Post-mortem and histological changes seen in the epithelial tissues of submerged wistar rats

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Abstract:

Background: Skin, the largest organ of living organisms, tends to separate the external surface which covers about 15% of the body weight of rats. Post-mortem diagnosis of hypothermia is challenging, therefore in this study we detected the histopathological changes that occur in adult albino rats. When compared to human skin, rat skin shows change in adipose tissue, nerves, mesodermal origin, etc after post-mortem analysis. Rats are efficiently used as models to assess nutritional deficiencies, aging, etc.

Aim: The aim of our study is to assess the histological changes seen in the skin and epithelial tissues seen in submerged wistar rats during post-mortem

Method: Albino strain of wistar rats weighing 180-210g were used in this study. The rats were divided into two groups. Group one contains 4 wistar rats and Group two contains 2 wistar rats. Group one wistar rats were killed by submerging them into water. Group two wistar rats are clinically sacrificed as normally done for any study. After a time duration of 72hrs, postmortem analysis is done where the skin and epithelial tissues are taken into account. Routine H and E stainings are performed and IHC staining was also done.

Results: Under histopathological analysis, necrosis of muscle and collagen decomposition is seen in various levels, necrosis of skin are seen and decreased vessel wall thickness was observed (SMA).The detachment of hair or hair sloughing and black discolouration of the ruptured skin was observed and layers of the epithelium are difficult to distinguish completely**.**

Conclusion: From our study we have observed changes like sloughing of hair, black discoloration of ruptured skin and also coagulation necrosis has been observed in the epithelial tissues during post-mortem analysis.

Key words: Post-mortem, histological changes, submerged wistar rats, skin, epithelial tissues

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1. Introduction

The period before death is termed as the ante-mortem period, but if the period is after death it is termed as post-mortem period. After death, the body undergoes tremendous changes in its physical and chemical composition, which is usually termed as post-mortem changes. These kinds of changes provide an interval known as post-mortem interval (PMI) (1). There have been numerous methods used in autopsy in order to get a systematically determined post-mortem interval and also the time of death. It includes the examination of both internal and external physical characteristics of the body (2) such as autolysis, post-mortem clotting, putrefaction, etc and chemical changes include detection of body fluids, using scene markers, etc. Extreme environmental factors will greatly influence the speed of decomposition. Environmental factors, humidity, temperature, etc seem to be very important variables in cutaneous decomposition(3).

Prior to a lot of environmental factors having a substantial impact on the outcome, the post-mortem period does a good job of accurately determining the time of death (4). Forensic pathology has always focused on and struggled with precisely determining the post-mortem interval (PMI), sometimes known as the time of death. Following a body's death, the skin goes through many stages of change. Investigations in the past have demonstrated that morphological changes in experimental animals and human skin after death correspond with time. However, there has not been much research up to this point on the histological alterations in human skin after death, and most earlier studies only focused on a relatively little period of time following death. When the body is post-mortem, there are significant skin changes that include, skin slippage, ulceration, discolouration and dehydration(4), have been recorded at the time of death under various conditions.Our team has extensive knowledge and research experience that has translate into high quality publications (5–14).

We can use histologic alterations to the skin or appendages to combat this issue. Similar to this, other research had noted results from the skin, sweat glands, or hair follicles alone. A simultaneous analysis of the histologic alterations in the skin and epithelial tissues of submerged wistar rats and a comparison to PMI were attempted in this study.

2. Materials and Methods

Animals

The Institutional Animal Ethics Committee (IAEC) adopted the National Guidelines and Protocols for the care and handling of animals (IAEC no. BRULAC/SDCH/SIMATS/IAEC/02-2019/015). In this investigation, healthy male albino Wistar strain rats (Rattus norvegicus) weighing 180–210 g (150–180 days old) were employed. At the Central Animal House Facility, animals were procured and kept in sterile polypropylene cages with a consistent 12 h light/12 h dark schedule under specified humidity (65 5%) and temperature (27 °C). They were given a typical rat pellet meal, and free access to clean drinking water was provided.

Experimental design

Healthy adult male albino rats were divided into two groups, where one group contains two wistar rats and the other group contains four wistar rats. Group 1: The rats have been submerged in water for death. Group 2: The rats are clinically sacrificed on a regular basis. In a time period of 72 hours the rats were sent to post-mortem analysis. During analysis the skin and epithelial tissues were studied.

Staining protocol:

The sections were deparaffinized using Xylene for 20 minutes and rehydration was done using alcohol for 10 minutes. Sections were washed in running tap water for 3-5 minutes and were stained with Harris's haematoxylin for 5 minutes, washed in running tap water; differentiation was achieved by dipping the slides in acid alcohol for one dip, then dipped in ammonia for one dip and washed in running tap water for bluing. Slides were transferred to eosin for a single dip after which the slides were dehydrated through descending grades of alcohol; the slides were cleared in xylene and mounted with DPX.

Data Collection:

The slides were viewed under the microscope by two independent blinded observers and the depth of invasion was noted and tabulated. The degenerative changes were measured and graded by experienced pathologists.

3. Results

In our study we have compared the epithelial degenerative changes such as epithelial thinning, necrosis, degeneration and edema. When compared to the controlled group the value of the induced group necrosis is observed to be 2.75±0.70711, induced group edema is 2±0.70711, induced group degeneration is 2.75±0.70711 and induced group epithelial thinning is 1.75±0.70711. Therefore there is a significant increase in all the epithelial tissues degeneration compared to the controlled group.

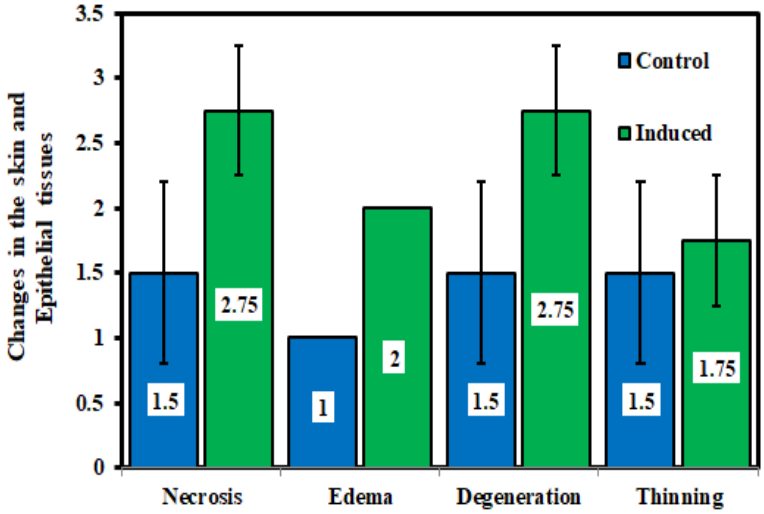

Figure 1: Changes in skin and epithelial tissues after a duration of 72 hour. The X-axis represents the cellular and epithelial degenerative changes and the Y- axis represents the mean values. The blue color denotes the control group and the green color denotes the induced or the test samples.

Table 1: The table represents the mean and standard deviation of the various degenerative changes seen in the samples and the control rat's epithelial tissue.

Group	Sig.	Mean	Std. Deviation
Necrosis Control Induced	.541	1.5000 2.7500	.70711 .50000
Degeneration Control Induced	.541	1.5000 2.7500	.70711 .50000
Thinning Control Induced	.541	1.5000 1.7500	.70711 .50000

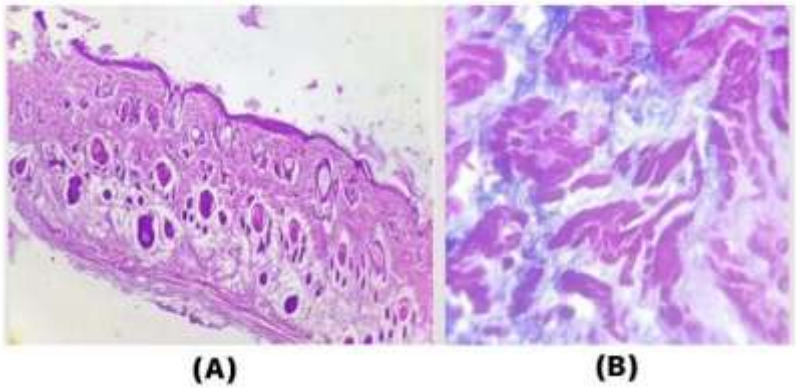

Figure 2: A) Control epithelial tissue samples showing well differentiated epithelial and connective tissues stroma showing the adnexal structures. B) The test sample's epithelial tissue and connective tissue stroma showed various signs of degeneration.

4. Discussion

The hypothesis and strategy of histology and pathology is the basis of classical forensic science. H&E staining is a simple, solid and conservative technical means to observe histomorphology. Rats have been used as models of human infections for quite a while. Indeed, they have many advantages when contrasted with other animals, permitting a basic and not costly display of human infections. Macrophages play a significant part in wound healing, and can create factors that stimulate angiogenesis and fibroplasias. Macrophages release growth factors, for example, PDGF and VEGF, which are generally vital for the triggering and proliferation of new tissue in the lesion area.

In a previous study, using L-arginine (L-ARG), a novel strategy for creating an adult rat model of epidermal necrosis was developed. Skin necrosis was detected in roughly 50 percent of the adult rats following a 5 to 7-day treatment with 500 mg/kg.b.w.d of L-ARG dissolved in their drinking water.Using L-ARG is simple and efficient to establish animal models of skin necrosis, yielding a high success rate within a relatively shorter period of time.(15)

Secondary necrosis, which can induce tissue injury, inflammatory and autoimmune responses, occurs in vivo when massive apoptosis overwhelms the available scavenger capacity or when the scavenger mechanism is directly compromised. In a study, the pathogenic effects of apoptosis were observed in multicellular animals with secondary necrosis. (1)
Gray to green discoloration, bloating, skin/hair loss, moist decomposition, insect activity, minimal bone exposure, mummification, and complete skeletonization with bone degradation were the observed postmortem changes and estimation of the postmortem interval in animal carcasses.(16)
In the region with the highest level of necrosis, flap shrinkage increased. According to perfusion data, the positive effects of VEGF and L-arginine on flap survival may be mediated by distinct mechanisms.(17)
The brain exhibits diffuse neuronal death and vacuolation in the brain parenchyma, the heart exhibits diffuse cardiomyocyte death and relatively increasing interstitial tissue, and the lung exhibits collapsed alveoli, proliferation of interstitial tissue, and hemorrhage, according to histopathological examination of rats that had been submerged in fresh water. Collagen deposition in the heart of freshwater and saltwater submersion exhibits blue-stained fibrous tissue surrounding the blood vessel wall, and lung exhibits blue-stained fibrous tissue that is restricted to the peribronchial tissue with no interalveolar proliferation. (9)

Very less samples are used, high amounts of samples may give higher significant values and the method which was used was very simple, methods like Special staining, immuno history chemistry and immuno fluorescence, etc can be used for better results.

5. Conclusion

From our study we have observed changes like sloughing of hair, black discoloration of ruptured skin and also coagulation necrosis has been observed in the epithelial tissues during post-mortem analysis. These findings show the changes in the epithelial tissues and how these can be used are markers for the time of death analysis.

Conflict of Interest:
The authors would like to declare no conflict of interest in the present study.

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