Introduction

Human cytomegalovirus (HCMV) is a member of the Herpesviridae family, beta-Herpesviridae subfamily (1). HCMV is linked to 70% of miscarriages and has a variety of clinical manifestations ranging from severe illness to death in immunocompromised people, such as organ and bone marrow transplant recipients, HIV and immunosuppressive patients, congenital infection of fetuses, newborns infected during pregnancy (perinatal infection), and non-hereditary sensorineural hearing loss in children (SNHL) (2-5). Over the past decades, many studies have proven the relationship between CMV infection and abortion during pregnancy, which can lead to miscarriage during pregnancy or defects at the birth of the fetus with up to 90% hearing loss, vision impairment, and mental retardation (6-8), and the mechanisms are CMV reactivation in pregnancy (9). Recurrent pregnancy loss or recurrent spontaneous abortion is the spontaneous loss of pregnancy in three or more non-consecutive miscarriages before 20-24 weeks, and its rate among women is about 1-5% (10). Vitamin D (VD) deficiency is associated with many global health problems due to its association with the immune system. It is a steroid hormone that facilitates calcium absorption, which is involved in calcium-phosphate homeostasis and has a vital role in skeletal health and growth after weaning (11,12). In addition to its role in regulating immune defenses and activating immune system cells, especially Th17 cells, regulatory T-cells, and secretion levels of the induced cytokines, its deficiency affects many autoimmune diseases (13-15). This study aims to determine the seroprevalence of CMV in miscarriage and investigate the effect of VD deficiency risks in pregnant women.
Participants
This study included 50 women suffering from recurrent miscarriage, aged between 15-40 years, and experienced several abortions (more than two to seven spontaneous miscarriages during the first trimester of pregnancy). The control group consisted of 25 women who had no previous miscarriage history, with at least one successful pregnancy, or were infertile.

Criteria for Inclusion and Exclusion
This investigation adopted the following criteria to identify the appropriate patients: recurrent miscarriages, VD deficiency, and CMV infection. Based on the mentioned criteria, we selected 50 patients. On the other hand, we excluded 25 patients due to the following exclusion criteria: no abortion history and lack of a dietary supplement.

Detection of VD Levels, CMV-IgG, and IgM Antibodies
Five milliliters of venous blood were drawn from each woman, dispensed into a gel tube for serological analysis, and processed immediately. Centrifugation treated the serum at 5000 RPM for five minutes at 4°C (16). VD, anti-CMV IgM, and anti-CMV IgG antibody samples (as tested by Cobas® e411, Roche, Germany) were analyzed by a fully automated analyzer (17,18). It is designed for qualitative and quantitative in vitro assays to determine various applications. We determined CMV-IgG, CMV-IgM, and VD levels according to the manufacturer’s instructions (Elecsys®, Roche Diagnostics, Germany).

Statistical analysis
We analyzed the data using Statistical Package for the Social Sciences (SPSS) software. The mean and standard deviation (SD) were calculated to characterize the study population. The t-test measured the statistical significance of the difference in data. P values < 0.05 are considered statistically significant.

Results
A total of 75 blood samples were drawn from women suffering from recurrent miscarriage based on age and the number of abortions, divided into abortion groups with two age groups (n = 50), 15-27 (56%) and 28-40 (44%), with the mean being 25 ± 5.8 years, and a control group (n = 25) with two age groups, 15-27 years (52%) and 28-40 years (48%), with the mean being 25 ± 6.19 years. CMV IgM and CMV IgG antibodies seropositive were 6% and 98%, respectively, with significant prevalence (P < 0.0001). The CMV IgM and IgG prevalence among females with abortions were higher, with 0.4 ± 0.21 for IgM antibodies and 259.5 ± 169.9 for IgG antibodies at higher levels compared to the control group, which was 0.18 ± 0.07 for antibodies of IgM and 98 ± 89.7 for antibodies of IgG. In addition, the IgG antibody prevalence in women within the group aged 15-27 years (58%) is very high compared to those in the group 28-40 years (40%), as shown in Table 1.

The VD level was evaluated in two groups divided into women with a history of recurrent miscarriages (7.1 ± 8.24 ng/mL and a control group (25.5 ± 6.02) ng/mL based on two age categories. 15-27 years, there were 28 (56%), and for the age group 28-36 years, there were 22 (44%) for the abortion group, with significant deficiency (P < 0.0001) for both groups. On the other hand, results for the control group were 15-27 years and 28-36 years, 13 (52%) and 12 (48%), respectively (Table 2).

The significant effect of VD deficiency on women who suffered fewer than two consecutive abortions was 16 (42.1%), while the effect was more significant (22) in women with more than two abortions (57.9%) (Table 3).

Discussion
The study showed that CMV IgM and IgG antibodies were seropositive at 6% and 98%, respectively. These results were in line with those of Koksaldi-Motor et al, in which the seropositivity of CMV IgG was 98.9% (19). Several previous studies, such as Aljumaili et al (20) in Iraq, found significant differences in the seropositive rate of CMV. They demonstrated a 95.7% increase in CMV IgG seroprevalence. In contrast, Falahi et al (21) found that the rates of CMV IgM and CMV IgG were 28.58% and 14.28%, respectively. Turbadkar et al (22) showed that the
positive rate of CMV IgM was 8.42% and CMV IgG was 91.05%, which is incompatible with our findings. In Iraq, Naame et al. (16) recorded the seropositivity of the CMV IgM at 2.5% and the CMV IgG at 30.8%. In another study, Jihad (23) discovered that CMV IgM seropositivity was 25% and CMV IgG seropositivity was 40%. Seropositivity for IgG and IgM antibodies was 58% and 2% in the 15-27 years group, respectively, compared to 40% and 4% in the 28-40 years group. Also, Jihad (23) recorded that antibodies of IgG were positive in the group of 20-30 years (75%), and IgM antibodies were 10%, but in the group of 31-40 years, the IgG and IgM antibodies were 70% and 20%, respectively, which inconsistent with the data of this study. The high level of CMV IgG at younger ages may be related to the possibility of pregnancy at younger ages. Also, the high rate of CMV IgG among women means that many are at high risk of developing infections and having major miscarriages during their pregnancy.

The findings revealed that a deficiency of VD is a significant cause of miscarriage in pregnant women. Approximately one-third of Iraqi women suffer an insufficient VD level (15 ng/mL) during pregnancy, with 32% having had fewer than two consecutive abortions and 44% having more than two abortions. Andersen et al (24) found that VD deficiency is related to first-trimester miscarriages, while a study in Australia found no difference between VD deficiency during weeks 10-14 of pregnancy (25). Chawla et al (26) showed that darker-skinned women have lower VD levels than white-skinned women. The researchers discussed the relationship between VD and sunshine exposure and how VD deficiency affects reproductive health due to its function in innate and acquired immunity and abortion protection (26, 27).

This study's clinical utility contains both practical and theoretical implications. The study's findings will add to past research by assessing the effects of CMV seroprevalence and VD levels on pregnancy. The study's findings will allow practitioners to develop strategies for assessing CMV-IgM and CMV-IgG antibodies, as well as VD levels, to reduce the risk of recurring miscarriage or abortion.

**Limitations**

As with any study, the current one has several limitations that open up prospects for future research. First, this study adopted a deductive approach. The longitudinal approach suggests many practical and theoretical contributions for practitioners and academics. The role of VD in female miscarriage was studied in women who had recurrent miscarriages. The adoption of miscarried females has shed light on the effects of abortion on pregnant women in Iraq. However, exploring the similarities and differences between cultural contexts advances new and innovative therapeutic approaches and broadens the contributions of practitioners.

**Recommendations**

The current study offers additional research or recommendations on this topic, such as:

- Adoption of routine screening for VD in women throughout pregnancy
- Use of molecular methods (RT-PCR) for CMV diagnosis
- The current study recommends conducting educational sessions for pregnant women about the risks of seroprevalence of CMV and VD deficiencies during pregnancy.

**Conclusions**

The high level of CMV IgG at younger ages is due to the high chances of pregnancy. VD deficiency has a target effect on recurrent miscarriages. The more VD deficiency, the higher risk in women suffering from more than two consecutive abortions. The contribution of this study includes the theoretical and practical implications that might be provided the clinical usage for practitioners to determine the CMV seroprevalence infection in miscarriage females by investigating the effect of VD deficiency in pregnancy and those who suffer from frequent miscarriages. Hence, the clinical use of these results is to reduce miscarriages by providing a treatment technique with low costs and fewer serious side effects.

**Authors’ Contribution**

Mays B. Jalil designed the study, developed the interview guides, data analysis, revised copy, and approved the final version. Mustafa S. Hassan: designed the study, analyzed the data, and revised it. Yarub A. Shnawa: designed the study and approved the final version.

**Conflict of Interests**

Authors declare that they have no conflict of interests.

**Ethical Issues**

The ethical committee of Al-Kunooze University College approved this study (No. 3012).

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