When do we need to perform a diagnostic lumbar puncture for neurometabolic diseases? Positive yield and retrospective analysis from a tertiary center

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Neurometabolic diseases diagnosed by cerebrospinal fluid (CSF) examination are GLUT1 deficiency, serine-deficiency syndromes, glycine encephalopathy, cerebral folate deficiency, neonatal vitamin-responsive epileptic encephalopathies, disorders of monoamine metabolism, and γ-amino butyric acid (GABA) metabolism.

We retrospectively analyzed and compared the demographic, clinical, laboratory, and neuroimaging features of 62 patients in whom CSF examination was performed. Of the 62 patients, 16 (25.8%) had a final diagnosis, including succinic semialdehyde dehydrogenase (SSADH) deficiency (n=4), aromatic amino acid decarboxylase (AADC) deficiency (n=4), L-dopa-responsive dystonia (n=3), glycine encephalopathy (n=2), pyridoxal-phosphate-dependent seizures (n=1), cerebral folate deficiency (n=1), and serine biosynthesis defect (n=1). Parental consanguinity was present in all patients except one. Positive yield of a diagnostic lumbar puncture (LP) for the diagnosis of inherited neurotransmitter metabolism disorder was 25.8% overall. Oculogyric crisis (50%), diurnal variation (81.8%) and consanguinity (93.8%) were the only statistically significant variables between patients with and without a specific diagnosis.

It is challenging to diagnose neurotransmitter defects, since there is no ideal set of clinical symptoms. In our cohort, consanguinity, diurnal variation and abnormal ocular movements were the most significant findings associated with a diagnosis of a specific neurometabolic disorder based on CSF examination. Early diagnosis is of great importance not only for specific treatment, but also for genetic counseling and prenatal diagnosis.

Key words: neurotransmitter metabolism, cerebrospinal fluid metabolites, diagnostic lumbar puncture.

Diagnostic yield of metabolic and genetic investigations in children with neurological impairment varies in different centers in parallel with experience and available laboratory investigations. Inherited disorders of neurotransmitters are a group of neurometabolic syndromes attributable to a primary disturbance of neurotransmitter metabolism and transport[1-3].

Neurotransmitters are involved in a wide spectrum of neurological diseases, and correct recognition and differentiation of clinical and neurological phenotype is essential for a structured diagnostic approach[1-6]. The primary aim is early recognition and diagnosis of treatable conditions. There is no standard set of signs and symptoms that can guarantee the presence of a pediatric neurotransmitter

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disease; however, the presence of any of the following may be a clue: neonatal metabolic encephalopathy and seizures; postural or action dystonia or tremor; progressive or fluctuating limb rigidity; diurnal variation of movement disorder, tone, or function; progressive gait disturbance; ataxia; hypotonia; expressive speech delay; oculogyric crises; convergence spasm; other intermittent ocular movement abnormalities; or autonomic symptoms, such as excessive sweating, temperature instability, hypoglycemia, hypothermia, or ptosis[4-8].

In addition to the presentation in different age groups and the clinical heterogeneity, the diagnosis of this emerging group of diseases is challenging, because measurement of metabolites in peripheral fluids is generally uninformative, and cerebrospinal fluid (CSF) must be collected in a strictly defined manner to obtain valid information from CSF metabolite profiles[8]. As a brief summary: a) There is a rostrocaudal gradient of neurotransmitter metabolites and BH4 in the CSF. The same fraction of CSF must be used for each metabolite analysis, and the values obtained must be compared to age-matched reference ranges established using the same collection criteria[9], b) Tetrahydrobiopterin in the CSF is very labile and rapidly oxidizes to nonbiopterin species: CSF must be either frozen immediately at the bedside on dry ice or be collected into an antioxidant mixture to protect the BH4[10], and c) Breakage of red blood cells into the CSF causes rapid oxidation of neurotransmitter metabolites: blood-contaminated samples, therefore, must be centrifuged immediately, with the clear CSF transferred to new tubes before freezing[8]. Evaluation of the results requires additional expertise, since the concentrations of all the metabolites decrease rapidly within the first few months of life and then continue to decrease, at a slower rate, into adulthood[11]. Similar CSF profiles can often be seen as a secondary phenomenon, so absolute diagnosis requires follow-up testing and confirmatory tests including molecular genetic analysis[8,12].

It is very clear that close interaction and multidisciplinary teamwork between pediatric neurologists and specialists in inborn errors of metabolism are essential to timely identify and treat these patients.

For these purposes, we evaluated our patients retrospectively, and compared the demographic, clinical and laboratory (electroencephalography [EEG] and neuroimaging) features of those both with and without a specific diagnosis, for whom a routine and metabolite CSF analysis was performed in Hacettepe University İhsan Doğramacı Children's Hospital, Metabolic Diseases Diagnosis and Screening Laboratory, based on assays done on CSF samples between March 2009-September 2010.

Material and Methods

We retrospectively analyzed the demographic, clinical, and laboratory (biochemical, electrophysiological, and neuroimaging) findings of 62 patients referred to the Departments of Pediatric Neurology and Metabolism outpatient clinics, or who were admitted to the hospital, in whom CSF examination was performed for a primary diagnosis of pediatric neurotransmitter disease. Inclusion criteria included otherwise undiagnosed patients presenting with: neonatal metabolic encephalopathy and seizures; postural or action dystonia or tremor; progressive or fluctuating limb rigidity; diurnal variation of movement disorder, tone, or function; progressive gait disturbance; ataxia; hypotonia; expressive speech delay; oculogyric crises; convergence spasm; other intermittent ocular movement abnormalities; or autonomic symptoms, such as excessive sweating, temperature instability, hypoglycemia, hypothermia, or ptosis.

A detailed history including family history was also evaluated, including anoxic/hypoxic insult at birth and complications, and a clinical phenotype according to neurological presentation and examination was defined. Patients with and without a final diagnosis in terms of CSF metabolite analysis were compared.

Cerebrospinal fluid (CSF) examination was done after a 4-hour fasting; serum samples for glucose, lactic acid and amino acids were taken first, and later, after appropriate sedation, lumbar puncture (LP) was performed. Special tubes (5 tubes individually marked) for sample collection were available at a site provided from the laboratory at -80°C, and after sample collection for CSF metabolites, an additional sample for CSF glucose, lactate and amino acids was obtained. CSF collected with the special tubes was then transferred to the laboratory at -80°C for analysis. CSF routine analysis included

CSF protein, glucose, lactate, and pyruvate values, and non-routine analysis included amino acids, biogenic amines and pterins. Biogenic amines and pterins were analyzed using high-performance liquid chromatography with a combination of electrochemical and fluorescence detectors.

DNA samples of the families are stored in Hacettepe University, Department of Medical Biology, TÜBİTAK/DNA Bank.

The Local Ethics Committee of Hacettepe University approved the study protocol (LUT 09/116-109), and informed consents were taken from the families.

Statistical Analysis

Categorical variables were defined with numbers and percentages (%), and for numerical variables, means ± standard deviations are given. Chi-square test was used for group comparisons of categorical variables. Numerical variables were analyzed by Shapiro-Wilk test and Mann-Whitney U test. A value of $p \leq 0.05$ was defined as indicating statistical significance. Analyses were done using the Statistical Package for the Social Sciences (SPSS) 15.0 program.

Results

Of the 62 patients (27 girls, 35 boys), 16 (25.8%) had a final diagnosis, including succinic semialdehyde dehydrogenase (SSADH) deficiency (n=4), aromatic amino acid decarboxylase (AADC) deficiency (n=4), L-dopa-responsive dystonia (n=3), glycine encephalopathy (n=2), pyridoxal-phosphate-dependent seizures (n=1), cerebral folate deficiency (n=1), and serine biosynthesis defect (n=1) (Fig. 1). Positive yield of a diagnostic LP, based on abnormal CSF metabolite profile, was 25.8% overall.

Demographic features and variables analyzed in patients with and without a specific diagnosis are presented in detail in Tables I and II. The median age of the patients and follow-up time (time between a diagnostic LP and first clinical examination) was 36.1 (0.4-192.8) months and 6 (0-147.8) months, respectively. The consanguinity rate was approximately 58% overall, and parental consanguinity was present in all but one patient with a specific diagnosis. In 4 of the patients in the group with a specific diagnosis (25%) and in 10 of the patients in the group without a specific diagnosis (21.7%), there was a history of a hypoxic-ischemic insult at birth, requiring neonatal intensive care in 14.5% in the whole study population, and this was not statistically significant between the two groups (p=0.176). There was a family history of a similar sibling in 25% (n=4) of the patients with a specific diagnosis and in 21.7% (n=10) of the patients without a diagnosis, and this difference was also not statistically significant (p=0.743). Clinical symptoms including seizures, ocular movements (oculogyric crisis, abnormal eye movements, nystagmus), autistic features, diurnal fluctuation, and neonatal and/or early infantile severe epileptic encephalopathy and features of the physical/neurological

Table I. Demographic Features of Patients

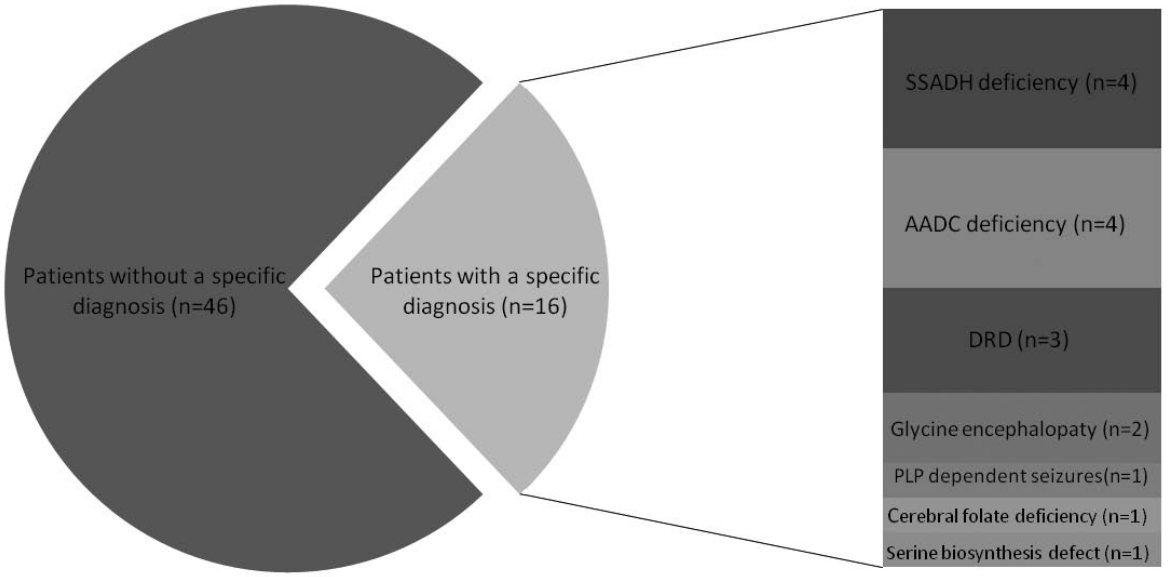
	Patients with a specific diagnosis (n=16) (%)	Patients without a specific diagnosis (n=46) (%)	Total (n= 62/p)
Median age (months)	29.8 (0.4-11.4)	36.1 (0.8-192.8)	
Gender			
Female	10 (37)	17 (63)	p= 0.138
Male	6 (17.1)	29 (89.9)	
Onset of symptoms (months), median (min, max)	4 (0-15)	4 (0-108)	
Follow-up time (months), median (min, max)	4.5 (0-28.8)	8 (0-147.8)	
Age at the time of diagnostic LP (months), median (min, max)	21.5 (0-105.4)	27.8 (0.7-156.7)	
Consanguinity			
(+)	15 (93.8)	21 (45.7)	p= 0.002
(-)	1 (6.3)	25 (54.6)	

Table II. Variables in Patients With and Without a Specific Diagnosis

	Patients with a specific diagnosis (n=16) (%)	Patients without a specific diagnosis (n=46) (%)	Total (n=62/p)
Oculogyric crisis			p= 0.000
(+)	8 (50)	0 (0)	
(-)	8 (50)	0 (0)	
Diurnal variation			p= 0.000
(+)	9 (56.3)	2 (4.3)	
(-)	7 (43.8)	44 (95.7)	
History of hypoxia			p= 0.176
(+)	4 (25)	10 (19.5)	
(-)	12 (75)	37 (80.4)	
Motor-mental retardation			
(+)	16 (100)	46 (100)	
(-)	0 (0)	0 (0)	
Family history			p= 0.743
(+)	4 (25)	10 (21.7)	
(-)	12 (75)	36 (78.3)	
Seizure			p= 0.616
(+)	11 (68.8)	36 (80)	
(-)	5 (31.3)	9 (20)	
Autistic findings			p=1.000
(+)	5 (31.3)	15 (32.6)	
(-)	11 (68.8)	31 (67.4)	
Intractable seizures (Neonatal/ infancy period)			p=0.520
(+)	3 (18.8)	14 (30.4)	
(-)	13 (81.3)	32 (69.6)	
Axial hypotonia			p=0.325
(+)	16 (100)	40 (87)	
(-)	0 (0)	6 (13)	
Peripheral hypertonia			p=1.000
(+)	8 (50)	24 (52.2)	
(-)	8 (50)	22 (47.8)	
Involuntary movement			p=0.408
(+)	8 (50)	18 (38.1)	
(-)	8 (50)	28 (60.9)	
Ataxia			p=0.162
(+)	6 (37.5)	8 (17.4)	
(-)	10 (62.5)	38 (82.6)	
Epileptiform activity (EEG)			p=0.949
(+)	8 (53.3)	27 (58.7)	
(-)	7 (46.7)	19 (41.3)	
Cranial MRI finding			p=0.439
Normal	4 (26.7)	7 (15.2)	
Abnormal	11 (73.3)	39 (84.8)	

examination including head circumference, body weight, axial hypotonia, peripheral hypertonicity, movement disorders (dystonia, chorea, tremor), ataxia, deep tendon reflexes, and pyramidal tract signs were evaluated in detail. Table II presents some of the data in terms of statistical significance.

All of the patients had motor and mental developmental delay. Head circumference was within normal limits in the majority of the patients (n= 43), while body weight of 37% (n=23) of the patients was ≤3p. One-fourth of the patients with a specific diagnosis had a history of anoxic/hypoxic insult and autistic



SSADH: Succinic semialdehyde dehydrogenase. AADC: Aromatic amino acid decarboxylase. DRD: L-dopa-responsive dystonia. PLP: Pyridoxal-5 phosphate.

Figure 1. Spectrum of patients with a specific diagnosis

features, neither of which was statistically significant between groups.

Oculogyric crisis (50%, p=0.000), diurnal variation (56.3%, p=0.000) and consanguinity (93.8%, p=0.002) were the only statistically significant variables between patients with and without a specific diagnosis.

Laboratory findings including EEG findings and cranial magnetic resonance imaging (MRI) were statistically not significant between the groups.

Discussion

The investigation of infants and children with suspected inborn errors of metabolism and specifically inherited disorders of neurotransmitter metabolism is challenging in pediatric neurology practice. Each infant/child deserves a detailed clinical and individual assessment. There is no standard battery of laboratory tests; thus, experience and multidisciplinary teamwork are of the utmost importance[1-3]. On the other hand, correct recognition and differentiation of complex clinical symptoms and presentations and of neuroradiological and biochemical phenotypes is extremely important for the timely introduction of advanced laboratory tests to allow early treatment, genetic counseling and prenatal diagnosis[4-8].

In the current study, we retrospectively analyzed in detail our patients suspected clinically of pediatric neurotransmitter disease. For this purpose, we reviewed our patients for whom a diagnostic LP was performed and analyzed in Hacettepe University İhsan Doğramacı Children's Hospital, Metabolic Diseases Diagnosis and Screening Laboratory. Our hospital is a tertiary referral center in Turkey.

Our primary aims were to define clues to perform a diagnostic LP study for a neurometabolic disorder and to evaluate the positive yield of a diagnostic LP in children with undiagnosed motor and mental retardation and otherwise undiagnosed specific clinical phenotypes, such as neonatal/early infantile severe epileptic encephalopathy and seizures, postural or action dystonia or tremor, progressive fluctuating dystonia, movement, tonus or functional disability fluctuating in nature, progressive gait disturbances, ataxia, hypotonia, delay in expressive language, abnormal eye movements, autonomic features, and ptosis.

Positive yield of a diagnostic LP in our cohort was 25.8% overall, i.e., 16 of 62 patients had a specific diagnosis based on abnormal CSF metabolite profile, after the procedure. This relatively high percentage can be explained by recognition of complex neurological/clinical

phenotypes by the same team, evaluation of patients in a multidisciplinary manner, a laboratory background, and the experience available at our center. It is also very clear that diagnosis based only on abnormal CSF metabolite profile should be supported by confirmatory tests such as enzymatic assays and genetic analysis, and definitive conclusions about the positive and negative yield of the CSF sample assay can be made thereafter.

The median time lag between the onset of symptoms and a diagnostic LP procedure in patients with a specific diagnosis was 21.5 (0-105.4) months, indicating the complex nature of symptomatology, which explains the prior diagnoses in these patients, such as cerebral palsy or childhood age-dependent epileptic encephalopathies. Another rate-limiting step is the availability of a laboratory experienced in evaluating CSF metabolite profile.

Positive yield of metabolic tests in children with motor and mental developmental delay has been evaluated in several studies[13]. In two class III studies evaluating 2655 patients with motor and mental developmental delay and mental retardation, positive yields of metabolic screening tests were 0.6% and 1.3%[14,15]. In a population-based study from Finland, involving patients with severe mental retardation who were born between 1969-1972 in a rather homogeneous and isolated population, the frequency of metabolic diseases was found to be 5%[16]. In a class III study from Israel and a class IV study from North America, even in the presence of a history and physical examination finding, positive yield of metabolic tests was approximately 1%[17,18]. The same group of investigators showed that diagnostic yield was less then 5% even in the presence of family history and consanguinity[19].

In our study, the diagnostic yield was high because a) for an invasive and more specific laboratory test, a homogeneous group of patients without a specific diagnosis was represented in the study, b) all patients had motor and mental developmental delay; however, they had additional features such as hypotonia, abnormal ocular eye movements, extrapyramidal and pyramidal signs, diurnal fluctuation, and refractory seizures, c) family history and consanguinity rate were high, and d) the patient population in our cohort was highly selected since our hospital is a tertiary referral center.

Looking at the detailed list of variables evaluated both in the history, birth and family history, symptoms and physical/neurological examination findings, diurnal variation, oculogyric crises and consanguinity were the most significant findings associated with a diagnosis of a specific neurometabolic disorder by CSF examination in our cohort.

One-fourth of the patients with a final diagnosis had a history of hypoxic birth, and this may lead to follow-up of patients with a diagnosis of static encephalopathy/cerebral palsy. Although it was not statistically significant, one- fourth of the patients with a specific diagnosis had autistic-spectrum disorder and stereotypical movements.

When we compared the physical/neurological examination findings of our patients with and without a specific diagnosis, there was no statistically significant variable, which also demonstrates the homogeneous nature of our patient population with the main findings of motor and mental developmental delay, axial hypotonia, peripheral hypertonicity, and pyramidal and extrapyramidal signs in combination. Parallel to these findings, there was no statistically significant variable in terms of laboratory tests (EEG, cranial MRI).

The diagnostic profile of the cohort with a specific diagnosis was heterogeneous: SSADH deficiency (n=4), AADC deficiency (n=4), L-dopa-responsive dystonia (n=3), glycine encephalopathy (n=2), pyridoxal-phosphate-dependent seizures (n=1), cerebral folate deficiency (n=1), and serine biosynthesis defect (n=1). Among the inherited disorders of neurotransmitter metabolism, there are a few exceptions that can be diagnosed based on abnormal metabolite profiles that can be detected using the universal methods for analyzing peripheral fluids[8]. For example, diagnosis of SSDH deficiency can be made by urine organic acid analysis or analysis of CSF for the presence of 4-OH-butyric acid, followed by confirmation of enzymatic activity level on lymphocytes and mutation analysis[20]. Elevation of vanillactic acid in the urine organic acid screen is another clue for AADC deficiency; however, detection of this metabolite is not routinely attempted, so the laboratory should specifically search for its presence[21]. Metabolic

tests such as urinary organic acid profile and/or tandem mass spectrometry (tandem-MS) were introduced at the same time as a diagnostic LP in some of our patients, and this explains why SSDH and AADC deficiencies are included in our diagnostic list.

Confirmatory tests such as enzymatic studies and molecular DNA analysis for SSADH deficiency and AADC deficiency and autoantibodies against membrane-bound folate receptors that are present on the choroid plexus[22] will be the second step in our cohort. Only after this second step will it perhaps be possible to calculate the positive and negative predictive values of the assays done on the CSF samples.

In conclusion, positive yield of diagnostic LP for a neurometabolic disorder should be considered especially in patients with abnormal eye movements and oculogyric crisis, diurnal fluctuation of symptoms and consanguinity. As there is no ideal set of metabolic screening tests, diagnosis of pediatric inherited neurotransmitter disorders depends on appropriate, individualized clinical, laboratory, neuroimaging, and further diagnostic investigations combined with a multidisciplinary teamwork.

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