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ORIGINAL ARTICLE

A Simple and Rapid Method for the Diagnosis of Mucopolysaccharidoses (MPS)

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Mucopolysaccharidoses (MPS) are hereditary, progressive disorders caused by the excessive intralysosomal accumulation of glycosaminoglycans (acid mucopolysaccharides) in various tissues. Glycosaminoglycans (GAGs) are long-chain, complex carbohydrates consisting of a variety of uronic acids, amino sugars and neutral sugars. They are usually linked to proteins to form proteoglycans, which form the major constituents of the ground substance of connective tissue. They are present in all membranes of the cell organelles [19].

The major Glycosaminoglycans are Chondroitin-4-Sulfate, Chondroitin-6-Sulfate, Heparan Sulfate, Dermatan Sulfate, Keratan Sulfate and Hyaluronic acid. These substances are normally degraded by the sequential action of lysosomal enzymes, leading to a stepwise shortening of the glycosaminoglycan chain.

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 herniae point to an interference of accumulated glycosaminoglycans with other metabolites such as collagen or fibronectin.

Different clinical forms of mucopolysaccharidoses are caused by different enzyme deficiencies, leading to the accumulation of biochemically different degradation products. As a general rule, the impaired degradation of dermatan sulfate, chondroitin sulfates and Keratan Sulfate result in mesenchymal abnormalities. Some of the salient features of MPS are summarized in [Table/Fig 1].

Table/Fig 1 - MUCOPOLYSACCHARIDOSES (MPS)

Number	Eponym	Chromosome locus and Inheritance	Enzyme Deficient	Glycosaminoglycan excreted in urine	Main Clinical Features
MPS I-H	Hurler	4p Autosomal Recessive	α-L-Iduronidase	Dermatan sulfate, Heparan sulfate	Mental retardation, Corneal clouding, organomegaly, Dysostosis multiplex, Childhood death.
MPS I-S	Scheie	4p Autosomal Recessive	α-L-Iduronidase	Dermatan sulfate, Heparan sulfate	Stiff joints, Corneal clouding, Normal intelligence, Survive to childhood
MPS I-H/S	Hurler-Scheie	4p Autosomal Recessive	α-L-Iduronidase	Dermatan sulfate, Heparan sulfate	Phenotype intermediate between I-H and I-S
MPS II	Hunter (Severe)	Xq X- Recessive	Idurono sulfate sulfatase	Dermatan sulfate, Heparan sulfate	Mental retardation, No corneal clouding, organomegaly, Dysostosis multiplex, Death before 15 years, Deafness
MPS II	Hunter (Mild)	Xq X- Recessive	Idurono sulfate sulfatase	Dermatan sulfate, Heparan sulfate	Normal Intelligence, short statue, survival to adulthood and often longer
MPS III-A	Sanfilippo A	17q Autosomal Recessive	Heparan N-sulfatase	Heparan sulfate	Profound mental deterioration, hyperactivity, behavioural problems, aggression
MPS III-B	Sanfilippo B	17q Autosomal Recessive	α-N-acetylglucosaminidase	Heparan sulfate	Phenotype similar to III-A, Progressive dementia, seizures
Number	**Eponym**	**Chromosome locus and Inheritance**	**Enzyme Deficient**	**Glycosaminoglycan excreted in urine**	**Main Clinical Features**
MPS III-C	Sanfilippo C	Not known Autosomal Recessive	Acetyl CoA: α-glucosaminide-N-acetyltransferase	Heparan sulfate	Phenotype similar to III-A, Survival to second or third decade of life possible, considerable
MPS III-D	Sanfilippo D	12q Autosomal Recessive	N-acetyl-glucosamine-6-sulfatase	Heparan sulfate	Phenotype similar to III-A, mild dysmorphism, coarse hair, clear cornea, usually normal height
MPS IV-A	Morquio A	16q Autosomal Recessive	Galactosamine-6-sulfatase	Keratan sulfate	Distinctive skeletal abnormalities, corneal clouding, odontoid hypoplasia, short trunk type of dwarfism, final height below 125cm
MPS IV-B	Morquio B	3p Autosomal Recessive	β-galactosidase	Keratan sulfate, Heparan sulfate	Phenotype similar to IV-A but milder, adult height over 120 cm
MPS V		NO LONGER USED			
MPS VI	Maroteaux-Lamy	5q Autosomal Recessive	N-acetylgalactosamine-4-sulfatase (arylsulfatase B)	Dermatan sulfate	Corneal clouding, Dysostosis multiplex, normal intelligence, survival to teens in severe form, milder form exist
MPS VII	Sly	7q Autosomal Recessive	β-glucuronidase	Dermatan sulfate, Heparan sulfate, Chondroitin-4,6-sulfate	Dysostosis multiplex, hepatosplenomegaly, wide spectrum of severity, including fetal and neonatal form
MPS VIII		NO LONGER USED			
MPS IX	-	3p Autosomal Recessive	Hyluronidase	Hyaluronan	Periarticular soft tissue masses, short statue

Urinary Excretion of Gags

The suspicion of MPS on the basis of clinical features will usually be followed by relatively simple tests like the histochemical staining of the cytoplasmic inclusions of circulating white cells and the demonstration of the excessive urinary excretion of GAGs. The normal urinary content of these substances range from 3 to 25 mg in 24 hours [28], [12], [27], [4] and consist of about 70% of low molecular weight chondroitin sulfates. Heparan Sulfate is present in significant quantities together with small amounts of other GAGs. There is a considerable day-to-day variation in thea) normal urinary excretion of GAGs. The amounts of GAGs excreted in urine also vary with age and sex. The highest values are usually observed in early childhood andb) during a major growth spurt [12], [24]. c)

In patients with Hurler disease, the daily excretion of GAGs is markedly increased and may exceed a value of 100 mg in 24 hours [11], [17]. Increased urinary excretion of GAGs has also been observed in a number of other pathological conditions such as hereditary chondroplasia [21], Marfan's syndrome [5], rheumatoid arthritis [9], [20], Scleroderma [29], [32] and Mongolism [3]. The differential clinical features of a patient with MPS and these other diseases, however, will allow the delineation of different diseases.

Several approaches have been used for the detection of MPS in urine. The important aspects of any diagnostic procedure should be a) to get the result quickly, b) the procedure should be simple and c) it should not be expensive. Lists of some common procedures used for the diagnosis of MPS are summarized below.

1. Spot Tests

Spot tests are carried out on urine samples to detect the presence of excreted mucopolysaccharides. The Toluidine blue and Azure-I Spot tests are the ones which are most commonly used [6], [8]. In these procedures, a spot of urine is placed on a filter paper, together with a spot of known mucopolysaccharide as a positive control and a spot of normal urine as a negative control. A drop each of the dye-Toluidine blue or Azure-I is superimposed on these spots and the change of colour of the spots is observed. The presence of mucopolysaccharides gives a pinkish colour which is seen in the standard spot. The original colour of the dye is retained in the negative control spot.

2. Turbidity tests

Two procedures are commonly used here.

a) The Acid albumin test in which the centrifuged urine sample is mixed with acid albumin. The development of turbidity indicates the presence of GAGs [10].

b) Another turbidity test is done by adding 7-8 drops of cetylpyridinium chloride to the centrifuged urine sample [22]. The development of turbidity indicates the excretion of GAGs in urine.

3. TLC Analysis

The qualitative analysis of urine for GAGs can be done by purifying them, separating them on DEAE cellulose, concentrating the eluted GAGs by lyophilization and then applying a small aliquot of the sample, together with standard onto a TLC of microcrystalline cellulose. The TLC is developed in a discontinuous fashion using 5 different solvent systems. The TLC is finally dried and stained to locate the separated GAGs [16]. This is indeed a cumbersome and a time consuming procedure.

Since enzyme defects have now been correlated with corresponding types of MPS [25], [7], [24], the importance of the extensive analysis of urinary GAGs has diminished. At present, mainly, the clinical features in the patient are being used as a guide for the biochemical analysis of the responsible enzyme defect. The procedures described above may also give false positive or false negative results, besides being insensitive [18], [26], [12].

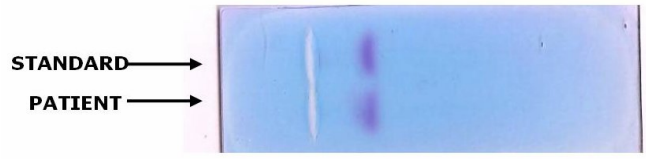
4. Our Method

We describe below a rapid and sensitive method for the diagnosis of MPS. This is based on the electrophoretic analysis of urine samples on an agarose gel prepared on a regular microscope slide. 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 procedure offers high sensitivity and rapidity. The result of the analysis provides a strong guideline for undertaking more detailed investigations of the patient.

Materials and Methods

1gm of agarose with a low electro-endosmosis (EEO) is dissolved in 100ml of pyridine-acetate buffer (10 ml pyridine + 1ml glacial acetic + 89 ml of dist. Water). 3.0 ml of agarose is poured carefully on to a regular microscope slide (7.5 cm x 2.5 cm) and is allowed to solidify. The slide is then transferred to a refrigerator for 10 min to allow the agarose to form a hard layer.

Two slits of 0.5 cm each, are made in the agarose, about 1.5 cms away from one end of the slide as shown in [Table/Fig 2]. Small filter paper strips of Whatman No.3 mm paper (0.4 cm x 0.3 cm), are inserted into the slits to aspirate the accumulated buffer. Another set of two filter paper strips of the same size are impregnated with 5 μL of the patient's urine sample and 5 μL of the standard heparin solution separately and are inserted into the slits. The slide is connected to the buffer compartments with the help of several folds of tissue paper. Electrophoresis is carried out at 30 volts for thirty minutes. At the end of the run, the slide is stained with 0.1% Toluidine blue for 20 min, the dye is poured out and the slide is destained by washing with repeated changes of water until the bands are visible. One such slide is shown in [Table/Fig 2].



(Table/Fig 2)

Discussion

Separating different types of GAGs is a difficult and time consuming process. Keratan Sulfate behaves anomalously and hence, raises special problems during the separation process. The quantities of the urine samples required for the purification procedure are large and also need to be maintained at subzero temperatures to prevent the degradation and the loss of MPS. As can be seen from [Table/Fig 1], the types of GAGs excreted in the urine and their relative proportions are useful parameters in the differential diagnosis of various MPS, [17], [23]. But the variation among patients with the same type of MPS may be considerable [33], [12], [4]. All of these factors make the diagnosis of MPS which is based on the separation procedures, rather ambiguous.

The importance of a simple electrophoretic analysis of urine provides an excellent benchmark as a lead to the diagnosis of MPS. This method can detect as low as 5 nanograms of MPS, revealing a clear visible band. The spot tests and the turbidity tests require at least ten times more concentrations of MPS to give us a positive result. Both spot tests and turbidity procedures are very much subjective and therefore, the result will be totally dependent on personal judgment.

As said before, since the elucidation and correlation of the enzyme defects with their corresponding MPS type [13], [24] and the presence of a band of MPS enables us to disregard all other approaches to the diagnosis of the disease of the patient, thus allowing us to concentrate on a single path of quantitative estimation of enzymes related to MPS. Three enzymes, α-Iduronidase, β-galactosidase, and β-Glucuronidase can be easily measured using fluorescent substrates. [22], [31], [30], [15]. Arylsulfatase B can be measured using chromogenic substrate [14].

Hunter's disease is sex-linked [34]. The sex of the patient automatically raises the diagnostic possibility and eliminates female patients. Lack of mental retardation eliminates the whole group of Sanfilippo syndromes. This covers the whole spectrum of MPS. We have introduced the electrophoretic analysis of urine samples as a routine general screening procedure for the past five years and have screened 2000 patients so far. The procedure has helped us in picking up 186 MPS patients whom we would have missed if we had gone by the presentation of clinical manifestations. The presence of a band of MPS led us to the quantitative estimation of the enzymes, which enabled us to identify the type of MPS in all of them. This procedure was also applied to another group of 90 previously diagnosed patients with a variety of MPS. In all these cases (100%), a strong band of MPS was obtained, thus proving the validity of the procedure. On the other hand, 8 patients out of 90 had produced negative spot tests. This could be due to the day to day variation in the excretion of MPS.

Application of the Apte procedure [1] to the diagnosis by the quantitative enzyme estimation enables one to classify the subjects into three categories - patient, carrier and normal. This procedure, together with the quantitative enzyme estimation, was successfully applied for the prenatal diagnosis of MPS VI by us [2].

We therefore very strongly feel that the procedure described here will be of immense help in arriving at the definitive diagnosis of MPS in all laboratories which are involved in the screening of patients with genetic diseases.

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