ACUTE PANCREATITIS AS AN OUTCOME
OF FAMILIAL SHAR PEI FEVER.
A CASE REPORT

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Abstract

A female dog of the Shar Pei breed, aged 28 months, was euthanised due to familial shar pei fever with amyloidosis. Microscopic and ultrastructural analyses demonstrated the dog to be affected by acute pancreatitis as an outcome of renal amyloidosis and morphological lesions occurring in the liver. The presented case report depicts the microscopic and ultrastructural pattern of the dog pancreas under the condition of acute inflammation and is a contribution to the knowledge on lesions in the kidneys and liver that induces disorders in the pancreas as well as on concomitant changes occurring in the spleen.

Key words: dog, acute pancreatitis, familial shar pei fever, amyloidosis, liver, spleen, kidneys, pathomorphology.

Although cases of pancreatitis in dogs are relatively often, its diagnosis still poses some difficulties (5, 6). Clinical signs, diagnostic imaging, and laboratory testing, even in combination, may be insufficient (6). Therefore, the most reliable diagnostic method remains histopathological examination (1, 5, 6). What is more, the knowledge of the aetiology and pathogenesis of pancreatitis is still insufficient and needs to be extended (1, 3, 5, 6). Though scientific input on the problem of pancreatitis is made by experiments with animals, case reports are of interest, especially those that cover not only microscopic but also ultrastructural analyses.

In the reported case, acute pancreatitis as an outcome of renal amyloidosis was demonstrated post-mortem and diagnosed at a microscopic and ultrastructural level in the course of familial shar pei fever (FSF).

Clinical description of the case

A female dog of Shar Pei breed (with body mass of 21.0 kg and at the age of 28 months) was euthanised due to FSF, which was diagnosed 13 months earlier. Two months before death, the dog was subjected to sterilisation. During this operation, fatty tissue section of the mesometrium was taken for the microscopic examination – there was detected amyloid. Before the operation, results of routine blood and urine tests were within standard values. Six weeks later, the dog suddenly felt bad. Clinical signs included: fever (40.8°C), conjunctival xanthosis and topical tenderness of the kidneys, anuria, vomitus, and depression. In addition, deviations from reference values were reported in haematological and biochemical parameters of serum as well as in the results of a routine urine test (Table 1). Worthy of notice is the fact that the serum levels of amylase and lipase were within standard values (686 U/L and 209 U/L, respectively) (14).

Material and Methods

Immediately after the dog’s euthanasia, instantaneous macroscopic examination was performed to collect material for microscopic and ultrastructural analyses.
Table 1
Selected results of haematological and biochemical analysis of blood serum as well as a routine urine test in the dog before euthanasia

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Leukocytes (x 10^9/L)</th>
<th>24.70</th>
<th>6.0-16.5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (x 10^12/L)</td>
<td>8.48</td>
<td></td>
<td>5.5-8.5</td>
</tr>
<tr>
<td>Haemoglobin (mmol/L)</td>
<td>11.50</td>
<td></td>
<td>7.45-11.27</td>
</tr>
<tr>
<td>Haematocrit (1/L)</td>
<td>0.50</td>
<td></td>
<td>0.37-0.55</td>
</tr>
<tr>
<td>Platelets (x 10^9/L)</td>
<td>141.0</td>
<td></td>
<td>200-580</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Aspartate transaminase (U/L)</th>
<th>266</th>
<th>1-45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>225</td>
<td></td>
<td>3-60</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>1495</td>
<td></td>
<td>20-155</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.3</td>
<td></td>
<td>3.9-6.7</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>470.0</td>
<td></td>
<td>79.6-150.3</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>27.7</td>
<td></td>
<td>3.32-8.3</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>303.3</td>
<td></td>
<td>5.1-15.4</td>
</tr>
<tr>
<td>Albumins (g/L)</td>
<td>22</td>
<td></td>
<td>33-56</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>686</td>
<td></td>
<td>300-1850</td>
</tr>
<tr>
<td>Lipase (U/L)</td>
<td>209</td>
<td></td>
<td>to 800</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Routine urine test</th>
<th>Relative density (1/L)</th>
<th>1.015</th>
<th>1.016-1.045</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/L)</td>
<td>5.6</td>
<td>below 0.03</td>
<td></td>
</tr>
<tr>
<td>Blood pigments (+)</td>
<td>trace</td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>Bile pigments</td>
<td></td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>Leukocytes (/in visual field)</td>
<td>5-15</td>
<td>0-4</td>
<td></td>
</tr>
<tr>
<td>Erythrocytes (/in visual field)</td>
<td>14-20</td>
<td>0-3</td>
<td></td>
</tr>
<tr>
<td>Tyrosine crystals (/in preparation)</td>
<td>single</td>
<td>absent</td>
<td></td>
</tr>
<tr>
<td>Waxy casts (/in preparation)</td>
<td>a few</td>
<td>absent</td>
<td></td>
</tr>
</tbody>
</table>

*Reference values according to Winnicka (14)

For microscopic analyses, 20 sections were collected from the pancreas as well as five sections from the liver, spleen, and kidneys. The sections were fixed in 10% neutralised formalin and after passing through the so-called “intermediate fluids”, they were embedded in paraffin blocks. The sections were stained with haematoxylin and eosin (HE). Microscopic analyses were conducted on five preparations from each section. Once amyloid concrements were suspected of occurring in the organ, the sections were stained with Congo red according to the Bennhold’s method and the resultant preparations were assessed under light and polarisation microscopes (2).

To assess the microscopic characteristics of the pancreas, an evaluation scale by Kyogoku et al. (8) was used. Following this method, the microscopic preparations were observed at a magnification of 200 x and determined as follows: for stromal oedema: 0 – lack, 1 – widening of interlobular septum, 2 – widening of interlobular septum, 3 – separation of single acinar cells; for leukocytic infiltration: 0 – lack, 1 – less than 20 cells in visual field (v.f.), 2 – from 20 to 50 cells in v.f., and 3 – more than 50 cells in v.f.; for necrosis: 0 – lack, 1 – less than 5% in v.f., 2 – 5 – 20% in v.f., 3 – more than 20% in v.f.; and for extravasations: 0 – lack, 1 – 1 - 2 in v.f., 2 – 3 - 5 in v.f., 3 – more than three loci in v.f.

Ultrastructural analyses were conducted for sections of the pancreas (one – from head segment, two – from body segment and one – from caudal segment). The material was fixed in 2.5% glutaraldehyde on a 0.2 mol/L phosphate buffer, pH 7.4, and embedded in Epon 812. Semi-thin sections were stained according to the method described by Levis and Knight’s (9) and assessed under a light microscope to identify the appropriate place for preparing ultra-thin sections. The ultra-thin sections were contrasted with uranyl acetate and lead citrate. Ultrastructural analysis was conducted using an Opton 900 PC TEM (Germany).

Results and Discussion

Macroscopic pattern. The macroscopic examination revealed small enlargement of the liver and spleen and distinct enlargement of the kidneys. The organs displayed a change in consistency towards hardness. In addition, the liver and kidneys were congested, whereas the pancreas did not display any pathological lesions.
Fig. 1. Pancreas: oedema with the presence of fibrin in lobular septa, sometimes separating pancreatic vesicles, and with infiltration of lymphocytic cells (arrows). HE, a – 150x, b – 500x.

Fig. 2a, b. Pancreas: necrosis of single acinar cells, congestion and extravasation (arrows). HE, 500x.

Fig. 3a, b. Liver – parenchymatous degeneration, necrosis of hepatocytes, leukocytic infiltration (long arrows), binuclear hepatocytes (short arrows), a – hyperplasia of connective tissue, b - haemostasis. HE, 500x.

Fig. 4a, b. Spleen: atrophy of lymphatic nodules, a – hyperplasia of walls of blood vessels, HE, 150x, b - amyloid in the wall of arteriole. Benhold’s staining, 750x.

Fig. 5a. Kidney: dystrophy. HE, 500x. b - amyloid in the wall of arteriole. Benhold’s staining, 750x.

Fig. 6a, b. Membraneous glomerulonephritis of renal glomeruli. a - HE, 500x, b – visible amyloid (arrows). Benhold’s staining, 750x.
**Fig. 7.** Acinar cells: a. excessively developed Golgi's apparatus with slightly widened cisterns (long arrow) and small acinar transformation of rough endoplasmic reticulum (short arrows). 12,000x. b – excessively developed rough endoplasmic reticulum with a tendency for acinar transformation (arrows) and oedema of mitochondria with breakdown of their crests (asterisks). 7,500x.

**Fig. 8.** Oedemotous mitochondria with myelin-like structures (asterisks) in acinar cells. 12,000x.

**Fig. 9.** Damaged mitochondria in acinar cells (asterisks), visible numerous zymogen granules and ductule lumen. 5,000x.

**Fig. 10.** Necrosis of acinar cell. 12,000x.

**Microscopic pattern**

**Pancreas.** Oedema was observed in preparations of each section and usually assessed as 1, and sporadically as 2 (Fig. 1). Infiltrations of single leukocytic cells assessed as 1 occurred in seven sections (Fig. 1). In most of the sections (especially those collected from the head segment), acinar cells showed characteristics of normal structure without abnormalities. Retrogressive lesions predominated in the sections collected from the body segment. Necrosis of acinar cells was observed in eight sections, it covered a few cells in the visual field and was assessed as 1 (Fig. 2). Vacuolar degeneration appeared sporadically in three sections. Congestion was reported in ¼ of the analysed material collected from the body and caudal segment, whereas extravasations occurred with a low intensity (usually assessed as 1) in three sections (Fig. 2). The presence of fibrin and proliferation of connective tissue in lobular septa were observed in 12 sections (Fig. 1). The structure of islets of Langerhans was correct.

**Liver.** Parenchymatous degeneration of various intensity was observed in all sections of the liver (Fig. 3), whereas vacuolar degeneration was reported considerably less frequently. Necrosis of single hepatocytes (Fig. 3) was observed in the specimens of two sections. The lesion was usually located near blood vessels. Disorders in circulation were manifested by haemostasis (Fig. 3b), congestion, and extravasation. Leukocytic infiltrations were relatively common (Fig. 3), whereas small proliferation of connective tissue was observed sporadically (Fig. 3a). Binuclear hepatocytes
were observed near the areas of parenchyma affected by retrogressive lesions.

**Spleen.** This organ was characterised by atrophy of lymphatic nodules and presence of amyloid concrements in walls of blood vessels (Fig. 4). Extravasations and haemosiderosis were observed locally.

**Kidneys.** This organ was affected by dystrophy (Fig. 5a). Observations revealed necrosis of tubules epithelium, parenchymatous and hyaline degradation, congestion, haemostasis, and haemosiderosis. The glomeruli were affected by membranous glomerulonephritis (Fig. 6). Amyloid concrements were observed in the wall of blood vessels and in the area of the glomeruli (Figs 5b, 6b). No morphological cells were observed that would indicate regeneration of the kidneys.

**Ultrastructural pattern of the pancreas.** Almost all acinar cells and cells of islets of Langerhans from the three segments of pancreas displayed a correct structure. In those cases, the proper structure was also confirmed for cells of excretory ducts epithelium. In turn, considerably more lesions were observed in the specimens collected from the second section of pancreas body. In addition, in the material originating from the body and caudal segments, lymphocytes and lymphoblasts as well as granulocytes and fibrin were observed to occur in the interstitial tissue. Sometimes, cells of blood vessels endothelium were oedematous.

In the sections collected from the body segment, lesions were often observed in the structure of acinar cell organella. They were also found, though less frequently, in specimens of the section from the caudal segment. Usually those lesions were linked with the rough endoplasmic reticulum (RER) and mitochondria. The RER indicated a tendency for acinar transformation (Fig. 7a) or hyperplasia (Fig. 7b). Relatively often mitochondria of the acinar cells were subject to destructive lesions. Observations revealed rarefaction of mitochondrial matrix, oedema of crests and sometimes their breakdown, and the presence of myelin-like structures in their interior (Figs 7b–10). Sometimes, the above-described lesions were accompanied by an excessive development of the Golgi apparatus (Fig. 7a).

On the area of most of the visual fields, in sections from the body segment and sporadically in other locations, single acinar cells showing characteristics of necrosis were observed (Fig. 10). Sporadically, necrosis affected a few such cells at one site. Occasionally, their fragments were observed in interstitial compartments.

Zymogen granules were numerous, with various sizes, usually round or oval (Figs 7a, 9, 10), sporadically expressed less distinctly (Figs 7b, 8). Sometimes they attained the form of condensing vacuoles.

The incidence of familial shar pei fever has recently been increasing, including Poland (5). In Shar Pei breed dogs, the disease has been demonstrated to be genetically-determined and to affect ca. 25 % of their population (5). In these dogs, it has been shown to proceed by the deposition of amyloid usually in the kidneys – especially in the medullary segment (3, 7, 11, 12), less frequently in other organs (7, 10), and sporadically in the spleen (3). It is estimated that every fourth dog affected by FSF suffers from amyloid deposition in the organs (5). Under those circumstances, as confirmed in the reported case, there is a risk of the occurrence of clinically-undiagnosed acute pancreatitis. Pancreatic lesions may be signalled by increased activity of amylase and lipase in the blood serum of a dog (6).

Worthy of note is the fact, also confirmed in this study, that acute pancreatitis is not diagnosed microscopically in the entire organ, but only locally. Hence, it is necessary to analyse samples of material collected from various sites of the organ.

The reported study depicts the microscopic and ultrastructural pattern of the pancreas in the course of acute pancreatitis. The lesions described are in part correlated with experimental observations made by Testas et al. (13) and Dlugosz et al. (4). In addition, the obtained results are a contribution to the knowledge on lesions in the kidneys and liver that induce disorders in the pancreas. In that respect, they expand, among others, the knowledge derived from observations made by other authors (1, 3, 7, 11, 12).

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**References**