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ASSAY OF α – L – IDURONIDASE AND DETECTION OF MUCOPOLYSACCHARIDOSIS I A REPORT IN SOUTH INDIAN POPULATION

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ABSTRACT

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive, progressive, multi system lysosomal storage disorder caused by mutations in the α – L – Iduronidase (IDUA) gene. These mutations lead to the deficiency of the glycosidase, α – L – Iduronidase (IDUA), which is required for the degradation of the glycosaminoglycans (GAGs) heparan sulphate and dermatan sulphate and hence, their storage in the lysosomes. At present the disease is not curable and has overlapping clinical features with other lysosomal disorders, hence definitive confirmation with enzyme assay is the service most often requested by the affected families, second only to the demand for effective therapy. The present study is a report of a study undertaken in this perspective, in south Indian population. 102 clinically diagnosed patients were biochemically analysed by urinary glycosaminoglycan levels. Of these, 11 patients were confirmed to be MPS I as they showed very low or almost nil activity of IDUA in their leukocytes ie 0.3± 0.6 nmol/hr/mg protein when compared to the activity of IDUA in leukocytes of 34 controls (14 adults and 20 children) who were found to have a mean specific activity of 27.2± 7.5 nmol/hr/mg protein in adults and 30.1± 9.7 nmol/hr/mg protein in children. The levels of the enzyme in 18 obligate heterozygotes were estimated and the mean specific activity was found to be 9.17± 4.07 nmol/hr/mg protein which was found to be little less than 50% of the mean in controls indicating their carrier status. Hence assay of IDUA in leukocytes of patients can be used for the definitive diagnosis of MPS I patients and an indication of carrier status of the heterozygotes. This is one of the first reports in south Indian population.

KEYWORDS: Mucopolysaccharidosis I, Lysosomal storage disorder, Glycosaminoglycans, α – L – Iduronidase, Carrier status.

Abbreviations used

GAGs – Glycosaminoglycans, IDUA - α – L – Iduronidase, MPS – Mucopolysaccharidosis.

INTRODUCTION

Mucopolysaccharidosis I (MPS I, MIM 252800) is an autosomal recessive disease that is caused due to the deficiency of the lysosomal glycosidase α – L – Iduronidase (IDUA, EC 3.2.1.76). It was one of the first of the ten disorders of the mucopolysaccharide metabolism described so far [1]. It is considered as the archetype MPS, and is the most common of the MPS in majority of the populations (between 1 in 100,000 and 1 in 150,000 live births [2, 3, 4]. MPS I is clinically delineated into three phenotypes namely Hurler, being the most severe Hurler-Scheie, with intermediate and Scheie with mild manifestations. However, currently the phenotypes are recognized as a continuous spectrum ranging from severe to mild phenotypes with progressive involvement of central nervous system, corneal clouding, skeletal abnormality, hepatosplenomegaly etc. These phenotypes are caused due to reduced or absence of IDUA activity required for degradation of the glycosaminoglycans dermatan and heparan sulphate in the lysosomes. Progressive accumulation of these GAGs in lysosomes results in multi organ dysfunction and excess of them are excreted in urine of the patients.[5,6]

Preliminary diagnosis of the patients is based on detection of GAGs excreted in urine by qualitative and quantitative methods. However, the definitive diagnosis is based on assay of the deficient enzyme IDUA, in leukocytes or fibroblasts. Families with MPS I often request for detection of carrier status. However, the analysis of IDUA activity does not provide definitive carrier information as there could be overlap between carrier and normal enzyme values. Unfortunately, heterogeneity of mutations that underlie MPS I and the technologies available to assess gene mutations do not currently allow for carrier detection by molecular methods.[7]

The present study is a report of a study undertaken in this perspective in south Indian population. 102 clinically diagnosed patients were biochemically analysed by detecting the GAGs excreted in their urine both qualitatively and quantitatively. Of these, 11 of them were confirmed to be IDUA deficient patients (MPS I) by assay if IDUA in their leukocytes. The

levels of the enzyme were also estimated in 18 obligate heterozygotes and were found to be little less than 50% of the mean activity in controls. The results give an indication that carrier detection of MPS I by assay of IDUA in leukocytes is possible and recommends the use of this method for confirmation of MPS I patients and also the examination of potential carrier status of this disorder in our population.

MATERIALS AND METHODS

Materials

Alcian Blue 8GX, chondroitin - 4 – sulphate (bovine trachea), chondroitin - 6 - sulfate (shark cartilage), heparan sulfate (bovine kidney), dermatan sulfate (bovine mucosa) and keratan sulfate (bovine cornea), 4 – methylumbelliferyl β – D – glucuronide, 4 –methylumbelliferone, p – nitrocatechol, p – nitrocatechol sulphate were purchased from Sigma chemicals Co, St Louis, MO, USA. 4- methylumbelliferyl-α-L-iduronide was procured from Calbiochem Novabiochem Corporation, CA, USA. Dextran grade A (MW 200,000 – 275,000) was from BDH laboratories, Poole, England. Cellulose acetate membranes were purchased from Schleicher and Schuell, New Hampshire, USA. Precoated cellulose plates (cat. no.5552) were obtained from E-Merck, Darmstadt, Germany. All other chemicals used were of analytical grade. Glass double distilled water was used in all experiments.

Subjects: Patients suspected to be suffering from MPS based on their clinical and radiological features were referred to our laboratory by different hospitals of Chennai city. These hospitals are national referral centres for patients with genetic disorders; hence the patient population were from different parts of south India and from different communities an economic background. Urine samples without any added preservative were collected from these patients and age and sex matched healthy children (Gospel Vision Ministry, Chennai; Meenakshi Clinic, Kanchipuram) and stored frozen at -70° C until analysis. Heparinized (15 IU/ml blood) venous blood (3-5 ml) was collected and transported to the laboratory at room temperature and processed immediately. Enzyme assay was carried out in patient blood samples. After confirming the MPS I patients by IDUA assay, heparinized venous blood was collected from the obligate heterozygotes (parents of MPS I patients) and sibs of the patients. Blood samples were also collected from age and sex matched normal children and normal adults to get the range of the enzyme values in normal individuals.

Urinary GAG analysis

Isolation of Urinary GAGs: Urinary GAGs were isolated from 8 ml of centrifuged urine of controls and patients as described.[8,9] The final GAG pellet was dissolved in 120 µl distilled water and stored frozen at – 20°C until analysis which was usually within a week.

Qualitative and quantitative analysis of urinary GAGs: The amount of GAGs isolated from urine was estimated by acid alcian blue complex formation method[8,9] and the results expressed as mg GAG/ mmol creatinine. The isolated GAGs were subjected to discontinuous cellulose acetate membrane electrophoresis[8] and sequential multi solvent thin layer chromatography [9,10] to identify the type of GAGs present in the urine and hence classify the patients based on it. The enzyme assays were carried out on patients who showed urinary excretion of heparan sulfate and dermatan sulfate.

Enzyme assays

Various lysosomal enzymes were assayed in the leukocytes. Leukocytes were isolated from heparinized blood by dextran sedimentation method.[11] The final leukocyte pellets were washed with 0.85% sodium chloride and suspended in 0.4 ml distilled water. For the assay, the frozen leukocytes were thawed on ice and sonicated using a 3 mm probe at 25,000 g for 30 sec pulses for four times with 10 sec intervals. 0.1 ml of the direct lysate was kept aside for IDUA assay and the remaining was centrifuged at 12,000 rpm for 15 min at 4ºC. The supernatant was used to assay arylsulphatase B, β – D- glucuronidase and acid phosphatase. Protein was estimated in both aliquots.[12]

The leukocyte IDUA was assayed by the fluorimetric method[13] using the substrate 4-methylumbelliferyl α-L- iduronide. Saccharic acid D – lactone was omitted in the assay and 15µg protein was used. β - D – Glucuronidase and acid phosphatase were also assayed using fluorimetric substrates namely 4- methylumbelliferyl β- D- Glucuronide and 4-methylumbelliferyl phosphate respectively.[11,14] With each experiment a standard graph of 4 – methylumbelliferone (MU) was run. Arylsulfatase B was estimated colourimetrically using p-nitrocatechol sulphate as substrate and p-nitrocatechol as standard.[11]

RESULTS AND DISCUSSION

Qualitative and quantitative analysis of urinary GAGs

To date, 11 enzyme deficiencies that result in 7 distinct types of MPS have been described.[7] However, it is not practical to examine all known enzyme defects in every suspected case,

considering the amount of patient's sample being available, cost of the substrates and time. A total of 102 clinically diagnosed patients were referred to our laboratory from different hospitals of Chennai city, over a period of five years. Urinary GAG analysis, both quantitative and qualitative analysis was carried out in all the patients. Based on the urinary GAG patterns obtained by cellulose acetate membrane electrophoresis and sequential TLC, the patients who excreted heparan sulphate and dermatan sulphate were suspected to be MPS I/II/VI/VII. A total of 15 patients showed this pattern of urinary GAGs.

Table 1: Mean specific activity of IDUA in normal and MPS I families.

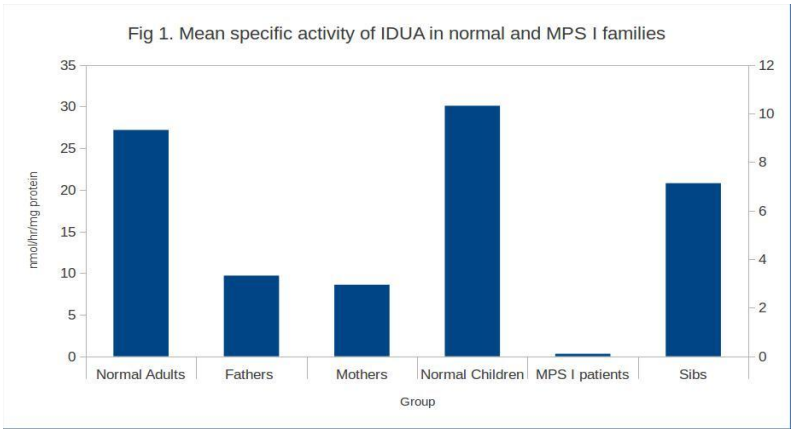
SL. No.	Group	Number Analysed	Mean Specific activity (nmol/hr/mg protein)
1	Normal Adults	14	27.2±7.5 (14.6 – 39.5)
2	Fathers	9	9.7±4.5 (6.2 - 21.01)
3	Mothers	9	8.6±3.1 (4.8 – 15.2)
4	Normal Children	20	30.1±9.7 (18.3 – 54.2)
5	MPS I patients	11	0.3± 0.6 (0 – 2.12)
6	Sibs	7	20.8±3.6 (16.1 – 26.4)

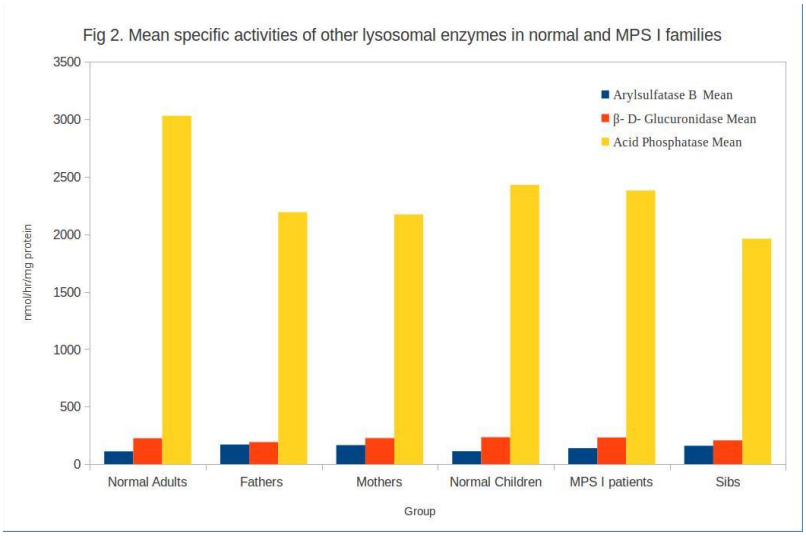
( ) Parenthesis indicates range in each group

Table 2: Mean specific activities of other lysosomal enzymes in normal and MPS I families.

SL. No.	Group	Number Analysed	Mean Specific activity and Range (nmol/hr/mg protein)		
			Arylsulphatase B	β- D-Glucuronidase	Acid Phosphatase
1	Normal Adults	14	110.9± 27 (85 – 175)	225.03 ± 79.7 (105 – 355.4)	3031.2 ±1343.7 (1412.6 – 6080.6)
2	Fathers	9	170.5±48.5 (80 – 220)	192.3 ±67.5 (122.5 – 345.2)	2191.8 ± 651.1 (1607.9 – 3400.3)
3	Mothers	9	165.4±41.3 (86 – 200)	226.5±122.7 (117.03 – 487.9)	2172.4 ± 880.7 (1414.9 – 4310.3)
7	Normal Children	20	112.4 ± 36.5 (55 – 175)	234.2 ± 113.7 (121.8 – 574.8)	2430 ±1287.3 (1049.2 – 4979.3)
5	MPS I patients	11	138.8±39.7 (80 – 200)	231.7 ± 45.9 (134.5 – 292.1)	2380.5± 736.5 (1341.3 – 3948.1)
6	Sibs	7	159.5 ± 38.5 (100 – 170)	207.1±77.5 (139.4 – 349.8)	1961.8 ± 979.3 (1254.4 – 3905.3)

( ) Parenthesis indicates range in each group





Estimation of lysosomal enzyme activities in patients

Although urinary GAG analysis helps in the differential diagnosis of MPS patients, the definitive diagnosis is based only on the lysosomal enzyme assays using artificial substrates in cultured fibroblasts or isolated leukocytes.[15] IDUA, arylsulphatase B (deficient in MPS VI), β-D glucuronidase (deficient in MPS VII) and acid phosphatase (control enzyme, marker enzyme of lysosomes) were assayed in the 15 patients suspected to be MPS I based on urinary GAG analysis. Of these, 11 patients showed very low or minimal residual activity of IDUA (0.3± 0.6 nmol/hr/mg protein) when compared to normal children and adults confirming them to be MPS I patients (Table 1, Fig.1). These patients showed activity in the normal range for the other lysosomal enzymes namely arylsulphatase B, β-D glucuronidase (deficient in MPS VI and VII) and the marker enzyme acid phosphatase (Table 2, Fig.2).

Estimation of lysosomal enzyme activities in obligate heterozygotes

Enzyme assays were performed in 18 obligate carriers, namely, parents of the nine enzymatically confirmed MPS I patients and seven sibs. From the results it is clear that, the

obligate heterozygotes showed mean IDUA activities one third of that of the normal adults indicating them to be confirmed carriers. These individuals showed activity in the normal range for the other lysosomal enzymes (Table 1 and 2 and Fig 1 and 2). The sibs also showed values lower than the normal children indicating them to be border line carriers.

CONCLUSION

All forms of MPS I have undetectable enzyme activity with currently available diagnostic assays. Hence, the residual enzyme activity cannot be used to predict the severity of the disease phenotype. The extent of clinical manifestation is thought to be related to the rate of turnover and the distribution of stored glycosaminoglycan in the body. Urinary glycosaminoglycan levels, although often higher in more severely affected patients, are not a reliable indicator of severity. It is widely accepted that mutational heterogeneity underlies the clinical heterogeneity of MPS I and that phenotype is largely determined by the type of mutation in the IDUA gene.[16] However, the large number of single-occurrence mutations underlying MPS I has limited the predictive value of genotype for many patients [6]. Similarly, mutation analysis gives the clear indication of the carrier status of individual. However, due to lack of data on hotspots mutations being available in the Indian population [17, 18] it is difficult to detect the carrier status of individuals, making enzyme assay the alternate option. Hence, the study provides considerable evidence and recommends the assay of IDUA for the definitive diagnosis of MPS I patients and to detect the carrier status of individuals in our population.

Informed Consent

All procedures followed were in accordance with the ethical standards and informed consent was obtained from all patients and family members for being included in the study.

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