Special Aspects of the Pathohistological Diagnostics of Familial Shar-Pei Amyloidosis

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Abstract

Introduction: Familial Shar-Pei amyloidosis is an autoinflammatory systemic disease characterized by pathological synthesis of the fibrillary protein in the cells of the reticuloendothelial system, followed by amyloid formation. The purpose of our research is mainly focused on the investigation of specific structural histological changes in kidneys, liver, and spleen of the Shar-Pei dogs suffering from familial amyloidosis. **Materials and Methods:** The studies included autopsy and post-mortem examination of the Shar-Pei dogs with the presumptive diagnosis of familial amyloidosis or other diagnoses. Samples of kidney, liver, and spleen tissues of all cadavers were collected for histological examination. **Results:** Our studies showed that amyloid was formed within the ground substance of the connective tissue. Early amyloid deposits were observed in the spleen samples, providing the pathomorphological marker of the initial stage of the process generalization, whereas during the later stages, amyloid was found in kidneys, liver, and myocardium. Gradually increasing amyloid deposits lead to compression and atrophy of the parenchymal cells, sclerosis, and multiple organ dysfunction syndrome, manifesting as a wide range of clinical signs. **Discussion:** As a result of the conducted post-mortem examination, we have revealed systemic amyloidosis in the cadavers of the animals, initially admitted with various pathologies, which proves the importance and relevance of timely diagnostics, detection of clinical manifestations, and latent forms of the condition. Histological examination is one of the most accurate diagnostic methods for this pathology.

Key words: Amyloidosis, autopsy, familial Shar-Pei fever, histological studies, Shar-Pei

INTRODUCTION

Shar-Pei amyloidosis is the most commonly used natural model for amyloidosis, developing as a result of familial Shar-Pei fever (FSF), which has the autosomal recessive inheritance pattern.[1] Diseases of this group are caused by the mutated genes, regulating the production of the proinflammatory cytokines, such as interleukins (IL). FSF manifests in fever, hot swelling of the dog's limb joints, and muzzle.[2,3] In general, in Shar-Pei dogs, the fever attacks occur more often during the 1st year of life. Apart from fever, the symptoms may include sickness, food refusal with consequent weight loss, frequent urination, often pain in the joints. The attacks last for 1-2 days. Fever may be accompanied by vomiting and diarrhea;[3,4] the proportion of dogs suffering from this condition reaches 23%.[5] Chronic FSF inflammation increases the risks of the reactive systemic amyloid A (AA) amyloidosis development, followed by renal or liver failure. Considering this, the investigation of the potential specific pathognomonic features of this disease is highly relevant.

Research objective was to investigate the specific structural histological changes in kidneys, liver, and spleen of the Shar-Pei dogs suffering from familial amyloidosis.

MATERIALS AND METHODS

The study was conducted at the premises of the Department of Veterinary Medicine of Agro-Technological Institute of Peoples' Friendship University of Russia. During the period from 2008 to 2016, the cadavers of the Shar-Pei dogs from the regions with low rates of infectious and invasive diseases were admitted to the laboratory of the autopsy room. Eight dogs were admitted with the presumptive diagnosis of familial amyloidosis; 6 Shar-Pei dogs with other diagnoses had, however, demonstrated the signs of amyloid deposits in the organs of the body in the course of post-mortem examination. The samples of kidney, liver, and spleen tissues were collected from all cadavers, according to the procedure described by Suleimanov and Shabunin for histological studies.[6] The slides were examined with Nikon Eclipse E100 microscope.

RESULTS AND DISCUSSION

The literature data analysis and our own observations suggest that amyloid is a complex glycoprotein, comprising fibrillar and globular proteins, linked to polysaccharides; moreover, the amino acid content in amyloid is different from that in serum and tissue proteins, hyaline, and collagen.[4] Amyloid protein and carbohydrate fractions are tightly bound to each other,[7] and morphologically, amyloid fibers are β-sheet-enriched structures, 10-20 nm in diameter, up to 1000 nm long.[7,8] They can be extended or twisted, can contain protofilaments, arranged in parallels with the main strands, or forming helices with central pockets.[9] The cells of the mononuclear phagocyte system usually inactivate amyloid; however, either decrease in their activity or amyloid hyperproduction result in the amyloid deposition in the tissues.[10] In dogs, renal amyloidosis primarily affects the glomeruli, whereas the involvement of the cortical and medullary interstitium is less common.[7] The initial stage of amyloidosis is characterized by glomerular amyloid deposits; then, the process spreads to the tunica propria of the tubules, whereas pronounced disease involves the interstitial connective tissue between the tubules. This process leads to atrophy of the tubules and glomeruli, extending to their disintegration. However, the literature data indicate that amyloidosis in Shar-Pei dogs has an interesting distinctive feature of prevailing medullar interstitial deposition of amyloid, whereas glomerular amyloidosis is common in other breeds of dogs.[7,11] Initial amyloid deposits have almost no clinical manifestations; therefore, the amyloidosis diagnostics in Shar-Pei dogs is extremely difficult at this stage. The clinical picture of renal pathology acquires distinctive features at later stages, with the involvement of the glomeruli and total substitution of the renal tissue with amyloid. At the early stages of amyloid deposition, low-magnification microscopy allows to discern the capillary network and endothelial nuclei. The formed elements are observed in the vascular lumens. Amyloid can be found between the glomerular vascular loops,

in the form of homogeneous bodies, weakly stained with eosin. In case of pronounced pathology, amyloid pervades the whole glomerulus, the vessel lumens are compressed and indiscernible, endothelial cells undergo atrophy and decrease in numbers. The connective tissue proliferates; the amyloid corrugation of kidneys develops. At the latest stage of the process, the glomeruli are homogenous, with a small number of nuclei, only a few endothelial nuclei can be observed among the amyloid bodies and masses [Figure 1].

If amyloid deposits are formed in the glomerular capsule, it thickens, and its structure may blend into the affected glomerulus. If amyloid deposition occurs in the tunica propria of the tubules, it becomes much thicker, appearing as homogenous rings, surrounding the tubules. The clinical manifestations of amyloid nephropathy include isolated proteinuria, progression is characterized by alternating proteinuria and nephrotic stages, subsequently resulting in chronic kidney disease [Figure 2].[12]

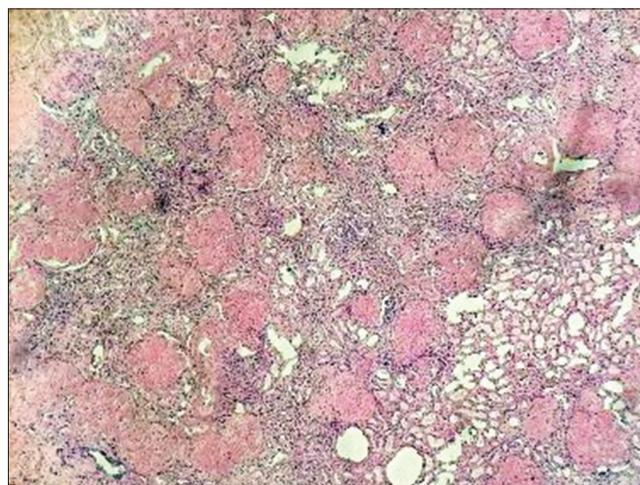The clinical signs of nephropathic amyloidosis include proteinuria, leukocyturia, and hematuria. These are followed

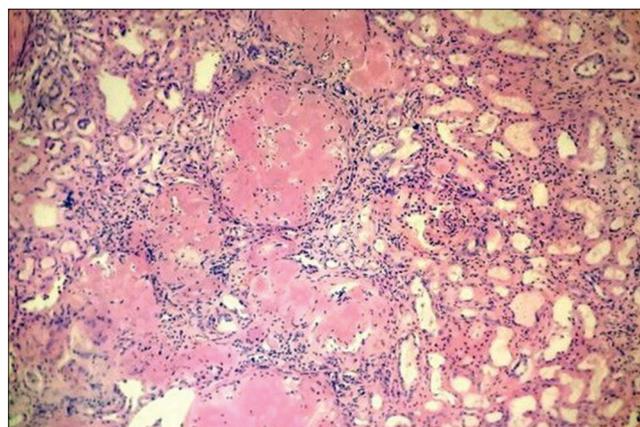**Figure 1:** Renal amyloidosis (hematoxylin and eosin staining, ×100)

Figure 2: Renal amyloidosis (hematoxylin and eosin staining, ×200)

by the nephrotic syndrome, marked by prominent proteinuria, dysproteinemia, hyperlipidemia, swelling, and declining glomerular filtration. The last stage of the nephrotic syndrome includes azotemic intoxication and hypertension, swelling, and hepatomegaly. Such pathologic signs as anemia, elevated erythrocyte sedimentation rate, and leukocytosis are also observed. However, the laboratory test results in familial amyloidosis are not pathognomonic and may indicate the pathological changes in certain organs rather than the cause of their impairment.[13] Morphological manifestations of nephropathic amyloidosis include amyloid deposits in glomeruli, stroma and blood vessels of the pyramids, and the intermedial area accompanied by hyaline droplet and lipid degeneration of the tubular epithelium. This process further results in pronounced glomerular amyloidosis, tubular atrophy, and stromal sclerosis.

According to a number of researchers, amyloidosis development depends on prolonged, abnormally high concentration of the serum amyloid A (SAA) protein in the blood serum, which is normally low. Apart from the N-terminal segments of SAA, amyloid fibrils contain the serum amyloid P-component (SAP). SAP binds to all types of amyloid precursors in a calcium-dependent manner, stabilizing their tertiary structure.[14] SAP is a physiological component of the glomerular basal membrane in kidneys, the basal membranes in skin, liver, lungs, blood vessels; it is a component of microfibrils lining the elastic membranes. These microfibrils structurally resemble amyloid fibrils. Several aspects of SAP influence on the amyloidosis pathogenesis have been described: SAP causes the formation of fibrils, binding the fibrillary protein; it protects amyloid fibrils from proteolytic degradation, it can be a structural component.[15]

Hepatomegaly, combined with hepatosplenomegaly, is most common in amyloidosis. Hepatomegaly is accompanied by jaundice, flatulence, and other usual symptoms of hepatic pathologies of any etiology. More robust sign, indicating hepatopathic amyloidosis, is combined impairment of liver and kidneys.[4] Histological examination of liver revealed massive amyloid deposits between the stellate reticuloendothelial cells of the sinusoids, along the reticular stroma of the lobes, in the walls of blood vessels, ducts, and in the connective tissue of the portal tracts. Amyloid deposition starts in the intertubular space, disturbing the tubular structure and leading to hepatocyte degeneration. The cells are then substituted with the fibrillar protein, completely replacing the hepatocytes of the lobe [Figure 3]. Figure 4 clearly demonstrates the amyloid deposition in the hepatic lobe, starting from the intertubular space and leading to degeneration of the hepatocytes.

AA-amyloidosis starts with the serum precursor SAA - the acute-phase protein, massively produced in the course of inflammatory response; therefore, AA-amyloidosis is referred to as reactive or secondary amyloidosis. Any chronic condition involving the activation of the markers of the acute stage of inflammation can be considered a risk factor for
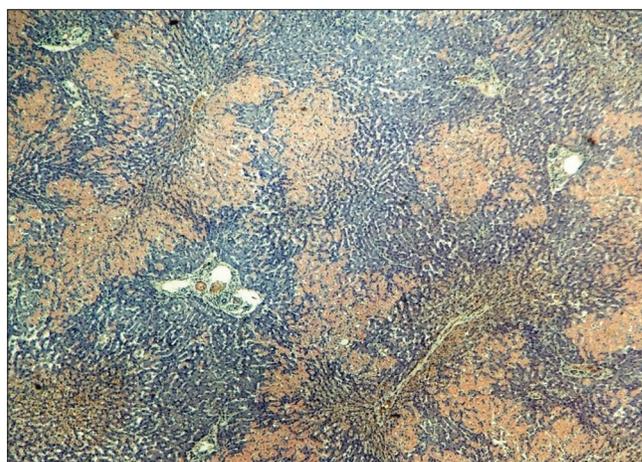

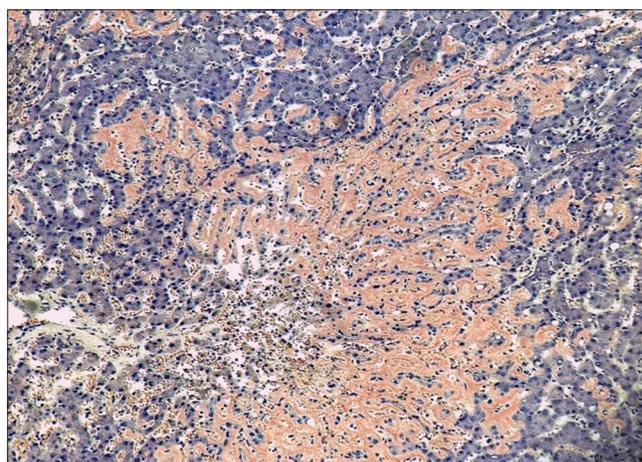**Figure 3:** Hepatic amyloidosis (hematoxylin and eosin staining, ×100)

Figure 4: Hepatic amyloidosis (hematoxylin and eosin staining, ×200)

AA-amyloidosis. In AA-amyloidosis, the protein of amyloid fibrils is synthesized by macrophages (amyloid oblasts) from its humoral precursor - SAA, the N-terminal part of its serum precursor with the molecular weight of 90,000.[16] SAA production in liver depends on a range of cytokines, including IL-1, IL-6, tumor necrosis factor.[4,11] The formation of the AA-amyloid fibrils is divided into two stages. The first stage includes synthesis of the precursor protein in the amounts, sufficient for the amyloid deposition. During the fever attack, the concentrations of the inflammatory mediators in the organism dramatically increase, in turn promoting the SAA synthesis and leading to elevated SAA concentrations in blood. This stage is often called preamyloid, and it can last from several days to several years without any pronounced amyloid deposition; its duration depends on the inflammatory stimulus and SAA levels. This stage is followed by the second one, called amyloid stage, which is characterized by enhanced intercellular SAA degradation and assembly of amyloid fibrils from the SAA fragments on the plasma membrane. The assembly is promoted by the amyloid-stimulating factor, primarily synthesized by the cells of spleen and liver tissues.[8,17] At the final stage of amyloidosis progression, the

fibrils are produced in the extracellular matrix.[4] Figure 5 clearly shows small bodies along the capillaries, mainly in the peripheral parts of the lobes as well as around the vessels of the interlobular connective tissue. The capillary lumens are narrowed, filled with the formed elements. In the cross-sections, the amyloid forms homogenous rings and sockets surrounding the capillaries.

In case of amyloid deposition in the spleen, one may assume generalization of the pathologic process and possible detection of the amyloid deposits in many different organs, which should also be confirmed by histological examination. Therefore, in contrast to other pathologies that do not directly target spleen,[18] splenectomy will not have the intended effect. There are two forms of splenic amyloidosis – sago and lardaceous. In the beginning of the process, i.e., in focal, or sago, form [Figures 6 and 7], amyloid deposits are observed in the white pulp follicles. The cross-section surface is covered with multiple small semi-transparent dense nodules, resembling boiled sago seeds. It leads to a dramatic increase in the follicle volume and the red pulp degeneration due to compression. Later on, amyloid deposition expands to the red pulp. The process becomes diffuse [Figure 8]; therefore, the cross-section surface of the organ looks waxy, red, and homogeneous. At the end of the process, the amyloid deposition becomes homogeneous with total degeneration of the cells. Only scarce, barely discernible patches of the parenchymal cells of the spleen can sometimes be observed.

In Figure 9, amyloid fills both the follicles and the rest of the pulp, making the follicle borders indiscernible. They can be sometimes identified by small clusters of lymphocytes and follicular arteries, where they are still preserved. There is only minor deposition of amyloid in the trabeculae, and they can be clearly seen as fibrillar cords or rounded islets (cross-section). The eosin staining of the trabeculae is heavier than that of the amyloid masses.

As a result of the conducted post-mortem examination, we have revealed systemic amyloidosis in the cadavers of the animals, initially admitted with various pathologies, which proves the importance and relevance of timely diagnostics, detection of clinical manifestations and latent forms of the
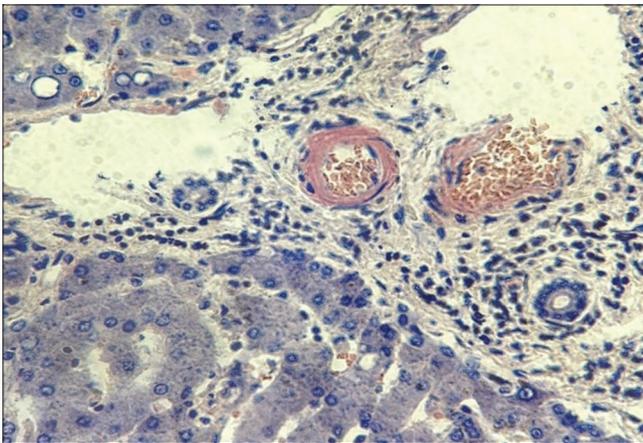

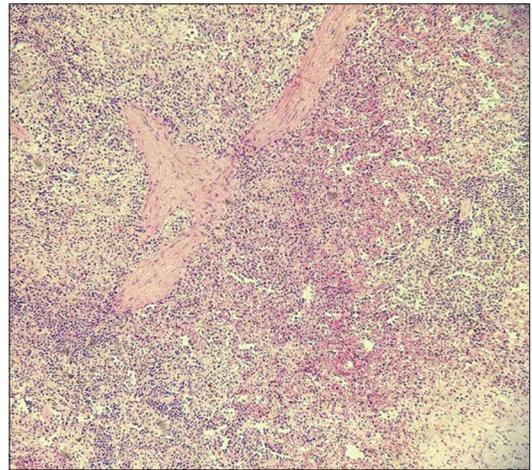**Figure 5:** Amyloid sockets surrounding the blood vessels (hematoxylin and eosin staining, ×400)

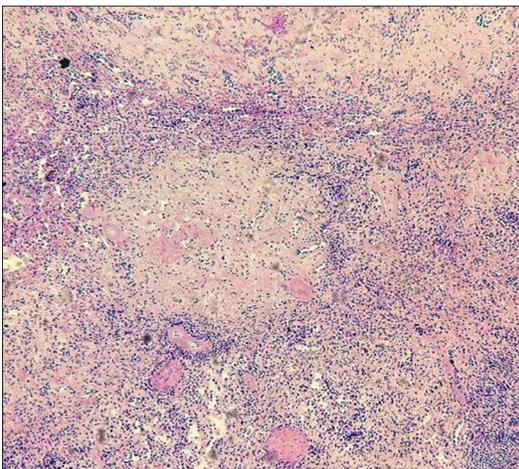**Figure 7:** Splenic amyloidosis, sago form (hematoxylin and eosin staining, ×200)

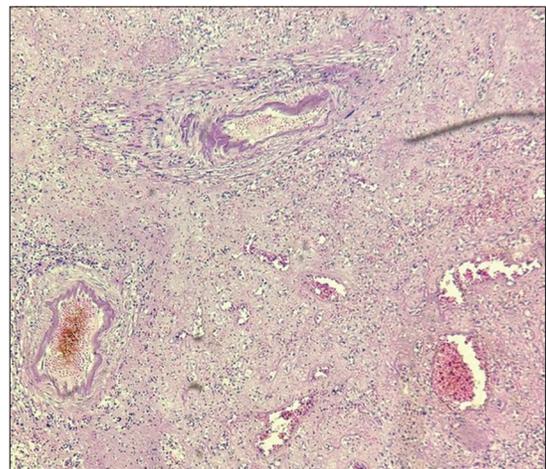**Figure 6:** Splenic amyloidosis, sago form (hematoxylin and eosin staining, ×100

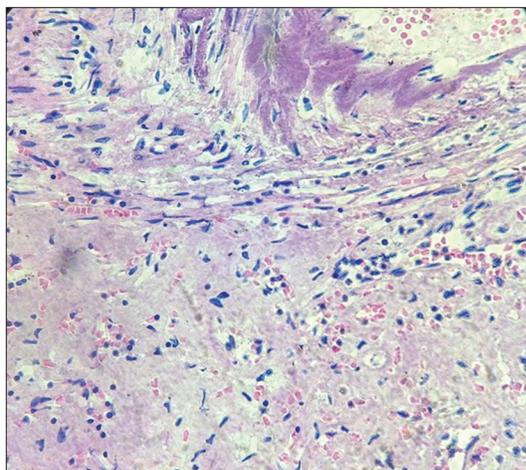**Figure 8:** Splenic amyloidosis, lardaceous form (hematoxylin and eosin staining, ×100)

Figure 9: Splenic amyloidosis, lardaceous form (hematoxylin and eosin staining, ×400)

condition. Being a systemic condition, familial amyloidosis affects all organs and tissues of the organism. Therefore, amyloid deposits are observed in liver, spleen, adrenal glands, pancreas, intestinal submucosa, myocardium, thyroid, prostate, lymph nodes of dogs.[1,7] However, the most severe damage is caused to kidneys and liver. Regarding the ability of wide distribution and impairment of the whole organism, one might say that amyloidosis resembles tumors.[19] We should remember that amyloid deposits can be observed in species other than dogs. This pathological process occurs in all animal species, disturbing multiple systems of the organism.[20,21]

CONCLUSIONS

Amyloid is formed within the ground substance of the connective tissue, closely connected to cells and fibers. Earlier and prevailing deposition of amyloid is observed in the spleen samples, providing the pathomorphological marker of the initial stage of the process generalization, whereas during the later stages, amyloid is found in kidneys and liver. Gradually increasing amyloid deposits lead to compression and atrophy of the parenchymal cells, sclerosis and multiple organ dysfunction syndrome. Histological examination is one of the most accurate diagnostic methods for this pathology.

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