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Case report

A case of familial amyloid polyneuropathy type I of Greek origin

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SUMMARY

Familial amyloid polyneuropathy is a rare hereditary disease. We present the case of a thirty-seven-year-old woman with sensorimotor neuropathy, which started five years ago accompanied by diarrhoea, urine incontinence and orthostatic hypotension during the last two years. This clinical constellation resulted in burns of the upper and lower limbs, walking disturbance and a loss of weight of ten kilograms. The patient's mother had a history of sensory neuropathy, heart failure and gastrointestinal problems and died at the age of forty. Our patient underwent a sural nerve biopsy, which showed amyloid deposits. A blood sample analysed further by polyacrylamide gel electrophoresis followed by isoelectric focusing revealed that our patient had a normal transthyretin band although present in decreased concentration and an extra band indistinguishable from the mutant transthyretin (Val30Met). Subsequent extraction of DNA, amplification of exon 2 and restriction by Nsi I confirmed our patient's heterozygosity for the amyloidogenic mutant transthyretin. We referred our patient for liver transplantation, the only available therapeutic modality to date known to halt the inexorable progression of the disease.

INTRODUCTION

Amyloidosis is a rare genetic disorder causing malabsorption and neurological symptoms, due to nerve infiltration by amyloid. So far, very few cases have been described in the international literature, although the exact incidence of the disease is unknown. Familial Amyloid Polyneuropathy type I is the commonest type of hereditary amyloidosis.[1-6]

We describe here a female patient of Greek origin with this disorder, in order to emphasise the need for careful investigation for the possibility of the existence of amyloidosis in every patient with neurological symptoms accompanied by malabsorption symptoms, as well as to stress the importance of nerve biopsy for the establishment of the correct diagnosis.[1-6]

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CASE REPORT

A thirty-seven-year-old woman was admitted to the hospital because of persisting diarrhoea, which started two years ago, along with tenesmus. The bowel motions were up to four daily, consisting mainly of mucus and were yellow in colour. Despite a good appetite she lost approximately ten kilograms in weight during this period. During the same time she had to wear diapers because of accompanying urine incontinence. Five years ago she had problems with standing on her toes, which she accidentally discovered during a fitness programme, and since then she had had gait disturbance. Requiring her to lift her feet high off the ground. In addition she could not feel the correct temperature and got burned several times during daily activities such as washing and preparation of meals. She did not smoke and consumed small amounts of alcohol only during social gatherings and was on no medication. She is married and has two children. Her mother who died at a young age (40 years old) had problems with sensation and burns as well as heart failure and gastrointestinal problems although the patient could not give a more detailed history of these.

On examination: Temperature $36^{3\ 0}$C, Pulse: 70 per minute/sinus rhythm, 12 respirations per minute. She

looked pale. Arterial pressure (systolic/diastolic) in the supine position: 100/70 mm Hg while arterial pressure in the erect position was 80/50 mmHg. Body weight: 40 Kgs. Height 1,55 m. Scars due to burns present on the right thumb and middle finger and in the dorsal aspects of both feet. Decreased sensation (pinprick, light sensation and proprioception) of the lower limbs extending from the feet up to the knees and from the fingers up to the forearms accompanied by diffusely decreased tendon reflexes and hypotonia. Decreased muscle power in upper and lower limbs. High-stepping gait with feet slapping on the ground. Personal history: Heterozygous for the í-thalassemia trait.

Laboratory investigations: hematocrite: 30,8%, haemoglobin: 9,4 gr/dl, MCV:70,1 fl, MCH: 21,3 pg, MCHC: 30,4 gr/dl, leukocytes: 6880/mm^3, platelets: 173000/mm^3, erythrocyte sedimentation rate: 12 (first hour). Blood glucose, urea, sodium, potassium, calcium, phosphate, uric acid, CK, lactic dehydrogenase, ?GT, alkaline phosphatase, AST, serum triglycerides, total serum protein and serum albumin were all normal. Alanine transferase was slightly increased (54 IU/lt) and serum cholesterol was at the lowest normal level (126 mg/dl). Total bilirubin: 2,9 mg/dl and indirect bilirubin: 2,52 mg/dl whilst corrected reticulocytes were 2%. The direct antiglobulin test was negative. Stool culture for bacterial pathogens was negative as was examination for ova and parasites. Antibodies against Yersinia enterocolitica, the Widal test, anti-gliadin and antiendomyscial antibodies were negative. Urine microscopy: 100 leukocytes per examination field, 5 erythrocytes per examination field. Urine specific gravity: 1015 with a pH of 5,0. Urine culture grew Escherichia coli >100.000 colonies/ml. **HBsAg**, antiHBs, HbeAg, antiHBe, antiHBcore, anti HCV, anti HIV,anti CMV were all negative. Serum iron, ferritin, serum folate and vitamin B12 were normal. Rheumatoid factor and cryoglobulins were negative. 24-hour urine collection for protein was negative. Bence-Jones protein was not present in the urine. Serum protein electrophoresis was normal. Electrocardiogram: sinus rate 75/minute. Chest X-ray was normal. Abdominal ultrasound showed mild dilatation of the right renal pelvis without depiction of renal stones. There was a significant amount of urine remaining in the trabeculated bladder after voiding. Quantitative assessment of IgG, IgA, IgM, ? and ? light chains, a_2 microglobulin, serum **ceruloplasmin, a_1 antithrypsin** were normal. Antinuclear antibodies were positive at a titre of 1/320 and had a speckled pattern. Anti-dsDNA antibodies, antimitochondrial antibodies, anti smooth muscle antibodies, pANCA and cANCA were negative. Bone marrow **biopsy** showed congo-red positive substance deposition on small arterial walls. An echocardiogram showed thickening of the left ventricle wall but no other lesion. Upper gastrointestinal tract endoscopy showed atrophy of the gastric mucosa and duodenal oedema with normal biopsies. Barium follow-through was normal. Colonoscopy showed oedema of the sigmoid and biopsies showed mild non-specific inflammatory lesions without any indication of malignancy. Sural nerve biopsy showed intense typical Congo-red positivity.

A blood sample was sent to Professor K. Altland (Biochemical Laboratory, Institute of Human Genetics, University of Giessen, Germany) for more detailed workup in order to identify the specific amyloid subtype. Polyacrylamide gel electrophoresis followed by isoelectric focusing under half denaturing conditions demonstrated the normal transthyretin (TTR) and an extra band indistinguishable from the mutant TTR (Valine for Methionine in position 30). TTR plasma concentration was 0,17gr/dl (normal: 0,2-0,4 gr/dl). Extraction of DNA, amplification of exon 2 and restriction by Nsi I confirmed that our patient was heterozygous for normal TTR and the amyloidogenic mutant mutant TTR (Val30Met). A penta dimercapto succinic acid scan showed increased deposition of the radionuclide on the abdominal wall, on the upper limbs and the spleen indicating a quite extensive amyloid involvement of these sites.

Having established a diagnosis of familial amyloid polyneuropathy we referred our patient for domino liver transplantation, but unfortunately even sixteen months later she has not yet received a transplant. We also advised family members of the disease (Figure 1).

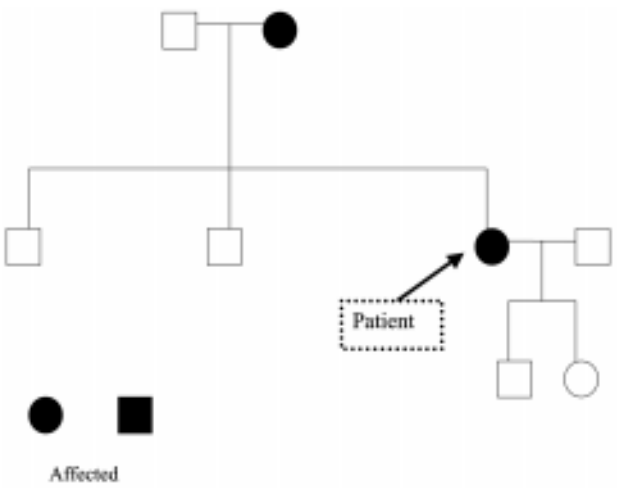

Figure 1. Genetic Tree

DISCUSSION

Amyloidosis consists of a heterogeneous group of diseases in which autologous proteins form insoluble fibrils, which subsequently accumulate in extracellular matrix thus causing structural and functional changes. Different types of amyloid are characterized by a universal positivity when stained with Congo red but they can be further classified in subtypes due to their unique tertiary structure.

Familial amyloid polyneuropathy (FAP) type I is the commonest form of systemic hereditary amyloidosis. It results from the deposition of a transthyretin mutation product, which takes the form of amyloid fibrils, in tissues such as the peripheral nerves, the autonomous nervous system, the heart, the eyes and the gastrointestinal tract. More than eighty different mutations have been described to date in the TTR gene, which is located in chromosome 18.[1] The commonest mutation is substitution of valine for methionine in position 30 and is inherited as an autosomal dominant trait. This mutation was documented in our patient as well.

Familial amyloid polyneuropathy type I is most prevalent in Portugal, Sweden and Japan but there are occasional reports from Italy and Greece.[2-5] Though this is an autosomal dominant disease, its penetrance is variable. Thus in northern Sweden 8000 individuals carry the trait but only 150 have overt disease.[6] Clinical manifestations become evident in the third or fourth decade, though they can start earlier and include: signs of sensorimotor neuropathy, orthostatic hypotension, erectile dysfunction, urine incontinence, arrhythmias including first and second-degree heart block, signs of heart failure, proteinuria and cachexia. Gastrointestinal involvement is common and occurs in 37-94%.[6] Severe constipation sometimes accompanied by nausea and vomiting are often the initial symptoms. As the disease progresses the constipation is relieved by bouts of diarrhea that subsequently become continuous and even take the form of incontinence of feces. Our patient had most of the aforementioned symptoms except for the proteinuria and the clinically overt cardiac disease (bearing in mind that she had an abnormal echocardiogram).

It must be emphasized that the liver is the source of the abnormal protein but there is no deposition of it in the liver and thus there is no hepatic involvement. Endoscopic and pathological manifestations of the gastrointestinal tract in FAP patients include: macroscopically fine granular appearance in the duodenum, lack of lustre and mucosal friability of the gastrointestinal tract whereas histopathological examination of tissues from FAP patients reveals a small amount of amyloid in the submucosa perivascular layer in contrast to a significant amount of amyloid around the nerves of the gastrointestinal tract but very little in Auerbach's nerve plexus. Another finding is a reduction of vasoactive intestinal peptide immunoreactive nerve fibres and neurons in myenteric plexus.[7-10] Our patient had malnutrition in association with an almost normal endoscopic appearance, so we attributed it to the gut motility dysfunction in accordance with recent data.[6] Less attention has been paid to ocular manifestations, although they are common, which include: abnormal conjuctival vessels, keratoconjunctivitis sicca, vitreous opacity, glaucoma and pupilary abnormalities such as decrease in the light reflex, deformity of the pupil, amyloid deposition in the papillary border and supersensitivity to 1,25% epinephrine but not to 2,5% metacholine.[11,12] The latter, which implies sympathetic involvement can be detected even before autonomic dysfunction, becomes evident. Death is usual ten years approximately after overt disease is evident and is related to heart failure or severe malnutrition.

A way to reach diagnosis is to initially employ one of the usual methods to find amyloid deposition.[13] However, establishment of diagnosis rests on the detection of the transthyretin mutant by one of the following methods a. isoelectric focusing in urea gradients b. matrix assisted laser desorption/ionisation time of flight mass spectroscopy c. multidimensional liquid chromatography coupled to electrospray ionisation spectrometry d. microextraction of the amyloid from minute tissue specimens with subsequent purification by SDS-PAGE, electroblotting onto PDVF membranes, excision and elution of amyloid protein related bands and reversed phase HPLC and e.non isotopic Rnase cleavage followed by direct DNA sequencing f. polymerase chain reaction- induced mutation restriction analysis.[14-21] Identifying this particular amyloid subtype with immunohistochemistry confirms the diagnosis as well and can be applied even to hair.[22] Scintigraphy with serum amyloid component or penta dimercaptoacetic acid (VDMSA) is helpful in determining the extent of tissue involvement and evaluating possible responses to therapeutic attempts.[23-25]

Ninety percent of normal transthyretin is produced in the liver and ten percent in the choroidal plexus. Thus the removal of the organ producing the transthyretin mutant frees the patient from the continuous amyloid burden. In addition the removed liver can be used in patients with primary or secondary liver malignancies because a latent period of twenty years is necessary for

the amyloid polyneuropathy to develop in the new recipient. This is the scientific concept of the so called domino or sequential orthotopic liver transplantation where the patient with the familial amyloid polyneuropathy is transplanted with a new liver but his own is given to a patient with liver malignancy. Orthotopic and partial liver transplantation from a living donor have been used as well. During a nine-year period approximately 490 orthotopic liver transplantations and 80 domino orthotopic liver transplantations have taken place worldwide for FAP type I with a reported 5-year survival rate of 78%. This holds true when transplantation takes place early after diagnosis and if possible sooner than seven years after disease onset. Within six months from liver transplantation there is an improvement in 15% of patients regarding electromyographic changes, in 60% percent regarding gastrointestinal complaints and nutrition and in 30% percent concerning erectile dysfunction. Improvement of cardiovascular problems and of urine incontinence was achieved in a smaller number of patients.[26-33] Investigational therapeutic attempts include use of molecules, which may inhibit the dissociation of the mutant transthyretin tetramer such as flufenamic acid, use of sulfite, scavenger therapy with vitamins C and E in conjunction with acetylcysteine and the use of a selective transthyretin-absorption column.[34-40] Dialysis is ineffective in transthyretin clearance.[39] L-threonine-3, 4 dihydroxyphenylserine has been used to treat orthostatic hypotension in these patients.[40] The neuroendocrine peptide therapy could be another complementary therapeutic option in the future regarding the gastrointestinal symptoms.[6]

In conclusion familial amyloid polyneuropathy type I is a rare type of hereditary amyloidosis with a grave prognosis if left untreated. Liver transplantation, either domino or partial from a living donor, can alter the natural course of the disease if instituted early after diagnosis. The physician should keep in mind that pathologic changes occur even in asymptomatic children of FAP patients and that phenotypic and genotypic disconcordance exists in addition to anticipation, and thus offer appropriate genetic counselling.[41-44]

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