H142 Cellular FLICE-Like Inhibitory Protein (C-FLIP) and Troponin-I: Promising Markers for the Determination of the Vitality in Suicidal Hangings

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Learning Overview: The goal of this presentation is the investigation of the potential use of C-FLIP (an intracellular protein involved in receptor-induced apoptosis) and Troponin I (fsTnI , fast skeletal muscle) in forensic practice to perform differential diagnosis between suicidal and simulated hangings.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by opening promising new horizons in forensic pathology, hopefully leading to further studies comparing these new molecules to classical immunohistochemical markers of vitality.

Differentiating between suicidal hanging and simulated hanging (suspension of the victim after murder) represents one of the most challenging problems for forensic pathologists. Beyond macroscopic findings, a diagnosis of suicidal hanging can only be carried out by investigating vitality signs of the ligature mark and underlying soft tissues. However, conventional macroscopic and histological findings may be unreliable; a great help in the determination of ligature marks' vitality can be provided by immunohistochemical methods. To date, immunohistochemical studies have shown good reliability with anti-myoglobin and anti-fibrinogen antibodies, as well as Anti-P-Selectin and anti-tryptase antibodies.

The expression of C-FLIP antibody and Troponin I, fast skeletal muscle (fsTnI) antibody in samples obtained from 21 subjects who died of hanging were examined in order to evaluate their potential use in forensic practice. C-FLIP acts as an apoptotic inhibitor, and its hyperexpression has proven to be related to human neoplasms. In ligation marks, compression-induced ischemia determines, via tissue hypoxia, under-regulation of C-FLIP and activation of caspase-8-related apoptotic paths. Troponin I, a muscle fast fiber-specific marker of skeletal muscle injury, is linked to the fact that the fast fibers have a lower concentration of ATP and, consequently, are more susceptible to ischemic damage due to constriction exerted by the noose.

Cases included 8 women and 13 men, mean age of 52.2 years, who died from suicidal hanging. The control group included six women and four men, mean age of 47.3 years, who died from opioid overdose ($n = 2$), car accident ($n = 3$) and sudden cardiac death ($n = 5$).

The skin samples of the neck for C-FLIP testing were taken diametrically opposite to the suspension point (where the maximum load occurs). As positive control for Anti-FLIP antibody (ab8421), neoplastic prostate and healthy kidney samples were selected. To perform negative controls, primary antibody was omitted and replaced with Phosphate-Buffered Saline (PBS). Slides were counter-stained with hematoxylin.

The analysis on fsTnI was carried out on sternocleidomastoid and infrahyoid muscles samples, at the same level as the skin samples. Positive testing for Anti-Troponin I antibody was carried out on a case of myocardial infarction.

All samples, after fixation in formalin, dehydration, and embedding in paraffin, were stained on an automated immunostainer, using polyclonal antibodies.

In all cases (21 out of 21) who died by hanging, a clear and evident intracytoplasmic depletion of C-FLIP was appreciated. No substantial differences were found in relation to type of hanging (complete or incomplete), knot position, or noose material. Only 19 out of 21 cases showed clear and evident intracytoplasmic depletion of Troponin I. The remaining cases showed lack of intensity reduction; this could either be due to noose material or to the fact that death occurred due to cardiac arrest, triggered by an inhibitory stimulation of neck's neurovascular bundles.

Results of this study suggest the possibility of discriminating between antemortem and postmortem hangings, especially when C-FLIP and fsTnI are tested in combination. The present study opens new possibilities in forensic pathology, hopefully leading to further studies comparing these new molecules to classical immunohistochemical markers of vitality.

Immunohistochemistry, Vitality, Hanging