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Original Article

Ultrastructure of Human Fertile *Hydatid Cysts* Using a Scanning Electron Microscope (SEM)

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Abstract

Introduction: Echinococcosis and hydatidosis caused by the metacestode of *Echinococcus granulosus* are among the most important zoonotic diseases in the world. This study aims to study the ultrastructure of fertile hydatid cysts that infect humans using a scanning electron microscope (SEM).

Materials and Methods: Twenty samples of human fertile hydatid cysts were collected from the human liver and lung after performing surgery operations and examined with an SEM.

Results: The results of the electron microscopy with different magnifications revealed that the laminated layer (LL) consists of sheets that appeared more compact and aligned. The brood capsules appeared, consisting of a net of finger-shaped structures that emerged from bulges of various sizes and shapes.

Conclusion: Under a transmission electron microscope, it was found that the LL had a coherent and flexible structure, settling on a three-dimensional microscopic network of hydrophilic fibers, with high humidity. These fibers were arranged irregularly and had a diameter of about 10 nm; therefore, the fibers adjacent to the germinal layer (GL) were possibly attached to microtriches of tegument, which reached a thickness of 1 mm in the LL.

Keywords: *Echinococcus granulosus*, Hydatid cysts, Scanning electron microscope (SEM)

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Introduction

The hydatid cyst is the larval stage of *Echinococcus granulosus* and is found in tissues and other structures of the intermediate host (1). It is spherical or almost spherical in shape and takes the shape of the affected organ, and its size varies with age. The growth rate of the hydatid cyst depends on the elasticity of the organ and surrounding tissues (2). Lung cysts grow faster than liver cysts, as the lungs are much softer than the liver. Negative pleural pressure may increase the rate at which cysts grow (3). Hydatid cysts grow in children at a faster rate and become larger than those in adults due to the fact that their lung tissues are highly elastic (4).

Carnivores of the Canidae family are the final hosts of *E. granulosus*, as these worms develop in the small intestine of the final host. The gravid segments that contain the eggs separate from the body of the parasite and pass through the feces to the external environment. The released eggs are characterized by their great ability to resist harsh environmental conditions for several months or even a year, depending on the environmental conditions (5). Infection occurs when the intermediate hosts (humans, camels, sheep, cows, buffaloes, pigs, etc.) eat food contaminated with the parasite eggs. Humans may also be infected when touching dogs, especially children, when the parasite eggs adhere to the dogs' hair (6).

The appearance of *E. granulosus* is one of the important criteria in taxonomic studies. Studies have differed in terms of the morphological features chosen in the study. Despite this, recent studies have focused on marshmallow calluses in both larvae and adults, in addition to ovarian shape and testicular distribution of granular echinococcosis to distinguish between breeds collected from different intermediate hosts in Europe (7) and Spain (8).

Materials and Methods

Hydatid Cysts Collection

Twenty samples of human fertile hydatid cysts were collected from the human liver and lung after performing surgeries, which was done in some hospitals in the central Euphrates governorates from January to December 2019. The samples were preserved with 10% formalin solution.

Scanning Electron Microscope

Samples were sent to the Department of Life Sciences, College of Science, University of Kufa, for examination with an scanning electron microscope (SEM). For investigating the outer shape of the protoscolices and layers of the hydatid cyst, electron microscope slides were

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prepared according to the following steps.

Fixation

The saturated solution previously prepared was placed in a scintillation bottle. The sample was chopped and placed into a scintillation bottle along with the solution. The bottle was kept closed so that no air could reach the sample. The sample temperature was kept at 4°C for 12-24 hours.

Osmium Tetroxide Step

The osmium tetroxide solution can be kept in a cold room or under freezing conditions for one month. It should be allowed to thaw at room temperature. This solution should be straw colored. It becomes unusable if it turns purple. The osmium tetroxide solution was diluted to a 1% solution in 25 mM phosphate buffer (25 mM as the final concentration of the phosphate buffer solution). The fixative was poured out and a 1% osmium tetroxide solution was added to it. It was kept in a cold room from 24 hours to several days until the osmium tetroxide solution turned black.

Dehydration

The solution of osmium tetroxide was poured, and the samples were washed 3 times with 25 mM phosphate buffer, making sure to discard the solution of osmium tetroxide. The samples were placed in a series of ascending concentrations of ethanol alcohol for 15 to 30 minutes in each step (30%, 50%, 65%, 75%, 89%, 95%, and 100% concentration four times). Then, the samples were placed in absolute alcohol for a day or two.

Critical Point Drying

The samples were placed in special baskets. They were fixed with alcohol. Drying was done in SEM facility.

Coating with Gold

Sputter coating of the samples was done with gold particles.

Results

The results of the electron microscopy with different magnifications showed precise morphological characteristics of the hydatid cyst. Under the SEM, sections of the hydatid cyst showed that the laminated layer (LL) consisted of plates that appeared more compact and aligned, and these plates showed different degrees of pressure adopted by a single type of ultrastructure (Figures 1A-1D).

The germinal layer (GL) of the cyst wall was shown to have the same surface ultrastructure as in the brood capsules. It appears that it is composed of a network of finger-shaped structures that emerge from bulges of various sizes and shapes without protrusions, and these bulges indicate the development of the laminar layer (Figures 2A-2D).

It invaginated inward protoscolices from the hydatid cysts of the human being, appeared from the liver. The lungs are small, oval-shaped, covered with a thin layer of wavy and shrunk tegument, and the rostellum area appears in a lower or furrow in the anterior part of the dented protoscolice and the stalk is not protruding.

In more advanced protoscolices, the protoscolice is linked to the GM by a small, clear neck, which is distinct from Figures 3A-3H.

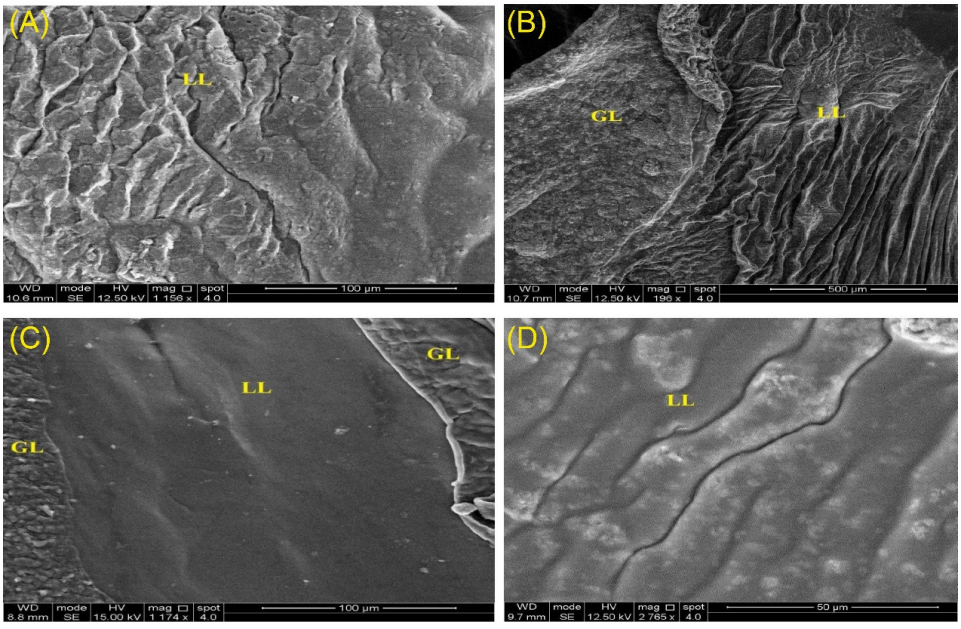

Figure 1. (A) Laminated Layer (LL) Composition and Uniform Layering (Magnification: 1156X). (B) LL Composition and Uniform Layering (Magnification: 196X). (C) LL Composition and Uniform Layering (Magnification: 1174X). (D) Composition of Sheets and Layering (Magnification: 2765X).

Discussion

The results of the electron microscopy with different magnifications showed accurate morphological characteristics of the hydatid cyst. Under the SEM, sections of the hydatid cyst showed that the LL consisted of sheets that appeared more compact and aligned, and these plates showed different degrees of pressure that adopted by a single type of ultrastructure.

Under a transmission electron microscope, it was found that the laminate layer had a coherent and flexible structure. It settled on a three-dimensional microscopic network of hydrophilic fibers, with high humidity. The fibers were arranged irregularly and had a diameter of about 10 nm (9-11). The fibers adjacent to the GM were possibly attached to microtriches of tegumental, which had a thickness of up to 1 mm in the lamellar layer (9).

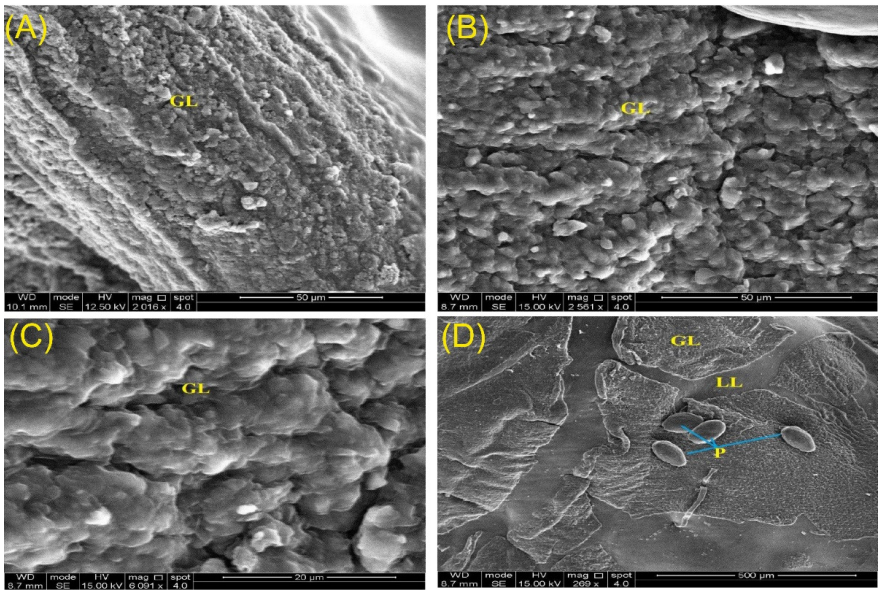

Figure 2. (A) The Composition of the Germinal Layer (GL) and the Laminated Layer (Adjacent LL) (Magnification: 794X). (B) GL and Finger Structures (Magnification: 2016X). (C) GL and Finger Overlays (Magnification: 2561X). (D) GL and Finger Overlays (Magnification: 6091X).

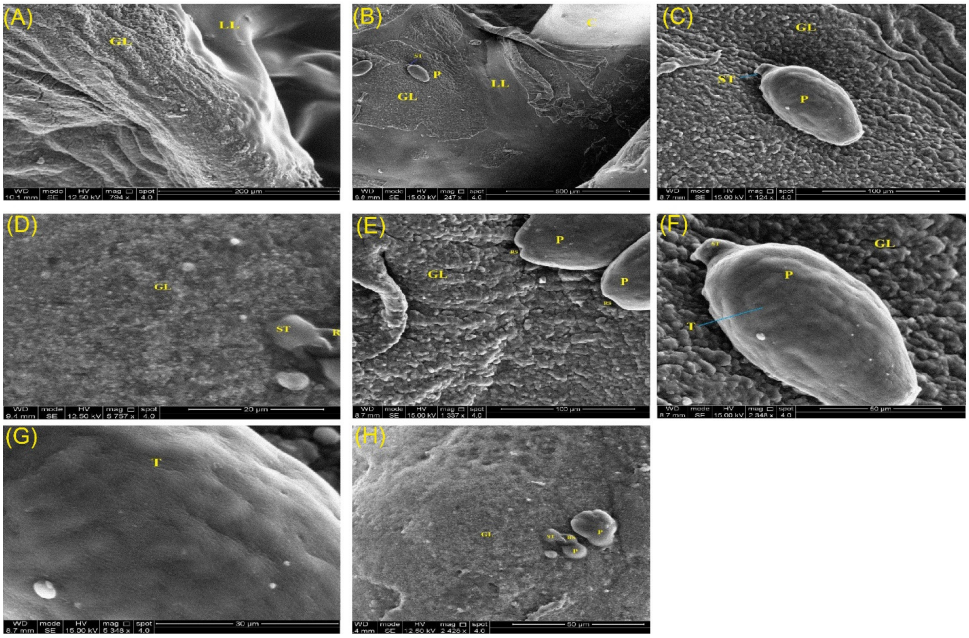

Figure 3. (A) Protoscolices (P,) Germinal Layer (GL), and Laminated Layer (LL) (Magnification: 269X).
(B). P, the GL, the LL, and a Part of the Capsule (C), Indicating the Attachment of the Head to the GL through the Stalk (ST) (Magnification: 247X). **(C)** P and GL Attached to the GL via the ST (Magnification: 1124X). **(D)** P Linked to the GL through the ST and also the Rostellum Cavity (RS) (Magnification: 5757X). **(E)** P, GL, and RS (Magnification: 1337X). (**F**) An Enlarged Portion of P Associated with the GL through the ST and the Tortuous Structure of the Tegument Layer (T) (Magnification: 2348X). (**G**) The Winding Composition of the T Layer in P (Magnification: 5348X). **(H)** P, GL, Neck (ST) and RS (Magnification: 2428X).

The laminar layer owes its name to the concentric laminations that appear under an optical microscope, while it appears under an SEM as an airtight layer in the *E. granulosa*, divided in an open book-like shape in *E. vogeli* (12). The origin of the plating is unknown, but it appears as a result of different degrees of pressure exerted by the host on the surface of the hydatid cyst when studied with a transmission electron microscope (10).

The present study also showed that the GM of the cyst wall has the same surface ultrastructure found in brood capsules. It appears that it is composed of a network of finger-shaped structures that appear as bulges of various sizes and shapes without protrusions, and these bulges indicated the development of brood capsules from the GM, which is consistent with previous studies (13-15).

This also agrees with another study (16) in which these structures were considered to represent different developmental stages of the protoscolices, from immature invaginated to mature evaginated protoscolices. The researcher clarified that the protoscolices collected from the livers and lungs of camels have similar structures in terms of morphological measurements. Additionally, in another study, it was observed (16) that the GM contains decomposed and non-dissolved protoscolices. The GM contains viable protoscolices (17). Moreover, embryonic or stem cells located on the GM possessed the ability to develop new brood capsules (18).

Conclusion

The results of the electron microscopy with different magnifications indicated that the GM of the cyst wall has the same ultrastructure found in brood capsules.

Conflict of Interests

The authors declared that no competing interests exist.

Ethical Issues

In this study, ethical considerations have been fully observed.

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Authors' Contribution

The authors did writing, editing of the manuscript, statistical analysis, and data collection.

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