Diagnosis of Sanfilippo Disease Correlating Clinical, Radiological and Biochemical Findings—A Case Report

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Abstract Mucopolysaccharidoses (MPS) are a group of genetic diseases and its diagnosis is a challenging task due to multiple differential diagnosis. We had combined clinical findings, radiological and ophthalmological features. Biochemical test for urine glycosaminoglycans (GAG) was done for confirmation of diagnosis in the patient. The case of Sanfilippo disease was characterized by slowly progressive, severe CNS involvement with mild somatic disease. Radiological features were suggestive of Sanfilippo disease and urine GAG test for MPS was positive in the case. With the clinical features we had multiple differential diagnoses. The radiological investigations minimized the list and the biochemical test confirmed GAG in urine. In this case the combination of clinical, radiological and biochemical findings confirmed the diagnosis of Sanfilippo disease.

Keywords Sanfilippo disease · Diagnosis · Clinical · Radiological · Biochemical

Introduction

Mucopolysaccharidoses (MPS) are a group of inherited storage diseases due to deficiency of lysosomal enzymes needed to degrade glycosaminoglycans (GAGs). GAGs are polymers of a disaccharide unit composed generally of uronic acid and sulphated amino or its *N*-acetylated monosaccharides [1]. GAGs are linked to proteins to form proteoglycans, the major constituent of the connective tissue as well as nuclear membranes. 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 intra-lysosomal accumulation of GAG. Distended lysosomes accumulate in the cell, interfere with cellular functions and that leads to a characteristic pattern of clinical, radiological and biochemical abnormalities.

We obtained the Institutional Ethical Committee's clearance before the start of the study. We also obtained the informed consent from the parents of the study subject and permission from the patient's parents for the publication of the pictures and the radiographs.

In the month of June one male patient of 13 years age presented to the pediatric department with the chief complaint of delayed developmental milestones since birth, regression of milestones since 6 years and distention of abdomen since 4 years.

Mother narrated the history that patient was a 3rd born male child. He was a full term normal vaginal delivered male child delivered by Dai at home. He cried immediately after the birth and his birth weight was 2.75 kg. There was no history of (h/o) any neonatal problems or admission to Neonatal-ICU at that time.

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Fig. 1 Spatula shaped ribs

Fig. 2 Irregular bullet phalanges

Fig. 3 Narrowing of acetabulum and ilium

Fig. 4 Calvarial thickening

Patient achieved neck holding at 4 months of age, sitting without support at 7 months and started walking at 14 months of age. Scribbling was present since 1.5 years, spoke bisyllables from 2 years of age and two words with meaning from 4 years of age. He achieved social smile and recognition of mother from 2 years of age and self-feeding was achieved at 3.5 years of age. As per parents the child was delayed in development and less intelligence as compared to other two siblings. At 7 years of age the patient developed difficulty in walking with initially falls and ultimately he was not able to walk without support. And now-a-days his speech also got affected.

At present he is able to sit without support, walk with support, monosyllables, palmer grasp and recognizes parents. At the time of admission patient also complained of distention of abdomen since last 4 years which was increasing gradually involving whole of the abdomen. On palpation, liver was 10 cm palpable in mid-clavicular line below costal margin having rounded border and smooth surface and was non-tender. Spleen was also palpable 5 cm below costal margin.

According to parents patient had behavioral problem in the form of he had tendency to beat someone close to him and tap his thighs in between whenever.

There was no h/o seizures, vision and hearing difficulty, nasal regurgitation, nasal twang, 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. Birth h/o was also normal. Patients received immunization as per his age according to the National Immunization Programme.

To differentiate this case from other neurodegenerative and dwarfing conditions, pathological, ophthalmologic as well as radiological examinations were done and the findings were as follows:

X-Ray Findings

Findings of the case was **a** chest: spatula shaped ribs (Fig. 1), **b** hands: irregular bullet phalanges (Fig. 2). **c** hip: narrowing of acetabulum and ileum (Fig. 3), **d** skull: calvarial thickening (Fig. 4).

Pathological Findings

In this case there was no abnormality in the peripheral smear except for mildly hypochromic RBCs. His routine urine report was normal.

Ophthalmologic Findings

In this case there was no corneal clouding.

With all these features and findings we could successfully distinguish dysostosis multiplex from other dwarfing conditions. Biochemistry is diagnostic to distinguish MPS from other dysostosis multiplex condition.

Biochemical Findings

His blood biochemistry was normal. The phosphate, calcium and vitamin D and parathromone levels were normal. For the detection of urinary GAG excretion, we performed cetylpyridinium chloride (CPC) test.

CPC Test for Mucopolysaccharidosis (2)

Collection and preparation of the sample: all the aseptic precautions were taken for the collection of urine sample of the patient. The specific gravity of the sample was measured by urinometer and adjusted to less than 1.020 with distilled water.

Principle of the Test

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.

CPC test: case (C-blank, S-standard, T-test) .

Reagents:

1. Citrate buffer pH 4.8.
2. CPC reagent. Dissolve 1 g of CPC in 1 l of the above buffer.
3. Standards. Prepare standard aqueous solutions of chondroitin sulphate (Sigma) to contain 50 and 100 mg/l. Store at -40°C .

Procedure:

1. Centrifuge the sample and use the clear supernatant for the test. Test was performed at room temperature. (This is extremely important as CPC comes out of solution below room temperature and thus give false high turbidity.)
2. Mix 1 ml of sample with 1 ml of citrate buffer as a blank.
3. Mix 1 ml of sample with 1 ml of CPC reagent for the test.
4. Mix 1 ml of standard with 1 ml of CPC reagent.

Interpretation of the Test

The CPC reagent is transparent solution. The CPC reagent become turbid with standard and/or MPS positive urine sample.

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

We had made the diagnosis of Sanfilippo disease on the basis of their clinical, pathological, ophthalmological, radiological and biochemical examinations.

Discussion

The purpose of this article was to discuss a simple biochemical test for diagnostic purpose having comparable outcome with clinical and radiological findings. A simple qualitative test in which CPC is added to urine and the

turbidity is recorded, was described by Manley and Hawksworth [2].

CPC test has been used for the screening purpose and following precautions improves its sensitivity. Infected samples should not be used, as some preservatives may interfere with the analyses, e.g., 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 [2].

The CPC test has the following advantages over other screening tests [2]:

1. It may be applied to random urine samples.
2. The effects of differences in ionic strength and pH of the sample are overcome.
3. The precipitate is stabilized so that timing of the reaction is less critical.
4. This test does not take urine concentration into account.
5. The test has not been known to give false negative results even in patients with Sanfilippo disease when other screening tests have failed [2].

Hence urine analysis for excessive and abnormal GAG excretion is important as a diagnostic test for Sanfilippo disease patients.

We had reported this case of MPS as it is a rare disease and diagnosed at our rural hospital. The overall incidence of MPS is between 3.5 and 4.5/1,00,000 [3]. The most common subtype is MPS III [3] whose incidence is 0.3/1,00,000 [4].

It takes its name from Dr. Sylvester Sanfilippo, who described the condition in 1963. Sanfilippo disease makes up a genetically heterogeneous but clinically similar group of all the other recognized types. The Sanfilippo syndrome caused by deficiencies of four different enzymes which are involved in degradation of heparin sulphate and their names are heparan *N*-sulfatase (type A), alpha-*N*-acetylglucosaminidase (type B), acetyl-CoA-glucosaminide acetyltransferase (type C) and *N*-acetylglucosamine-6-sulfatase (type D) [1].

Phenotypic variation exists in MPS III patients but to a lesser degree than in other MPS disorders. Patients with Sanfilippo 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. Sometimes patients may have had severe behavioral

problems [3]. All four enzymes were involved with the breakdown of heparan sulfate. Heparan sulfate 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 Sanfilippo disease patients didn't have much skeletal deformity [5, 6] and their ophthalmological examination was also normal, there was no corneal clouding as seen in other MPS patients [7]. In all tissues there would be accumulation of heparan sulphate. This phenomenon was much more pronounced in the viscera, especially in the liver [8].

The case we mention here also had all the mentioned typical features of Sanfilippo disease like coarse facial feature, delayed development, progressive mental retardation, aggressive behavior and hepatosplenomegaly.

This case mimics other dwarfing condition on clinical presentation. Radiologically, we could differentiate this case as dysostosis multiplex from other dwarfing conditions [9, 10]. But for the diagnosis of MPS, biochemical test is the definitive diagnostic tool [5, 9, 10].

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

The urinary detection of GAG to distinguish MPS from other dysostosis multiplex by this CPC test not only helps in making the correct diagnosis but also it is rapid and inexpensive. There is no need of any costly equipment to do the test. Also the CPC reagent could be stored at room temperature for at least 1 year. This rapid and inexpensive test is effective for the diagnosis of MPS at rural hospitals.

Conclusion

The diagnosis of Sanfilippo disease can be confirmed by simple, rapid and inexpensive CPC test along with clinical and radiological features. The test can be opted for the screening of the patients of Indian rural community having clinical and radiological suspicion of MPS, so that they can diagnose early and refer to the tertiary centre for the treatment.

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Conflict of interest The author(s) declare no conflict of interests.

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