obtained from those patients who were exclusively positive for IgM antibodies were also positive by PCR, suggesting a primary CMV infection that could have had spread to the intrauterine cavity. Interestingly, the viral DNA was also detected by PCR in more than 50% of those cases with equivocal reaction for CMV IgM antibodies, supporting the notion that PCR is a more sensitive and specific tool for the diagnosis of CMV infection than serological tests [15, 17]. Additionally, 59.6% of those patients with dual IgM & IgG positive serology were also positive by PCR, advocating possible reactivation, reinfection or persistent intrauterine viral infection.

Although the majority of the previous studies focused mainly on the neonatal neurological manifestations following in utero CMV infection, the sequelae of CMV infection during pregnancy is far more complex and could result in other complications including pregnancy

loss [13]. CMV-induced pregnancy adverse outcomes are trimester-dependent and the earlier the infection the more severe the complications [27]. In this regards, the acquisition of CMV during peri-conceptional or early weeks of pregnancy has been associated with miscarriage [29]. Spano et al. have also shown that 75% of archived placental tissues collected from first trimesteric abortion were positive for CMV by immunofluorescence [32]. Another study from Japan has also demonstrated a rate of 7.1% of CMV infection by using Real-Time PCR and vaginal swab samples obtained from 848 healthy pregnant women and CMV infection was associated with 7 folds greater relative risk for spontaneous abortion [10]. A later study has also shown significantly greater prevalence of CMV DNA by PCR in placental tissues obtained from 62 cases of foetal deaths compared with 35 controls [33]. Similar results have also been reported by more recent serological studies in which the rates of CMV IgM seropositivity were significantly higher than controls and increased the risk of abortion [11, 34].

The findings of the present study correlate with those previously reported from a variety of populations since they showed significantly higher rates of CMV DNA and IgM antibodies in the case group compared with control. Additionally, these observations provide further support to the notion that, CMV infection during pregnancy is associated with higher risks of spontaneous abortion as shown by the results of logistic regression. The mechanisms by which CMV is disseminated into the intrauterine cavity are still largely illusive [22, 29]. However, it is believed that in utero CMV infection is initiated in the endometrium before the foetus and could result in impaired placentation and subsequently abortion and/or foetal death [29]. In this context, CMV induced villitis in infected placental tissues in vivo [35, 36] and inhibited the invasion activities of cytotrophoblasts in vitro, together with increased trophoblastic cell damage lead to the production of inflammatory cytokines [22, 37-39]. Hence, it could be postulated that, intrauterine dissemination of CMV during the peri-conceptional time and/or early weeks of pregnancy increase the risk of spontaneous abortion by inducing trophoblastic damage and compromising the process of placentation. However, further studies are needed to explore the effects of CMV infection on placental tissue integrity and invasiveness during early pregnancy.

A variety of risk factors associated with the acquisition of CMV infection during pregnancy have been identified and they include lower income [15], low level of education [28], maternal age [40], increased parity [41] and close contact with young children [40], which are in agreement with the findings of the present study. Therefore, those women at high risk could benefit from a screening program to avoid/prevent CMV associated morbidity and mortality during pregnancy. Nevertheless, the implementation of CMV screening programs is debatable and not recommended in many developed and undeveloped countries [18, 19]. Hence, large prospective cross-sectional multi-centres studies are still required to investigate the precise CMV infection rates among pregnant women and neonates in Saudi Arabia. Such research could provide strong evidence on the magnitude of this viral infection and its related adverse events during pregnancy. In the meanwhile, the introduction of preventive programs (e.g. counselling-based interventions) for those women at high risk of acquiring CMV during their pregnancy could result in lowering the rates of CMV-induced pre- and postnatal sequelae [1, 5, 14, 21, 42].

## 5. Conclusion

The majority of pregnant women in the Western province of Saudi Arabia were previously exposed to CMV and could be at risk of viral reactivation during subsequent pregnancies. CMV infection during the first trimester with the possibility of viral dissemination into the intrauterine cavity, also appears to be an independent risk for the development of spontaneous abortion. The initiation of preventive programs during antenatal consultations, especially for those women at high risk, could be beneficial in controlling the associated adverse events during pregnancy. Further studies are needed to measure the actual prevalence of CMV and its associated sequelae during pregnancy in the different regions of the kingdom.

- ***Conflict of Interest***

The authors declare that they have no competing interests

- ***Ethics approval***

Ethical approval was agreed upon by the Faculty of Applied Medical Sciences Ethics Committee (AMSEC 10-15-9-2013). All procedures performed in studies involving human participants were in accordance with the ethical standards of Umm Al-Qura University research committee and with the 1964 Helsinki declaration and its later amendments.

- ***Informed consent***

Informed consent was also obtained from all participants included in the study.

- ***Availability of data and materials***

All data generated or analysed during this study are included in this published article.

- ***Acknowledgment***

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