



H23 Immunohistochemical Detection of Fibronectin, P-Selectin, FVIII, HSP-70, and MRP-8 in the Skin of Ligature Marks of Suicidal Hangings

Fiorella Caputo, MD*, Department of Legal and Forensic Medicine, Genova 16132, ITALY; Rosario Barranco*, University of Genova, Department of Legal Medicine, Genova 16132, ITALY; Francesco Ventura, MD*, University of Genova, Department of Legal Medicine, Genova 16132, ITALY; Tony Fracasso, MD, PhD, University Center of Forensic Medicine, Geneva 1211, SWITZERLAND

Learning Overview: After attending this presentation, attendees will understand that the determination of the vitality of skin injuries is one of the most central research areas in forensic pathology because it is often necessary to discriminate antemortem wounds from postmortem damage. Typical is the case of hanging in which real hanging has to be distinguished from postmortem suspension of a body. Despite the fact that researchers have intensively investigated this topic in the past, reliable ancillary testing methods are still lacking.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by highlighting the importance of the identification of early markers of tissue vital reaction in cases of hanging.

Materials and Methods: Fifteen human skin samples from ligature marks and 15 uninjured skin samples from deaths by suicidal hanging were analyzed (11 males and 4 females, mean age of 43.46 years). Fifteen skin samples from ecchymoses in victims of homicide with a short survival interval (9 males and 6 females, mean age of 43.26 years) were studied as control group. Sections were stained with hematoxylin and eosin and were also processed for immunohistochemistry to detect the expression of fibronectin, p-selectin, Factor VIII (FVIII), Heat Shock Protein-70 (HSP-70), and Myeloid-Related Protein 8 (MRP8.) Immunohistochemical reactions were classified semiquantitatively. For statistical analysis, Student's *t*-test was performed and a level of $P < 0.05$ was considered as statistically significant.

Results: Fibronectin—In ligature marks, fibronectin was significantly less expressed compared to ecchymoses. Its expression was similar in hanging marks and uninjured skin. P-selectin—In both ligature marks and ecchymoses, the expression was significantly increased in the endothelial cells and in the perivascular spaces compared to uninjured skin. FVIII:—In both ligature marks and ecchymosis, expression of FVIII was significantly decreased in the epidermis compared to uninjured skin. In the epidermis of ligature marks, FVIII was significantly less expressed compared to ecchymoses. HSP-70—In the epidermis of both ligature marks and ecchymoses, the expression was decreased compared to uninjured negative controls. In the epidermis of ligature marks, the expression was significantly lower compared to ecchymoses. MRP8—In both ligature marks and ecchymoses, the expression of this factor was significantly increased in blood vessels of dermis and hypodermis and also in the hemorrhagic areas compared to uninjured skin.

Discussion and conclusions: In uninjured skin, fibronectin is physiologically expressed in the epidermal basement membrane, around skin appendages, and in blood vessels. In the ligature marks, it is just a little more expressed in the hemorrhagic areas, so its detection is not useful in the determination of vitality. P-selectin plays a role in the early binding of leukocytes to endothelium during inflammation and in the recruitment of platelets at areas of vascular injury. In ligature marks, it is extensively more expressed compared to uninjured controls so it could be useful in the determination of vitality. FVIII is a blood coagulation factor that mediates the adhesion of thrombocytes to subendothelial connective tissue. In uninjured skin, it is expressed in the epidermis and in the blood vessels. The decreased expression of FVIII in epidermis of ligature marks could be due to the compression of the skin that reduces the diffusion of the factor in the epidermis; the marker is instead more expressed in the areas of dermal hemorrhage, and it could be useful in the determination of vitality. HSP-70 play an important role in cell responses to stress, and its expression is very high in epidermis of uninjured skin. Mechanical injury due to hanging can reduce the expression of this marker in the epidermis, so it is valuable in the study of vitality of ligature marks. Finally, MRP8 is an early marker of inflammation and because of its increased expression, it could be useful in the study of vitality of ligature marks.

In conclusion, the results of this study show that immunohistochemical study of early inflammatory and coagulation factors could be valuable to determine the vitality of ligature marks. In particular, the combined analysis of p-selectin, FVIII, HSP-70, and MRP-8 can be considered for this purpose while fibronectin seems not to be useful.

Suicidal Hanging, Vital Wounds, Immunohistochemistry