Association of Parvovirus B19 Infection and increased Some immunological markers among spontaneous abortion Women

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Abstract

The purpose of this study was to see if parvovirus (B19) was present in abortion women and if certain cytokines were involved in abortion induction. between August 2021 and January 2022 (2022). A total of 60 women who had previously had abortions were included in the study, as well as 25 healthy pregnant women who showed no evidence of chronic inflammatory disease, were gathered from Al Rifai hospital in Dhi Qar city. The patients range in age from 19 to 50 years. Parvovirus was examined on ELISA in blood samples taken from abortion women and healthy controls. A total of 60 samples were taken, with 17(28.3percent) positive results for parvovirus B19, And 43(71.6%) were negative for parvovirus while pregnant women's samples yielded 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). the results appeared as follows at first 8(13.3%), second and third trimesters 7(11.6%) 2(3.3)respectively.Interleukin-2 levels were measured using an ELISA test aborted +parvovirus women show a significant (p=0.0483) were (54.66 ±29.97) compared to pregnant women in the control group (2.312±0.6295).the results revealed there are no significant differences(p=0.1207) in the levels ofC3 (131.0±6.049)in Positive parvovirus B19 were found in when compared to the control group(118.4±5.107). As for the results of complete blood count, they were as follows, lower significant white blood cells levels (3.534±0.0776) compared to the control group were (7.412±0.4661), and lower significant lymphocytes levels(1.128±0.1103) compared to control group were (2.816±0.2416), as well as lower significant haemoglobin (Hb) levels (8.941±0.5584) compared to the control group(11.68±0.1695), on the other hand, the results related to the neutrophil showed that there are no significant differences between the patients and the control group(4.111±0.5345)(3.224±0.2375)respectively, also PLT, results showed lower significant levels(163.7±22.55) in +Parvovirus aborted women compared to control group(273.6±14.59).

Keywords: parvovirus (B19), pregnant abortions, ELISA test

1. Introduction

Abortion is defined as the procedure of terminating a pregnancy by removing an embryo or fetus from the uterus before it can live outside of it [1]. Morbidity and mortality among mothers associated with unsafe abortion complications have been identified as serious public health issues. Furthermore, all hazardous abortions are common in impoverished countries, where 98 percent relating to abortion deaths occur. Each year, around 56 million abortions are performed worldwide, with less than half of them occurring in an unsafe manner [2]. Recurrent pregnancy loss (RPL) is caused by a variety of organisms that can be transmitted in utero at various stages of pregnancy, including Toxoplasma gondii, herpes viruses, CMVand rubella virus [3]. There are several causes of abortion, including viral ones, and the important virus that cause abortion is Parvovirus.

Parvoviruses are a group of single-stranded DNA viruses that infect invertebrates and vertebrates, ranging from insects to mammals, and have a wide cellular tropism and host range. Although several parvoviruses are major veterinary infections, the family only contains two human pathogens: human parvovirus B19 and the recently discovered human bocavirus [4]. B19V belongs to the Erythroviruses genus, which is pathogenic for humans and can infect the placenta. B19V infection causes fetal death and hydrops fetalis, which usually occurs during the second trimester [5].

2. Methods and Materials

The samples were taken in their entirety Dhi Qar's AL Rifai Teaching Hospital 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 distinct groups, The initial 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 through venous puncture from each patient and gathered in two tubes groups, one of which included anticoagulant to assess total count of blood as well as the other group, which did not have an anti-coagulant as well as a basic tube to use for serum preparation, to collect serum, they were centrifuged for 5 minutes at 3000 rotations per minute, which was then contained in different tubes before being transported to sterile tubes using aM micropipette with sterile disposable tips. The serial number was assigned to the

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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 parvovirus IgG antibodies in human serum (SUN LONG, Chain).

A- Procedure for testing

1. Two wells were left empty in the Microelisa strip plate as a negative control, two wells were left empty as a positive control, and one well was left empty as a blank control. Negative control and positive control 2 holes, CK 1 hole: the sequential number, a corresponding sample of the microporous hole 2 per board (CK hole without samples and HRP-Conjugate reagent, the rest of the same step operation).
2. Added samples: A volume of 50 µl of negative and positive control is added to the negative and positive control wells, respectively. 40 µl Sample dilution buffer and 10 l sample are introduced to sample wells. Without touching the good wall, samples should be loaded into the bottom. With careful shaking, thoroughly combine the ingredients.
3. After being sealed with Closure plate membrane, incubated for 30 minutes at 37°C.
4. distilled water was used to diluted the concentrated washing buffer (30 times for 96T).
5. Washing: Removed the Closure plate membrane with care, aspirated, and refilled with wash solution. After 30 seconds of relaxing, discarded the wash solution. The washing method was repeated five times
6. Added 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubated according to Step 3.
8. Washing: as described in Step 5.
9. Coloring: To each well, added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B, mixed gently, and incubated at 37 °C for 15 minutes. When coloring, stay away from light
10. Termination: To stop the reaction, each well was filled with 50 µl stop solution. The well's color should shift from blue to yellow.
11. Using a Microtiter Plate Reader, readed the absorbance O.D. at 450nm. The blank control well's OD value is set to zero. After adding the stop solution, the assay should be completed within 15 minutes

Calculation of results

average value of the positive control is 1.00, whereas the average value of the negative control is 0.10. Calculation of the critical value (CUT OFF): critical value = average value of negative control + 0.15
Negative judgment: the sample is Human B19-IgG negative if the OD value is CUT OFF.
Positive judgment: the sample is Human B19-IgG positive if the OD value is CUT OFF.

3. Result and Dissection:

Distribution of Parvovirus B19 based on the age of the aborted and expectant ladies

According to the findings of the current research, the samples were dispersed as follows, according to age:18 (30%) at16-22 years,19 (31.6 %) at23-29 years,16(26.6 %) at 30-36 years,4 (6.6 %) at 37-43 years, and 3(5 %)at 44 -50 years. Pregnant ladies, can on the other hand be proven 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 (1), 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. [6], who found the highest occurrence at the same age. While the current findings contradict those of Mohammad and conducted a previous study 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 [7]. 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 if there is fetal loss or fetal abortion occurs after 10 weeks of pregnancy and results in the loss of an embryo, or first-time abortion if it occurs before ten weeks of pregnancy [8].

Table(1): shows the age abortion distribution and control (pregnant) women

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

Distribution of Parvovirus B19 infection according to the pregnancy trimesters, there is an infection difference between aborted and controlled women (pregnant).

According to the stages of pregnancy trimesters, the majority of abortions occurred in the first trimester (34/60), and positive instances of parvovirus 8(31%), other abortions happened in the second and third trimesters were reached7(11.6%),2(3.3%) respectively. this outcome was in agreement with 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 [9]. However, the conclusions of this study differ from those of the study by Feldman et al. [10] which they 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.

Table(2): Parvovirus infection rates in aborted and control (pregnant) women by trimester of pregnancy:

Pregnant women +parvo	No Pregnant women	Aborted women+parvo	No Aborted women	Trimesters
Nil (%)	14	8(13.3%)	34	First trimester
Nil (%)	7	7 (11.6%)	21	Second trimester
Nil (%)	4	2(3.3%)	5	Third trimester
Nil (%)	25(29.4%)	17(28.3%)	60(70.5%)	Total

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 parvovirus IgG using (Enzyme-Linked Immunosorbent Assay) technique. the results of abortion patients showed that 17 samples were positive (parvovirus) IgG, It shows a significant level and was as follows 7(11.6%)at 16-22 years,3(5%) at23-29 years,5(8.3%)at30-36 years,2(3.3%)at 37-43 years and zero(0%) at 44-50, 43 samples were negative(71.66%) (parvovirus) IgG, While all of the control group were given a negative(parvovirus) IgG as seen in the figure(1), This result was in line with what had been predicted where they noticed 30% to 50% of pregnant women are susceptible to B19, only a small percentage of them will be infected with this virus.

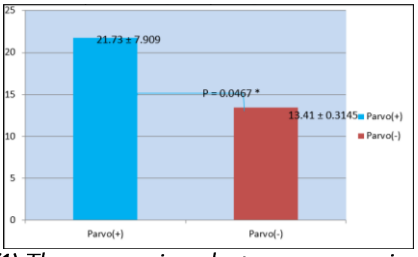


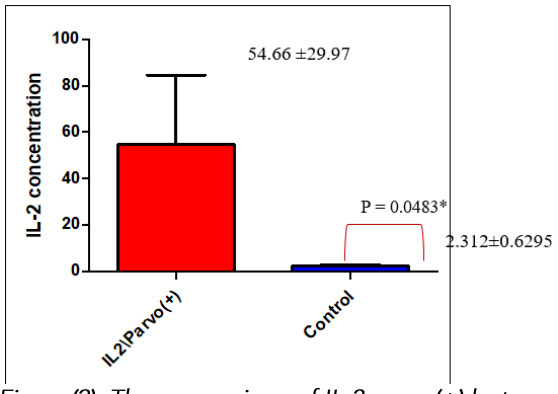
Figure:(1) The comparison between parvovirus(+) and parvovirus(-)in aborted women

Cytokines detection

Interleukin 2 (IL-2) measurement level:

As indicated in the image, the findings of this research demonstrated a significan IL-2 Levels in the Serum of aborted women who were +parvoviruses (54.66 ±29.97) compared to pregnant women (2.312±0.6295) depicted in Figure (2)These findings corroborate previous findings [11]. While disagreeing with Saleh and [12] who suggested that Aborted women's serum had low amounts of IL-2 who have had multiple abortions could be linked to a lack of T-cell and B-cell inducing actions growth, which could lead to a lack of induction in the gestation to protect women's immune response and could be the cause.

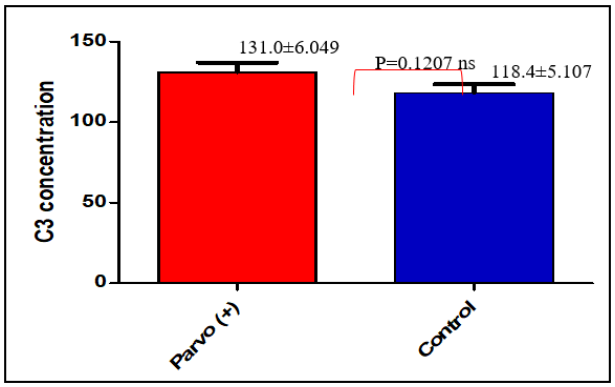
Low IL-2 levels in the serum can be reduced by immunizing the mother against ancestral antigens. IL-2 uses its influence on the immune response by boosting the differentiation of primitive CD4+T cells into Thelper cells. iL-2 takes advantage of prostaglandin E2 induction (PEG2), which is released from chorion tissue during cyclooxygenase pathways in women. PEG2 is involved in avoiding luteolysis, which is vital for the preservation of pregnancy [13]. 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 [14].



Figure(2): The comparison of IL-2 parvo(+) between patients and control.

Radial Immunodiffusion to Complement C3 Protein (RID)

The findings revealed there are no significant differences in the levels of C3(131.0±6.049)Positive parvoviruses were found when compared to the control group(118.4±5.107) as evidenced by the diagram(3) these results are consistent with Yang et al. [15], they noticed C3 levels may be artificially "normal" as a result of an acute C3 elevation followed by immune complex ingestion and return to normal. C3protein regarded as a key particle in the complement system The largest levels of C3 originate from induction of pathways represented by alternative and classical pathways, which is necessary for all beneficial functions that occurred via the complement system [16] 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.

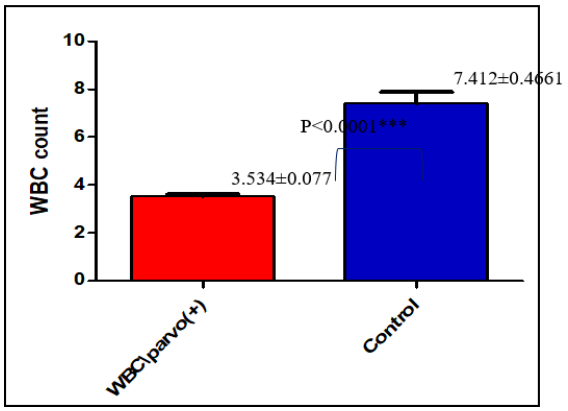


Figure(3): The comparison of parvo(+) between patients and control

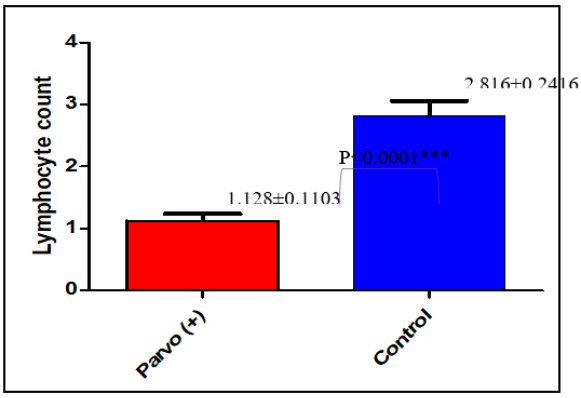
Figure(4): Show the Complement C3 protein plate with an gel agarose and a noticeable ring around the well that holds the serum sample.

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 [17]. The findings of our investigation demonstrated a considerable decrease in white blood cell numbers (WBCs) (3.534±0.0776) Among women who were aborted and had the +parvovirus when compared to the control group(7.412±0.4661), Significantly decreased lymphocyte counts were found in +parvovirus aborted mothers(1.128±0.1103) when compared to the control group(2.816±0.2416). Figures (5) and (6) show this (5)

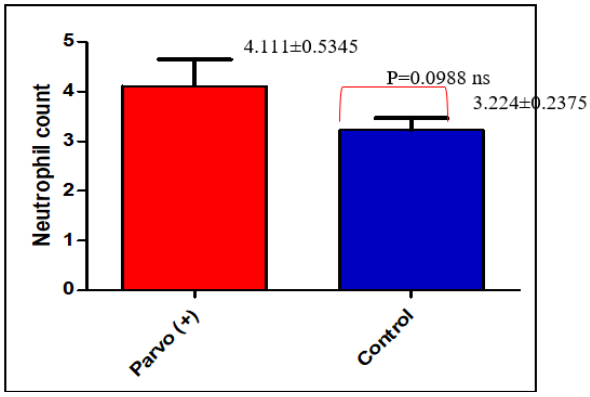


Figure(5) The comparison of WBC parvo (+) between patient and control



Figure(6) The comparison of Lymphocytes between Parvo (+) patients and control.

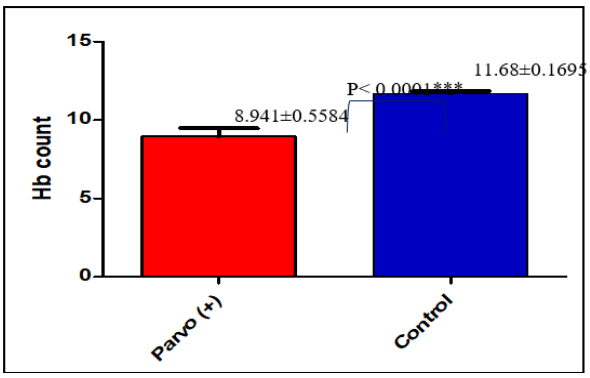
but, disagreeing with the [18] who found a high level of WBCs in their investigation, these are due to the nature of dual mechanisms that can be considered a critical point needed for the embryo's survival and immunotolerance, which noted stress is specialized in the pregnancy also inhibits antibody formation via lymphocyte activation and is linked to pregnancy loss [18, 19]. The findings, on the other hand, were consistent with those of [20], who found lower levels of lymphocytes during the first and second trimesters of pregnancy There are no significant differences in the levels of neutrophil (4.111±0.5345)Positive parvoviruses were found in when compared to the control group(3.224±0.2375) as evidenced by the diagram(7)



Figure(7) The comparison of Neutrophil between parvo(+) patients and control.

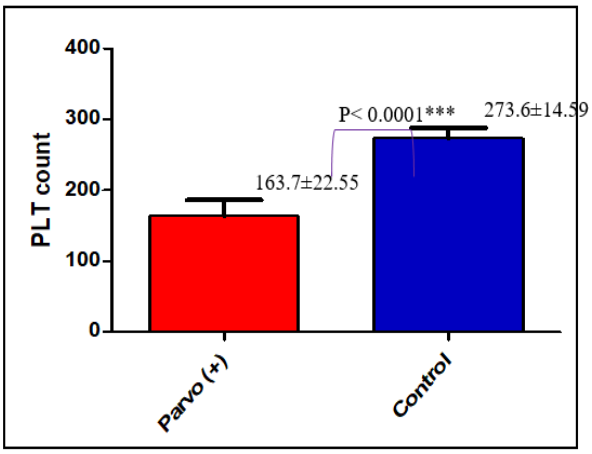
In the current study, the levels of haemoglobin (Hb) in aborted women were significantly lower (8.941±0.5584)

than in pregnant women (11.68±0.1695), as shown in Figure (8). The outcomes were in agreement with Chisaka et al. [21] who noticed that B19V is a cytolytic virus with an apoptosis-inducing component and the potential to arrest cells in the G1 or G2 cell cycle phases. As a result, B19V can destroy a large number of erythroid progenitor cells, resulting in anaemia.



Figure(8) The comparison of Hb between parvo(+) patients and contro

the levels of PLTs were significantly lower in aborted women (163.7±22.55) than in pregnant women (273.6±14.59), which was consistent with previous research by Aktepe et al. [22] Thrombocytopenia is caused by viral nonstructural-1 proteins, which are cytotoxic to megakaryocytes.



Figure(9) The comparison of PLT between parvo(+) patients and control

4. Conclusions

The findings showed that parvovirus (B19) was found in higher concentrations in the serum of aborted women than in pregnant women, raising the possibility that this virus is one of the leading causes of miscarriage. Interleukin-2 (IL-2) levels in aborted women are higher than in pregnant women. The findings also demonstrated that there were no significant differences in C3 levels in Positive parvovirus patients (B19), the aborted women with +parvovirus were lower rates of white blood cells, lymphocytes, haemoglobin (Hb) and PLT, on the other hand, the results related to the neutrophil showed that there are no significant differences between the patients and the control group.

Ethical Clearance

Before enrolment, all subjects submitted their written informed consent after the protocol was approved by the Ethical Review Board for human studies at the Faculty of Nursing/University of Kufa/Iraq (No. 10-04/01/2015).

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