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Immunohistochemical Evaluation of Syndecan-1 Expression in the Liver of Small Ruminants with Natural Liver Fluke Infection

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Abstract: This study was conducted to investigate the pathomorphology of damage in liver tissues of ruminants infected with liver flukes and the role of syndecan-1 protein in this damage. In the study, liver tissue samples from 62 ruminants with liver parasites were used. Histopathological examination of these tissues was performed using hematoxylin-eosin staining. An immunohistochemical staining procedure was performed through the streptavidin-biotin-peroxidase (ABC) method to determine the syndecan-1 protein. Upon the macroscopic examination, congestion, hemorrhage, necrosis, and fibrosis were observed in infected livers. Hemorrhage, inflammation, degeneration, necrosis, and hyperplasia of the bile ducts were observed in severe liver sections upon microscopic examination. Syndecan-1 protein immunohistochemically exhibited a strong immunopositive reaction in infected liver tissues. This study concluded that the release of syndecan-1 protein increased liver damage induced by liver flukes.
Keywords: *Immunohistochemistry, Liver, Small ruminant, Syndecan-1.*

Karaciğer Kelebekleriyle Doğal Enfekte Küçük Ruminant Karaciğerlerinde Sindekan-1 Salınımının İmmunohistokimyasal Olarak Değerlendirilmesi

Özet: Bu çalışmada karaciğer kelebekleri ile enfekte olan ruminnatlara ait karaciğer dokularında meydana gelen hasarın patolomorfolojisi ve bu hasarda sindekan-1 proteinin rolü araştırıldı. Çalışmada karaciğer paraziti tespit edilen 62 ruminnata ait karaciğer doku örneği kullanıldı. Elde edilen dokularda histopatolojik inceleme amacıyla hematoksilen eosin boyaması yapıldı. Sindekan-1 proteinin belilenmesi amacıyla streptavidin-biotin-peroksidaz (ABC) yöntemiyle immunohistokimyasal boyamalar yapıldı. Makroskobik incelmede enfekte karaciğerlerde konjesyon, kanama, nekroz ve fibrozis görüldü. Mikroskobik incelmede ise şiddetli karaciğer kesitlerinde kanama, inflamasyon, dejenerasyon, nekroz ve safra kanallarında hiperplazi gözlendi. İmmunohistokimyasal olarak enfekte karaciğer dokularında sindekan-1 proteinin şiddetli immunopozitif reaksiyon verdiği tespit edildi. Bu çalışma sonucunda karaciğer kelebeklerinin neden olduğu karaciğer hasarında sindekan-1 proteinin salınımının arttığı belirlenmiştir.
Anahtar Kelimeler: *İmmunohistokimya, Karaciğer, Küçük ruminant, Sindekan-1.*

Introduction

Liver fluke infections, also called fasciolosis or distomatosis, are parasitic zoonotic diseases prevalent in many species across the world. It produces significant economic losses since it causes weight loss, reduction in milk yield, susceptibility to bacterial illnesses, and especially morbidity and mortality in ruminants (Boray et al., 2017; Kaplan et al., 2001; Mas-Coma et al., 2009; Schweizer et al., 2005; Slifko et al., 2000). The disease is caused by the gastrointestinal trematodes *Fasciola* spp (*Fasciola hepatica*, *Fasciola gigantica*) and *Dicrocoelium dendriticum* (*D. dentriticum*). Drinking water contaminated with the feces of infected animals or eating aquatic plants with parasitic metacercaria cause infection. Young parasites developing in the metacercariae in the intestines migrate through the peritoneum to the liver and bile ducts, where they mature. During migration, these parasites in the liver and bile ducts cause significant hemorrhage, necrosis, and fibrosis in the liver (Dar et al., 2018; Khan et al., 2015; Mendes et al., 2012; Okoye et al., 2015: Talukder et al., 2010; Xia et al., 2015).

Syndecans are cell surface proteoglycans with a transmembrane domain, a C-terminal cytoplasmic intracellular domain, and an extracellular domain containing a core protein and bound to glycosaminoglycan (GAG) chains. Proteoglycans are located as hydrogels in the extracellular matrix (ECM) and enable the tissue to acquire mechanical strength (Roskams et al., 1996; Zvibel et al., 2009). These proteins are family members of four distinct genes (Syndecan 1, Syndecan 2, Syndecan 3, and Syndecan 4), each of which has different GAG chains and exhibits tissue-specific release. Syndecan 1 is a heparan sulfate proteoglycan (HSPG) released

primarily from the basolateral surface of liver cells and functions as a receptor of triacylglycerol-rich lipoproteins (TRLs) in the liver. It binds heparin sulfate in the extracellular structure and provides cell-cell and cell-matrix interaction as a cell surface protein. They have different functions, including co-receptor roles in chemokines and growth factors, and cellular migration and proliferation. Recent studies have reported that they have a role in liver cirrhosis, chronic cholestatic liver disease, bile duct proliferation, liver malfunction, and liver pathologies such as hepatocellular carcinoma (Hayashida et al., 2008; Matsumoto et al., 1997; Nam et al., 2017; Regos et al., 2020). This study aims investigates the role of the Syndecan-1 gene in liver damage induced by liver flukes, which are prevalent in ruminants.

Material and Methods

Sample Collection: The liver tissues of 62 ruminants with liver fluke were used in this study. The tissue samples were fixed in neutral (Ph-7.0) formaldehyde for histopathological and immunohistochemical analyses. Healthy liver tissues from the slaughterhouse were used as the control group. All stages of the study were carried out with the approval of the national ethics committee and under the supervision of the local ethics commission (Approval No: 2022/001-01.11).

Histopathological Analysis: Tissue samples were fixed in 10% neutral buffered formalin (pH 7.2–7.4) and taken for a routine pathology process. The tissue sections were passed through the xylol and alcohol series and stained with Hematoxylin and Eosin (HE). Samples were examined under a light microscope (Olympus DP–73, Olympus BX53–DIC microscope; Tokyo, Japan).

Immunohistochemical Staining: Formalin-fixed paraffin-embedded sections, performed on standard streptavidin biotin peroxidase complex (ABC) technique protocol (Zymed, Histostain Plus Kit, California, USA), after deparaffinization and rehydration procedures. The section was pre-treated using heat-mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 mins. The sections were incubated with Syndecan-1 (DL-101, sc-12765, Santa Cruz, USA), diluted as 1:50 overnight at 4 °C. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary antibody and visualized using an HRP conjugated ABC system. Diaminobenzidine (DAB, Dako/Denmark) was used as chromogen. Finally, the section was stained with hematoxylin for counterstain.

Results

Identification of Parasite Species: The detected parasites' species were identified based on their morphological structures and adult or immature forms of *Fasciola hepatica*, *Fasciola gigantica*, and *D. dentriticum* defined.

Macroscopic Findings: In the necropsy of the cadavers brought with suspicion of endoparasite, yellow contents ranging from 100-400 ml were observed in their abdominal cavity. Fibrin particles were found on the liver and in the abdominal cavity in some cases. It was remarkable that livers with fasciolosis were larger than usual and congested. Their surfaces include large areas of pale necrotic foci. The parasite migration routes in the shape of strips were bleeding. Around these sites, oedema and severe hyperaemia were observed. Fibrosis was noteworthy in some areas. There were severe hemorrhagic regions and necrosis and fibrosis in the parenchyma on the section surface. In some hamorrhage regions, parasitic forms were observed. In all the cases, the fullness of the gall bladder has drawn attention. Bile was found to be viscous with a blackish color. The bile ducts were dilated, and some cases encountered parasitic forms were in the duct.

Microscopic Findings: It was remarkable that there were large hemorrhage regions in the liver tissues where parasites were detected. Inflammatory cell reactions involving many neutrophils, leukocytes, eosinophils, lymphocytes, and macrophages were observed around these hemorrhage sites. Additionally, multinucleated giant cells were identified among these cells. Hemosiderin pigment was detected in some macrophage cytoplasms. The remark cords were enlarged and filled with erythrocytes and had an irregular shape. Some hepatocyte cytoplasms locally had bile-colored pigments. In some hepatocytes, degenerative alterations were observed upon cytoplasmic vacuolization. Parasitic forms drew attention in some locations. However, it was pointed out that large areas of connective tissue were grown resulting from the destruction caused by the migration of the parasite in a significant part of the liver (Figure 1a, 1b).

It was immunohistochemically observed that syndecan-1 gave a mild cytoplasmic reaction in hepatocytes around the parasite migration routes with hemorrhage. Likewise, hepatocytes with degeneration and necrosis were slightly positive. Also, hepatocytes in areas where fibrosis was formed exhibited a more severe immune reaction. The staining had a granular structure and concentrated mainly at the sites close to the cell

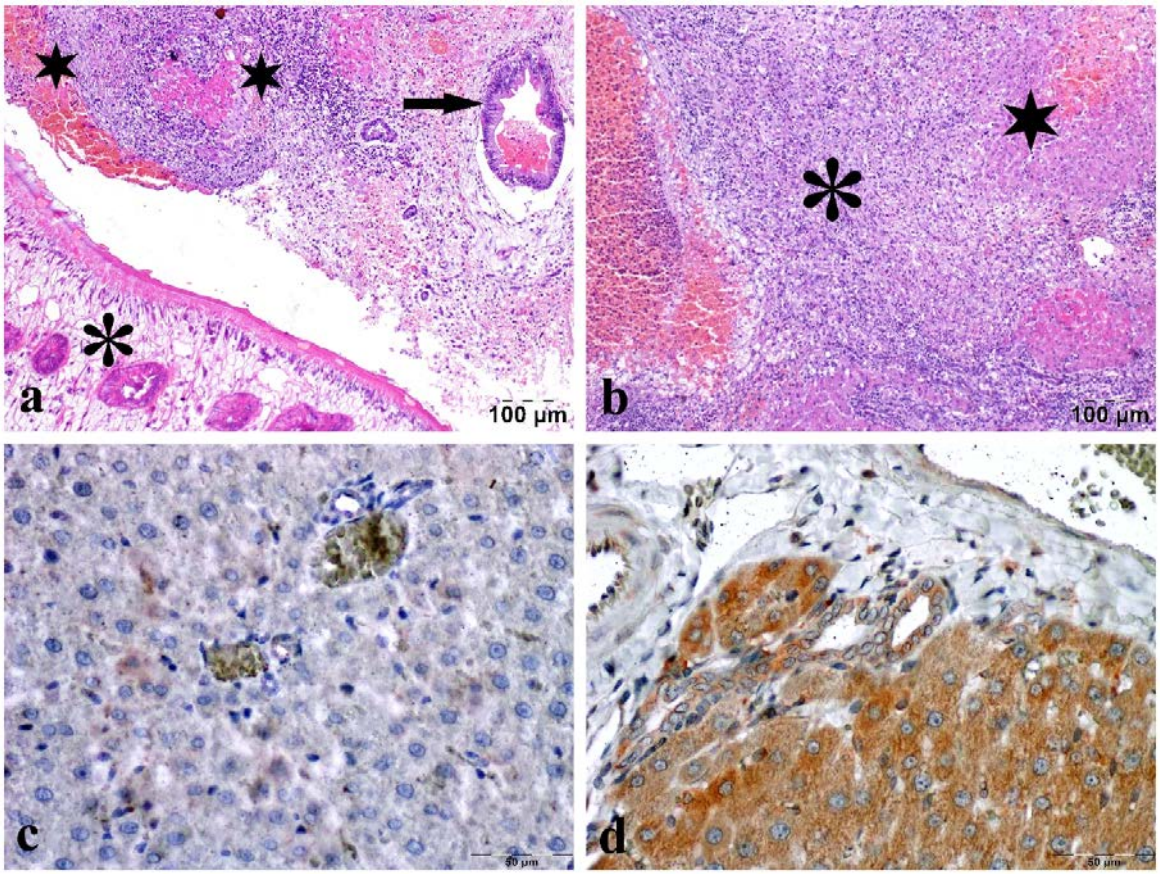

Figure 1. a) Immature fluke (asterisk) producing hemorrhage and necrosis (stars) and irregular proliferation of bile ductuli (arrow). b) Fibrosis and inflammatory response (asterisk) producing necrosis (star), HE. c) In control liver tissue mild immunpositive cells, ABC, DAB chromogen. d) Severe positive reaction of syndecan-1 in infected liver, ABC, DAB chromogen.

membrane. On the other hand, liver tissues from healthy animals utilized as a control group showed a mild reaction (Figure 1c, 1d).

Discussion and Conclusion

The studies on liver fluke parasites have revealed that they are common in many parts of the world, particularly in ruminants. The fight against this parasite results in significant economic losses (Schweizer et al., 2005; Slifko et al., 2000). These parasites, in particular, settle in the liver and cause mechanical and toxic damage to the organ, impairing vital functions. This liver damage causes the chemical mediators to release. These mediators result in the formation of inflammation and immune response. However, parasites that settle the liver migrate in the liver parenchyma and bile ducts and cause damage to the liver, including hemorrhage and necrosis (Dar et al., 2018; Khan et al., 2015; Talukder et al., 2010; Xia et al., 2015). The study by Dar et al. (2018) reported severe necrosis and fibrosis in liver tissues due to fasciolosis. In their study, Mendes et al. (2012) identified necrosis in acute hemorrhage and active granulomatous sites. Rahko (1969) examined the parasite's early and late pathological damage in the liver tissue and concluded that it resulted in cirrhosis due to hemorrhage and macrophage proliferation in the early period. They determined in the same study that thrombosis occurred in the blood veins of the parasite migration routes. The study by Oyarzún-Ruiz et al. (2014) identified hyperplasia and hypertrophy in the bile ducts and inflammation in some bile ducts. In the same study, they detected fibrosis and granulomatous response in the liver parenchyma.. Al-Khafaji et al. (2020) detected hemorrhage cholangitis, and pericholangitis associated with fibrosis caused by adult parasite migration in the liver parenchyma. In their study on goats, Talukder et al. (2010) observed fatty alterations with atrophy in infected liver tissues. In their study on cattle, Okoye et al. (2015) identified degeneration in infected liver cell walls and deformation in the nucleus. Belina et al. (2015) reported that fat necrosis, multifocal necrosis, inflammation in the bile ducts, cholangitis, pyogranulation, and fibrosis and cirrhosis in the portal areas histopathologically formed in the liver tissues. These damages in the liver affected the body's biochemical values, resulting in systemic

infections. Deger et al. (2008) compared the oxidant/antioxidant values in the liver tissues of healthy and infected animals. In their study, they determined that the MDA concentration and GPx activity in the liver tissues of animals infected with liver flukes were much higher than those in the control group. However, in the infected group, the Cu, Zn-SOD, CAT activities, and GSH, vitamin C concentrations were significantly lower. The same study reported that ALT and AST serum activities were extremely high in the infected group in the blood biochemical evaluation. In their similar study, Kitila and Megersa (2014) biochemically determined that serum ALT, AST, and ALP values increased in parallel with the severity of pathological damage. The pathomorphology of liver tissues infected with live fluke was evaluated in this study. The findings of the study are compatible with previous studies. Pathological findings of acute inflammation and hemorrhage were observed, particularly in the early phase of infection. Fibrosis was prevalent in the late infection phase, along with the findings on chronic inflammation.

Syndecan-1 enables cell-cell and cell-matrix interactions by being released by many epithelial cells, particularly hepatocytes. These genes have been found in metabolic disorders such as infectious, fibrotic, and diabetes. Recent studies have reported that they have a role in liver pathologies, such as cirrhosis, infectious diseases, and different liver cancers (Roskams et al., 1996; Zvibel et al., 2009). Li et al. (2016) observed that syndecan-1 release increased in liver damage caused by ischemia-reperfusion in liver tissue in rats experimentally induced hepatic ischemia-reperfusion model. Matsumoto et al. (1997) investigated the release of syndecan-1 in the liver tissue of 57 HCC patients by immunohistochemistry and protein expression methods. Their study determined that the immunopositive reaction was histopathologically more severe in cases diagnosed with well-differentiated HCC than less differentiated HCC tissues. In the same study, they observed a widespread positive reaction in hepatocytes and bile duct epithelial cells in cases with HCC developing with hepatitis. Metwaly et al. (2012) determined that the serum syndecan-1 level elevated considerably in patients with cirrhosis, and this increase was positively correlated with ALT and AST values. Zvibel et al. (2009) determined that syndecan-1 levels were significantly higher, particularly in patients with hepatitis and cirrhosis than healthy individuals. Roskams et al. (1996) determined the presence of syndecan-1 positivity in chronic cholestatic liver disease. Regos et al. (2020) conducted a study on tissue samples taken from patients with various liver disorders and determined that syndecan-1 was significantly immunopositive around hepatocytes and on proliferating bile duct surfaces in the cirrhotic liver. On the other hand, some researchers reported in their experimental studies that inflammatory response and tissue damage were more severe depending on the syndecan-1 deficiency. Hayashida et al. (2008) observed that in experimentally induced infectious toxic shock, mortality rates were higher in $Sdc1^{-/-}$ (syndecan-1-negative) rats than in normal rats and that liver tissue damage was severe along with vascular permeability. The same study reported that TNF-alpha and IL-6 significantly increased during the inflammatory response, and the inflammatory response got delayed. Nam et al. (2017) also determined that in $Sdc1^{-/-}$ rats with acetaminophen-induced liver damage, the mortality rate due to toxicity were higher, the serum ALT and AST levels elevated significantly, and histomorphologically, necrosis and apoptosis in the liver were more pronounced compared to normal rats. However, in the same study, they determined that treatment of syndecan-1 reduced serum ALT levels and histomorphological damage in these rats, and syndecan-1 had a role in liver tissue repair. The present study revealed that Syndecan-1 release was severe in fluke-induced liver damage, particularly in cases with late symptoms, fibrosis, and bile duct proliferation. Additionally, immunopositive reactions were found in the hepatocyte cytoplasms in areas with acute inflammatory reactions. Syndecan-1 positivity, on the other hand, was weak in areas with severe hemorrhage and necrosis. Based on the findings of this study, it was concluded that syndecan-1 release increased in liver damage induced by liver flukes, and these proteins might have a role in that damage. However, we believe that more comprehensive related studies would clarify the pathogenesis of the damage caused by liver flukes.

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Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

The authors declared that Research and Publication Ethical rules were followed.

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We declare that the similarity rate of the article is 10% as stated in the report uploaded to the system.

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