SPONTANEOUS AMYLOIDOSIS AND DIABETES MELLITUS IN A RHESUS MACAQUE (MACACA MULATTA)

HANDAN HILAL ARSLAN, CEVAT NISBET1, AND TOLGA GUVENC2

Department of Internal Medicine, 1Department of Biochemistry, 2Department of Pathology, Faculty of Veterinary Medicine, University of Ondokuz Mayis, 55139, Samsun, Turkey
hharslan@omu.edu.tr

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Abstract

Clinical signs and necropsy findings were described in a four-year-old female rhesus macaque with diabetes mellitus, resulting from spontaneous amyloidosis. Weakness, polyphagia, polydipsia, and polyuria, which lasted for a few weeks, were determined from anamnesis and clinical investigations. Highly increased serum BUN, AST, ALP levels, glycosuria, and proteinuria was detected in the biochemical analysis. According to these clinical, haematological, and biochemical data, it was thought that the monkey was affected with diabetes mellitus. Despite all efforts to cure the animal, the patient died. Diffuse amyloidosis was detected in the kidney and pancreas at the necropsy and histopathological examinations.

Key words: monkey, spontaneous amyloidosis, diabetes.

Diabetes mellitus has been described in numerous non-human primates. Most of these descriptions derived from experimental data, in which diabetes was induced by β-cell destruction either chemically with streptozotocin or surgically (15, 20). However, spontaneous diabetes has been reported in cynomolgus and rhesus monkeys but availability of these reports is limited (16).

Amyloidosis is a term used for the description of a group of diseases, which are characterised by the excessive deposition of ultrastructurally identical but biochemically different protein fibrils either focal or multifocal in various tissues (12). Amyloid may be deposited in many organs and tissues but it is most frequently found in the spleen, liver, kidneys, and adrenals. Generally, it is a chronic, progressive, insidious disease of unknown origin. The disease may not become clinically apparent until major organ dysfunction develops due to displacement, atrophy, or necrosis (1, 12). Thus, generally, the disease is usually diagnosed histopathologically (7). If critical organs such as the kidneys, liver, or heart are involved, the disease may be fatal (1). Spontaneously occurred deposition of amyloid and gradual degeneration of all cells have been detected in pancreatic islets of Langerhans in some macaque species and the lesions have been evaluated as a reason of diabetes mellitus (9). Similar spontaneous amyloidosis occurs in many non-human primate species, including rhesus monkeys, but the clinical diagnosis and treatment is difficult. The main clinical signs of amyloidosis are hyperglycaemia and cachexia, but these signs cannot be detected in all cases (10, 20).

In this case, diabetes mellitus was diagnosed clinically and spontaneous pancreatic amyloidosis was demonstrated histopathologically in a four-year-old rhesus macaque. It was a very unusual case for Turkey, because Turkey is a subtropical country in the northern hemisphere of the world, and there are no normal living examples of this species. Therefore, it is known that this is the first report of spontaneous pancreatic amyloidosis and diabetes mellitus in a rhesus macaque in Turkey.

Material and Methods

A four-year-old female rhesus macaque who was brought with complaints of polyphagia, polyuria, and polydipsia that lasted for a couple of weeks was the subject of the investigations. The blood samples were collected from the jugular vein into EDTA-containing and empty tubes for haematological and biochemical evaluations. The serum was separated by centrifugation of the tubes at 1550 x g for 10 min. The blood cells were counted by a cell-counter (Ermax-18, Erma Inc., Japan). Serum concentrations of glucose and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN) levels were analysed with commercially available kits according to the manufacturer’s instructions by autoanalyzer (Autolab, AMS Srl, Selective Access, Netherlands).

The early diagnosis was diabetes mellitus and the treatment was immediately started. Firstly, 0.5 IU/kg of insulin (Humulin M 70/30, Eli Lilly and Company) was administered subcutaneously (the total dose was divided into two equal parts and was given at 12 h
intervals). Additionally, intravenous 0.9 % NaCl and lactated Ringer’s solutions were administered. Additionally, intravenous 0.9 % NaCl and lactated Ringer’s solutions were administered.

Macroscopic, histopathological, and immunohistochemical investigations were performed after the patient died. After the necropsy, tissue samples were fixed in 10% neutral buffered formalin, cut into 4 to 5 µm thick sections that were then stained with haematoxylin-eosin (HE) and Congo-red technique. Additional sections, placed on 3-aminopropyltriethoxysilane (Sigma, USA) coated slides, were stained by the streptavidin/biotin/peroxidase complex technique using monoclonal antibody (Clone Bam-10, Sigma, USA) for β-amyloid. The slides were then dipped in freshly prepared absolute methanol containing 3% hydrogen peroxide (v/v) for 5 min to block endogenous peroxidase activity. All the sections were then pre-incubated in a 10% normal goat serum for 30 min at room temperature, to block non-specific binding of the second-step antibody. The sections reacted with a primary antibody for 60 min at room temperature, and then they were rinsed with PBS. Next, the sections reacted with biotin-conjugated second-step antibody for 10 min at room temperature, and then they were rinsed in PBS. Afterwards, the sections were treated with streptavidin/biotin/peroxidase complex for 10 min at room temperature. After another washing with PBS, the sections were incubated with diaminobenzidine for 10 min and then counterstained with Mayer’s haematoxylin. Negative control tissue sections were incubated with normal rabbit serum.

Results

All findings were summarised in Table 1. At the clinical examination, the monkey was found to be lethargic, severely dehydrated, hypothermic, and cachectic. The mucous membranes were pale and the capillary refill time was prolonged.

In comparison with reference values (17, 18) complete blood cell count showed a marked leukopenia (5.4x10³/mm³) and monocytosis (14.8%), and serum biochemical results showed significant increase in serum glucose (352 mg/dL), AST (235 U/L), ALT (158 U/L), and BUN (52 mg/dL) levels. Urine analysis revealed significant glycosuria and proteinuria. On the basis of the findings, diabetes mellitus was suspected and the treatment was immediately started. Despite all therapy efforts, the patient died within 24 h after the treatment started.

The liver and pancreas were found to be yellowish and crusty at necropsy. The kidneys were swollen and their capsules were easily peeled. Homogenic pink coloured amyloid accumulations in wider areas of the pancreas were observed microscopically after HE and Congo-red staining (Figs 1 and 2). Immunohistochemically, β-amyloid deposits were seen in the pancreas and renal glomeruli (Fig. 3). Degeneration of hepatocytes and disruption of Remark cords were found in the liver.

Discussion

Diabetes mellitus has been reported in some non-human primates. Although most of these descriptions derived from experimental data, it has been also noted that diabetes mellitus may occur in non-human primates spontaneously (20).

Hubbard et al. (12) observed hyperglycaemia and cachexia in 12 of the 30 monkeys with pancreatic islet amyloidosis. Furthermore, the researchers reported that it was difficult to determine spontaneous pancreatic islet amyloidosis clinically, but they could do it histopathologically.

Table 1: Clinical and pathological findings

| Anamnesis | Polyphagia, polydipsia and polyuria. |
| Clinical signs | Lethargy, severe dehydration, hypothermia, cachexia, prolonged capillary refill time |
| Haematological findings | Leukopenia, monocytosis |
| Biochemical findings | High glucose, AST, ALT and BUN levels |
| Urine analysis | Glycosuria, proteinuria |
| Necropsy | Yellowish and crusty liver and pancreas, swollen kidneys, and easily peeled kidney capsule |
| Histopathology and Immunohistochemistry | Amyloid accumulations in pancreas and glomeruli of kidneys, β-amyloid deposits in pancreas and renal glomeruli, degeneration of hepatocytes and disruption of Remark cords |
**Fig. 1.** Amyloid accumulation in Langerhans islets in the pancreas (HE, 90x).

**Fig. 2.** Amyloid accumulation in Langerhans islet in the pancreas (Congo-red staining, 180x).

**Fig. 3.** Amyloid accumulation in renal glomeruli (Streptavidin/biotin/peroxidase complex technique, 180x).
Bohm et al. (6) detected significantly decreased leukocyte number in an immune-suppressed Macaca mulatta. In addition, pathologic changes, such as tissue destruction, and acute and chronic inflammations, and immune disturbances, can be counted among the reasons of monocytosis. Thus, monocytosis is considered to be a possible alteration in the blood profile of a patient with amyloidosis, which is defined as a chronic and immune-mediated disorder (4). Leukopenia and monocytosis were detected in this case.

The most important clinical and biochemical findings of diabetes mellitus in monkeys are hyperglycaemia, glycosuria, hyperlipidaemia, polyuria, polydipsia, polyphagia, weight loss, anorexia, and weariness (20). In this case, polyphagia, polydipsia, and polyuria already existed but anorexia, weariness, and severe weight loss followed these findings. A severe hyperglycaemia was found at biochemical analysis. Walzer (20) reported that serum glucose values were persistently higher than 150 mg/dL in diabetic primates and higher than 300 mg/dL in severely diabetic primates. In this case, serum glucose value was 352 mg/dL. Glycosuria is one of the very important classical clinical symptoms of diabetes mellitus (16). Severe glycosuria was found in the urine analysis. Blood and Studdert (5) noted that the kidneys are the organ most often affected by amyloidosis and the amyloid is most often deposited in glomeruli. Glomerular amyloidosis usually leads to marked proteinuria (8). In this case, proteinuria was detected and this was connected with amyloid accumulation in the glomeruli. All these clinical and laboratory data, which supported the diagnosis of diabetes mellitus were compatible with the previously reported findings.

BUN increases in the kidneys with chronic amyloidosis (4). The BUN level was determined high in this case, and amyloid accumulations were seen in the periphery of capillary balls of the glomerulus in the histopathological examinations. The cause of a high BUN level resulted from chronic amyloidosis of the kidneys.

Intensive amyloid accumulation was observed in the pancreas after necropsy (Fig. 1). Similarly, with our findings, Palotay and Howard (14) found amyloid accumulation in Langerhans islets of the pancreas of 21 monkeys with diabetes mellitus. Howard and Palotay (11) also noted β-cell loss in Langerhans islets, due to massive amyloid infiltration in the pancreas in two monkeys with hyperglycaemia. Wagner et al. (19) reported that due to pancreatic amyloidosis, type II diabetes mellitus occurred in monkeys.

There is a connection between diabetes and liver destruction. It has also been reported that hepatomegaly and abnormalities of liver enzymes could occur as a result of hepatocellular glycogen accumulation (2). AST is an enzyme, localised in cytosol and mitochondria, and it is not organ specific. Its highest concentrations are in hepatocytes, myocardium, skeletal muscles, kidney tissue, and placenta cells. Serum AST concentrations increase due to necrosis or destruction of these tissues (2, 3). ALT is a cytoplasmic enzyme and its serum level increases in hepatic diseases with hepatocyte destructions (16).

Serum ALT and AST levels were found to be remarkably higher in this rhesus macaque than the reported reference levels (17, 18). In addition, degeneration in hepatocytes and disruption of Remark cords were seen in the histopathological examinations of the liver. These findings were considered compatible with the hepatic degeneration, due to the disorders in carbohydrate metabolism in diabetes mellitus related to pancreatic amyloidosis.

Wagner et al. (19) examined diabetes mellitus and islet amyloidosis in 6 cynomolgus monkeys aged between 15 and 20 years. They concluded that amyloidosis was the common symptom in the old age. Similarly, to the case described, pancreatic islet amyloidosis was determined as the cause of diabetes mellitus in those cases. However, in contradiction with that report, the monkey in our study was 4-year-old. Thus, this is a valuable finding; because it is proof that spontaneous severe islet amyloidosis in the pancreas is not only seen in old monkeys.

Naumenko and Krylova (13) detected amyloidosis in 88 monkeys of 2 588 autopsied macaques. In their study, amyloidosis was more often seen in the medullary stroma and vascular walls and more rarely under tubular basal membranes and in glomeruli of the kidneys. Amyloidosis was detected in glomeruli of the kidneys in this case. The prevalence of amyloidosis was determined only in 3.2% in 14-year-old Macaca mulatta and this ratio increased to 23.2% in 17-20-year-old ones and to 44.1% in those older than 20 years. (13). Therefore, this data also showed that spontaneous glomerular and pancreatic amyloidosis is unusual in a 4-year-old Macaca mulatta.

In summary, diabetes mellitus was observed in a 4-year-old rhesus macaque with typical history and clinical and laboratory findings. Similarly, to the previous data, increased blood glucose levels, glycosuria, polyuria, polydipsia, polyphagia, dehydration, weight loss, anorexia, and weariness were detected. Unfortunately, the patient was lost despite all therapy efforts. Evaluation of this case can be valuable due to spontaneous occurrence of diabetes mellitus following severe pancreatic islet amyloidosis. Additionally, the case showed that although it contradicted with many reports, the disease could take place in early courses of life in monkeys. Furthermore, it is known that this is the first reported case of spontaneous pancreatic amyloidosis and diabetes mellitus of a monkey in Turkey.

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References


