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RESEARCH REPORT

Screening Mucopolysaccharidosis Type IX in Patients with Juvenile Idiopathic Arthritis

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Abstract Mucopolysaccharidosis is a group of lysosomal disorders of a deficiency of specific enzyme required for glycosaminoglycan degradation. Mucopolysaccharidosis type IX is the rarest form of mucopolysaccharidosis. To date, only four patients have been reported. The first reported patient had mild short stature and periarticular soft tissue masses; the other reported patients are clinically indistinguishable from juvenile idiopathic arthritis. In the present study, we screened mucopolysaccharidosis type IX among patients with juvenile idiopathic arthritis with hyaluronidase enzyme assay. One hundred and eight patients with JIA and 50 healthy age-matched control subjects were enrolled in the study. Among all patients, none had deficient hyaluronidase activity. Though serum Hyal-1 activity was significantly increased in JIA patients, compared with control subjects ($p < 0.000$), no correlation was found between CRP, ESR, and Hyal-1 activity ($p = 0.187$). In conclusion, the data reported in our study indicates that systemic metabolic investigation for hyaluronidase activity is not recommended in all patients with JIA.

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Introduction

Mucopolysaccharidosis (MPS) is a rare group of lysosomal disorders of glycosaminoglycan (GAG) catabolism, caused by a deficiency of a specific enzyme required for GAG degradation (Wraith 1995). These disorders are associated with a progressive accumulation of different types of GAGs in the lysosomes of various organs compromising their function. The clinical features of MPS differ depending on the specific enzyme deficiency, but major clinical features are mainly facial dysmorphism; hepatosplenomegaly; cardiac, respiratory, and skeletal involvement; and neurological, hematological, and ocular symptoms (Neufeld and Muenzer 2001).

Mucopolysaccharidosis type IX is caused by the deficiency of enzyme hyaluronidase 1 (Hyal-1) which degrades hyaluronan (hyaluronic acid) (HA). MPS type IX is the rarest form of MPS, and to date only four patients were reported. The first patient was reported in 1996. She had periarticular soft tissue masses, mild short stature, and acetabular erosions without classical MPS features like neurological or visceral involvement (Natowicz et al. 1996). Other three patients were the children of consanguineous Middle Eastern parent, and all present as juvenile idiopathic arthritis (JIA) (Imundo et al. 2011).

All reported patients with MPS type IX were presented with joint and skeletal problems; therefore, MPS type IX can be easily misdiagnosed as JIA. There is no information in the literature concerning this prevalence investigation. The aim of the present study is to assess the prevalence of

MPS type IX among a group of Turkish patients diagnosed with JIA.

Material and Method

Study Design and Population

This was a cross-sectional study of 108 JIA patients attending the Outpatient Pediatric Rheumatology Clinic of Cerrahpasa Medical Faculty Children's Hospital. Patients' JIA diagnosis was confirmed by a child rheumatologist using ILAR criteria (Petty et al. 2004). The patients were selected by random sampling. The patients with other diagnoses except JIA, patients who were not definitely diagnosed with JIA, those diagnosed with systemic JIA, and those who refused to join the study were excluded from the study. Age, gender, age at diagnosis, detailed family history (including consanguinity, additional affected siblings), subtype of JIA, medications, and response to medical treatment were recorded. All patients underwent a careful physical examination including height, weight, arthritis, periarticular masses, scoliosis, hepatosplenomegaly, dysmorphic features, and ophthalmologic evaluation. Last laboratory investigations including complete blood count, glucose, liver transaminases, urea, creatinine, creatinine phosphokinase, C-reactive protein (CRP), erythrocyte sedimentation ratio (ESR), antinuclear antibodies (ANA), anti-double-stranded DNA (anti-ds-DNA), rheumatoid factor (RF), and HLA B27 were recorded.

Serum samples for hyaluronidase analyses from patients were collected at the time of attendance to outpatient clinic and were stored at −80°C until analysis. 50 age-matched healthy volunteers served as control group.

Hyaluronidase Assay

Serum Hyal-1 activity was assessed for both patient and control groups. Hyal-1 activity was measured as described by Natowitz and Wang (Natowicz and Wang 1996). Ten microliter serum was incubated with 250 µl buffered substrate solution (0.10 mol/l sodium formate, pH 3.9, containing 0.1 mol/l sodium chloride, 250 mg/l HA, and 1.5 mmol/l saccharic acid 1,4-lactone) for 4 h at 37°C. The enzyme reaction was specifically terminated by addition of 50 µl 0.8 mol/l potassium tetraborate at pH 9.1 to each sample. The tubes were heated for 3 min in a boiling water bath and cooled in tap water. 1.5 ml *p*-dimethylaminobenzaldehyde reagent, prepared as described by Reissig et al., was added, then vortexed, and heated at 37°C for 20 min, briefly centrifuged and read using a colorimeter at 585 nm (Reissig et al. 1955). Consequently, the amount of reaction product by reducing *N*-acetylglucosamine termini was determined. Blanks for the reaction consisted of tubes in which the buffered substrate was incubated for 4 h at 37°C in the absence of serum which subsequently received potassium tetraborate and then serum and were then treated as described above. A standard curve was formed by using known concentrations of *N*-acetylglucosamine. For this method, 1 unit of Hyal-1 activity was defined as the production of 1 µmol/min of reaction product (reducing terminal *N*-acetylglucosamine) at 37°C.

This study was approved by the Local Ethics Committee of the University of Istanbul Cerrahpasa Medical Faculty (protocol: 83045809/604/02-15538, 05/06/2014), and an informed consent was obtained from all parents of the children included in this survey.

Statistical Analysis

Data were expressed using descriptive statistics such as mean and SD for continuous variables and number and percentage for categorical variables. A one-sample *t*-test was carried out for the quantitative estimation of Hyal-1 activity. Comparison between the groups was carried out by independent sample *t*-test. Statistical analyses of the parameters were performed by using the Statistical Package for the Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). *p*-value <0.05 was considered statistically significant.

Results

One hundred and eight patients with JIA were enrolled to the study. Patients' clinical and demographic characteristics are given at Table 1. Among all patients enrolled in the study, none had deficient hyaluronidase activity. Though serum Hyal-1 activity was significantly increased in JIA patients, compared with control subjects ($p < 0.000$), no correlation was found between CRP, ESR, and Hyal-1 activity ($p = 0.187$) (Table 2).

Discussion

In the present study, we screened for Hyal-1 deficiency for the diagnosis of MPS type IX among patients diagnosed with JIA. Among 108 patients with JIA, no patient totally lacked serum Hyal-1 activity, so we were unable to detect any patients with MPS type IX. Despite that, the abnormality found was the increased level of plasma Hyal-1 level related to the control group. These results suggest that the prevalence of screened MPS type IX in association with JIA is low.

Only four patients were reported with MPS type IX to date. Natowitz described the first patient in 1996 with

Table 1 Demographic and clinic characteristics of JIA patients and control subjects

Variables	Patients	Controls
Sex (male/female)	46/62	23/27
Age (months)	129.01 ± 54.51	129.67 ± 51.25
Oligoarticular JIA (%)	55/108 (52.3)	
Polyarticular JIA (%)	43/108 (39.8)	
Enthesitis-related JIA (%)	10/108 (9.2)	
Responsive to medication (%)	104/108 (96.2)	
Scoliosis (%)	4/108 (3.7)	
Recurrent otitis media (%)	3/108 (2.7)	
Minor dysmorphic appearance (%)	12/108 (11.1)	
Mental retardation (mild) (%)	3/108 (2.7)	

Table 2 Hyaluronidase enzyme activity of JIA patients and control group

	JIA patients	Control subjects
Age (month)	129.89 ± 53.6	133 ± 51.5
Patients	108	50
Hyaluronidase activity (mU/l)	3,127.7 ± 564.5	2,525.4 ± 669.3
p-value	$p < 0.000$	

Values are mean ± SD

multiple periarticular soft tissue masses, popliteal cysts, joint effusions, acetabular erosions, mild short stature, flat nasal bridge, bifid uvula, submucosal cleft palate, and recurrent episodes of otitis media (Natowicz et al. 1996). The other three patients, reported by Imundo et al. (2011), were siblings of consanguineous Middle Eastern parents. These patients were clinically indistinguishable from JIA; none of the patients had cutaneous swelling, otolaryngeal problems, or short stature (Imundo et al. 2011). Triggs-Raine demonstrated the molecular basis of MPS type IX that is caused by mutations in HYAL1, a gene encoding hyaluronidase (Triggs-Raine et al. 1999). Mouse models of human MPS type IX revealed that HYAL1 mutations resulted in osteoarthritis and not surprisingly that all mice appeared normal with no evidence of skeletal defects and no organomegaly (Martin et al. 2008). Another study revealed that HYAL2-deficient mouse exhibited skeletal and hematological abnormalities, especially on frontonasal and vertebral bone formations (Jadin et al. 2008). Because of the indistinguishable clinical features of the disease and lack of the diagnosing criteria of MPS type IX, all subtypes of JIA patients were included in the study, randomly. MPS type IX is the rarest form of MPS, but apparently the disease characterizes rheumatologic features, and none of the reported patients showed any of MPS findings. Although high consanguinity rate and some skeletal abnormalities including scoliosis were defined in our study group, we were unable to identify any patient with deficient Hyal-1 activity, therefore with MPS type IX.

Studies exploring plasma or synovial fluid HA levels can be a marker for rheumatoid arthritis (RA), especially as a reflection of synovial involvement and inflammation in adult patients. Deficient hyaluronidase enzyme activity was not detected in any of these patients (Goldberg et al. 1991; Paimela et al. 1991; Nagaya et al. 1999). Enhanced serum hyaluronidase activity was also described in patients with spondyloarthropathies that was most evident in psoriatic arthritis (Kunder 2010). In another study of 48 patients with bone or connective tissue abnormalities, some with specific diagnosis (mucopolysaccharidoses, Ehlers–Danlos syndrome, achondroplasia, osteogenesis imperfecta, etc.) and some without any specific diagnosis (osteoporosis, dysplasia, skeleton deformities, etc.) were tested for hyaluronidase enzyme levels, and deficient enzyme activity was not detected in these patients, either (Fiszer-Szafarz et al. 2005). Serum Hyal-1 activity was also enhanced in this survey, which was consistent with previous studies. We were unable to define any relationship between serum Hyal-1 activity and other laboratory or clinical findings including the activity, the subtype of JIA, or response to treatment.

Limitations of this study should be underlined. The failure to detect any MPS type IX in our group of patients might be due to the limited sample size; much larger study groups including those with other rheumatologic diseases with therapy-resistant joint and skeletal involvement would be warranted to detect extremely rare MPS type IX.

Conclusions

As being an extremely rare inherited progressive lysosomal storage disorder, there are no definite criteria about which patients should be screened. Ill-defined clinical findings of MPS type IX led us to investigate whether there are unrecognized patients along JIA patients, although only oligoarticular and acetabular involvement has been reported previously

Failure to describe new patients within JIA patients led us to conclude that screening of hyaluronidase activity should be more limited to patients with poor response to standard therapies, with mono-oligoarticular involvement, with additional skeletal manifestations, and having a family history of multiple individuals affected and a history of consanguinity before deciding for the population to be screened for further studies.

Take-Home Message

Systemic metabolic investigation for hyaluronidase activity is not recommended in all patients with JIA.

Details of the Contributions of Individual Authors

Ertugrul Kiykim serves as the guarantor for the article. He accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. He has been involved in conception, design, analysis, and interpretation of the data and also drafting the article.

Kenan Barut has been involved in conception, design, analysis, and interpretation of the data.

Mehmet Serif Cansever has been involved in analysis and interpretation of the data.

Cigdem Aktuglu-Zeybek has been involved in conception, design, analysis, and interpretation of the data.

Tanyel Zubarioglu has been involved in conception, design, analysis, and interpretation of the data.

Ahmet Aydin has been involved in revising the article critically for important intellectual content.

Ozgur Kasapcopur has been involved in conception, design, and interpretation of the data and revising the article critically for important intellectual content.

Compliance with Ethics Guidelines

Conflict of Interest

Ertugrul Kiykim, Kenan Barut, Mehmet Serif Cansever, Cigdem Aktuglu-Zeybek, Tanyel Zubarioglu, Ahmet Aydin, and Ozgur Kasapcopur declare that they have no conflict of interest.

The authors confirm independence from the sponsors; the content of the article has not been influenced by the sponsors.

All procedures followed were in accordance with the ethical standards of the Local Ethics Committee of Cerrahpasa Medical Faculty and with the Helsinki Declaration of 1975, as revised in 2000.

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