Iraqi Journal of Veterinary Sciences

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Histochemical study of the hepatic metacestodes in sheep infected with hydatidosis

B.H. Al-Sabawi[1], H.S. Sadoon[2] and M.G. Saeed[3]

[1]Department of Anatomy, College of Medicine, [2]Department of Biology, College of Education for Pure Science, [3]Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received March 28, 2022
Accepted June 11, 2022
Available online June 12, 2022

Keywords:
Hydatid cysts
Liver
Histopathological changes
Sheep

Correspondence:
M.G. Saeed
mgsaeed@uomosul.edu.iq

Abstract

This study aimed at detecting the liver histopathological changes of hydatid cysts sheep infestation. Seventeen sheep liver's samples, infested with hydatid cysts, were collected from the local butchers. The specimens sectioned, processed, and stained with hematoxylin and eosin and some special stains such as Gomori's aldehyde fuschin, Van Gieson, Toluidine blue, Alcian blue, Periodic Acid Schiff (PAS) and Mansso's trichrome. The results of haematoxylin and eosin stain of infected groups revealed the presence of the laminated membrane of hydatid cyst in different spots of hepatic tissue encircled by infiltration of inflammatory cells, an increase in fibrous tissue and severe necrotic hepatic tissue. The special stains as in Gomori's aldehyde fuschin revealed the protoscolex in green colour and elastic fibres of connective tissue surrounding hydatid cyst with its laminated membrane in purple colour. Van Gieson stain showed dark brown-purple colour of the elastic fibres reduplication of connective tissue surrounding hydatid cyst, while Toluidine blue stain sections revealed the dark blue stain of acidic components of hydatid cyst laminated membrane and necrotic hepatocytes. Alcian blue stain revealed the blue colour reactivity of the proteoglycans of the connective tissue surrounding hydatid cyst. PAS reaction stain revealed the magenta colour reaction of the intact hepatocytes in the control group comparing with depletion of magenta colour in necrotic hepatocytes surrounding hydatid cyst. Masson's trichrome stain revealed increased collagen fibres of the connective tissue. At the conclusion, it's evident that infection with hydatid cysts causes a variety of histopathological alterations that appeared through several tissue stains.

DOI: 10.33899/ijvs.2022.133402.2222,

Introduction

Cystic echinococcosis is one of the most important transitional zoonotic parasitic diseases that affect humans and animals, and it is a widespread disease (1). The transmission of disease is attributed to the inability to discover the disease in early stages since there are no symptoms shown but following the increasing size of cyst that causes pressure on the tissue and adjacent members (2). The canine family is regarded as the definitive host of *Echinococcus granulosus*, whereas herbivores such as sheep and cows serve as intermediate hosts, while humans deemed occasional ones (3). The larval form (hydatid cyst) is found in liver and other viscera of Man and other herbivores. The adult worms parasitized in dog's intestine. Hydatid cyst primarily generated in the intermediate host's liver and lungs. It is noted that the liver is the most infected organ, followed by the lungs. The direct mass effect of hydatid cysts, as well as consequences associated to cyst rupture or super infection might produce symptoms (4). For determining the disease's pathophysiologically, we need to understand the nature of a hydatid cyst. Best's Carmine stain showed the pale stain of hepatic tissue as depletion of glycogen in the necrotic hepatocytes.

The aim of the current study is to determine the histological changes in hydatid cysts, which are one of the most important diagnostic criteria for this disease. The study of this aspect is considered one of the most important priorities in understanding the development of the disease and defining the type of infection and pathological lesion that is formed by using many special dyes.

Materials and methods

Seventeen sheep, with hydatid cysts in their livers, were collected. Samples were taken from Sadoon slaughterhouse and a butcher's shop in Mosul, Iraq's Nineveh Governorate. The samples were taken to the Anatomy Department's Research and Postgraduate Laboratory at the University of Mosul's College of Medicine. The specimens were cut into approximately 1cm-size pieces. After fixation in 10 % formalin, the samples were processed using standard procedures and embedded in paraffin. The samples were then sectioned at a thickness of 4-6 microns and stained with hematoxylin and eosin (5), and some special stains such as PAS, Best carmine, Alcian blue, Toluidine blue, Van giesons, Gomori's aldehyde fuschin, and Mansso's trichrome for histological inspection (6). The slides were filmed with a TOUPCAM camera with different magnifications and diagnosed by a specialist in veterinary pathology.

Ethical approve

Liver samples infected with the hydatid cysts parasite were obtained from sheep naturally infected at Al-Saadoun slaughterhouse located in the Kokjali area in Mosul city, Iraq from August -December 2021, and after obtaining the parasite, the infected livers were burned to prevent the spread of infection. Of course, we did not use any experimental animals in this research.

Results

The results of histopathological changes of haematoxylin and eosin stain sections were those of infected groups showing presence of laminated membrane of hydatid cyst in different areas of hepatic tissue, infiltration of inflammatory cells in surrounding and portal areas as polymorph nuclear and mononuclear cells, an increase in fibrous tissue as a capsule surrounding hydatid cyst, necrotic hepatic tissue and severe necrosis of hepatocytes and dilatation of sinusoids in comparison to the liver of control group which appeared normal (Figures 1 and 2).

The results of histochemistry special stains showed lesions evident in the liver of infected groups. Sections stained by haematoxylin, eosin as well as other special stain like Gomori's aldehyde and fuschin stains showed the *Echinococcus granulosus* protoscolex in green colour and elastic fibres of connective tissue capsule surrounding hydatid cyst with its laminated membrane in purple colour and the necrotic hepatocytes in dark green compared with control liver group (Figure 3). Van Gieson- stained sections of infected groups showed the elastic fibres reduplication of connective tissue surrounding the hydatid cyst laminated membrane in dark brown-purple colour and encircling necrotic hepatic tissue in yellow colour compared with control liver sections of yellow-red colour (Figure 4).

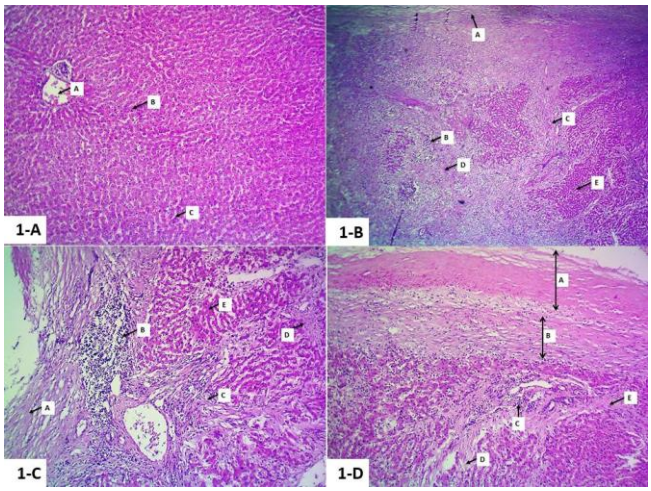

Figure 1: Photomicrographs of liver. (1-A): control group shows normal architecture represented by portal area (A), hepatocytes (B) and sinusoids (C), 100X. (1-B) & (1-C): infected groups shows presence of laminated membrane of hydatid cyst (A), infiltration of inflammatory cells (B), increase fibrous tissue (C) and severe necrosis of hepatocytes (D) with few normal hepatic tissue (E), 40X & 100X. (1-D): infested group shows presence of laminated membrane of hydatid cyst (A), surrounded by increased fibrous tissue (B), infiltration of inflammatory cells in portal area (C) and necrosis of hepatocytes (D) with increased fibrous tissue between it (E), 100X. H&E stain.

Toluidine blue stained sections of infected groups revealed the dark blue colour of acidic components of hydatid cyst laminated membrane and the dark blue colour of necrotic hepatocytes in lytic nucleic acids in the interstitial tissue in comparison with normal hepatic tissue of control group which appeared in blue colour (Figure 5). Best's Carmine stain of infected groups showed the pale-yellow colour of hepatic tissue depleting of glycogen in the necrotic hepatocytes surrounding the hydatid cyst with pale pink-yellow colour of its laminated membrane in contrast with liver control group in the pink colour of glycogen contents of the intact hepatocytes (Figure 6). Alcian blue stain of infected groups revealed the blue colour reactivity of mucopolysaccharides of the proteoglycans of the connective tissue surrounding hydatid cyst in comparison with liver control group of the intact hepatocytes (Figure 7).

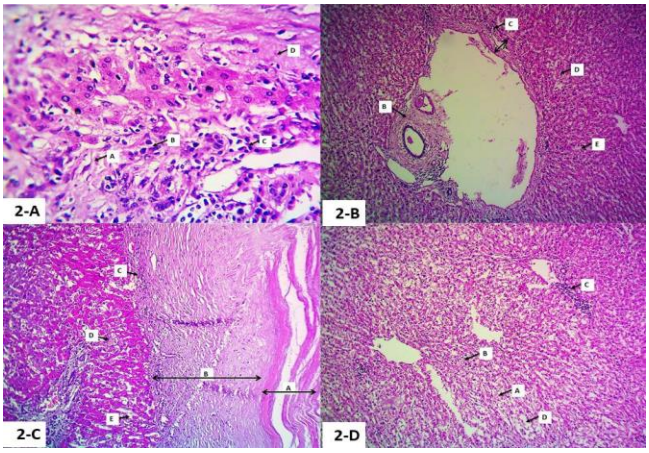

Figure 2: Photomicrographs of liver. (2-A): infected group shows presence of increased fibrous tissue (A), infiltration of inflammatory cells in portal area as polymorph nuclear (B) and mononuclear cells (C) with necrosis of hepatocytes (D), 400X. (2-B) & (2-C): infected groups show presence of laminated membrane of hydatid cyst in portal area (A), surrounded by increased fibrous tissue (B), infiltration of inflammatory cells (C), necrosis of hepatocytes (D) and dilatation of sinusoids (E), 100X. (2-D): infected group shows massive necrosis (A) and degeneration (B) of hepatocytes, infiltration of inflammatory cells in portal area (C) and dilatation of sinusoids (D), 100X, H&E stain.

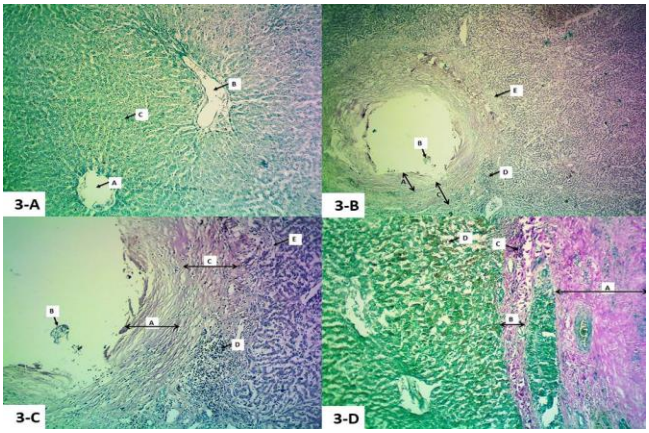

Figure 3: Photomicrographs of liver. (3-A): control group shows normal architecture represented by central vein (A), portal area (A) and hepatocytes (C), 100X. (3-B) & (3-C): infected groups show presence of laminated membrane of hydatid cyst (A) containing *Echinococcus granulosus* protoscolex (B), surrounded by the fibrous tissue (C), infiltration of inflammatory cells (D) and necrosis of hepatocytes (E), 40X &100X. (3-D): infected group shows presence of laminated membrane of hydatid cyst (A) surrounded by fibrous tissue (B), infiltration of inflammatory cells (C) and necrosis of hepatocytes (D), 100X. Gomori's aldehyde fuschin stain.

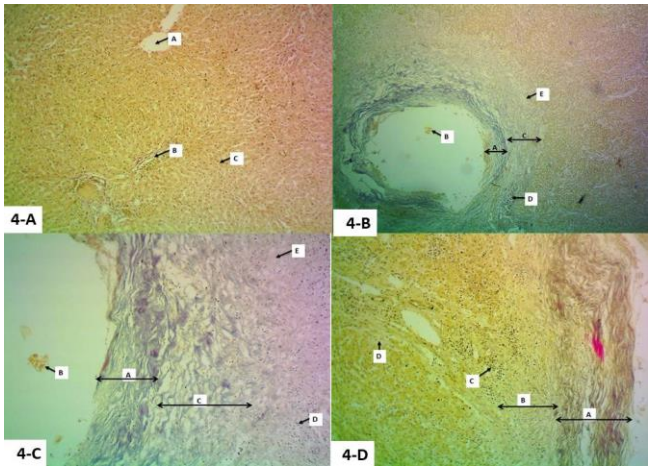

Figure 4: Photomicrographs of liver. (4-A): control group shows normal architecture represented by central vein (A), portal area (A) and hepatocytes (C), 100X. (4-B) & (4-C): infected groups show presence of laminated membrane of hydatid cyst (A) containing *Echinococcus granulosus* protoscolex (B), surrounded by fibrous tissue (C), infiltration of inflammatory cells (D) and necrosis of hepatocytes (E), 40X & 100X. (4-D): infected group shows presence of laminated membrane of hydatid cyst (A) surrounded by fibrous tissue (B), infiltration of inflammatory cells (C) and necrosis of hepatocytes (D), 100X. Van Gieson stain.

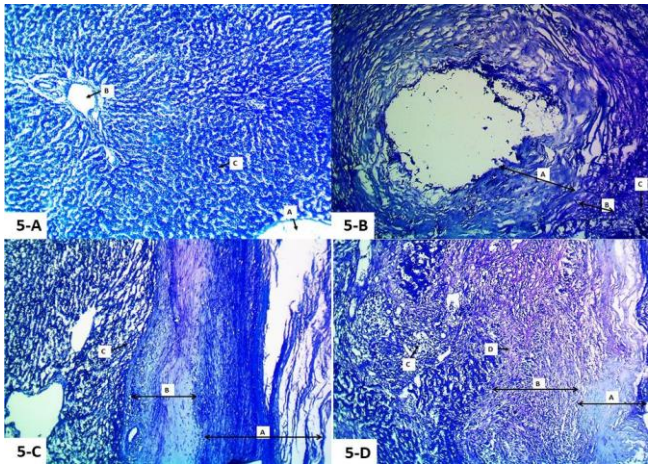

Figure 5: Photomicrographs of liver. (5-A): control group shows normal architecture represented by central vein (A), portal area (A) and hepatocytes (C), 100X. (5-B) & (5-C): infected groups show presence of laminated membrane of hydatid cyst (A) surrounded by fibrous tissue (B) and necrosis of hepatocytes (C), 100X. (5-D): infected group shows presence of laminated membrane of hydatid cyst (A) surrounded by fibrous tissue (B), infiltration of inflammatory cells (C) and necrosis of hepatocytes (D), 100X. Toluidine blue stain.

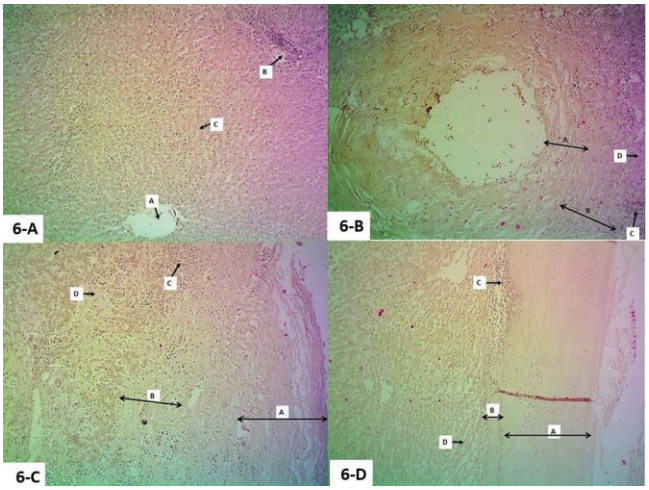

Figure 6: Photomicrographs of liver. (6-A): control group shows normal architecture represented by central vein (A), portal area (A) and hepatocytes (C), 100X. (6-B), (6-C) & (6-D): infected groups show presence of laminated membrane of hydatid cyst (A) surrounded by fibrous tissue (B), infiltration of inflammatory cells (C) and necrosis of hepatocytes (D), 100X. Best's Carmine stain.

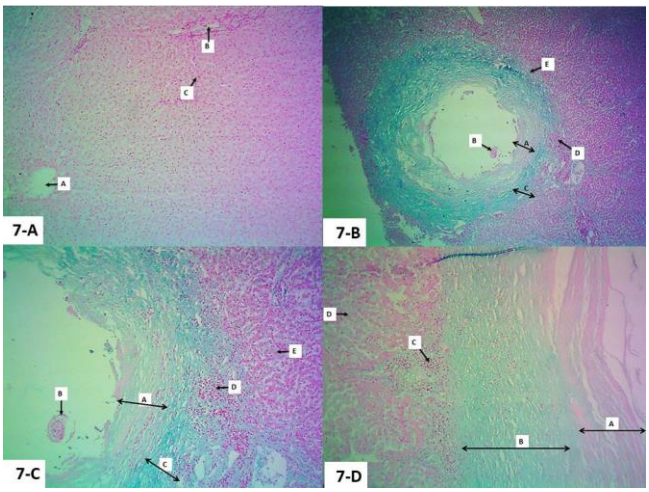

Figure 7: Photomicrographs of liver. (7-A): control group shows normal architecture represented by central vein (A), portal area (A) and hepatocytes (C), 100X. (7-B) & (7-C): infected groups show presence of laminated membrane of hydatid cyst (A) containing *Echinococcus granulosus* protoscolex (B), surrounded by fibrous tissue (C), infiltration of inflammatory cells (D) and necrosis of hepatocytes (E), 40X & 100X. (7-D): infected group shows presence of laminated membrane of hydatid cyst (A), surrounded by fibrous tissue (B), infiltration of inflammatory cells (C) and necrosis of hepatocytes (D), 100X. Alcian blue stain.

PAS reaction stain showed the magenta colour reaction of the intact hepatocytes in the control group in contrast with depletion of magenta colour in necrotic hepatocytes surrounding hydatid cyst in the infected groups (Figure 8). Masson's trichrome stain of infected groups revealed the reaction of increased collagen fibres of the connective tissue in the interstitial hepatic tissue and surrounding hydatid cyst in the green colour compared with liver of control group in the normal connective tissue (Figure 9).

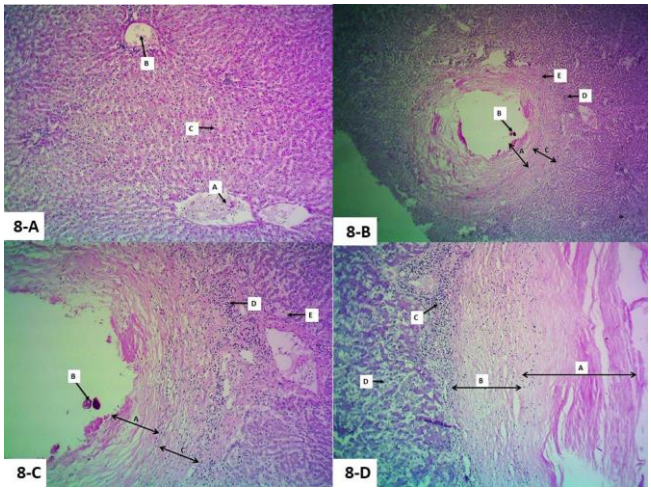

Figure 8: Photomicrographs of liver. (8-A): control group shows normal architecture represented by central vein (A), portal area (A) and hepatocytes (C), 100X. (8-B) & (8-C): infected groups show presence of laminated membrane of hydatid cyst (A) containing *Echinococcus granulosus* protoscolex (B), surrounded by fibrous tissue (C), infiltration of inflammatory cells (D) and necrosis of hepatocytes (E), 40X & 100X. (8-D): infected group shows presence of laminated membrane of hydatid cyst (A), surrounded by fibrous tissue (B), infiltration of inflammatory cells (C) and necrosis of hepatocytes (D), 100X PAS reaction stain.

Discussion

Our results showed, using a number of special stains, the occurrence of clear histopathological changes represented by dilation of sinusoids, the presence of elastic fibers surrounding the hydatid cyst, necrosis in hepatocytes, accumulation of acidic components of hydatid cyst laminated layer, depletion of glycogen by the parasite for the purpose of ensuring its survival inside the host, as well as the accumulation of proteoglycans surrounding the hydatid cyst. These molecules are large glycoconjugate complex that are found in high concentration within the extracellular matrix of connective tissue compared with the uninfected liver with hydatid cyst which appeared normal.

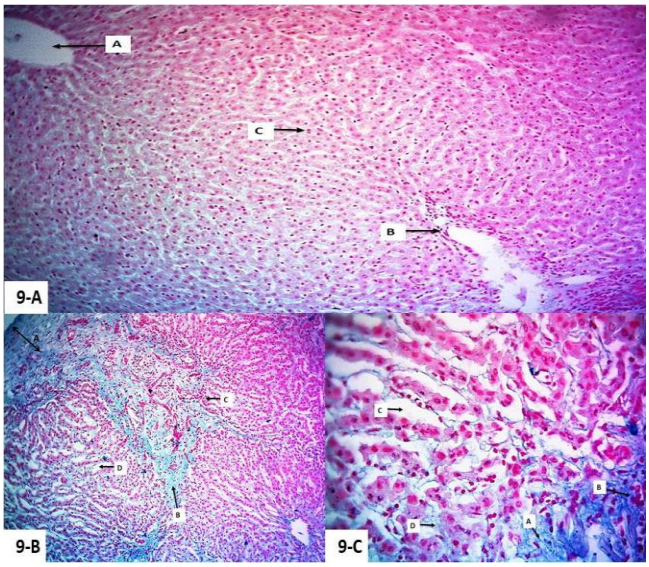

Figure 9: Photomicrographs of liver. (9-A): control group shows normal architecture represented by central vein (A), portal area (A) and hepatocytes (C), 100X. (9-B): infected groups show presence of laminated membrane of hydatid cyst (A) surrounded by increased fibrous tissue (green colour) (B) with infiltration of inflammatory cells (C) and dilation of sinusoids (D), 100X. (9-C): infected group shows presence of increased fibrous tissue between hepatocytes (green colour) (A), infiltration of inflammatory cells (B), dilation of sinusoids (C) and necrosis of hepatocytes (D), 400X Masson's trichrome stain.

Histopathological damage of hydatid cyst occurs as a result of the replacement of the host tissue with a developing cyst, causing deterioration of organ tissue (7). This happens gradually due to the increase in the size of the developing cyst (8). A pressure congestion portal vein may occur due to the grown cyst which compresses liver cells, in addition mice infected lead to histological changes resulting in inflammatory cells infiltration (9). A few hours after infection, the parasitic infection damages the infected host tissues, causing signals to be released that cause macrophages, dendritic cells, eosinophils, basophils, and mast cells to be recruited (10). Both the paranasal sinuses and the central veins were found to be dilated (11). In addition, the comprehensive, in certain cases, fibroplasia and cirrhosis were found attributed to the host tissue's immunological reactions (12). Hyperplasia of the bile duct and degenerative biliary disease alterations in the biliary epithelium, as well as bile infiltration, inflammatory cells were seen in several livers that were impacted (13).

Necrosis, degeneration, atrophy, fibrosis, and cellular infiltration of mostly macrophages, lymphocytes, and plasma cells were documented in liver sections taken from the areas adjacent to the cyst wall, as well as the presence of hypertrophy in the sinus, and fibrosis was seen. Also hypothesized that liver cell necrosis may be caused by the wounded cells' internal enzymes gradually acting on them or by a metabolic disruption preventing DNA and protein production (14).

The periodic acid-Schiff stain is useful for recognizing glycogen; however, diastase digestion improves detection of non-digested material such as alpha-1-antitrypsin globules, basement membrane, macrophage debris, and fungal pathogens by eliminating glycogen. The existence of glycogen deposition can be confirmed by PAS with and without digestion, as seen in many glycogens' storage disorders (15), and immunological reactions of the host tissue were to blame in certain cases.

The laminated layer is a carbohydrate-protein complex with galactose, galactosamine, and glucosamine as the polysaccharide portion's main components (16). It stains strongly with Schiff's reagent (PAS) and is a good diagnostic marker in many histological studies (17). Alcian blue at pH 2.5 also revealed the presence of sulfate containing acidic mucopolysaccharides (18).

The Masson's trichrome stain is one of the most widely used special stains on liver specimens. Collagen takes on a blue tint against a red background of hepatocytes and other structures. It emphasizes the existence and distribution of reactive fibrosis due to a liver injury by staining type 1 collagen, which is normally found in the portal tracts and vessel walls. It's most commonly utilized for fibrosis evaluation and measurement as well as being used to determine the stage of chronic liver disease (19).

Collagen fibers were visible in the cyst wall of liver sections stained with Masson's trichrome. In sheep, fibrous tissue is related to hydatidosis. The presence of collagen suggests the inflammatory response to chronic irritation, which can be attributed to the hydatid cyst's constant, gradual exosmosis (16). This is the basic defensive reaction that leads to the creation of the cyst wall and the confinement of the parasite. As previously stated, the host tissue may be to blame for the significant fibrosis and cirrhosis seen in some cases (20).

To identify hooklets, Wheatly's adaptations to Gomori's trichrome stain, fibrinoid material and mucin (periodic acid-Schiff stain positive), demonstrating pseudolamellar formations, are the differential diagnosis when the laminated membrane fragments are diagnostic (21). Silver methenamine and Best's carmine stain were particularly useful in identifying laminated layer fragments. Mast cells secrete a large number of mediators with proteolytic, growth, and proangiogenic properties, making them an essential cell type in the pathophysiology of a variety of diseases (22). Metachromatic regions and mast cell buildup were found in and surrounding fibroblastic areas around the cyst in our investigation (23).

Oncosphere infected animal leads to death in battle zone and followed by inflammatory cell infiltration; however, parasite trans up into cyst to survive these harsh conditions.

Next, a layer of fiber-producing cells develops adjacent to the lamellar layer, surrounded by mast cells, eosinophils, histiocytes, and fibrillating cells (24). This is what we discovered in our new investigation utilizing the van Gieson stain (25).

In our research, we studied metachromatic regions and mast cells. The increase of cells was seen in and around the cyst. There are fibroplastic regions in chronic instance of mast cell proliferation in the perivascular space, its consequences increased secretory activity. Also, the presence of many mast cells, in fibro-cellular contact next to the cyst wall, was discovered in our current investigation employing sections stained with toluidine blue. Mast cells were discovered. Inside, the granules were dyed metachromatic and outside the cells next to them (16). Mast cells play an important function in a variety of inflammatory illnesses and are also engaged in tissue remodeling (24).

Conclusion

Hydatid cyst disease is one of the health problems facing animals and human alike, which causes health and economic problems. Therefore, we need an intensive study to help reduce it and push out health awareness about the seriousness of this disease.

Acknowledgements

We would like to thank the Deanships of the colleges of Medicine, Education for pure science, and Veterinary Medicine at the university of Mosul for assisting us with the study, as well as the staff at the Sadoon slaughterhouse in Mosul for providing us with research samples.

Conflict of interest

There are no conflicts of interest declared by the authors.

References

1. Zhang J, Ye B, Kong J, Cai H, Zhao Y, Han X and Li F. In vitro protoscolicidal effects of high-intensity focused ultrasound enhanced by a superabsorbent polymer. Parasitol Res. 2013; 112:385-391. DOI: 10.1007/s00436-012-3176-3
2. Moazeni M, Larki S, Saharkhiz MJ, Oryan A, Ansary Lari M, Mootabi Alavi A. In vivo study of the efficacy of the aromatic water of Zataria multiflora on hydatid cysts. Antimicrob Agents Chemother. 2014;58(10):6003-8. DOI: 10.1128%2FAAC.02963-14
3. Wang J, Wang N, Hu D, Zhong X, Wang S, Gu X, Peng X, and Yang G. Genetic diversity of *Echinococcus granulosus* in Southwest China determined by the mitochondrial NADH dehydrogenase subunit 2 gene. Sci World J. 2014; 867839. DOI: 10.1155/2014/867839
4. Cristian B, Bogdan M, and Traian P. Hepatic hydatid cyst - diagnose and treatment algorithm. J Med Life. 2018;11(3): 203-209. DOI: 10.25122/jml-2018-0045
5. Slaoui, Mohamed, and Laurence Fiette. Histopathology procedures: from tissue sampling to histopathological evaluation. Drug safety evaluation. Humana Press. 2011:69-82. DOI: 10.1007/978-1-60761-849-2_4
6. Smith YU. Special stains and techniques: Dermatopathology. CRC Press; 2001. 347-52. DOI: 10.1201/9781498713665-30
7. Derbel F, Ben M, Hadj Hamida MB, Mazhoud J, Youssef S, Ben A. Hydatid cysts of the liver - diagnosis, complications and treatment. Abdominal Surg. 2012;78:101-109. DOI: 10.5772/48433
8. Webster GA, Cameron TWM. Observation on experimental infections with Echinococcus in rodents. Canadian J Zool. 2011;39:877-891. DOI: 10.1139/z61-082
9. Chen X, Zhang R, Alji T, Shao MY, Chen Y, and Wen H. novel interventional management of hypatic hydatid cyst with nanosecond pulses on experimental mouse model. Sci Rep. 2017;7:4491. DOI: 10.1038/s41598-017-04873-5
10. Battelli G. Echinococcosis: Costs, losses and social consequences of a neglected zoonosis. Vet Res Commun. 2009;33:47-52. DOI: 10.1007/s11259-009-9247-y
11. Ibrahim SA, and Gameel AA. Pathological, histochemical and Immunohistochemical studies of lungs and livers of cattle and sheep infected with hydatid disease. The 5th Annual Conference-Agricultural and Veterinary Research. 2014;2:1-17. [available at]
12. Khadidja H, Achour Y, Houcin B, and Vasile C. Histological appearance of *Echinococcus granulosus* in the camel species in Algeria. Bulletin UASVM Vet Med. 2014;71:79-84. [available at]
13. Solcan C, Solcan G, Ionit AM, Hristescu DV, Mitrea IL. Histological aspects of cystic echinococcosis in goats. Sci Parasitol. 2010;11:191-198. [available at]
14. Sołtysiak Z, Rokicki J, Kantyka M. Histopathological diagnosis in parasitic diseases. Ann Parasitol. 2014;60(2):127-131. [available at]
15. Fairbanks KD, Tavill A. Liver disease in alpha 1-antitrypsin deficiency: A review. Am J Gastroenterol. 2008;103(8): 2136-41. DOI: 10.1111/j.1572-0241.2008.01955.x
16. Beigh AB, Dazzi A, Shah A, Shah SA. The pathology of cystic echinococcosis and structural details of hydatid cyst and protoscolex. Indian J Vet Pathol. 2021;42(1):8-14. DOI: 10.595810973-970X.2018.00002.0
17. Solcan C, Solcan G, Ioniþă M, Hristescu DV, Mitrea IL. Histological aspects of cystic echinococcosis in goats. Sci Parasitol. 2010;11:191-198. DOI: 10.1007%2Fs12639-017-0929-z
18. Al-Hyali FQ. Histological and histochemical studies on developmental *Echinococcus granulosus* protoscolices. Raf Sci J. 2004;15(4):37-44. [available at]
19. Murli MD. Role of special stains in diagnostic liver pathology diagnostic. Clin Liver Dis. 2013;2(S1):12. DOI: 10.1002/cld.148
20. Anwar Z, Tanveer A, Bashir S. *Echinococcus granulosus*: Histopathology of naturally infected sheep liver. Punjab Uni J Zool. 1999;14:105-111. [available at]
21. Hira PR, Shweiki H, Lindberg LG. Diagnosis of cystic hydatid disease: role of aspiration cytology. Lancet. 1988;2(8612):655-657. [available at]
22. Vercelli-Retta J, Manana G, Reissenweber NJ. The cytologic diagnosis of hydatid disease. Acta Cytologica. 1982;26(2):159-168. [available at]
23. Jakubowski MS, Barnard DE. Anaphylactic shock during operation for hydatid disease. Anesthesiol. 1971;34:197-199. DOI: 10.1097/00000542-197102000-00029
24. AL Malki J, Ahmed N. Epidemiological and histomorphic studies in sheep infected with hydatid cyst in Taif area. Saudi J Biol Sci. 2022;29(2):886-893. DOI: 10.1016/j.sjbs.2021.10.017
25. Hidalgo C, Stoore C, Baquedano MS, Pereiro I, Franco C, Hernández M and Paredes R. Response patterns in adventitial layer of *Echinococcus granulosus* sensu stricto cysts from naturally infected cattle and sheep. Vet Res. 2021;52:66:1-7. DOI: 10.1186/s13567-021-00936-8

دراسة نسيجية كيميائية لإصابة الكبد بالطور اليرقي للديدان الشريطية في الأغنام المصابة بالأكياس العدرية

بثينة حاتم السبعاوي[1] و حنان صديق سعدون[2] و محمد غسان سعيد[3]

[1]فرع التشريح، كلية الطب، [2]قسم علوم الحياة، كلية التربية للعلوم الصرفة، [3]فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

هدفت الدراسة الحالية إلى تحديد التغيرات المرضية النسيجية لكبد الأغنام المصابة بالأكياس العدرية. تم جمع سبعة عشر عينة من أكباد الأغنام الحاوية على الأكياس العدرية والتي تم الحصول عليها من الجزارين المحليين. جرى تحضير المقاطع النسيجية ثم تم صبغها بصبغة الهيماتوكسيلين والأيوسين وبعض الصبغات الخاصة مثل صبغة كوموريس اليهايد وفوشين وفان جايسن وتولودين بلووالسيان بلو وباس وصبغة مانسوس تكروم. أظهرت نتائج التغيرات المرضية النسيجية لصبغة الهيماتوكسيلين والأيوسين للأكباد المصابة وجود الغشاء المصفح للكيس العدري في مناطق مختلفة من الكبد محاط بارتشاح الخلايا الالتهابية وزيادة النسيج الليفي ونخر شديد للخلايا الكبدية. واظهرت الصبغات الخاصة لأكباد الأغنام المصابة والمتمثلة بصبغة كوموري وجود طفيلي المشوكة الحبيبية باللون الأخضر والألياف المرنة للنسيج الليفي المحيط بالكيس العدري باللون الأرجواني. هذا وأظهرت صبغة فان جايسون اللون البني الأرجواني الغامق للألياف المرنة المتكاثرة حول الكيس العدري، أما صبغة تولودين بلو فأظهرت اللون الأزرق الغامق للمكونات الحامضية للغشاء المصفح للكيس العدري بالكبد المصاب. وأظهرت صبغة السيان بلو التفاعل الشديد باللون الأزرق الغامق للبروتيوكلايكان بالنسيج الليفي المحيط بالكيس العدري للكبد المصاب. وأظهرت صبغة باس بوضوح اللون الأرجواني الغامق للخلايا الكبدية الحاوية على الكلايكوجين في مجموعة السيطرة مقارنة مع الكبد المصاب الذي يفقد هذا اللون في الخلايا المتنخرة. وأخيرا أظهرت صبغة ماسونس تريكروم اللون الأخضر لألياف النسيج الليفي المتكاثر حول الكيس العدري في الخلايا الكبدية المتنخرة. نستنتج أن الإصابة بالأكياس العدرية في أكباد الأغنام تسبب تغيرات مرضية نسيجية متنوعة تم الكشف عنها بواسطة عدة صبغات خاصة.