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Original Article

Claustral neurons are vulnerable to ischemic insults in cardiac arrest encephalopathy

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Abstract: Cardiac arrest encephalopathy is the major cause of global brain ischemia which is the main cause of death in these cases. Morphological assessment of the ischemic changes is important to prove the extent of the brain injury and its effects. Selective vulnerability is the common form of ischemic injury in these cases and commonly affects the CA1 of hippocampus, cerebral cortex in watershed zone and Purkinje cells of the cerebellum. In this retrospective study, we reviewed the clinical and pathological data for the 135 retrieved cases and only 16/135 cases met the selection criteria (i.e. confirmed cardiac arrest episode with known survival time, autopsy performed in less than 24 hours after death, compatible gray matter ischemic changes with the survival period, and no pathological changes of other diseases that could confound the result). We found that the claustrum is the most sensitive area for ischemic changes. This represents a novel finding, as up to our knowledge, the neurons of the claustrum have not been considered before as common area to show selective vulnerability. In the 3 cases with a short survival (6-9 hours), the hippocampus showed no or mild ischemic changes and as such was not a good area for assessment of global ischemia. Another unexplained novel finding was prominent vacuolar changes predominantly around both sides of the gray-white matter junction of the cerebellar dentate nucleus and focally inside the dentate nucleus.

Keywords: Cardiac arrest, hypoxic-ischemic encephalopathy, claustrum, global brain ischemia, dentate nucleus

Introduction

Cardiac arrest encephalopathy is the major cause of global brain ischemia, which is the main cause of death in these cases, as it counts for 67.7% of the out-of-hospital and 22.9% of the in-hospital cardiac arrests [1]. There are many variable factors that should be thought of before any conclusion about the effect of cardiac arrest on the brain, which include: duration of the cardiac arrest, temperature, pH, and glucose levels [2]. As such, morphological detection of ischemic changes cannot be overemphasized to confirm the extent of the brain injury as a consequence to cardiac arrest.

There are variable forms of morphological changes post cardiac arrest which could be classified to selective neuronal necrosis and pancellular necrosis [3]. Areas of selective vulnerability to global brain ischemia include the hippocampus, the cerebral cortex, and the cerebellar Purkinje cells. Delayed neuronal death that is preferentially apparent in the hippocampal neurons [4], the wide possible area for the watershed zone, and the possible deceptive nature of scattered neuronal necrosis may affect the assessment of the ischemic neuronal changes in some cases. In this work, we compare the sensitivity or the tolerance of variable gray matter structures to global ischemia as a consequence of cardiac arrest.

Materials and methods

This retrospective study was approved by the University of Western Ontario's research ethics board. Formal hospital autopsy consents were obtained for all cases prior to autopsy.

In this work, we used the same data set used before to study the nature of white matter pathology post cardiac arrest [5]; however, our interest in this work is the vulnerability of differ-

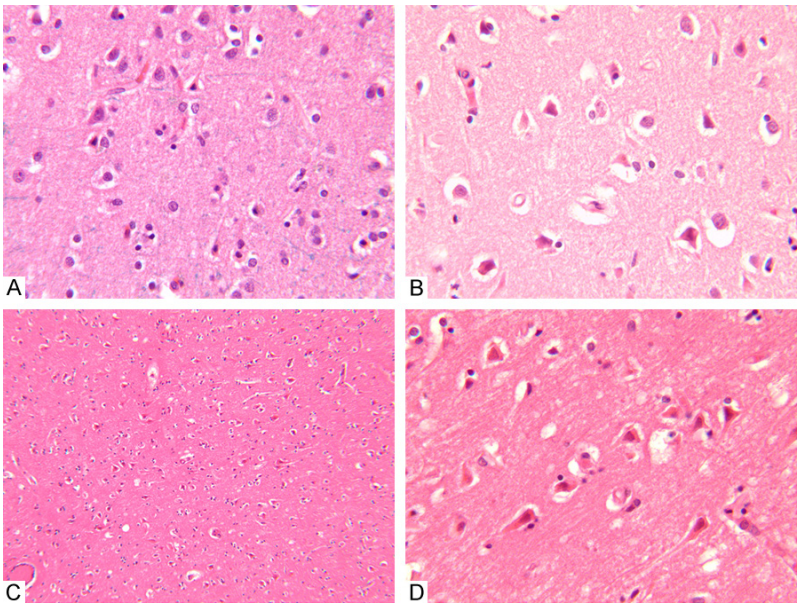

Figure 1. The scoring system of the severity of ischemic neuronal changes. A: Scattered ischemic neurons affecting less than 50% of all neurons in the examined area is considered mild and assigned score 1. B: Ischemic neurons affecting 50-90% of all neurons in examined area is considered moderate and assigned score 2. C, D: Ischemic neurons affecting more than 90% of all neurons in examined area is considered severe and assigned score 3. H & E, original magnification: (A: 400×), (B: 400×), (C: 100×), (D: 400×).

ent gray matter structures to global ischemia in cardiac arrest. These cases were retrieved from London Health Sciences Center's archives for the period of 12 years starting from 2002 that included a diagnosis of cardiac arrest or hypoxic ischemic encephalopathy.

We reviewed the clinical and pathological data for the 135 retrieved cases. Only 16/135 cases met our selection criteria, which were: confirmed cardiac arrest episode with known survival time, autopsy performed less than a day post death, compatible gray matter ischemic changes with the survival period, and no pathological changes from other diseases that could confound the result.

The brain fixation (in 4% formaldehyde for 10-14 days) and examination (coronal sections for cerebral hemispheres, sagittally/parasagittally sectioned for the cerebellar hemispheres and horizontal for the brainstem) were performed similar to other routine cases. The routine brain sampling for these kind of cases included: cortex and underlying white matter from frontal (middle frontal including watershed zone between anterior and middle cerebral artery), temporal (middle and superior), and occipital lobes (calcarine area), hippocampus at the coronal level of the lateral geniculate nucleus, basal ganglia and claustrum near the level of anterior commissure, thalamus at the level of mammillary bodies, midbrain from brainstem, cerebral cortex and another section from the cerebellar dentate nucleus, and additional sections for any gross abnormal areas. The part of the claustrum that was examined in all sampled cases was in the coronal slices of the basal ganglia at the coronal where the putamen and the 2 parts of the Globus pallidus could be visualized.

In this part of the study we only used slides stained with hematoxylin and eosin and luxol fast blue with hematoxylin and eosin (LFB-HE). The degree of ischemic neurons in different gray matter areas (cortex, claustrum, lentiform nucleus, thalamus, CA1 of the hippocampus, brainstem, Purkinje cells and dentate nucleus of the cerebellum) was assigned 4 scores according to the severest focus of the involved gray matter structure: No ischemic neurons or unaffected area was assigned score 0. Scattered ischemic neurons (less than 50%) of all neurons in affected area was considered mild and assigned score 1 (**Figure 1A**). Ischemic neurons affecting 50-90% of all neurons in the examined focus was considered moderate and assigned score 2 (**Figure 1B**). Ischemic neurons affecting more than 90% of all neurons in examined focus was considered severe and assigned score 3 (**Figure 1C**, **1D**).

Results

The degree of ischemic neurons for each case in different gray matter areas is summarized in (**Table 1**) for the 16 included cases. These cases were distributed among both sexes: 9 males and 7 females. The ages at time of death ranged from 39-79 years, and the survival duration after cardiac arrest ranged from 6 hours to

Table 1. Clinical history and distribution of ischemic neurons in gray matter structures

N.	A/S	S	Clinical history preceding cardiac arrest	Cr	Cl	LN	Th	CA1	BS	PC	D
1	52 M	6 h	Acute myocardial infarction (MI)	1	3	2	NS	1	0	0	NS
2	65 M	7 h	Upper gastro-intestinal bleeding	2	2	2	NS	1	0	1	1 & PV
3	61 M	9 h	One week history of dyspnea, cough and unsteadiness	2	2	2	1	0	1	2	2 & PV
4	44 M	14 H	Hypokalemia secondary to tumor lysis syndrome (previous history of melanoma).	3	NS	NS	3	3	1	2	2 & PV
5	39 F	2 D	Acute chest infection	3	3	2	3	2	0	3	3 & PV
6	65 M	2 D	Acute MI	2	NS	NS	1	2	1	2	0 & PV
7	67 M	3 D	Previous history of MI, DM2, and COPD	2	2	2	3	2	2	2	2 & PV
8	76 F	3 D	Pneumonia.	3	3	3	NS	3	2	2	2 & PV
9	41 F	3 D	Previous history of recurrent supraglottitis	3	3	3	3	3	0	3	2 & PV
10	62 M	4 D	Acute MI	2	3	3	NS	3	1	2	1 & PV
11	57 F	4 D	Recent history of liver failure	1	2	1	2	2	1	3	2 & PV
12	72 F	4 D	Cardiac arrest at home	1	3	1	3	1	0	0	UR
13	52 F	5 D	Shortness of breath and fever. Past medial history (PMH) indicated epilepsy and depression for 2 years.	3	3	3	2	3	0	2	1 & PV
14	59 F	6 D	Acute MI. PMH: COPD, Congestive HF, and epilepsy	3	2	2	2	3	1	3	0 & PV
15	79 M	9 D	Post right hip revision surgery	3	3	3	2	3	1	2	2 & PV
16	68 M	14 D	Five days post cholecystectomy	3	3	3	3	3	1	NS	NS

A/S: age/sex, BS: brainstem, CA1: Cornu ammonis 1 of hippocampus, Cr: cortex, Cl: claustrum, D: dentate nucleus, LN: lentiform nucleus, N.: case number, NS: not sampled, PC: Purkinje cells, PV: peridentate vacuoles, S: survival period after the arrest, Th: thalamus, UR: unremarkable, V: vacuoles, Score 0: no pathological change, 1: mild, 2: moderate, 3: severe.

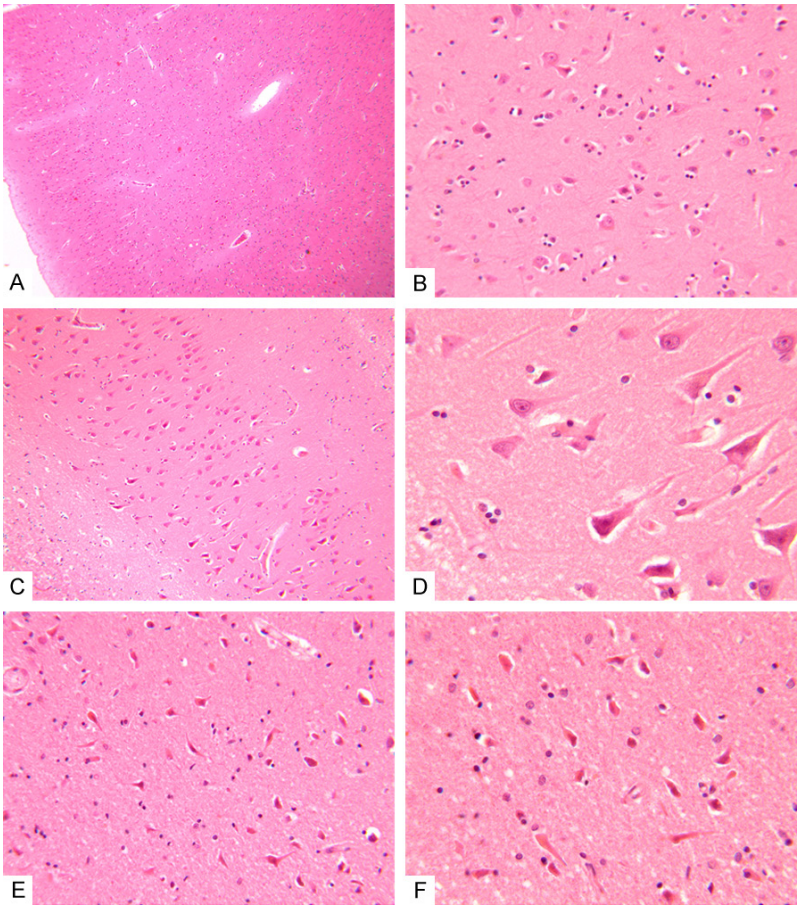

Figure 2. The degree of ischemic neurons in different areas in Case 1. A, B: Mild (score 1) ischemic changes in the most severe affected cortex. C, D: The hippocampus shows only scattered focal "shrunken" neurons. These shrunken neurons represent a very early stage of "ischemic" neurons (mild, score 1). E, F: Severe (score 3) ischemic changes in the claustrum. H & E, original magnification: (A: 40×), (B: 250×), (C: 100×), (D: 400×), (E: 250×), (F: 400×).

14 days. No regaining of consciousness after cardiac arrest episode was observed in any of the cases.

Claustrum was the most sensitive area to global ischemic injury out of all the gray matter areas, and in most of the cases it showed the most severe ischemic changes. Lentiform nuclei tended to show comparable sensitivity but in general less prominently than the claustrum. The most severe cortical area in the watershed zones did show comparable sensitivity as well. The CA1 of the hippocampus showed comparable sensitivity only in cases with a 14-hour or more survival duration, while it was inferior in cases with a 6 to 9-hour survival duration (3 cases). For example, in the first case with the shortest survival (6 hours), the changes was only mild (score 1) in the cortex (**Figure 2A**, **2B**) and hippocampus (**Figure 2C**, **2D**), and severe (score 3) in the claustrum (**Figure 2E**, **2F**). The thalamic nuclei and Purkinje cells of the cerebellum were also a sensitive area for neuronal ischemic changes but less prominently than the claustrum. The sampled brainstem was the least sensitive area for neuronal ischemic changes. Multifocal areas of complete infarcts were observed in a few cases only.

Neurons of the dentate nucleus showed moderate sensitivity for ischemic changes, but a peculiar change was observed in almost all the sampled cases except for case 12. This change involved vacuolar changes predominantly in the gray-white junction of the dentate nucleus from both sides (**Figure 3A**), hence we call it a peri-dentate vacuolar change. These vacuoles were not associated with ischemic changes in the dentate nucleus (**Figure 3B**, **3C**) in 2 cases but were associated with ischemic neuronal changes (**Figure 3D-F**) in 11 cases. The vacuoles were large and similar in size to the adjacent neurons and were empty most of the time but can be associated with traversing axons. Even in cases associated with ischemic neurons, the ischemic areas did not show significant edematous changes. We do not have a conclusion about the nature of these vacuoles.

Discussion

We found that the claustrum is the most vulnerable gray matter structure for neuronal ischemic changes in cardiac arrest/global ischemia. Selective neuronal changes were most apparent in the claustrum even in cases with a 6-hour survival. This was much superior to the Cornu Ammonis 1 (CA1) area of the hippocam-

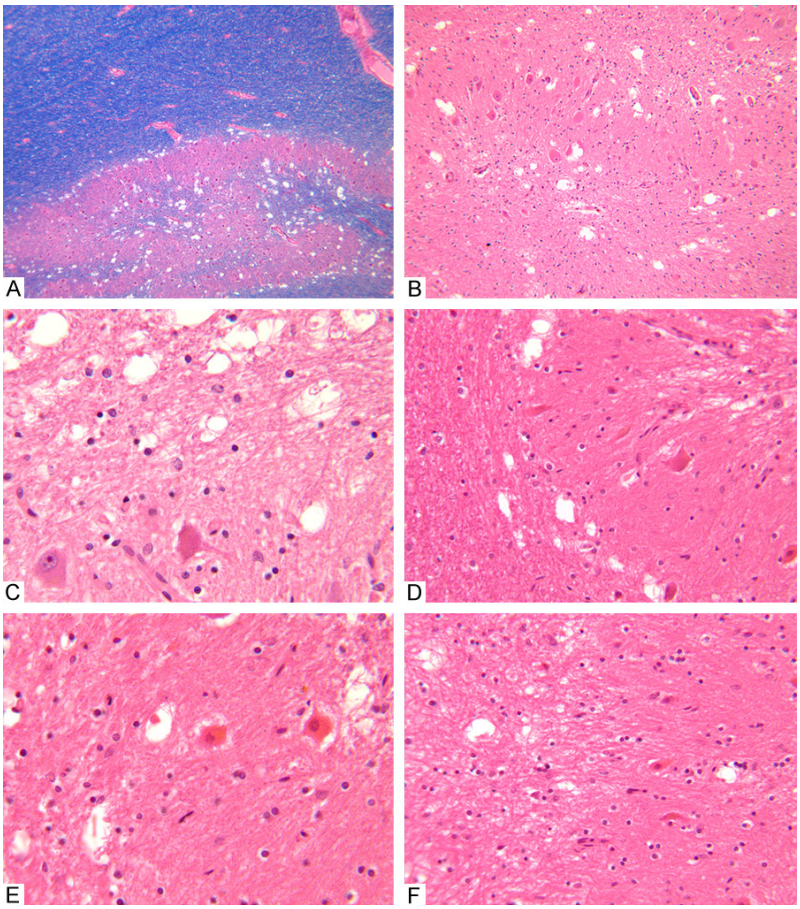

Figure 3. Cerebellar peri-dentate vacuoles. A: Prominent vacuoles in the junction between the gray and white matter in both sides of the cerebellar dentate nucleus. Case 14 (LFB-HE original magnification: 40×). B, C: These vacuoles are large (similar in size to adjacent neurons but unrelated to them) with regular smooth edges. No ischemic changes in the neurons, and no edematous changes in the background. Case 14, H & E, original magnification: (B: 100×), (C: 400×). D: Similar vacuoles but associated with ischemic neuronal changes in the dentate nucleus. Case 5 (H & E, original magnification: 250×). E, F: The larger vacuoles are associated with fine vacuoles in the background, indicating mild edematous changes in the background most likely secondary to ischemia. Case 5, HE original magnification: (E: 400×) & (F: 250×).

pus where ischemic neuronal changes were not observed or only showed mild ischemic changes in the 3 cases with a 6 to 9-hour survival. This finding is in agreement with previous observations in cardiac arrest cases with less than 18 hours survival, where the hippocampus showed minimal changes while the cerebral cortex and the putamen showed moderate ischemic changes [6]. Other examined gray matter structures showed variable neuronal ischemic changes but were similar to what was predicted from previous studies. The cerebral cortex in the watershed zone, the Purkinje cells of the cerebellum, and lentiform and thalamic nuclei were prominently affected, while the brainstem was the least affected.

Cardiac arrest is a prototypical cause of global brain ischemia. In this form of ischemia, selective vulnerability phenomenon become apparent as the ischemic changes will first become apparent in the areas most sensitive to global ischemia [7]. The vulnerable areas in this global ischemia are the hippocampus, the cerebral cortex, the striatum and the Purkinje cells of the cerebellum [2, 8]. Additional studies also showed other important areas like the thalamus, and the cerebellar vermi [9]. This is reflected in common neuropathological practice, as the most commonly examined areas for global ischemia are the CA1 of the hippocampus, the Purkinje cell layer and Layers 3 and 5 of the cerebral cortex, especially in water-shed zones. In the basal ganglia, the striatum is more commonly affected (more sensitive) than the Globus pallidus [2], while the claustrum was not specifically mentioned. This may indicate that the claustrum was not specifically studied before.

Selective neuronal death in vulnerable areas represents one of the two forms of ischemic changes in gray matter resulting from cardiac arrest [3]. The other form of ischemia that could happen in cardiac arrest is pancellular necrosis with the involvement of all types of cells in the affected area and usually associated with spongiotic changes in the neuropil. This form of injury is considered a secondary event due to microvascular dysfunction which may lead to diffuse microthrombi and result in multi focal infarcts [10]. Another source of emboli could be small fat emboli complicating rib fractures as result of the act of cardiorespiratory resuscitation. Other forms of secondary injury may result

from the complicated process and various vascular changes that can occur with reperfusion [11].

The other important finding is the presence of predominant vacuoles in the gray-white matter junction of the cerebellar dentate nucleus on both sides. The vacuoles were empty most of the time but occasionally contain transversed axons. These vacuoles were present in all sampled cases (14 cases) except case 12 which also did not show ischemic changes in the dentate nucleus and not even in the Purkinje cells, which may indicate that the blood supply to the cerebellum was not reduced enough to cause ischemic changes. In the remaining 14 cases where the vacuoles were present, only 2 cases were not associated with ischemic neurons in the dentate nucleus. Most of the vacuoles had regular edges, and their sizes were similar to the size of the adjacent neurons. The background in some cases showed accompanying small vacuoles with separation of myelinated axons indicating mild edematous changes. However these edematous changes were not present in the other cases. There is no significant expansion of perivascular spaces and only a few ischemic neurons had a slight expansion of the perineuronal space. The adjacent white matter away from the gray-white matter junction did not show similar findings, and as such it is unlikely to represent an artifact or an autolytic change. The pathogenesis of these vacuolar changes remains enigmatic, and it seems further study with ultrastructural examination is indicated to better understand the nature of these vacuoles. We are not aware of any similar cerebellar description of these vacuoles in the literature.

Global brain ischemia is an important contributor if not the main cause of death in cardiac arrest [1] and the morphological documentation of ischemic changes in these cases cannot be overemphasized. The common sampled areas were less sensitive for ischemic changes, and they have their pitfalls in morphological assessment. Cortical areas at the watershed zone showed comparable changes to the claustrum, although slightly less sensitive. However, the exact watershed zone is usually difficult to localize during brain cutting and is slightly different between individuals. The deceptive nature of scattered ischemic neurons may be overlooked in routine cases.

The other common area to sample is the CA1 of the hippocampus. This small area could be easily localized and sampled. However, in our study and in agreement with previous studies [6], ischemic changes may not be present in cases with short survival. This has been attributed to a “maturation” or delayed neuronal death phenomenon, as hippocampal neurons may show delayed neuronal death [2, 8]. This phenomenon is not unique to the hippocampus and it has been demonstrated, in a trivial form, in Purkinje cells of the cerebellum but not in the cerebral cortex [4]. All other known vulnerable areas did show ischemic changes but were less sensitivity than the claustrum. As such, we concluded that the claustrum is the ideal area to look for the selective neuronal changes which support the occurrence of ischemic brain injury as the result of global brain ischemia in cardiac arrest. This conclusion is based on the low threshold for ischemic changes of the claustral neurons, and the small area that has to be examined.

The claustrum is a small sheet-like nucleus beneath the insular cortex that runs longitudinally (parasagitally). In coronal sections (a common practice in brain cutting) it appears as a thin area between the external and extreme capsules. Although it is a small gray matter structure, it has complex reciprocal connections to almost all areas of the brain, so it has a wide range of functions especially in regulation of consciousness [12]. It is difficult to know with certainty the effects of selective neuronal loss in the claustrum, as no previous studies have dealt with this pattern of injury on the claustrum selectively. All subjects in this cohort did not regain consciousness after cardiac arrest. It is unclear whether claustral involvement in all included cases could have an impact on the level of consciousness, or if there was selection bias in this cohort, as it only included cardiac arrest cases in which the patient did not regain consciousness and thus had prominent claustral involvement. Further study comparing another set of cases in which the patients had regained consciousness after cardiac arrest is necessary to answer this question.

The major limitation of this study is its retrospective nature as we limited it to the previously routinely sampled areas, and in few cases some areas were not sampled. This limitation made it not possible to study other areas not

routinely sampled for ischemic changes. However, our conclusion about the claustrum and the peri-dentate vacuolar changes in ischemia should not be affected by these limitations.

In conclusion, we found that the claustrum is one of the most sensitive areas for selective neuronal ischemic changes and as such represents the best area to sample in order to confirm global brain ischemia in cases with at least a 6-hour survival. Another unexplained novel finding was the prominent vacuolar changes predominantly around both sides of the cerebellar dentate nucleus and focally inside the dentate nucleus. More researches with prospective design would be necessary to confirm these conclusions and to illustrate the nature of these vacuoles in the peri-dentate areas.

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Disclosure of conflict of interest

None.

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