Clin. Lab. 2019;65:XXX-XXX


## CASE REPORT

# Clinical Case of Parvovirus B19 Infection in Pregnant Woman with B-Thalassemia in Bulgaria

S. Voleva [1], V. Manolov [2], St. Krumova [1], B. Marinov [3], V. Vasilev [4], S. Shishkov [5], L. Nikolaeva-Glomb [1]

[1] *National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria*
[2] *Department of Clinical Laboratory and Clinical Immunology, MU, Sofia, Bulgaria*
[3] *University Obstetrics and Gynecology Hospital "Maichin Dom", Sofia, Bulgaria*
[4] *Clinical Laboratory and Clinical Pharmacology, Aleksandrovska Hospital, Sofia, Bulgaria*
[5] *Sofia University "St. Kl. Ohridski", Laboratory of Virology, Faculty of Biology, Sofia, Bulgaria*

### SUMMARY

**A pregnant 30-year-old female in the 34th gestational week was admitted at University "Maichin Dom" Hospital prior to childbirth. The patient is diagnosed with β-thalassemia. During laboratory screening hemoglobin of 98 g/L was established. Blood smear shows mild microcytic hypochromic anemia: RBC 5.15 x $10^{12}$/L, HGB 98 g/L, MCV 65.8 fL, MCH 19.4 pg, MCHC 295 g/L. Serum iron concentration is 12.9 µmol/L and ferritin 17.5 µg/L.**
**For the delivery process cesium was considered. Two days after procedure a rash presented on face, hands and breasts.**
**Although the mother was positive for parvovirus B19 infection, the baby was negative. This was confirmed by serological and molecular investigations. We discovered only the mother's B19V IgG antibodies in the newborn. In connection to the main disease, namely β-thalassemia, acute virus infection could cause aplastic crisis. After consultation with a hematologist, serum hepcidin concentration (an iron homeostasis regulator) was quantified: 19.4 µg/L.**
**ELISA test was used to prove B19V IgM antibodies in the mother. PCR analysis shows the presence of B19V DNA.**
**During infection, inflammatory cytokines increase hepcidin secretion, leading to iron deposition into cells.**
**(Clin. Lab. 2019;65:xx-xx. DOI: 10.7754/Clin.Lab.2018.181023)**

**Correspondence:**
Stefka Krumova Ivanova
44 A, General Stoletov Blvd
1233 Sofia
Bulgaria
Phone: +359 878 854 203
Email: stefka.krumova@gmail.com

Case Report accepted October 30, 2018

### KEY WORDS

pregnancy, β-thalassemia, anemia, hepcidin, parvovirus B19

### CASE PRESENTATION

A pregnant 30-year-old female in the 34th gestational week was admitted at University "Maichin Dom" Hospital prior to her second forthcoming childbirth. The patient is diagnosed with β-thalassemia. During laboratory screening hemoglobin of 98 g/L was established. CBC results were: RBC 5.15 x $10^{12}$/L, HGB 98 g/L, MCV 65.8 fL, MCH 19.4 pg, MCHC 295 g/L. Blood smear shows aniso-macro-microcytosis with presence of single target cells. RBCs were hypochromic. The reticulo-

cytes were 19%. Serum iron concentration is 12.9 μmol/L, ferritin 17.5 μg/L. Electrolytes (sodium, potassium, calcium, phosphates, and magnesium) were within reference ranges. Total bilirubin of 15.0 μmol/L was normal, most of it was conjugated (direct) (7.4 μmol/L); LDH was slightly increased (321 U/L), which indicates intravascular hemolysis. Fasting glucose was 5.1 mmol/L; kidney and liver function parameters were also within the reference ranges.

## DISCUSSION

The patient gave birth by c-section. Post-partum period was normal. Two days after the procedure a rash presented on face, hands and breasts. Due to the specific rash, parvovirus B19 infection was suggested.

Serological test (indirect immune-enzyme analysis by Anti-Parvovirus B19 IgM/IgG ELISA kits, Euroimmun, Germany) proved specific IgM/IgG antibodies against parvovirus B19 (B19V). By PCR assay (KAPA Taq PCR Kits) two B19V-DNA regions were detected: conventional NS1 region and NS1/VP1u "junction" region. The primer pairs which we used had a concentration of 20 p/mol according to Servant's protocol [1].

NS1 region (103 bp) was confirmed by standard PCR:

Forward Primer (e1905f):

5' TGCAGATGCCCTCCACCCA 3'

Reverse Primer (e1987r):

5' GCTGCTTTCACTGAGTTCTTC 3'

For NS1/VP1u, B19V region (994 bp) was confirmed by nested PCR:

Forward Primer (e1855f**):**

5' CACTATGAAAACTGGGCAA 3'

Reverse Primer (**e**2960r**):**

5' ACAATTCTTCATCTGCTAC 3'

Forward Primer (e1863f**):**

5' AAACTGGGCAATAAACTACAC 3'

Reverse Primer (e2953r**):**

5' CTTCATCTGCTACCGTCCAA 3'

The PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide. Presence of virus nucleic acid was proven.

On the fifth day after rash occurrence, a second specimen was taken. Again, specific IgM/IgG antibodies to B19V were proven along with B19V-DNA, which confirms etiology of virus infection.

Although the mother presented with perinatal B19V infection, the newborn was missing such data, and this was confirmed by applying serological and molecular examinations. Only the mother's B19V-IgG antibodies were discovered. This proves that parvovirus B19 is not a teratogenic agent, affecting embryogenesis (8 - 10 gestational week); therefore, it is not an indication for interruption of pregnancy [2] and 85% have a successful outcome of pregnancy [3].

Sequencing was performed on PCR products (994 bp) from the second-round of the nested NS1/VP1u - PCR and genotype 1a was shown (Figure 1).

All sequence techniques included series of consecutive operating protocols according to standard operating procedure "LNS-II-SOP052_Sequencing ABI Capillary sequencer".

The B19V 1a genotype detected has been circulating in European countries, including Bulgaria, for the past 10 years [4]. BLAST analysis in Genbank shows more than 90% similarity in results from Switzerland, Germany, Luxemburg, Holland, Italy, Belarus, and Georgia. Immunostimulators as therapy were prescribed to mother.

## CONCLUSION

Thalassemic syndromes are a heterogeneous group in hereditary diseases. The lack of or reduced synthesis of one or more normal polypeptides in the hemoglobin molecule is observed. Most frequent forms involve α- and β-chains. These conditions are the result of mutations in genes, which are inherited autosomal-recessively. β-thalassemia is more often seen in the Mediterranean area - Greece and Cyprus, including Bulgaria. Nearly 2.5% of the Bulgarian population is diagnosed with this disease [5]. This is equal to 200,000 persons that carry the β-thalassemia gene.

Parvovirus B19 shows not only cytolytic, but apoptotic activity. The virus can stop the cell's cycle, which may lead to death of erythroid progenitor cells and can unlock anemic syndrome [6]. In view of the patient's concomitant illness, namely β-thalassemia, acute viral infection could cause an aplastic crisis. Aplasia, also known as erythroblast hypoplasia, erythroblastopenia, erythrocyte agenesis can occur in cases without anomalies of leucopoietic or thrombocytopoetic systems. A similar case is originally described by Casalsan in 1922. Aplastic anemia may be the result of the influence of external factors, including infectious agents (B19V infection) [7] and drugs.

Usually in iron-deficiency anemia and β-thalassemia, serum hepcidin concentrations are decreased, probably caused by iron deposition. In our case, estimated serum hepcidin is within the reference ranges, which is caused by the presented inflammation. This is due to inflammatory cytokines [8]. Probably increased hepcidin concentration plays a protective role against the microorganisms' growth by reducing extracellular iron. On the other hand, increased hepcidin may lead to iron deficiency blocking the element's absorption by duodenal enterocytes. Supplementation with iron in case of inflammation may cause side effects by stimulated proliferation of latent pathogens [9].

Perinatal mothers' parvovirus B19 infection went on benign and recovery ad integrum with no infection and any risk for the newborn. The reported clinical case demonstrates that early diagnosis and timely therapy can be a blocking factor for complications, namely aplastic crises due to B19 infection in the presence of pathological conditions as β-thalassemia.

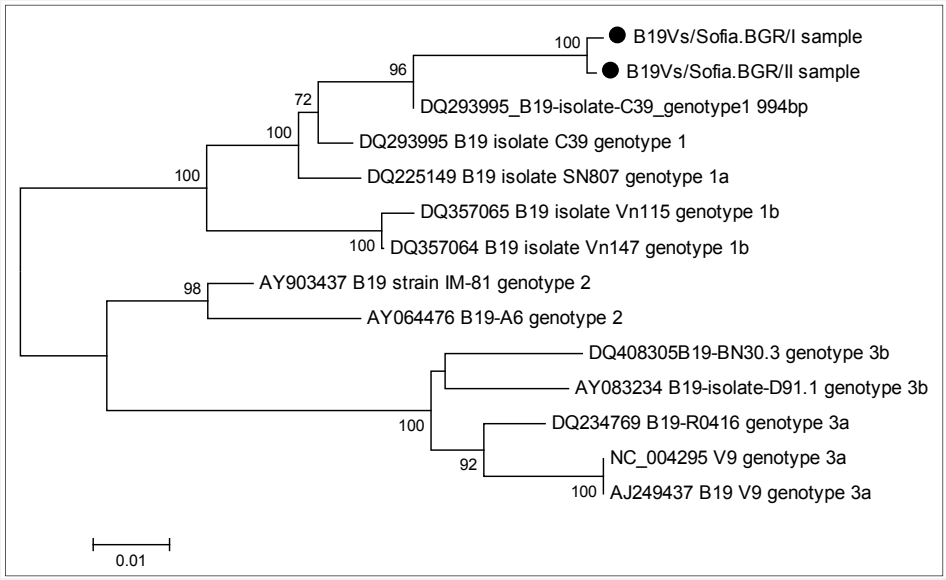


**Figure 1. Phylogenetic analysis of B19V sequences: 14 sequences (2 included in our study and 12 from GenBank).**

**Positive NS1/VP1u PCR product is marked by symbol black dot and is related to B19V genotype 1a. Phylogenetic analyses were performed using the Molecular Evolutionary Genetics Analysis (MEGA) version 5.0.5 software. The Kimura 2-parameter model and the neighbour-joining algorithm of MEGA 5 were applied and only bootstrap values ≥ 70% (1,000 replicates) were considered significant and are shown in the trees.**

**Acknowledgment:**
This trial was sponsored by the Medical University Sofia, Bulgaria - Grant No. Д-124/2017.

**Declaration of Interest:**
The authors declare that they had no conflicts of interest with any organization or institute during preparation of materials. All patients included in the trial have signed an informed consent according to respective requirements from The Code of Ethics of the World Medical Association (Declaration of Helsinki).
This article was prepared after sample collection from pregnant females in the department of University Obstetrics and Gynecology Hospital "Maichin Dom", Sofia, Bulgaria. During this period no pharmaceutical or other company was involved in the trial.
Medical University in Sofia, Bulgaria was involved in this trial by giving its agreement from the Ethics Committee.

**References:**

1. Servant A, Laperche S, Lallemand F, et. al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol 2002;76(18):9124-34 (PMID: 12186896).
2. Crane J; Society of Obstetricians and Gynaecologists of Canada. Parvovirus B19 infection in pregnancy. J Obstet Gynaecol Can 2002;24(9):727-34 (PMID: 20638583).
3. Levy R, Weissman A, Blomberg G, Hagay Z. Infection by parvovirus B19 during pregnancy: a review. Obstet Gynecol Surv 1997;52:254-9 (PMID: 9095492).
4. Ivanova St, Mihneva Z, Toshev A, et. al. Insights into epidemiology of human parvovirus B19 and detection of an unusual genotype 2 variant, Bulgaria, 2004 to 2013. Euro Surveill. 2016;21(4): (PMID: 26847955).
5. Petkov GH, Efremov GD, Efremov DG, et al. Beta-thalassemia in Bulgaria. Hemoglobin. 1990;14(1):25-33 (PMID: 2200762).
6. Chisaka H, Morita E, Yaegashi N, & Sugamura K. Parvovirus B19 and the pathogenesis of anaemia. Rev Med Virol 2003;13(6): 347-59 (PMID: 14625883).
7. Young NS, Kaufman DW. The epidemiology of acquired aplastic anemia. Haematologica 2008;93:489-92 (PMID: 18379007).
8. De Mast Q, Syafruddin D, Keijmel S, et al. Increased serum hepcidin and alterations in blood iron parameters associated with asymptomatic P. falciparum and P. vivax malaria. Haematologica 2010;95:1068-74 (PMID: 20133896).
9. Kemna E, Tjalsma H, Podust V, Swinkels D. Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. Clin Chem 2007;53:620-8 (PMID: 17272487).