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Suicidal ligature strangulation without an auxiliary mechanism. Reports of two cases with a cotton rope or a T-shirt and staining results of the brains using anti HSP-70, CIRBP, RBM3, HIF1-α, SIRT 1 and p53 antibodies

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Abstract: It is still often described in forensic textbooks that suicidal strangulation by ligature without auxiliary device to remain tightening the ligature after unconsciousness of the victim. We report two cases of suicidal ligature strangulation without auxiliary mechanism. One occurred in a closed room and other was carried out in a police cell. These two cases reveal that the suicidal ligature strangulation is possible without auxiliary mechanism, however it was difficult to distinguish from homicide only from forensic autopsy findings, although death scenes and existence of suicidal notes produced clear verdicts of suicide in two cases. The death mechanism in these cases was discussed. The nucleus of the neuronal cells showed good reactivity with hypoxia or ischemia related antibodies, such as cold inducible RNA-binding protein (CIRBP), RNA-binding motif protein 3(RBM3), heat shock protein 70(HSP-70) and hypoxia-inducible factor 1(HIF-1), however the cells showed no reactivity with anti Sirtuin 1(SIRT 1) and p53 antibodies which are related energy metabolism or apoptosis. This is the first report in which the expression of hypoxia and /or ischemic related antigens described above was confirmed even in post-mortem human brains.

Key Words: suicidal ligature strangulation, death mechanism, Hypoxia related antigens.

The majority of strangulation cases are homicides. Suicidal ligature strangulations are rare, since we encountered only several cases in last twenty years. In our experience suicidal strangulations were usually performed utilizing an elastic band, an elastic bandage, a cable tie, and/or a rubber tube. Some authors and criminal investigators seem to continue to believe that suicidal self-strangulation without a device for keeping the compression of one's neck after one's death, is not possible and that strangulation must therefore represent homicide [1].

In this presentation we show two suicidal ligature strangulation cases in which no auxiliary mechanism was used to prevent the loosing of tension by the ligatures after losing their consciousness. One case occurred in a closed room of housing for prisons workers and another occurred in a police cell. We reported the results obtained from histochemical staining of the cells in the basal ganglia and the hypothalamus, using hypoxia, ischemia or apoptotic related antibodies, such as anti CIRBP, RMB3, HSP-70, HIF-1α, SIRT 1 and p53 antibodies.

CASE REPORTS

Case 1

Case history

A middle aged-man with worries about changing of working position before 3 months of his death. He formerly worked as a prison guard and was changed as a clerical employee for accounts. His death was found by

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his superior officers in a room of his residence, because of his absence from office. The room was locked and secured and the superior officers gained entry using a master key and cutting door-chain. His superior officer found that the decedent fall prone on a tatami, Japanese floor mattress. He covered his neck with a thin towel and wound a cotton rope two times on his neck. The rope was crossed on his frontal neck using overhand knot (Figure 1A). The suicide note to his family was remained in his mobile phone. A small amount of nosebleed was remained on the mattress.

Autopsy findings

Postmortem lividity was recognized on his back, showing his death was found in three or four hours after his death. There were two ligature marks with 0.5cm width and tiny bullas between two traces on his neck were detected on the skin. The upper side ligature mark was located at 10.5 cm below from right side earlobe, 10 cm from left side earlobe and at 10cm below from the chin. The severe congestion over the ligature mark (Figure 1B), and small blisters were observed between two ligature marks (Figure 1C). A large number of petechiea in the conjunctiva and mouth mucosa was also recognized. Tow pale lines on the palm of the both hands were detected, seeming to be due to the compression by the rope when he strangled his neck with the rope.

As internal findings, there were layers bleeding in the tongue (Figure 1D), severe congestion with speck bleeding in pharynx-larynx and no fracture in the thyroid cartilage and the hyoid bone. The severe pulmonary edema was observed. The internal organs including the brain showed severe congestion and no bleeding was microscopically detected in the brain which changed slightly softening. The morphology of the Willis circle was usual but the posterior communicating arteries were slightly narrow in both sides. The location of bifurcation of both carotid arteries was at level of third cervical vertebrae (C3 level) that was usually in Japanese [2].

No alcohol and no drugs in the blood were detected with GC-Mass.

Case 2

Case history

A man who was in his mid-thirties has been taken into custody of the police for examination of fraudulent practices for 1 week. One early morning a prison guard found his death in his bedding in a prone position. He was covered from head to foot with a futon, Japanese quilt. He wound a sleeve of his T-shirt around his neck and crossed the shirt on his nucha making overhand knot (Figure 2A). A small amount of nosebleed was remained on the futon, Japanese mattress. The suicide note was found under his body. The left side sleeve of the T-shirt has gotten 6cm long than right side.

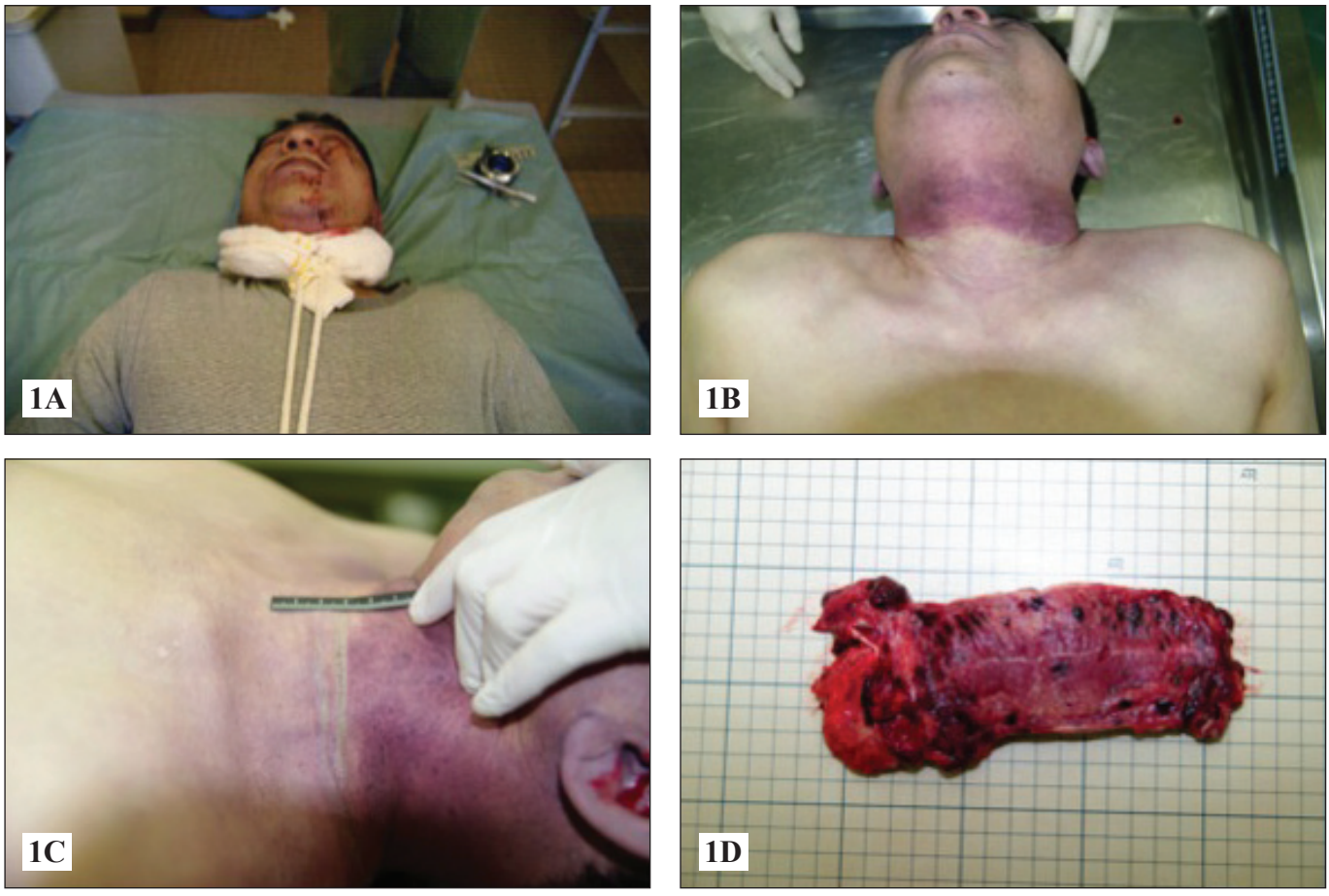

Figure 1. A victim died in his room with strangulation by a rope. **1A.** He covered his neck with a thin towel and wound a cotton rope two times on his neck. The rope was crossed on his frontal neck using overhand knot; **1B.** The severe congestion over the ligature mark; **1C.** Small blisters were observed between two ligature marks; **1D.** The layers bleeding in the tongue were observed.

Figure 2. A victim died in a police cell with strangulation by T-shirt. **2A.** He wound a sleeve of his T-shirt around his neck and crossed the shirt on his nucha making overhand knot; **2B.** The single pale ligature mark with about 3 cm width was recognized on his neck and the severe congestion was observed over the ligature mark; **2C.** A large number of petechiea in the conjunctiva was recognized; **2D.** Several spots bleeding were detected in the mucosa of the inside of the larynx.

Autopsy finding

He had a samurai tattooed, a Japanese warrior, on all over his back. Postmortem lividity was recognized on the face and upper chest, showing that he died before at least 10 hours of finding his body and after short hours of the last dinner. The single pale ligature mark with about 3 cm width was recognized on his neck (Figure 2B). The upper ligature margin was located at 7cm below from both side earlobe and at 6.5cm below from the chin. The severe congestion and petechia on his face were detected over the ligature mark. A large number of petechiea in the conjunctiva and mouth mucosa was also recognized (Figure 2C). There was no finding on his palms.

As internal findings, there were a spot bleeding in the tongue, two spots bleeding in the surface of the thyroid, several spots bleeding in the mucosa of the inside of the larynx (Figure 2D), and no fractures in the thyroid cartilage and the hyoid bone. The severe pulmonary edema was observed. The internal organs including the brain showed severe congestion and no bleeding was microscopically detected in the brain. The morphology of the Willis circle was usual but the posterior communicating arteries were slightly narrow in both sides. The location of bifurcation of both carotid arteries was at level of C3. No alcohol and no drugs in the blood were detected with GC-Mass.

HISTOCHEMICAL EXAMINATION

We tried immunohistochemical staining of the basal ganglia and the hypothalamus obtained from two suicidal cases presented in this report and additional two homicidal ligature strangulated victims. Antibodies against HSP-70 (purchased from Santa Cruz Biotechnology, USA), CIRBP (purchased from Proteintech, USA), RBM3 (purchased from Proteintech, US), HIF1-α (Santa Cruz Biotechnology, USA), SIRT1 (purchased from Novus Biologicals, USA) and p53 (purchased from Santa Cruz Biotechnology, USA) antigens were used under the indications from each company and SAB kit (Nichirei, Japan) technique. The folmalin fixed and paraffin embedded brain tissues were cut into 3 μm and immunohistochemically stained using antibodies described above and according to previous report by us [3].

We selected the basal ganglion and hypothalamus for histochemical study, since the hippocampus, which was usually selected in the study on brain asphyxia, was supported blood supply through the vertebral arteries in human. CIRBP and RBM3 were intensively expressed in the neural cells and/or astroglias in the basal ganglion and the hypothalamus, and HSP-70 was moderately but clearly expressed, and HIF-1 was weakly expressed in

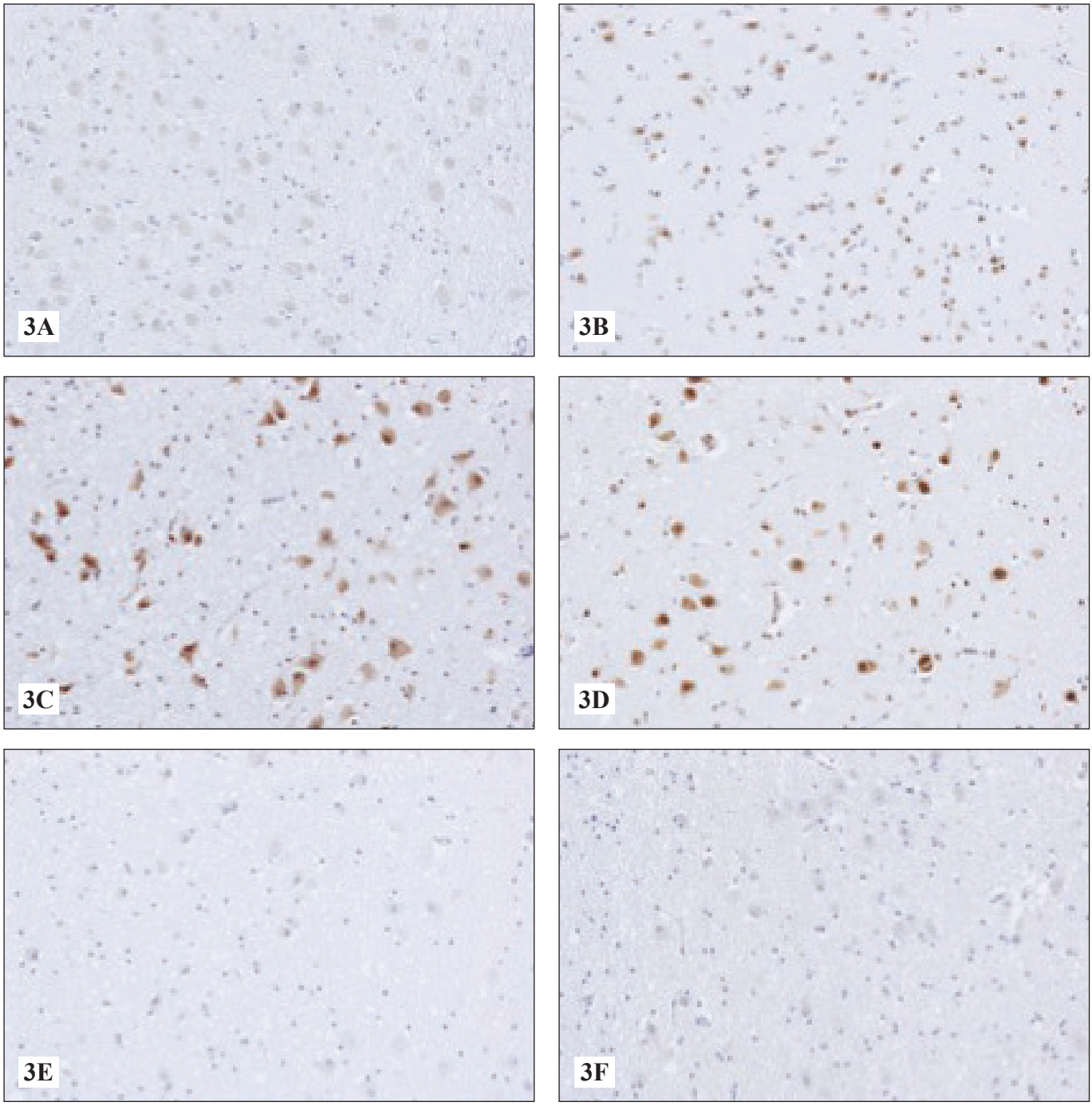

Figure 3. The staining results with antibodies. The brain tissues were obtained from the case 2. 200×magnification. **3A.** Anti HIF-1 antibody showed weak reactivity with the cytoplasm of the neurons; **3B.** Anti HSP-70 was moderately but clearly expressed in the cytoplasm and nucleus of the neurons; **3C.** Anti CIRBP were intensively expressed in the nucleus of the neural cells and astroglias; **3D.** Anti RBM3 were intensively expressed in the nucleus of the neural cells and astroglias; **3E.** AntiSIRT 1 did not show the reactivity with the neurons; **3F.** Anti p53 did not show the reactivity with the neurons.

these legions, however anti SIRT1 and p53 antibodies showed no reactivity with neural cells and astroglias, as shown in Figure 3.

DISCUSSION

The majority of strangulation cases are homicides. Suicidal self-strangulations are rare. Suicidal self-strangulation requires use of a ligature that can be locked in place by some mechanism [4]. Sorokin *et al.* [5] described that if the ligature is only tightened manually by the individual and there is no locking mechanism then the tension applied will be lost upon loss of consciousness with associated muscle relaxation, flowing by resumption of cerebral blood flow and the regaining individual consciousness. In cases of ligature strangulation, the importance of distinguishing self-inflicted death from homicide is crucial. This entails objective scene investigation, autopsy and anamnesis in order to elucidate the manner of death correctly [6].

In the first case the victim was found in a complete closed room with setting door chain. He covered his neck with thin towel and strangled his neck by a rope. This act indicted that he strangled his neck by

himself. His suicidal note for his wife was remained in his mobile phone, and in the second case the victim died in a police cell and his suicidal note was also found under his body. Since the ligature marks from both bodies were located under the position of bifurcations of them, their deaths were not due to carotid sinus reflex. There was no fracture in the thyroid cartilage and hyoid bone from two individuals. It is well known that pressure with over 14 kg, was necessary to obstruct the trachea, showing their death were not due to asphyxia [7].

When the jugular veins are blocked by the compression of the ligature with over 2 kg (about 147mm Hg that is a blood pressure value measured by a tonometer), which are enough to close the jugular veins by a tension in the rope, disturbing of the cerebral circulation and a rapid rising of venous pressure in the head are occur. Since a tension of 5 kg (about 368mm Hg) on ligature blocks carotid arteries, pressure with over 5 kg on the neck by compression of the ligature which results in stoppage of the cerebral circulation through carotid arteries and both internal and external jugular veins, and fall of blood flow and coma, although the blood supply is continued through vertebral arteries [8].

When two men in the present cases could keep the tensions of their ligature at 5 kg after losing their consciousness, two routes to the brain could be stopped and the tension with 2 kg could stop the blood flow to return to the heart and increase intracranial pressure. Shoening *et al.* described that the mean±SD values of flow volumes in the common, internal, and external carotid and vertebral arteries were 470±120, 265±62, 160±66, and 85±33ml/min on either side, respectively in healthy adults [9].

Normal cerebral blood flow (CBF) is approximately 50-to 60ml/100g/ min and varies in different parts of the brain. In response to ischemia, the cerebral autoregulatory mechanisms compensate for a reduction in CBF by local vasodilatation, opening the collaterals, and increasing the extraction of oxygen and glucose from the blood.

However, when the CBF is reduced to below 20 ml/100g/min, an electrical silence ensues and synaptic activity is greatly diminished in an attempt to preserve energy stores. The less than 10ml/100g/min of CBF causes brain ischemia and irreversible neuronal injury [10]. Buettner *et al.* reported that bilateral occlusion of the rat carotid arteries immediately caused a significant decrease of cerebral blood flow to ischemic values [11].

It seems in some individuals to be difficult to supply the above volume to the brain through vertebral arteries. Ischemia causes brain damage by activating the ischemic cascade, which progresses to local depletion of oxygen or glucose, causing failure of production of high-energy phosphate compounds, like adenosine tri-phosphate (ATP). This adversely affects energy-dependent processes necessary for tissue cell survival, and sets off a series of interrelated events culminating in cellular injury and death. The extent of damage usually depends on duration, severity, and location of ischemia. It has been observed that energy failure do not precipitate immediate cell death, but 5–10 min of occlusion may lead to irreversible brain injury [12].

ATP primarily provided by oxidative metabolism of glucose plays important role in the maintenance of normal neuronal activity in the brain. The compromise of oxygen and substrate supply as a result of cerebral blood flow reduction may cause neuronal damage within minutes to a few hours depending on the severity of ischemia [13]. Buettner *et al.* reported that the brain's response to ischemia is a dynamic but well-coordinated process that comprises distinct phases [11]. They obtained HSP-70 was a most strongly up-regulated gene within 15 min occlusion of bilateral carotid arteries and after at one hour reperfusion HSP-70 gene increased 69-fold compared with sham operation level. Although CIRBP and RBM3 were known as cold stress proteins, these were also up-regulated in response to hypoxia and the regulation did not require HIF-1 [14]. It was also described that SIRT1 was located in the nucleus and cytoplasma, and its expression was up-regulated during low energy states and SIRT 1 regulated expression of p53 that related apoptosis [15].

The staining results obtained in this study reveal that CIRBP and RBM3 were intensively expressed in nucleus of the neural cells and astroglias in the basal ganglion and the hypothalamus, and HSP-70 was moderately expressed, and HIF-1 was weakly expressed in the cytoplasm. However anti SIRT1 and p53 antibodies showed no reactivity in the brains obtained from ligature strangulation cases. The staining results may be influenced by the period of ischemia of the brain during strangulation and status of each gene regulation. Staining with antibodies may be useful to understand of patho-physiological mechanism and diagnosis of the brain hypoxia or ischemic status.

After experience of the two events we obtained an information from police and colleagues in our hospital concerning an attempted suicide case in which an old man strangled his neck by a rope, however he did not die and called an ambulance. His face showed severe congestion and small amount of bleeding from nose and ears was observed. Although not all attempter is able to die by self-strangulation, some of them can certainly die by self-strangulation.

CONCLUSIONS

The autopsy finding obtained from present cases reveals that the distinguish of suicidal ligature strangulation from homicide one is very difficult, since there was no remarkable finding concerning to specific and/or particular one related with suicidal ligature

strangulation, except the thin towel inside the rope and the traces on the palm of the hands due to the compression by the rope in the first case. Detailed examination of the scene and of the victims, along with investigation of the circumstances leading to the death is of high importance.

The cause of death of two men seemed to be the brain ischemia and energy consume due to obstruction of the carotid arteries and the internal and external jugular veins.

This is the first report in which the expression of hypoxia and /or ischemic related antigens described above was confirmed even in post-mortem human brains. Further examination is necessary, concerning to the expression of the genes related with ischemia and hypoxia in the brains.

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