Mucopolysaccharidosis type I: clinical and biochemical study

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خلاصة: أحيل إلى عيادة الوراثة البشرية 1240 مريضاً خارجياً بين سنة 1997 وسنة 1998. وكان لدى 248 (20%) منهم أخطاء استقلابية (أيضية) خلقية. ومن بين هؤلاء تم تشخيص 36 (14%) مريضا بداء عديد السكاريد المخاطي. وقد وجد في 82% من هؤلاء المرضى أن الوالدين كانا من الأقارب. واكتشف في 17 مريضاً عَوَز في الإنزيم ألفا-ل-إديو رونيديز (IDUA) في الكريات البيضاء وزيادة في إفراغ عديدات السكاريد المخاطية في البول. وكان اختبار البقعة البولية غير حاسم في اكتشاف غلوكوزأمينوغليكان في أربع من الحالات السبع عشرة. وأظهرت النتائج وجود ارتباط بين النشاط الكيمياتي الحيوي للإنزيم في الكريات البيضاء وبين مقدار عديدات السكاريد المخاطية المفرزة، والنمط الفرعي والمسار الذي يتخذه داء عديد السكاريد المخاطي من النمط الأول. وخلاصة القول إن تقدير نشاط الإنزيم IDUA في الكريات البيضاء يمكن أن يميز بين الحالات المتداخلة سريريا من النمطين الأول والثاني من داء عديد السكاريد المخاطي. وبالنظر إلى المظاهر السريرية للنمط الأول، فإنه يعتبر طريقة تشخيصية مؤكدة بينما يعتبر اختبار البقعة البولية غير حاسم.

ABSTRACT Of 1240 outpatients referred to the Human Genetics Clinic between 1997 and 1998, 248 (20%) had inborn errors of metabolism, 36 (14%) of which were diagnosed as mucopolysaccharidoses. Parental consanguinity was present in 82% of these patients. Deficiency of α-L-iduronidase (IDUA) enzyme in leukocytes and increased urinary mucopolysaccharides excretion were detected in 17 patients. The urinary spot test for glucosaminoglycans was inconclusive in 4 of the 17 cases. Results showed a correlation between the biochemical enzyme activity in leukocytes, the amount of excreted mucopolysaccharides and the subtype and course of mucopolysaccharidosis type I. We conclude that estimation of IDUA enzyme activity in leukocytes can differentiate between clinically overlapping cases of MPS I and MPS II and given the clinical manifestations of MPS I is a definitive and unequivocal method of diagnosis while the urinary spot test is inconclusive.

La mucopolysaccharidose de type I: étude clinique et biochimique

RESUME Sur 1240 patients ambulatoires orientés vers la Clinique de Génétique humaine entre 1997 et 1998, 248 (20%) avaient des erreurs innées du métabolisme dont 36 (14%) ont été diagnostiquées comme mucopolysaccharidoses. La consanguinité des parents était présente chez 82% des patients. Un déficit de l'enzyme α-L-iduronidase dans les leucocytes et une excrétion urinaire accrue en mucopolysaccharides ont été détectés chez 17 patients. Le test ponctuel dans les urines à la recherche de glucosaminoglycans était peu concluant chez 4 des 17 patients. Les résultats ont montré une corrélation entre l'activité de l'enzyme biochimique dans les leucocytes, la quantité de mucopolysaccharides excrétés et le sous-type et l'évolution de la mucopolysaccharidose de type I. Nous concluons que l'estimation de l'activité de l'enzyme α-L-iduronidase dans les leucocytes permet de différencier entre les cas de mucopolysaccharidose de types I et II qui se chevauchent sur le plan clinique et, étant donné les manifestations cliniques de la mucopolysaccharidose de type I, qu'il s'agit d'une méthode de diagnostic définitive et sans équivoque tandis que le test ponctuel dans les urines est peu concluant.

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Received: 30/05/99; accepted: 05/07/99

Introduction

Mucopolysaccharidoses are a group of hereditary progressive disorders caused by degradative enzyme(s) deficiency, which leads to an excessive intralysosomal accumulation of glycosaminoglycans (GAGs) in various tissues. Distended lysosomes accumulate in the cell and interfere with normal cell function. Mucopolysaccharidosis type I (MPS I) is considered the prototypical mucopolysaccharidosis disorder, occurring at a frequency of approximately 1 per 100 000 in most populations [*1*]. It includes three subtypes: Hurler, Scheie and Hurler–Scheie syndromes [*2*]. MPS I is caused by a deficiency of α-L-iduronidase (IDUA) enzyme. Its clinical features range from severe mental retardation with hepatomegaly, dysostosis multiplex, corneal clouding, cardiac involvement and death in early childhood, to milder symptoms, such as corneal clouding and/or mild visceral involvement with normal intelligence and a normal life span. Hurler syndrome (MPS IH) is the most common and severe form of α-L-iduronidase deficiency, while Scheie syndrome (MPS IS) is a milder form of the disease. In addition, many patients have an intermediate phenotype between Hurler and Scheie syndromes called Hurler–Scheie syndrome (MPS IH-S) [*3*].

The IDUA gene has been localized to 4p16.3, and various mutations have been identified in different populations [*4–8*]. Genotype/phenotype correlation exists for many of the mutations identified [*9,10*]. Hurler and Scheie syndromes are allelic mutations of the IDUA gene resulting in varying degrees of residual activity of the IDUA enzyme toward its natural substrate [*11*]. Hurler–Scheie syndrome is an intermediate phenotype and is caused by homozygous mutations or double heterozygosity of different IDUA gene mutations [*12,13*].

Normally MPS I is suspected on the basis of clinical manifestations. However, MPS I can be confirmed by demonstrating an elevated urinary excretion of mucopolysaccharides and IDUA deficiency [*14*].

Subjects and methods

Between 1997 and 1998, 1240 patients were referred to the Human Genetics Clinic, National Research Centre, Cairo, Egypt. Various inborn errors of metabolism were diagnosed in 248 patients and mucopolysaccharidoses were suspected in 36 (14%) of these. The urinary spot test for GAGs was positive in 32 patients, but the IDUA enzyme in leukocytes was deficient in only 17. For all 17 patients, the following investigations were conducted:

- clinical examination and pedigree analysis
- skeletal survey
- intelligence quotient (IQ) estimation by Stanford–Binet test
- spot test for urinary GAGs, as per Meyer et al. [*15*]
- determination of the level of activity of IDUA enzyme in leukocytes, as per Hopwood et al. [*16*] (normal values = 0.105–0.327 μmol phenol per 18 hours per mg protein)
- determination of the level of mucopolysaccarides (MPS) in urine, as per Di Ferrante and Rich [*17*] (normal values = 116.4–324.4 mL MPS/g creatinine).

A group of 20 individuals, matched by age and sex, were selected as controls.

Results

The study included 36 patients diagnosed with mucopolysaccharidoses on the basis of clinical manifestations, the spot test for

GAGs and radiological findings. Of these, 17 patients (47.2%) whose leukocytes were deficient in IDUA enzyme, (although a spot test for GAGs was inconclusive in 4 cases) and who had excessive excretion of MPS in their urine were diagnosed with MPS I. On the basis of clinical, radiological and biochemical findings, 7 (41.2%) of the 17 patients were diagnosed as having Hurler syndrome, 6 (35.3%) with Hurler–Scheie syndrome and 4 (23.5%) with Scheie syndrome (Table 1).

Patients with Hurler syndrome had a urinary MPS range of 1103–1698 mL MPS/g creatinine, the highest levels of which were found in the 2 most severely affected patients, who subsequently died. IDUA activity in the leukocytes of the patients with Hurler syndrome ranged from no detectable activity to very low activity (0.015 µmol phenol per 18 hours per mg protein). The 2 most severely affected patients had no detectable IDUA activity in their leukocytes.

Patients with Scheie syndrome had a urinary MPS range of 447–974 mL MPS/g creatinine. The range of IDUA activity in their leukocytes was 0.058–0.086 µmol phenol per 18 hours per mg protein.

Patients with Hurler–Scheie syndrome had a urinary MPS range of 545–852 mL MPS/g creatinine and IDUA activity in their leukocytes in the range of 0.012–0.032 µmol phenol per 18 hours per mg protein.

The other 36 patients (excluding the 17 with MPS I) were diagnosed with MPS II-XR (Hunter disease), MPS IV (Morquio disease), MPS VI (Maroteaux–Lamy disease) and MPS III (Sanfilippo disease).

Discussion

A deficiency of IDUA underlies a group of autosomal recessive lysosomal storage disorders termed MPS I. The syndrome is thought to follow a continuum of clinical presentations from the severe form, known as Hurler syndrome, to the milder form, Scheie syndrome. Our results concur with those of Lowry et al., who found MPS I to be the most common MPS subtype [*1*].

However, the clinical manifestations of MPS I and MPS II overlapped in 5 patients and it was difficult to make a differential diagnosis even after pedigree analysis and corneal slit-lamp examination. Evaluation of IDUA activity in their leukocytes detected a deficiency in enzyme activity in 2 patients (patients no. 2 and no. 5), which confirmed a diagnosis of MPS I. The urinary spot test for GAGs was positive for 13 patients but inconclusive for 4, although the main clinical features of MPS I, i.e. coarse features, short stature, hepatomegaly, dysostosis multiplex and mental subnormality, were present. The measurement of IDUA activity in their leukocytes, however, confirmed the clinical diagnosis of MPS I in these 4 patients. The combination of clinical and biochemical data pointed to the diagnosis of Hurler syndrome in patient no. 6 and Hurler–Scheie syndrome in patients nos. 13, 16 and 17. Therefore, the urinary spot test is a good screening test but not a diagnostic one. Evaluation of IDUA activity should be the specific test for diagnosis of MPS I [*14*].

Parental consanguinity was noted in 14 of the 17 patients (82%), which is higher than the consanguinity rate of 28.9% documented in the Egyptian population [*18*]. This increased parental consanguinity is in line with the autosomal recessive mode of inheritance in MPS I.

Hurler syndrome

In our study, Hurler syndrome was the most common MPS I detected, present in 7 (41.2%) patients. The age at onset of mani-

festations was under 2 years in all patients; a finding similar to that of Cleary and Wraith [*19*]. Two patients died at 8 years and 11 years of age. They also had the highest levels of excreted MPS and non-detectable levels of IDUA activity in their leukocytes.

The IQ of patients with Hurler syndrome ranged from 50 to 71. As severe mental retardation usually develops with advancing age, the young age of our patients may explain their mild degree of mental retardation [*20*]. The affected children had the characteristic coarse features (Hurler phenotype), hepatomegaly and short stature. Umbilical hernia and dysostosis multiplex with gibbus were detected in 6 patients. Stiffness of joints, especially interpalangeal joints, were detected in 5 patients and clouding of the cornea was found in a further 3. Patient no. 5, who was 18 months old, did not show any radiological changes, hernia, stiffness of joints or corneal clouding.

A review of the literature revealed that, while coarse features and skeletal abnor-

Table 1 **Results of clinical and biochemical tests in 17 patients with iduronidase deficiency**

Patient number	Sex	Age (years)	Age at onset (years)	IQ	Coarse features	Short stature	Corneal opacity	Organomegaly
1	F	8	1.75	70	Yes	Yes	No	Yes
2	M	1	1	65	Yes	Yes	No	Yes
3	M	12	1	54	Yes	Yes	Yes	Yes
4	F	5	1	59	Yes	Yes	Yes	Yes
5	M	1.5	1.25	50	Yes	Yes	No	Yes
6	F	3.5	1	71	Yes	Yes	Yes	Yes
7	F	11	2	53	Yes	Yes	No	Yes
8	F	6.25	3	90	Yes	No	Yes	Yes
9	F	4.25	3	98	Yes	No	Yes	Yes
10	M	7.5	4	100	Yes	No	Yes	Yes
11	M	4	4	94	Yes	No	Yes	Yes
12	M	3	1	100	Yes	Yes	No	Yes
13	M	4	2	60	Yes	Yes	No	No
14	M	8	1	95	Yes	Yes	No	Yes
15	M	6.5	1	86	Yes	Yes	Yes	Yes
16	M	8	2	63	Yes	Yes	No	Yes
17	F	2.5	2	70	Yes	Yes	Yes	Yes

There were two deaths; patient no. 1 died at 8 years and patient no. 7 died at 11 years
Normal urine MPS levels = 116.4–324.4 mL MPS/g creatinine
Normal α-L-idurinidase levels = 0.105–0.327 µmol phenol per 18 hours per mg protein
MPS = mucopolysaccharide; H-S = Hurler–Scheie

malities with gibbus appear by the end of first year, growth stature is still normal at that age, slowing down thereafter [*14*]. Gibbus develops in the lower thoracic and upper lumbar regions. The vertebral bodies become ovoid in shape and develop beak-like projections on their lower anterior margins, while their upper portions remain hypoplastic. This results in the gibbus deformity commonly present in MPS IH and might even be used as an early diagnostic sign for Hurler syndrome [*21,22*].

Although hepatomegaly is not the presenting feature in the majority of cases, it was found in all our patients, and in most cases (5 out of 7), it was the main complaint [*23*]. Stiffness of joints and corneal clouding are frequently reported with MPS IH [*24*]. Hernias, either inguinal or umbilical, are an early manifestation of MPS I and appear at around 6–8 months of age [*20*]. The early appearance of hernia and gibbus deformity may be used as distinctive signs for investigating and identifying suitable

Table 1 **Results of clinical and biochemical tests in 17 patients with iduronidase deficiency** *concluded*

Patient number	Hernia	Stiff joints	Abnormal X-ray	Urinary MPS (mL MPS/ g creatinine)	Enzyme activity (µmol phenol/ 18 h/mg protein]	Diagnosis
1	Yes	Yes	Yes	1694	0.000	Hurler
2	Yes	Yes	Yes	1681	0.000	Hurler
3	Yes	Yes	Yes	1545	0.000	Hurler
4	Yes	Yes	Yes	1631	0.000	Hurler
5	No	No	No	1602	0.000	Hurler
6	Yes	Yes	Yes	1103	0.015	Hurler
7	Yes	No	Yes	1698	0.000	Hurler
8	No	Yes	No	447	0.086	Scheie
9	No	Yes	Yes	510	0.073	Scheie
10	No	Yes	No	889	0.058	Scheie
11	Yes	Yes	Yes	974	0.068	Scheie
12	No	No	Yes	824	0.016	H–S
13	No	No	Yes	736	0.027	H–S
14	No	No	Yes	723	0.017	H–S
15	No	No	Yes	831	0.029	H–S
16	Yes	No	Yes	545	0.012	H–S
17	Yes	No	Yes	852	0.032	H–S

There were two deaths; patient no. 1 died at 8 years and patient no. 7 died at 11 years
Normal urine MPS levels = 116.4–324.4 mL MPS/g creatinine
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cases for bone marrow transplantation or enzyme therapy [*25–27*]. However, patient no. 5 had no hernia and Cleary and Wraith have reported that the presence of hernia, although an important early manifestation, is not a mandatory sign in Hurler syndrome [*19*]. Levels of urinary excretion of MPS were markedly increased in all our patients with Hurler syndrome (> 1100 mL MPS/g creatinine). Excretion of MPS was also highest in those patients with severe manifestations (patients no. 1 and no. 7). IUDA activity in leukocytes was very low in all the patients with Hurler syndrome, in fact non-detectable in most cases, including the most severe.

Scheie syndrome

Scheie syndrome (MPS IS) was diagnosed in 4 patients (23.5%). We observed the age at onset of clinical manifestations to be from 3 to 4 years, while Matalon noted that clinical features usually appear around 5 years of age [*24*]. Scheie syndrome is milder than Hurler syndrome; stature is normal, as is intelligence, and there is normal survival into adulthood. All these characteristics were detected in our patients. In common with previously published reports [*14*], the major determining clinical features in these 4 patients were coarse, but not Hurler-like, features, mild hepatomegaly, corneal clouding and severe joint stiffness in the hands (to the extent of clawing in patient no. 10). Half of the patients had radiological abnormalities, but without gibbus changes, and 1 patient had umbilical hernia. Cleary and Wraith noted that mild dysostosis multiplex, without the vertebral changes or gibbus deformity, is present in Scheie syndrome [*19*]. Mild levels of MPS were excreted in the urine of all 4 patients (< 1000 mL MPS/g creatinine) and IDUA activity in their leukocytes was mildly deficient (0.058–0.086 µmol phenol per 18 hours per mg protein).

Hurler–Scheie syndrome

Hurler–Scheie syndrome (MPS IH–S) was detected in 6 patients (35.3%). The characteristic clinical manifestations were coarse, but not Hurler-like, features, short stature, mild skeletal changes and normal–moderate IQ scores, findings which are similar to those of Roubicek et al. [*12*]. Hepatomegaly was detected in 5 of the patients and corneal clouding and hernia were present in another 2 patients. No joint stiffness was noticed in any of the patients, which is in agreement with previous reports [*28,29*]. Urinary excretion of MPS was moderate (515 852 mL MPS/g creatinine) and IDUA activity levels in leukocytes was moderately low in all cases (0.012 0.032 µmol phenol per 18 hours per mg protein).

The ranges of urinary MPS and IDUA activity in leukocytes were clearly different in patients with Hurler syndrome and those with Scheie syndrome; but while patients with Hurler–Scheie syndrome had intermediate levels of IDUA enzyme activity in their leukocytes, their range of excreted urinary MPS overlapped with that of Scheie syndrome. Therefore, determination of IDUA activity in leukocytes is a more reliable test than urinary MPS for diagnosing different subtypes of MPS I and might even predict the course of the disease.

Conclusion

Our study suggests that there is a correlation between biochemical phenotype, clinical manifestations and radiological findings in the subtypes of MPS I. Coarse features, gibbus deformity, very low IDUA activity in leukocytes and very high excre-

tion levels of urinary MPS are characteristics of Hurler syndrome. In Scheie syndrome, normal stature, joint stiffness, mild IDUA deficiency in leukocytes and only a moderate increase in excreted MPS are the main features. Hurler–Scheie syndrome, however, has intermediate clinical and biochemical manifestations. Its accurate diagnosis depends on clinical expertise and molecular analysis.

In future work, the assessment of IDUA is recommended for carrier detection among relatives of an affected individual and for prenatal diagnosis.

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