How to Cite:
AL-Koufi, M. K. K., & AL-Khilkhali, H. J. B. (2022). Study of some immunological markers associated with cytomegalovirus among spontaneous abortion women. *International Journal of Health Sciences*, *6*(S3), 8296–8309. https://doi.org/10.53730/ijhs.v6nS3.7897

Study of some immunological markers associated with cytomegalovirus among spontaneous abortion women

Mohammed K. K. AL-Koufi
Faculty of Science, University of Kufa, Iraq
*Corresponding author email: aswq3338@gmail.com

Huda Jameel Baker AL-Khilkhali
Faculty of Science, University of Kufa, Iraq
Email: huda.alkhilkhali@uokufa.edu.iq

***Abstract*---**The goal of this study was to investigate the existence of CMV in abortion women and also to observe if particular cytokines played a role in abortion induction. during the time between August (2021)to January(2022).total of 60 women who have previous abortions, and the control group consisted of 25 healthy pregnant women with no signs of chronic inflammatory disease were gathered from Al Rifai hospital in Dhi Qar city. The patients range in age from 19 to 50 years. CMVwere examined on ELISA in blood samples taken from abortion women and healthy controls. A total of 60 samples were taken,60(100%) were positive results for CMV while the study of pregnant women revealed nil (zero per cent) negative results for every 25 samples using the ELISA test. The distribution of aborted women according to their abortion frequency was revealed the first abortion was the highest percentage (34/60). and, most importantly, CMV positive samples at a rate of one per cent 34(56.6%) demonstrates how important this virus is in the forecast the second and third abortions resulted in a total of 21 being aborted (35%) and 5(8.3%) respectively. Interleukin-2 levels were measured using the ELISA test and had a high significance ($p<0.0001$) in aborted + CMV women (23.22±2.867) compared to pregnant women in the control group (2.312±0.6295). The results revealed a significant degree ($p<0.0001$) in aborted + CMV women in the levels of C3was (146.9±3.696) in contrast to the control (118.4±5.107). The current study showed lower significant white blood cells levels (5.203±0.8281) in aborted women with +cmv compared to the control group were (7.412±0.4661)and lower significant lymphocytes levels(1.336±0.08859) in +cmv aborted women compared to control group were (2.816±0.2416), also lower significant haemoglobin levels (9.984±0.2357) compared to the control group(11.68±0.1695) and significant level of neutrophil (4.254±0.2670)

Manuscript submitted: 27 March 2022, Manuscript revised: 9 April 2022, Accepted for publication: 18 May 2022

in +cmv aborted women in contrast to the control(3.224±0.2375), On the one hand, PLT. The results showed lower significant levels (199.4±11.88) in +cmv aborted women compared to control group (273.6±14.59). The current study's findings indicated that the risk of infection with cytomegalovirus (CMV) In pregnant women, increases the danger of abortion in addition to an increase in the levels of some interleukins increasing the possibility of miscarriage. This gives more importance to detecting viruses and the level of interleukins specific to IL-2 and decreases some haematological Parameters such as haemoglobin (Hb) levels, PLT, and lymphocytes which led to an abortion.

***Keywords*---**CMV, pregnant abortions, ELISA test.

Introduction

Abortion is defined as the procedure of terminating a pregnancy by removing a fetus or embryo from the uterus before it can live outside of it (Grimse and Stuart,2010). Maternal morbidity and mortality associated with unsafe abortion complications have been identified as serious public health issues. Furthermore, all hazardous abortions occur in developing nations, where ninety-eight per cent of abortion-related deaths occur. Each year, around 56 million abortions are performed worldwide, with less than half of them occurring in an unsafe manner (Yezlankyzy and Niazbekov, 2015). Recurrent pregnancy loss (RPL) is caused by a variety of organisms that can be transmitted in utero at various stages of pregnancy, including herpes viruses, cytomegalovirus, rubella virus, and Toxoplasma gondii (Hussan, 2013). There are several causes of abortion, including viral ones, and the important virus that causes abortion is cytomegalovirus. Cytomegalovirus (CMV) is a DNA virus that belongs to the Herpesviridae family's Betaherpesvirinae subfamily. Congenital CMV infection, which is more common in low-income families Colugnati et al.,(2007), can have major clinical effects (Dreher *et al.*,2014). The risk of primary CMV infection during pregnancy is significant, given the relatively high number of CMV seronegative women of reproductive age (Naing *et al.,*2016). Furthermore, unlike other infectious diseases, the risk of fetal infection from CMV infection during pregnancy is higher in the general population, due to the high incidence of serological positive in women of reproductive age. CMV can produce reactivations in pregnant women after a first infection, allowing for the detection of fetal infection (Britt, 2015). Transmission can happen at any time during pregnancy, but it is most common in the first trimester (Enders *et al* .,2011).

Materials and Methods

Whole samples were collected from Al Rifai teaching hospital in Dhi Qar city between August and January of 2021. (2022). A total of 60- women have had previous abortions, with another 25 cases of normal pregnancies(parentallyhealthy) serving as a control group, with ages ranging from 19 to 50. All of the samples were divided into two groups, The first group

contains sixty samples and represents women who have had recurrent abortions while the second group contains twenty-five samples and represents a control.

Collecting blood samples

Blood samples were obtained in the virus section of Al Rifai hospital in Dhi Qar city, with about five millilitres of blood extracted from each patient via vein puncture and collected in two groups of tubes, one of which included anti-coagulant to assess total blood count and the other group, which did not have an anti-coagulant as well as a simple tube to use for serum preparation, were centrifuged for five minutes at three thousand rotations per minute to obtain serum, which was then contained in other tubes before being transported to sterile tubes using aM micropipette with sterile disposable tips. The serial number was assigned to the samples, and the full samples were kept in the refrigerator in a degree frozen case at (-20°C) until they were.

ELISA kit for detecting CMV IgG antibodies in human serum (ACON, Chain)
A-Assay procedure

- Added 100 µl of calibrator 1 in wells B1 and C1 (yellow reagent)
  - Added 100 µl of calibrator 2 in wells D1and E1(blue reagent)
  - Added 100 µl of calibrator3 in wells F1and G1(blue reagent)
  - Added 100 µl of calibrator4 in wells H1andA2(blue reagent)
- Added 100 µl of specimen diluent to assigned wells starting at B2(Green reagent)
- Added 5 µl of the specimen to assigned wells starting at B2 Then colour change from green to blue will occur to verify that the
- Mixed gently by swirling the microwell plate on a flat bench for 30 seconds.
- Covered the microwell plate with the plate sealer and incubated in a water bath or incubated at 37C for 30 minutes.
- Removed the plate sealer.
  - Washed each well 5 times with 350 µl of working wash buffer per well, then removed the liquid.
  - Turned the microwell plate upside down on absorbent tissue for a few seconds .ensured that all wells have been completely washed and dried .
- Added 100 µl of the conjugate to each well except for the Blank well. (Red reagent)
- Covered the microwell plate with the plate sealer and incubated in a water bath or incubated at 37C for 30 minutes.
- Washed each well 5 times with 350 µl of working wash buffer per well, then removed the liquid.
- Turned the microwell plate upside down on absorbent tissue for a few seconds and ensured that all wells have been completely washed and dried .
- Added 50 µl of substrate A to each well. (clear reagent)
- Added 50 µl of substrate B to each well. (clear reagent)
- Then blue colour should develop in wells containing positive specimens.
- Mixed gently then covered microwell plate with a plate sealer and incubated in a water bath or incubated at 37Cfor 10 minutes.
- Removed the plate sealer.

- Added 50 µl of stop solution to each well. (clear reagent)
- Then a yellow colour should be developed in wells containing positive specimens.
- Readed at 450/630-700nm in 30 minutes.

Calculation of results

Create a calibration curve and calculate the quantitative specimen result.

- Subtract the blank absorbance from the mean absorbance of each calibrator, then plot them on the Y-axis on the linear graph paper against their concentration in U/ml on the X-axis to build the calibration curve.
- Using the calibration curve, obtain quantitative specimen data from their absorbance.

Table 1
Analysis of Results

	Concentration
Negative	< 13.5 U/ml
Positive	> 16.5 U/ml
Equivocal	13.5-16.5 U/ml

If the results are ambiguous, the specimens should be re-tested in triplicate and the average value calculated before making a decision. Consistently ambiguous re-testing specimens should be done using a different approach. If the results are still ambiguous, take another sample in two weeks. The specimen is believed to be positive if the new specimen is positive.

Result and Dissection

Distribution of CMV according to the age group of aborted and pregnant women

According to the findings of the current study, the samples were dispersed according to age as follows:18 (30%) at 16-22 years,19 (31.6 %) at 23-29 years,16(26.6 %) at 30-36 years,4 (6.6 %) at 37-43 years, and 3(5 %)at 44 -50 years. Pregnant ladies, on the other hand, can be demonstrated as:5 (8.3%) at 16-22 years,8 (13.3 %) at 23-29 years, 9 (15%) at 30-36 years,1 (1.6 %) at 37-43 years, and 2 (3.3 %) at 44 -50 years, as shown in the table (2), The findings in aborted and pregnant women aged 23-29 years (31.6%) and 30-36 years(26.6%) it were consistent with the researcher's findings of A Naqid *et al.*,(2020), who found the highest occurrence at the same age. While the current findings contradict those of a previous study by Mohammad and Salman,(2014), who found that the most abortions occurred in those aged 42 and up, the reasons for the increased prevalence in people between 23 and 29 are discussed. The first explanation is the relationship between the highest risk of transmission to women infected with The herpes virus during pregnancy, particularly in the last trimester. The second reason is that the illness spreads from person to person

through contact, particularly between partners, without the persons being aware of their infection status, as well as women who are in a reproductive condition at this age. an infection could have occurred as a result of virus shedding or periods of minor outbreaks Wood,(2011). Certainly, increases range between 9% and 12% in adult females under the age of 35 but climb to 15% in adult females above the age of 40. Due to features connected to the differences of each, miscarriage will be more classified as fetal loss or fetal abortion if it occurs after ten weeks of gestation and results in the loss of an embryo, or first-time abortion if it occurs before ten weeks of pregnancy (Kolte *et al.*, 2015).

Table 2
Shows the age distribution of abortions and control (pregnant) women

Age group	Aborted women No	Per cent %	Control(Pregnant) women No	Per cent %
16-22	18	30%	5	20%
29-23	19	31.6%	8	32%
30-36	16	26.6%	9	36%
37-43	4	6.6%	1	4%
44-50	3	5%	2	8%
Total	60		25	

Distribution of CMV infection between aborted and controlled Women (pregnant) according to the pregnancy trimesters

According to the stages of pregnancy trimesters, the majority of abortions occurred in the first trimester (34/60), and positive instances of CMV 34 (56.6%), other cases of abortion occurred in the second and third trimesters were reached (21/60) and (5/60), respectively, and positive cases of CMV 21(35%),5(8.3%) sequentially. this outcome was in agreement with Picone *et al.*,(2013) who stated that When compared to subsequent infections, perinatal outcomes are worse with first-trimester maternal infection. Not only is there a higher rate of aberrant outcomes, but the consequences are also more severe. The probability of an unfavourable perinatal outcome in fetuses confirmed to be infected ranges from 20% to 45 per cent for first trimester infections to 6% to 17% for second-trimester infections(Enders *et al.*,2011). However, the conclusions of this study differ from those of the study Feldman *et al.*,(2011) referenced. The incidence of vertical transmission is affected by the time of infection, ranging from about 30% in the first trimester to up to 70% in the third trimester.

Table3
Distribution of Infection Parvoviruses in aborted and control (pregnant) women by trimester of pregnancy

Trimester	No Aborted women	Aborted women +parvo	No Pregnant women	Pregnant women +parvo
First trimester	34	8(13.3%)	14	Nil (%)
Second trimester	21	7 (11.6%)	7	Nil (%)

Third trimester	5	2(3.3%)	4	Nil (%)
Total	60(70.5%)	17(28.3%)	25(29.4%)	Nil (%)

The frequency of abortion affects the distribution of CMV and parvovirus B19 infection among aborted women. According to the data, the distribution of aborted women based on their abortion frequency revealed that the first abortion was the most common 21(35%), while the second documented abortion19(31.6%), The third and fourth miscarriages were recorded12(20%) and8(13.3%) respectively. as shown in Figure(1)

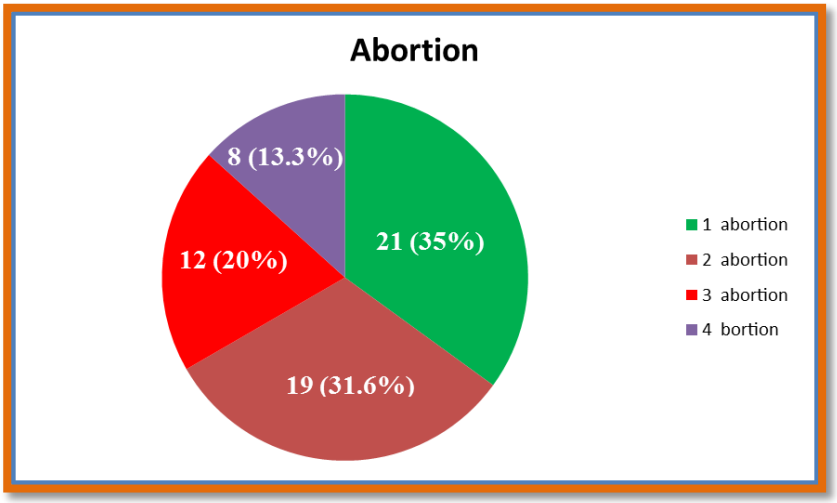

Figure 1.Abortion cases in women are distributed according to the number of abortions

This is in line with the work by Munro *et al.*,(2005), which emphasized the importance of CMV Approximately 10% to 15% of congenitally infected babies have detectable infection Sensorineural hearing loss, organomegaly, microcephaly, cerebral calcification, and chorioretinitis are the most prevalent symptoms caused by a direct viral cytopathic influence on the fetus. The viral pathogen parvovirus B19 causes fetal losses during pregnancy.

Detection of CMV and parvovirus B19 IgG among abortions patients and control groups by ELISA Technique

All sixty samples from abortion patients and Twenty five samples were examined for CMV IgG using (Enzyme-Linked Immunosorbent Assay) technique. the rate of infection is (100%) and they are infected, at 18 (30%) at 16-22 years,19 (31.6 %) at 23-29 years,16(26.6 %) at 30-36 years,4 (6.6 %) at 37-43years, and 3(5 %)at 44 -50 years, while all of the control group were given negative(CMV) IgG, as in the figure(2), The findings in aborted women aged 23-29 years (31.6%) and 30-36 years(26.6%) were consistent with those of A Naqid *et al.*,(2020), who found the highest occurrence at the same age.

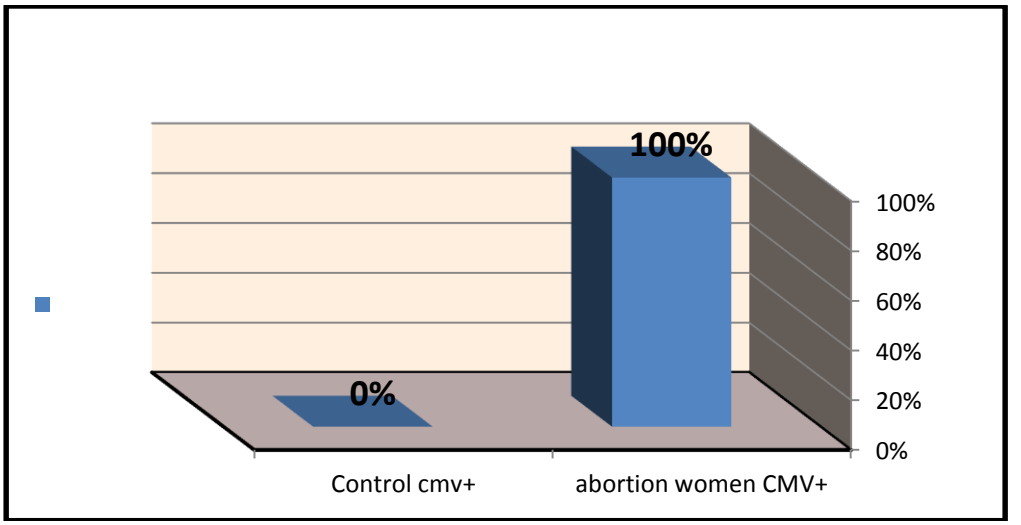

Figure2. CMV distribution among aborted and non-aborted pregnant women

Immunological research
Cytokines detection
Interleukin 2 (IL-2) measurement level

As indicated in the image, the results of this study demonstrated a significant level of IL-2 in the serum of aborted women who were positive for CMV (23.22±2.867) compared to pregnant women (2.312±0.6295) depicted in Figure(3). These findings corroborate previous findings (Rezaei and Dabbagh,2002; Pandey *et al.*2005). While disagreeing with Saleh and Kharibet,(2015) who suggested that low levels of IL-2 in the serum of aborted women who have had multiple abortions could be linked to a lack of inducing effects for T-cell and B-cell growth, which could lead to a lack of induction in the gestation to protect women's immune response and could be the cause low levels of IL-2 in the serum can be reduced by immunizing the mother against ancestral antigens. IL-2 uses its influence on the immune response by boosting the differentiation of primitive CD4+T cells into T helper cells. IL-2 takes advantage of the induction effect on prostaglandin E2 (PEG2), which is released from chorion tissue during cyclooxygenase pathways in women. PEG2 is involved in avoiding luteolysis, which is vital for the maintenance of pregnancy (Liao *et al.*,2013). PGE2 is also involved in the prevention of luteolysis, which aids in the protection of the fetus during pregnancy. Th1 cells generate cytokines including IL-2 and interferon-gamma (IFN-), which activate inflammatory and cell-mediated cytotoxicity responses. Th1 cytokines are normally harmful to successful pregnancies, and significant levels of Th1 cytokines have been detected in pregnant women who have been exposed to abortion (Zhang *et al.*,2019).

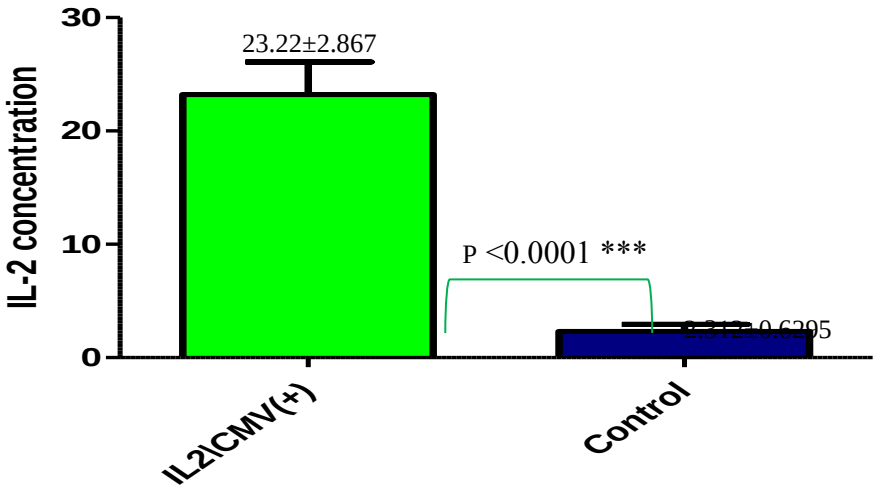

Figure 3. The comparison of IL-2/CMV(+) between patients and control

Radial Immunodiffusion to Complement C3 Protein (RID

The levels of C3 were found to be highly significant(146.9±3.696)+CMV were found in when compared to the control group(118.4±5.107) as seen in the diagram(4) So, researchers Al-Sheikh *et al.*,(2007) remembered that the highest levels of C3 proteins in the serum of aborted women, C3protein considered a central particle in the complement system that the induction represents fundamental for all benefits functions occurred via complement system, the highest levels of C3 come from induction of pathways represented by alternative and classical pathways Miller *et al.*,(2000) Where C3 protein plays various tasks, including inducing phagocytosis, providing local inflammatory responses against pathogens, and directing adaptive immune responses to appropriate antigens for humoral immunity responses (AL-Fatlawi and Sultan, 2016). Complete activation of the complement system derived from the mother's circulation and local creation via several cell sources found in the placenta. As a result, the tissues of the embryo frequently form alloantibodies and semi-allogenic in the mother, and the placenta is likely exposed to complement that mediates the immunological interface, potentially resulting in pregnancy loss (Holers *et al.*,2002).

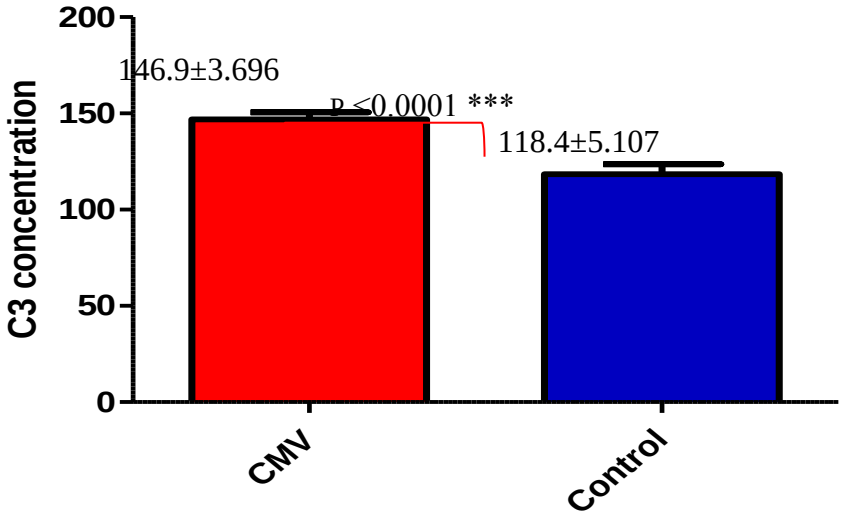

Figure 4. The comparison of C3 between CMV(+) patients and control

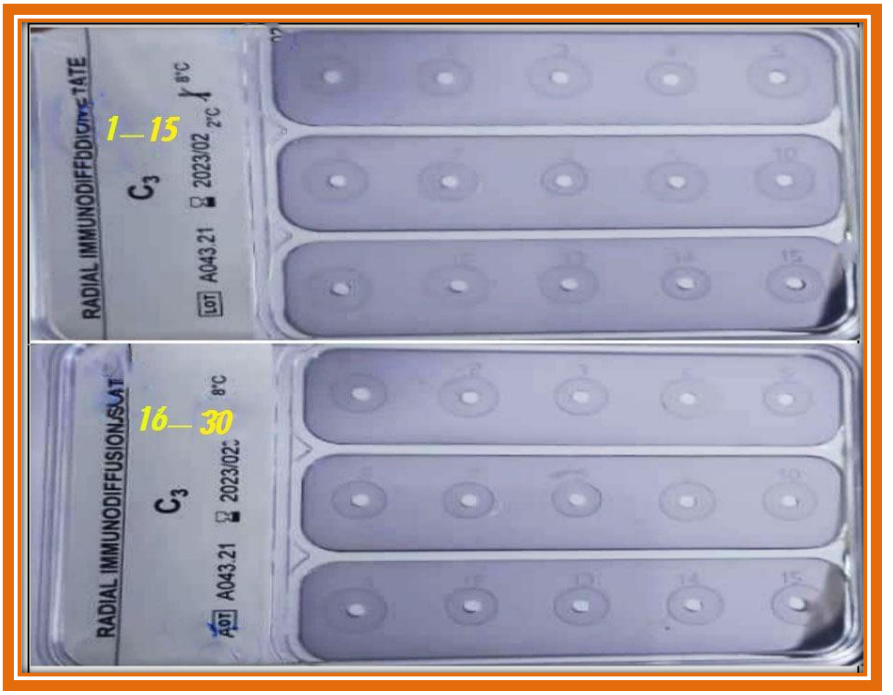

Figure 5. Show the Complement C3 protein plate with an agarose gel and a noticeable ring around the well that holds the serum sample

Complete Blood Count (CBC)

The complete blood count is a blood test that is used to assess overall health and diagnose a wide range of abnormal conditions in the blood system, such as leukaemia and anaemia. The assay includes white blood cells, red blood cells,

hematocrit, and haemoglobin, as well as another physiological parameter that provides a complete picture of the blood system's problems (Asaduzzaman *et al.*,2018). When compared to the control group (7.412±0.4661), our data revealed a significant drop in white blood cell numbers (WBCs) (5.203±0.8281) among women who were aborted and had the +CMV, When compared to the control group (2.816±0.2416), +CMV aborted moms had significantly lower lymphocyte counts (1.336±0.08859). as seen in Figures 6 (7) The outcomes were in agreement with Sindre *et al.*,(2000) who made the observation the presence of CMV infection could indicate that the virus caused the leukopenia. This is a viable option. as multiple publications mention such a complication during CMV infection.

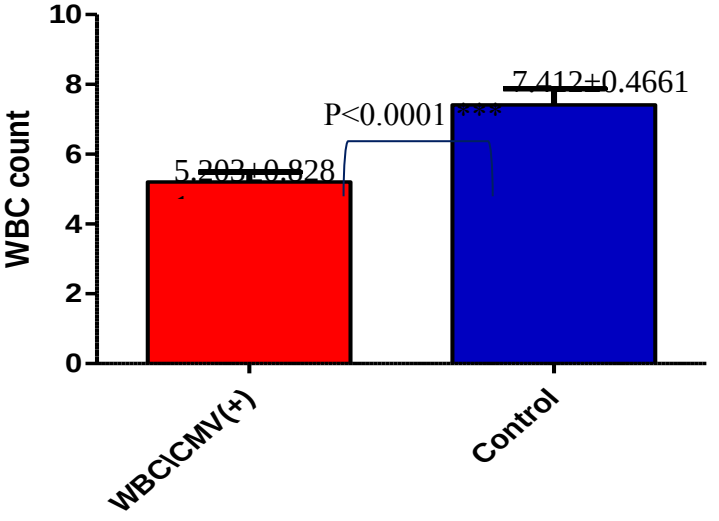

Figure 6. The comparison of WBC/CMV(+) between patients and control

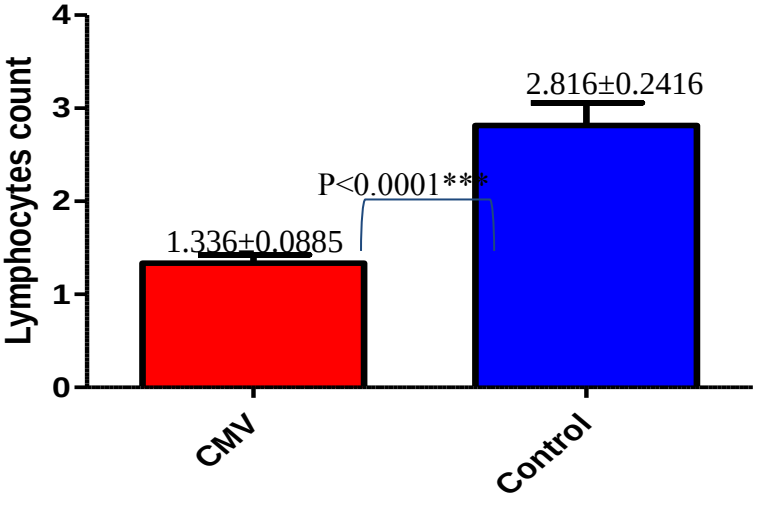

Figure 7. The comparison of Lymphocyte between CMV(+) patients and control

When comparing women who were aborted and had the +CMV to the control group (11.68±0.1695), our findings revealed a significant drop in Hemoglobin level (Hb) (9.984±0.2357).as shown in figure(8) The results were consistent Betjes *et al.*,(2009) They noticed CMV has been linked to haemoglobin levels being negatively regulated.

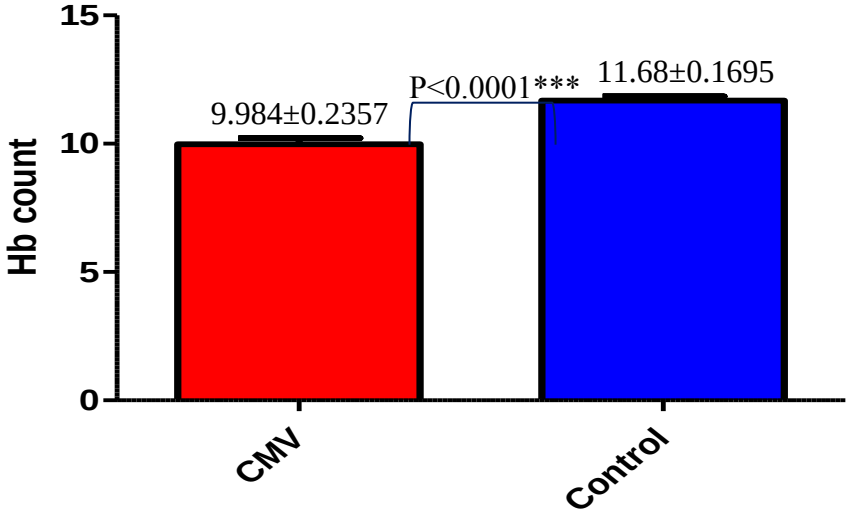

Figure 8. The comparison of Hb between CMV(+) patients and control

Significantly decreased PLT counts were found in +CMV aborted mothers(199.4±11.88) when compared to the control group(273.6±14.59) as seen in the diagram(9) The outcomes were in agreement with Yenicesu *et al.*,(2002) Crapnell *et al.* characterized the numerous theories of CMV infection-induced thrombocytopenia as CMV-induced direct cytotoxicity to hematopoietic cells, immune-mediated elimination of infected cells, or disruption of bone marrow stromal function.

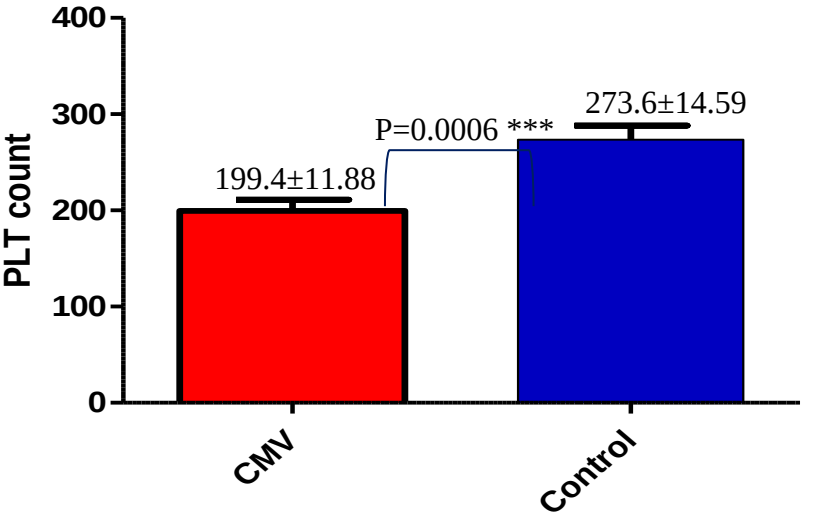

Figure 9. The comparison of CMV(+) between patients and control

As shown in the diagram, +CMV aborted moms had significantly quite high neutrophil counts (4.254±0.2670) than the control group (3.224±0.2375). (10) The results were all in agreement, Drescher and Bai, (2013), neutrophils have been demonstrated to perform antiviral functions for a variety of viruses via ROS and herpesviruses, such as HCMV, have been shown to cause ROS in phagocytic cells during lytic infection.

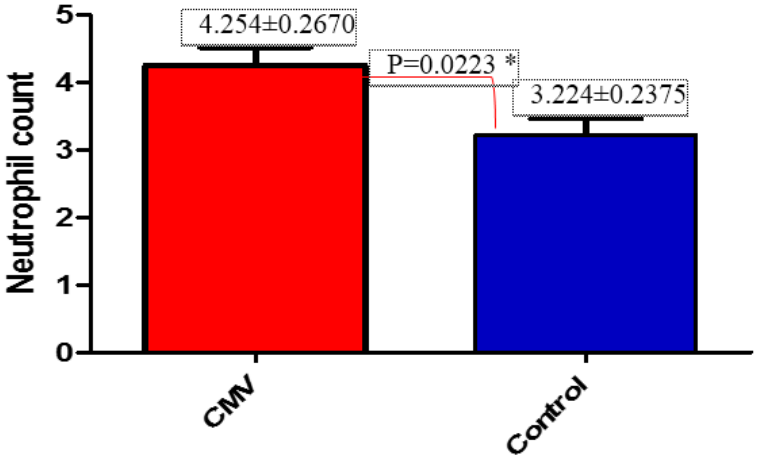

Figure 10. The comparison of Neutrophil between CMV(+) Patients and control

Conclusion

The findings demonstrated that human cytomegalovirus (CMV) were found in larger concentrations in the aborted women's serum than in the pregnant women's, raising the idea that these viruses are one of the main causes of

miscarriage, Aborted women have higher levels of interleukin-2 (IL-2) than pregnant women. The levels of C3 were found to be significantly greater in aborted + CMV women, The study revealed lower interest rates of white blood cells, lymphocytes, haemoglobin (Hb) and PLT in aborted women with + CMV compare to the control group, on the other hand, the results related to the neutrophil showed significant differences between the patients and the control group.

Ethical Clearance

After the approval of protocol by the Ethical Review Board for human studies, Faculty of Nursing/University of Kufa/Iraq (No. 10-04/01/2015) and before enrollment, all the subjects gave their written informed consent.

References

Al-Samarrae, E. A. A. (2010). Study the Relationship of Toxoplasma IgG Titer with Some Other Immunoglobulin and Complement Components. *Iraqi J. Comm. Med*, *23*(2), 127-129.

A Naqid, I., H Yousif, S., & R Hussein, N. (2020). Seroprevalence of Rubella and Herpes Simplex Virus in Women with Miscarriage and Stillbirth in Zakho City, Kurdistan Region, Iraq: A Cross-Sectional Study. *Women's Health Bulletin*, *7*(1), 18-22.

Asaduzzaman, M., Mrida, A. H., Hasan, N., Faruq, M. O., Juliana, F. M., Islam, M. J., & Kabir, M. S. (2018). The effect of herpes simplex virus infection on different blood parameters: A transverse study. *IOSR-JDMS*, *17*(9), 81-89.

Colugnati, F. A., Staras, S. A., Dollard, S. C., & Cannon, M. J. (2007). Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. *BMC infectious diseases*, *7*(1), 1-10.

Dreher, A. M., Arora, N., Fowler, K. B., Novak, Z., Britt, W. J., Boppana, S. B., & Ross, S. A. (2014). The spectrum of disease and outcome in children with symptomatic congenital cytomegalovirus infection. *The Journal of pediatrics*, *164*(4), 855-859.

Drescher, B., & Bai, F. (2013). Neutrophil in viral infections, friend or foe?. *Virus research*, *171*(1), 1-7. Enders, G., Daiminger, A., Bäder, U., Exler, S., & Enders, M. (2011). Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection about gestational age. *Journal of Clinical Virology*, *52*(3), 244-246.

Feldman, B., Yinon, Y., Oikawa, M. T., Yoeli, R., Schiff, E., & Lipitz, S. (2011). Pregestational, periconceptional, and gestational primary maternal cytomegalovirus infection: prenatal diagnosis in 508 pregnancies. *American journal of obstetrics and gynaecology*, *205*(4), 342-e1. Grimes, D. A., & Stuart, G. (2010). Abortion jabberwocky: the need for better terminology. *Contraception*, *81*(2),93-96.

Hussan, B. M. (2013). Study the prevalence of ACL, APL, CMV, HSV, Rubella and Toxoplasma gondii in aborted women in Baghdad. *Medical Journal of Babylon*, *10*(2), 455-464.

Holers, V. M., Girardi, G., Mo, L., Guthridge, J. M., Molina, H., Pierangeli, S. S., ... & Salmon, J. E. (2002). Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *The Journal of experimental medicine*, *195*(2), 211-220.

Kolte, A. M., Bernardi, L. A., Christiansen, O. B., Quenby, S., Farquharson, R. G., Goddijn, M., & Stephenson, M. D. (2015). Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. *Human reproduction*, *30*(3), 495-498.

Munro, S. C., Trincado, D., Hall, B., & Rawlinson, W. D. (2005). Symptomatic infant characteristics of congenital cytomegalovirus disease in Australia. Journal of paediatrics and child health, 41(8), 449-452 Naing, Z. W., Scott, G. M., Shand, A., Hamilton, S. T., van Zuylen, W. J., Basha, J., ... & Rawlinson, W. D. (2016). Congenital cytomegalovirus infection in pregnancy: a review of prevalence, clinical features, diagnosis and prevention. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *56*(1),9-18.

Picone, O., Vauloup-Fellous, C., Cordier, A. G., Guitton, S., Senat, M. V., Fuchs, F., ... & Benachi, A. (2013). A series of 238 cytomegalovirus primary infections during pregnancy: description and outcome. *Prenatal diagnosis*, *33*(8), 751-758.

Rezaei, A., & Dabbagh, A. (2002). T-helper (1) cytokines increase during early pregnancy in women with a history of recurrent spontaneous abortion. *Medical Science Monitor*, *8*(8), CR607-CR610.

Saleh, D. S., & Kharibet, K. E. (2015). Detection of Cytomegalovirus, Rubella virus, and IL-2 Levels in a Sample of Recurrently Aborted Iraqi Women. *Iraqi Journal of Science*, *56*(3A), 1890-1894.

Yenicesu, İ., Yetgin, S., Özyürek, E., & Aslan, D. (2002). Virus-associated immune thrombocytopenic purpura in childhood. *Pediatric haematology and oncology*, *19*(6), 433-437.

Yezlankyzy, T., &Niazbekov, S. (2015). Abortion Is the Same as a Murder of an Innocent Human Being. *International Student's Journal of Medicine*, *1*(4).