



IN VITRO RESEARCH OF THE ALTERATION OF NEURONS IN VAGAL CORE IN MEDULLA OBLONGATA AT ASPHYXIC DEATHS

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ABSTRACT

The aim of this study was to research the morphological changes of neurons in the vagus nerve nuclei in medulla oblongata in asphyxia related death cases. Morphological changes that were investigated were mainly in the dorsal motor respiratory center (DMRC), nucleus tractus solitarius (nTS) and nucleus ambiguus (nA) in the medulla oblongata. In our research, the autopsy material from asphyxia related death cases was used from various etiologies: monoxide carbon (CO), liquid drowning, strangulation, electricity, clinical-pathological death, firing weapon, explosive weapon, sharp and blunt objects and death cases due to accident. The material selected for research was taken from medulla oblongata and lungs from all lobes. The material from the medulla oblongata and lungs was fixed in a 10% solution of buffered formalin. Special histochemical methods for central nervous system (CNS) were employed like: Cresyl echt violet, toluidin blue, Sevier-Munger modification and Grimelius. For stereometrical analysis of the quantitative density of the neurons the universal testing system Weibel M42 was used. The acquired results show that in sudden asphyxia related death cases, there are alterations in the nuclei of vagal nerve in form of: central chromatolysis, axonal retraction, axonal fragmentation, intranuclear vacuolization, cytoplasmic vacuolization, edema, condensation and dispersion of substance of Nissl, proliferation of oligodendrocytes, astrocytes and microglia. The altered population of vagus nerve neurons does not show an important statistical significance compared to the overall quantity of the neurons in the nuclei of the vagus nerve ($p < 0,05$).

KEY WORDS: death, strangulation, asphyxia, nuclei, vagus, stereometry

INTRODUCTION

Asphyxic lesions cause lethal lesions or nonlethal lesions of health due to respiratory disorders. Hypoxia appears due to lack of proper supply of tissues with oxygen (O₂), whilst total lack of oxygen flow in tissues causes anoxia. As a consequence of an accumulation of carbon dioxide (CO₂) hypercapnia appears in the organism, and at once final metabolic products are accumulated as well and finally, along with hypercapnia, cause the paralyzes of respiratory centres which brings to general strangulation or asphyxia. ^(1,2,3) Respiratory control is a result of interactive complexes in between neurons and medulla oblongata. Three neuronal groups enable normal breathing: dorsal respiratory group (nucleus dorsalis nervus vagus – DMNV, tractus and nucleus solitarius - nTS), ventral respiratory group (Botzinger complex, nucleus retroambigualis and nucleus ambiguus), pontin respiratory group (Klikler – Fuse complex and nucleus parabrachialis medialis). Differentiated neurons of these groups are held responsible for the activity of respiratory cycle (Figure 1).

Coordination of activities of these neurons in a group manner generates the respiratory rhythm. ⁽⁴⁾ Cell hypoxemic manifestations are well emphasized in hippocampus and in cores of cranial nerves. Early changes in neuronal cells caused by hypoxias in animal models are evident following a couple of seconds. Brain ischemia or cerebral trauma is considered as cause of brain secondary lesion by producing edema, damaging of *hematoencephalic barrier* and exposure of the brain perivascular tissue. Conditions with prolonged hypoxia cause irreversible functional changes and cell damage (cell death), but they can be avoided for a certain time period. Neurons show a huge number of morphologic changes,

which, either specific or non-specific ones, shows different pathologic component of CNS conditions. Chromatolysis is noticed at lower motoric neurons (frontal ribs of spinal cord and cranial nerve nuclei). Ischemic changes of neuron (eosinophilic degeneration) represent an often morphologic change, found in ischemia, anoxia and hypoglycemia. Satellitosis may show degenerative changes. Neuronophagia is often found in rapid deaths of nerve cells (4,5,6,7,8). Axonal degeneration appears during cases of toxic and metabolic disorders, that attacks the neuron in whole, respectively attacks body and its extensions. Segmental axonal demyelination appears usually in Schwann cell necrosis in the respective segment. Astrocytosis usually associates the acute neuronal lesion (7-9). *Microglial cells*, in pathologic conditions, transform into cane cells and participate in the creation of glial noduluses (9,10). The aim of this study was to research the morphological changes of neurons population in the vagal motoric dorsal nuclei, fasciculi and nucleus tractus solitarius as well as nucleus ambiguus and the study of numeric density of normal and pathologic neurons in respiratory cores of nervus vagus in different asphyxic conditions.

MATERIAL AND METHODS

Research was conducted in cooperation with the Department of Forensic Medicine, Pathologic Anatomy Institute and Experimental Unit of Medical Faculty in Prishtina, with permission of the Ethic Commission by respecting principles of Helsinki Declaration. In our researches, material from autopsies of asphyxic deaths with different etiologic cause was utilized. Material was taken from autopsies performed in the Institute of Forensics in Prishtina. Case grouping was done by the collocation as follows (Table 1):

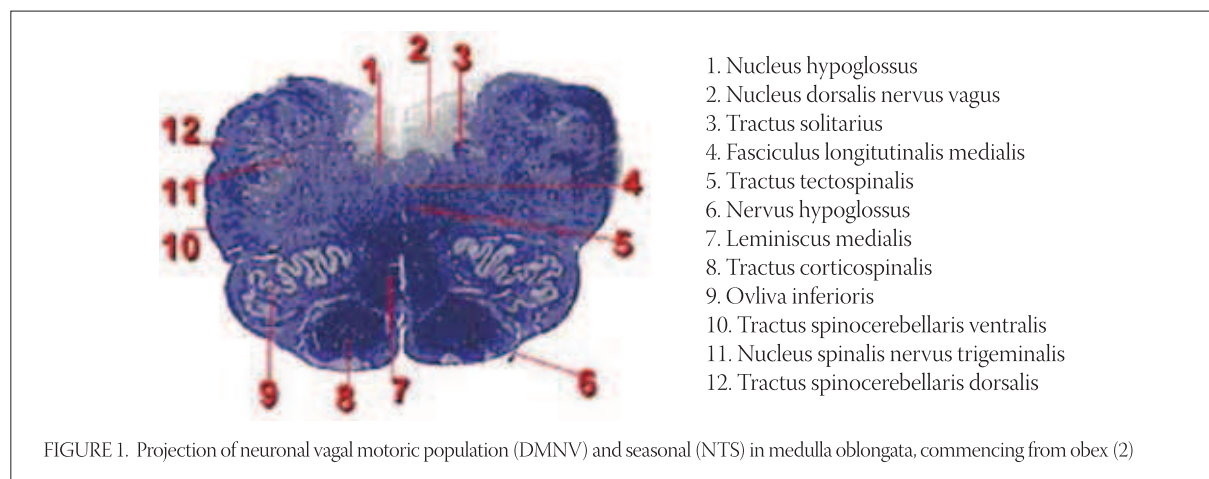


FIGURE 1. Projection of neuronal vagal motoric population (DMNV) and seasonal (NTS) in medulla oblongata, commencing from obex (2)

Group	Death cause	Number of cases
I	Intoxication with carbon monoxide (ICM)	5
II	Liquid drowning (LD)	5
III	Deaths from hanging – lasso strangulation (DH)	5
IV	Deaths from electricity (DE)	5
V	Clinical – pathological deaths (CPD)	5
VI	Firing weapon deaths (FWD)	5
VII	Explosive devices deaths (ED)	5
VIII	Sharp objects deaths (SOD)	5
IX	Blunt objects deaths (BOD)	5
X	Accident deaths (AD)	5

TABLE 1. Death causes

Material selected for research was taken from these parts of the organs:

Medulla oblongata and lung tissue from all lobes. Horizontal incisions were done in the medulla oblongata in a serial manner in 10µ thickness initially commencing

from obex in ± 4 mm thickness, where projection of dorsal vagal nuclei is done (dorsal motoric nucleus of nervus vagus and tractus solitarius nucleus), and ventral group of respiratory neurons (Böttinger complex, retroambigial and ambigial core), as per stereometric analysis of different authors. Material from medulla oblongata with pulmonary tissue was fixed in a 10% solution of buffered formalin. Research methods as follows are used: histochemical method – staining with *hematoxylin and eosin*, staining with cresyl echt violet for nerve and glial cells, staining with cresyl echt violet for Nissl substance, staining with argyrophilic grain (Grimelius), Sevier-Munger modification for nerve endings staining). Stereometric methods: for stereometric research, universal testing system Weibel M 42 was used. Following the extension of the system with adequate micrometer

	K.Q.	R.A.	FA.	V.I.N.	V.C.	E.T.	K.N.	SH.N.	PA	PO.	P.M.
I.C.M.											
1.DMNV	2+	1+	1+	0	0	2+	2+	2+	1+	2+	1+
2.nTS	3+	2+	2+	2+	1+	3+	3+	3+	1+	2+	1+
3.nA	1+	1+	0	0	0	2+	2+	2+	0	1+	0
L.D.											
1.DMNV	1+	1+	0	0	0	2+	1+	1+	0	1+	0
2. nTS	2+	2+	1+	1+	0	3+	2+	2+	0	2+	1+
3. nA	1+	1+	0	0	0	2+	1+	1+	0	1+	0
D. H.											
1.DMNV	1+	1+	1+	1+	0	2+	1+	1+	0	1+	1+
2.nTS	2+	2+	1+	1+	0	3+	2+	2+	0	2+	1+
3.nA	1+	1+	1+	1+	0	1+	1+	1+	0	1+	1+
D. E.											
1.DMNV	2+	1+	2+	1+	0	2+	2+	1+	0	2+	1+
2.nTS	3+	2+	3+	1+	0	3+	3+	2+	1+	3+	2+
3.nA	1+	1+	1+	1+	0	2+	2+	2+	1+	1+	1+
C. P.D.											
1.DMNV	2+	1+	2+	1+	0	2+	2+	2+	1+	2+	1+
2.nTS	3+	2+	3+	2+	1+	3+	3+	3+	2+	3+	2+
3.nA	2+	1+	2+	1+	1+	2+	1+	1+	1+	1+	1+
FW/D											
1.DMNV	1+	1+	0	0	0	2+	1+	1+	0	1+	1+
2.nTS	2+	2+	0	0	0	1+	1+	2+	0	2+	1+
3.nA	1+	1+	0	0	0	2+	1+	1+	0	1+	0
E.D.											
1.DMNV	1+	1+	0	0	0	2+	1+	1+	0	2+	1+
2.nTS	2+	1+	0	0	0	3+	2+	2+	1+	3+	1+
3.nA	1+	2+	0	0	0	2+	1+	1+	1+	2+	1+
S.O.D.											
1.DMNV	1+	0	0	0	0	1+	1+	1+	0	0	0
2.nTS	1+	0	0	0	0	2+	2+	2+	0	1+	0
3.nA	1+	0	0	0	0	1+	1+	1+	0	1+	0
A.D.											
1.DMNV	1+	1+	0	0	0	2+	1+	1+	0	1+	0
2.nTS	2+	1+	0	1+	0	3+	2+	2+	1+	2+	1+
3.nA	1+	1+	0	1+	0	2+	1+	1+	0	1+	0
V.M.M.b.											
1.DMNV	1+	1+	0	0	0	1+	1+	0	0	1+	0
2.nTS	2+	1+	0	0	0	2+	2+	1+	0	1+	0
3.nA	1+	1+	0	0	0	1+	1+	0	0	1+	0

Legend: C.C. - central chromatolysis; A.R. – axonal retraction; A.F. – axonal fragmentation; I.N.V. – intranuclear vacuolization; C.V. – cytoplasmic vacuolization; B.E. – brain edema; C.N. – condensation of Nissl substance; M.N. – melting of Nissl substance; P.O. – proliferation of oligodendroglial cells; P.A. – proliferation of astroglial cells; P.M. – proliferation of microglial cells; I.C.M. – Intoxication with carbon monoxide; L.S. – Lasso strangulation; D.H. – Deaths from hanging; D.E – Deaths from electricity; C.P.D. - Clinical Pathological deaths; FW/D – Firing weapon deaths; E.D. – Deaths from explosive devices; S.O.D. - Sharp object deaths; A.D – Accidental deaths; B.O.D – Blunt object deaths. DMNV – Dorsal motoric nuclei of vagus; nTS – Nucleus tractus solitarius, nA – Nucleus ambiguus.

TABLE 2. Morphologic altering changes in nervus vagus nuclei in medulla oblongata at violent asphyxic deaths

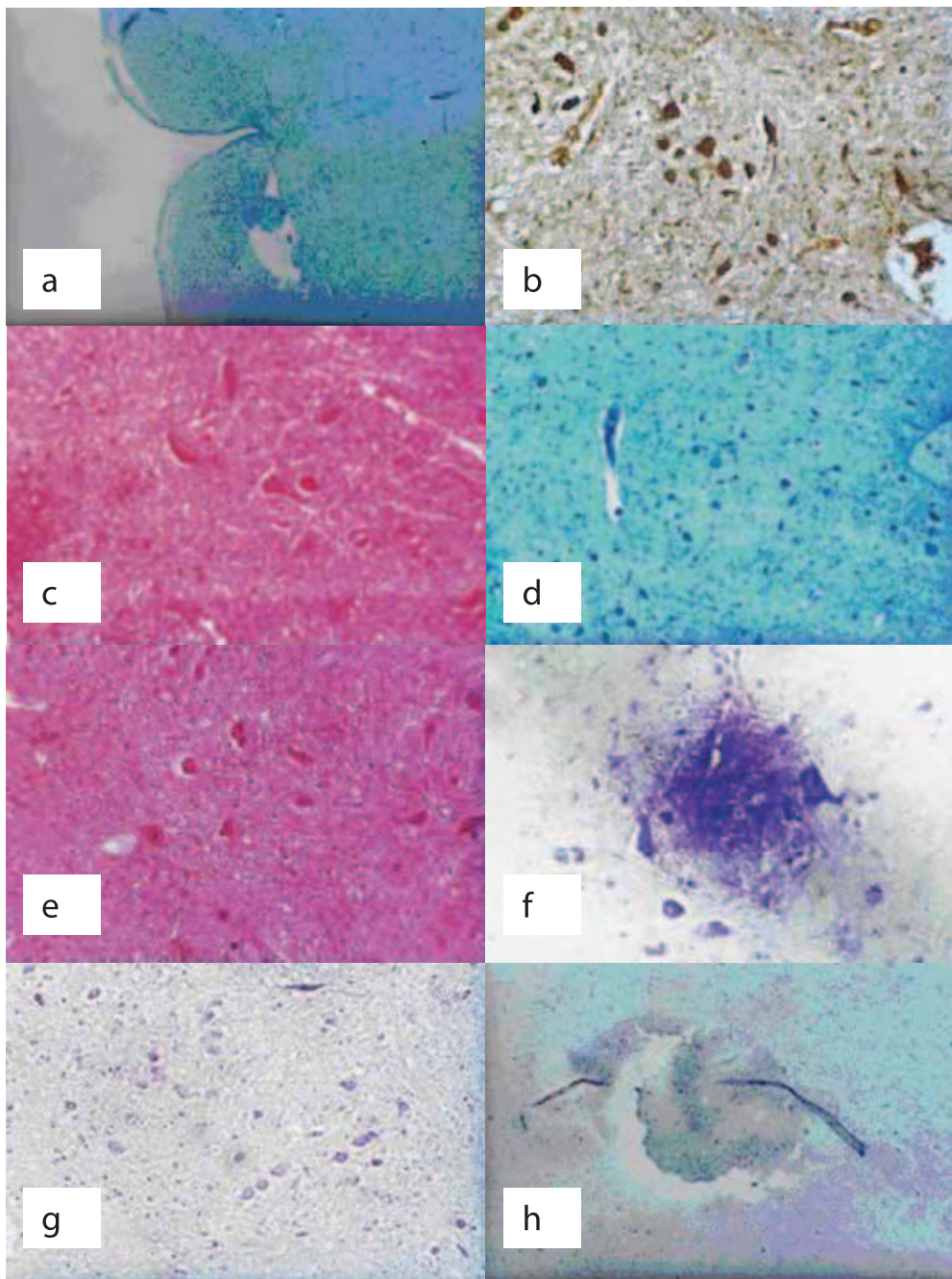


FIGURE 2. a) Exploration of neurons in motoric dorsal vagal nuclei, commencing from obex of medulla oblongata (staining - Toluidin blu,100 x); b) Population of neurons in motoric dorsal vagal nuclei where initial changes of Nissl substance can be noticed, axonal retraction, proliferation of glial cells and emphatic edema of brain tissue (staining - Grimelius; 400 x); c). Population of neurons in motoric dorsal vagal nuclei where emphatic changes of central chromatolysis and reactive astrocytes can be noticed (staining - Safranin; 400 x); d) Population of neurons in vagal core nucleus ambiguus with emphatic changes of axonal retraction and central chromatolysis (staining - Cresyl echt violet; 200 x); e) Population of neurons in nucleus ambiguus with changes in intracytoplasmic vacuolization, central chromatolysis, degeneration of Nissl substance, axonal retraction and proliferation and hyperplasia of glial cells (staining - Safranin; 400 x); f) in the fasciculus of nTS, emphatic changes of demyelination and axonal fragmentation with proliferation of oligodendrocytes and emphatic focal necrotic changes can be noticed (staining - Cresyl echt violet; 400 x); g) Population of neurons in nTS with emphatic changes of central chromatolysis, central focal softening, proliferation and hyperplasia of oligodendrocytes (staining - H & E; 200 x); h) emphatic altering changes in nTS, increased reaction of oligodendrogliosis (staining - Cresyl echt violet; 200 x).

of the object (40 x), we have had values as follows of the measuring diameter (D) and the measuring field (At).

$$D = 2,5 \times 10 \mu = 25 \mu\text{m}$$

$$At = 0,23 \text{ mm}^{-2}$$

Researches were done in five testing points (Pt) and in tissue serial cuts of 7 and 10 μ .

Determination of numeric density (neurons).

Numeric density is a relative stereologic variable, which shows that there are grains (neurons) in the voluminous unit. This variable has dimension with exponent (-3). Calculation is done as per Abercrombie formula:

$$Nv = Na : t + D$$

Nv = numeric density, Na = number of neurons in incision, t = thickness of incisions in series, D = average diameter of neurons.

Na = Nf: At

Nf=number of points in the observed phase, At=0,23 mm²

Results gained statistically are calculated with the assistance from computer statistic system INSTAT 2 with T-testin of reliability (GraphPad Instat tm Copyrin ©, 1990-93,Graph Pad software V2,02; Dr Ernberger, Case Western Reserve U.930952

RESULTS

Morphologic research was performed in tissue serial incisions of medulla oblongata, commencing with obex with diameter ± 4mm. Serial incisions were done in 7 and 10 μ (micron). Continually, within horizontal incisions, visualisation of dorsal vagal nuclei is done (DMNV, nTS) and population of ventral vagal nuclei (nA). Most often lesions in the population of nervus vagus are presented in below figures. In Table 2, different rate of altering morphology of nervus vagus nuclei are presented, beginning from: central chromatolysis, axonal retraction, axonal retraction fragmentation, intranuclear vacuolization, cytoplasmic vacuolization, brain swelling, condensation of Nissl substance, proliferation of astroglial, oligodendroglial, and microglial cells. In Table 2, different altering stages of population of vagal neurons in dorsal and ventral nuclei of nervus vagus in medulla oblongata at violent asphyxic deaths. Altering changes are scaled from 0 – 3+. 0 – does not represent apparent optical changes in examined neurons, 1+ - represent mild neuronal altering changes, 2+ - represent neuronal altering changes of mid scale, and 3+ - represent emphatic neuronal altering changes. In cases with intoxication with carbon monoxide (ICM), edema of brain in the DMNV nucleus, condensation of Nissl substance, melting of Nissl substance, central chromatolysis, and proliferation of oligodendroglial cells are emphatic. In nTS, higher scale of altering changes is evident, commencing with condensation and melting of Nissl substance, central chromatolysis, axonal retraction and fragmentation and proliferation of oligodendroglial, astroglial, and microglial cells. Intensity of abovementioned changes is lower in nA nucleus. At lasso strangulation in nTS, higher scale of brain swelling, condensation and melting of Nissl substance, central chromatolysis, and proliferation of oligodendrocytes in comparison to neurons of DMNV and nA nuclei are evident. At deaths from hanging in nTS neurons, mid

	DMNV	nTS	nA
Total number of neurons (T.n.n)	16,8 ± 0,85	20±1,84	15,77±1,3
Pathologic number of neurons (P.n.n)	11± 1	8,29±1,14	6,3±1,37
Normal number of neurons (N.n.n)	5,75±0,49	11,9±1,29	9,47±0,4

TABLE 3. Numeric density of normal and pathologic respiratory vagal neurons in vagal nuclei in violent asphyxic deaths (X ±SEM) N.50 mm²

scale of condensation and chromatolysis of Nissl substance, central chromatolysis, axonal retraction and fragmentation and proliferation of oligodendrocytes in comparison to neurons of DMNV and nA nuclei are noticed. At deaths due to electricity, emphatic changes of brain swelling, condensation and melting of Nissl substance, central chromatolysis, axonal fragmentation, proliferation and hyperplasia of oligodendrocytes are noticed. At clinical – pathological deaths, morphologic altering changes are evident in all of the respiratory nuclei, commencing with brain swelling, condensation and melting of Nissl substance, central chromatolysis, axonal retraction and fragmentation, and degenerative vacuolar, intranuclear and intracytoplasmic changes. Glial reaction is also emphatic. In Table 3, cases of total neuronal numeric density, morphologically pathologic and normal ones, in DMNV, nTS and nA nuclei in medulla oblongata in asphyxic deaths with various causes are presented. Following the statistical calculations done between different groups of asphyxic deaths in vagal nuclei DMNV, nTS and nA, important statistical significance between the population of normal and pathologic neuron was not gained, (p<0,05). In Figure 3, density of neurons in DMNV, nTS and nA nuclei are presented by column diagram. Total number of neurons, number of neurons with normal appearance and neurons with pathologic altering changes are also presented. No statistical sig-

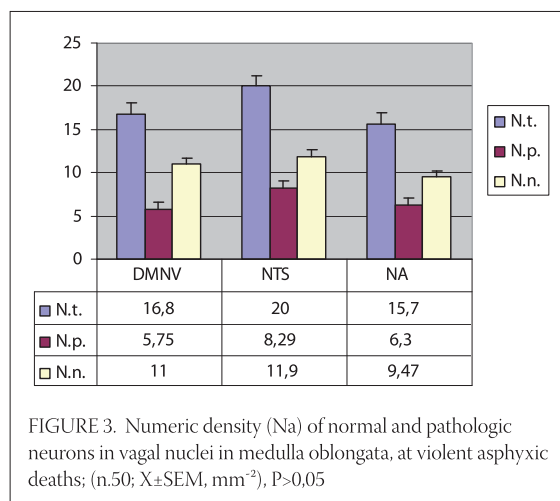


FIGURE 3. Numeric density (Na) of normal and pathologic neurons in vagal nuclei in medulla oblongata, at violent asphyxic deaths; (n.50; X±SEM, mm²), P>0,05

nificant change can be obtained from the column diagram between total, normal and pathologic neurons. In Figure 4., numeric density (Na) of normal and pathologic neurons in vagal nuclei in medulla oblongata, at violent asphyxic deaths from different cause is presented. From obtained data, it is noticed that there is no statistical important difference of normal and pathologic neurons in vagal nuclei in medulla oblongata, at asphyxias with different etiologic factors ($p>0,05$).

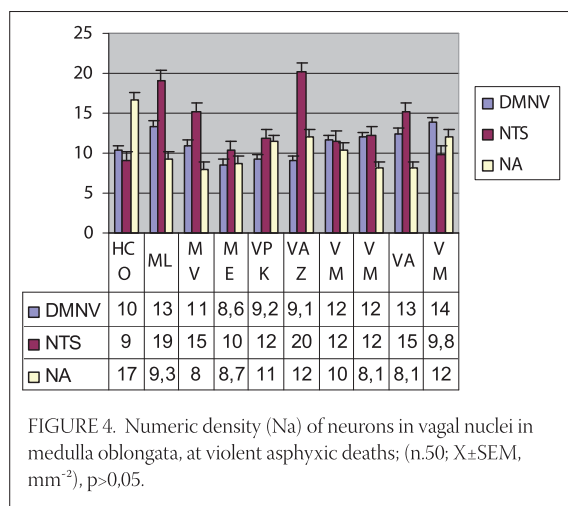


FIGURE 4. Numeric density (Na) of neurons in vagal nuclei in medulla oblongata, at violent asphyxic deaths; (n.50; X±SEM, mm⁻²), $p>0,05$.

In Figure 5., numeric density (Nn) of normal, pathologic, and total neurons in dorsal vagal nucleus in violent asphyxic deaths are presented. Following the statistical calculations, the existence of emphatic significance in terms of prominence of neurons with normal appearance in nervus vagus nuclei is noticed (total Vs normal $p<0,001$; total Vs pathologic $p<0,001$).

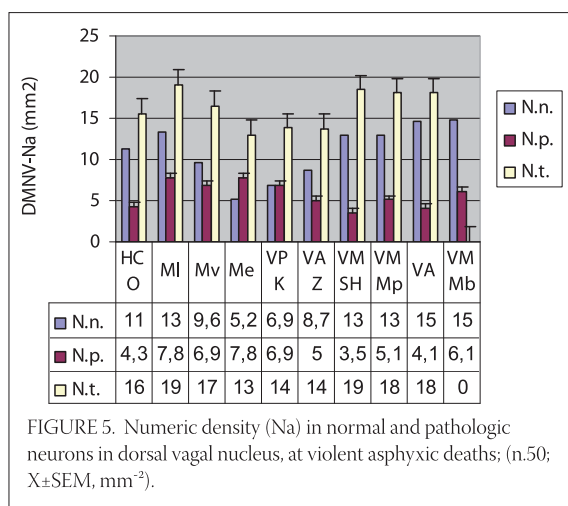


FIGURE 5. Numeric density (Na) in normal and pathologic neurons in dorsal vagal nucleus, at violent asphyxic deaths; (n.50; X±SEM, mm⁻²).

In Figure 6, numeric density (Na) of normal, pathologic, and total neurons in nucleus tractus solitarius, in violent asphyxic deaths with different etiology are presented. From obtained statistical calculations, no statistical important significance of pathologic neurons in nuclei of nTS is noticed (total Vs normal $p<0,001$; total Vs pathologic $p<0,001$; normal Vs pathologic $p>0,05$).

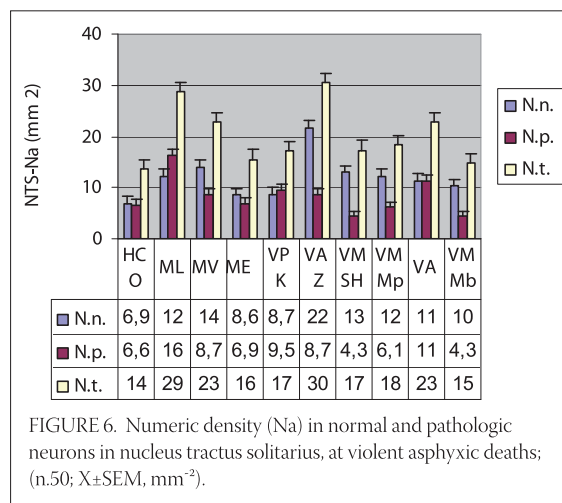


FIGURE 6. Numeric density (Na) in normal and pathologic neurons in nucleus tractus solitarius, at violent asphyxic deaths; (n.50; X±SEM, mm⁻²).

In Figure 7, numeric density (Na) of total, normal, and morphologically altered neurons in the ventral vagal nucleus (nucleus ambiguus), at violent asphyxic deaths with different etiology factors. Following the statistical calculations in between different groups of asphyxias, it is noticed that pathologic neurons takes no important statistical place (total Vs normal $p<0,001$; total Vs pathologic $p<0,001$; normal Vs pathologic $p>0,05$).

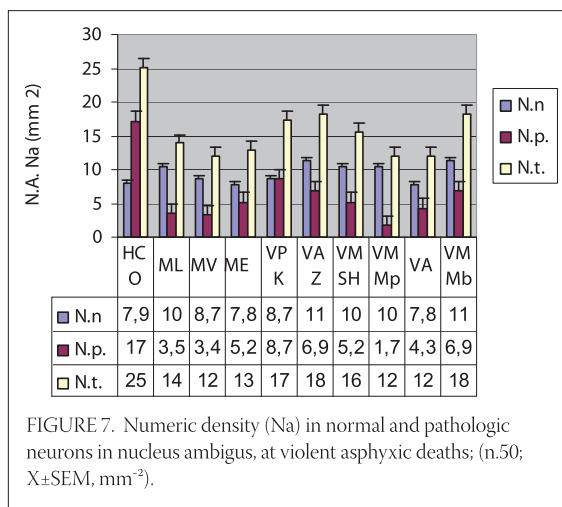


FIGURE 7. Numeric density (Na) in normal and pathologic neurons in nucleus ambiguus, at violent asphyxic deaths; (n.50; X±SEM, mm⁻²).

DISCUSSION

“*In vitro*” studies of preparations enables to examine cell and sub-cell levels of the respiratory control. During the severe hypoxia and anoxia, progressive changes in respiration appear, from hyperpnea to respiration break, with a period of expiratory apnea followed by a wheeze (dispnea). Respiratory dispnea is characterized with a series of shorter eruption of frenic activity with immediate beginning followed by a rapid fall and the lack of expiratory activity (10-14). At intoxication with carbon monoxide (ICM) cases, brain swelling, condensation of Nissl substance, melting of Nissl substance, central chromatolysis, and

proliferation of oligodendroglial cells are emphatic in DMNV nucleus. In nTS, larger scale of altering changes is evident, starting from condensation and melting of Nissl substance, central chromatolysis, axonal retraction and fragmentation, and proliferation of oligodendroglial, astroglial and microglial cells. Intensity of abovementioned changes is lower in nA nucleus.

At lasso strangulation, higher scale of brain swelling, condensation and melting of Nissl substance, central chromatolysis, and proliferation of oligodendrocytes in nTS are evident in comparison to neurons of DMNV and nA nuclei. At deaths from hanging, mid scale of condensation and chromatolysis of Nissl substance, central chromatolysis, axonal retraction and proliferation of oligodendrocytes in nTS neurons are noticed in comparison to neurons of DMNV and nA nuclei. At deaths due to electricity, emphatic changes of brain swelling, condensation and melting of Nissl substance, central chromatolysis, axonal fragmentation, proliferation and hyperplasia of oligodendrocytes are noticed. At clinical – pathological deaths, morphologic altering changes are evident in all of the respiratory nuclei, commencing with brain swelling, condensation and melting of Nissl substance, central chromatolysis, axonal retraction and fragmentation, and degenerative vacuolar, intranuclear and intra-

cytoplasmic changes. Glial reaction is also emphatic. At homicide with hand firing weapons, average altering lesions are noticed in the population of neurons in nTS, in the form of brain swelling, central chromatolysis, and proliferation of oligodendroglial cells. Similar morphologic altering changes are seen at cases of homicide with explosive devices, homicide with sharp devices, accidental deaths and deaths with blunt devices.

Mechanisms of brain parenchyma regeneration are activated by lesions of brain protective mechanisms but some of them moves to the direction of nerve cell necrosis or apoptosis. Many studies regarding the role of glial cells in the prevention of neuronal death and role of the perivascular space in the hemostasis of traumatized brain are done. Reactive hyperplasia and astrocyte hypertrophy are concomitant phenomenon in central nervous system at the traumatic tissue destruction. *Hematoencephalic barrier* is open at venules for influx of cells and blood elements, mainly for mononuclear phagocytes. At asphyxic deaths, no important statistical significance was gained between population of normal and pathologic neurons in vagal nuclei of DMNV, nTS and nA ($p < 0,05$). Total number of neurons in DMNV, nTS and nA nuclei, with pathologic altering changes, does not show significant statistical changes between total, normal and pathologic neurons.

CONCLUSION

Following the overall histochemical and stereometric analysis of neuronal populations in nervus vagus nuclei in medulla oblongata at violent asphyxic deaths, we can conclude as follows:

1. At intoxication with carbon monoxide (ICM) cases, brain swelling, condensation of Nissl substance, melting of Nissl substance, central chromatolysis, and proliferation of oligodendroglial cells are emphatic in DMNV nucleus. In nTS, larger scale of altering changes is evident, starting from condensation and melting of Nissl substance, central chromatolysis, axonal retraction and fragmentation, and proliferation of oligodendroglial, astroglial and microglial cells. Intensity of abovementioned changes is lower in nA nucleus.
2. At lasso strangulation, higher scale of brain swelling, condensation and melting of Nissl substance, central chromatolysis, and proliferation of oligodendrocytes in nTS are evident in comparison to neurons of DMNV and nA nuclei.
3. At deaths from hanging, mid scale of condensation and chromatolysis of Nissl substance, central chromatolysis, axonal retraction and proliferation of oligodendrocytes in nTS neurons are noticed in comparison to neurons of DMNV and nA nuclei.
4. At deaths due to electricity, emphatic changes of brain swelling, condensation and melting of Nissl substance, central chromatolysis, axonal fragmentation, proliferation and hyperplasia of oligodendrocytes are noticed.

5. At clinical – pathological deaths, morphologic altering changes are evident in all of the respiratory nuclei, commencing with brain swelling, condensation and melting of Nissl substance, central chromatolysis, axonal retraction and fragmentation, and degenerative vacuolar, intranuclear and intracytoplasmic changes. Glial reaction is also emphatic.
6. At homicide with hand firing weapons, average altering lesions are noticed in the population of neurons in nTS, in the form of brain swelling, central chromatolysis, and proliferation of oligodendroglial cells. Similar morphologic altering changes are seen at cases of homicide with explosive devices, homicide with sharp devices, accidental deaths and deaths with blunt devices.
7. Total number of neurons in DMNV, nTS and nA nuclei, with pathologic altering changes, does not show significant statistical changes between total, normal and pathologic neurons.
8. From obtained data, it is noticed that there is no statistical important difference of normal and pathologic neurons at asphyxias with different etiologic factors ($p > 0,05$).
9. Numeric density (Nn) of normal, pathologic, and total neurons in dorsal vagal nuclei in violent asphyxic deaths shows the existence of emphatic significance in terms of prominence of neurons with normal appearance in nervus vagus nuclei (total Vs normal $p < 0,001$; total Vs pathologic $p < 0,001$).
10. Numeric density (Na) of normal, pathologic, and total neurons in nucleus tractus solitarius, in violent asphyxic deaths with different etiology shows an important significance of pathologic neurons in nuclei of nTS (total Vs normal $p < 0,001$; total Vs pathologic $p < 0,001$; normal Vs pathologic $p > 0,05$).
11. Numeric density (Na) of total, normal, and morphologically altered neurons in the ventral vagal nucleus (nucleus ambiguus), at violent asphyxic deaths with different etiology factors shows that between different groups of asphyxias, it is noticed that pathologic neurons takes no important statistical place (total Vs normal $p < 0,001$; total Vs pathologic $p < 0,001$; normal Vs pathologic $p > 0,05$).

List of Abbreviations

DMRC	-	Dorsal motor respiratory center
nTS	-	Nucleus tractus solitarius
DMNV	-	Dorsal motor nervus vagus
nA	-	Nucleus ambiguus
ICM	-	Intoxication with carbon monoxide
LD	-	Liquid drowning
DH	-	Deaths from hanging – lasso strangulation
DE	-	Deaths from electricity
CPD	-	Clinical – pathological deaths
FWD	-	Firing weapon deaths

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