

REVIEW

SPINAL MUSCULAR ATROPHY (WERDNIG-HOFFMANN ATROPHY/DISEASE): TWO CASE PRESENTATIONS AND LITERATURE REVIEW

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ABSTRACT

Introduction. Spinal muscular atrophy type 1 is an autosomal recessive neuromuscular disorder characterized by degeneration of the anterior horn cells in the spinal cord, leading to symmetric muscle weakness and atrophy. 95% of affected children die before 2 years of age. The annual incidence in the world has been estimated at around 1/11 000. The errors (mutations) in the *SMN1* gene prevalence vary from 1: 38 to 1: 70 in the population. The disorder is primarily caused by the homozygous deletions of the gene (5q12.2-q13.3). The *SMN* gene mutation is primarily caused by a homozygous deletion in exons 7 or 8.

Case presentations. 2 clinical cases of children with the Werdnig-Hoffmann disorder will be presented, and a literature review of this pathology. Two cases of spinal muscular atrophy diagnosed in Chernivtsi region, Ukraine, are presented. In both children, a molecular genetic analysis found the homozygous deletions of *SMN1* gene in exons 7 and 8. Most affected children die within 2.3- 1.3 years of age. These two cases ended lethally due to subinfection. Material was collected in accordance with ethical standards of work person under Helsinki Declaration (World Medical

RÉSUMÉ

Atrophie musculaire spinale de Werdnig-Hoffmann: deux rapports de cas et revue de la littérature

Introduction. L'atrophie musculaire spinale de type I appartient au groupe des maladies neuromusculaires autosomiques récessives caractérisées par la dégénérescence des cellules des cornes antérieures de la moelle épinière, qui entraîne à son tour une faiblesse musculaire symétrique et une atrophie musculaire. Chez 95% d'enfants, on observe une mortalité précoce jusqu'à l'âge de 2 ans.

Rapports de cas. Nous présentons 2 cas cliniques de trouble de Werdnig-Hoffmann chez les enfants de la région de Tchernihivtsi, en Ukraine, et faisons une analyse de la littérature sur cette pathologie. Deux cas d'atrophie musculaire sont présentés. Le matériel a été compilé conformément aux normes éthiques applicables aux essais cliniques (Association médicale mondiale, Déclaration d'Helsinki, Principes éthiques pour les recherches médicales humaines). On a utilisé des méthodes généalogiques et moléculaires génétiques, des tests sanguins biochimiques, ENMG. La maladie devrait être suspectée chez les bébés si l'anamnèse indique

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Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects). Genealogical analysis of families, biochemical analysis of blood, ENMG were carried out. The molecular genetic method was used: DNA was extracted and the deletions of 7 and 8 exons of the telomeric SMN gene were tested by PCR method. The disorder usually manifests in young children, if mother has a history indicating a weak fetal movement during pregnancy. Hypotension and hypotrophy of muscles, absence of tendon reflexes on lower extremities, fibrillation of the muscles of the tongue and fingers are observed in the neonatal period. Children with this pathology can poise their heads, but never turn over and do not sit. They are characterized by a „frog“ position: the limbs are laid in the shoulder and femoral joints and bent in elbow and knee joints. Chest distortions are pathognomonic. The main cause of death is respiratory distress associated with intercurrent respiratory disorders.

Conclusion. Based on the literature data and our experience of monitoring children with SMA type I, the disorder has a malignant rapidly progressing course.

Keywords: Werdnig-Hoffmann disease, spinal muscular atrophy, SMN1 and SMN2 gene, children.

Abbreviations. PCR – polymerase chain reaction; ENMG – electroneuromyography; SMA – Spinal Muscular Atrophy.

INTRODUCTION

Spinal muscular atrophy (SMA) is a group of hereditary disorders characterized by progressive degeneration of motor neurons of the anterior horns of spinal cord¹. There are proximal forms, which occur more often, and distal spinal muscular atrophy^{1,2}. The frequency of proximal forms of SMA is 80-85%, and distal – about 10%¹.

At present, 5 types of proximal SMA are distinguished, depending on the time when clinical manifestations appeared:

- SMA 0, the most severe form, which manifests itself intrauterinely;
- SMA I, severe form of Werdnig-Hoffmann disorder (OMIM 253300), clinically manifested in the first 6 months of life and which ends lethally in the first two years;
- SMA II, intermediate form (OMIM 253550), patients live longer than 4 years;
- SMA III, Kugelberg-Welander disorder, juvenile form (OMIM 253400) progressive muscle weakness develops after 2 years;
- SMA IV, adult form (OMIM 271150)^{1,3,4}.

un faible mouvement foetal pendant la grossesse. Une hypotension prononcée et une hypotrophie des muscles, une absence de réflexes tendineux sur les membres inférieurs, une fibrillation des muscles de la langue et des doigts sont notées dans la période néonatale, La principale cause de décès est l'insuffisance respiratoire dans le contexte de maladies respiratoires intercurrentes. L'article présente deux cas d'atrophie musculaire spinale de Werdnig-Hoffmann. La délétion des 7ème et 8ème exons du gène SMN1 à l'état d'homozygote a été détectée chez les deux enfants. La durée de vie des enfants a été de 2,3 et 1,3 ans. **Conclusions.** Ces deux cas se sont terminés par la mort des enfants en rapport avec l'addition d'une infection intercurrente.

Mots-clés: maladie de Werdnig-Hoffmann, amyotrophie spinale, SMN1 et SMN2, enfants.

SMA was first described by G. Werdnig in 1891, who presented a clear description of pathomorphological changes in various groups of muscles, peripheral nerves and symmetric atrophy of the cells of anterior horns of spinal cord and ventral roots, and also made assumptions about the hereditary nature of the disorder. A year later, J. Hoffmann identified SMA as an independent nosological unit. In 1893, G. Werdnig and J. Hoffmann presented a description of 7 cases from 4 families and showed that the cause of the disorder is the degeneration of the cells of the anterior horn of the spinal cord⁵.

The genes responsible for the disorder development are localized in the region of the chromosome 5q12.2-q13.3. Four genes, whose damage causes the development of the disorder and determines its severity, are localized here¹.

The most common mutation in the SMN gene is presented as a deletion of the 7 or 8 exons in the homozygous state⁶. The mutation in telomeric copy of the SMN gene is a necessary, but insufficient condition for the disorder, as the descriptions of healthy people with such a mutation in a homozygous state are known¹.

Gene – *NAIP* (a neuronal apoptosis inhibitor gene, OMIM: 600355), whose deletions of one or more exons in homozygous state occur in 40-70% of patients with SMA type I^{6,7}. In patients with deletion of the 7 and 8 exons of the *SMN*-gene in a homozygous state, a violation in the *NAIP* gene is detected simultaneously^{8,9,10}. The third gene – *H4F5* is located next to the *SMN* gene. It is assumed that its deletion or mutations are responsible for the severity of the clinical course of various types of SMA².

BTF2p44 is the fourth gene responsible for emergence of the disorder. About 15% of patients with SMA have the deletion of this gene in the heterozygous state.

The factors determining the severity of the clinical picture are: a) the number of centromeric copies of the *SMN* gene (2 – for type I of SMA and 3-5 – for II and III types of SMA); the simultaneous presence of deletion in the *NAIP*, *H4F5*, *BTF2p44* genes¹¹.

Most of the SMA are inherited by an autosomal recessive type. Gene *SMN* (survival motor neuron gene), represented by two highly homologous copies (telomeric – *SMN1* and centromeric – *SMN2*). In 90% of patients with different types of spinal atrophy, the deletion of the *SMN1* gene in 7 and 8 exons is recorded. The coding sequence of *SMN2* differs from *SMN1* with one nucleotide in exon 7 (840C-T), its replacement results in a decrease in the transcription and deficiency of the normal stable *SMN* protein. Approximately 94% of SMA people lack both copies of the *SMN1*-exon 7, which leads to significant protein loss. Loss of exon 7 may be the result of deletion or duplication of 840C-T, which, in essence, transforms *SMN1* into *SMN2* (by genetic conversion). Losing *SMN1* can also occur from other reasons, such as large deletions or point mutations. Most of the *SMN* protein is synthesized from the *SMN1* gene. The *SMN2* gene provides about 1/10 of the total amount of functional protein in the cell^{6,8}.

The protein encoded by the *SMN* gene consists of 294 amino acids and is expressed in all body tissues. The vast majority of protein is found in the motor neurons of the anterior horns of the spinal cord. *SMN*-protein of peripheral motor neurons has the following functions: participates in the process of mRNA; participates in the transport of mRNA in the axons of motor neurons; modulates the growth of axons and the dynamics of the cytoskeleton; *SMN* also plays an important role in maturation of axon terminals in muscles after childbirth; as a result of mutations in the *SMN1* gene, peripheral motor neurons lose the ability to control the transition from pre-mRNA to mRNA and produce the proteins necessary for their survival and functioning^{8,12,13}.

The authors¹⁴ have shown that the reduced level of *SMN* proteins changes the expression of the microRNA and their distribution in neurons. In particular, the levels of microRNA-183 are increased in *SMN*-deficient neurons. The depression of the expression of microRNA-183 in the spinal cord in the model of SMA mice increases life expectancy and improves the function of motor neurons.

It has been shown that in different human populations, the incidence of SMA type 1 varies from 1 to 6,000-11,000, or approximately 7.8-10 per 100,000 live births. The estimated incidence of the disorder in the world is 1 per 11,000 individuals. According to some data, the frequency of mutation of the *SMN1* gene in the human population ranges from 1:38 to 1:70¹². The reason for such a low morbidity rate may be that the genotype of some fetuses is characterized by the ratio of copies of the *SMN1*/*SMN2* genes as 0:0 (that is, the *SMN* protein is not synthesized at all), which is known to cause fetal death in other species^{15,16}. The Werdnig-Hoffmann SMA in Saratov region occurs with a frequency of 1.6 per 100,000 population, in Uzbekistan – 0.26 per 100,000 population, in Kazakhstan – 0.87 per 100,000 population. The prevalence of Werdnig-Hoffmann SMA in Switzerland is 1:17000, in Denmark – 1:20 000, in Toronto – 1:16000³.

The population-genetic research of Werdnig-Hoffmann SMA was also conducted among the Ukrainian population recently. In particular, it was found that the frequency of mutation carriers in exon 7 of the *SMN1* gene (840 C-T) was 3.24% (1:31)¹⁷. It should be noted that Ukraine is a multiethnic state¹⁸ with a number of effects of population dynamics, such as migration^{19,22}. This can lead to changes in the population frequencies of normal and pathological signs and the need for their continuous monitoring, as is the case, in particular, with respect to the SMA in other countries of the world²³.

THE PURPOSE OF THIS RESEARCH is to supplement the obtained population data with two clinical cases of Werdnig-Hoffmann disorder, in particular, their detailed description, which were discovered in the inhabitants of Northern Bukovina.

CASE PRESENTATIONS

We present 2 clinical cases of children with the Werdnig-Hoffmann disorder, which are determined by the genetician of OCCH in Chernivtsi, Ukraine, in 2016.

The material was collected in accordance with ethical standards of working person under Helsinki Declaration (World Medical Association Declaration

of Helsinki, Ethical Principles for Medical Research Involving Human Subjects).

The commission of biomedical ethics of the Bukovina State Medical University has not revealed violations of moral and legal rules in the conduct of medical scientific research.

Genealogical analysis of families, biochemical analysis of blood, ENMG were carried out. The molecular genetic method was used: DNA was extracted and the deletions of 7 and 8 exons of the telomeric SMN gene were tested by PCR method.

For the purpose of molecular genetic analysis, DNA from the blood leukocyte nuclei was taken from patients with spinal muscular atrophy and their family members. The informed consent for the study was obtained from all the patients. DNA was obtained by standard method – hydrolysis of cell lysates with proteinase K, followed by phenolic extraction²⁴. The presence of deletions of the 7 and 8 exons of the *SMN1* and *SMN2* genes was performed after hydrolysis of the in vitro amplification products of the corresponding sequences of the restriction endonucleases of *DraI* and *DdeI*, respectively, at a temperature of 37°C for 3-5 hours in 2% agarose gel²⁵. Both amplification products of the 7 exon of the *SMN1* and *SMN2* genes have the same size – 188 bps. They are distinguished by the product of amplification of the 7 exon of the *SMN2* gene, which has a site recognized by the endonuclease restriction of *DraI*. In patients with SMA, with a homozygous deletion of the 7 exon of the *SMN1* gene, only the hydrolyzed amplification products (fragments of 164 and 24 bps.) are detected on the electrophoresis, corresponding to the 7 exon of the *SMN2* gene. If the electrophoresis showed an unhydrolyzed PCR product of 188 bps, this indicated a deletion of the 7 exon of the *SMN2* gene in this patient. Hydrolyzed (corresponding to the sequence of the *SMN2* gene), as well as non-hydrolyzed amplification products (corresponding to the sequence of the *SMN1* gene), have been identified in patients with at least one copy of the *SMN1* or *SMN2* genes. The amplification products of the 8 exon of the *SMN1* and *SMN2* genes also have the same size (189 bps). They differ only due to the fact that in the 8 exon of the *SMN2* gene there is a cognition site for the restriction endonuclease *DdeI*. Therefore, if only these amplification products were determined on the electrophoresis, this indicated that the patient had no sequence of the 8 exon of the *SMN1* gene (homozygotes, patients with SMA). The presence of only one non-hydrolyzed fragment of 189 bps meant the absence of the sequence of the 8 exon of the *SMN2* gene in this individual. If, on the electrophoresis, both the hydrolyzed (corresponding to the *SMN2* gene) and the non-hydrolyzed (corresponding to the *SMN1* gene) amplification products were observed, this patient

was a heterozygote and had at least one copy of the sequences of the 8 exon of the *SMN1* and *SMN2* genes.

First case. A family with a two-year-old child, who was born from the third uncomplicated pregnancy, ended with physiological delivery, presented to the genetician. The mother's maternal grandmother and mother's blood brother are heterozygous carriers of mutations in the 7 and 8 exons of the telomeric SMN gene; the mother's brother born from the second marriage has been diagnosed with the type III SMA. The weight at birth – 4000 g, body length – 54 cm. From the history, we found out that in 3 months she began to poise her head, did not sit, did not stand. By the end of 9 months, the mother drew attention to a decrease in motor activity, muscle weakness. On examination, muscle tone is reduced. Tendon reflexes from the lower extremities were not activated. Abdominal reflexes are absent, there are no pathological reflexes. Muscle hypotension is detected. At 4.5 months, the girl stopped poising her head. She doesn't bear against her legs. The abdominal respiration type, the intercostal muscles almost do not participate in the act of breathing. The cranial nerves: III, IV, VI pairs – without pathology, bulbar disturbances. The diffuse muscular hypotonia, arreflexia, the „frog“ position are found, active movements in the limbs are not found. The osteoarticular system: „chicken breast“ chest distortion, hip dysplasia.

From the age of 1 month, anemia was diagnosed, hemoglobin levels were low until the age of one year. The girl was breastfed, the weight gain corresponded to the age. At the age of 5 months, she suffered acute nasopharyngitis, at the age of 6 months – acute pharyngitis. First teeth from 6 months.

Vaccinations: BCG, against viral hepatitis B (three times), against infantile paralysis (three times), DTP (twice). The child has been regularly observed by pediatrician and examined by other specialists.

In the biochemical blood analysis: an increase in the activity of creatine phosphokinase up to 9.10 $\mu\text{C/L}$ (normal value up to 3.4 $\mu\text{g/L}$), the lactate is normal (1.54 mmol/l). The results of electroneuromyography (ENMG) revealed the signs of diffuse motor neuropathy.

Indicative ENMG-picture allowed the suspicion of a hereditary disorder of the nervous system. The Werdnig-Hoffmann spinal atrophy was diagnosed phenotypically.

During the genetic testing, the deletion of exons 7-8 of SMN gene in homozygous state was revealed, which confirmed the diagnosis.

At the age of 2.2 years, she was admitted to the pediatric department with respiratory failure on the background of acute respiratory infections, followed by arrhythmic breathing, so the patient was transferred to the intensive care unit on the artificial lung

ventilation apparatus. During the whole hospitalization time, the child was in a clear consciousness. Despite the treatment, the state remained without any positive dynamics. Constantly, she was on ALV, lung-heart failure was constantly progressing. The child died at the age of 2 years and 3 months.

Second case. A one-year and two months old girl was admitted as emergency to the department of intensive care of the Children's Municipal Clinical Hospital in Chernivtsi, Ukraine, with complaints of shortness of breath, lack of movements in the legs, hypotrophy of the lower extremities.

Genealogical history – parents are healthy, from the first pregnancy in the same marriage a girl whose illness had similar symptoms was born and died at the age of 1 year and 4 months (pathological diagnosis: Werdnig-Hoffmann spinal muscular atrophy, acute cardiovascular insufficiency).

From the history: child from the 2nd pregnancy, without pathology, 2nd delivery at 39 weeks of gestation. She was born with a weight of 3500 g. From birth, mother drew attention to silent crying, reducing muscle tone, the child (lying on her stomach) did not raise her head and arms above the shoulder level.

Starting from 2 months of age, the child experienced a progressive decrease in motor activity, weight loss, loss of motor skills, ceased to poise her head.

There was diffuse muscular hypotension, absence of tendon reflexes from the lower extremities, the „frog“ position. After neurological treatment, there was a short-term positive dynamics in the form of improvement of motor activity.

According to the conclusion of a board of doctors, with the involvement of pediatricians and resuscitators, the condition of the child was treated as an unspecified myopathic disorder with respiratory failure.

Neurological status: clear consciousness, quiet crying. Cranial nerves: the fixed sight, followed the subject, bulbar disturbances, hypomimia. There were fasciculations of the tongue, diffuse muscular hypotension to atony in the legs, areflexia, the „frog“ position. The chest almost didn't participate in the act of breathing, transferred into artificial lung ventilation. Pulmonary exam – pneumonia.

Due to the critical condition of the child, it was not possible to perform ENMG. The DNA analysis revealed deletions of the 7 and 8 exons of the telomeric *SMN1* gene in the homozygous state. Conclusion: spinal muscular atrophy, type I. In the dynamics, the child's condition deteriorated due to the progression of respiratory and cardiovascular insufficiency, neurological symptoms, intoxication. The fatal outcome was due to the development of multiple organ failure at the age of one year and three months.

In the first and second case, the anatomopathological diagnosis was: Werdnig-Hoffmann spinal muscular atrophy. Dystrophic degenerative changes in the neurons of the nuclei of the medulla oblongata, the anterior and posterior horns of the spinal cord. Pneumonia. Polyorganic insufficiency.

DISCUSSION

The pathogenesis of SMA type I is associated with progressive degeneration of the motor neurons of the anterior horns of the spinal cord (in some cases, the motor cornea of the brain stem too). Due to the genetic defect, the programmed cell death emerges, as well as violations of axonogenesis. Loss of motor neurons leads to the development of flaccid paralysis and denervation atrophy of transversally strained muscles. In most cases, symmetrical lesions of the proximal muscles of the extremities are detected. Distal amyotrophy, lesions of bulbar musculature and deficiency asymmetry develop less frequently. Central motor neurons are intact. There are no sensory disturbances. The intelligence is saved^{5,17,18}.

Type I SMA manifests itself up to 6 months of age and has a rapidly progressing course.

From the medical history, the mother indicates a weak fetal movement during pregnancy. In the neonatal period, marked hypotension and hypotrophy of the muscles, absence of tendon reflexes, fibrillation of the muscles of the tongue and fingers are detected. Children with this pathology can poise their heads, but never turn over and do not sit. They are characterized by a „frog“ position: the limbs are laid in the shoulder and femoral joints and bent in elbow and knee joints. The muscles of the proximal parts of the lower extremities are the first to be involved into the pathological process. Then, hypotonia of muscles extends in upward type. Indicative bone deformities (saddle-shaped, sunken chest, as well as scoliosis and kyphosis in the chest and lumbar spine). In evolution, with the progression of the lesion, it extends to the muscles innervated by the bulbar group of the cranial nerves. Often, vegetative disorders appear in the form of moderate distal hyperhidrosis.

If muscle weakness occurs immediately after birth, death occurs at about 6 months of age, whereas when the first symptoms appear after 3 months of life, the life span can be about 2 years, only 10-12% of SMA children live longer than 5 years¹⁹. The main cause of death is respiratory failure on the background of intercurrent respiratory disorders.

The genetic method, which consists in determining the deletion of 7 and/or 8 exons in the *SMN1* gene, is the „gold standard“ for diagnosis^{13,20}. The deletions in the specified areas of the gene, in a patient in a homozygous state, confirm the diagnosis of SMA.

In the absence of deletion, a quantitative analysis of the number of copies of the SMN genes, by a multiplex ligation reaction method, with subsequent amplification, should be conducted². The following electrophysiological methods are used: needle electromyography, the study of muscle response caused by electrical stimulation and the estimation of the number of motor units. These methods are proposed for monitoring the course of SMA¹. Electromyographic (EMG) SMA marker: characteristic signs of denervation due to motor neuron defeat: spontaneous rhythmic activity („rhythm of the palisade“), potentials of fibrillation and fasciculations, positive sharp waves, changes in the potentials of motor units, with formation of gigantic polyphase potentials, and a decrease in the number of motor units¹⁹.

Biochemical markers in patients with SMA are: activity of creatine phosphokinase in blood serum, which can exceed the normal value by 2-4 times, but not more than 10 times. In the morphological study of muscles, specific signs of muscle damage are revealed: clusters of diminished fibers (beam atrophy), which alternate with sections of hypertrophic fibers¹.

The current treatment method for SMA is the use of anti-sense oligonucleotides (ASOs) directed against sequences that usually suppress the inclusion of the 7th exon of the SMN2 gene. This is one of the promising therapeutic approaches to SMA treatment. The ASOs demonstrated the ability to increase the inclusion of 7 exon in the transcript of the matrix RNA (mRNA) SMN2 and the development of a complete SMN protein.

Isis Pharmaceuticals (USA) has already completed the Phase I-II ASO trials (SPINRAZA medication) at SMA, which indicates the safety and potential efficacy of this therapeutic approach. SPINRAZA is administered by an intrathecal injection that provides drug penetration directly into the cerebrospinal fluid (CSF). At present, the company is working on two clinical trials of Phase III²¹⁻²³.

CONCLUSIONS

Thus, based on the literature data and our experience of monitoring children with SMA type I, the disorder has a malignant rapidly progressing course.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study“

REFERENCES

- Evtushenko SK, Morozova TM, Shestova EP, Omel'yanenko AA, Simonyan VA, Lutskiy KS. Spinalnyie myishechnyie atrofii i bokovoy amiotroficheskiy skleroz kak proyavlenie boleznii dvigatel'nogo neyrona u detey. *Mezhdunar.nevrolog. zhurn* 2013; 60(6): 15-28. (in Russian)
- Zabnenkova VV, Dadali EL, Polyakov AV. Proksimal'naya spinal'naya myishechnaya atrofiya tipov I-IV: osobennosti molekulyarno-geneticheskoy diagnostiki. *Nervno-myishechnyie boleznii* 2013; 3: 65-74. (in Russian)
- Arupova DR. Rasprostranennost i nozologicheskii spektr nervno-myishechnyih zabolovaniy v razlichnyih populyatsiyah (obzor literatury). *Nauka, Novyie tehnologii i innovatsii Kyrgyzstana* 2016; 7: 72-5. (in Russian)
- Hua Y, Sahashi K, Hung G, et al. Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model. *Genes Dev* 2010; 24(15): 1634-44.
- Pobedinskaya AI, Buyanova GV, Maslennikova NV, Smirnov DS. Spinalnyie myishechnyie atrofii detskogo vozrasta. *Pediatricheskii vestnik Yuzhnogo Urala* 2015; 2: 78-82. (in Russian)
- Fang P, Li L, Zeng J, Zhou WJ, et al. Molecular characterization and copy number of SMN1, SMN2 and NAIP in Chinese patients with spinal muscular atrophy and unrelated healthy controls. *BMC Musculoskelet Disord* 2015; 16: 11.
- Zeng G, Zheng H, Cheng J, et al. Analysis and carrier screening for copy numbers of SMN and NAIP genes in children with spinal muscular atrophy. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2014; 31(2): 152-5.
- Theodorou L, Nicolaou P, Koutsou P, et al. Genetic findings of Cypriot spinal muscular atrophy patients. *Neurol Sci* 2015; 36(10): 1829-34.
- Zarkov M, Stojadinovic A, Sekulic S, Barjaktarovic I, Peric S, Kekovic G, et al. Association between the SMN2 gene copy number and clinical characteristics of patients with spinal muscular atrophy with homozygous deletion of exon 7 of the SMN1 gene. *Vojnosanit Pregl* 2015; 72(10): 859-63.
- Jedrzejowska M, Milewski M, Zimowski J, et al. Phenotype modifiers of spinal muscular atrophy: the number of SMN2 gene copies, deletion in the NAIP gene and probably gender influence the course of the disease. *Acta Biochim Pol* 2009; 56(1): 103-8.
- Peeters K, Chamova T, Jordanova A. Clinical and genetic diversity of SMN1-negative proximal spinal muscular atrophies. *Brain* 2014; 137(Pt 11): 2879-96.
- Darras BT. Spinal muscular atrophies. *Pediatr Clin North Am* 2015; 63(3): 743-66.
- Qu YJ, Bai JL, Cao YY, et al. A rare variant (c.863G>T) in exon 7 of SMN1 disrupts mRNA splicing and is responsible for spinal muscular atrophy. *Eur J Hum Genet* 2016; 24(6): 864-70.
- Kye MJ, Niederst ED, Wertz MH, et al. SMN regulates axonal local translation via miR-183/mTOR pathway. *Hum Mol Genet* 2014; 23(23): 6318-31.
- Prior TW, Snyder PJ, Rink BD, et al. Newborn and carrier screening for spinal muscular atrophy. *Am J Med Genet* 2010; 152A(7): 1608-16.
- Ogino S, Wilson RB. Spinal muscular atrophy: molecular genetics and diagnostics. *Expert Rev Mol Diagn* 2004; 4(1): 15-29.
- Eggert C, Chari A, Lagerbauer B, Fischer U. Spinal muscular atrophy: the RNP connection. *Trends Mol Med* 2006; 12(3): 113-21.

18. Gregoretta C, Ottonello G, Chiarini Testa MB, et al. Survival of patients with spinal muscular atrophy type 1. *Pediatrics* 2013; 131(5): e1509-14.
19. Giannopoulou EZ, Martin T, Wirth B, Yilmaz U, Gortner L, Meyer S. Tongue fasciculations in an infant with spinal muscular atrophy type 1. *Clin Case Rep* 2015; 3(10): 832-4.
20. Alias L, Bernal S, Fuentes-Prior P, Barceló MJ, et al. Mutation update of spinal muscular atrophy in Spain: molecular characterization of 745 unrelated patients and identification of four novel mutations in the SMN1 gene. *Hum Genet* 2009; 125(1): 29-39.
21. Faravelli I, Nizzardo M, Comi GP, Corti S. Spinal muscular atrophy - recent therapeutic advances for an old challenge. *Nat Rev Neurol* 2015; 11(6): 351-9.
22. Zhou H, Janghra N, Mitropant C, et al. A novel morpholino oligomer targeting ISS-N1 improves rescue of severe spinal muscular atrophy transgenic mice. *J Hum Gene Ther* 2013; 24(3): 331-42.
23. Evers MM, Toonen LJ, van Roon-Mom WM. Antisense oligonucleotides in therapy for neurodegenerative disorders. *Adv Drug Deliv Rev* 2015; 87: 90-103.
24. Maniatis T, Fritsch EE, Sambrook J. *Molecular Cloning. A laboratory Manual*. Cold Spring Harbor Laboratory 1982; 480 p.
25. Hahnen E, Forkert R, Marke C, et al. Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. *Hum Mol Genet*.1995; 4: 1927-33.