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Research on alteration of neurons in vagal nuclei in medulla oblongata in newborns with respiratory distress

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Abstract

Neuronal and axonal degenerative changes in motor vagal neurons (DMNV) and sensory vagal neurons (nTS) in the medulla oblongata in newborns were studied. Material was taken from the autopsies of newborns, live and dead newborns, in different gestational weeks (aborted, immature, premature and mature). 46 cases were studied. Material for research was taken from the medulla oblongata and lung tissue. Serial horizontal incisions were made in the medulla oblongata (± 4 mm), commencing from the obex, where the DMNV and nTS vagal nuclei were explored. Fixed cuttings in buffered formalin (10%) were used for histochemical staining. Serial cuttings were done with a microtome (7 µm). Pulmonary infections, being significant ($p < 0.05$), have an important place when studying respiratory distress (RD) in newborns. Morphological changes of nerve cells in DMNV and nTS nuclei in the medulla oblongata in newborns in different gestational weeks are more emphasized in matures in comparison to aborted and immature ($p < 0.05$). Depending on the lifetime of dead newborns, neuronal morphological changes in vagus nerve nuclei are significant ($p < 0.05$).

Therefore, it can be concluded that pulmonary infections are often caused due to dramatic respiratory distress in newborns, while hypoxaemic changes in the population of vagus nerve neurons in respiratory distress are more emphasized in matures.

Key words: *medulla oblongata, DMNV, nTS, lungs of a newborn.*

Introduction

Respiratory pathological conditions associated with dyspnoea and cyanosis, which appear within the first hours following birth, manifest mainly as respiratory distress (RD). Respiratory distress is defined as a disease of the hyaline membranes due to

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the lack of secretion of surfactant and immature pulmonary structures [21].

Many factors of this complex syndrome are confirmed, but pathogenesis of this disease in newborns is not yet definitely clarified despite many studies.

Respiratory distress is a severe state in premature newborns, with a high rate of mortality up to 60%. As per Kumar *et al*., overall incidence in newborn is 6.7% [23]. In the USA, RD manifests in 40 000 newborns per year and it is considered that it causes about 20% of deaths in newborns [18,32].

Respiratory distress usually appears in premature infants (< 35 weeks of gestation) but it can also appear following the 35th week of intrauterine life in newborns with late lung maturity. Respiratory distress incidence is higher, as the weight is lower in newborns, which depends on gestational weeks of intrauterine growth [13].

All these pathological conditions are assumed to be related to the immaturity of respiratory centres, a process which remains unclarified [9-11].

Damage of these respiratory centres is deemed to have an effect on their maturity, which results in disorders of the respiratory system [7,8,19].

According to some authors, one of the causes of damage to these centres is hypoxia, which causes neuronal damage of the nuclei of cranial nerves, respectively of the dorsal motor nucleus of the vagus (DMNV), from where emerge nerve fibres that innervate respiratory, cardiovascular and gastrointestinal organs, ending at the parasympathetic intramural ganglions of these organs [16,20].

Information from the vagal sensory neurons ends up in the *nucleus tractus solitarius* (nTS), which is in close relation to DMNV and together with the *area postrema* (AP) forms the circumventricular complex [5].

Many authors describe the role of the *medullary raphe nuclei* (MRN) – *raphe pallidus* (RPa), *magnus* (RMa) and *obscurus* (Rob), which are implied in a series of cardiorespiratory physiological functions [3,4].

Stimulation of neurons in the *raphe nuclei medulla* produces deep effects in the phrenic nerve, by causing the state of apnoea [30].

Biondo *et al.* observed changes in neuronal and glial cells in nTS of newborns with sudden infant death syndrome (SIDS). Brain tissue damage stimulates activation of astrocytes and microglia, which has neuronal damage in nTS as a consequence [6].

Different authors have also described a connection between exposure of the fetus to hypoxia and RD manifestation. Hypoxia oversets mechanisms for the control of respiration by also including the sensitivity of respiratory centres in different stimulations and in the process of brain memory of breathing, which is dependant on the first cycles of breathing immediately following infant birth [2].

Description of the different degenerative changes and other pathological alterations in these nerve centres as a consequence of hypoxic-ischaemic lesions, that mainly manifest in the form of gliosis, which include the *nucleus olivary inferior,* and in the form of aplasia or hypoplasia of the *nucleus arcuatus* in the ventral surface of the *medulla oblongata* is in favour of this ascertainment [17].

The *nucleus arcuatus* is involved in the adjustment of the breathing cycle against the stimulation reply of carbon dioxide in centres for regulation of breathing [15].

Therefore, presentation of histopathological changes in these nerve centres involved in the process of regulation of breathing in early stages of prenatal and postnatal development have importance for the explication of pathophysiological mechanisms of breathing process disorders in respiratory distress syndrome (RDS).

The aim of this work consists in the study of degenerative neuronal and axonal changes in vagal motor neurons and sensory vagal neurons in the medulla oblongata of newborns who died due to respiratory distress syndrome in different gestational ages.

Material and methods

The study was performed with the approval of the Ethic Commission by respecting the principles of the Helsinki Declaration. The study used material from live and dead newborn autopsies, due to different causes in different gestational weeks. Case grouping was done as follows in Table I.

Selected material for the study was taken from the:

- medulla oblongata,
- ung tissue from all of the lobes.

Series of horizontal cuttings were done in the medulla oblongata with initial orientation from the obex at the depth of ± 4 mm, at the projection of the dorsal motor nucleus of the vagal nerve (DMNV) and sensory nucleus tractus solitarius (nTS), according to brain stereo-metric atlases. Material was fixed with a solution of buffered formalin (10%), for histochemical staining. Lung tissue was also fixed with

a solution of buffered formalin (10%), for histochemical studies. Preparation cuttings were made with a microtome (7 µm).

Special histochemical research methods were used for the central nervous system: haematoxylin-eosin staining, Cresyl echt violet staining (for nerve and glial cells), Cresyl echt violet staining for the Nissl substance, argyrophilic granule staining, argentophile (Grimelius), chromaffin reaction, Sevier-Munger modification (for nerve endings), Luxol fast blue – MBS (for myelinated fibres).

Calculation of statistical data was done by the statistical computer system GraphPad Instat 3.06. One-way analysis of variance (ANOVA), Tukey-Kramer multiple comparisons test, unpaired *t*-test, and the two-tailed *P* value were used also.

Results

The study analysed 46 cases, from which 11 samples were eliminated due to autolysis while 20 samples were eliminated due to lack of data on the survival period of newborns.

The pulmonary pathological changes in different gestational ages in newborns are presented in Table II.

The analysis of pulmonary pathological changes in different gestational ages commencing with the aborted, immature, premature and mature revealed that bronchopneumonias are the most common pulmonary pathologies in newborns (Fig. 1). Testing of the significance of parameters was also done by one-way analysis of variance (ANOVA). The *P* value 0.0449 is considered significant. Analysis shows that pulmonary parenchyma inflammations are foregoing to respiratory insufficiency at all of the gestational ages.

Neuronal morphological changes in the nuclei of the vagus nerve in the medulla oblongata are presented in Table III. The neurons of sensory nucleus nTS with advanced degenerative changes of central chromatolysis were observed, as well as axonal degeneration and reactive astrogliosis.

The morphological changes of dorsal motor nucleus of the vagal nerve (DMNV) and sensory nucleus tractus solitarius (nTS) lesions in cases with pulmonary pathology are presented in Fig. 2. Higher

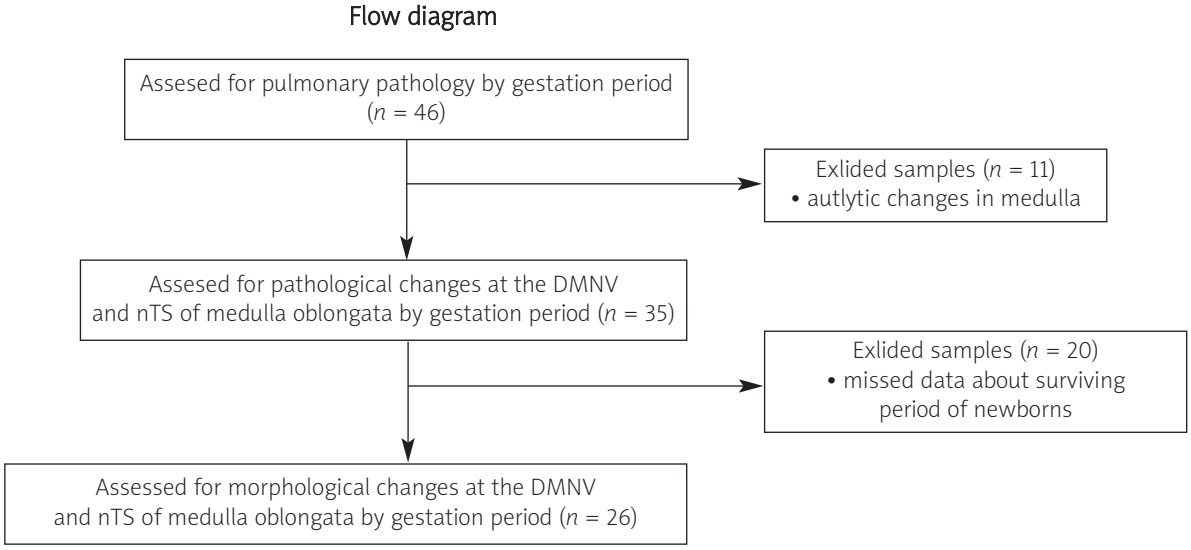

Table I. Case grouping of newborn based on weight and gestational weeks

Group	Pathological term	Weight (g)	Gestation weeks
I	Aborted	< 500	< 22
II	Immature	500-1100	23-29
III	Premature	1100-2500	30-37
IV	Mature	> 2500	> 38

frequency of neuronal axonal degeneration is obvious. Neuronal morphological changes are significant in mature newborns in comparison to aborted and immature ($p < 0.05$).

Table IV presents neuronal morphological changes in motor and sensory vagal nuclei depending on the period of newborns' lifetime.

In Fig. 3 morphological neuronal changes in DMNV and the nTS nuclei in newborns are presented. Considerable significance of neuronal changes in nuclei of dead newborns is seen ($p < 0.05$). Pathology of special morphological neuronal changes (CC, AD, RA, NFD) is not considered as significant ($p > 0.05$).

Table II. Pulmonary pathological manifestation in different gestational weeks in newborns ($n = 46$)

	IP	MHP	ALAM	BPPB	Total
Aborted	2	0	0	2	4
Immature	4	1	0	5	10
Premature	0	6	3	10	19
Mature	0	2	2	9	13
Total	6	9	5	26	46

IP – imaturatio pulmonum, MHP – membranae hyalinea pulmonum, ALAM – aspiratio liquoris amnii et meconialis, BPPB – bronchopneumonia purulenta pulmonum bilateralis

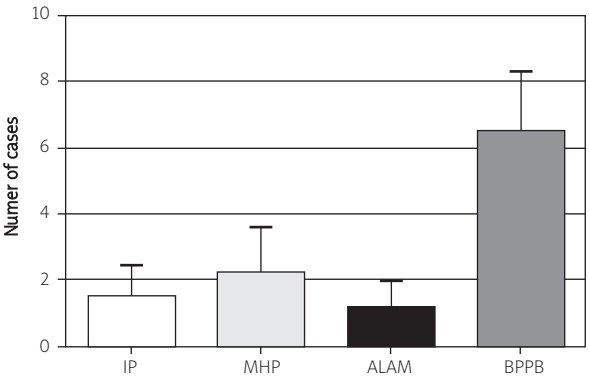

Fig. 1. Frequency of pulmonary pathology in newborns depending on age ($n = 44$).

IP – imaturatio pulmonum, MHP – membranae hyalinea pulmonum, ALAM – aspiratio liquoris amnii et meconialis, BPPB – bronchopneumonia purulenta pulmonum bilateralis

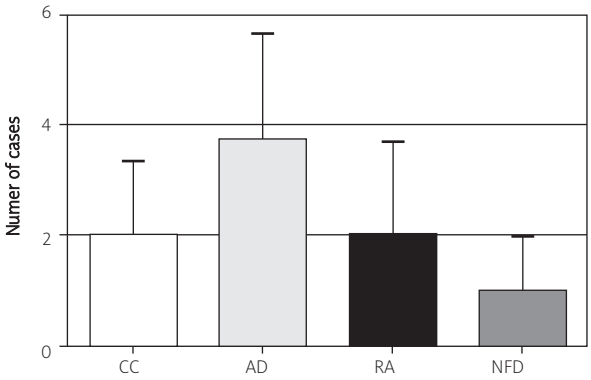

Fig. 2. Frequency of neuronal changes in DMNV and nTS nuclei in newborns depending on age ($n = 35$).

CC – central chromatolysis, AD – axonal degeneration, RA – reactive astrocytosis, NFD – neurofibrillary degeneration

Table III. Pathological manifestations of nerve cells in DMNV and nTS nuclei in medulla oblongata depending on age ($n = 35$)

Neuronal changes	Aborted (< 500 g)	Immature (500-1100 g)	Premature (1100-2500 g)	Mature (> 2500 g)	Total
CC	0	1	1	6	8
AD	0	1	6	8	15
RA	0	0	1	7	8
NFD	0	0	0	4	4
Total	0	2	8	25	35

CC – central chromatolysis, AD – axonal degeneration, RA – reactive astrocytosis, NFD – neurofibrillary degeneration

Table IV. Morphological neuronal changes in DMNV and nTS nuclei in newborns depending on age (n = 26)

	Live born	Dead born	Total
CC	18	8	26
AD	11	15	26
RA	18	8	26
NFD	22	4	26

CC – central chromatolysis, AD – axonal degeneration, RA – reactive astrocytosis, NFD – neurofibrillary degeneration

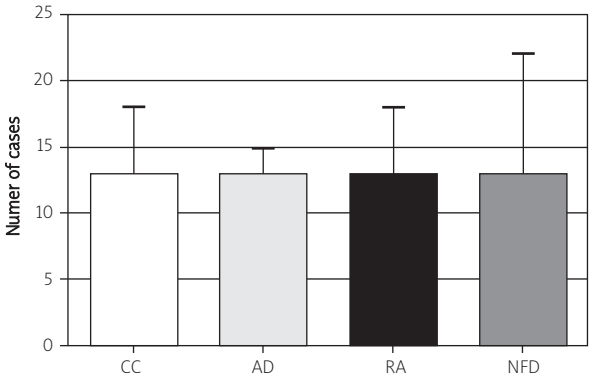

Fig. 3. Frequency of morphological neuronal changes in DMNV and nTS nuclei in newborns depending on their lifetime (n = 26).

CC – central chromatolysis, AD – axonal degeneration, RA – reactive astrocytosis, NFD – neurofibrillary degeneration

Different neuronal changes in the vagal nuclei in the medulla oblongata observed in newborns with respiratory distress are shown in Fig. 4. The advanced degenerative changes of central chromatolysis in neurons of sensory nucleus nTS and in motor neurons of the vagal nerve DMNV as well as focal necrotic changes are demonstrated.

Discussion

Despite numerous studies, RD's aetiology remains controversial. According to many authors, intranatal hypoxia is a central cause in manifestation of this syndrome. However, different studies have confirmed that other aetiological factors, such as aspiration of amniotic fluid, meconium fluid and infections of the respiratory tract, can also be the cause of this syndrome [1].

In our research, one of the most frequent pathologies that preceded RDS was disease of the hyaline membrane, which is in agreement with the results of other authors [31].

However, lack of the surfactant, which causes hypoxia and acidosis, is considered to be the initial factor of this syndrome.

Lack of the surfactant in RDS can be primary (lung immaturity) or secondary (aspiration of meconium and blood) [22].

Moss, in his study, presents the hypothesis by which pulmonary changes that are characteristic for RDS, such as lung shock, respiratory hypoxia and disease of the hyaline membrane, have central neurogenic aetiology. This author concludes that cerebral isolated hypoxaemia stimulates stagnant hypoxia of the shock state that initiates the hypoxic hypoxia with RDS as a consequence [25].

Following the appearance of hypoxia and acidosis, cascade of the degenerative changes is initiated, which firstly includes degeneration of vagal neuronal cells in the medulla oblongata located in DMNV and nTS.

Ischaemic changes as the result of hypoxia result in morphological changes also to the central nervous system, especially in the neural vagal cell density in the medulla oblongata, where there are vagal nuclei (DMNV) motor and sensory vagal nuclei (nTS), which are responsible for respiration. Information from the sensory vagal neurons usually ends up in nTS, found immediately nearby DMNV, and together with the area postrema (AP) form the circumventricular organ.

Neurons show a large number of specific and non-specific morphological changes in the CNS, such as central chromatolysis (tigrolysis), which represents loss of the Nissl substance. They can be central and peripheral ones. Melting of the Nissl substance appears at central chromatolysis, which persists only in the periphery of cytoplasm, puffing of perikaryon and dislocation of the nucleus in the periphery. This condition is noted in the inferior motor neurons (frontal ribs of the spinal cord and cranial nerve nuclei), where there appears the perikaryon reaction in the lesion of the axon, "retrograde degeneration". Peripheral chromatolysis differs from the central type in terms of the presence of Nissl substance,

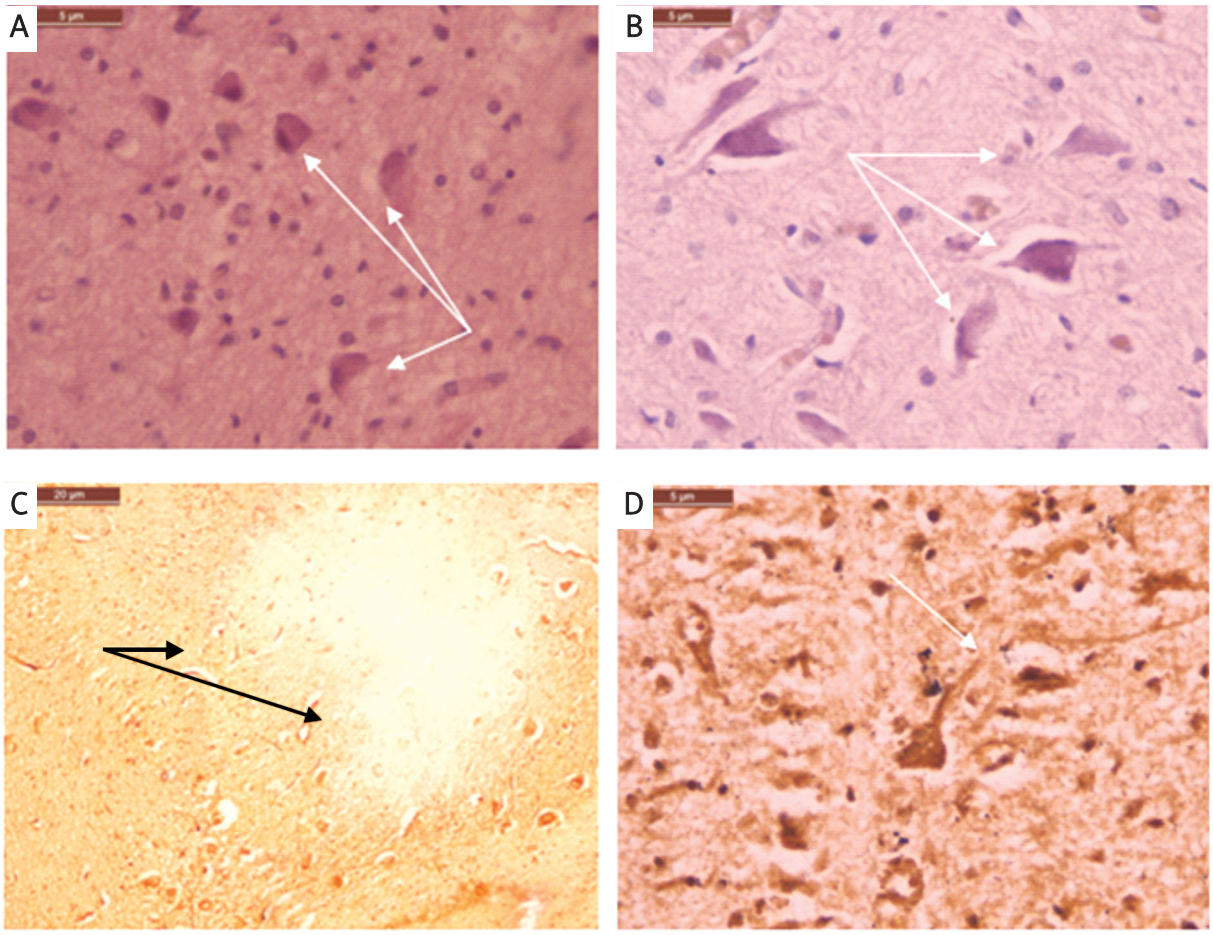

Fig. 4. Neuronal morphological changes in the nuclei of vagal nerve in newborns with different gestational ages (n = 35). (**A**) Neurons of sensory nucleus nTS with advanced degenerative changes of central chromatolysis (arrows; increasing × 400, hue – H & E). (**B**) Central chromatolysis in motor neurons of the vagal nerve DMNV. Nissl substance melts, cytoplasm puffs becomes eosinophilic and the core shifts on the periphery (arrows; magnification 400 ×, hue – cresyl violet). (**C**) Focal ischaemic reduction of the neuronal area of nTS (arrows; magnification 100 ×, hue – modified Sevier-Munger with silver salts). (**D**) Axonal demyelisation of motor nucleus neurons population of vagal nerve DMNV (arrows; magnification 100 ×, hue – modified Sevier-Munger with silver salts).

only in the middle part of the perikaryon. This represents the stage of neuron regeneration [12].

Machaalani presents an increase of neuronal apoptosis in SIDS in comparison to patients without SIDS. Furthermore, the author concludes that mechanisms of apoptosis in SIDS are dependent on age, gender, and exposure to different risk factors of newborns [24].

Onai T, in his study, presents two functional subdivisions in nTS in cats. The inspiratory zone is sensitive to exposure to hypoxia [27].

Actually, hypoxia permanently endangers the development of brain centres by commencing with the early fetal period up to birth. It is the main factor that causes cell damage, neurodegeneration and death of neuronal cells, mainly through the disorder of haemostasis of intracellular Ca^{2+} which is followed by damage in the cell and also of excitatory amino acids (EAAs) [26].

Neurons, as a consequence of this condition, manifest a large number of specific and non-specific morphological changes, such as central (tigrolysis) and peripheral chromatolysis.

Based on our morphological observations, we have ascertained that the structures most susceptible to anorexia are central motor neurons, firstly the

group of neurons DMNV and sensory nTS in the medulla oblongata (Fig. 3) which manifests with central chromatolysis (CC), axonal degeneration (AD), reactive astrocytosis and neurofibrillary degeneration. In mature ages, we have recorded different forms of metabolic degenerative damage and axonal toxicity in peripheral axons in the form of segmental demyelisation, neurotubular fragmentation, proliferation of Schwann cells and protuberance of neurotubules and axonal neurofilaments. These pathological manifestations are mainly comparable to the observations of other authors.

Axonal and neurofibrillary degeneration can be observed in many CNS degenerative diseases which are in the form of argentaffin degeneration. In our observations, these degenerations have the associating frequency similar to other morphological changes of the density of neural cells in vagal nuclei (Fig. 2).

Takashima *et al.*, during the description of changes in 15 cases of death of newborns due to sudden death syndrome, present these changes: gliosis in *nucleus reticularis magnocellularis* in the medulla oblongata and also nTS and *nucleus dorsalis vagus* in 50-80% of cases. Also, in 20-40% of cases, they also describe reactive reactions of astrocytes with antibody in the glial fibrillary acidic protein with the presence of astrogliosis [28,29].

Meanwhile, Folkerth *et al*. described changes in 22 dead newborns from different causes. They describe histopathological changes in 63.6% of cases. In 54.5% of cases, acute neuronal necrosis was identified in the *nucleus arcuatus* with or without histopathological changes (hypereosinophilia and nuclear pyknosis or karyorrhexis) [14].

In mature ages, we have found different forms of metabolic degenerative damage and axonal toxicity in peripheral axons, such as segmental demyelisation, neurotubular fragmentation, proliferation of Schwann cells and fragmentation and protuberance of neurotubules and axonal neurofilaments.

The above-mentioned observations suggest the direct impact of cardiorespiratory insufficiency in the population of nerve cells, dorsal motor nucleus of the vagal DMNV and sensory vagal nTS, so-called "neurogenic inflammation", a pathology which should be studied even more in the future.

Conclusions

Based on our morphological observations, in the simultaneous examined material of the lungs and vagal nerve nuclei in the medulla oblongata in newborns with respiratory distress, we can ascertain as follows:

1. Bronchopneumonia is the most common morphological pulmonary pathology in newborns, in different gestational weeks, which causes respiratory distress ($p < 0.5$).
2. Morphologic changes of nerve cells in the DMNV and nTS nuclei in the medulla oblongata, in newborns in different gestational weeks, are more emphasized in mature ages compared to aborted and immature ($p < 0.5$).
3. Depending on the survival of newborns, neuronal morphological changes in vagus nerve nuclei are significant ($p < 0.5$).

From these conclusions it can be seen that pulmonary infections are the most common causes of dramatic respiratory distress in newborns, while hypoxaemic changes in the population of vagus nerve neurons in respiratory distress are more emphasized in matures. In dead newborns, neuronal hypoxaemic changes in vagus nerve nuclei in the medulla oblongata are significant.

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