The medico-legal value of histopathological examination in hanging

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Abstract: Asphyxia deaths due to constriction of neck is common in all parts of the world, prospective studies in different setups to examine the profile of neck structure injuries are needed to differentiate the suicidal or homicidal nature of such deaths with a greater certainty. The purpose of this paper is to establish the cause of death from the histopathological examination of the neck’s soft tissues, as well as ante mortem versus post-mortem aspects of such cases. The study is made on 66 hanging cases, for which medicolegal autopsies were conducted in the Departments of Forensic Medicine Bihor, Cluj and Bistrita-Nasaud in the period 01.01.2015 to 31.12.2015 to study the histopathological changes in neck structures. Results show the importance of the histological and immunohistochemistry, examination in these cases of mechanical asphyxia.

Key Words: asphyxia deaths, histopathological examination, hanging, IHC.

Hanging is one of the forms of the death by asphyxia that is produced by the complete or incomplete suspension of the body by a ligature placed around the neck; the constricting force is the weight of the body [1]. Worldwide, in any country, hanging is between the first three methods of committing suicide.

During the year 2015 hanging was the most common method of committing suicide in Romania: 79% of the suicide cases were performed by this method [2]. There were also reported rare cases of accidental hanging and situations in which hanging was a murder, but all hangings are considered suicides until otherwise it is proved contrary [3]. The intra vitam character of the hanging is proven by congestion and by hemorrhagic collection of underlying anatomical structures [4], otherwise there is the possibility of a homicide dissimulation by post-mortem hanging. The hemorrhagic collection is located at the soft tissues of the neck, most commonly at the sternocleidomastoid muscles, at the prevertebral muscles and at the thyroid lobes [5]. The Amussat’s sign, that consist of transverse laceration of the intimal layer of carotid arteries can be caused by manual strangulation, by hanging or by other type of trauma as whiplash or blunt neck trauma. In hangings the Amussat’s sign have a relative frequency of 16.1% at macroscopic examination [6], but a significant relative frequency of 90% into the microscopical analysis [7]. The histopathological examination of the ligature mark provides limited informations for the differential diagnosis of the intra vitam or post mortem moment of the hanging [5]. There are authors that performed studies that concluded that “the results of histological examination of tissues were all incidental to the cause, mechanism, and manner of death” in the cases of routine histological examination in hanging deaths [8]. We have to note that the quoted study performed histological evaluation of the samples from the liver, thyroid, lung, and rectum. Excepting the lung and the thyroid, the rest
of the harvested samples were inappropriated or had a limited significance for the death by hanging evaluation.

Regarding the difference between hanging and strangulation, the histopathological examination of the skin and subcutaneous tissues is mandatory [9]. According to the quoted authors, congestion and compression were found in 100% cases of strangulation and only in 43.95% cases of hanging. Wrinkling of the skin was not found in the strangulations cases, but it was present in 46.15% cases of hanging.

The macroscopic examination of the noose mark is very important, but not very relevant regarding the intra vitam character of the lesion: the 23 experiments that Casper performed to determine the effect of hanging on a corpse, led to the conclusion that the noose marks on the neck could be produced within 2 hours after death [10].

The congestion is considered the safest vital reaction, despite its late appearance: 10-15 minutes is the minimum survival time that allows this sign to appear [11].

The aim of this paper is to establish the cause of death from the histopathological and immunohistochemistry examination of the neck's soft tissues, as well as ante mortem versus post-mortem aspects of such cases.

**MATERIALS AND METHODS**

The study was performed on the 66 cases of hanging that occurred in Bihor, Cluj and Bistrita-Nasaud Countries in the period 01.01.2015 to 31.12.2015. According to the Romanian Penal Law, there were performed forensic autopsies in all the 66 cases. The current study did not interfere with any of the autopsies performed into the analyzed cases, regarding the legal part, and the decisions to collect organ samples for histopathological examination purpose were independent to the present study, mainly to support the cause of death.

We had determinate analyzing the 66 cases, the percentage of the cases with skin and soft tissue samples for histopathological examination, the type of soft tissue from which the samples were collected and the histopathological diagnostic that emerged from the examinations.

The tissue was preserved 48 hours in buffered formalin. The sections prepared from formalin-fixed paraffin embedded tissue were cut at 4μm thickness and after that were stain by hematoxylin-eosin [12, 13].

On the second step the vessels slides were analyze from immunohistochemical point of view. The analysis was performed on 4 μm-thick sections prepared from formalin-fixed paraffin-embedded tissue by using an automated immunostainer (Bechmark GX, Ventana Medical Systems Inc., Tucson, AZ, USA) according to the manufacturer's instructions provide by package insert. Omni Map DAB (3,3'- diaminobenzidine) detection kit (Ventana Medical Systems, Inc.) and counterstained with Hematoxylin and blueing was used to highlights the reaction on tissue. The vessels were stain with Smooth Muscle Actin (1A4 clone) mouse monoclonal antibody. The positive control was done by using the SMA for a uterus sample [14].

**RESULTS**

The histopathological examination of the neck subjacent tissues was performed only for 16 cases from the total of 66, meaning an amount of 24.24%. The examined tissues were mostly skin from the noose marks, muscle, connective tissue and adipose tissue; in a single case we analyzed larynx, neck vessels including carotid artery, hyoid bone and trachea. The larynx, the neck vessels, the hyoid bone and the trachea were taken as samples in a case when there was a suspicion of manual strangulation with consecutive post-mortem hanging with the purpose of concealing the murder. The suspicion was found on macroscopic assessments and was confirmed by microscopical findings.

![Figure 1](image1.png)  Noose mark: epidermis and dermis compression and epidermis abrasion, hematoxylin-eosin 100X.

![Figure 2](image2.png)  Muscular layer and adipose tissue with hemorrhagic lesions due by hanging, hematoxylin-eosin 40X.
Into the 16 hanging cases, the microscopical lesions at the site of noose marks were represented by wrinkling of the skin, congestion and compression (Fig. 1), with hemorrhagic infiltration of the muscular and adipose tissue (Fig. 2). The hemorrhagic reaction of the subjacent tissues was seen at 11 cases, meaning 68.75% from the total, and the epiderm compressed at the noose mark was seen at 15 of the cases as well as the abrasion of the epiderma shallow layer, meaning 93.75% from the histopathological evaluated cases. In one case (6.25%), when there was a suspicion of manual strangulation, we found hemorrhagic infiltration of the neck elastic and muscle type artery (Fig. 3) and hemorrhagic infiltration of the hyoid bone (Fig. 4).

We compared two muscle type vessels from the same area. One of them was considering to be the normal image and express Smooth Muscle Actin antibody for the muscle layer (vessel media) where the muscle fascicles are brown, compact packed with spare spaces between theme (Fig. 5). The compress artery show that part of the smooth muscle fibers are fragmentized with an important edema around the fiber in the media layer. Meantime the nuclei are large than normal proved that is an injury at this point (Fig. 6).

**DISCUSSION**

Usually, hanging is a suicidal act, but it may be also accidental or homicidal [12]. There are situations when post-mortem hanging is a method of dissimulation of a homicide act. Inclusion criteria were to include the cases with asphyxia deaths due to constriction of neck, where there was a history of hanging or strangulation by hand. A detailed dissection was performed of the neck tissue to detect injury of deeper structure other than skin. In hand compression the degree and character of injury to the deeper tissue are dependent of the amount of pressure applied in this case on a small surface, like the fingertips. Also regarding the deeper tissue injury in hanging an important matter is represented by the type of noose used, because a thin one will provide a greater depth and a small change in the skin tissue.

![Figure 3. Hemorrhagic infiltration of the carotid artery, hematoxylin-eosin 40X.](image1)

![Figure 4. Fracture of hyoid bone with hemorrhage; hematoxylin-eosin 100X.](image2)

![Figure 5. The immunohistochemistry analysis shows the brown positive SMA normal reaction on the smooth muscle fiber of the artery, x 10.](image3)

![Figure 6. The immunohistochemistry analysis shows the brown positive SMA reaction on the smooth muscle fiber of the compressed artery, x 20.](image4)
The histopathological examination of the soft tissues of the neck is not a routine exploration in hangings, regarding the trend of minimum sampling in Romanian forensic pathology. This trend is opposite to the Council of Europe’s Recommendation no. R (99)3 of the Committee of Ministers to member States on the harmonization of medico-legal autopsy rules, adopted in February 1999, that specifies that even if the scope of sampling is case-dependent, in all autopsies there is a basic sampling scheme. This scheme “includes specimens from the main organs for histology” and when there is a violent death, sampling must include the injuries [15]. The forensic Romanian authors included the R (99) Recommendation into the forensic textbooks [16] and the Health Ministry adopted a Minister Directive no. 321/06.04.2005 that is making mandatory the methodology of the autopsy from the recommendation in question [17]. However, the forensic medicine practitioners are forced by the economic issues to a minimum histopathological sampling that is often insufficient for a well-documented scientific confirmation of the macroscopically findings [18-20]. Into the studied lot of 66 cases, the 24.24% of the hanging cases with sampling from the skin and from the soft tissue of the neck is a relative low amount. The usefulness of the extended histopathological examination was proven by the microscopic confirmation of the manual strangulation in one of the 16 cases with skin and soft tissue sampling. The fact that by immunohistochemistry analysis can be identify more aspects to identify exact the place where the hanging take place can help in the end the forensic judgment [21, 22]. The fragmentation of the muscle layer in the blood vessels and the nuclei modification proof that the exts sm is intra vitam and not post-mortem. The various features of mechanical cutaneous alteration either alone or in combination are highly suggestive to determine ante mortem aspects since these histopathological features indicate that the violent compression of neck took place.

CONCLUSIONS

The vital character of the noose mark can be revealed with accuracy only by histopathological examination of the skin and subjacent soft tissues.

All the microscopic changes must be correlated to the macroscopic findings on the autopsy and with the report issued by the criminal investigation institution.

Even that some extra-costs the immunohistochemistry stain can be the most important tool to set if is hanging or strangulation. There is a relative discrepancy between the macroscopically findings and the histopathological findings of examination of the neck structures among hanging deaths.

There is an economic issue that lead the forensic pathologist in Romania to under sample when performing a autopsy causing a loss of information, with negative consequences to the quality of the medico-legal autopsy report, which could be a possible source to generate judicial errors.

There is a major professional risk for the forensic doctors that are disregarding the Health Ministry's Directive and the R (99)3/1999 Council of Europe's Recommendation to be accused of medical mistake and to be prosecuted.

A detailed evaluation of the gross and histopathological findings of the neck structures, if undertaken as a routine would be more conclusive in establishing the cause and manner of death.

Conflict of interest. The authors declare that there is no conflict of interest.

References
9. Yadav A, Gupta BM. Histopathological changes in skin and subcutaneous tissues at ligature site in cases of hanging and strangulation, Journal of Indian Academy of Forensic Medicine, 2009, 31(3): 200-204.