

Cow's-milk–induced Infant Apnoea With Increased Serum Content of Bovine β -Casomorphin-5

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Suddenly occurring life-threatening events with symptoms such as apnoea, changes in skin colour (eg, blue or cyanotic), altered muscle tone (eg, floppy, stiff), coughing, choking, or gagging are called apparent life-threatening events (ALTE). About 7.4% to 10% of infants with ALTE, mostly in its recurring form, cannot be saved and they die of sudden infant death syndrome (SIDS) (1–3). A number of different and independent causes of ALTE pathogenesis have been described. Those causes include diseases of various organs and systems, infections, nonaccidental traumas, and Münchausen syndrome by proxy (1,4,5). A coexistence of digestive tract disorders is also diagnosed in >50% of infants with ALTE. Gastro-oesophageal reflux is 1 of the most often diagnosed disorders; however, its role in the genesis of infantile apnoea has not been fully explained (6). ALTE aetiology is explainable and diagnosable only in half of all of the cases (2,3). The causes of ALTE described before do not include an opioid action of the exogenous peptides released from milk β -caseins. We report a case of a breast-fed infant with recurrent apnoea episodes, which have always been preceded by his mother's consumption of fresh cow's milk. A biochemical examination has revealed a high level of β -casomorphin-5 (BCM-5) in the child's serum. We speculate that it is an opioid activity that may have a depressive effect on the respiratory centre in the central nervous system and induce a phenomenon called milk apnoea.

A full-term male infant from a rural family, age 7 weeks, was referred to a macroregional SIDS prevention centre to diagnose the cause of his recurrent ALTE. Apnoea had been occurring since the child was 3 weeks old and its clinical course kept getting more serious. The infant's mother noticed that those events occurred only after her consumption of cow's milk, particularly when she had consumed large amounts of it (up to 2 L/d). Before and during her pregnancy, the mother drank milk with no adverse effects. The boy's ALTE occurred during breast-feeding or directly after that, and was manifested as sudden, 20-, 30-, or 40-second-long apnoea with generally lowered muscle tone. Most of the episodes required resuscitating the child. He did not demonstrate any clinical symptoms between the episodes, and his prenatal and perinatal life had no

complications. The boy was born from a first pregnancy, by natural ways and forces—37 hdb, Apgar score 10, and body mass 2850 g. The child was not exposed to tobacco smoke in his lifetime. The family history of ALTE, SIDS, and atopic diseases was negative. On admission to the hospital, the boy did not demonstrate any symptoms during a physical examination. Differential diagnostics (Table 1) was carried out according to the ALTE diagnostic guidelines recommended by the European Society for the Study and Prevention of Infant Death (2). Negative cultures were obtained from the boy's urine, faeces, and a smear from his throat. Toxoplasmosis, other, rubella, cytomegalovirus, herpes simplex infections were also excluded. Biochemical indicators of liver and kidney functions were normal. A complex screening for metabolic disorders was negative. The ionogram, blood glucose, ammonium, lactic acid, pyruvic acid, and gasometric examination were normal. Also, the oxymetric examination, COHb, MetHB, and HHb were normal. Specialist consultations, cardiologic (electrocardiography [ECG], echo, creatine kinase-MB fraction), neurologic (neurologic responses, electroencephalography, head ultrasound), ophthalmic examinations, and oto-rhino-laryngology provided normal results as well. Abdominal ultrasound was also normal. No allergic and atopic markers were found in the peripheral blood; eosinophilia was 267.8 cells/ μ L, total serum IgE was 2 IU/mL, antibody IgE against casein, α -lactalbumin, and β -lactoglobulin were in class 0; IL-4 serum (Quantikine High Sensitivity enzyme-linked immunosorbent assay; R&D Systems, Minneapolis, MN) was below the sensitivity of the method. The content of BCM-5 in the blood of the infant with ALTE was measured. A control group consisted of 10 infants, selected according to their age, 8 of whom were in contact with cow's milk via their mother's food. A local ethical committee granted its consent for the research (act no. R-I-003/290/2006). The content of BCM-5 was defined using a competitive enzyme-linked immunosorbent assay test with polyclonal antibodies against BCM-5. The BCM-5 content in the blood of the infant with ALTE was 15147.2 ng/mL when compared to the control group median—57.05 ng/mL (interquartile range 43.35–411.05 ng/mL) ($P < 0.05$) (Table 2 and Fig. 1). According to the ALTE diagnostic standard, a 15-hour-long polysomnographic examination was performed (Alice 5, Respironics, Pittsburgh, PA). The recorded channels included ECG, ECG-derived beat-to-beat heart rate, thoracic and abdominal breathing movements, nasal air flow measured by a nasal thermistor, chin electromyogram to detect nutritive and non-nutritive sucking, an electrooculogram to detect sleep states, and electroencephalogram. The obtained results were normal: respiratory disturbance index was 2.5/1 hour of sleep, the maximal duration of the recorded central apnoea was 10 seconds, no obstructive apnoea was observed, the total duration of periodic breathing was 0 minutes, saturation mean (SpO₂) during the sleep was 96%, the SpO₂ nadir was 91%, and the oxygen desaturation index was 1.3/1 hour. The average breathing frequency during the inactive sleep was 23/1 minute and 27/1 minute during the active sleep. The average heartbeat rate was 129.9 beats/min. In a simultaneously performed pH-metry, no events of acid gastro-oesophageal reflux were

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TABLE 1. Diagnostic examinations of the newborn with ALTE according to the ESPID protocol (20)

Procedures	Results
Complete child history	Pregnancy, birth, and neonatal period were undisturbed; neonatal screening toward phenylketonuria, hypothyreosis, and cystic fibrosis negative
Family history	ALTE and SIDS history negative
Laboratory tests and consultations	
Blood count and Hb	Hb—11.2 g/dL, hematocrit—31.9%, MCV 88.4 fL, MCH—31 pg, MCHC—35.1 g/dL, PLT— $364 \times 10^3/\mu\text{L}$, OxyHb—91.5%, COHb—2.5%, MetHb—0.3%, HHb—5.7%
Inflammatory tests	CRP—0.0 mg/L, WBC— $8.37 \times 10^3/\mu\text{L}$, TORCH screening—negative Bacterial screening: urinalysis: specific gravity—1005 g/cm ³ , pH—6.0, albumin—negative, glucose—negative, ketones—negative, nitrates—negative, urobilinogen—0.2 Ehrlich units/dL, pus cells 0–2/hpf, red blood cells—0–1/hpf, epithelial cells—negative, bacteria—negative, urine culture—negative, stool culture— <i>Streptococcus faecalis</i> (++) (physiological flora), cultures of the throat smear— <i>Streptococcus mitis</i> (++) (physiological flora)
Biochemical tests—metabolic workup	Blood electrolytes: Na—135 mmol/L, K—4.9 mmol/L, Cl—104 mmol/L, Ca ²⁺ —1.34 mmol/L (pH—7.432), P—6.15 mg/dL, Mg—2.14 mg/dL Urea—15 mg/dL, creatinine—0.17 mg/dL, uric acid—2.3 mg/dL Pyruvic acid—0.9 mg/dL, lactic acid—10.3 mg/dL Enzymes: aminotransferases AST—35 IU/mL, ALT—25 IU/mL, GGTP—34 IU/mL, ALP—270 U/L, CK—139 U/L, CK-MB isoenzyme—25 U/L blood diastase—7 U/L, blood glucose—79 mg/dL, ammonia—56 $\mu\text{mol/L}$, total bilirubin—0.6 mg/dL, direct bilirubin—0.12 mg/dL, proteins: total—6.1 g/dL, albumin—3.86 g/dL, prealbumin—19.17 mg/dL Tandem mass spectrometry and gas chromatography/mass spectrometry—negative
Blood gas analysis	Acidity (pH)—7.432, bicarbonate—21.1 mmol/L, carbon dioxide pressure—35.2 mmHg, BE(vt)—2.2 mmol/L, AG—9.9 mmol/L
Allergic studies	Eosinophilia—267.8 cells/ μL ; total serum IgE—2 IU/mL; specific serum IgE antibodies to casein (f78)—class 0, to α -lactalbumin—class 0, β -lactoglobulin—class 0 (Pharmacia CAP System); serum IL-4—not detected (R&D)
Imaging studies of abdominal cavity	Reviewing image and ultrasound scan of the abdominal cavity—no pathological chances detected
Cardiological study	Physical examination, chest radiography, electrocardiography (QTc = 0.36)—no deviations, echocardiography—normal myocardial dimensions and contractility normal: RVD (mm)—8.8, IVS (mm)—3.7, LVDd (mm)—26.3, LVPW (mm)—3.4, LVDs (mm)—17.5, Ao (mm)—13, LAD (mm)—17, PA (mm)—8, %SF—33.3, Vp (cm/s)—110, Va (cm/s)—75
Neurological study	Physical examination, EEG, and video-EEG during the spontaneous dream, ultrasound examination of the head, nondiluted and diluted fundoscopy—no deviations
Laryngological study	No abnormalities detected during a specular examination of nasopharyngeal cavity and ears
Sleep study with video	Polysomnographic study with gastric pH probe to evaluate for acid gastroesophageal reflux—no abnormalities

AG = anion gap; ALP = alkaline phosphatase; ALT = alanine aminotransferase; ALTE = apparent life-threatening events; Ao = aorta; AST = aspartate aminotransferase; BE(vt) = base excess (voiding time); CK = creatine kinase; COHb = carboxyhaemoglobin; CRP = C-reactive protein; EEG = electroencephalography; ESPID = European Society for the Study and Prevention of Infant Death; GGTP = γ -glutamyl transpeptidase; Hb = haemoglobin; HHb = deoxy (reduced)-haemoglobin; IVS = interventricular septal thickness; LAD = left atrium dimension; LVDd = left ventricular end-diastolic dimension; LVDs = left ventricular end-systolic dimensions; LVPW = left ventricular posterior wall; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; MetHb = methaemoglobin; OxyHb = oxygenated haemoglobin; PA = pulmonary artery; PLT = platelets; QTc = heart rate-corrected QT interval; RVD = right ventricular diastolic dimension; SF = shortening fraction; SIDS = sudden infant death syndrome; TORCH = toxoplasmosis, other, rubella, cytomegalovirus, herpes simplex infections; Va = aortic velocity; Vp = pulmonary velocity; WBC = white blood cells.

observed: the fractional reflux time (pH <4.0) was 2.4% (normal), the longest event of acid reflux was 3.48 minutes, the average pH in the oesophagus during sleep time was 5.7. The boy's mother did not grant written consent for his oral provocation with cow's milk during the polysomnographic examination because of her fears for the child's life. Until his 12th month of age, the child was monitored

at home with an apnoea monitor. The alarm rang only once, when the boy was 4 months old and his mother attempted to provoke him with milk (200 mL of fresh cow's milk), after which an ALTE episode occurred. Presently, the 21-month-old boy is kept on a milk-free diet and does not demonstrate any clinical symptoms. No analysis on the infant's serum was performed when the mother was

TABLE 2. General characteristics of the studied group and the BCM-5 contents in the blood sera for the infant with ALTE and 10 healthy controls

Case/control	Gestational age, wk	Age, wk	Sex	Feeding	BCM-5 content in the serum, ng/mL
Case	37	9	Male	Breast-fed + fresh CM*	15147.2
Control-1	38	7	Male	Breast-fed + CM [†]	53.7
Control-2	40	7	Male	Breast-fed + CM	33
Control-3	41	9	Male	Breast-fed + fresh CM	56.8
Control-4	38	13	Female	Breast-fed + CM	864
Control-5	37	12	Male	Breast-fed – CM [‡]	1
Control-6	39	14	Male	Breast-fed + CM	57.3
Control-7	41	11	Male	Breast-fed + fresh CM	81
Control-8	40	12	Female	Breast-fed + CM	741.1

ALTE = apparent life-threatening events; BCM-5 = β -casomorphin-5; CM = cow's milk.

* + fresh CM—the infant's mother consumes fresh cow's milk.

[†] + CM—the infant's mother consumes milk bought from a shop.

[‡] – CM—the infant's mother does not like milk.

on a cow's-milk-free diet and no analysis of the content of opioid peptides in the mother's milk with and without previous milk consumption was performed.

DISCUSSION

Milk is the first and the only food of infants in their primary life stages. Lactating women's milk contains substances that contribute to the proper development of the child (7). BCMs are a specific group of biologically active milk peptides with opioid activity (8). The occurrence of BCMs in human milk (9,10) and in milk formulas has been proved (11). The influence of BCMs interacting with the μ -opioid receptor on the digestive, immune, circulatory, and central nervous systems has been widely discussed in the literature. BCMs are peptides rich in proline, and thus they are resistant to most proteases with wide specificities. Therefore, those peptides may reach the intestine in their nonaltered forms and have physiological effects (8,12–14). Just after birth, the infant's digestive system is not colonized by microflora, which is connected with

weakened stomach acidity, immature mucous membranes, and a decreased exertion of gastric juice, and it is the immature immune system that makes the infant's intestine more permeable for proteins and for low-molecular-mass molecules. Exogenous opioid peptides are attributed to a share in the infant's adaptation to the external environment after the birth—they decrease postnatal stress and ease pain. Some researchers suggest that they may also play an important role in functional development and regulation of the infant's digestive system (7,15). In newborn mammals, the existence of BCM precursors has been demonstrated not only in their digestive systems but also in their blood sera (16). Moreover, the abilities of both bovine and human BCM-5 and BCM-7 to permeate through the human intestinal epithelium have been shown in model experiments *in vitro* with the Caco-2 cell line (17,18).

The exogenous opioid peptides, present in the blood serum, may have an influence on the infant's immature central nervous system, including its' inhibitory actions on the respiratory centre. Hedner and Hedner studied the influences of morphine and BCMs on the central nervous system in rabbit newborns and adult rats (19). In both cases, these substances caused depression of the respiratory system after intracerebroventricular administration; however, BCM-5 was 10 times more potent than morphine. All of the ventilatory effects induced by the BCMs were reversed or prevented by naloxone, which confirmed the way of influence via the opioid receptors (19). The possible connection of BCMs with the central respiratory depressive effects were widely reviewed by Sun et al (20).

In our infant with ALTE, a highly increased level of BCM-5 in serum was the only observed aberration. It significantly exceeded the mean for the blood sera of healthy infants in the control group. That result and multiple time relations of apnoea seizures with his mother's consumption of cow's milk (a positive result of the oral provocation) and a total stoppage of the seizures after removing milk from the infant's mother's diet (a positive result of the elimination attempt) suggest the relation between the opioid actions of milk-released peptides and the pathomechanism of the apnoea seizures and atony. A progressive nature of the seizures, their recurrence, the need for parents' interventions, and the age of the infant allowed for qualification of the child to the SIDS risk group. One of the hypotheses is that SIDS occurs because of a developmental brainstem abnormality in ventilatory and/or autonomic control during sleep, which may be subjected to various

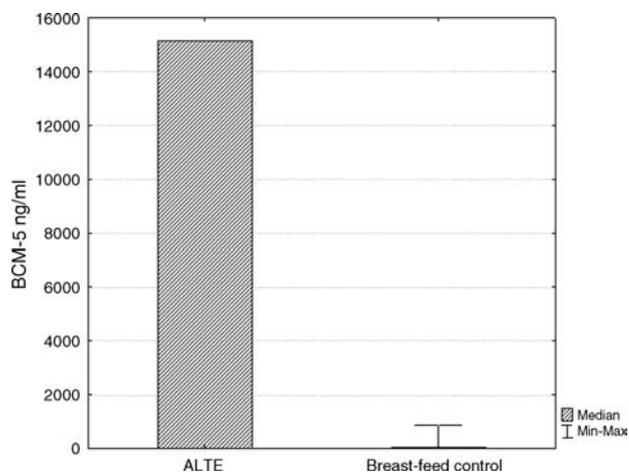


FIGURE 1. Content of bovine β -casomorphin-5 in the blood sera for the infant with apparent life-threatening events and 8 healthy breast-fed controls.

interactions, including those with endogenous opioid or food-derived ones (BCMs) (20). Numerous diseases may manifest through ALTE symptoms, and therefore, if the causes cannot be identified in infants, they are diagnosed as having idiopathic ALTE. The discussed case could be included in that group if the content of BCM-5 in his blood serum was not known.

CONCLUSIONS

The aim of the present report was to draw researchers' attention to the possibility of occurrence of a systemic reaction with an apnoea seizure on the infant's exposure to the proteins in cow's milk. We are convinced that such a clinical situation occurs rarely; however, it is accompanied by a real threat to the infant's life that can be avoided when applying a simple and not costly dietetic intervention. It should be stressed, however, that the research is based on 1 case only and a single collection of the child's serum. In the future, the research spectrum should be broadened to several collections of the serum and an analysis of the opioid peptide level in the mother's milk.

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REFERENCES

1. Adams SM, Good MW, Defranco GM. Sudden infant death syndrome. *Am Fam Phys* 2009;79:870–4.
2. Kahn A. Recommended clinical evaluation of infants with an apparent life-threatening event. Consensus document of the European Society for the Study and Prevention of Infant Death. *Eur J Pediatr* 2003;163:108–15.
3. Kiechl-Kohlendorfer U, Hof D, Peglow UP, et al. Epidemiology of apparent life threatening events. *Arch Dis Child* 2005;90:297–300.
4. Altman RL, Li KI, Brand DA. Infections and apparent life-threatening events. *Clin Pediatr (Phila)* 2008;47:372–8.
5. Edner A, Wennborg M, Alm B, et al. Why do ALTE infants not die in SIDS? *Acta Paediatr* 2007;96:191–4.
6. Semeniuk J, Kaczmarek M, Wasilewska J, et al. Is acid gastroesophageal reflux in children with ALTE etiopathogenic factor of life threatening symptoms? *Adv Med Sci* 2007;52:213–21.
7. Weaver LT. Breast and gut: the relationship between lactating mammary function and neonatal gastrointestinal function. *Proc Nutr Soc* 1992;51:155–63.
8. Kostyra E, Sienkiewicz-Szlapka E, Jarmolowska B, et al. Opioid peptides derived from milk proteins. *Pol J Nutr Sci* 2004;13:25–35.
9. Sidor K, Jarmolowska B, Kaczmarek M, et al. Content of beta-casomorphin in milk of women with a history of allergy. *Pediatr Allergy Immunol* 2008;19:587–91.
10. Jarmolowska B, Sidor K, Iwan M, et al. Changes of β -casomorphin content in human milk during lactation. *Peptides* 2007;28:1982–6.
11. Jarmolowska B, Szlapka-Sienkiewicz E, Kostyra E, et al. Opioid activity of humana formula for newborns. *J Sci Food Agric* 2007;87:2247–50.
12. Meisel H, FitzGerald RJ. Opioid peptides encrypted in intact milk protein sequences. *Br J Nutr* 2000;84:27–31.
13. Phelan M, Aherne A, FitzGerald R, et al. Casein-derived bioactive peptides: biological effects, industrial uses, safety aspects and regulatory status. *Int Dairy J* 2009;19:643–54.
14. Teschemacher H. Opioid receptor ligands derived from food proteins. *Curr Pharm Des* 2003;9:1331–44.
15. Lucas A, Bloom SR, Green AA. Gastrointestinal peptides and the adaptation to extrauterine nutrition. *Can J Physiol Pharmacol* 1985;63:527–37.
16. Kost NV, Sokolov OY, Kurasova OB, et al. Beta-casomorphins-7 in infants on different type of feeding and different levels of psychomotor development. *Peptides* 2009;30:1854–60.
17. Iwan M, Jarmolowska B, Bielikowicz K, et al. Transport of μ -opioid receptor agonists and antagonist peptides across Caco-2 monolayer. *Peptides* 2008;29:1042–7.
18. Sienkiewicz-Szlapka E, Jarmolowska B, Krawczuk S, et al. Transport of bovine milk-derived opioid peptides across a Caco-2 monolayer. *Int Dairy J* 2009;19:252–7.
19. Hedner J, Hedner T. Beta-casomorphins induce apnea and irregular breathing in adult rats and newborn rabbits. *Life Sci* 1987;41:2303–12.
20. Sun Z, Zhang Z, Wang X. Relation of beta-casomorphin to apnea in sudden infant death syndrome. *Peptides* 2003;24:937–43.