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Gaucher disease in North Macedonia: Unexpected prevalence of the N370S GBA1 allele with attenuated disease expression

Nevenka Ridova [a], Sanja Trajkova [a], Biljana Chonevska [b], Zlate Stojanoski [a], Martin Ivanovski [a], Marija Popova-Labachevska [a], Simona Stojanovska-Jakimovska [a], Venko Filipche [c], Aspazija Sofijanova [d], Irina Panovska-Stavridis [a,*]

[a] Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, University Clinic for Hematology, 1000 Skopje, Republic of North Macedonia
[b] Acibadem Sistina Hospital – Skopje, Department of pediatric Hematology/Oncology, Skupi 5A, 1000 Skopje, Macedonia
[c] Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, University Clinic for Neurosurgery, 1000 Skopje, Republic of North Macedonia
[d] Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, University Clinic for Pediatric Diseases, 1000 Skopje, Republic of North Macedonia

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ABSTRACT

The majority of Gaucher Disease (GD) cases result from pathologic mutations in the GBA1 gene. A rich mutational spectrum of about 500 identified variants has been recognized. The disease is characterized by phenotypic diversity. Data regarding the genotype-phenotype correlation are scanty and inconclusive. Here, we summarize the genetic and phenotypic "portraits" of 14 patients with GD type 1 in the Republic of North Macedonia, 4 of Macedonian and 10 of Albanian origin. Altogether, 6 variants were detected, compounding 6 different genotypes. All genotypes contained the N370S variant, which was detected with an overall prevalence of 60.7%. Other frequent variants included the 1263del55 deletion and the double mutant allele D409H;H255Q, each with a prevalence of 14.2%. We detected two rare mutations: W92* - a pathogenic nonsense mutation and D399N – a single nucleotide variant of uncertain pathogenicity. The most common genotypes were N370S/1263del55 and H255Q;D409H/N370S, both present in 4/14 patients, followed by N370S homozygosity (3/14). Splenomegaly was the most common clinical manifestation, identified in all patients. Hepatomegaly was less frequent and was present in 50% of cases. Thrombocytopenia was present in 9/14, while half of the patients had anemia. Bone pathology was demonstrated in 8 patients. Patients with different genotypes displayed a high degree of phenotypic heterogeneity, suggesting that the other allele determines the onset and severity of the disease in patients with the N370S mutation. Longer follow-up, bigger cohorts of patients and multicentric studies should be conducted to further define the association between the genotypic and phenotypic expression in GD.

1. Introduction

Gaucher Disease (GD) is a rare autosomal recessive disorder of lipid metabolism and is the most prevalent lysosomal storage disease. There is considerable variability in incidence rate among different populations. The estimated annual incidence in the general population is 1/40,000 to 1/60,000 births, however, it reaches up to 1/800 in Ashkenazi Jews [1].

GD is caused by decreased activity of the lysosomal enzyme β-glucocerebrosidase, which catalyzes the breakdown of the glycolipid glucosylceramide (GlcCer) to ceramide and glucose. β-glucocerebrosidase is a 497 amino acid glycoprotein, encoded by the glucocerebrosidase gene (GBA1), located on chromosome 1 (1q21), which spans 7.6 kb of genomic DNA divided into 11 exons [2]. The majority of GD cases result from pathologic mutations in this gene. According to the Human Genome Mutation Database (HGMD), around 500 mutations have been described up to date, with variable prevalence in different ethnic populations [1].

Molecular heterogeneity of the disease and notable variation in allele distribution among different populations have been noticed. However, four mutations have been observed more frequently, including N370S, IVS2 + 1 + 1G > A, 84GG and L44P, accounting for 95% of the pathogenic alleles in individuals of Ashkenazi Jews. In non-Jewish populations these four mutations account for 50%–60% of the pathogenic alleles [3]. Among these, the N370S mutation is most frequent, and has

* Corresponding author at: Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, University Clinic for Hematology, 1000 Skopje, Republic of North Macedonia.
E-mail addresses: stojanoskiza@t-home.mk (Z. Stojanoski), dr_irina@yahoo.com (I. Panovska-Stavridis).

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been observed with a prevalence of 40% to 45% in Non-Jewish patients with GD and around 70% among Ashkenazi Jews GD patients [4].

The genotype-phenotype correlation in GD has still not been fully defined [5]. However, gathered knowledge has shown that the N370S mutation is associated with an attenuated disease expression, with N370S homozygotes typically presenting with mild symptoms, indolent clinical course and delayed disease onset. On the other hand, certain mutations, such as the L44P mutation, have been associated with neurological involvement and a severe phenotype [6]. In addition, patients with a heterozygous N370S mutation have been reported to have increased risk of developing Parkinsonism, whereas homozygosity for the N370S mutation has been linked with increased risk of monoclonal gammopathy [7].

We present here the clinical and genetic findings of 14 patients with GD type 1 from the Republic of North Macedonia. We report an unexpectedly high prevalence of the N370S mutation and investigate the possible association between genotype and phenotype expression in our GD cohort.

2. Methods

We evaluated 14 adult patients with GD type 1 followed and treated at The University Clinic for Hematology – Skopje. They originate from 7 various regions of the Republic of North Macedonia. Table 1 lists patients' individual characteristics, initial clinical manifestations, diagnostic procedures and types of therapy. Genetic profiles of these patients are shown in Table 2. Molecular genetic analyses were performed at The Research Centre for Genetic Engineering and Biotechnology at the Macedonian Academy of Sciences and Arts. Various molecular techniques were applied for detection of GBA1 mutations. DNA was extracted from EDTA peripheral blood mononuclear cells. In most cases, Gaucher Disease Strip Assay (ViennaLab Diagnostics GmbH, Vienna, Austria) was used for screening and detection of the most common mutations (N303S, IVS2 + 1 + 1G > A, 84GG, L44P, D409H, V394L, R463C and R496H) and the two most common recombinant alleles RecNciI, RecTL, following the manufacturer protocol. The assay is based on Polymerase Chain reaction (PCR) amplification and reverse hybridization of the amplification (biotinylated) PCR products to a strip containing allele-specific oligonucleotide probes. Affected alleles were detected by streptavidin-alkaline phosphatase and color substrates and identified as homozygotes, heterozygotes, and normal genotypes. DNA Sequencing (Sanger Method), followed by automated system capillary electrophoresis was applied for detection of point mutations in GBA gene exons, while Multiplex ligation probe amplification analysis with subsequent capillary electrophoresis was used for deletions and larger duplications in the exons. Samples that were not completely characterized by this approach were further analyzed by Next Generation Sequencing (NGS).

3. Results

3.1. Demographic characteristics

The study group consisted of 14 adult patients. There were 8 females and 6 males. The mean age of the patients was 39 (range: 19–55), while the mean age at the time of diagnosis was 30 (range 4–53). All patients were born to non-consanguineous marriages. Four patients were of ethnic Macedonian origin and 10 of Albanian ethnicity. Criteria for ethnic affiliation were based on personal identification of the patients. There were 3 relationships of first degree: patients 2 and 3; 7 and 8 and 9 and 10 (brother-sister; brother-brother and sister-sister respectively). There were no other interpersonal relationships and family linkages in both groups. Based on family history, none of the patients had any Jewish ancestry.

Six patients were diagnosed with GD in childhood (mean age 9; range: 4–14) at the University Clinic for Pediatric Diseases and later referred to our institution. The remaining seven were diagnosed in adulthood at our institution (mean age 43; range: 37–53).

3.2. Clinical and hematological findings

All patients, in whom enzyme replacement therapy (ERT) was initiated before puberty, have reached expected mid-parental height. In addition, all of them have normal body mass index (BMI). Majority of the patients diagnosed in adulthood (62%) have normal BMI. Females in this group are of short stature with an average height of 154.5 cm, which

Table 1
Individual characteristics of patients diagnosed with GD type 1 in Republic of North Macedonia.

No	Sex	Ethnicity	Age	Age at dg	Initial presentation	Diagnostic procedure	Treatment
1	F	Albanian	19	11	Massive splenomegaly, hepatomegaly, thrombocytopenia, anemia, abdominal pain	Genetic analysIs; BMB	Imiglucerase
2**	M	Albanian	19	4	Massive splenomegaly, hepatomegaly, thrombocytopenia, anemia, swollen knee	Genetic analysIs; enzyme assay, BMB	Imiglucerase *Splenectomy*
3**	F	Albanian	22	8	splenomegaly, hepatomegaly, bone pains	Genetic analysIs; enzyme assay, BMB	Imiglucerase
4	M	Albanian	44	7	Massive splenomegaly, hepatomegaly, thrombocytopenia, anemia, bone pains, fracture of the left hip, mobility impairment	Genetic analysIs; enzyme assay, BMB	Imiglucerase *Splenectomy*
5	M	Albanian	49	44	Massive splenomegaly, hepatomegaly, anemia, severe thrombocytopenia,	Genetic analysIs; BMB	Imiglucerase
6	F	Macedonian	34	7	Splenomegaly, bone pains, Rib fractures.	Genetic analysIs; enzyme assay, BMB	Imiglucerase, 11/2019 – Eliglustat tartrate
7**	M	Macedonian	50	37	Splenomegaly, thrombocytopenia, bone pains, lytic lesions in the spine and in the right hip.	Genetic analysIs; BMB	Taliglucerase alfa
8**	M	Macedonian	43	14	Splenomegaly, bone pains, fracture of the left hip	Genetic analysIs; BMB	Taliglucerase alfa
9**	F	Albanian	46	46	Splenomegaly, anemia, bone pains	Genetic analysIs; BMB	Velaglucerase alfa
10**	F	Albanian	42	40	Splenomegaly, thrombocytopenia, anemia	Genetic analysIs; BMB	Taliglucerase alfa
11	F	Albanian	42	41	Massive splenomegaly, hepatomegaly, thrombocytopenia, anemia, bone pains, osteoporosis	Genetic analysIs; BMB	Velaglucerase alfa *Splenectomy*
12	M	Albanian	49	47	Splenomegaly, thrombocytopenia, bone pains, fracture of the left hip	Genetic analysIs; BMB, liver biopsy	Taliglucerase alfa
13	F	Macedonian	55	53	Splenomegaly, thrombocytopenia	Genetic analysIs; BMB	Taliglucerase alfa
14	F	Albanian	38	37	Massive splenomegaly, accessory spleen, hepatomegaly, anemia, bone pains, fracture of the left hip	Genetic analysis; Bone biopsy	Velaglucerase alfa *Splenectomy*

Legend: BMB - bone marrow biopsy, F-female, M-male.
** Patients number 2&3; 7&8 and 9&10 are siblings.

Table 2
Genotypes and allele variations of patients diagnosed with GD type 1 in Republic of North Macedonia* Columns 2 and 3 use the cDNA nomenclature, according to which nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the first ATG translation initiation codon in the reference cDNA sequence. Columns 4 and 5 use HGVS recommended nomenclature, per which first ATG is numbered as codon 1 (http://hgvs.org/varnomen). Columns 6 and 7 use the traditional nomenclature, which considers as amino acid 1 the first amino acid after the signal peptide.

Pat.*	cDNA sequence variant		HGSV – Protein reference sequence		Protein change – traditional name		Molecular consequence	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 1
1	c.392A > G	c.1226A > G	p.Trp131Ter	p. Asn409Ser	W92*	N370S	Nonsense	Missense
2**	c.1226A > G	c.1263-1317del	p. Asn409Ser	p. Leu422ProfsX4	N370S	1263del55	Missense	Frameshift
3**	c.1226A > G	c.1263-1317del	p. Asn409Ser	p. Leu422ProfsX4	N370S	1263del55	Missense	Frameshift
4	c.1226A > G	c.1312G > A	p. Asn409Ser	p.Asp438Asn	N370S	D399N	Missense	Missense
5	c.882 T > G; 1342G > C	c.1226A > G	p.His294Gln; p.Asp448His	p. Asn409Ser	H255Q/D409H/	N370S	Double missense	Missense
6	c.1226A > G	c.1226A > G	p. Asn409Ser	p.Asp438Asn	N370S	N370S	Missense	Missense
7**	c.1226A > G	c.1263-1317del	p. Asn409Ser	p. Leu422ProfsX4	N370S	1263del55	Missense	Frameshift
8**	c.1226A > G	c.1263-1317del	p. Asn409Ser	p. Leu422ProfsX4	N370S	1263del55	Missense	Frameshift
9**	c.1226A > G	c.1226A > G	p. Asn409Ser	p.Asp438Asn	N370S	N370S	Missense	Missense
10**	c.1226A > G	c.1226A > G	p. Asn409Ser	p.Asp438Asn	N370S	N370S	Missense	Missense
11	c.882 T > G; 1342G > C	c.1226A > G	p.His294Gln; p.Asp448His	p. Asn409Ser	H255Q/D409H/	N370S	Double missense	Missense
12	c.882 T > G; 1342G > C	c.1226A > G	p.His294Gln; p.Asp448His	p. Asn409Ser	H255Q/D409H/	N370S	Double missense	Missense
13	c.115 + 1G > A	c.1226A > G	Not determined	p. Asn409Ser	IVS2 + 1G > A	N370S	Splice junction	Missense
14	c.882 T > G; 1342G > C	c.1226A > G	p.His294Gln; p.Asp448His	p. Asn409Ser	H255Q/D409H/	N370S	Double missense	Missense

** Patients 2 & 3; 7 & 8 and 9 & 10 are siblings.

is not the case with male individuals. The most frequent clinical finding was splenomegaly. Massive splenomegaly, defined as spleen reaching the iliac crest or crossing the midline, or spleen diameter > 18 cm as measured by abdominal ultrasonography, was present in 6 patients. Four of them underwent splenectomy at the age of 5, 7, 40 and 6, respectively (Table 1). Concomitant hepatomegaly was found in 7 patients. The most common laboratory abnormality was thrombocytopenia, which was moderate in 8 patients (platelet counts of 50 to 100 $\times$ 10^3 per μL) and severe in one patient (platelet counts below 50 $\times$ 10^3 per μL). Anemia was documented in 7 patients. We found a high prevalence of bone pathology in our patients. All but one of the patients diagnosed in adulthood had osteoporosis or severe osteopenia, assessed by bone densitometry. Bone fractures were confirmed in six patients - one patient had a pathological rib fracture as a child; five adults had hip fractures and two of them underwent total hip endoprothesis implantation due to extensive hip arthrosis.

3.2.1. Neurological status

All our patients were considered to a have non-neuronopathic disease-GD type 1, based on the absence of neurological complications associated with abnormal accumulation of glucocerebroside in the brain. However, emerging scientific reports indicating increased incidence of Parkinsonism and peripheral neuropathies in patients with type 1 disease question the suitability of the conventional classification and many authors consider GD as a continuum of phenotypes [8]. Therefore, our patients were evaluated for the presence of neurological symptoms or signs that could be present in GD type 1 such as Parkinsonism, peripheral neuropathies, abnormal eye movements and psychiatric symptoms [9,10]. By the time of the follow-up, only one patient who carries the D399N mutation had peripheral motor neuropathy on both legs, which was secondary to the marked bone disease and deformities of the legs. He also experienced hip fractures on several occasions and suffered from anxious depression.

3.3. Molecular genetic analyses

Genetic analysis identified 6 different mutations that were distributed in 6 different genotypes. The N370S mutation was most frequent and was identified in 17/28 alleles (60.7%). Other frequent mutations included the 1263del55 frameshift deletion and a double mutant allele of two *in cis* missense mutations (H255Q/D409H), which were both detected in 4/28 alleles. The IVS + 1G > A mutation, which has been detected at a relatively high frequency in other populations, was detected in only 1/28 alleles. The remaining two mutations included D399N and W92*, and were also detected in one allele each.

One patient of Macedonian origin was a homozygote for N370S and three patients were compound heterozygotes for N370S and IVS + 1G > A (one patient) or 1263del55 – (two brothers). We found 5 different mutations and 5 different genotypes in patients of Albanian origin, including N370S/N370S, N370S/1263del55, H255Q;D409H/N370S, W92*/N370S and N370S/D399N.

The most common genotypes were N370S/1263del55 and H255Q; D409H/N370S, each accounting for 28.6% (4/14 patients), followed by the N370S homozygosity (3/14 patients). The IVS + 1G > A/N370S; W92*/N370S and N370S/D438N genotypes were present in one patient each (1/14).

3.4. Genotype-phenotype correlations

Data on genotype-phenotype correlations are presented in Table 3. One of the patients with N370S homozygosity was diagnosed as a child presenting with hepatosplenomegaly, bone pains and rib fractures. The other two patients were sisters and were both diagnosed at an age of >40. They had a similar clinical presentation, including splenomegaly, moderate thrombocytopenia and anemia, but in contrast to patients with compound N370S heterozygosity had only mild bone pathology (osteopenia without osteoporosis and pathological fractures).

Among patients harboring the N370S/1263del55 genotype, the first two patients were diagnosed at the age of 4 and 8, respectively, presenting with hepatosplenomegaly. These two patients had been placed on enzyme replacement therapy (ERT) and had no signs of bone disease. In contrast, the most dominant feature in the other two patients, both diagnosed in adulthood was severe bone disease.

All compound heterozygotes for N370S and H255Q;D409H were diagnosed at adult age with advanced bone disease and pathological fractures. Three of them presented with massive splenomegaly and cytopenia. The patient carrying the W92* mutation was diagnosed at the age of 11 with organomegaly and thrombocytopenia. ERT was promptly initiated and she had normal hematological parameters and no signs of organomegaly at presentation in our institution. The patient carrying D399N, who was also diagnosed with GD as a child, due to poor compliance was irregularly monitored and treated.

4. Discussion

GD is considered to be an underdiagnosed disease due to the variable symptomatology and the lack of specialized referral centers. Approximately one in six patients reports a diagnosis delay of seven years from

Table 3
Genotype/phenotype correlation in patients with GD type 1 from the Republic of North Macedonia.

Genotype	Age of onset	Splenomegaly No. (%)	Hepatomegaly No. (%)	Thrombocytopenia No. (%)	Anemia No. (%)	Bone disease No. (%)
c.[1226A > G;1263-1317del]	Mean: 15,75 Range: 4–37	4 (100%)	2 (50%)	2 (50%)	1 (25%)	3 (75%)
c.[882 T > G;1342G > C; 1226A > G]	Mean: 42,25 Range: 37–47	4 (100%)	2 (50%)	3 (75%)	3 (75%)	3 (75%)
Homozygous c.[226A > G]	Mean:31 Range: 7–46	3 (100%)	0	2 (66.6)%	3 (66.6%)	1 (33.3%)
c.[392A > G;1226A > G]	1 patient					
c.[1226A > G;1312G > A]	1 patient					
c.[115 + 1G > A;c.1226A > G]	1 patient					

the first medical consultation [11]. Assuming an annual birth rate of 19.000 (State Statistical Office of Republic of North Macedonia), we expect an annual incidence rate of GD in our population of 0.31–0.47. We have data for a total of 20 diagnosed patients with GD type 1. There is anecdotal data for 2–3 suspected type 3 GD patients before the year of 2000, which were never confirmed, and no medical documentation is available. All adult patients had no or mild symptomatology at a younger age and symptoms developed after the third decade of life. As described above, our patients manifested various degrees of hematological and skeletal involvement. Our results are in line with the results from a large survey from 11 international centers [11], that reported splenomegaly, associated with hepatomegaly and thrombocytopenia as the most common presenting feature, occurring in 60% of 668 patients; followed by anemia (40%) and bone pains (50%).

We compared our molecular findings with the studies from neighboring and other European countries. N370S is present at a higher prevalence (60.7%) in our population compared to other countries in the region, including Albania [12] and Romania [13], with a prevalence of 50%, and Hungary, with a prevalence of 40.7% among GD patients [14]. Dimitrou and colleagues [15] recently reported a prevalence of 49.2% in a large cohort of 141 Greek GD patients, of which 120 with type 1 GD, with similar frequencies between patients of Greek and non-Greek origin (mainly Albanians). In contrast, recent data [16] on 114 Turkish GD patients (62 with type 1) place this mutation with a frequency of 32.6% in second place after L444P. The high frequency of N370S in our cohort, a mutation commonly associated with Ashkenazi Jews, as well as the similar observations in neighboring countries may suggest some related migratory history. From what is known, the first Jews settled in the Balkan Peninsula in the 4th century BCE, but were predominantly of Romaniote origin. However, around 1360 many Ashkenezi Jews settled in Bulgaria, Greece and North Macedonia, which could account for the high prevalence of this mutation in the region. Still, the majority of our patients were compound heterozygotes for N370S and a rate mutation, as reported for Non-Jewish populations [3].

Concerning the most prevalent genotypes in our population, compound heterozygotes carrying N370S/1263del55 genotype are believed to have a less favorable form of the disease in terms of age of diagnosis and symptomatology, because deletion of 55 bp leads to frame shift mutation and full loss of function [17]. Patients diagnosed in childhood had massive organomegaly and adult patients had marked bone disease.

D409H is another common mutation that was detected in 4 patients. This mutation can be found alone or in association with H255Q, which is typical for the Balkan region [18]. The presence of the H255Q in cis mutation has been in vitro reported to have a detrimental cumulative effect on the glucocerebrosidase enzymatic activity, leading to more severe phenotype in patents homozygous for the double mutant allele, compared to the patients carrying D409S in homozygous status [19,20]. On the other hand, the clinical phenotype of patients who are compound heterzoygots depends on the pathogenicity of the other allele [18,21]. In our patients the D409H/H255Q allele was always associated with the N370S allele, which could account for the diagnosis in later life and the milder disease characterized mainly by bone disease and moderate cytopenia. All of the patients with this mutation were of ethnic Albanian origin, which further supports previous data from haplotype analyses suggesting that this mutation originated in the Albanian part of the Balkans or among Albanian descendants in the Adriatic area of Italy [21].

Our analysis uncovered two rare mutations, W92* and D399N. The W92* or c.392A > G (p.Trp131Ter) mutation results in a premature termination codon and consequent truncation or absence of the encoded protein due to nonsense mediated decay. Several mutations in the same codon have been described. It is shown to be a pathogenic mutation, but data on its clinical implication are scarce [10,16,22]. In our patient, a young girl of Albanian ethnicity, the presence of this mutation resulted in massive splenomegaly and hepatomegaly, accompanied with cytopenia.

The D399N or c.1312G > A (p.Asp438Asn) missense mutation is located in the catalytic domain and has been reported to decrease the GBA enzymatic activity [23]. This mutation has been identified in only a few patients and data on its clinical significance in type 1 GD are lacking. It was described in a GD type 2 in association with L444P in a stillborn baby, and later in a heterozygous status with R463C mutation in a 24-year-old girl with organomegaly, bone disease and abnormal eye movements [24]. Previous data on the association of the missense substitutions in proximity with congenital heart disease expose the probable severity of this variant [10]. However, our patient had no evidence of cardiac disease, neither primary neurological and ocular involvement.

5. Conclusions

This is the first comprehensive overview of the molecular genetics and clinical phenotype of patients diagnosed with GD type 1 from the Republic of North Macedonia. The majority of detected mutations are well described variants, but our analyses detected two rare variants, whose clinical significance should be further studied. Our analysis revealed an unexpectedly high prevalence of the N370S allele. Patients with this mutation had relatively mild symptoms and no neurological involvement, consistent with an attenuated disease expression. A high degree of phenotypic heterogeneity was observed, suggesting that the other allele also influences the clinical course of patients with the N370S mutation. To better clarify the phenotypic consequences of specific mutations, other than N370S, and to further investigate the genotype/phenotype correlation in type 1 GD patients, data on larger GD populations would be required. Multicentre genotype/phenotype studies should be encouraged.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding authors on a demand.

Author contributions

Conceptualization: Irina Panovska-Stavridis*, Nevenka Ridova*. Data curation: Irina Panovska-Stavridis, Nevenka Ridova, Sanja Trajkova, Biljana Chonevska, Zlate Stojanoski, Martin Ivanovski, Marija Popova-Labachevska, Simona Stojanovska-Jakimovska, Venko Filipche, Aspazija Sofijanova.Writing: Nevenka Ridova* and Irina Panovska-Stavridis* wrote the original draft of the manuscript. Manuscript editing: Irina Panovska-Stavridis, Nevenka Ridova, Sanja Trajkova, Biljana Chonevska, Zlate Stojanoski, Martin Ivanovski, Marija Popova-Labachevska, Simona Stojanovska-Jakimovska, Venko Filipche, Aspazija Sofijanova. Supervision: Irina Panovska-Stavridis. Formal analysis: Irina Panovska-Stavridis, Nevenka Ridova, Martin Ivanovski.

Ethics approval

Not required.

Patient consent for publication

Informed consent was obtained from all patients for being included in the study.

Declaration of Competing Interest

The authors declare that they have no conflict of interest. All authors have provided ICMJE disclosure forms.

Data availability

Data will be made available on request.

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