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HISTOCHEMICAL CHANGES OF THE PULMONARY HYDATID CYSTS IN SHEEP INFECTED WITH CYSTIC ECHINOCOCCOSIS

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Abstract.

Background: Hydatid cyst (Hydatidosis) is considered one of the parasitic diseases that pose a threat to both animals and humans alike.

Objectives: The present study was aimed to investigate cystic echinococcosis in sheep. By employing a variety of specific stains, we sought to uncover the mesmerizing histochemical changes associated with this condition.

Methods: Twenty lung samples infected with hydatid disease were obtained. The samples were fixed in 10% formalin and processed and then stained with haematoxylin and eosin and some special stains such as Periodic Acid Schiff (PAS), Gomori's aldehyde fuschin, Best Carmine, Van Gieson, Toluidine blue and Alcian blue to investigate the histochemical changes that occurred in the lung tissue.

Results: Chemical changes were observed in the lung tissue affected by hydatid cysts, revealing insights into the underlying pathological processes. The presence of hydatid cysts was characterized by encapsulation within the lung tissue, consisting of a laminated layer surrounded by a fibrous membrane. Severe pneumonia was evident, accompanied by the infiltration of inflammatory cells and the presence of necrotic cells containing lytic nucleic acids. These changes resulted in atelectasis (partial or complete lung collapse) and emphysema (destruction of alveolar walls). Despite these pathological changes, intact elastic fibers were observed surrounding the bronchiole, indicating preserved structural integrity. Necrosis of epithelial cells in the bronchiole was also observed, disrupting normal respiratory system functioning.

Conclusions: The hydatid cyst has a clear effect on the lung tissue, represented by the histochemical changes that certainly cause damage to the lung and this is reflected in its vital function inside the body.

Key words: Histopathological changes, Hydatid cysts, Lung.

Introduction.

Echinococcus granulosus is a tapeworm that infects the canine family as a final host, causing echinococcosis, while its larval stages infect mammals as intermediate hosts, causing hydatidosis [1]. Hydatid cyst is considered one of the diseases of health and economic importance, especially in developing countries. Because of the slaughter of livestock outside the slaughterhouses and the throwing of infected guts that are within the reach of loose dogs, this type of unsanitary behaviour contributes significantly to the transmission of disease to humans and domestic animals [2].

Infection of animals with hydatid disease leads to a decrease in milk and wool production, fertility, and the slow growth of animals, in addition to the damage to the affected organs [3].

The liver is the first organ to be affected by a hydatid cyst, followed by the lungs. Any other organ, on the other hand, can be affected by this disease. The lung is one of the most critical and important organs for all animals [4]. Hydatid cyst usually develops quietly over years, if not decades, before presenting with clinical symptoms. Clinical signs are mostly determined by the location, size, and the number of lesions. Cough is the most prevalent symptom of a pulmonary hydatid cyst, followed by chest pain, dyspnoea, expectoration, fever, haemoptysis, and allergic reactions [5]. The current study was focused on knowing the histochemical changes in the sheep lung tissue infected with this disease by using many special stains.

Methods.

In a research study conducted from September to November 2021, twenty lung samples infected with hydatid cyst disease were collected from sheep at local slaughterhouse in Mosul city. After slaughtering the sheep, the lung samples were carefully obtained and stored in a refrigerated cork container to maintain their integrity during transportation to the laboratory. Upon arrival, the specimens were prepared for analysis by being chopped into approximately 1 cm long pieces. Standard laboratory techniques were employed to process the samples, which involved fixation in 10% formalin and subsequent embedding in paraffin. To facilitate histological evaluation, the samples were sectioned into thin slices with a thickness ranging between 4 to 6 microns. These sections were then stained using a variety of specific stains, including hematoxylin and eosin, periodic acid-Schiff (PAS), Best carmine, Alcian blue, Toluidine blue, Van Gieson's, Gomori's aldehyde fuschin, and Mansso's trichrome. These staining techniques allowed for the identification and characterization of various cellular components and structures within the lung samples, aiding in the comprehensive assessment of the hydatid cyst disease [6].

Results.

In comparison to the control groups, which appeared normal, our current findings revealed the presence of hydatid cysts in the lung tissue with a laminated layer surrounded by a fibrous membrane and severe pneumonia with infiltration of inflammatory cells, atelectasis, and necrosis of epithelial cells of the bronchiole in sections stained with hematoxylin and eosin stain.

PAS stain revealed lesions in the lung of the affected groups represented by bronchopneumonia, with infiltration of peribronchial inflammatory cells surrounding the bronchiole and in the alveolar walls without magenta colour, atelectasis, and emphysema.

The infected sections showed destruction and necrosis of the bronchiole, pneumonia, and bronchopneumonia. There was a severe infiltration of inflammatory cells and inflammatory exudate in the bronchi. The sections stained with Gomori's aldehyde appeared dark green compared to the control group, which appeared normal. Sections stained with Best’s Carmine stain showed a laminated membrane of a hydatid cyst with a
fibrous tissue capsule surrounding the lung. There was also bronchopneumonia with severe infiltration of inflammatory cells and emphysema in the alveolar walls.

Through sections stained with Van Gieson stain, it indicates the presence of severe pneumonia with inflammation in the cells, bronchopneumonia with extensive inflammation around the bronchioles and alveolar walls, and emphysema when compared to the control group.

Infected groups sections stained with Toluidine blue stain, showed the presence of dark blue colour of acidic components of the laminated membrane of hydatid cyst with necrotic cells of lytic nucleic acids in the interstitial tissue with increased fibrous connective tissue in pink colour.

Through sections stained with Alcian blue stain, the architecture of the control group was normal. While the affected groups had blue mucopolysaccharide reactivity in the proteoglycans of the hydatid cyst laminated surrounding the lung, they also had severe infiltration of inflammatory cells surrounding the bronchiole and in the alveolar walls (Table 1 and Figure 1).

Discussion.

The lung represents the second station after the liver for hydatid cyst stability. The hexacanth embryo in eggs of this parasite turns into a hydatid cyst that gradually grows in the lung, and its growth rate is related to the softness of the organ and the elasticity of the surrounding tissues. The low resistance of lung tissue makes it an ideal environment for the rapid formation of hydatid cysts [7].

The host's reaction to the injury begins with the formation of fibrous tissue around the cyst as a result of the inflammatory effect of the host, and this is evident in this study. The density of this tissue depends on the location and size of the tissue, as well as the period of growth and the ability of this organism to form alveolar-epithelial injury and apoptosis [8].

Our current findings have shed light on the presence of fibrous tissue surrounding the hydatid cyst. This fibrous tissue develops as a result of the host's contact with the parasite's inflammatory effect, which initiates early in the cyst's growth. The severity of the reaction plays a role in influencing the extent of the fibrous tissue formation. In addition to this, our examination of lung tissue samples has revealed significant tissue response and infiltration of inflammatory cells surrounding bronchiole and alveolar walls. Notably, there was an absence of magenta color, atelectasis, or emphysema. Furthermore, we observed the proliferation of fibrous tissue capsule surrounding the lung, as well as degeneration of the lung tissue accompanied by the spread of necrotic areas of the interstitial tissue with severe broncho-pneumonia. It is worth mentioning that these findings have been previously confirmed by similar studies that focused on investigating pathological cases of hydatid cysts [9-15].

In addition to damaging the infected host tissues, the parasite infection causes the production of signals that trigger the recruitment of many cells, including mast cells, eosinophils, basophils, and dendritic cells. When these parasites are present, the cells have the capacity to secrete immunological components that control the immune response processes [16].
Recent investigation has found evidence supporting the notion that inflammatory cells, specifically eosinophils and mononuclear cells, quickly enter the cyst layer in hydatid cysts found in the lungs. Additionally, these cells often spread into the nearby alveoli and bronchioles, which is consistent with previous observations made by Beigh et al. in 2017 [10]. This finding suggests that the immune response is important in the development of hydatid cysts in the lungs. Eosinophils, a type of white blood cell involved in allergies and parasitic infections, tend to gather in tissues affected by helminthic infections like hydatid cysts.

Atelectasis, a condition characterized by the collapse or incomplete expansion of the lung, has been observed to occur due to various factors. These factors include pressure on the lung or obstruction of the airways, specifically the bronchioles or bronchi. One of the main causes of atelectasis is the presence of foreign bodies or mucus that block the airways, preventing proper airflow. In a recent study conducted by Al-Malki and Ahmed [15], in a study analyzed pathological lesions in infected sheep to determine the prevalence of various conditions such as bronchiectasis, alveoli atelectasis, areolar emphysema, bronchiolar epithelial hyperplasia, thickening of interalveolar septa, collagen fiber amount, and pulmonary vessel congestion. The results showed a significant increase in these parameters in infected sheep compared to uninfected ones, indicating the impact of infection on the development of atelectasis. This research emphasizes the importance of understanding the mechanisms and pathophysiology of atelectasis and its potential implications for animal and human health.

In several histological studies, PAs, and Masson's trichrome are excellent prognostic markers. As in our current study, the cyst wall was made up of three layers: an exterior fibrous layer, a laminated layer and the innermost germinal layer. The lung tissue appeared as a very dark purple shade using PAS, with a laminated area consisting of acid mucopolysaccharides. Notably, in the case of sheep, the region between the pericyst and the ectocyst exhibited the precipitation of both neutral and acidic polysaccharides. These findings align with the observations made in the current study. The identification of these components and their distribution within the pericystic area further contributes to our understanding of the intricate biological processes occurring in this specific context. Such discoveries shed light on the complex nature of these interactions and provide valuable insights for future research in this field [18].

The content of myosin blue, a specific marker, was found to exhibit distinguishable patterns in certain cells of lung tissue that were affected by hydatid cysts. This distinction was observed through the utilization of Alcian blue staining, which allowed for the visualization and differentiation of these cells from the control group. This finding aligns with the results reported by Arabi et al. in their study conducted in 2013 [19]. The use of Alcian blue staining as a diagnostic tool in the examination of lung tissue affected by hydatid cysts has proven to be consistent with their findings, further supporting the relevance and accuracy of this staining technique in identifying and characterizing pathological changes in cellular composition. This discovery contributes to our understanding of the cellular alterations occurring in lung tissue affected by hydatid cysts and emphasizes the importance of utilizing staining techniques such as Alcian blue to aid in the accurate diagnosis and characterization of such conditions [19].

In this study, Toluidine blue-stained sections of lung tissue revealed the presence of mast cells in the fibro-cellular reaction near the cyst wall. These mast cells contained granules that were labeled both inside and outside of them. Additionally, the alveolar parenchyma of the lungs affected by hydatid cysts showed a significant mast cell response, which is supported by a previous study conducted by Beigh et al. [10]. This study found that lung tissue sections stained with toluidine blue showed a fibro-cellular reaction near the cyst wall. This reactive area contained a significant number of mast cells. What made our observation even more interesting was the presence of

![Table 1](image_url)

**Table 1.** The reaction of the intensity of lung tissue infected with hydatid cysts using specific stains.
granules within and outside the cells neighboring the mast cells, which exhibited metachromatic labeling. This metachromatic labeling suggests the presence of substances or molecules within these granules that have the ability to change the color of the stain, providing valuable insights into the cellular and molecular environment surrounding the hydatid cyst. Furthermore, our investigation extended to the alveolar parenchyma of the lungs affected by the hydatid cyst, and we discovered a robust mast cell response. This finding aligns with the research conducted by Beigh et al. [10], further supporting the significance of mast cells in the context of cystic lung diseases. By utilizing toluidine blue staining and carefully examining the lung tissue sections, we were able to shed light on the significant presence of mast cells and the associated fibro-cellular reaction near the cyst wall. These findings contribute to our understanding of the cellular and molecular dynamics involved in hydatid cysts in the lungs. Further research in this area could explore the mechanisms underlying mast cell activation and their potential as therapeutic targets for managing cystic lung diseases [10].

Tissue hypoxia, a condition characterized by inadequate oxygen supply to the cells, is a crucial factor that triggers mast cell activation [20]. Mast cells, a type of immune cell found in connective tissues, play a pivotal role in the immune response and are known for their ability to release various bioactive substances [21]. Mast cells, when activated, release various substances including proteolytic enzymes, angiogenic factors, and growth factors. These substances can lead to tissue deterioration and remodeling in both normal and pathological conditions. The release of proteolytic enzymes by mast cells, such as tryptase, chymase, and matrix metalloproteinases, can degrade components of the extracellular matrix, causing tissue damage. Mast cells also release angiogenic factors, promoting the formation of new blood vessels, which is important for wound healing but can contribute to diseases like cancer and chronic inflammation when excessive. Additionally, mast cell activation results in the secretion of growth factors that play a role in cell proliferation, migration, and tissue remodeling. This can lead to changes in tissue structure and function, and excessive remodeling can contribute to the development of fibrosis [22]. The study discovered color changes and an abundance of mast cells in certain areas. These mast cells release various mediators that have proteolytic, proliferative, and angiogenic effects. These findings were observed in and around the fibroblastic regions [23].

Pulmonary fibroplasia is a condition characterized by excessive connective tissue formation in the lungs. Mast cells, immune cells found in the respiratory system, play a crucial role in tissue remodeling, and are closely associated with fibroblast foci and alveolar type II cells. The interaction between mast cells and fibroblasts is important in the development and progression of lung fibrosis and inflammation caused by hydatidosis, a parasitic infection. Mast cells release mediators such as cytokines, growth factors, and chemokines, which stimulate fibroblast activation and proliferation, leading to the excessive production of collagen and other components of the extracellular matrix. These changes disrupt lung function, resulting in respiratory symptoms and complications. Understanding the role of mast cells and their mediators is important for developing targeted therapies to alleviate symptoms and improve the quality of life for individuals with pulmonary fibroplasia [22,24,25].

Masson's trichrome stain is a commonly used technique in pathology and histology to differentiate between collagen and muscle tissues. It helps evaluate collagen in various diseases and is particularly useful in distinguishing collagen fibers from muscle fibers. A recent study using Masson's trichrome stain identified collagen fibers in the cyst wall of lung tissue, providing insights into the structure affected by certain conditions or diseases. This stain aids researchers and pathologists in understanding the underlying pathology, assessing fibrosis, or scarring in lung tissue. Masson's trichrome stain has also been used to demonstrate fibrous tissue associated with hydatidosis in sheep, contributing to a better understanding of the disease's pathogenesis and potential treatments [17,26,27]. Collagen is a sign of inflammation in response to chronic stimulation, which could be a result of the regular, sluggish exosmosis that takes place in hydatid cysts. This is essentially the fundamental defensive reaction used to contain the parasite that leads to the development of the cyst wall.

Several trichrome techniques can show fibrin, which is useful for histologists to provide important information on the pathology. In tissue sections, fibrin is a byproduct of coagulation, and its location and structure provide important information on the pathological state of the tissue. For example, fibrin spots will appear on the lung with lobar pneumonia. Lung tissue shows fibrinogen and hemorrhage in specific areas where fibrin usually appears in pink color, and this is what saw in our current study.

The use of a modified Verhoeff's elastin stain to stain the thin elastic fibers of pulmonary blood vessels. This staining technique enables histomorphometric imaging analysis of vessel wall thickness in small arteries and intra-acinar vessels. [28].

Conclusion.

Through this study, it has been concluded that hydatidosis, a parasitic infection caused by the larvae of Echinococcus granulosus, has a significant impact on lung tissue. This conclusion is based on the findings obtained by using several stains that revealed clear histochemical changes in the infected lung tissue. These changes indicate a disruption in the normal structure and function of the lungs, which are vital organs responsible for respiration and oxygen exchange in the body. The presence of hydatid cysts in the lung tissue can lead to various complications, including compression of adjacent structures, impaired lung function, and even potentially life-threatening situations such as rupture or secondary infection. The histochemical changes observed in this study highlight the destructive nature of hydatidosis and emphasize the need for early diagnosis and appropriate treatment to mitigate the detrimental effects on lung tissue and overall respiratory health. Understanding the impact of hydatidosis on the lung is crucial for healthcare professionals to provide effective management strategies and improve patient outcomes.

REFERENCES