CASE REPORT

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Our experience in the treatment of botulism

Naše iskustvo u lečenju botulizma

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Abstract

Introduction. Botulism is a neuro -intoxication caused by a toxin secreted by *Clostridium botulinum*. Due to extremely high toxicity and lethality, this toxin can be used as an agent in a biological warfare. **Case report**. We presented six patients, mean age 28.8 years, who ate canned food and in whom the diagnosis of disease was made based on the typical clinical picture. Predominant symptoms were blurred vision, double vision (diplopia), dry mouth and constipation which were present in all patients. The patient whose disease was recognized only after 23 days and who did not receive the anti botulinum serum underwent the longest hospital treatment. All the patients received antibiotics and 4 patients received antitoxin. Neostigmine and enemas were used for the treatment of the disorder of intestinal motility and constipation. **Conclusion**. The diagnosis of botulinum was made based on afebrility, preserved states of consciousness, double vision, dry mouth and history data on consumption of suspicious food. Polyvalent serum anti botulinum should be applied as soon as possible because it reduces the occurrence of complications, length of hospital stay and mortality rate.

Key words:
botulism; decontamination; botulinum antitoxin; treatment outcome; bioterrorism.

Apstrakt

Uvod. Botulizam predstavlja neurointoksikaciju izazivanu toksinom koji luči *Clostridium botulinum*. Zbog visoke toksičnosti i smrtnosti, ovaj otrov se može koristiti kao biološko oružje. **Prikaz bolesnika**. Prikazano je šest bolesnika, srednje starosti 28,8 godina, koji su konzumirali konzervisanu hranu i kod kojih je dijagnoza postavljena na osnovu tipične kliničke slike. Najčešći simptomi, prisutni kod svih bolesnika bili su: zamagljen vid, dupla slika (diplopija), suva usta i opstipacija. Najduže lečenje primenjeno je kod bolesnika kod kojeg je bolest prepoznata tek posle 23 dana i koji nije primio serum antibotulinum. Svi bolesnici su primili antibiotike, a četvoro je primilo antitoksin. Neostigmin i klistir su korišćeni za lečenje poremećaja pokretljivosti creva i opstipacije. **Zaključak**. Dijagnoza botulizma bila je postavljena se na osnovu afebrilnosti, očuvanog stanja svesti, duple slike, suvih usta i podataka o konzumiranju sumnjive hrane. Polivalentni antibotulinski serum treba da se primeni što pre, jer smanjuje pojavu komplikacija, dužinu bolničkog lečenja i stopu smrtnosti.

Ključne reči:
botulizam; dekontaminacija; botulin, antitoksin; lečenje, ishod; bioterorizam.

Introduction

Botulism is a neuro -intoxication which is characterized by gastrointestinal, nervous and secretory disorders induced by a toxin secreted by *Clostridium botulinum– C. botulinum* (rarely C. Baratii F, and C. butyricum E) [1], a Gram-positive, anaerobic bacterium. There are 8 known antigenic types: A, B, C alpha, C beta, D, E, F and G. As a disease cause, the most frequently isolated are serotypes A, B and E [2] while serotype F is rarely isolated as a disease cause. Spores of *C. botulinum* can be found in the environment, they are resistant to heat, so they can survive a few hours at 100°C. If they are exposed to moist heat at a temperature of 120°C, they can be destroyed within 30 min. On the other hand, toxins are easily destroyed by heat, therefore, heat treatment of food at 80°C for 30 min can protect from botulism. Toxin production (in particular of type E) is possible at lower temperatures, even at 3°C [3], which is the temperature inside the refrigerator.

Spores of *C. botulinum* are everywhere in the environment and the disease can be caused by the ingestion or inhalation of dust, by absorption through the eyes and through the damaged skin. Botulism usually occurs in three forms: botulism transmitted by food, botulism entered through a wound and infant botulism (usually infants younger than 6 months). Some authors mention the inhalation botulism as the fourth type of botulism [4].

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Botulism transmitted by food occurs after consuming contaminated food in which the toxin is produced; in the other two forms, *C. botulinum* produces a neurotoxin *in vivo*, in the infected tissue or in the colon. Today, it is known that the botulinum neurotoxin on peripheral nerve endings binds to two receptors on neurons by its synaptic vesicle R subdomain with a low affinity for the ganglioside and high for a synaptic vesicle protein known as a synaptic vesicle (SV2A) and SV2B and synaptotagmin II (Syt II). After that, a toxin that is bound to the receptor by endocytosis enters the cell, [5, 6] and leads to the blockage of acetylcholine release at the level of neuromuscular synapse, which results in the appearance of paralysis.

Infant botulism occurs most often in infants younger than 6 months [3]. Since botulinum is one of the strongest toxins, it is considered that 1 ng / kg is lethal to humans, while 200 g in the form of crystalline is enough to kill all of the humanity. It can be used in bio-warfare [7, 8] and it belongs to class A of biological agents [9, 10]. It can be used by spraying into the air or injecting into food.

Since botulism is a rare disease, early recognition of clinical symptoms is highly important for serum anti botulinum application. Furthermore, it is also necessary to report the disease and epidemiological treatment of each case.

Case report

We reported 6 patients diagnosed with botulism, who were treated during the period of ten years (from January 2004 to January 2014) in the Clinic for Infectious Diseases, the Clinical Center Niš, Serbia. The diagnosis of botulism was made based on the clinical picture and the epidemiological surveys, and according to the disease evolution and further paraclinical tests, differential diagnostic capabilities (CDC recommendations - USA) were excluded [11].

Case 1

A 44-year-old patient, an electrical engineer, presented with nausea, vomiting and watery excrement. The disease occurred in November 2007. Two days after the initial symptoms, in the evening hours, diplopia, dryness of the mouth, fatigue, difficulty in swallowing and speaking occurred. He gave the epidemiological data that he had eaten a can of squid seven days before the onset of symptoms and smoked meat the day before the onset of the symptoms. He was hospitalized three days after the illness had occurred, with signs of dryness of mucous membranes and mydriasis. Lung X-ray showed vague contours of the left dome of the diaphragm, which may correspond to the left, basal pulmonary infiltrate. The control lung X-ray done five days later was normal. The test of neuromuscular transmission was also performed and it showed a slight increase, up to 10%, on the system *nervus medianus- musculus abducens* poll left of evoked muscular responses I-IV, while there was no increase in the amplitude of evoked muscular responses in other tested systems. During the hospitalization for constipation, deep enema with sodium chloride solution 0.9% (NaCl) was also applied to the patient on two occasions. He was treated with anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E.), neostigmine (0.5 mg im for 7 days) and metronidazole 500 mg/8 h for 10 days. The patient was cured and discharged after fourteen days.

Case 2

A 26-year-old male patient, a student, presented with vomiting and constipation in mid-July 2008. He gave an indication that the previous day he had eaten "a can of meat". Diplopia, dryness of mucous membranes in the mouth and difficulties swallowing and speaking occurred two days after consuming the suspected food. On admission, four days after the onset of symptoms, mydriatic pupils, non-responsive to light, dehydration signs, left tonsil arch elevation and speech difficulty were noticed. He was treated with neostigmine 0.5 mg im for 7 days, lactulose and penicillin G, 10 million IU daily for 10 days. During hospitalization, despite the application of lactulose and deep enemas with 0.9% NaCl, the constipation persisted for few days after discharge.

Case 3

A 19-year-old female patient, a student, presented with nausea and vomiting in October 2009, the day after she consumed pate of unusual taste and sardines. The next day, blurred vision, dryness of oral mucous membranes, sore throat and difficult swallowing occurred. She was referred to a neurologist who prescribed her medication for "circulation". She was hospitalized four days after the onset of clinical symptoms, with signs of adynamia, dehydration, mydriatic pupils which poorly responded to light and accommodation, as well as with decreased strength. On admission, laboratory analysis of blood verified leukocytosis 11.6×10^9/L [normal range (nr) 4–10.5 $\times$ 10^9/L], with dominance of polymorphonuclears 80% (nr 50–70%), C–reactive protein (CRP) 45.3 (nr less than 3 my/L, fibrinogen 7.4 g/L (nr 1.5–3 g/L (she was also diagnosed with concomitant urinary tract infection for which she was treated with ceftriaxone). During hospitalization, the enema was applied on several occasions and stool became regular 15 days after the treatment. She was treated with neostigmine 0.5 mg im for 7 days, penicillin G, 10 million IU daily for 10 days, and received the anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E). Clinically rehabilitated, she was discharged from the clinic after 17 days.

Case 4

An 11-year-old patient, a pupil, presented with nausea and vomiting in June 2010, the same day he consumed pate. The next day, he felt pain in the lower leg and forearm muscles. The disease showed further clinical progression and after 48 h of onset of symptoms he presented with diplopia and vision difficulties, watery excrement, sore throat, difficult

swallowing, hoarseness. He was afebrile during the whole course of illness. He was hospitalized on the fifth day of the illness and on admission he had eyelid ptosis, mydriasis, dryness of mucous membranes. The patient was adynamic and arrived in a wheelchair. The patient had elevated sedimentation 80 mm/h (nr 0–22 mm/h), with leukopenia 4.2 × 10^9/L, normal findings of leukocytes formulas and CRP 17.3.g/L. He was treated with neostigmine 0.5 mg im for 7 days, ceftriaxone 2.0 g daily for 10 days, and has received the anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E). The problem of constipation was solved by enema (0.9% NaCl) which was performed on the fourth day of hospitalization, and thereafter the patient had regular excretion of feces. The patient was cured and released after eleven days of hospital treatment.

Case 5

A 45-year-old patient, a journalist, presented with nausea, pain in bowels, vomiting and mild diarrhea in mid-July 2010, a day after he had eaten pate in the casing of dubious quality. Dryness of the palms and soles, difficulties in swallowing, "blurred vision", hoarseness and constipation occurred after two days. His chosen doctor treated him with metoclopramide hydrochloride and he was also treated by a neurologist and ophthalmologist. He was admitted six days after the first symptoms appeared and on admission, he had anisocoria, dysphagia, dysphonia, adynamia, dehydration, and tachycardia 98 beats/ min. He was treated with neostigmine 0.5 mg im for 7 days, metronidazole 500 mg/8 h for 10 days and the anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E.). Due to constipation, enema with 0.9% NaCl was done only on the day of admission and later he had regular stools. Clinically cured, he was released after fourteen days.

Case 6

A 31-year-old patient, an electrical engineer, presented with nausea, belching, vomiting and 5-6 watery excrements in February 2013. After 72 h he developed blurry vision regardless of distance, difficult swallowing and constipation, tiredness and was unstable in standing position. During the treatment, he passed stool on the third day and after that his stool was regular so there was no need for using enemas. He was treated with penicillin G, 10 million IU daily for 10 days, neostigmine 0.5 mg im for 7 days and infusion solutions with vitamins B and C. The patient was cured and released after thirteen days of treatment. Table 1 summarizes the different symptoms and signs in patients with botulism.

Discussion

Botulism can occur regardless of age. Sobel et al. [12] showed that in a sample of 263 patients, the average age was 48 years. Leclair et al. [13] tested a sample of 205 patients from Canada during a period 1985–2005 and showed that mean age was 45 years (3–83 years), with 48.4%. They also stated that the prevalence of type E botulinum toxin was 86.2%. These findings are consistent with our case series. The patients hospitalized in our Clinic were aged from 11 to 45 years, with a mean age of 28.8 years. Four patients were male and two were female.

Table 1

Review of clinical symptoms and signs of botulism

Symptoms	Patients, n (%)
Gastrointestinal tract	
nausea	6 (100)
vomiting	6 (100)
diarrhoea	4 (50)
Autonomic nervous system	
constipation	6 (100)
dryness of mouth	6 (100)
dryness of palms\ soles	3 (50)
Neurological system	
mydriasis	3 (50)
blurred vision	6 (100)
diplopia	2 (33.3)
eyelid ptosis	1 (16,6)
hoarsness	2 (33.3)
difficulty swallowing	6 (100)
general weakness	4 (66,6)

Based on epidemiological surveys, the most likely route of infection in four patients was the pate, in one patient the luncheon meat, and in one it was most likely dried meat. Therefore, the route of infection in all cases was alimentary. It was not possible to prove the presence of *C. botulinum* toxins in suspicious food samples, considering that they had not been preserved. Apart from the patients, no one else had consumed the suspected food so there was no control group.

Besides the most frequently mentioned antigenic types that cause botulism, Bouvet et al. [14], described the case of an 83 year-old-person with clinical signs of severe sepsis in whom *C. botulinum* type III, which had a "mosaic structure" of genetic parts of C and D types, was isolated from the blood cultures.

The incubation period ranged from 24 h to four days. Arnon et al. [15] showed that the first symptoms, in the form of descending symmetric paralysis, bulbar paralysis, diplopia, dysarthria, dysphagia and dysphonia occur 12 to 72 h after exposure. The youngest patient was 11 years old and had the shortest incubation period because he stated that 30 min after eating pate, he felt nauseated and vomited abundantly, and the next day he got the neurological symptoms.

The most predominant symptom, which aroused general practitioner's suspicion of botulism, was a disturbed vision. All patients had blurred vision, double vision and could not read. In all cases the dryness of mouth, difficulties in swallowing and speaking were recorded. With the youngest patient, in addition to mydriasis, the ptosis of the eyelids was registered. Some authors state that the clinical picture is characterized by the triad – symmetric descendent paralysis and bulbar paralysis, afebrility and preserved sensorium [16, 17]. Aurora et al. [18] give an overview of the case of a 35-year-old patient from India, who, 4 days after consuming food, deve-

loped eyelid ptosis, blurred vision, difficult swallowing, progressive weakness of the lower limbs and fasciculation of the muscles of the whole body, which is rarely seen.

Describing the occurrence of 30 cases of patients suffering from botulism poisoning after eating in a restaurant in El Paso, Texas, Angulo et al [19] said that in 18 of them the diagnosis was confirmed, with 5 it was likely, and in 2 cases it was suspicious. The most common symptoms were fatigue, diplopia, blurred vision and dizziness. They specifically point out two cases with the asymptomatic clinical picture. With one, the diagnosis was made on the basis of positive coprocultures, and in the other the findings of electromyography were specific. Sobel [20] pointed out that in a case of an epidemic, all patients did not have the same clinical picture because the toxin was not equally distributed in all contaminated groceries.

Khakshoor et al. [21] described the case of a 37-year-old Iraqi who had a binocular horizontal diplopia as the only sign of the disease. Three days later, the patient began to vomit, and two days later he developed other symptomatology of botulism.

As it can be seen from the mentioned cases, the diagnostic wanderings are frequent, whether the patients are treated with suspicion of other diseases or whether the referral to virologist was belated, even three weeks after the onset of the symptoms.

Vossen et al. [22] pointed out the rare frequency of the disease and the importance of early recognition of the clinical picture indicates. They described two cases of botulism in Vienna that were diagnosed after a period of 21 years in which there was no botulism. Besides the selected physician, an ophthalmologist, laryngologist, and gastroenterologist were consulted and on the eighth day of the disease, when a neuro-ophthalmologist was consulted, an infectologist was included and the disease was diagnosed. Early diagnosis is needed for timely application of serum which is the first 36 h. Sobel [20] points out that it would be ideal to apply the anti botulinum serum in the first 24 h of the symptom onset.

Kotan et al. [23] state that in addition to the characteristic clinical picture, it is also possible to use electromyoneurography (EMNG) for the purpose of diagnosis. They presented the case of a 43-year-old female patient who developed neurological disorders and indicated the specific findings of EMNG in botulism. The response to repeated stimulation of the ulnar nerve with low-frequency (3 Hz) is lowered, while at the repeated stimulation with high frequencies (10–50 Hz), the response is facilitated [23, 24].

In two patients the therapy did not include the serum anti botulinum, because one of them appeared 23 days from the onset of symptoms and the other after 5 days. Both patients were treated by antibiotics, neostigmine, lactulose, and infusion of electrolytes and vitamins. Lonati et al. [25] presented the case of a nine–month- old infant with a severe clinical picture of botulism who, in addition to serum anti botulinum 250 mL / 25 mL / h, was also orally given 5 g activated charcoal through a nasogastric tube and prostigmine 0.05 i.v. mg/kg. The same group of authors believes that the application of prostigmine inhibits the enzymatic degradation of acetylcholine and therefore may be useful in preventing ileus [26].

Other four patients were given trivalent serum anti botulinum - Botulism Antitoxin Behring® from the company Novartis. The serum contains horse proteins but since the patients were not previously treated with serums of animal origin, the premedication was not carried out. Antibotulinum serum was applied in the form of a slow i.v. infusion of 250 mL, and then sequenced with another 250 mL. After 4–6 h another 250 mL of the serum was infused. There was no reaction in any of the patients. With the use of neostigmine, the patients were also given antibiotics, lactulose, and other symptomatic therapy. Robinson et al. [27] believe that early use of serum anti botulinum prevents the progression of paralysis but it does not cure it. A group of authors believes that there are certain doubts about the application of serum anti botulinum since it can cause anaphylaxis, and as additional treatment options, they listed corticosteroids, plasmapheresis, and implementation of immunoglobulins [18]. Tacket et al. [28] describe the evaluation of the effect of using trivalent horse serum in 132 patients with clinical signs of botulism and note that it has a positive effect on the survival and shortens the course of the disease.

Some authors believe that passively applied animal serum only leads to neutralization of toxins in circulation by binding antitoxins to receptors of neurons, where it cannot directly inhibit the proteolytic activity of toxin which entered the cell. Humanized monoclonal antibodies bind and block the action of zinc metalloprotease intracellularly. In that case, botulinum neurotoxin cannot cause specific and direct adverse effects. Accordingly, humanized monoclonal antibodies should be immunotherapeutic medicine for botulism [29].

Lonati et al. [26] say that Dr. Zamani recommends the sterilization of hose by using sorbitol and does not recommend the use of magnesium salts as they can lead to a deterioration of neuromuscular blockades.

A group of Japanese authors described the case of a 69-year-old married couple suffering from severe forms of botulism after eating vacuum packed food. They tested the presence of toxin and *Clostridium* in the feces and toxin in the serum. For epidemiological reasons, it is essential that, at the beginning of their disease, Clostridium in feces was isolated in both patients and toxin was detected in serum and feces. After three months, the toxin was not detected in the serum, in one patient it was detected in the feces, and in both patients *Clostridium* was isolated in the feces [30].

The role of antibiotics in the treatment of botulism remains unclear but penicillin G has been recommended to treat wound botulism. Any other antibiotics that treat *Clostridium* species, such as cefalosporins or metronidazole could also be administrated. Antibiotics are useful in wound botulism, but they have no role in foodborne botulism. Antibiotics are also useful in the treatment of secondary infections. In infant botulism, antibiotics are used only to treat secondary infections because lysis of intraluminal *C. botulinum* may increase the amount of toxin available for absorption [31].

The length of hospitalization ranged from 11 to 20 days, noting that two patients were treated for 14 days, and one 13

and 17 days. The longest treatment had the patient who was admitted 21 days after the first symptoms since the disease was not recognized on time and he had not received anti botulinum serum. All patients were cured without sequelae.

Conclusion

Botulism is a neurointoxication which is characterized by afebrility, the appearance of visual disturbances, difficult swallowing, dryness of mucous membranes in the mouth, constipation and preserved sensorium. The main clinical symptoms that aroused the doctor's suspicion of the disease are diplopia and visual disturbances. *Clostridium botulinum* can be used in bioterrorism as a biological poison and, therefore, there is an imperative need to report every case of suspicion of the disease. Treatment of patients must be carried out in intensive care units. Anti botulinum serum should be applied as soon as possible because it reduces the occurrence of complications, length of hospital stay and mortality rate, but the problem is the fact that, in any case when the serum was required it could not have been obtained in the country and had to be commissioned from abroad.

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