International Journal of Molecular Biology and Biochemistry

ISSN Print: 2664-6501
ISSN Online: 2664-651X
Impact Factor: RJIF 5.4
IJMBB 2024; 6(1): 07-12
www.biologyjournals.net
Received: 05-11-2023
Accepted: 09-12-2023

Faten Abed AL-Kadhem Khalaf Aldawmy
Faculty of Pharmacy, University of Alzahraa, Karbala, Iraq

Ahmed S Abed
Faculty of pharmacy, Jabir Ibn Hayyan University of Medical and Pharmaceutical Sciences, Najaf, Iraq

Saif Jabbar Yasir
Faculty of Medicine, University of Kufa, Najaf, Iraq

Corresponding Author:
Faten Abed AL-Kadhem Khalaf Aldawmy
Faculty of Pharmacy, University of Alzahraa, Karbala, Iraq

Correlation between human parvovirus and anemia in pregnant women in Najaf governorate

Faten Abed AL-Kadhem Khalaf Aldawmy, Ahmed S Abed and Saif Jabbar Yasir

DOI: https://doi.org/10.33545/26646501.2024.v6.i1a.57

Abstract
Introduction: Due to the parvovirus B19's hazardous effects on expectant mothers and the developing fetus, it warrants special care. It may invade the bone marrow and infect RBC progenitors, and it can undergo latent infection and reactivation.
Objective: The purpose of this study was to ascertain the severity of parvovirus infection in the anemia of pregnant women.
Methods: In the case-control study, parvovirus was detected in blood serum samples using the ELISA technique, and positive samples were tested using the nested PCR technique. A total of 252 samples were tested by ELISA, and 63.4 % of the samples for anti-Human IgM antibodies against parvovirus. Where 71.4% were positive for anti-HPV B19 IgG antibodies. In a sample of anemic pregnant women, both groups' sample diagnostic utilizing PCR technique for Parvovirus B19 DNA findings revealed that 61.1% had positive DNA.
Results: A total of 126 control groups of only 6.3% provide a positive result for parvovirus DNA. In subgroup sample diagnosis by PCR technique for the detection of viral DNA, there are also 40.1 % of the first subgroup's PCR approach yielded a mildly positive result, while in moderate anemia it is 50.6 %. The other group (severe anemia) showed that 76.9 % had a positive viral infection.
Conclusion: The relationship is directly proportional between infection with the virus and the increase in anemia in pregnant women suffering from anemia. They showed more viral infections than pregnant women, who did not suffer from anemia. Furthermore, as anemia severity increased, so did virus infection rates.

Keywords: Human parvovirus, pregnant women, serum, ELISA test, anemia, nested PCR

Introduction
A disorder known as anemia occurs when the body does not produce enough healthy red blood cells. The body's tissues receive oxygen from red blood cells. Various forms of anemia consist of: Anemia brought on by a lack of vitamin B12. Anemia brought on by a lack of folate (folic acid). [3]

Parvoviruses belong to *Parvoviridae* family, these viruses have been taken their name from Latin *parvum* or *parvus* denoting to tiny or small virions of parvovirus [1]. While Yvonne Cossart who discovered parvovirus first time in 1974 in human during checking of surface antigen of hepatitis virus type B in serum sample and microscopically testing showed abnormal results, This virus was termed B19 depending on code of serum sample with number 19 in board B, then in 1985 International Committee on Taxonomy of Viruses (ICTV) documented B19 as its relationships with numerous diseases [2].

The genomes of Parvoviruses have single-stranded DNA (ss DNA) naturally have two genes named VP/cap gene and NS/rep gene, the viral protein (VP) encoded by VP gene which consist viral capsid while non-structural (NS) protein NS1 encoded by NS gene for protein replication which have HUH superfamily endonuclease [3]. The interfering of open reading frames with encoding slight number of promoting proteins contained in variant parts in life cycle of this virus [1].

Parvovirus B19 was a common infection that affected 1% to 5% of pregnant women, with a normal pregnancy result [4]. Infection during the first two trimesters of pregnancy is associated with a higher risk of an adverse fetal outcome, although it can arise at any time

during pregnancy. Severe fetal anemia can result from infection of fetal erythroid progenitor cells with a reduced erythrocyte half-life, which can lead to non-immune hydrops fetalis and high output cardiac failure [5]. Normal people have been shown to experience transient leucopenia, thrombocytopenia, and lymphocytopenia after hPV B19 infection [6]. Transient aplastic crisis is more likely to improve in pregnant women with hematological abnormalities such sickle cell disease [7].

Material and methods

Patients

About 252 pregnant patients at AL-Sader Medical City who participated in the case-control retrospective research were divided into two groups, one group suffering from anemia and the other group not yet suffering from anemia. Patients were between 15 and 55 years of age in Najaf governorate from January 2020 to January 2021.

Anemia Diagnosis

Hemoglobin, red blood cells, and other components of your blood are measured by a complete blood count (CBC) test. Following the CBC, your doctor will inquire about your medical and family histories. A blood smear or differential to count your white blood cells, an examination of the morphology of your red blood cells, and a search for abnormal cells are likely among the procedures they will perform. To detect immature red blood cells, measure the reticulocyte count.

Samples

Two types of samples, including blood serum. Whole blood was involved in complete blood count (CBC) test, while serum was used to detect parvovirus IgG and IgM antibodies by ELISA technique, and virus DNA by PCR technique.

Serological examination

Parvovirus B19 IgM and IgG was determined by ELISA (IBL – international, GMB, D-22335Hamburg-Germany (according to the manufacturer's instructions.

All patients provided written informed permission before having their entire medical histories and physical examinations performed. This study's subjects were all treated to the following procedures: Complete blood count and hematological analysis (CBC).

Serological examination

Studying parvovirus B19 required measuring specific IgM and IgG using enzyme linked immune sorbent assay. (IBL (international), GMB (generally recognized as a trade mark), D-22335 Hamburg, Germany (manufacturer's instructions).

Viral DNA Extraction

Viral DNA was extracted from serum samples by using Genomic DNA mini kit (gSYNC™ DNA, Geneaid Biotech Ltd. USA).

DNA amplification

The Nested PCR primers for Human Parvovirus was done as a previous study described by Schennach *et al*., (2002) [8]. Primer was supplied according (macrogen company, Korea) as following

Table 1: Amplification Primers

Primer	Sequence 5´- 3´	Amplicon
N-PCR	Forward, AATGAAAACTTTCCATTTAATGA	591bp
	Reverse, TCCTGAACTGGTCCCGGGGATGGG	

Genetic assessment Nested PCR assays were carried out to identify Human Parvovirus B19 in blood samples, after which the purity of the DNA was verified using a Nanodrop spectrophotometer (THERMO.USA) that reads absorbance in at (260 /280 nm). The procedure used to conduct this experiment was reported in detail by (Schennach *et al*., 2002) [8]. The following table 2 details the parameters used by a conventional PCR thermocycler setup.

Table 2: Conventional PCR Reaction Thermal Profile

PCR step	Temp. °C	Time	Repeat (cycle)
Initial Denaturation	94	5-min	1
Denaturation	94	30-sec.	35
Annealing	55	30-sec.	
Extension	72	2-min	
Final extension	72	5-min	1
Hold	4	Forever	-

After that, the previously mentioned components of the nested PCR master mix were added to a standard Accu Power PCR Pre Mix Kit, which also contains primers, probes, and dNTPs (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, stabilizer, and tracking dye) along with all other components required for a PCR reaction. Subsequently, place every PCR tube inside an Exispin vortex centrifuge and spin it for three minutes at 3000 rpm. After that, a PCR thermo cycler (Mygene, Bioneer, Korea) was used.

Statistical analysis

All data analysis was conducted in SPSS 17 (Inc., Chicago, IL, USA) from the Statistical Package for the Social Sciences. The chi-square test was used to examine the differences between the groups, and a significance level of P 0.05 was considered to indicate a significantly Authors Al-Ukaelii, S. A, and Al- Shaeb, S. M. (1998) [9].

Results

There were two main groups in the study: First group consisted of 126 pregnant women out of 252 with anemia, while second group consisted of 126 pregnant women out of 252 without anemia. Five age groups were created from the two groups. Figure.

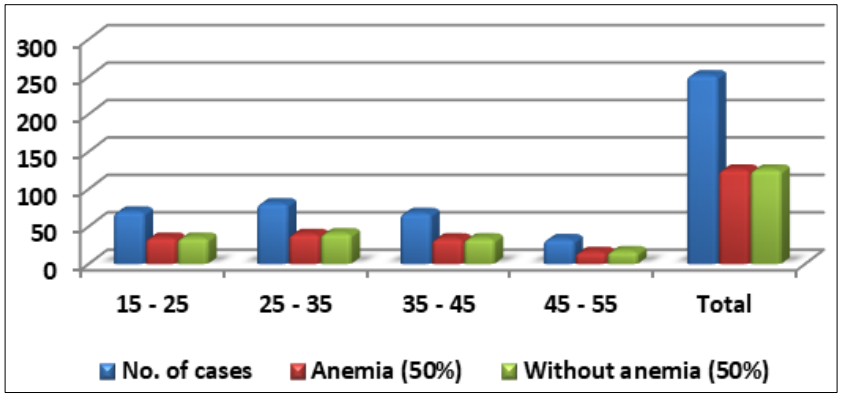

Fig 1: Anemia and non-anemia groups according to age groups

Depending on the level of anemia in pregnant women, the anemia in this study was categorized into three subgroups: mild anemia (54.3%), moderate anemia (29.5%), and severe anemia (15.4%). Figure 2.

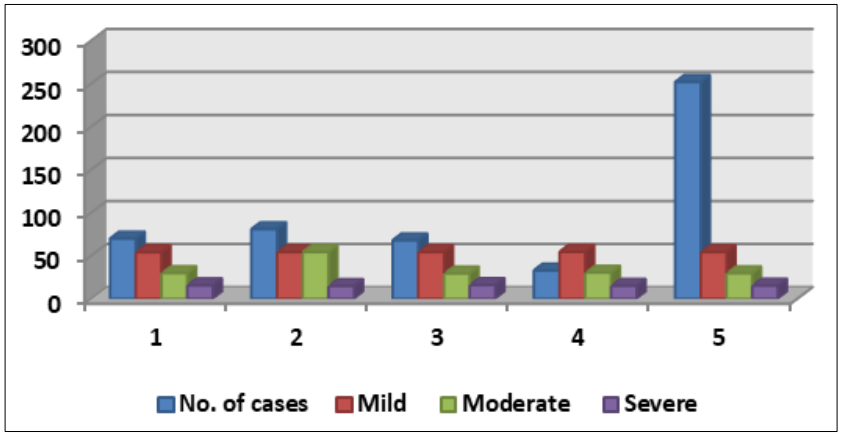

Fig 2: Degree of anemia cases

A total of 252 samples were tested, ELISA test showed that 80 out of 126 sample (63.4%) were positive Anti-Human Parvovirus IgM antibodies. Whereas 90 samples (71.4%) were positive for anti-HPV B19 IgM antibodies. Tables 3 and 4 show that only 10 (7.9%) of the 126 samples of pregnant women without anemia had anti-HPV IgG antibodies. However, only 26 samples (20.6%) tested positive for IgM antibodies against HPV. Table 3 and 4.

Table 3: Anti-Human Parvovirus IgM antibodies seropositivity in anemia and non- anemia group

Antibody	Anemia group	Control group	Total
IgM +	80 (63.4%)	10 (7.9%)	90 (35.7%)
IgM -	46 (36.5%)	116 (92%)	162 (64.2%)
Total	126	126	252

Table 4: Anti-Human Parvovirus IgG sero positivity in anemia and non- anemia group

Antibody	Anemia group	Control group	Total
IgG +	90 (71.4%)	26 (20.6%)	116 (46%)
IgG -	36 (28.2%)	100 (79.3%)	136 (53.9%)
Total	126	126	252

The results of the PCR technique used in both groups' sample diagnosis for the purpose of detecting viral DNA in pregnant women with anemia revealed that 77 out of 126 samples had a DNA positive result (61.1%).

In the same figure, a total of 126 control group only 8 sample out of 126 sample give positive result of parvovirus DNA (6.3%). The difference between study group and control group was significant (P>0.01).

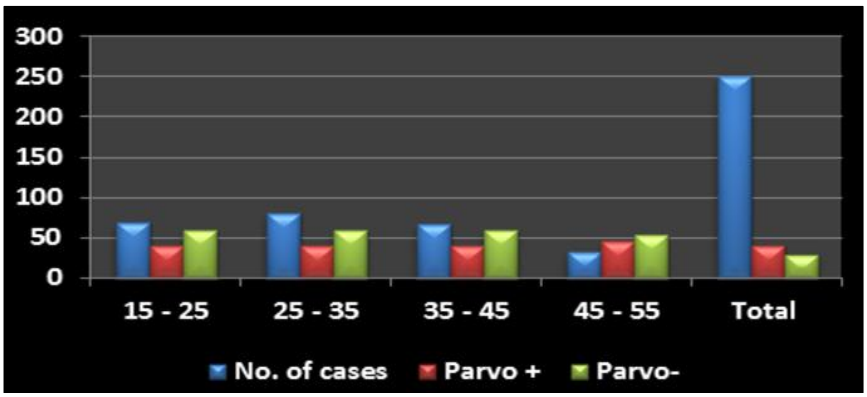

Fig 3: Parvovirus infection according to age groups.

As seen in figure 4, subgroups sample diagnosis using PCR technique with the aim of viral DNA identification. According to this number, 55 out of 137 (40.1%) samples had positive results for the PCR technique in the first subgroup (mild anemia), whereas 38/75 (50.6%) samples had moderate anemia. The other group (severe anemia) showed that 30 out of 39 (76.9 %) give positive result for PCR technique. there is significant deference (p <0.01). between the both sub group according to PCR result positive for HPV-B19 DNA.

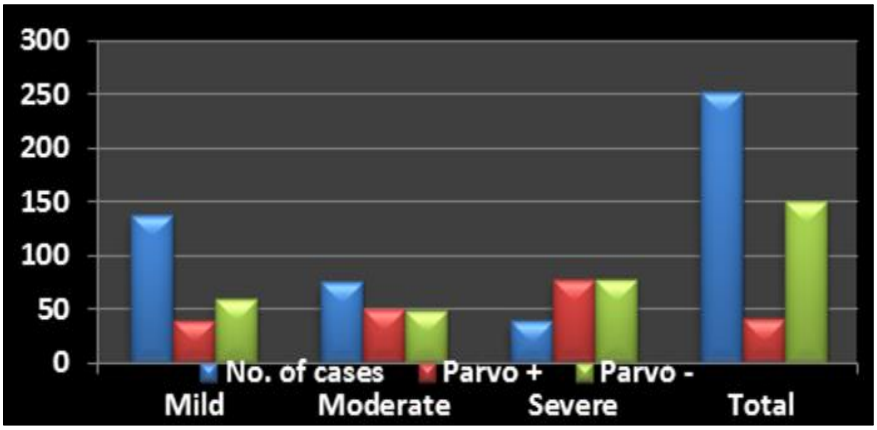

Fig 4: Association of parvovirus infectivity with the degree anemia cases according to age groups

The present study demonstrates about 54 samples (42.8%) that have both antivirus (IgM & IgG Ab) positive result while 10 samples (7.9%) was antivirus (IgM & IgG Ab) negative result. Table 5.

Table 5: Anti-Human Parvovirus IgM sero positivity according to IgG results

Antibody	IgG +	IgG -	Total
IgM +	54 (42.8%)	26 (20.6%)	80
IgM -	36 (28.5%)	10 (7.9%)	46
Total	90	36	126

The table 6 included the correlation between molecular and serological technique of pregnant women with anemia through detection of anti-B19 IgM antibodies and viral DNA, among the sample with IgM sero-positivity there were 75 sample (93.7%) with PCR result, while 5 sample (6.2%) give PCR negative result. At the same time only 3 sample (6.5%) with IgM negativity give positive PCR result.

Table 6: Correlation between HPV DNA findings and the prevalence of anti-HPV IgM sero positivity in pregnant women with anemia

Antibody	Viral DNA +ve	Viral DNA -ve	Total
IgM +	75 (93.7%)	5 (6.2%)	80 (63.4%)
IgM -	3 (6.5%)	44 (95.6%)	46 (36.5%)
Total	77 (62%)	49 (38%)	126
P value	< 0.00001 Sign. Dif. at p < 0.01		

In table 7. The correlation between molecular and serological technique of pregnant women without anemia through detection of anti-B19 IgM antibodies and viral DNA, among the sample with IgM sero-positivity there were 6 sample (60%) with PCR result, while 4 sample (40%) give PCR negative result. At the same time only 2 sample (1.7%) with IgM negativity give positive PCR result.

Table 7: Correlation between sero prevalence of parvovirus B19 detected by anti-HPV B19 IgM antibodies and DNA detected by Nested PCR technique control group

Control group	Viral DNA +ve	Viral DNA -ve	Total
IgM +	6 (60 %)	4 (40 %)	10 (7.9%)
IgM -	2 (1.7%)	114 (98.2%)	116 (92%)
Total	8 (7%)	118 (93%)	126
P value	< 0.00001		

Discussion

Human parvovirus B19 infection during pregnancy is common. The virus is not immune to between 30 and 50 percent of pregnant women, which is important throughout the child's wide years, which are between the ages of 3 and 6. While the majority of babies are healthy, about 30% of unwell pregnant mothers experience vertical transmission to the fetus. Placentitis and fetal confusion as severe anemia may result from the feto-placental characteristics of inflammation and loss of red blood cell precursors, in contrast to the frequently transient symptoms experienced by the mother [10].

The aim of distributing samples in this manner in the study was to compare pregnant women who developed anemia and those who had not yet developed anemia, as well as to demonstrate the role of viral infection in these cases. Owing to continuous care and their immune status, pregnant women have been exposed to many kinds of infection.

ELISA test showed that 63.4% were positive for anti-HPV IgM antibodies. Whereas 71.4 % were positive for anti-HPV B19 IgM Abs. in these results there was no statistical difference (P>0.05) between anti- IgG and IgM antibodies in study group. But when examining the control group, it gave much less results than the first group, 7% and 20.6%, respectively, which indicates that patients who suffer from anemia have much more infections than patients who have not developed anemia yet.

In an Indian study by [11], parvovirus B19 IgM and viral DNA were discovered in 40.7% and 37% of aplastic anemia

patients, respectively, suggesting a connection between parvovirus infection and aplastic anemia.

Since serological responses are less common in immune compromised and pregnant patients, molecularly based techniques may be useful in diagnosing acute or chronic infection [12]. On the other hand, human parvovirus B19 IgM can remain in the blood for up to 3-4 months after infection and is usually detectable 10-12 days after infection, as demonstrated by [13]. The present result disagrees with [15], which demonstrated that infection with B19V cannot be relied upon as adangerous factor in adult immune compromised, especially HIV-infected individuals. Circulating IgG remains forever with slowly diminishing titers until increased by further viral exposure.

Young *et al*., 2004 [16] indicate that the human erythroid progenitor cell is the only known host cell of parvovirus B19. Glob side (erythrocyte P antigen), which serves as a virus receptor, is responsible for this tropism.

Gaskell *et al*., (2008) [17] revealed that the frequent anemia more accurse in older age, with prevalence about ~17% in the group of older persons >65 years of age.

Our results in table 4 indicate that from 252 samples there are 137 samples are mild while 75 are moderate and about 39 samples with severe case while table 5 showed relation between parvovirus and age interestingly. The prevalence of Parvovirus infection in table 6 was slightly higher in age moderate at 25 to 35 years than what has been reported before and after this age and sever case of anemia was detected at 39 years.

Information on the relationship between the severity of the disease and the viral load in the mother's peripheral blood, umbilical cord blood, amniotic fluid, placental and extra placental tissue, can be used to predict the short- and long-term fetal outcome. Although HPV B19 infections can be detected by serology and polymerase chain reaction (PCR), more precise suggestive testing is needed to distinguish between acute, recent, and chronic HPV B19 illness.

The DNA of the virus was investigated in the three levels of anemia of a group of condition women who suffer from anemia. The three levels of mild, moderate and severe gave high proportions of the presence of the virus, and the last age group was the most affected, especially at the acute level of anemia, followed by the intermediate level. This indicates that the rate of infection with the virus and the presence of the virus is directly proportional to the level of anemia, and the greater the severity of anemia, the higher the percentage of the virus in the body.

Pregnant women and those with impaired immune systems have less pronounced serological reactions, and enhanced Because of the P antigen, parvovirus B19 prefers to bind to erythroid progenitor cells and less so to leukocyte and megakaryocyte cell lines. When the virus infects bone marrow-derived red blood cell lines, it causes hemolysis and red blood cell aplasia [19].

The present study showed in table 8 IgM in 80 pregnant women while IgG was detected in 90 pregnant women from 126 samples suffering from anemia and without anemia in table 9 then in table 10 appeared the correlation between IgM and IgG antibodies when about 12 samples detected with IgG also have IgM while 78 samples didn't have. Then table 11 shows the correlation between HPV DNA findings and the prevalence of anti-HPV IgM sero positivity in lymphoma patients with anemia depending on PCR technique there are 75 (93.7%) of samples detected with IgM while only 3 samples didn’t have IgM this result compatible with [21] Particularly relevant to the investigation of potentially abnormal pregnancies is the finding of fresh infection indicated by the presence of specific IgM antibodies in conjunction with viral DNA testing.

Zajkowska *et al*. 2015 [22] detect specific B19V-IgG antibodies in pregnant women and newborns at 40.4% percentage this shows that in spite of the prevalent diffusion of the antibody in the people, the detection of viral DNA and viremia is rare. In present study table 7 showed the correlation between sero prevalence of parvovirus B19 detected by anti-HPV B19 IgM antibodies and DNA detected by Nested PCR technique control group about 6 (60%) have IgM while 2 (1.7%) without. this result less than results conducted by [23] who showed (15.4%) of B19V-IgM antibodies in patients while B19-IgG antibodies were detected in (40.4%) women Lastly, serology and PCR are primarily used for diagnosis; however, additional selective diagnostic tests are essential to distinguish between acute, novel, and persistent hPV B19 disease.

In 1996, Young [24] provided a description of The connection between parvovirus B19 infection and the development of aplastic anemia is unclear. One of the two hypotheses that might be put up is that parvovirus B19 has an immediate impact. This virus's cellular receptor has been identified as a P group antigen in blood, which is also expressed by megakaryocytes and fetal liver cells.

Osaki *et al*. (1999, [25] and Muir *et al*. (1992, [26] provide explanations. The second theory postulates that immunological mechanisms serve as a mediator. Virus-associated haemophagocytic syndrome, brought on by acute parvovirus B19 infection, would be characterized by pancytopenia and/or reduced haematopoiesis due to increased cytokines such as interferon, which would impair regulation of the phagocytic system.

According to [27], those who have low erythrocyte counts as a result of diseases such as thalassemia, HIV, sickle cell disease, spherocytosis, or iron deficiency anemia are more prone to experience a transient aplastic crisis in the event that they contract parvovirus B19. The virus prevents the formation of red blood cells.

Ashish *et al*., 2012 [27] concluded that even in immune competent people, B19 can cause severe marrow aplastic effects, according to Ashish. As a consequence, it should be considered as one of the differential diagnoses in acquired aplastic anemia patients. Because all of the patients in this trial were part of a high-risk demographic, the exposure ratio of B19 was much higher than in earlier studies (haematological malignancy, immune compromised status, etc.). Malignant lymphoma patients were shown to have high rates of B19 infection. The research investigation was carried out to shed light on the connection between B19 infection and anemia. [28]. Moreover, viral infections, and particularly PVB19, are among the most prominent pathogenic agents in autoimmune illnesses and may play a crucial role in the onset of pathogenesis in these situations. No significant association between PVB19 prevalence and SLE was seen across age ranges or sexes. [29].

Conclusion

The relationship is directly proportional between infection with the virus and the increase anemia, as pregnant women suffering from anemia showed more viral infections than pregnant women who did not suffer from anemia. Also, the rates of infection with the virus increased with the increase in the degree of anemia, as between severe and moderate anemia rates of infection with the virus More than the mild degree of anemia.

Ethical Approval
After gaining both verbal and written agreement from the participants, blood samples were collected. The research plan has been given the go light by the Kufa College of Medicine's ethics board.

Conflict of Interested and Authors Contribution
It's confirmed with the attached file.

Funding
Funding from Personal Savings.

References

1. Cotmore SF, Agbandje-McKenna M, Canuti M, Chiorini JA, *et al*. ICTV Virus Taxonomy Profile: Parvoviridae. J Gen Virol. 2019;100(3):367-368.
2. Heegaard ED, Brown KE. Human parvovirus B19, Clin Microbiol Rev. 2019;15(3):485-505.
3. Anemia: Practice Essentials, Pathophysiology, Etiology". 9 November 2021. Retrieved 8 February 2022.
4. Mietzsch M, Pénzes JJ, Agbandje-McKenna M. Twenty-Five Years of Structural Parvovirology, Viruses. 2019;11(4):362.
5. Darvishi M, Forootan M, Nazer MR, Karimi E, Noori M. Nosocomial Infections, Challenges and Threats: A Review Article. Iranian journal of medical microbiology. 2020;14(2):162-181.
6. Feldman DM, Timms D, Borgida AF. Toxoplasmosis, parvovirus, and cytomegalovirus in pregnancy. Clin Lab Med. 2010;30(3):709-20.
7. Ergaz Z, Ornoy A. Parvovirus B19 in pregnancy Reprod Toxicol. 2006;21(4):421-35.
8. Schennach H, Lanthaler AJ, Mayersbach P, Human Parvovirus B19. Detection in Asymptomatic Blood Donors: Association with Increased Neopterin Concentrations. 2002;186:(10):1494-1497.
9. Potter CG, Potter AC, Hatton CS, Chapel HM, Anderson MJ, Pattison JR, *et al*. Variation of erythroid and myeloid precursors in the marrow and peripheral blood of volunteer subjects infected with human parvovirus (B19) J Clin Invest. 1987;79(5):1486-1492.
10. Beigi RH, Wiesenfeld HC, Landers DV, Simhan HN. High rate of severe fetal outcomes associated with maternal parvovirus b19 infection in pregnancy. Infect Dis Obstet Gynecol; c2008. 524601.
11. Danesh F, Ghavidel S. Coronavirus: Scientometrics of 50 Years of global scientific productions. Iranian Journal of Medical Microbiology. 2020;14(1):1-16.
12. Al-Ukaelii SA, Al-Shaeb SM. Statically Analysis by used SPSS Program. Al-Shoroq house for Publishers and advertisement Amaan Jordan; c1998.
13. Ronald F, Lamont, Sobel J, *et al*. Parvovirus B19 Infection in Human Pregnancy BJOG. 2011;118(2):175-186.
14. Mishra B, Malhotra P, Ratho RK, Singh MP, Varma S, Varma N. Human Parvovirus B19 in patients with aplastic anemia. Am J Hematol. 2005;79:166-167.
15. Knoll A, Louwen F, Kochanowski B, *et al*. Parvovirus B19 infection in pregnancy: quantitative viral DNA analysis using a kinetic fluorescence detection system (TaqMan PCR) J Med Virol. 2002;67(2):259-266.
16. Barton RB. Parvovirus B19: twenty-five years in perspective. Pediatr Dev Pathol. 1999;2(4):296-315.
17. Ghiasi SS, Esmaeili D. Rapid Detection of COVID-19 by RT-LAMP PCR Technique and its Comparison with Real-Time RT-PCR Method. مجله میکروب شناسی پزشکی ایران, 2-2.
18. De-Jong EP, De-Haan TR, Kroes AC, Beersma MF, Oepkes D, Walther FJ. Parvovirus B19 infection in pregnancy. J Clin Virol. 2006;36(1):1-7.
19. Nouri M, Kamakifar P, Khodabandehlou N, Sadri J, Nahand, Tavakoli A, *et al*. 2019;33:137.
20. Young NS, Brown KE. Mechanisms of disease: Parvovirus B19. N Engl J Med. 2004;350:586-597.
21. Gaskell H, Derry S, *et al*. Prevalence of anaemia in older persons: systematic review. BMC Geriatr. 2008;8:1.
22. Alger LS. Toxoplasmosis and parvovirus B19. Infect Dis Clin North Am. 1997;11:55-75.
23. Levy R, Weissman A, Blomberg G, Hagay ZJ. Infection by parvovirus B19 during pregnancy: a review. Obstet Gynecol Surv. 1997;52:254-9
24. Voleva S, Manolov V, Angelova S, Vasilev V, *et al*. Research Article - Clinical Practice. 2018;15(1).
25. Servant-Delmas A, Lefrère JJ, *et al*. Advances in human B19 erythrovirus biology. J. Virol. 2010;84(19):9658-9665.
26. Zajkowska A, Garkowski A, Czupryna P, *et al*. Seroprevalence of parvovirus B19 antibodies among young pregnant women or planning pregnancy, tested for toxoplasmosis. Przegl. Epidemiol. 2015;69(3):479-482, 597-600.
27. Young NS. Parvovirus infection and its treatment. Clin Exp Immunol. 1996;104(1):16-30.
28. Ghorani M. Antiviral Effects of Probiotic Metabolites. Iranian Journal of Medical Microbiology. 2022;16(2):83-97.
29. Osaki M, Matsubara K, Iwasaki T, *et al*. Severe aplastic anemia associated with human parvovirus B19 infection in a patient without underlying disease. Ann Hematol. 1999;78:83-6.
30. Muir K, Todd WT, Watson WH, *et al*. Viral-associated haemophagocytosis with parvovirus-B19-related pancytopenia. Lancet. 1992;339:1139-40.
31. Smith-Whitley K, Zhao H, Hodinka RL, Kwiatkowski J, Cecil R, Cecil T, *et al*. Epidemiology of human parvovirus B19 in children with sickle cell disease. Blood. 2004;103:422-7.
32. Sehgal A, Jain D, Sen R, Gupta A. Acute Parvovirus B19 Infection Leading to Severe Aplastic Anemia in a Previously Healthy Adult Female. Indian J Hematol Blood Transfus. 2012 Jun;28(2):123-126.
33. Qasim MD, Yasir SJ. ASSOCIATION OF PARVOVIRUS B19 INFECTION WITH ANEMIA IN LYMPHOMA PATIENTS. Biochem. Cell. Arch. 2019;19(1):1975-1981.
34. Saadat N, Vakilimofrad H, Khazaei S, Ansari N, Amini R, Azizi-Jalilian F. Serum Amyloid A (SAA) as early diagnosis of Covid-19 disease: A systematic review and meta-analysis. مجله میکروب شناسی پزشکی ایران, 3-3.
35. Mahmood TA, Abbas TS, Yasir SJ, Hashim MS. Parvovirus infection in systemic lupus erythematous patients in Najaf governorate, Iraq. © Annals of Tropical Medicine & Public Health. 2021;24(5):424-430.