Histomorphological Studies of Schistosomiasis in the Liver of Mouse
Illahi Bux Kalhoro* and Hameeda Kalhoro1
Department of Anatomy and Histology, Sindh Agriculture University, Tandu Jam, Pakistan
1Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro, Pakistan

Abstract
The aim of this study was to observe the histomorphological alterations in the livers of mice infected with Schistosoma (S) mansoni and S. margrebowiei after 35 and 42 days post-infection (DPI). The most significant changes were the appearance of single or multiple eggs and female worm in the blood vessels of the liver of mice. Some of the eggs were enclosed by miracidium, which were surrounded by degenerated red blood cells and few connective tissue cells. Few eggs were surrounded by granuloma and in some of the areas of liver early necrosis were also seen. Few blood vessels were blocked, heavily infiltrated with inflammatory cells, thrombotic and many eggs trapped in them. Infiltration of cells consisted of a few basophils, plasma cells, monocytes, giant cells, migratory neutrophils, some lymphocytes, dividing cells and eosinophils in both parasite infections. The endothelial cells were enlarged, swollen walls of blood vessels and increased sinusoidal spaces. Some of the hepatocytes around granuloma were shrunken, atrophy, fatty degeneration, pyknosis and karyorrhexis was also visible. It was concluded that after 35 DPI, due to the presence of eggs infiltration of cells granuloma, early necrosis, increased sinusoidal spaces; the shrunken, atrophy and fatty degeneration of hepatocytes were seen. In addition to these blood vessels were mainly blocked and heavily infiltrated in both parasite infections.

Keywords: Histomorphology, Liver, Mouse, Schistosoma mansoni, Schistosoma margrebowiei

Introduction
Schistosomiasis, known as bilharziasis, a parasitic disease that leads to chronic ill health and is caused by genus Schistosoma. It is the major health risk in the rural areas of Central China, Egypt and continues to rank high in other developing countries (Wiwanitkit, 2005). The disease spread by egg-laying adult parasites that reside in the mesenteric veins of their vertebrate hosts (Ross et al., 2002). It causes significant morbidity, mortality and severe disease is associated with defective regulation in the human population that it affects (Wynn et al., 1998). This disease is widespread in domestic ruminants in the Indo sub-continent and adjacent areas (Franeeen et al., 1990).

In the case of S. mansoni, mature female worms produce hundreds of eggs a day, of which many exit the body through the gut. A substantial number of eggs are trapped in host tissues such as the liver and intestine of the mouse (Grzych et al., 1991, Pearce et al., 1991, Wynn et al., 1993, Kaplan et al., 1998). Among the affected organs, the lungs, intestines (Hirata et al., 1993), and spleen are the major sites of egg deposition in human schistosomiasis (Andrade and Andrade, 1965). During infection of vertebrate hosts with S. mansoni, trapped eggs induce tissue damage and granulomatus lesions that interfere with normal organ functions (Muller et al., 2001). Acute schistosomiasis or Katayama fever is a clinical syndrome that occurs within three to six weeks after infection with S. mansoni and S. japonicum (Khalil et al., 1993). It is marked by hepatomegaly and eosinophilia usually begins before large number of eggs continues to be deposited in host tissue (Von Lichtenberg, 1964). In Pakistan, the urinary schistosomiasis is also sporadic. It is noted in Pakistan that schistosomiasis should be included as a viable differential for hematuria in travelers (Khalid and Mahmood, 2001). In control mice hepatic changes includes portal preiphlebitis, vesicular obstruction, infarction leading to arterIALIZation and preferential sinusoidal channeling. Whereas, deprived mice showed greatly reduced eggs reactions composed principally of macrophages, monocytes and occasional neutrophils, and only minimal alteration of liver architecture; however, focal and disseminated hepaticellular lesions became prominent as the infection progressed, and by day 18 virtually every hepatocyte was effected (Byram et al., 1979). Macroscopic and histopathological studies showed multiple liver abscesses around granulomas of S. mansoni in the acute and chronic phases of schistosomiasis. Infection of mice with S. mansoni is associated with hepato-intestinal disease, which is
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characterized by the formation of granulomas around the parasite eggs that become lodged in host tissues. The egg-induced liver pathology evolves through an acute phase at approximately 8 weeks post-infection, which is characterized by vigorous granulomatous inflammation (Andrade and Warren, 1964). The following study described the histomorphological changes in the structures of liver of mouse due to appearance of *S. mansoni* and *S. margrebowieiei* eggs.

**Materials and Methods**

Age matched 12 female mice of the Bantam and Kingman Tyler’s Original (BKTO) strain, weighed approximately 20-35g each were infected with cercariae of either *S. mansoni* (Puerto Rican Strain) maintained in albino Biomphalaria glabrata snails and random-bred to mice or *S. margrebowieiei* (originally obtained from Lochinar National Park, Zambia) and maintained in Bulinus natalensis intermediate host snails (the original stock was obtained from the Experimental Taxonomy unit of the British museum of natural history, London). Before administrating the cercariae, the experimental animals were anaesthetized with sodium pentobarbitone (Nembutal). The abdominal skin was shaved and the mice were placed on their backs in wooden holding racks and gently secured with sellotape. The shaved skin was moistened with aquarium water and cercarial suspension (1 ml/animal) was placed through plastic ring. All 12 mice were killed at day 35 and 42 and autopsies were performed immediately after the animals were killed by dislocation of neck region. The liver from each animal was fixed in Heidenhain’s Susa fixative, washed, dehydrated in ethanol, infiltrated and embedded in histoiresin. Selected 4μm thick sections were stained in polychrome method (Blackstock, Pers. Comm.) and 1% toluidine blue in 1% borax staining methods for general histomorphology. The morphometric study included at least 5 measurements for length and width of the granuloma and infiltration of cells was measured in micron with occlumeter. The sections of liver of mouse were interpreted on Ernst Leitz Wetzler Light Microscope (Model No.786554).

**Results**

**Appearance of the *S. mansoni* and *S. margrebowieiei* eggs in the liver of mouse**

Eggs at different stages of maturation, i.e. both immature and mature eggs were observed on 35 and 42 DPI in the center of granulomas and inside the capillaries and branches of veins of the liver of mice in both parasite infections. Intrauterine eggs were also seen in the enlarged muscular wall of the uterus of *S. mansoni* female worm on day 35 (Fig. 1). The granuloma with single and multiple eggs surrounded by various types of connective tissue cells. In some areas of liver the granulomas with various stages of eggs were surrounded by mainly eosinophils, some macrophages, lymphocytes and neutrophils. The mean diameter of granuloma appeared in *S. mansoni* infected liver was 150.89 μm and in *S. margrebowieiei* it was 148.70 μm after 42 DPI. At the center of granulomas are eggs; some containing a developing miracidium and others are empty eggshells. In the blood vessels of the liver of mice *S. mansoni* egg contained developed miracidium which was surrounded by eggshell and several vacuolated red blood cells were prominently visible close to it (Fig. 2). In *S. mansoni* infection some of the eggs with minimum and moderate types of cellular reaction, but in *S. margrebowieiei* few eggs were seen without these reactions. Miracidia of various morphological features were prominent in both parasite infections. **Histomorphological responses due to the schistosome eggs in the livers of mice**

Normal liver showed blood vessels, sinusoids and hepatocytes stained light blue foamy cytoplasm and pale nuclei with 1% toluidine blue (Fig. 3). After 35 DPI it was occasionally observed that hepatocyte nuclei were irregular in shape in *S. mansoni* infection. In *S. margrebowieiei* increased areas of marked infiltration showed some hepatocytes with granular cytoplasm darkly stained. The hepatocytes were shrunken, irregular and diminished in size (Fig. 2). The pyknotic nuclei stained dark and foamy cytoplasm with patches of light red color. Few areas of fatty degeneration and early necrosis of hepatocytes were observed in *S. mansoni* infection. The some of endothelial cells lining the walls of the blood vessels were swollen, enlarged and yellow granules were seen in the sinusoids. The nuclei of hepatocytes, endothelial cells and some of inflammatory cells were stained dark with polychrome and pale blue with 1% toluidine blue. The nuclear membrane (envelope) appears as a dark blue-purple line due to deposit of granular chromatin scattered along the inner surface. The spaces of sinusoids were increased, yellow material; red blood cells, macrophages and granulocytes were prominent in both parasite infections. The blood vessels were inflamed and heavily infiltrated with increased numbers of connective tissue cells in both parasite infections. Numerous mast cells stained blue with uniform granules were observed in both parasite infections. Infiltration of cells consisted of a few basophils, plasma cells, monocytes, giant cells, migratory neutrophils, some lymphocytes, dividing cells and eosinophils predominated. The mean diameter of infiltration of cells at 35 DPI was 122.35
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Fig 1 Intrauterine S. mansoni eggs were visible after 35 DPI. Stain: Polychrome.

Fig 2 Mature S. mansoni egg containing miracidia in the blood vessel after 35 DPI.
Note: red blood cells in blocked blood vessel, infiltration of various connective tissue cells, irregular and pyknotic nuclei of shrunken and atrophied hepatocytes on both sides of blood vessel. Stain: Polychrome.

µm. The blood vessels containing eggs were infiltrated with connective tissue cells, thrombotic and some eggs were also observed in both parasite infections. Due to presence of eggs in blood vessels their walls were swollen and enlarged endothelial cells which contained yellow material. After 42 DPI granulomas had increased in size and were surrounded by various egg developmental stages and increasing yellow material in sinusoids in both parasite infections (Fig. 4).

Fig 3 Normal liver histology showing hepatocytes, sinusoids and blood vessels
Stain: 1% Toluidine blue in 1% borax.

Fig 4 Granuloma with single S. margrebowiei egg surrounded by various connective tissues cells after 42 DPI. Note increased spaces of sinusoids close to granuloma
Stain: 1% Toluidine blue in 1% borax.

Discussion
In this study histomorphological changes caused by S. mansoni and S. margrebowiei were observed in experimentally infected livers of mice. In the present study after 35 DPI S. mansoni and S. margrebowiei eggs were observed in the liver of mice. Whereas, Bogitsh (1975) had reported that the following oviposition by the female worm, the S. mansoni eggs usually can be observed within the liver of the vertebrate host. This finding is close agreement with present study of S. mansoni only. Amer (1994) has reported that the eggs after being laid by the worm, and developed embryos. Similar result was also noticed in the present study.
In the present study, eggs were deposited in the blood vessels of the liver of mice. The blood vessels were
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heavily infiltrated with inflammatory cells, thrombotic and some eggs trapped in them. Varying degrees of granulomatous reaction were also observed around the eggs in both parasite infections. These results are also agreement with the Cook et al., (1974) Saber et al., (1983), and Hirata et al., (1993). Eggs are deposited in venules and small vein of the viscera and may be extruded into the lumen of these hollow viscera may become trapped in the visceral wall, or may be dislodged from the venules and enter the venous blood to micro-embolize to distant organ. Eggs produce varying degrees of tissues lesions, such as a granulomatous response (Cook et al., 1974, Rocklin et al., 1980, & Saber et al., 1983). In present study formation of granuloma comprises large numbers of eosinophils, some macrophages, lymphocytes, few neutrophils and giant cells. Numerous mast cells stained blue with uniform granules were observed in both parasite infections. Whereas, Rocklin et al., (1980) and Khalil et al., (1993) have reported that during acute schistosomiasis stage in the liver with formation of acute granuloma comprising of eosinophils, neutrophils (Rocklin et al., 1980) and a few mononuclear cells around the eggs of S. mansoni (Khalil et al., 1993).

In the present study most of hepatocytes present close the granulomas were showing fatty degeneration and early necrosis in the both parasite infections. Whereas, Fransen et al., (1990) reported in natural infection of S. spindale the hepatocytes adjacent to the granulomas were necrotic. In the present study most of hepatocytes were shrunken, diminished in size and vacuolated nuclei and foamy cytoplasm was observed close to the granulomas. The results reported by Popper et al. (1961) that hepatocellular injury due to a wide variety of causes can stimulate secondary response of the liver, such as biliary-ductular proliferation, vascular alterations, and fibroblastic activity; these reactions may terminate in hepatic cirrhosis.

It was concluded from the present study that after 35 DPI due to the presence of various eggs granuloma, infiltration of cells and blood vessels were mainly blocked in both parasite infections. However, in S. mansoni infected liver intrauterine eggs were visible in the body of female worm. In addition to these, the shrunken, atrophy, fatty degeneration of hepatocytes and early necrosis in some of the areas of liver were seen. The nuclei of the hepatocytes close the egg contained embryo were pyknotic and in severely infected areas of liver karyorrhexis was also visible.

Abbreviation:- A = Apical gland, BV = Blood vessel, G = Penetration gland, GC = Germinal cells, GR = Granuloma, H = Hepatocytes, IUE = Intrauterine eggs, M = Miracidium, NM = Neural mass, RBC = Red blood cells, SI = Sinusoids, W = Worm.

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References


