Generalized AA amyloidosis and fibrino-hemorrhagic pancreatitis in a *Gazelle subgutturosa*: a case report

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ABSTRACT: Generalized AA Amyloidosis and fibrino-hemorrhagic pancreatitis were diagnosed in a 6-year-old, male *Gazelle subgutturosa* submitted for necropsy from a Wild Animal Production Station in Malatya, a province from eastern Turkey. Adhesions between the visceral surfaces of abdominal organs including the liver, pancreas, diaphragma and spleen were observed in the necropsy. Microscopically, the mass consisted of fibrino-hemorrhagic pancreatic tissue. Chronic inflammatory reactions characterized by mononuclear cell infiltration and fibrous connective tissue proliferation were found on the serosal surface in the liver. Amyloid depositions were detected and confirmed by Congo red stained sections of the pancreas, liver, kidneys, and spleen viewed under a polarized light microscope. Generalized AA amyloidosis was thought to responsible for a chronic inflammation characterized by adhesions in *Gazelle subgutturosa*. Generalized AA amyloidosis along with pancreatic involvement and fibrino-hemorrhagic changes are described for the first time in the *Gazelle subgutturosa* species.

Keywords: AA amyloidosis; *Gazelle subgutturosa*; gazelle; pancreatitis

Amyloidosis is characterized by pathological deposition of fibrillar protein subunits in various organs. Chemical classification of amyloidosis is generally in two major classes (Cheville, 1988; Cotran et al., 1999). In animals, Amyloid Associated (AA) type of amyloid is the most frequently found (Cheville, 1988; Dunlop, 2004). AA amyloid is derived from an acute-phase protein called serum amyloid A that plays a role as a chemoattractant in the inflammatory process (Cheville, 1988; Cotran et al., 1999). In most species, Generalized AA amyloidosis occurring sporadically is typically secondary to chronic inflammation such as tuberculosis, bacterial osteomylelitis, *Actinomyces pyogenes* infections or neoplasia (Cheville, 1988; Rideout et al., 1989; Schulze et al., 1998). AA Amyloidosis manifests as pink translucent areas by hematoxylin and eosin (HE) staining (Cheville, 1988). Diagnosis of AA amyloidosis is dependent on the microscopic demonstration of Congo red-binding material and thereafter green birefringence under polarized light (Putchler et al., 1962). In AA-amyloidosis, amyloid deposition is usually widespread in many organs, especially in the spleen, liver, and kidneys (Cheville, 1988). AA-amyloidosis has been documented in various wild mammalian species such as cheetah (Papendick et al., 1997), Siberian tigers (Schulze et al., 1998), and mink (Nieto et al., 1995). Although this type of amyloidosis has been reported in the *Dorcas gazelle* (Rideout et al., 1989), and the mountain gazelle (Linke et al., 1986), to the best of authors' knowledge it has not been reported in *Gazelle subgutturosa (G. subgutturosa)*.

This report describes generalized AA amyloidosis characterized by amyloid deposition in the kidneys, liver, pancreas and spleen in a 6 year old, male *G. subgutturosa*, submitted by the Wild Animal Production Station in Malatya-Turkey. Approximately 13 gazelles, 44 mountain goats and 13 wild bighorn sheeps grazed there in semi-wild condition. Four gazelles died and could not be necropsied due to autolyses over past two years. The gazelles were originally acquired from another wild animal station in the Sanliurfa province. According to personal information, the animal was clinically inappetent, and exhibited progressive weight loss over the course of its life. The gazelle was brought for necropsy to our laboratory where a systemic necropsy was performed.

Tissue samples from the lungs, heart, kidneys, liver, adrenal gland, gastro-intestinal tract and

central nervous system were fixed in 10% neutral buffered formalin, embedded in paraffin, and cross sections were stained with hematoxylin and eosin (HE). Selected tissue sections were also stained by Congo red according to the Puchtler method and examined by light and polarized light microscopes (Putchler et al., 1962). Sections were also treated with the potassium permanganate procedure (van Rijswijk and Heusden, 1979). Microbiological cultivation from the tissue samples were carried out on blood agar.

Macroscopically, the gazelle was emaciated. Adhesions between the visceral surfaces of abdominal organs including the liver, pancreas, diaphragma and spleen were observed. The pancreas, which had a fibrino-hemorrhagic appearance, was also attached tightly to the spleenic capsula (Figure 1). The inner renal medulla and papilla had a pale cut surface and a waxy appearance. The liver was dark brown in colour and had a thickened capsular surface. Parasitologically; *Multiceps multiceps* spp. were isolated from the small intestinal content.

Microscopically, fibrino-hemorrhagic pancreatitis characterized by fibrinous exudate, hemorrhage, edema and a few mononuclear cell infiltrations was detected (Figure 2). Islets of Langerhans cells were clearly seen. The pink deposit was defined as an amyloid deposition because of Congo red positive staining. No fat necrosis was seen. Amyloid depositions were also observed in many perivascular areas among the exocrine acinar cells and Islet of Langerhans cells (Figure 3).

Chronic inflammatory reactions characterized by mononuclear cell infiltration, connective tissue cell and fibrous proliferation were detected on the serosal surface of the abdominal organs.

The amyloid depositions were also seen in the liver, kidneys, and spleen. Hepatocellular atrophy caused by amyloid depositions in the spaces of Disse in the liver was apparent. Depositions of amyloid were observed primarily in centrilobular regions (Figure 4). Subsinusoidal amyloid was mildy Congophilic. Portal areas were markedly fibrotic consisting of mononuclear cell infiltrations and connective tissue proliferations.

Marked chronic glomerulonephritis as well as chronic interstitial nephritis with multiple cortical and medullary scarring was found in the kidneys. Amyloid depositions were seen intensely on the renal cortex as well as the renal medulla. Global glomerular deposits occupying the entire glomerular tuft compressed and distorted the normal architecture. Tubules were dilated and atrophic and contained considerable eosinophilic material resembling thyroid colloid (Figure 5). Inflammatory cells consisted predominantly of lymphocytes and plasma cells. In the glomerulus and medullar intersititium, hyalinic material was deposited primarily in the glomerular tuft and around the tubular basement membranes, in the walls of blood vessels, where deposits were demonstrated by Congo red staining (Figure 6). These exhibited green birefringence under polarized light (Figure 7). However, thyroidisation in proximal tubules was consistently Congo red-negative. In the renal papilla, amyloid depositions were diffuse. Focal tubular and interstitial mineralization was detected in the medulla.

Further, amyloid was present within the splenic germinal centers, forming an "amyloid ring" around the lymphoid follicles, causing follicular atrophy.

No amyloid deposit was detected in the heart, the lungs, the gastrointestinal tract, the lymph nodes

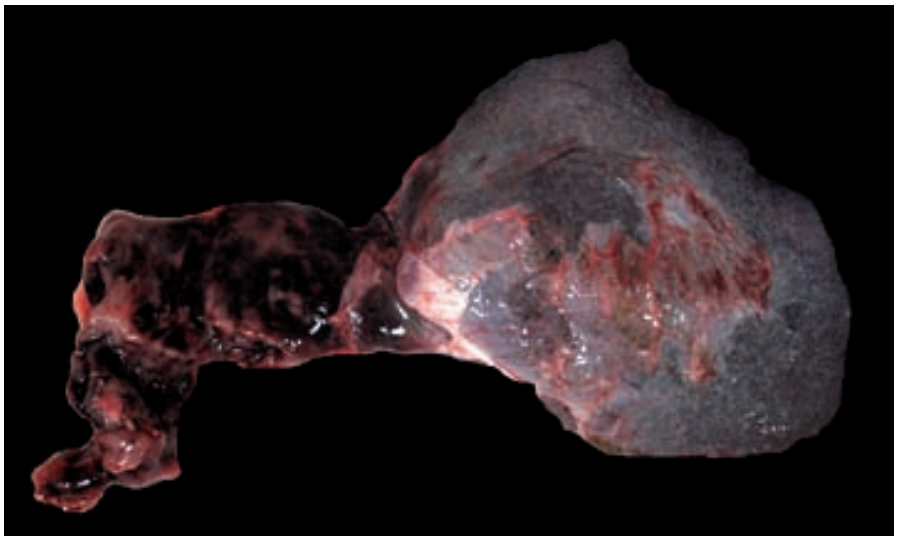

Figure 1. Pancreas; gazelle. Hemorrhagic and flesh like mass attached to spleen capsula

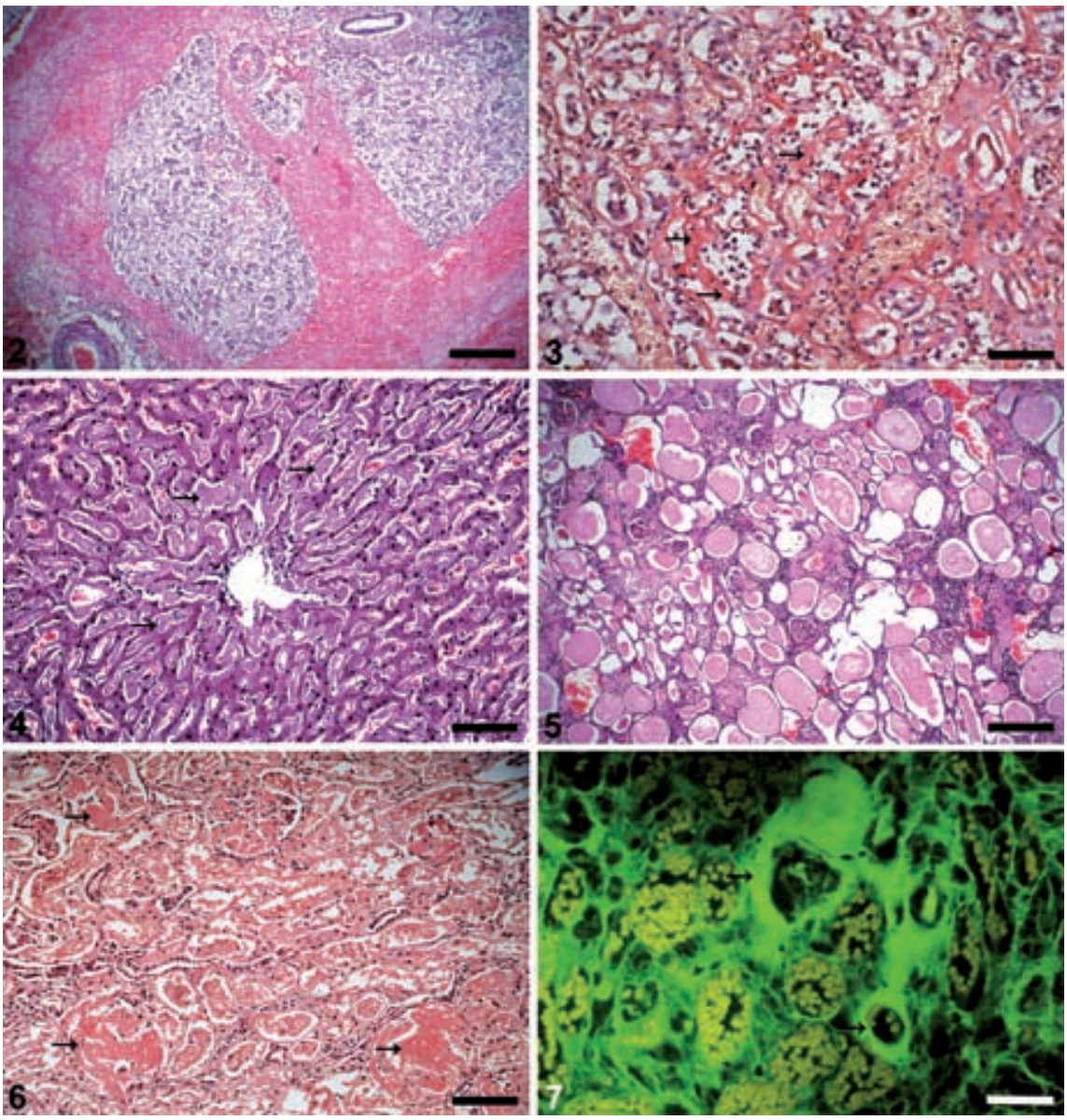

Figure 2. Pancreas; gazelle. Acute inflammatory reaction characterized by severe fibrinous exudate and hemorrhagia in septal connective tissue of pancreas. HE stain, bar = 325 μ

Figure 3. Pancreas; gazelle. Amyloid depositions (arrows) among the islet of Langerhans cells. Congo red stain, bar = 50 μ

Figure 4. Liver; gazelle. Amyloid depositions (arrows) in space of Disse. HE stain, bar = 100 μ

Figure 5. Renal cortex; gazelle. Tubules were dilated and contained eosinophilic material. HE stain, bar = 325 μ

Figure 6. Renal cortex; gazelle. Glomerular amyloid deposits (arrows). Congo red stain, bar = 100 μ

Figure 7. Renal medulla; gazelle. Amyloid deposits (arrows) were demonstrated by the Congo red stain and showed green birefringence under polarized light, bar = 50 μ

and the central nervous system. No agents were isolated from any organs examined bacteriologically.

In the present case, amyloid depositions were detected in Islet cells. These depositions were positive by the Congo red stain. It has been reported that amyloid depositions in the pancreas are caused by generalized AA amyloidosis or type II diabetes in humans and by diabetes syndrome and aging in

some animal species (Cheville, 1988; Cotran et al., 1999; Dunlop, 2004). It was found that diabetes in cats is characterized in ß cells by progressive loss of function. Loss of ß cells was reported to be caused by glucose, fat toxications and amyloid depositions in Islets (Dunlop, 2004). The amyloids which deposit in islets are referred to as islet amyloid polypeptides or amyline (Cotran et al., 1999; Dunlop, 2004). Anomalies in β cells and irregular insulin hormone production are considered to be the cause of amylin formation (Dunlop, 2004). In the present case, no biochemical or hormonal analysis was conducted as the gazelle was dead. No pathologic change was observed in islet cells. The fact that amyloid depositions were also observed in the liver, spleen and kidneys, in addition to the islets in the pancreas, indicates that the problem in this case might be caused by a generalized amyloidosis rather than diabetes.

The cause of death in the present case could be explained by both renal and pancreatic failure. Previous studies in mountain and *Dorcas gazelle* identified amyloid nephrosis as the most prevalent lesion and clinical findings and weight loss were regarded as the outcome of the nephrosis (Linke et al., 1986; Rideout et al., 1989). In the present case, considerable pathological changes including amyloid deposition and inflammatory changes were also present in the pancreas. Both hemorrhage and inflammatory changes were regarded as secondary to amyloidosis due to the chemoattractant features of serum amyloid AA and increased vascular permeability. Similar to the present finding, acute pancreatitis has also been reported as a rare complication of generalized amyloidosis in human beings and it has been demonstrated that this complication might be fatal because generalized amyloidosis usually involves dysfunction in multiple organs (Matsuda et al., 2003). Although the question remains open as to the cause of adhesions in the intra-abdominal organs, there is, however, little doubt that chronic inflammation and altered vascular permeability give rise to serosal adhesions. Furthermore, in a study performed on human patients with chronic renal failure and amyloidosis, elevated levels of intercellular adhesion molecules were observed in serum (Bilezikci et al., 2004). It seems likely that the increased amount of adhesion molecules plays a role in maintenance or progression of adhesions. In our case, it was concluded that the fibrino-hemorrhagic changes observed in the pancreas were caused by the lesions related to the chronic inflammation and amyloidosis, which emerge with the adhesions in the serous of the organs in the abdominal cavity.

Some researchers have suggested that renal papillary necrosis observed in the renal medulla is connected with amyloid depositions (Rideout et al., 1989; Schulze et al., 1998), and that this relationship was due to the decrease in blood flow in the renal papilla as a result of the compression of the vasa recta due to interstitial amyloid depositions (Dibartola and Benson, 1986; Rideout et al., 1989). In the present case, the fact that significant perivascular amyloid depositions were observed in the renal medulla confirms the relationship between acute papillary necrosis and perivascular amyloid depositions.

A familial form of generalized amyloidosis has been defined in Chinese Shar Pei dogs and Abyssinian cats (Dibartola and Benson, 1986; Dibartola et al., 1990). Researchers have not considered renal amyloidosis observed in the Dorcas gazelle as the familial form and have reported that chronic or repetitive *Actinomyces pyogenes (A. pyogenes)* infections, which has a high prevalence in this gazelle species, may be an important factor in the development of generalized amyloidosis (Rideout et al., 1989). In the present case, to mention the familial form seems irrelevant because the necropsy material came from only one animal. Microbiological tissue analyses revealed no *A. pyogenes* agent. Therefore, it was thought that the AA amyloidosis detected in *G. subgutturosa* cannot be attributed to *A. pyogenes*. Generalized AA amyloidosis was evaluated as an idiopathic type in mountain gazelle due to a lack of macroscopic and microscopic findings related to any specific disease (Linke et al., 1986). Another amyloid form in sheep and goats is the Amyloid Prion Protein detected in the central nervous system of animals affected by scrapie (Wood and Done, 1992; van Keulen et al., 1995). In our case, because no Congo red positive areas were observed in the central nervous system parenchyma, no relationship could be established with this form of amyloid. In the present case, AA amyloidosis was thought to be caused by a chronic inflammation characterized by adhesions in the abdominal cavity organs.

In this study, amyloid depositions staining positive for Congo red were confirmed by a polarized light microscope. Following a published procedure, Congo red staining was removed by the oxidation of the tissues with potassium permanganate and thus, as reported in other animal species, it was found that amyloid depositions were of AA type

and sensitive to permanganate (van Rijswijk and Heusden, 1979).

These have been defined as acute tubular dilatation in the kidneys and renal thyroidization characterized by hyaline cylinder in *G. subgutturosa*. In humans, renal thyroidization is a common finding of chronic pyelonephritis (Cotran et al., 1999). In our case, these kinds of changes could be explained by pressure atrophy of amyloid depositions in nephrons and scarification. Similar lesions have also been reported in the Dorcas gazelle, possibly due to inflammatory reactions (Rideout et al., 1989).

To conclude, generalized amyloidosis together with fibrino-hemorrhagic pancreatitis is described here for the first time in the *G. subgutturosa* species and we believe that this a valuable contribution to the literature on this topic.

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Received: 2009–07–02
Accepted: 2009–07–27

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