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Post-Mortem Alterations in Spleen Tissue of Submerged Wistar Rats: A Histological Examination

Rupa Devi R[1], Dr. Palati Sinduja*[2]
[1]Department of Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[2]Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. E-mail: rupa2002raja@gmail.com, sindujap.sdc@saveetha.com

ABSTRACT
Background: The post-mortem diagnosis of hypothermia presents a significant challenge in forensic practice. This study aims to identify histopathological changes that occur in adult Wistar rats under conditions of submersion. The spleen, as the largest secondary immune organ, plays a vital role in initiating immune responses against blood-borne antigens and filtering the blood of foreign material and damaged red blood cells. These functions are attributed to two distinct compartments within the spleen: the white pulp and the red pulp, characterized by differences in architecture, vascular organization, and cellular composition. **Aim:** Our study focuses on assessing histological changes observed in the spleen tissues of Wistar rats from Group 2, which were subjected to submersion following standard clinical euthanasia procedures. **Methods**: We utilized albino Wistar rats weighing between 180-210g for this investigation. The rats were categorized into two groups: Group 1, consisting of 4 Wistar rats, and Group 2, which included 2 Wistar rats. In Group 1, the rats were humanely euthanized by submersion in water for a duration of 72 hours. Post-mortem analyses were conducted, with particular attention given to spleen tissue. Routine hematoxylin and eosin (H&E) staining was employed alongside immunohistochemistry (IHC) staining. **Results:** Histological analysis revealed significant disruptions, collagen degeneration, edema, and vasoconstriction observed at varying levels in the spleen tissues. **Conclusion:** Previous studies primarily focused on the splenic layers, but our investigation highlights disruptions, degeneration, and vasoconstriction as noteworthy histopathological changes in this context.
Keywords: Post-mortem, histological changes, submerged Wistar rats, spleen tissues.

INTRODUCTION:

Post-mortem examinations, commonly referred to as autopsies, are vital procedures in forensic medicine and pathology, conducted to determine the cause of death, ascertain the nature of death (natural or unnatural), and estimate the time elapsed since death.(1) These examinations provide critical insights into the complex cascade of post-mortem changes that occur within the human body.(2)

The spleen, the largest secondary immune organ in the body, plays a pivotal role in initiating immune responses against blood-borne antigens and filtering the bloodstream, removing foreign materials and damaged red blood cells.(3) This indispensable organ consists of two primary compartments: the white pulp and the red pulp, each distinguished by unique architectural, vascular, and cellular characteristics .(4)

In the systemic circulation, the red pulp functions as a blood filter, removing foreign matter and dysfunctional erythrocytes while serving as a storage reservoir for iron, erythrocytes, and platelets. (5)Encased in a capsule comprising dense fibrous tissue, elastic fibers, and smooth muscle, the spleen's outermost layer consists of mesothelial cells, which may not be apparent in histological sections .(6)

The splenic arterial system bifurcates into trabecular arteries, which penetrate the splenic parenchyma. Within the red pulp, a complex network of splenic cords and venous sinuses forms a three-dimensional meshwork.(7) These cords, consisting of reticular fibers, reticular cells, and associated macrophages, play a vital role in splenic contraction. The white pulp is subdivided into the periarteriolar lymphoid sheath (PALS), follicles, and the marginal zone, housing lymphocytes, macrophages, dendritic cells, plasma cells, and a network of arterioles and capillaries ensconced in a reticular framework akin to the red pulp.(8). In the rat spleen, the venous sinuses (often referred to as pulp venules) differ significantly from those in mice and are more prominent. The spleen originates as a cluster of primitive reticular cells in the dorsal mesogastrium (9). Functionally, the rat spleen shares similarities with its human counterpart in cleansing the bloodstream of both endogenous and exogenous particulate matter.(10) In rats, this organ presents as a dark red to blue-black structure located in the left cranial abdomen within the omentum, resembling an elongated bean. (11)The rat spleen is enclosed by a robust connective tissue capsule comprising dense collagenous connective tissue interspersed with reticular fibers and scattered smooth muscle cells.(12). Various methods have been employed in forensic autopsies to systematically determine the post-mortem interval and estimate the time of death. (13)These methods encompass the examination of internal and external physical characteristics of the body, including autolysis, post-mortem clotting, and putrefaction, as well as the analysis of chemical changes involving the detection of body fluids and the use of scene markers. (14)This study aims to contribute to our understanding of post-mortem changes by assessing histological alterations in spleen tissue. When a body is submerged in water, decomposition may follow a different pattern compared to bodies exposed to air. (15)Environmental factors such as water temperature, microbial activity, dissolved oxygen, and aquatic organisms can influence the rate of tissue degradation. These factors may accelerate or modify the process of decomposition, making it essential to study the histological changes that occur in submerged tissues.(16). The spleen is an important lymphoid organ involved in immune defense, blood filtration, and removal of aged erythrocytes. (17)Structurally, the spleen consists of red pulp and white pulp. The red pulp is responsible for blood filtration, while the white pulp contains lymphoid follicles that participate in immune responses. Due to its soft consistency and rich vascular supply, the spleen is particularly susceptible to rapid post-mortem degradation.(18). Histological examination is a valuable method for studying microscopic structural changes in tissues. (19)By observing cellular and tissue alterations under a microscope, researchers can identify patterns of degeneration and decomposition that occur after death. Studying the histological changes in spleen tissue during submersion may provide useful insights into the process of tissue decomposition and may assist in forensic investigations.(20). Animal models, particularly Wistar rats, are commonly used in biomedical and forensic research because of their physiological similarities to humans and their ease of handling in experimental studies. Observing post-mortem changes in Wistar rat organs under controlled experimental conditions helps researchers understand how tissues respond to environmental factors such as water immersion.

MATERIALS AND METHODS:

Animals : Animals were maintained as per the National Guidelines and Protocols approved by the Institutional Animal Ethics Committee (IAEC no: BRULAC/SDCH/SIMATS/IAEC/02-2019/015). Healthy male albino rats of Wistar strain (Rattus norvegicus) weighing 180–210 g (150–180 days old) were used in this study. Animals were obtained and maintained in clean polypropylene cages under specific humidity (65±5%) and temperature (27±2 °C) with a constant 12 h light and 12 h dark schedule at the Central animal house facility, Saveetha Dental College and Hospitals, Chennai-77. They were fed with a standard rat pellet diet (Lipton India, Mumbai, India), and clean drinking water was made available and lithium.

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 hematoxylin 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

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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 dependent blinded observers and the depth of invasion was noted and tabulated. The degenerative changes were measured and graded by experienced pathologists.

RESULTS: In our study we have compared the histological degenerative changes such as disruption, vasoconstriction, degeneration and edema. When compared to the controlled group the value of induced group disruption is 1.5, induced group Oedema is 2, induced group degeneration is 1.7, induced group vasoconstriction is 2.2. Therefore there is a significant increase in all the spleen tissues compared to the controlled group.

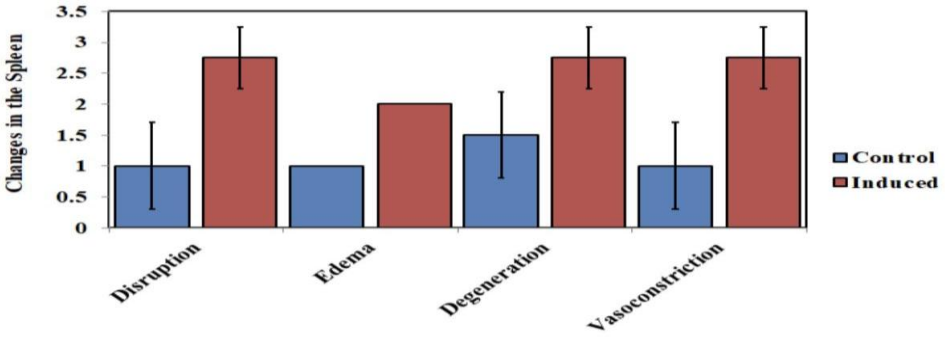


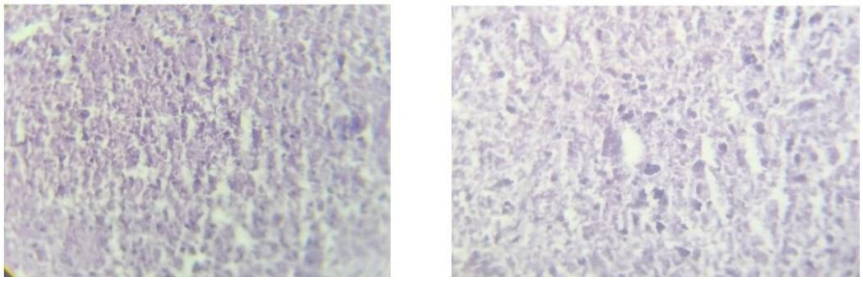
Figure 1: Changes in wistar rat spleen tissues after a duration of 72 hour. The X-axis represents the cellular changes in spleen tissues and the Y-axis represents the mean values. The blue color denotes the control group and the red color denotes the induced or the test samples.

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

	Mean		Standard Deviation		Significance
	Control	Induced	Control	Induced	
Disruption	1	1.5	0.0	0.5	
Oedema	1	2	0.0	0.0	
Degeneration	1.5	1.7	0.7	0.5	
Vasoconstriction	1	2.2	0.0	0.5	

Figure 2: A) Control spleen tissue samples, B) The test samples spleen tissue

A B



DISCUSSION:

The hypothesis and strategy of histology and pathology is the basis of classical forensic sciences. H and E staining is a simple, solid, and conservative technical means to observe histomorphology. Rats have been used as a model 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. Treatment of splenic infarct is based primarily on the underlying causative disease state. Splenic infarct the non infectious setting may be treated with analgesics, hydration,anti -emetics and other means of supportive care. In a previous study, pus significantly prolonged the survival duration of rats with severe intra-abdominal sepsis. This treatment may be an effective, noninvasive therapy that dampens detrimental immune responses.

Histopathological of fresh water submersion of rats revealed that the brain is showing diffuse neuronal death and vacuolation in the brain parenchyma, heart is showing diffuse death off cardiomyocytes and relatively increasing in the interstitial tissue and lung displays collapsed alveoli, proliferation of interstitial tissue and hemorrhage. Collagen deposition in the heart of fresh and saltwater submersion is showing blue-stained fibrous tissue limited to peribronchial tissue with no interalveolar proliferation. Gray to green discoloration, bloating, skin/hair loss, moist decomposition, insect activity, minimal bone exposure, mummification, and complete skeletonization with bone degradation were observed postmortem changes in animal carcasses and estimation of the postmortem interval. The present study provides further insight into the microscopic changes that occur in the spleen following post-mortem submersion. The spleen is considered one of the more delicate internal organs due to its soft parenchymal structure and high vascularity. Because of these characteristics, it is particularly prone to rapid autolytic changes after death. The findings observed in this study demonstrate a gradual deterioration of splenic architecture as the post-mortem interval increases.During the early post-mortem stage, mild histological alterations such as cellular swelling, congestion of splenic sinusoids, and slight disruption of lymphoid follicles were observed. These changes may be attributed to the cessation of cellular metabolism and the depletion of adenosine triphosphate (ATP), which leads to failure of ion pumps in the cell membrane. As a result, water accumulates within cells, causing cellular swelling and early degeneration.As the post-mortem interval progressed, more significant structural changes became evident. The white pulp, which consists mainly of lymphocytes and lymphoid follicles, showed progressive disintegration. Lymphocytes are particularly sensitive

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to hypoxic conditions and enzymatic degradation, which explains the early destruction of lymphoid tissue. The red pulp also exhibited degenerative changes such as disruption of splenic cords and dilation of vascular sinusoids.

Another important factor contributing to these histological changes is the process of putrefaction, which occurs due to the activity of microorganisms. After death, bacteria present in the gastrointestinal tract and surrounding environment invade tissues and accelerate decomposition. In submerged bodies, water may facilitate the spread of microorganisms and promote bacterial growth, thereby increasing the rate of tissue degradation.Environmental conditions also play a significant role in the decomposition process. Factors such as water temperature, pH, oxygen levels, and microbial content can influence the rate at which tissues break down. Warmer temperatures generally accelerate enzymatic activity and microbial proliferation, leading to faster tissue decomposition. Conversely, colder water conditions may slow down the process of decomposition.

The results of this study are consistent with previous research that has demonstrated similar patterns of post-mortem histological changes in soft organs. Such observations support the usefulness of histological examination in forensic investigations, particularly in estimating the time since death in submerged bodies.

However, it is important to acknowledge certain limitations of the study. The experimental conditions were controlled within a laboratory environment, which may not completely replicate the complexity of natural aquatic environments. In real forensic cases, additional variables such as water movement, aquatic organisms, and varying environmental conditions may alter the pattern and rate of tissue decomposition.

Future studies could focus on examining longer post-mortem intervals, studying additional organs, and analyzing the influence of different environmental conditions on tissue degradation. Such investigations may provide more comprehensive information and improve the accuracy of post-mortem interval estimation in forensic science.

CONCLUSION

From our study we have observed changes like vasoconstriction, oedema, degeneration, disruption in the spleen tissues during post-mortem analysis. These findings show the changes in the spleen tissues and how these can be used as markers for the time of death analysis.

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CONFLICT OF INTEREST: The authors would like to declare no conflict of interest in the present study.

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