# ANEMIC SYNDROME ASSOCIATED TO PARVOVIRUS B19 INFECTION IN PATHOLOGY PREGNANCY WOMEN

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**Summary**

Viral infections during pregnancy on a world scale are one of the main reasons for severe complications and mortality of the mother and fetus. The post-infectious anemic syndrome is characterized by low serum iron and increased hepcidin, which is the cause for iron accumulation in the endothelial macrophage system and iron deficiency for the needs of erythropoiesis. Probably the increased hepcidin plays a protective role against the growth of microorganisms by reducing extracellular iron. On the other hand, the increased hepcidin may lead to iron deficiency and to inability for effective compensation upon oral supplementation because it suppressed intestinal iron absorption.

This study aims to determine the involvement of parvovirus B19 in the anemic syndrome development in the course of/during pathological pregnancy.

**Materials and Methods:** In total 47 serum samples of pregnant women with anemia hospitalized in University Obstetrics and Gynecology Hospital "Maichin Dom",were tested. Three newborn babies were also included in the study. Serological (ELISA), molecular (PCR), and immunological (CLIA) methods were used. The statistical processing of the results is based on paired Student's t-test and Pearson's correlation.

**Results and discussion:** 9/47 (19.1%) of patients showed presence of B19V-IgM antibodies. B19-IgG antibodies were detected in 19/47 (40.4%) women. The PCR analysis showed presence of viral DNA in all patients with positive B19V-IgM antibodies. B19V-IgM antibodies were proved in one of the newborn and viral DNA was detected. All three babies were positive for B19V-IgG antibodies. In four of the positive patients with the three diagnostic markers for acute infection, the anemia was determined as iron-deficiency according to the low serum levels of hepcidin 2.54 ± 0.4 μg/L compared to control group of pregnant women without anemia (21.7 ± 3.1 μg/L: $P<0.001$). In the remaining women with a proven acute B19V infection, we found a statistically significant increased level of serum hepcidin (65.3 ± 5.7 μg/L; $P<0.001$) compared to non anemic pregnant women.

**Conclusion:** Assessment of the frequency and the grade of involvement of parvovirus B19 in the anemic syndrome development during pregnancy and determination of the serum level of hepcidin would contribute to the etiological clarification of the occurred anemic syndrome and would prevent improper iron supplementation by pregnant women.

***Key words**: parvovirus B19, anemia, hepcidin, iron, pregnancy*

## Introduction

In view of the various transmission of parvovirus B19, the transplanted pathway of the infection's transmission in pregnant women and the possible complications resulting from the infection, the screening of women of childbearing age (18-40 years) and seronegative pregnancies in the 1st and 2nd trimesters of pregnancy for presence of this viral agent is an important approach for compiling a correct differential diagnosis and outcome by the patients in risk.

The infections reach the fetus perinatally (from vaginal secret or blood) or after birth (with mother's milk). The clinical manifestations of neonatal infections vary depending on the viral agent and the gestational age of obtaining.

Parvovirus B19 is a single-chained DNA virus and is responsible for the development of

erythema infectiosum [1] or fifth childhood disease. Infection with this pathogen in the past has often been diagnosed as "rubella". Although in most cases by adults, the infection passes asymptomatically or with an atypical clinical picture, including influenza-similar illnesses, arthralgia and arthropathy, toxo-infectious syndrome, etc., the effects on the fetus are serious and include miscarriage, fetal anemia, myocarditis and/or intrauterine fetal death. Starting from the B19V tropism, the fetus and the placenta are its target due to the presence of specific P antigen, situeted on their surface. The frequency of vertical transmission by maternal infection is estimated by different authors between 17 and 33% [2,3]. Parvovirus B19 infection is associated with non-immune fetal hydrops [4,5].

The possible mechanisms in this connection include anemia, due to the viral particles which cross the placenta, inhibits fetal erythropoiesis, and as a result progressing to aplastic crises and congestive anemia, hypoxia and heart failure, which leads to fetal hydropathy. Another possible reason is fetal viral myocarditis, which results in heart failure and damage to liver function, caused by direct hepatocyte damage due to the hemoglobin accumulation [6,7]. The probability of this after 20 gestation's week is 2.3% [3].

A number of authors report neonatal complications after maternal B19 infection, including transfusion-dependent anemia [6,8], liver failure [9], myocarditis [10].

Literary data indicate a favorable outcome for the fetus at infecting a seronegative mother in 85% of cases [1]. Parvovirus B19 is not considered as teratogenic agent, affecting embryogenesis (8-10 weeks), therefore there is no indication for the interruption of pregnancy [3].

Parvovirus B19 has affinity to the hematopoietic system, including erythroid progenitor cells and, to a lesser extent, leukocytes and megakaryocytic cell lines [1]. The virus attacks the cell of the red blood cell line in the bone marrow, which results in hemolysis and erythrocyte aplasia[1].

Anemia is common during pregnancy and is associated with higher perinatal maternal morbidity and mortality in developing countries [11]. Iron deficiency is the cause of much of anemia among pregnant women [12]. Hepcidin, a peptide composed of 25 amino acids, is considered to be a major regulator of iron metabolism and anemia of chronic inflammation [13]. Hepcidin is synthesized mainly in the liver. It regulates the metabolism of iron by the inhibition of iron absorption in the duodenum at the level of the intestinal epithelium and by affecting mobilization of iron from the liver. Hepcidin associated with intracellular iron exporter, ferroportin, causing its internalization and degradation [14]. Ferroportin is required for materno-fetal transfer of iron from the duodenal enterocytes, macrophages and hepatocytes [15].

## Materials and methods

Clinical samples

In this study, a total of 47 serum samples from patients with anemia and progressing a pathological and/or risky pregnancy, as well as 3 serum samples of newborns, tested for possible maternal-fetal infection, are included. The following methods are used:

Serological analysis

An immunological method (ELISA) is used to demonstrate the presence of specific Parvovirus B19 IgM/IgG antibodies (commercial test for indirect immunoassay analysis Euroimmun Parvovirus B19 IgM/IgG). ELISA method was used for serum hepcidin quantification.

Established results were compared to age matched healthy controls – females with normal pregnancy, with no Parvovirus B19 infection and with no anemia.

**Molecular biological analysis**

From source material (serum samples) using a commercial test (Invitrogen), viral nucleic acid - DNA was extracted. To demonstrate the presence of parvovirus B19 DNA, PCR analysis was performed using a commercial kit (KAPA Taq PCR Kits) and the following consensus primers (at a concentration of 20 p / mol).

*Forward Primer (**e1905f**): 5' TGCAGATGCCCTCCACCCA 3'*

*Reverse Primer (**e1987r**): 5' GCTGCTTTCACTGAGTTCTTC 3'*

The amplification reaction for amplification of the NS1-B19 conserved region included: 1 cycle - 94 ° C for 6 min; 5 cycles - 94 ° C for 30 sec, 55 ° C for 1 min, and 72 ° C for 1 min followed by 45 cycles - denaturation at 94 ° C for 30 sec, aniling - 60 ° C for 30 sec, elongation - 72 ° C for 30 sec, final elongation - 72 ° C for 7 min.

**Agarose gel electrophoresis**

To visualize the obtained PCR products, electrophoresis is performed on a 2% agarose gel. Positive controls and a molecular weight marker are used thought 100 base pairs.

Statistical processing of the received results

The indicators for relative portions/shares are expressed as a percentage (%). Graphics and table analyses, Student's t-test и Pearson's correlation are also used.

Results and Discusion

Specific primary-reactive B19-IgM are proved in 9/47 (19.1%) of the patients. All analyzed samples are subjected to molecular-genetic analysis. The results show that all patients with proven early B19-IgM antibodies, viral DNA is also isolated and a positive PCR signal is detected (Figure 1)

Figure 1. Electrophoretic analysis 2% agarose gel: lines 1, 18 – MM (100bp); lines 2-6 и 9-14 – positive samples; lines 7, 8 – negative samples; line 15 – NC extr.; line 16 – negative PCR control; 17 – positive PCR control

In 19/47 (40.43%) patients, the results of serological and molecular-biological analyses show negative result. Based on this result, the patients have been recommended to re-examine a serum sample at a later stage, within one month of the last test or earlier at occurrence of symptoms and/or illness, as well as careful pregnancy monitoring by the monitoring obstetrician.

Protective B19-IgG antibodies, evidences of past infection, are found in 19/47 (40.4%) of women.

By one of the newborn B19V-IgM antibodies are proved and viral DNA is detected. All three babies are positive for B19V-IgG antibodies (maternal).

Figure 2 shows the percentage distribution of examined patients regarding to the three diagnostic markers.

Figure 2. Distribution of the examined patients on a test diagnostic mark

Patients with presence of a marker for acute viral infection (IgM and / or pathogenic DNA) are recommended monitoring the pregnancy (FV monitoring of the fetal and Doppler screening) and laboratory testing with traceable serum samples (1-3-6 months).

In four of the positive patients with the three diagnostic markers for acute infection, the

anemia was determined as iron-deficiency according to the low serum levels of hepcidin 2.54 ± 0.4 μg/L compared to control group of pregnant women without anemia (21.7 ± 3.1 μg/L: $P<0.001$). In the remaining women with a proven acute B19V infection, we found a statistically significant increased level of serum hepcidin (65.3 ± 5.7 μg/L; $P<0.001$) compared to non anemic pregnant women (Fig. 3).

Figure 3. Quantified serum hepcidin levels in pregnant women with IDA, with Parvovirus B19 infection and in control group (presented in μg/L, and as mean value ± SD).

*P – pregnancy, IDA – iron-deficiency anemia, Pv B19 – Parvovirus B19 infection

The study is of a start-up character and gives only initial data in view of the insufficient number of patients herein involved. The results obtained up to the moment turn the attention of the medical specialists concerned to the etiological role of parvovirus B19 in the development of anemic syndrome in patients with pathological and risky pregnancies. Viral infections are associated with significant changes in iron homeostasis. Anemia in infectious diseases is characterized by low serum iron and increased hepcidin, which is the cause of iron accumulation in the endothelial macrophage system and deficiency within the erythropoiesis. Probably the increased hepcidin plays a protective role against the growth of microorganisms by reducing extracellular iron. On the other hand, the increased hepcidin may lead to iron deficiency and an inability to effective compensation upon oral supplementation because it suppresses iron absorption through the intestinal mucosa. Inappropriate iron supplementation of children from these regions without deficiency in landfills can lead to severe side effects due to stimulated proliferation of latent pathogens [16,17,18].

Testing for the presence of specific IgM antibodies in combination with the presence of viral DNA is evidence of fresh infection, which is of particular importance in the monitoring of cases of pathological pregnancy. To this criterion in our study reply 19.1% of the patients surveyed.

Among the examined patients with anemia, although there is no clear clinical picture for this virus, including fever-rashes syndrome, the affinity of B19V appears to erythroid progenitor cells, leading to anemia. This may be another marker in differential diagnosis in pregnant women with severe, undiagnosed to the moment anemia and a possible viral agent, namely parvovirus B19 involved in the development of pathological pregnancy.

One of the newborns is with inborne infection and positive for all serological and molecular-biological markers (positive B19V-IgM and -IgG antibodies, positive result of PCR result).

Regarding the protection of the examined group of pregnant women and newborns towards B19V (presence of specific B19V-IgG antibodies), a percentage ratio of 40.4% is calculated. This indicates that despite the widespread diffussion of the antibody in the population, viraemia and the detection of viral DNA is rare. These data correspond to a small number of studies [19,20]. High attention should be exercised in seronegative pregnant women, which were 40% of the examined group of patients. Among the investigated clinical cases of women with anemia, the presence of acute B19 infection does not dominate, provided that the number of tested patients was small and the study was of a start-up character.

The results from different studies indicate that hepcidin is lower during pregnancy than in non-pregnant women, presumably to provide greater bioavailability of iron for both mother and fetus. Pregnant women with undetectable levels of hepcidin transfer a greater amount of iron taken from the mother to the fetus when compared to women with a detectable hepcidin, indicating that the levels of maternal hepcidin partially determine the bioavailability of the iron to the fetus. However, inflammatory conditions, including preeclampsia, malaria infection, and obesity are associated with a higher hepcidin pregnancies compared to healthy controls, suggesting that the bioavailability of the iron in the mother and the fetus may deteriorate under such conditions[21].

Patients with inflammatory and reduced hepcidin are expected to have an iron deficiency. In contrast, those with high level of hepcidin are diagnosed with ACI.
Using serum hepcidin levels would help in assessing the need for the application of preparations containing iron. The results suggest that patients with IDA may be subjected to treatment with such drugs, while patients with ACI do not need them.
Conclusion: Assessment of the frequency and the grade of involvement of parvovirus B19 in the anemic syndrome development during pregnancy and determination of the serum level of hepcidin would contribute to the etiological clarification of the occurred anemic syndrome and would prevent improper iron supplementation by pregnant women.

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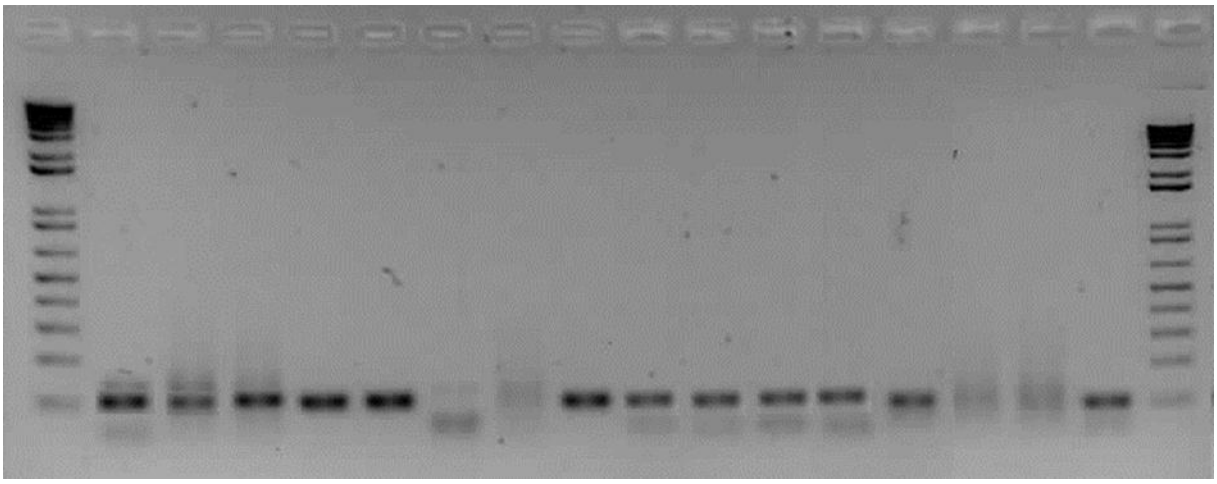

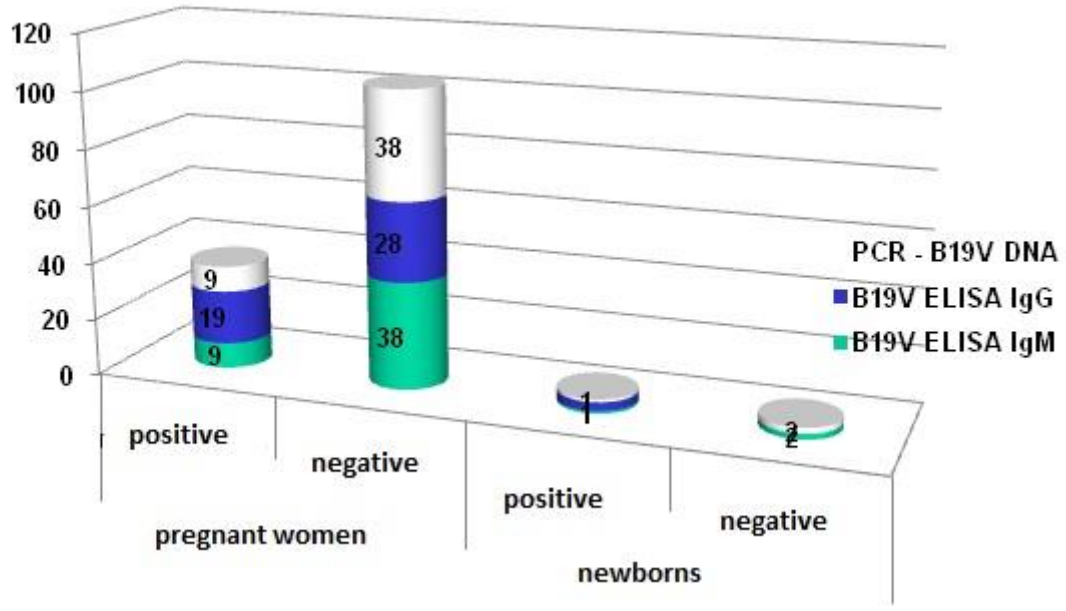

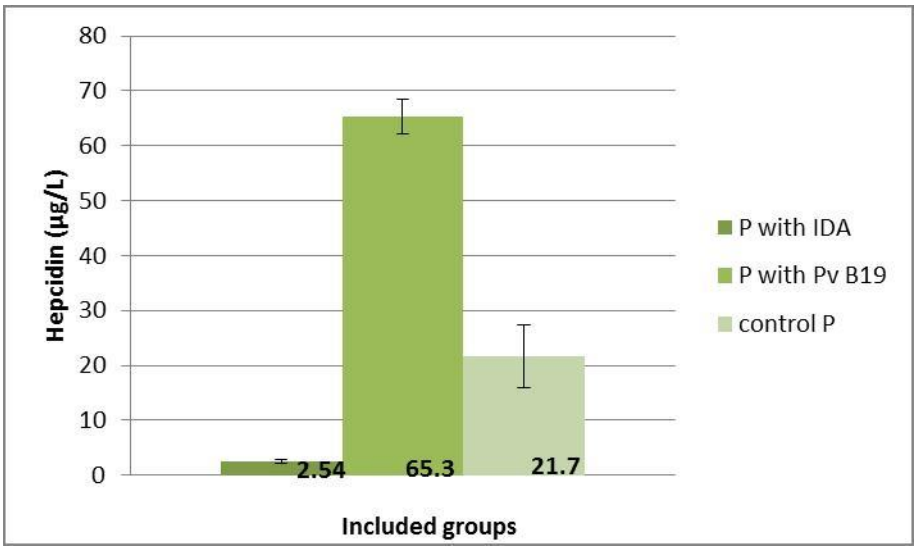
80
70
60
50
40
30
20
10
0
Hepcidin (µg/L)
2.54
65.3
21.7
Included groups
P with IDA
P with Pv B19
control P