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THE DIFFICULTIES OF EARLY DIAGNOSIS AND TREATMENT OF BOTULISM

Abstract

The popularity of home canning contributes to a sufficiently high incidence of botulism worldwide. The canned products containing botulinum toxin do not change neither color, taste, nor smell of contents of canned food. A distinctive feature of the paralytic syndrome in botulism is its symmetry and the absence of a violation of sensitivity. The criteria for the severity of the course of botulism are considered a violation of swallowing liquid food and symptoms of difficulty breathing. A violation of swallowing liquid food and the severity of acute respiratory failure are considered criteria of the severity of the course of botulism. The paper presents the features of the therapy of the patients with botulism in the intensive care unit. Clinical examples illustrate the difficulties in recognizing botulism at the early stage of the disease, which are due to the polymorphism of the clinical picture of botulism and the similarity of symptoms with other diseases. Most commonly, patients with botulism are diagnosed with acute intestinal infection or the neurological pathology. Patients are not hospitalized in a timely manner, which can affect the outcome of the disease. The ability to recognize botulism at the prehospital stage is necessary for all doctors.

Key words: *botulism, botulism therapy, early diagnosis of botulism, anti-botulinum serum, hyperbaric oxygenation, mechanical ventilation*

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HBO — hyperbaric oxygenation, VC — vital capacity, GIT — gastrointestinal tract, MV — mechanical ventilation, IDH — infectious disease hospital, ARF — acute respiratory failure, CVA — cerebral vascular accident, ICU — intensive care unit, BFP — bacterial food poisoning

Introduction

Botulism is a serious infectious disease caused by bacterial toxin, known to humankind since ancient times. Historically, the name “botulism” comes from the Latin “*botulus*” — sausage — a product that

previously caused botulism and where the pathogen was first isolated [1–3]. However, meat products are now a rare cause of botulism. In Moscow, for example, home-preserved mushrooms and vegetables are now the number one cause, followed by salted and smoked home-produced fish [2, 3].

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Epidemiology

The dangers of home canning are well known and obvious: the heat resistance of the spores allows them to withstand boiling for up to 6 hours, while the concentration of salts and vinegar used in canning does not prevent toxin formation, and the sealing of cans at home leads to the creation of anaerobic conditions. Toxin-containing canned foods do not change color, taste, or smell [1–3, 8]. Industrially manufactured canned food is relatively safe, since the technology of their production should provide for compliance with technical conditions and standards established by regulations in force in the state that adopted the standard [2, 6]. Toxin formation in home-produced canned products is uneven, that is, it occurs in “pockets”, leading to selective infection of the people consuming said product, which corresponds to the literature [2, 3, 8, 9]. All of the above combined with the popularity of home canning explains the relatively high incidence of botulism worldwide. For example, in the Russian Federation about 300 cases of botulism are registered annually [2].

The introduction of therapeutic anti-botulinum serum (ABS) into clinical practice, the use of mechanical ventilation (MV), the use of hyperbaric oxygenation (HBO), the rational use of broad-spectrum antibiotics to prevent the activation of opportunistic microflora contributed to the improvement of patient outcomes and the reduction of the number of adverse outcomes in this disease [3–5]. Mortality from botulism according to the literature currently ranges from 7 to 9 % [2].

Pathogenesis

Seven serotypes of botulinum toxin — A, B, C, D, E, F and G — are known, but the disease in humans is mainly caused by three of them — A, B and E. Immunity after the disease is type-specific, so reinfection is possible [2, 3, 7, 9].

The incubation period is on average from 2–4 hours to 2–3 days, with maximum of 5 days, which almost always depends on the dose of the toxin.

Botulinum toxin with food enters the gastrointestinal tract (GIT) and penetrates into the neuromuscular synapses by hematogenous way. It has been proven that botulinum toxin selectively affects

the motor neurons of the anterior spinal horns, motor nuclei of the cranial nerves and peripheral nervous system by stopping the release of acetylcholine from acetylcholine-containing bubbles. The block of nerve impulses transmission leads to myasthenia gravis and pseudo-paralysis without anatomical damage, which are restored when the toxin is neutralized. The cause of death in patients is respiratory arrest secondary to progressive acute respiratory failure (ARF) and cardiac arrest. The process of a patient's recovery is due to the gradual breakdown of the botulinum toxin in the places of its fixation, possible aspiration of vomit, the addition of secondary bacterial infection and ventilation hypoxia [2–5, 8].

Clinical course

Human botulism symptoms are specific and consist of several main syndromes: paralytic, gastrointestinal and intoxication. In 30–50 % of cases, the disease begins with gastrointestinal syndrome [1–3, 5, 8]. The onset of the disease with the appearance of nausea, vomiting, loose stool, dry mouth is often the basis for the diagnosis of acute intestinal infection (bacterial food poisoning (BFP), acute gastroenteritis, salmonellosis, etc.) or acute pathology of GIT (irritable bowel syndrome, exacerbation of chronic gastritis, acute pancreatitis, etc.). It is important to remember that the phenomena of gastrointestinal lesions in botulism are short-term (up to 1–2 days) and disappear by the time of the appearance of neurological symptoms [1–3]. Often the first signs of botulism are the patient's complaints of visual impairment (cloudy vision, diplopia, ptosis, inability to read the text), which is the cause of erroneous visit of patients to an ophthalmologist. There is also a need to differentiate botulism with true neurological pathology (cerebral vascular accident (CVA), encephalitis, myasthenia gravis, etc.). It is important to remember that the distinctive features of the paralytic syndrome in botulism are symmetry, bilaterality and the absence of sensitivity disorders [2, 4, 8].

It should be noted that the assessment of the severity of neurological symptoms is largely subjective and depends on the doctor's qualification, which can often cause errors in determining the true severity of the process.

Impaired swallowing of a liquid of any severity is recognized as a criterion of severe botulism. This symptom is easily determined by any practitioner with minimal knowledge of the epidemiological history of the disease and clinical signs. This criterion is an absolute indication for urgent hospitalization of patients in the intensive care unit, even bypassing the emergency department.

Complaints of patients on a feeling of not getting enough air, difficulty to inhale, dyspnea, the so-called “respiratory discomfort”, regardless of the severity of other neurological symptoms, are also an indication for the administration of appropriate resuscitation treatment for the patient. It should be noted that external respiration disorder often occurs after the symptom of impaired swallowing of liquid. Thus, severe botulism is considered two states, when, regardless of the presence and severity of all other signs of botulism, dysphagia with liquid food and “respiratory discomfort” take place [2, 4].

Features of respiratory failure in patients with botulism

The development of ARF in patients with botulism is associated with paresis of the respiratory muscles, paresis of the abdominal muscles, soft palate and epiglottis. In turn, this can lead to impaired mechanism of coughing, getting saliva into the respiratory tract, and in some cases, aspiration of gastric contents in case of vomiting. Aspiration pneumonia occurs most often in patients with severe forms of botulism, but itself is rarely the cause of ARF [2, 4, 5, 8]. In addition, a number of patients with botulism have a high position of the diaphragm secondary to paresis of the gastrointestinal tract, which also aggravates the external respiration function. Thus, ARF in patients with botulism develops by hypoventilation type, which complicates the differential diagnosis of ARF caused by other causes, clinical assessment of respiratory failure and the choice of therapeutic measures.

Treatment

All patients with suspected botulism are subject to mandatory hospitalization, since the development of ARF is possible. The primary task of the doctor

at the prehospital stage is the quickest possible removal of the unabsorbed toxin from the patient's body by washing the stomach and intestines with a 5 % solution of sodium bicarbonate (NaHCO_3). Due to the danger of vomit aspiration in patients with impaired fluid swallowing, primary gastric lavage is carried out only by means of a gastric probe, in the volume of 2–3 liters.

The next stage of therapy is the introduction of anti-botulinum serum (ABS) to neutralize the circulating toxin in the blood as soon as possible from the onset of the disease [1–3]. Before the introduction of serum, blood should be sampled in the amount of 10 ml, as well as urine, gastric lavage liquid (vomit) for testing for botulinum toxin and the causative agent of botulism. A food product that is believed to have caused the disease is also sent for analysis. After diagnosis with an undetermined type of toxin, anti-botulinum serum with ABE types must be administered: type A — 10,000 U, type B — 5,000 U, type E — 10,000 U. In case of known type of toxin, monovalent serum should be used. One dose of serum is given, fractionally, according to the instructions dated 17.02.2000 (Besredka method). To avoid possible allergic reactions, 60–90 mg of prednisolone is administered to the patient prior to intravenous infusion of serum [4].

In addition to specific treatment, pathogenetic therapy is carried out to eliminate pathological changes caused by botulinum toxin, including secondary ones; detoxification therapy and proper care are provided [2, 3, 5, 7, 8]. Enteral nutrition through the nasogastric tube should be begun as soon as possible.

Due to the development of tissue hypoxia, hyperbaric oxygenation (HBO) should be included in the set of therapeutic measures whenever possible [2, 5].

Secondary bacterial complications are treated with antibacterial drugs.

The development of neurological lesions in botulism has a fairly clear direction “from top to bottom”: vision impairment → difficulties with swallowing (solid food → liquid) → respiratory impairment, i. e. ARF occurs after the complete disappearance of the ability to swallow liquid. Therefore, all patients with aphagia (complete inability to swallow even liquids) are recommended routine nasotracheal

intubation [3, 4]. With nasotracheal intubation, the possibility of gastric content aspiration is excluded, the bronchoalveolar lavage is facilitated and it is possible to transfer the patient to MV in a planned manner, i. e. before the development of severe ARF events.

The experience of the authors of this work, accumulated in the management of patients with a variety of nosological forms, shows that nasotracheal intubation can be carried out for a long time (in our experience in the case of botulism — up to 81 days) without any severe side effects and allows to avoid a tracheostomy [5].

The final extubation is carried out only with the full restoration of liquid food fraction swallowing. Transfer of the patient to the general infectious department is carried out not earlier than 2–3 days after extubation.

Complications

Non-specific complications of botulism include various secondary microbial complications, among which pneumonia is distinguished both in the frequency of occurrence and in the influence on the outcomes of the main process [2, 5]. The combination of pneumonia with bronchitis, laryngitis and sinusitis is possible. Several complications in the urinary system (pyelonephritis, pyelitis, etc.) are less common. Also, botulism promotes the activation of any chronic inflammatory processes, especially in female genitals [4, 2]. In addition, with botulism, iatrogenic complications are often detected, which include all cases of drug allergy (and primarily serum disease), dysbiosis during prolonged use of antibacterial agents, post-injection infiltrates, abscesses, bedsores, etc.

Cardiac lesion in the form of myocardiodystrophy can be considered as a specific complication of botulism — botulinum myocarditis that occurs on day 7–15 of the disease [5]. The specificity of this process is confirmed by the direct relationship of its incidence with the severity of the main process (i. e., with toxin dose) and the absence of such with the presence of secondary microbial complications and/or the intensity of drug therapy (in particular — with doses of ABS), i. e. with purulent intoxication and nonspecific allergization [2, 5].

Procedure for discharge of patients from hospital

There are no clear terms of discharge of patients with botulism from the hospital, because they are strictly individual and depend only on the rate of reverse development of symptoms during the main process and the complications. It is believed that the patient can be discharged after complete recovery of swallowing, phonation and articulation, and complete resolution of the manifestations of secondary complications. At the same time, asthenic syndrome and moderate visual impairment (inability to read small print) may persist for several months (5–6) and are not indications for prolonged stay of patients in the hospital [3].

These data indicate the need for this work, the purpose of which is to assess the clinical course of botulism and analyze errors in diagnosis.

Materials and methods

The analysis of 27 medical records of patients diagnosed with botulism in IDH No. 1 for the period 2016–2017 was carried out. Statistical data processing was carried out using the standard STATISTICA 6.0 MS Office application package.

Study results and discussion

We have analyzed 27 medical records with diagnosis of botulism during 2016–2017. The mean age of the patients ranged from 30 to 87 years. Among the patients, 20 (74 %) were women. It should be noted that the majority of patients (67 %) were admitted to the hospital on the 4th–5th day of the disease and only 9 (33 %) patients were hospitalized before the 2nd day of the disease, which affected the severity of the course and the duration of hospitalization. At the initial visit for medical attention, botulism was diagnosed only in 8 patients, which amounted to 29 % of cases, another 8 patients were diagnosed with BFP, and 8 (29 %) more patients were hospitalized with suspected CVA. It was not possible to exclude alcohol intoxication in two patients, and 1 patient was hospitalized with the diagnosis of acute pancreatitis.

Based on the data of epidemiological history, it was found that in 13 patients (48 %) the disease was

associated with the use of home-preserved mushroom, 5 (19 %) — smoked fish and 9 (33 %) — other home-produced canned products. Thus, the consumption of canned food and smoked products on the eve of the disease was noted in 100 % of patients, but only in 59 % it was revealed at the pre-hospital stage, and only in 41 % — on the 2nd-4th day from admission to the hospital, which led to an incorrect diagnosis.

It should be noted that all patients on the first day of the disease complained of nausea, weakness and dryness of oropharyngeal mucous. Most of the patients with complaints of visual impairment (gray fog, blurry vision, and double vision), weakness, hoarseness, dysarthria were examined by a neurologist on the first and subsequent days of hospitalization, but botulism was not suspected. In 62.5 % of patients (5 patients out of 8) with primary diagnosis of CVA, previous gastrointestinal syndrome (nausea, vomiting, abdominal pain, loose stool) was not diagnosed, which caused erroneous diagnosis.

Two patients before hospitalization were examined by several specialists (ophthalmologist, surgeon and neurologist), which also did not allow timely diagnosis. The above data testify to insufficient knowledge of epidemiological history and symptoms of botulism among health care personnel that is required for early diagnosis of botulism.

Taking into account the criteria of primary disease severity, out of 27 patients, a severe course was reported in 13 (48 %), 5 patients had a mild course and 9 patients had a moderate course. Seventeen patients were hospitalized in the ICU, 13 of them required mechanical ventilation (MV). The duration of MV ranged from 4 to 55 days. Twenty-six patients were discharged with recovery. One fatal outcome in an 87-year-old female patient was due to late admission.

The diagnosis of botulism does not require 100 % laboratory verification of the diagnosis, and so the diagnosis is usually based on clinical and epidemiological data. All patients underwent gastric and intestinal lavage with the sampling of biological material to identify the toxin before ABS administration, and only 10 (37 %) patients had the diagnosis confirmed by laboratory tests. Type A toxin was detected in 6 patients, type B and E — in 2 patients, respectively. No complications of

ABS administration were reported in any patient. Immediate administration of ABS is recommended for all patients with suspected or diagnosed botulism. The reason for refusal of serum administration can be only late admission of the patient with a clear reverse development of disease symptoms. The above is confirmed by the following clinical cases.

Clinical case No. 1

Patient G., 58 years old, was delivered by the ambulance team to IDH No. 2 on 11.09.2017 with a diagnosis of acute gastroenteritis of unspecified etiology on the second day of the disease. From the epidemiological history it is known that on September 9 the patient ate home-produced smoked fish brought from Astrakhan. The next day he felt heaviness in his stomach, weakness, nausea; vomiting with eaten food occurred 5 times; and he noted a single episode of loose stool without pathological impurities. The patient self-induced vomiting, irritating the back wall of the oropharynx using two fingers, but the general state remained the same. On September 11, the patient called an ambulance and was hospitalized with diagnosis of acute gastroenteritis in IDH No. 2. On 12.09.2017 the patient's condition worsened; there were complaints of double vision, dry mouth, hypernasal voice, and, in this regard, the diagnosis of intestinal infection was withdrawn, and suspected botulism was the reason for the patient's transfer to IDH No. 1.

The state on admission was considered as severe. There are complaints of weakness, double vision, dry mouth, difficulties with liquid food swallowing. The patient has complaints of heaviness in the abdomen, hoarseness, and a feeling of not getting enough air. During examination the skin is pale. Respiratory rate is 16 breaths per minute. Bronchial breathing is auscultated in the lower parts. Pulse is rhythmic with satisfactory filling and tension, 70 beats per minute. Blood pressure is 125/70 mm Hg. Heart sounds are rhythmic without pathological sounds. Tongue is dry with white coating. During the examination of the oropharynx palatine velum sagging was visualized. During palpation, the abdomen is not distended and painless in all departments. Liver and spleen are not enlarged. Patient had no stool since 11.09.2017. Urination is free. Facial expressions are

preserved, there are bilateral hemiptosis of upper eyelids, bilateral mydriasis, and pharyngeal reflex is not triggered. The clinical diagnosis was severe botulism.

At the department, blood was taken for testing for botulinum toxin. In order to empty the stomach a nasogastric tube was introduced, and the washing was collected for laboratory testing. Also, the bowel was cleaned using a high enema with 5 % sodium bicarbonate. A single dose of polyvalent ABS (type A — 10,000 IU, type B — 5,000 IU, type E — 10,000 IU) was intravenously administered in accordance with the Guidelines 2000. The presence of aphagia and the risk of sudden respiratory arrest in patient were the reasons for the patient's transfer to the ICU with a nasotracheal intubation. MV was performed in the BIPAP mode taking into account physiological parameters.

The patient was prescribed detoxification therapy, and intravenous ciprofloxacin was administered at a dose of 0.4 g twice a day for 10 days to prevent complications caused by secondary bacterial flora. Enteral nutrition was carried out through the nasogastric tube until the restoration of independent swallowing. On September 19 (7 days of MV) after complete recovery of breathing and swallowing, the patient was extubated and two days later was transferred to the diagnostic department to the general ward. The set of therapeutic measures included 5 sessions of HBO. The patient was discharged on the 21st day of the disease (29.09.2017) in a satisfactory condition under the supervision of a primary care doctor.

This clinical case demonstrates the difficulties of botulism diagnosis at the prehospital stage. The onset of the disease with gastroenteritis symptoms was the basis for the diagnosis of intestinal infection of unknown etiology, and only the symptoms of paralytic syndrome that appeared from the 3rd day allowed to suspect botulism. The disease was regarded as severe, since there was aphagia, bulbar paralysis and impaired breathing.

Clinical case No. 2

Patient A., 48 years old, was transferred to IDH No. 1 from the neurological department of the therapeutic hospital on 04.12.2016 with a diagnosis of botulism.

From the epidemiological history, it is known that on December 2, he alone ate canned fish in a glass container, sausage, pork in a vacuum pack bought at a country fair.

Deterioration of general state occurred on the next day, 03.12.2016, when he felt discomfort in the abdomen, vomited twice, and had loose stool without pathological impurities — up to 6 times. He did not seek medical attention and did not take any medications. On the next day (December 4), he noted a decrease in visual acuity, double vision, slight weakness, dizziness. Loose stool did not appear. He visited the ophthalmologist in an outpatient department, who revealed paralysis of the medial rectus muscle of the right eye, and with suspected CVA the patient was sent for hospitalization in the neurological department of a therapeutic hospital in Moscow, where after examination, the diagnosis of ischemic stroke with bilateral gaze paresis and bulbar vestibulocerebellar ataxia raised doubts. The patient was examined by an infectious disease specialist who suggested botulism. After gastric lavage and enema with 5 % sodium bicarbonate, the patient was transferred to IDH No. 1 on the same day. Upon admission, the patient's condition was regarded as moderate, as there were complaints of double vision, dry mouth, dizziness, moderate general weakness. The gastrointestinal syndrome has regressed.

Examination results: skin is of normal color, clean, there are no edema and hemorrhages. With auscultation, breathing in the lungs is vesicular. Respiratory rate is 16 breaths per minute. Pulse is rhythmic with satisfactory filling, 68 beats per minute. Blood pressure is 120/80 mm Hg. Heart sounds are rhythmic without pathological sounds. Tongue is dry with white coating. Abdomen is moderately distended and painless during palpation in all parts. Liver and spleen are not enlarged. There was no stool during hospitalization. Urination is normal. Facial expressions are preserved, bilateral hemiptosis of upper eyelids, bilateral mydriasis, significant divergent strabismus, gaze paresis, the pharyngeal reflex is reduced. The state of consciousness was normal. Upon admission, the patient was immediately administered with one dose of polyvalent ABS (type A — 10,000 IU, type B — 5,000 IU, type E — 10,000 IU) in accordance with Guidelines for use of purified concentrated liquid anti-botulinum serums

with types A, B, C, E and F, approved on 17.02.2000. Adequate detoxification therapy was carried out in the patient, and intestinal stimulation was performed using enema with 5 % sodium bicarbonate. To prevent complications caused by opportunistic bacterial flora, Ceftriaxone at a dose of 1.0 g twice a day was intravenously administered for 10 days.

The patient was discharged in a satisfactory condition under the supervision of an infectious disease specialist at the place of residence on the 13th day of the disease.

In this clinical case, the incorrect interpretation of the paralytic syndrome in botulism and underestimation of the epidemiological history, which were the cause of the erroneous diagnosis of stroke, were demonstrated.

Summary

1. The knowledge of epidemiological history and clinical presentation of this disease will allow any physician in the shortest possible time to hospitalize a patient in an infectious diseases hospital to prevent death.
2. Impaired liquid swallowing of any severity indicates a severe course of the disease and is an indication for urgent hospitalization of the patient in the intensive care unit.
3. As specific therapy, all patients with botulism need intravenous administration of one dose of ABS according to the directions for use (fractional administration of serum by Besredka method).
4. Respiratory failure in botulism is manifested by a feeling of compression in the chest, impaired rhythm of breathing, difficulties in inhaling and exhaling due to damage to the respiratory muscles and diaphragm and has hypoventilation nature.
5. Efficacy of complex therapy in patients with severe botulism is affected by the maintenance of close psychological contact of medical staff with the patient, which is necessary to create optimism in the patient about the ongoing therapeutic measures.

Conclusion

Thus, despite the clearly expressed clinical pattern of botulism, a large number of diagnostic errors can be because the disease is relatively rare, doctors

are not familiar with it and ignore the epidemiological history.

The clinical and epidemiological study, analysis of the literature data allowed to establish that at the prehospital stage, acute intestinal infection, acute cerebrovascular accident, hypertensive crisis, encephalitis, myasthenia gravis are most often diagnosed instead of botulism.

At the same time, the general practitioner must have the necessary and sufficient knowledge to timely suspect botulism, and should be able to provide emergency aid, since early diagnosis and timely pathogenetically justified treatment reduce the frequency of possible complications and deaths in botulism.

References:

1. Nikiforov V.N., Nikiforov, V.V. Botulism. M. Medicine. 1985; 200p. [in Russian].
2. Nikiforov V.V. Botulism. Infectious diseases: national leadership, ed. Yushchuk N.D., Vengerova Yu.Ya. M. GEOTAR-media. 2018;558-568 [in Russian].
3. Sanin B.I. Botulism. Selected lectures on infectious diseases and epidemiology. ed. Luchsheva V.I., Zharova S.N. M. RGMU, MIMSR. 2004;219-239 [in Russian].
4. Popelansky J.D., Fokin M.A., Pak S.G. The defeat of the nervous system in botulism. M. Medicine. 2000;192p. [in Russian].
5. Nikiforov V.V., Tomilin Yu.N., Davydov A.V. Case of severe botulism: 127 days of artificial lung ventilation. Epidemiology and infectious diseases. 2013; 6: 49-57 [in Russian].
6. GOST R 51740-2016 Technical specifications for food products. General requirements for development and design. 2018. In touch: <http://docs.cntd.ru/document/1200142432> [in Russian].
7. Wendt S, Eder I, Wölfel R, Braun P, Lippmann N, Rodloff A. Botulism: Diagnosis and Therapy Dtsch Med Wochenschr. 2017 Sep;142 (17):1304-1312. doi: 10.1055/s-0043-112232. Epub 2017 Aug 29 (in German).
8. Sobel J. Botulism. Clinical Infectious Diseases. 2005; 41(8):1167-1173. doi.org/10.1086/444507
9. James G. C., Rashmi A., Miller J.E. Clostridium botulinum and the Clinical Laboratorian: A Detailed Review of Botulism, Including Biological Warfare Ramifications of Botulinum Toxin. Archives of Pathology & Laboratory Medicine. 2004; 128 (6): 653-662.