A Case of Sanfillippo's Disease Correlating Clinical and Biochemical Findings

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Received: 5 August 2013 / Accepted: 15 January 2014 / Published online: 31 January 2014
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Abstract The mucopolysaccharidoses (MPSs) are a heterogeneous group of rare inherited disorders caused by deficiency of lysosomal enzyme necessary to break down mucopolysaccharides or glycosaminoglycans (GAGs). We had combined clinical findings and ophthalmological features. Biochemical test for urine glycosaminoglycans was done for confirmation of diagnosis in the patient. The case of Sanfillippo's disease was characterized by delayed development, hyperactivity with aggressive behaviour. Coarse facial feature, hirsutism and sleep disorder. Urine GAG tests for MPS was positive in the case. Based on clinical findings and biochemical tests for MPS, this case was diagnosed as a type III mucopolysaccharidoses. Urinary GAG's electrophoresis is an important screening test for MPS suspected cases.

Keywords Sanfillippo's · Mucopolysaccharidoses (MPSs) · Glycosaminoglycans (GAG)

Introduction

Mucopolysaccharidoses (MPS) are a group of inherited storage diseases due to deficiency of lysosomal enzymes needed to degrade glycosaminoglycans (GAGs). Degradation of proteoglycans starts with proteolytic removal of the proteins followed by the stepwise degradation of the GAG

moiety. Failure to degrade due to absence or grossly reduced activity of mutated lysosomal enzymes results in the intralysosomal accumulation of GAGs leads to a characteristic pattern of clinical, radiological and biochemical abnormalities [1].

Some clinical manifestations of the mucopolysaccharidoses such as coarse facial features, thick skin, corneal clouding and organomegaly can be regarded as the direct expression of glycosaminoglycan accumulation in tissues. Others such as mental retardation, growth deficiency and skeletal dysplasia are the result of defective cell function. Joint contractures and hernia an interference of accumulated glycosaminoglycans with other metabolites such as collagen or fibronectin [2].

Case Presentation

One female patient of 10 years age presented to the paediatric department with the chief complaint of delayed developmental milestones since birth, Dysmorphic features, Aggressive behaviour, Hyperactivity, Inability to speak in sentences, Nocturnal enuresis. No history of self injury.

Mother narrated the history that patient was a 2nd born female child. She was a full term normal vaginal female child delivered by Doctor at hospital. She cried immediately after the birth and his birth weight was 2.5 kg. There was no history of (h/o) any neonatal problems or admission to Neonatal-ICU at that time. Baby of 3rd degree consanguineous parents. No family history of developmental delay.

Child achieved neck holding at 4 months of age, gross motor at 4 yrs of age, fine motor at 2 years of age, and she achieved social smile and recognition of mother from 1.5 years of age and self-feeding was achieved at 4 years

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of age. Nowadays her speech also got affected. At present she is able to monosyllables words, recognizes parents. There was no h/o seizures, vision and hearing difficulty, nasal regurgitation, constipation, rough skin, bleeding from any site, rash over the body and jaundice. No h/o blood transfusion. Past and family h/o was insignificant. Patient received immunization as per her age according to the National Immunization Programme.

Physical Examination

Coarse facial features, low anterior hair line, hypertrichosis, epicanthic folds, and bilateral clinodactyly. height 120 cm (88 % of the expected) Weight 23 kg (76 % of the expected) and IQ of baby was 21 (profound retardation).

Pathological Findings

In this case there was no abnormality in the peripheral smear normochromic normocytic RBCs. Haemoglobin was 11gm/dl. Urine routine examination was normal.

Ophthalmologic Findings

In this case there was no corneal clouding.

Biochemical Findings

Her blood biochemistry was normal. The calcium, vitamin D and thyroid function test were normal.

The child was given the provisional clinical diagnosis of mucopolysaccharidosis and urine sample was sent for screening for IEM.

Screening of the Urine Sample for IEM Test for Mucopolysaccharidosis

Toluidine Blue Spot Test

- Spot tests are carried out on urine samples to detect the presence of excreted mucopolysaccharides. In this, a spot of urine is placed on a filter paper, together with a spot of known chondroitin sulphate as a positive control. A drop dye-Toluidine blue superimposed on these spots and the change of colour of the spots is observed. GAGs react with toluidine blue, a cationic dye, to yield a violet colored compound. Spot results suggesting the presence of MPS [3].

CPC Turbidity and Quantification Test

Principle

Under controlled conditions of pH and electrolyte concentration cetylpyridinium chloride (CPC) reacts with GAGs to form an insoluble precipitate. Interaction between cationic quaternary ammonium compound, CPC and polyanionic glycosaminoglycans results in turbidity in test (T) which can be compared with standard (S) (chondroitin sulphate). Appearance of turbidity in test as in standard suggest significant amount of GAG in urine.

In the presence of a citrate buffer at pH 4-8 this precipitate is sufficiently stabilized and dispersed to measure the absorbance at 680 nm in a spectrophotometer [4].

CPC test for mucopolysaccharidosis (urinary GAG) was positive in the urine of this patient.

486 CPC units/gm creatinine (normal up to 150 CPC units for 9–10 year) found.

Assay of Total GAG in Urine by Using 1, 9-Dimethylene blue

1, 9-dimethylene blue, DMB is a cationic dye more frequently applied due to its higher sensitivity, forms a complex with sulphated GAGs present in urine that can be measured at 520 nm in a spectrophotometer [5].

- Result : 227 mg/gm of creatinine

(Reference range for 10 year age group is 4.5–6.3 mg/gm of creatinine)

Identification of Gag Excretion Pattern by Electrophoresis

Principle

This is based on the electrophoretic analysis of urine samples on any type of cellulose acetate membrane. The presence of a band of MPS provides a definite, positive and a strong evidence for carrying out further analysis of the patient's leukocyte enzymes so that the type of MPS of the patient can be diagnosed.

The membrane is soaked in the electrolyte solution for at least 20 min before use. The membrane is then blotted between Whatman No. 1 filter paper to remove excess electrolyte and then placed across electrophoresis tank. Electrophoresis is done in 0.1 M barium acetate at 2 mA/cm constant current for three hours. Separated GAG Electrophoretic strips are stained for alcian blue in acetic acid. Electrophoresis in barium acetate buffer gives separation with the following order of decreasing mobility- chondroitin sulphates (CS), keratan sulphate (KS), dermatan sulphate (DS), and heparan sulphate (HS), Heparin. [4]

Cellulose acetate electrophoresis: presence of heparan sulphate.

We had made the diagnosis of Sanfillippo's disease on the basis of the clinical, pathological, ophthalmological and biochemical examinations.

Discussion

The purpose of this article was to discuss a simple biochemical test for diagnostic purpose having comparable outcome with clinical findings.

A simple qualitative test in which CPC is added to urine and the turbidity is recorded, was described by Manley at Hawksworth [4]. CPC test has been used for the screening

purpose and following precautions improves its sensitivity. Thymol interferes with the test and toluene lowers the efficiency of precipitation in the experiment. Specimen of urine should be collected without preservative and stored frozen until analysed. Precipitation of GAG is affected by ionic strength and pH, if these factors are not taken into account, urine samples may give false positive or false negative results. The effects of ionic strength and pH can be overcome by buffering the CPC solution and the use of citrate buffer at pH 4.8 [4].

Pitfalls of DMB test

Among these are patients with rickets, malabsorption syndrome with gross osteomalacia, malignant disorders with extensive secondary deposits (including leukaemia), patients with disseminated lupus erythematosus, rheumatoid arthritis [6], and some patients with Marfan syndrome [7]. In addition, heparin and acrylic acid polymer used in paper diapers for babies will interfere with the assay [8]. In all of these cases, follow-up analysis by electrophoresis will reveal a normal GAG pattern.

The importance of a simple electrophoretic analysis of urine provides an excellent tool as a lead to the diagnosis of MPS, revealing a clear visible band. Abnormal presence of dermatan sulphate and heparan sulphate is an indication of MPS I or MPS II, while a prominent heparan sulphate spot points to MPS III. The subtypes of MPS III can only be differentiated by enzyme studies. The spot tests and the turbidity tests require at least ten times more concentrations of MPS to give us a positive result [9].

The incidence of Sanfillippo's disease is 1: 100,000 populations. It takes its name from Dr. Sylvester Sanfillippo, who described the condition in 1963. The Sanfillippo's syndrome caused by deficiencies of four different enzymes which are involved in degradation of heparan sulphate heparan *N*-sulfatase (type A), alpha-*N*-acetylglucosaminidase (type B), acetyl-CoA-glucosaminide acetyltransferase (type C) and *N*-acetylglucosamine-6-sulfatase (type D) [1]. Patients with Sanfillippo's disease were characterized by slowly progressive, severe CNS involvement with mild somatic disease. Presenting features include delayed development, hyperactivity with aggressive behaviour, coarse hair, hirsutism, sleep disorders and mild hepatosplenomegaly. Delay in diagnosis of MPS III is common due to mild physical features, hyperactivity and slowly progressive neurologic disease. Heparan sulphate is primarily found in the central nervous system and its accumulation in the brain is responsible for the numerous problems that affect individuals with all types of MPS III.

Others report showed that Sanfillippo disease patients didn't had much skeletal deformity [10] and their ophthalmological examination was also normal, there was no

corneal clouding as seen in other MPS patients [11]. In all tissues there would be accumulation of heparan sulphate.

The case we mention here also had all the mentioned typical features of Sanfillippo's disease like coarse facial feature, delayed development, progressive mental retardation, Aggressive behaviour, sleep disorder.

Genetic analysis and/or enzymatic assays are more reliable for the diagnosis of Sanfillippo's disease which could not be performed due to limited infrastructure.

Conclusion

The diagnosis of Sanfillippo's disease can be confirmed by simple, rapid and inexpensive CPC test, DMB and electrophoresis along with clinical features. The CPC test can be opted for the screening of the patients of Indian rural community having clinical suspicion of MPS, so that they can diagnose early and electrophoresis can be done to confirm the diagnosis.

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