**Case Report**

**An Atypical Carbohydrate-Deficient Glycoprotein (CDG) Syndrome Patient in South Africa**

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The carbohydrate-deficient glycoprotein (CDG) syndrome caused by congenital defects in glycoprotein anabolism is a relatively recently discovered group of diseases, with autosomal recessive inheritance and multisystemic involvement. Seven different subtypes of this syndrome have been described to date and clinical symptoms may vary within these subtypes. Very little is known about the genetic basis of these defects, but the enzyme defect has been determined for three of the subtypes, namely types IA, IB and II, and the enzyme assays can be used to assist in the diagnosis of these variants.

A common characteristic of the subtypes is a deficiency in the carbohydrate moiety of glycoproteins, which are involved in various biochemical processes. The clinical features of patients with defects of the glycoprotein anabolism are less distinct and no abnormal oligosaccharides are present in the urine of these patients. These deficiencies can, however, be readily observed in the serum glycoprotein transferrin, although several if not all other glycoproteins are affected by this defect. Isoelectric focusing (IEF) of transferrin, followed by immunodetection, shows a cathodal shift as a consequence of partial deficiency of sialic acid and is commonly used as a reliable diagnostic test for the CDG syndrome. The partial deficiency of sialic acid is the result of a lack of two or four of the terminal trisaccharides normally present on the transferrin molecule. Transferrin is widely used as a marker for the CDG syndromes, and preliminary results indicate a relatively high incidence of this disease in European countries.

Both the nervous system and other organs are involved in the pathology of these diseases. The neurological picture comprises mild to severe psychomotor retardation, olivopontocerebellar atrophy, retinal degeneration, seizures, stroke-like episodes and peripheral neuropathy. The heart is also affected and patients may show cardiomyopathy or pericardial effusions. Hepomegaly has also been described in these patients. Facial dysmorphism, skeletal abnormalities and abnormal subcutaneous adipose tissue distribution, together with inverted nipples, are also frequently found in CDG patients. Coagulation defects such as thrombosis or excessive bleeding are common, along with failure to thrive and feeding difficulties such as vomiting and diarrhoea. Infantile spasms and abnormal skin pigmentation have also been reported.

In general, serum glycoprotein concentrations or enzyme activities are decreased, though a few (follicle-stimulating hormone (FSH) and arylsulphatase A) may be increased and others (transferrin) may be normal. Serum transaminases may be increased, while levels of coagulation factors and plasma albumin may be decreased. Serum levels of growth hormone and insulin may also be increased, while some patients may be anemic.

The most common biochemical manifestation of the CDG syndromes is raised levels of carbohydrate deficient transferrin (CDT) in serum. In healthy controls serum transferrin consists mainly of tetrasialotransferrin, with an isoelectric point (pI) of 5.4, and to a lesser extent of pentasialotransferrin with a pI of 5.3 and tri- and disialotransferrin with pIs of 5.6 and 5.7 respectively. Patients suffering from the CDG syndromes show decreased levels of anodal transferrin isofoms and pronouncedly increased levels of cathodal transferrin isofoms, e.g. disialotransferrin with a pI of 5.7 and asialotransferrin with a pI of 5.9.

**Case Report**

The patient, female and an only child, was born by normal delivery after a normal pregnancy and weighed 3 370 g at birth. At birth she was 53 cm in length, with a head circumference of 34 cm. No immediate neonatal difficulties arose. At 3 months of age a squint was observed during a spell of otitis media. She smiled reciprocally at 6 - 8 weeks of age and laughed aloud at 3 months. Axial hypotonia had been noticed and therapy was administered. At 4½ months clearer deviances were noted, namely floppy neck, poor eye contact,
poor visual pursuit and poor motor activity from lying prone. At 5½ months epileptic seizures set in, with prolonged clonic jerking of the right shoulder and arm, occurring three times in 24 hours. Medication-refractory seizures of generalised tonic-clonic nature occurred repetitively over 6 days. Apnoeic spells also occurred and she showed poor throat clearance, with pharyngeal pooling. She fed poorly and by that time skull growth had decelerated.

At 6½ months the patient was admitted to hospital in a stuporous state. Her muscle mass was low and pronounced hypotonia was present. Upper motor neuron dysfunction was observed, and she was puffy and mildly anaemic. No visceromegaly was seen and magnetic resonance imaging (MRI) scanning showed only mild cortical atrophy. She was of small stature, weighing 6 600 g, 67 cm in length and with a head circumference of 41.5 cm. Her visually evoked potentials were greatly impaired, but the electroretinogram (ERG) was normal. On fundoscopy a severe maculopathy was observed. Over time seizure control remained difficult, in spite of various polypharmacological regimens. There was progressive growth impairment of the skull and spasticity set in, affecting mainly the lower extremities. Scoliosis developed and articular hypermobility of the upper limbs was noted. Her distal limbs became long and narrow; while breast tissue developed some mass with inverted nipples. No cardiac or liver dysfunction was recognised.

At age 4 years and 10 months she has gone for 3 months without seizures and greater responsiveness has been observed. She is functionally blind, with intact pupil responses and blink-to-menace response. Severe psychomotor retardation has set in and she can sit independently, but cannot crawl. Blood samples from the patient were obtained and IEF of transferrin was performed.

INVESTIGATIONS

Samples from the patient were obtained as blood spots on filter paper cards and transferrin was eluted from the paper in 100 μl of water at 4°C for 24 - 36 hours. Of this eluate, 50 μl was incubated with 200 μM iron (III) citrate at 37°C for 20 minutes, after which 1.5 μl of the incubation mixture was loaded in the sample applicator and placed on a Phastgel IEF, pH 4 - 6.5. Iso-electric focusing of the samples was performed according to the IEF programme used by Hackler and co-workers. After electrophoresis, 50 μl of goat-antihuman transferrin diluted with 50 μl of sodium chloride was spread evenly over the gel and left at room temperature for 40 minutes. The gel was washed overnight in 0.15M sodium chloride. Afterwards the gel was washed in water for 30 minutes, the sample was fixed in the gel for 30 minutes with 20% tri-chloroacetic acid and washed in water for 30 minutes. The gel was stained with Coomassie Brilliant Blue for 30 minutes and destained with ethanol and acetic acid for 30 minutes, after which the gel was dried.

RESULTS

Fig. 1 shows the results of IEF of samples from controls (lanes 1 and 6), positive controls for CDG type I (lanes 2 and 3), a positive control for CDG type II (lane 5) and the patient (lane 4). In the control samples the normal transferrin pattern can be observed clearly and shows high levels of tetrasialotransferrin, with lower levels of penta-, tri- and disialotransferrin. The positive control for CDG type II shows lower levels of tetrasialotransferrin and increased levels of tri- and disialotransferrin. The transferrin pattern of the patient shows a pronounced increase in the levels of di- and asialotransferrin, a pattern that corresponds with the positive controls for CDG type I. Fig. 2 shows a photograph of the patient at age 5.
It will take some time to determine the evidence of these syndromes in South Africa and to make a comparison with other countries. It will also take some time to create sufficient physician awareness to ensure speedy and accurate diagnosis of patients with this disease. Because it is a relatively new disease, it is also possible that many more subtypes may be discovered. A note of caution is appropriate at this point, since alcohol abuse, galactosaemia and some liver disease may also cause abnormal glycosylation of transferrin. General clinical symptoms suggesting the presence of the CDG syndromes are psychomotor retardation, growth retardation, liver dysfunction, ataxia, cerebellar hypoplasia, skeletal abnormalities, feeding difficulties, peripheral neuropathy and other unexplained neurological symptoms. Testing for these syndromes can be done on both blood and serum samples dotted on filter paper cards, which can be sent by mail.

Fig. 2. The patient, suffering from CDG syndrome, at 5 years of age.

+mannose therapy (100 - 150 mg/kg body mass five times per day). This patient displayed normal enzyme activities for both enzymes used to distinguish between CDG IA and IB, i.e. phosphomannomutase (PMM) and phosphomannose isomerase (PMI) respectively.

Since an extensive search of the literature failed to provide any information on the incidence of this disease in South Africa, it can be said that this is the first description of a South African patient presenting with the CDG syndrome. Judged on the transferrin pattern, this patient suffers from the type I form of the CDG syndrome, but in an atypical form according to the enzyme analyses. This opens up a whole new chapter in the study of genetic and metabolic diseases in this country, as the mere presence of this patient indicates that this disease may occur more frequently than expected, and since it is a recessive autosomal disease, several carriers may also be undiagnosed. In addition, preliminary studies have revealed that in a local institution for mentally retarded people, out of 15 patients who fitted the clinical description for the CDG syndrome, 5 revealed abnormal transferrin patterns.