JIMD Reports
DOI 10.1007/8904_2015_484

RESEARCH REPORT

Screening for Attenuated Forms of Mucopolysaccharidoses in Patients with Osteoarticular Problems of Unknown Etiology

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Received: 13 January 2015 / Revised: 29 May 2015 / Accepted: 09 July 2015 / Published online: 28 August 2015

Abstract *Introduction*: The mucopolysaccharidoses (MPS) are a group of 11 inborn errors of metabolism (IEM) which are part of the lysosomal storage diseases (LSDs). The MPS are multisystemic conditions that affect the entire body, with variations in the clinical presentation, having specific treatments available depending on the type of MPS. Nearly all MPS disorders compromise the osteoarticular system in different ways, and virtually all patients have abnormal urinary excretion of glycosaminoglycans (GAGs). MPS are rare diseases that are underdiagnosed due to health-care professionals' lack of awareness, to poor access to screening and diagnostic methods, and to their extensive clinical heterogeneity. Attenuated forms may occur, which can make diagnosis of MPS even more difficult.

Methods: This study was conducted prospectively from March 2012 to January 2014 and included 55 patients at rheumatology and/or orthopedic services in Porto Alegre, Brazil. The screened patients presented with articular manifestations with no defined etiology. These patients were screened by quantitative and qualitative assessment of urinary GAGs.

Results and Discussion: Among the 55 cases investigated, one 15-year-old patient exhibited increased urinary GAG excretion; this patient was subsequently diagnosed with an attenuated form of MPS II, which was previously undetected.

Conclusion: Although the proportion of patients with MPS identified in the study sample was small (1/55), this study shows that these diseases are underdiagnosed and that systematic screening can help identify patients who may benefit from specific treatments already available for several MPS types.

Communicated by: Maurizio Scarpa, MD, PhD

Competing interests: None declared

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Introduction

The mucopolysaccharidoses (MPS) are inborn errors of metabolism involving the glycosaminoglycans (GAGs), which are formed by sugar chains bound to a protein core structure and are a major component of the connective tissue matrix. Deficiencies in the enzymes involved in GAG degradation lead to the accumulation of GAGs in the lysosomes, particularly in organs such as the liver, spleen,

heart, and cornea, among others, and result in marked osteoarticular impairment (Neufeld and Muenzer, 2001).

With the exception of MPS II (Hunter syndrome, inherited recessive X-linked mode), all MPS are autosomal recessive. Currently, there are 11 different enzyme deficiencies known to result in MPS. MPS are rare conditions, affecting an estimated 1 in 22,000 individuals (Poorthuis et al. 1999; Meikle et al. 1999). Currently, enzyme replacement therapy is available for 3 MPS types (I, II, and VI) and in development for 4 other types (IIIA, IIIB, IVA, and VII).

The clinical manifestations of each condition vary greatly, which may be related not only to the varied underlying genotypes but also to other poorly understood factors (Giugliani 2012). Because of the clinical heterogeneity of these conditions, early diagnosis is most likely for patients who exhibit severe phenotypes, and diagnosis may be delayed in the attenuated forms (Hendriksz 2011).

Stiffness occurs in patients with all types of MPS, except MPS IV, which is associated with ligament laxity. In cases of attenuated disease, specialists in rheumatology and orthopedics are often sought before a diagnosis is reached because of the common osteoarticular problems in MPS patients, which are often the first symptoms to appear. These specialists should be prepared to recognize and diagnose MPS or rule out a MPS diagnosis and refer the patient to an appropriate management. However, recognition and diagnosis can be a challenge because the attenuated forms of MPS manifest with subtle and/or nonspecific symptoms. Incorrect diagnoses common to these cases include autoimmune diseases, muscular dystrophies, connective tissue disease, osteogenesis imperfecta, polymyositis, dermatomyositis, polyneuropathy, fibromyalgia, rheumatoid arthritis, scleroderma, juvenile idiopathic arthritis, spondyloarthritis, Legg–Calve–Perthes disease, and other systemic rheumatic conditions including "growing pain" (Hendriksz 2011). One study performed by Cimaz et al. (2009) showed that only 13% of pediatric rheumatologists and 19% of rheumatologists were able to detect MPS when clinical cases of an 8-year-old girl and a 23-year-old woman were presented, respectively, in which both had classic symptoms and articular manifestations of MPS.

The goal of the present study was to evaluate the frequency of MPS in a sample of rheumatology and orthopedic patients with joint complaints of unknown etiology after a typical clinical and laboratory investigation was performed.

Materials and Methods

This study was conducted prospectively from March 2012 to January 2014 in rheumatology and orthopedic outpatient clinics in Porto Alegre, Brazil, and was previously approved by the research ethics committee of Hospital de Clinicas de Porto Alegre (university hospital).

This study had a cross-sectional design, and the main inclusion criterion was the presence of osteoarticular manifestations (usually articular pain, joint stiffness, joint contractures, noninflammatory increased articular volume, secondary osteoarthrosis) that had unknown etiology after a typical clinical and laboratorial investigation was performed. In addition to a known etiology, the presence of inflammatory arthropathy or primary osteoarthrosis was also considered an exclusion criterion.

The study included 55 patients with symptoms that could not be explained by any diagnosis. Data were collected from these patients. Despite available for many patients, X-ray results were not considered as inclusion or exclusion criteria and were not analyzed in this study.

From all patients, a random urine sample was collected, which was evaluated for measurement of the excretion of total GAGs using a quantitative di-methylene blue colorimetric assay (De Jong et al. 1992) and electrophoresis for qualitative evaluation of the GAG species (Pennock 1976; Cappelletti et al. 1979).

Results and Discussion

Of the 55 patients included in the study, 28 were male and 27 were female, with ages ranging from 3 to 21 years (mean age of 9 years). All patients reported discomfort or joint pain, and 2/3 of the patients also complained of stiffness.

The results of the urinary GAG analysis allowed us to identify six cases with increased GAG excretion when reference values were adjusted for age. In five of these cases, the results could not be confirmed in a second sample, and the qualitative analysis of the GAG species was normal; thus, a MPS diagnosis was ruled out. False positives were more frequently observed in childhood, when urinary GAG excretion is relatively higher (data not shown). This 10% false positive rate was considered acceptable because, as in the present study, additional tests are available to confirm each diagnosis. We should point out that false negatives may occur and that more refined tests should be performed when a specific clinical suspicion of MPS is raised, especially of MPS III or MPS IV, even if the result of the screening is negative.

In one case, an increase in urinary GAGs was observed along with an altered qualitative pattern of GAG species. This patient, a 15-year-old male, was referred to the Pediatric Orthopedics Service with joint limitation in his hands and a limitation of extension in his fingers. An evaluation of GAG

excretion indicated a urine level of 147 μg/mg creatinine (reference value for this age, 13–59 μg/mg creatinine). The electrophoresis analysis showed an abnormal pattern of urinary GAGs in the presence of dermatan sulfate and heparan sulfate. As this pattern may be indicative of MPS I, II, or VII, further diagnostic tests were performed to analyze the activities of the specific enzymes deficient in these three MPS types (alpha-iduronidase, iduronate sulfatase, and beta-glucuronidase, respectively).

Iduronate sulfatase levels within the leukocytes of this patient were lower than normal (1.3 μmol/h/mg protein compared with the reference range of 31–110 μmol/h/mg). This result, coupled with the normal activity of another sulfatase (arylsulfatase B), ruled out the possibility of multiple sulfatase deficiency and confirmed the diagnosis of Hunter syndrome (MPS II).

Although our study included only 55 patients, the finding of a positive MPS case can be considered quite significant as the frequency of MPS is estimated to be 1 in 22,000 in the general population (Poorthuis et al. 1999; Meikle et al. 1999).

The attenuated forms of MPS have less systemic involvement than the classical forms, but over time, the osteoarticular manifestations can be significant. Joint pain, joint limitation, mild bone deformities, and necrosis of the femoral head can occur. Because of these symptoms, the demand for rheumatology and/or orthopedic services is common in these cases, making the patients treated by these specialists target groups for the detection of attenuated forms of MPS (Hendriksz 2011).

The history of the diagnosed patient indicated that he weighed 3.2 kg and was 49.0 cm long at birth, which is considered normal for babies born at term. The patient was the child of healthy, non-consanguineous parents with no known family history of MPS. At 2 months of life, the patient began to suffer from recurrent infections of the upper airways, which became increasingly less frequent until the age of 6, when the infections became rare. As recorded in this study, his weight at age 15 was 43.4 kg, and his height was 1.56 m, both of which are below average for his age. A closer examination revealed the presence of a winged scapula, palpable liver 5 cm below the costal margin, and a slightly infiltrated face. The results of additional tests, such as spirometry, resting electrocardiogram, pulmonary function tests, abdominal ultrasound, and a 6-minute walk test, were normal for his age. Together with the biochemical results, these findings established the clinical diagnosis of a non-neuronopathic (attenuated) form of MPS II, a condition for which enzyme replacement therapy with idursulfase is indicated and may positively modify the natural history of the disease in this patient.

Conclusion

Although the proportion of patients with MPS identified in this study sample was small (1/55), this study indicates that underdiagnosis of these diseases can occur and that systematic screening in patients with articular changes of unknown etiology may help to identify patients with MPS. This screening, which could be performed in a random urine sample, could be included in the evaluation work-up and requested directly by the rheumatologist or orthopedic surgeon. If clinical concerns remain, the referral to a genetic/metabolic specialist could be considered. Diagnosed patients may have access to available treatment measures, and their families may benefit from genetic counseling, prenatal diagnosis, and the early detection of new cases.

Acknowledgments The authors are grateful to CNPq (Brazilian National Council for Scientific and Technological Development); to FIPE/HCPA; to the Medical Genetics Service of Hospital de Clinicas de Porto Alegre, particularly its Laboratory of Inborn Errors of Metabolism (LEIM); and to the MPS Brazil Network.

Synopsis

As attenuated forms of mucopolysaccharidoses may be difficult to identify, selective screening in patients with osteoarticular disease of unknown etiology may help to identify affected cases who could benefit from the specific therapies already available for several MPS types.

Contributions of the Individual Authors

TCRS interviewed the patients, obtained informed consent, collected the samples, and drafted the first version of the manuscript. CFMS, PL, MP, and IS referred patients for inclusion in the study, provided clinical information, and reviewed the final version of the manuscript. FB and MB performed the laboratory tests needed for screening and diagnosis and reviewed the final version of the manuscript. RG supervised the project, provided guidance in all steps, and revised the final version of the manuscript.

Compliance with Ethics Guidelines

Competing Interest Statement

RG received travel grants, speaker honoraria, and/or investigator fees from Actelion, Amicus, BioMarin, Genzyme, Shire, and Synageva; all other authors have no competing interests to disclose.

Details of Funding for All Authors

This study was supported by an institutional grant from FIPE/HCPA and by a research grant from CNPq/Brazil. RG received educational and research grants from Actelion, Amicus, BioMarin, Genzyme, Shire, and Synageva, which are not related to the present study. All other authors have no funding sources to disclose.

Details of Ethics Approval

This study was approved by the Institutional Review Board (GPPG/HCPA) in 17/07/2012, with the number 11-0557.

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