

Association Between Parvovirus B19 Infection, Interleukin-6, and Tumor Necrosis Factor-Alpha Levels in Pregnant Women with Spontaneous Abortion in Kirkuk, Iraq

Sangol Mohammed Najeeb, Mohammed Yawoz Noraldeem¹

Medical Laboratory Techniques Department, Kirkuk Directorate of Health, Kirkuk, Iraq, ¹Medical Laboratory Techniques Department, College of Health and Medical Techniques, Northern Technical University, Kirkuk, Iraq

Abstract

Background: Severe fetal anemia, intrauterine fetal mortality, and spontaneous abortion are only a few of the pregnancy issues that human parvovirus B19 can induce. Several immunological factors, like viral infection and changes in cytokine signaling, have a role in abortion, which is a regular occurrence. This means cytokines are crucial for regulating and increasing immune cell numbers in response to viral infections. **Objective:** The objective of the study was to evaluate the levels of Interleukin-6 and Tumor necrosis factor-alpha in parvovirus B19-infected women who had spontaneous abortions in Kirkuk city. **Materials and Methods:** Blood samples were taken from 135 pregnant women who had abortions were attended Gynecological and Pediatric Hospital and private clinics in Kirkuk city from November 2022 to April 2023. The study also included 45 healthy women who had successful pregnancies as a control group. The sera obtained from the study samples were tested to measure the levels of IL-6 using Electrochemiluminescence Immunoassay and tumor necrosis factor-alpha (TNF- α) using ELISA technique, B19 infection was confirmed by polymerase chain reaction (real-time PCR). **Results:** The result indicated that out of the 135 cases of aborted women samples evaluated, 12 cases (8.89%) were found to be positive for HPV B19 DNA through RT-PCR testing, while 123 (91.11%) of the cases classified negative for HPV B19 DNA. The study also revealed that the highest mean IL-6 and TNF- α were recorded among the aborted women with B19 infection, followed by aborted women without B19 infection and the lowest mean was in the control group. The provided *P* value of <0.001 indicates a statistically significant difference in IL-6 and TNF- α levels across the studied groups. **Conclusion:** B19 virus infection stimulates an inflammatory response characterized by increased IL-6 and TNF- α production, which may contribute to pregnancy complications associated with B19 infection.

Keywords: Human parvovirus B19, interleukin-6, tumor necrosis factor-alpha

INTRODUCTION

The human parvovirus B19 (HPVB19) virus was found for the first time in 1975.^[1] Additionally, this virus may kill a fetus during pregnancy and develop serious anemia in people with hemolytic conditions. It also causes disease that does not manifest on a medical test. Additionally, human parvovirus B19 can be passed from the mother to the fetus by nasal and throat secretions. Pregnancy-related complications from a B19V infection include hydrops fetalis, miscarriage (before week 22), and intrauterine fetal mortality (at or after week 22).^[2] Pregnant women are more likely to get an acute parvovirus B19 infection, which has been linked to a number of factors. Women

who have only one kid, for instance, are three times more likely to contract an illness than women who have never given birth. Additionally, the risk increases by around 7.5 times for women who have had three or more children. Additionally, it appears that working in daycare facilities, after-school programs, or nursery schools raises the risk. Most of the time, parvovirus B19 cannot be grown

Address for correspondence: Ms. Sangol Mohammed Najeeb, Medical Laboratory Techniques Department, Kirkuk Directorate of Health, Kirkuk, Iraq. E-mail: sangol92@ntu.edu.iq

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in normal culture conditions. Histologically, it could be diagnosed by looking for viral particles or intranuclear inclusions that are unique to the disease.^[3]

Cytokines are important parts of how the immune system is controlled, and they do a lot of important immunoregulatory things. An abnormal pro-inflammatory cytokine profile or a change in the balance of the Th1/Th2 cytokine response^[4] may affect how the virus is treated and how long it stays in the body. Parvovirus also makes the immune system work better by making cytokines like tumor necrosis factor-alpha (TNF- α), interferon-gamma (IFN- γ), and Interleukin-6 (IL-6). After a person gets an acute infection, these cytokines are found in their blood. The cytotoxic non-structural-protein of parvovirus B19 acts on the promoter regions of the TNF- α and IL-6 genes to make them more active. This makes the TNF- α and IL-6 genes more active.^[5] IL-6 boosts the body's natural defenses and gets rid of pathogens. TNF- α is a key mediator of both short-term and long-term inflammatory responses in the body.^[6] It additionally has a role in a number of physiological functions, including the immune system's homeostasis, responses to tumors, and the control of inflammation.^[7] In the past, a primary B19 infection has been linked to high levels of TNF- α and IL-6. Also, a link was found between the rise of different cytokines that cause inflammation and the symptoms of B19 infection.^[4] So, the goal of this study was to look at if there was a link between having parvovirus B19, high levels of IL-6, and high levels of TNF- α in pregnant women with a spontaneous abortion.

MATERIALS AND METHODS

A cross-sectional hospital and private clinics study involving 135 pregnant women who underwent spontaneous abortions and visited gynecological and pediatric hospitals and private clinics aged 15–45 years was conducted in Kirkuk city between November 2022 and April 2023. The study also included 45 healthy women who had successful pregnancies as a control group. This study excluded all patients with chronic illnesses, Rubella, Cytomegalovirus (CMV), Herpes simplex virus (HSV), and toxoplasma parasites, as well as all women with abortion-related bacterial or viral infections. Five milliliters of blood were drawn from each participant's vein using a syringe after a vein puncture in the study. Blood samples were centrifuged at 3000 rpm for 15 min in sterile test tubes to create serum. Afterward, the sera were sucked using an automated micropipette and put into Eppendorf tubes before being put in a deep freezer at -20°C . HPV B19 DNA was extracted from patient's serum according to commercial DNA extraction kit (Magnetic Bead Method) No. 6 (Hamburg, Germany). HPV B19 DNA in human serum was detected using Human Parvovirus B19 Kit (Bosphore, Turkey). Then used for evaluation of the levels of IL-6 using Electrochemiluminescence Immunoassay

(Nipigon Health Corp., Canada) kit and TNF- α using (SunLong Biotech, China) kit by ELISA technique.

Calculation of IL-6 results

The system software automatically calculates the analyte concentration using a particular algorithm, and the resulting unit is picograms per milliliter.

Calculation of TNF- α results

Plots of known human TNF-standard concentrations on the log scale (x -axis) and their corresponding reading on the log scale (y -axis) are displayed. The amount of human TNF- α in the sample can be calculated by plotting the optical density of the sample on the y -axis. To calculate the original concentration, multiply the dilution factor.

Statistical analysis

The Chi-square test was used with conventional equations to compare the results and reported with P value of <0.001 as the accepted level of significance accordingly.

Ethical consideration

Before each woman signed up for the study, her husband or other family members also received informed consent. Each person was extensively briefed on the procedure prior to the blood sample being obtained to ensure they understood every step. Kirkuk's health directorate has accepted this according to document number 48025 on October 31, 2022.

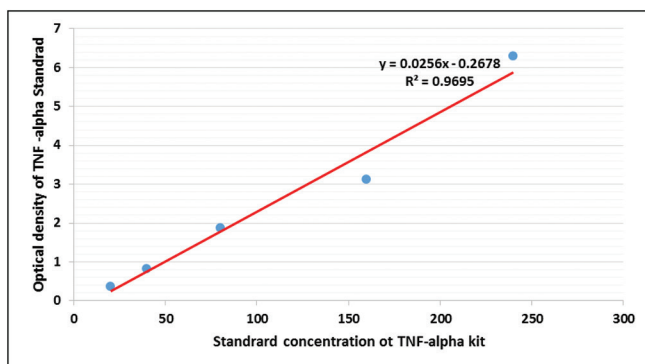
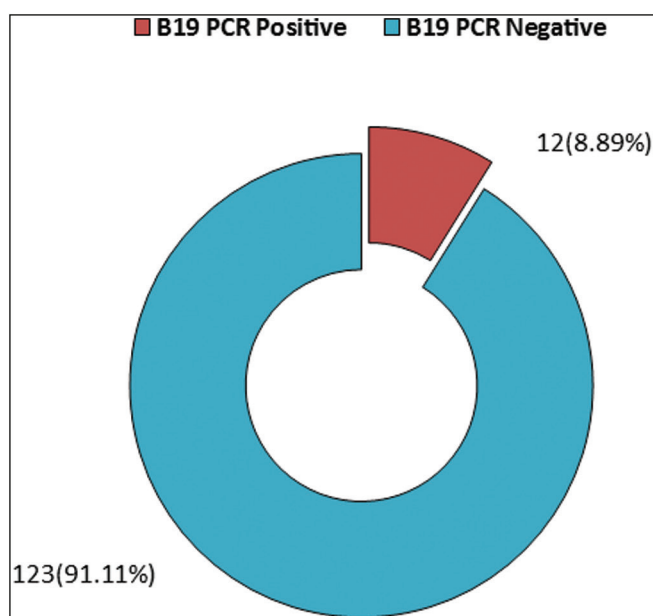
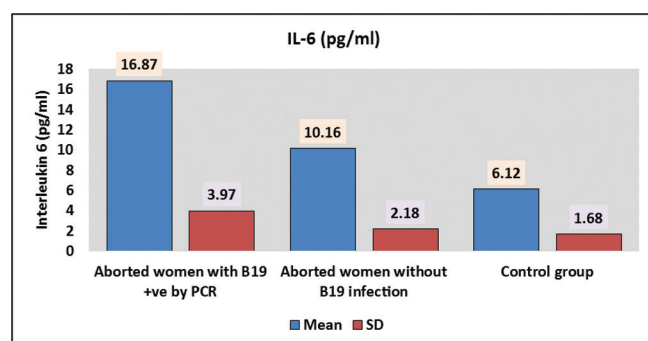
RESULTS

A total of 135 aborted women were enrolled in this study, the study revealed that a large percentage of women who had spontaneous abortions were found between the ages of 26 and 35 years, were of normal weight, and had abortions more than twice, were from urban areas, and had done so during the first trimester of pregnancy. The result indicated that out of the 135 cases of aborted women samples evaluated, 12 cases (8.89%) were found to be positive for HPV B19 DNA through RT-PCR testing. On the other hand, 123 (91.11%) of the cases were classified negative for HPV B19 DNA. The presented study revealed that the highest mean of IL-6 level (16.87 ± 3.97 pg/mL) was recorded among the aborted women with parvovirus B19 infection (positive for HPV B19 DNA through RT-PCR testing), followed by aborted women without B19 infection (negative for HPV B19 DNA through RT-PCR testing) (10.16 ± 2.18 pg/mL) and the lowest mean was recorded in the control group (6.12 ± 1.68 pg/mL). The provided P value of <0.001 indicates a statistically significant difference in IL-6 levels across the studied groups [Table 1]. On the other hand, the highest mean of TNF- α level was recorded among the aborted women with parvovirus B19 (positive for HPV B19 DNA through RT-PCR testing) (33.8 ± 4.2 pg/mL) followed

Table 1: Level of IL-6 in aborted women with and without B19 virus and the control group

Studied groups	No.	IL-6 level (pg/mL)
Aborted women with B19 +ve by PCR	12	16.87 $\pm$ 3.97
Aborted women without B19 infection	33	10.16 $\pm$ 2.18
Control group	45	6.12 $\pm$ 1.68

P value = <0.001

**Figure 1:** Standard curve of the human TNF- α standard**Figure 2:** Prevalence HPV B19 DNA among aborted women**Figure 3:** Level of IL-6 in aborted women with and without HPV B19 and the control group
أعلى النموذج**Table 2: Level of TNF- α in aborted women with and without B19 virus and the control group**

Studied groups	No.	TNF- α level (pg/mL)
Aborted women with B19 +ve by PCR	12	33.8 $\pm$ 4.2
Aborted women without B19 infection	33	22.7 $\pm$ 3.9
Control group	45	13.6 $\pm$ 3.4

P value = <0.001

by aborted women without B19 infection (negative for HPV B19 DNA through RT-PCR testing) (22.7 $\pm$ 3.9 pg/mL) [Figures 1–3] and the lowest mean was recorded in the control group (13.6 $\pm$ 3.4 pg/mL). The provided P value of <0.001 indicates a statistically significant difference in TNF- α levels across the studied groups [Table 2].

DISCUSSION

Molecular-based techniques, such as PCR, have proven valuable in diagnosing various infectious diseases caused by different agents.^[8] Serum samples can detect low levels of viral DNA up to 4 months postinfection, while in other tissues, this period can extend to years of 40. To diagnose chronic or acute infections, a combination of standard real-time PCR along with serological evaluations is recommended.^[9] The study shows that women who had an abortion and had B19 PCR positive have higher levels of IL-6 than the control group. The fact that aborted women with B19 infection had higher levels of IL-6 than those without B19 infection and the control group suggests that B19 virus may cause an inflammatory response that leads to more IL-6 being made. In line with what has been found so far, Al-Fahad *et al.*^[10] Plasma levels of the inflammatory cytokine IL-6 have been determined to be significantly higher in B19-positive women who had an abortion than in women who did not have the infection and in a control group. In our study, higher IL-6 levels in women who had an abortion and had a positive PCR test could mean that the abortion process is still causing inflammation or that there is an infection underneath.^[11] Miscarriages can be caused by several immune factors, such as changes in the way cytokines signal and viral infections.^[12,13] So, cytokines are a key part of how immune cells work and grow to fight off viral disease, for example, T-lymphocytes (CD4 and CD8) and natural killer cells make IFN- γ on their own. This causes dendritic and neutrophilic leukocytes to wake up at the start of an infection.^[14] When compared to healthy women, women who aborted had higher levels of TNF- α , IFN- γ , IL-2, IL-4, IL-6, and IL-6.^[15] This shows that in recurrent miscarriage, cytokines have a role. These levels of pro-inflammatory TNF- α and ILs seem to be the most important parts of cytokine systems linked to pregnancy. Several studies have shown that the levels of TNF- α in the blood of women who have miscarriages are high, and high levels of cytokine have been found in the fluid that comes from trophoblast-activated peripheral blood mononuclear cells.^[16,17] It has been said that cytokines like IL-6 and

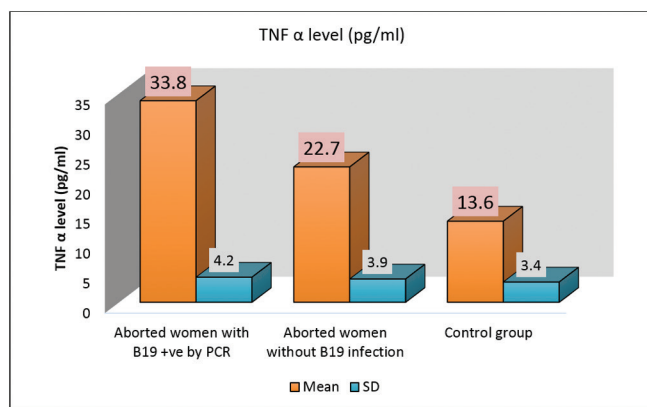


Figure 4: Level of TNF- α in aborted women with and without HPVB19 and the control

IL-8, which are involved in inflammatory and infection responses, have more than one job. For example, IL-6 is a marker of inflammation for COVID-19 and IL-8 is a regulator of embryo implantation.^[18] They are found in large amounts in the reproductive system in women and send signals in women who have a lot of miscarriages. This means that not enough IL-6 and IL-8 in the area can result fetal death. Women who have a lot of miscarriages have less IL-6 in their endometrium.^[19,20]

The current results about the high levels of TNF- α in women who had an abortion and were infected with the B19 virus are similar to those of earlier studies that looked at the role of cytokines in recurrent miscarriage. These earlier studies have found that women who have had miscarriages have higher levels of TNF- α and other cytokines like IFN- γ , IL-2, IL-4, and IL-6 than healthy women.^[21,22] High levels of pro-inflammatory cytokines like TNF- α and ILs have been found to be important parts of cytokine networks linked to pregnancy. Several studies have shown that miscarriages are linked to higher levels of TNF- α in the blood.^[23] High levels of cytokines have also been found in the fluid that comes from trophoblast-activated peripheral blood mononuclear cells.^[24] This shows that cytokines are involved in miscarriage. All of these results suggest that misregulation of cytokines, like TNF- α and other interleukins, may play a role in how miscarriages happen. Higher levels of pro-inflammatory cytokines were found in both the current study and earlier research. This shows that immune and inflammatory pathways are being activated, which could affect how the pregnancy turns out. The fact that a B19 virus infection is linked to high levels of TNF- α suggests that there may be a link between the virus and the immune-inflammatory processes that cause pregnancy complications and abortions. If you can figure out how this link works, you can learn a lot about how complications during pregnancy can be caused by B19^[25,26] [Figure 4].

CONCLUSIONS

B19 virus infection stimulates an inflammatory response characterized by increased IL-6 and TNF- α production, which may contribute to pregnancy complications associated with B19 infection.

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Conflicts of interest

There are no conflicts of interest.

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