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ECHINOCOCCUS GRANULOSUS: HISTOPATHOLOGY OF NATURALLY INFECTED SHEEP LIVER*

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Abstract: Histological sections of both uninfected and naturally infected sheep liver with hydatidosis were studied. Intensity of histopathological changes was found proportional to the size of cyst. Small cysts (1-2 mm diameter) showed pathological changes only close to the cyst wall. The hepatocytes were deformed due to reduced cytoplasmic contents and large gaps were seen around the fibrous cyst wall. Sinusoidal spaces were however expanded forming aster like channels around the cyst wall. Some of the hepatic cells were also found swollen. Medium (1 cm) and large (3-4 cm) cysts showed prominent changes near to the cyst wall, like pyknotic nuclei, heavy leucocytic infiltration and fibrosis. Hepatocytes also showed cytoplasmic and nuclear vacuolation near the wall of medium and large cysts. Some of the cysts also showed pressure atrophy, which was more common in larger cysts. Hepatic architecture was completely destroyed while prominent changes near the cyst wall included increase in sinusoidal spaces and bile canaliculi. Portal veins and lymphatics became indistinguishable. The histopathology of hepatocytes surrounding the cyst were noted at various distances from the cyst wall with the findings that the damage was inversely proportional to the distance from the cyst.

Key words: Echinococcus granulosus, sheep, liver, histopathology.

INTRODUCTION

Pandey (1971) has reported that the damage produced by the hydatid cyst in liver, lungs and spleen of goats was both mechanical and toxic. Svilenov *et al.* (1978) studied histopathology of *Echinococcus multilocularis* cysts in the liver of *Meriones unguiculatus* and reported that connective tissue membrane around the cysts was rich in lymphocytes, histiocytes and occasional eosinophils. Remaining liver tissue showed dystrophic-granulomatous changes. Logachev and Bat'kaev (1979) studied multilocular hydatids in 6 to 130 *Marmota bobac* in Kazakh (U.S.S.R.). In 2 cases, all the organs and the serous membrane of the abdominal cavity were affected. In the remaining 4 animals, the cyst almost entirely filled the liver and included numerous chambers of varying size, up to 3

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cm in diameter. Khan (1982) worked on incidence in sheep (8.27%), goat (7.55%), cow (9.59%) and buffalo (12.25%) in Lahore district, Pakistan and reported that females were more susceptible than males among all the four groups of animals. Singh *et al.* (1988) studied the pathology of experimental *E. granulosus* in sheep, guineapigs, hamsters and mice. The gross pathological changes seen were congestion and haemorrhages in the internal organs. Hepatomegaly and splenomegaly were observed in the guinea pigs and mice. Microscopic examination showed cut sections of the cysts in internal organs, possessing a laminated cyst wall and an inner layer of germinal epithelium. The cyst wall was found surrounded by a zone of necrotic tissue and a connective tissue capsule infiltrated mostly with mononuclear cells and few polymorphs. Matto *et al.* (1990) reported that in buffaloes liver cysts were usually large in size and surrounded by infiltration of foreign body giant cells, macrophages, lymphocytes and a thick fibrous capsule. Markovics *et al.* (1992) studied four large and several smaller cysts of *E. granulosus* in the liver of a baboon. Three of the large cysts had an atypical structure and were packed with a semi-solid mass of collapsed daughter cysts, small cysts containing fluid and involuted membranes. Present study was aimed to work out the histopathological changes in sheep liver and also to investigate the effective range of hydatid cyst fluid (HCF) and cystic pressure towards the damage of hepatic parenchyma.

MATERIALS AND METHODS

Out of one thousand livers examined at local slaughter house, only five uninfected and ten which harbored unilocular hydatid cysts of various sizes were collected and fixed quickly after the animal was slaughtered. The infected tissue was cut in long strips of 0.5 x 0.5 x 3.0 cm in such a way that a portion of the cyst was also included at one end of the strip, while the other end was farthest from the cyst. The sections (4-5 μm thick) were cut and stained in hematoxylin and eosin. Both progressive and regressive methods were used. The stained sections were studied to observe pathological changes.

RESULTS

Fig.1 showed normal hepatic architecture of sheep. The cysts were divided in three categories according to their diameter, small (1-2 mm), medium (1 cm) and large (3 cm).

Small cysts

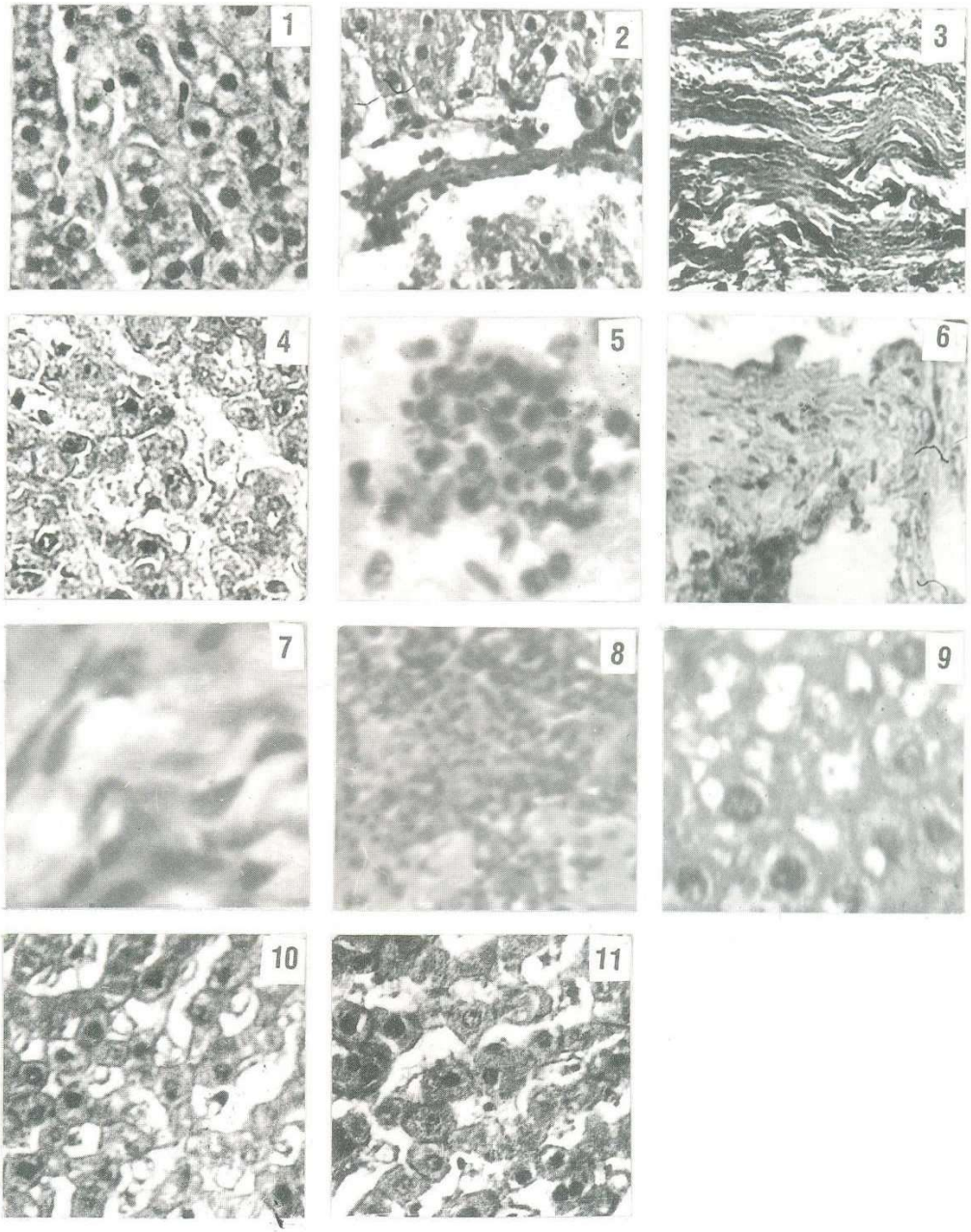
Figure 2 showed a tissue containing very small cysts. The germinal layer was covered by laminated layer and well pronounced fibrous tissue layer surrounded the cyst. This layer seemed to be formed by highly compressed cells in which the membranes had been obliterated. The hepatic nuclei were elongated, dark and solid showing indications of pyknosis. The depth of the fibrous area extended from 0.022 to 0.047 mm (Fig.3). There were some indication of abnormal cytoplasmic vacuolation in the area beyond the fibrous tissue along with slight disturbance in the arrangement of hepatic cords and with no signs of any active repair process. Some of the hepatocytes were deformed due to reduced cytoplasmic contents. Large gaps in the tissue were seen around the fibrous cyst wall. Sinusoidal spaces were however, expanded forming aster like channels around the cyst wall (Fig.2).

Medium cysts

The histopathology of such cyst was noted at the distance of zero to 0.5, 0.5-1, 1-1.5 and 1.5-2.0 cm from the cyst. Zone of zero to 5 cm showed that considerably thickened cyst wall in which laminated layer was covered by fibrous area measuring 0.136-0.181 mm. The nuclei (not visible in compact cyst wall but in loose tissue) in the fibrous areas were definitely pyknotic and the cell membranes not discernible. The cells near fibrous layer seemed to be compressed with disturbed arrangement of hepatic cords, occasional cytoplasmic vacuolation, heavy infiltration of leukocytes along with visible change in the nuclear size of hepatic cells. In the zone of 0.5-1.0 cm the nuclei of the hepatocytes appeared comparatively normal and hepatic cords were better arranged with reduced cytoplasmic vacuolation while the blood vessels were packed with lymphocytes. However, some of the cells were slightly swollen. In the zone 1.0-1.5 cm, arrangement of hepatic cords was unaltered with almost normal nuclei. Number of lymphocytes in the blood vessels were less as compared to previous zone. There was no sign of pressure atrophy. At distance 1.5-2.0 cm from the cyst the arrangement of hepatic cords, hepatocytes and their nuclear sizes were almost normal. Structurally, cells and the nuclei were functional while number of lymphocytes in the blood vessels was higher than normal but lesser than those found in the previous zone (Figs.4,5).

Large cysts

Largest cysts processed during present study were between 3.0-4.0 cm in diameter. Their cyst wall was well developed, thick, fibrous and stained poorly with eosin. Main zone, 0-0.5 cm was subdivided into three sub zones: Subzones SZ1, 0.0-0.1 cm, SZ2 0.1-0.4 cm, SZ3 0.4-0.5 cm from cyst. SZ1 (Figs.6-8) consisted of a germinal (cellular) and laminated (acellular) layer which was again covered by a fibrous layer that extended from 0.036 to 0.091 mm (Fig.6). The cyst wall was followed by a narrow cellular area in which cell membrane had been lost completely. Parenchyma in the close vicinity of the cyst wall was completely lost which formed very large intercellular spaces that were exwardly directed (in which the tissue had disappeared). Cells where present, were very small in size and lobular pattern was entirely missing. In some regions majority of the nuclei were deshaped probably due to pressure and/or toxic atrophy. The number of nuclei per cell were not countable in most of the cells. Some of the nuclei were much elongated. Sinusoidal spaces, bile canaliculi, protal veins and lymphatics were not visible in the close vicinity of cyst wall. A large number of lymphocytes, polymorphs and eosinophils were present in this region. In SZ2, the cells were highly compressed (Fig.9) with deshaped nuclei. Some of them were pyknotic, the cell membranes were diffused, blotted and vague. The arrangement of hepatic cords were greatly disturbed. Sinusoidal spaces, bile canaliculi, portal veins and lymphatics were not distinct. The cytoplasm showed vacuolation and colloidal disintegration. Portal veins were visible, hepatic lobules damaged but with visible boundaries. Kupffer cells were present representing the phagocytic action. SZ3 showed recognizable cells but with poor hepatic architecture and large sinusoidal spaces, representing oedemic condition. Majority of the nuclei were pyknotic but portal areas were visible. Hepatic arteries and veins in this area were thick walled with smaller diameter and tightly packed cells in some areas. Kupffer cells were present indicating on going of the repair process. Number of the nucleoli per nucleus was not countable in most of the nuclei because of pyknosis. Cytoplasm was highly granulated with diffused cell membranes. Most of the veins and arteries were broken and degenerated in this zone. Erythrocytes were also seen in the sinusoidal spaces (Fig.10).



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In zone 0.5-1.0 cm most of the nuclei were pyknotic, others possessed 2-3 nucleoli per nucleus. The nuclear membranes were clear, solid and the nuclei were large in size. Vacuolation was only present in some of the nuclei. Large number of lymphocytes were present in the blood vessels while a few hepatocytes showed division representing a little repair activity. Condition of cell membrane was still very poor, while the cells were spherical to oblong. Arrangement of hepatic cord and general condition of the cell was very poor. The size of the cell was however, improved, while bile ducts were thick walled, dilated with free bile patches, visible in sinusoidal spaces. Kupffer cells were occasionally present and lymphatic vessels were large in this zone. Condition of bile canaliculi were very poor due to desolated and dilated hepatic cords. Blood vessels were however, compact thick walled and no pressure atrophy was seen in the hepatocytes. Sinusoidal spaces were expanded representing oedemic condition (Fig.11).

Zone 1.0-1.5 cm showed slight improvement in the arrangement of hepatic cords. The number of nucleoli were usually two per nucleus. Nuclear and cytoplasmic presence vacuolation. Nuclear membranes were clear and well demarcated. Only a few nuclei were undergoing mitosis. Nuclear size was larger than the nuclei present in the previous zone but smaller than control. Condition of cell membrane was improved, cells were spherical to oval but their typical hexagonal condition was not maintained. Arrangement of hepatic cord was in better position. Bile ducts were close to the normal. Kupffer cells were present but lymphatic vessels still expanded. No pressure atrophy was seen in this zone but sinusoidal spaces were still expanded and free bile patches were seen. Hepatic arteries and veins were thick walled but not compressed.

In zone 1.5-2.0 cm proportion of pyknotic nuclei was comparatively less. The degree of vacuolation and colloidal disintegration was unchanged. The cells were swollen but in some cells there were three nucleoli per nucleus. Some of the nuclei were hypertrophied and the cells were hexagonal, hepatic cord were improved and hepatocytes were arranged according to hepatic architectural. Bile canaliculi were visible in this zone but were highly expanded. No pressure atrophy was seen in this zone. Sinusoidal spaces were packed with erythrocytes, polymorphs, eosinophils, monocytes and lymphocytes.

Fig. 1: Showing a normal hepatic architecture of sheep with sharp and well demarkated cell membrane, cytoplasm clear and large & active nuclei (X1000); Fig. 2: Showing a small cyst (1.0 mm diameter). Fibrotic layer around the cyst is formed by highly compressed cells. Large spaces in the tissue are seen around the fibrous cyst wall. Sinusoidal spaces have been expanded forming aster like channels around the cyst wall (X400); Fig. 3: Closest to a small cyst showing fibrosis in the cyst wall. Many macrophages are penetrating in the cyst wall (X1000); Fig. 4: Showing a medium cyst (1.0 cm diameter). Some of the hepatic nuclei are pyknotic with prominent, cytoplasmic vacuolation. The arrangement of hepatic cords is remarkably disturbed. Sinusoidal spaces have been enlarged and expanded (X1000); Fig. 5: Close to a medium cyst (1.0 cm diameter) showing pyknotic nuclie, cytoplasmic disintegration, numerous lymphocytes, polymorphs and kupffer cells (X1000); Fig. 6: Showing a large cyst (4.0 cm diameter) with visible fibrosis in thick cyst wall alongwith many macrophages penetrating in the thick cyst wall (X1000); Fig. 7: 1.0 mm from the cyst (4.0 cm diameter) showing hepatocytes surrounding the cyst wall have undergone necrosis, while nuclear and cell membranes are dissolved. Both hepatocytes and nuclei have been elongated due to pressure atrophy and also by toxic invasion of HCF (X1000); Fig. 8: 0.01-0.10 cm from a large hydatid cyst (4.0 cm diameter) showing hepatic nuclei are pyknotic. Cytoplasm shows colloidal disintegration. The arrangement of hepatic cords has been disturbed. Numerous defensive cells are visible (X400); Fig. 9: 0.1-0.4 cm from the large cyst (4.0 cm diameter) showing hepatic nuclei are pyknotic. Cytoplasm shows colloidal disintegration and vacuolation. The arrangement of hepatic cords has been disturbed (X1000); Fig. 10: 0.4-0.5 cm from a large hydatid cyst (4.0 cm diameter) showing hepatic nuclei are with dissolved membranes. Cytoplasm shows colloidal disintegration. The arrangement of hepatic cords has been disturbed. Cells have almost lost their shapes (X1000); Fig. 11: 0.5-1.0 cm from the large cyst (4.0 cm diameter) showing hepatic nuclei are pyknotic. Both cytoplasm and nucleoplasm shows colloidal disintegration and vacuolation. The arrangement of hepatic cords has been disturbed while sinusoidal spaces dilated (X1000).

In zone 2.0 to 2.5 cm nuclear and cytoplasm vacuolation was absent, nuclear and cell membranes were clear and well demarcated. Hepatic lobules were intact while, sinusoidal spaces were packed with erythrocytes and lymphocytes. Hepatic arteries and veins were normal and filled with blood cells.

Zone 2.5-3.0 cm was close to that of normal. The nuclei were spherical, with no nuclear or cytoplasm vacuolation. Bile ducts, arteries, veins, lymphatic vessels and hepatic lobules were all normal. A few Kupffer cells were however, present.

DISCUSSION

The appearance of vacuole, atrophy of some of the hepatic cells and extensive hypertrophy of hepatic nuclei were the typical signs of hepatotoxicity. The atrophy of the hepatic cells indicate some abnormalities in cell membrane permeability. Hypertrophy of some of the hepatic nuclei at 1.00 cm of largest cyst may be because of increased nucleic acid content. This has also been supported by increased nucleic acid content (Anwar and Tanveer, 1996) in rabbits infected with hydatidosis. Two facts were already known that the cyst wall was permeable and that when released in the body the hydatid fluid can induce a severe anaphylactic reaction in the body. The growth rate of the cyst was very slow (Chandler and Read, 1961). The present findings showed that in case of small cysts there was practically no prominent effect on the host tissue and cells and nuclei were also normal and functional. This is expected because at this stage neither the size of the cyst is large enough nor the host tissue has been exposed to high quantities of HCF. As the cyst grow in size the cells in the immediate vicinity of the cyst showed a pressure atrophy. The cell membranes of hepatocytes were destroyed and nuclei become elongated and pyknotic and the progressive fibrosis started. There was heavy leukocytosis which showed damage to the tissue. In large growing cysts in addition to this the host tissue showed fibrosis, cirrhosis, even upto a distance of 2.0 cm from cyst. The HCF and/or autolysis based proteolytic enzymes were definitely in action and damaged the plasma membranes of hepatocytes and interfered the permeability of the plasma membrane, which probably resulted in the seepage of such cytoplasmic enzymes, metabolites and plasma constituents out of cell membrane. The remarkable damage in the hepatocytes upto 2.0 cm from the large cyst can be attributed to the pressure atrophy as well as to the toxins present in the fluid that had been continuously seeping out of the cyst in quantities varying according to the cyst size. From Pakistan no significant work has so far been done on this aspect of hydatidosis which is of great medical and veterinary importance.

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