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Selective Screening for Inborn Errors of Metabolism Using Tandem Mass Spectrometry in Newborns of the West Kazakhstan: Pilot Study

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Abstract: Tandem mass spectrometry can detect and quantify many metabolites in a single blood spot to diagnose amino acid disorders, organic acids, fatty acid oxidation, and urea cycle disorders. The use of tandem mass spectrometry (MS/MS) is expanding for the implementation of newborn screening programs for inborn errors of metabolism and for selective screening of children of different ages. In Kazakhstan, the use of MS/MS in metabolic screening programs is not yet developed due to the high cost of equipment and consumables and the lack of special screening centers and specialists. Data on the prevalence of most inborn errors of metabolism in Kazakhstan are not presented in the literature. Aim: to perform selective screening for hereditary metabolic diseases among newborns in western Kazakhstan using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Methods: Selective screening was performed among 250 newborns with suspected hereditary metabolic disorders using tandem mass spectrometry. Results: The results of selective newborn screening were interpreted by comparison with reference values established for this group. Diagnosis was based on clinical signs, blood levels of amino acids, acylcarnitines, succinylacetone, urine organic acids, and gene mutation tests. An assessment of 37 inborn errors of metabolism frequencies in high-risk newborns was performed. Conclusion: The research will further develop the national as selective as expanded newborn screening programs.

Keywords: Inborn errors of metabolism, Tandem mass spectrometry, Screening, Newborns

Introduction

Selective screening is an essential tool for diagnosing various types of inborn errors of metabolism (IEM). IEM are a group of phenotypically and genotypically heterogeneous metabolic disorders caused by mutations in genes encoding enzymes of metabolic pathways or receptors. Deficiency or change in the activity of necessary enzymes or other proteins in intermediate metabolic pathways leads to the accumulation or deficiency of the corresponding metabolites in cells or body fluids, manifesting in a wide range of diseases with clinical heterogeneity, thus complicating their diagnosis (Mak et al., 2013). Many IEM do not have specific clinical signs and are difficult to diagnose using only clinical manifestations or routine laboratory tests (Champion et al., 2010). Most often, IEM occur in early infancy and childhood, and the prevalence within different racial and ethnic groups is not the same. Hence, there are population differences in the incidence of IEM (Champion et al., 2010; Lampret et al., 2015; Shibata et al., 2018; Sarker et al., 2019). Tandem mass spectrometry can detect and quantify many metabolites in a single blood spot to diagnose amino acid disorders, organic acids, fatty acid oxidation, and urea cycle disorders (Chace et al., 2009; Lampret et al., 2015; Kaysheva et al., 2022; Gelb et al., 2022). The use of tandem mass spectrometry is expanding for the implementation of newborn screening programs (NBS) for inborn errors of metabolism and for selective screening of children of different ages (Landau et al., 2017; Shibata et al., 2018). The results from these expanded NBS programs provided information

on the prevalence of these diseases in the USA (Landau et al., 2017), some countries in Europe (Scolamiero et al., 2015; Messinaet al., 2018; Lampretet al., 2020; Loeber et al., 2021), and Asia (Yunus et al., 2016; Yang et al., 2020; Deng et al., 2021).

In Kazakhstan, the use of MS/MS in metabolic screening programs is not yet developed due to the high cost of equipment and consumables and the lack of special screening centers and specialists. Data on the prevalence of most inborn errors of metabolism in Kazakhstan are not presented in the literature.

Study Objectives

To perform selective screening for hereditary metabolic diseases among newborns with suspected IEM in western Kazakhstan using the LC-MS/MS method.

Tasks

1. to assess the burden of metabolic disorders detected by LC-MS/MS in western Kazakhstan by examination of newborns at clinical risk in pediatric clinics throughout the region;
2. to analyze prevalence, and age of onset for each identified IEM, further comparing the obtained findings with those from previously published reports in other populations.

Methods

Study Design and Ethics

In this observational study, a cross-sectional design is used due to its screening nature. The data for the present research will be derived from a selective LC-MS/MS IEM screening of 250 clinical-risk newborns.

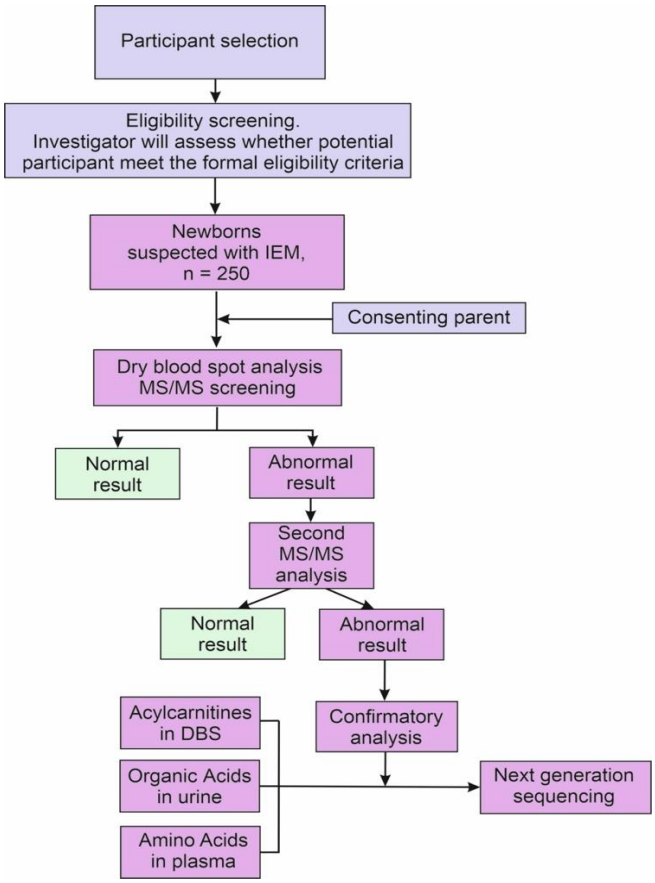

Figure 1. Flowchart of the study. (DBS means Dried Blood Spot analysis)

This research poses no risk to participating individuals. All study procedures are conducted according to the principles of the Declaration of Helsinki (2013), and patient rights are observed. Participation in the study is voluntary. Informed consent is obtained from all parents and/or legal guardians of children involved in the study. The study was approved by the Bioethics Committee of the West Kazakhstan Marat Ospanov Medical University (Ref. No. 7, 09/09/2020.).

Criteria for Inclusion in the Study

The inclusion criteria for IEM selective screening are presented in Table 1.

Table 1. Criteria for inclusion in the selective screening for IEM.

Main criteria (symptoms)	Sudden deterioration in the clinical condition of the child after a period of normal development (days, weeks, months): acute metabolic encephalopathy lethargy (coma) seizures resistant to antiepileptic therapy Hepatomegaly (hepatosplenomegaly) Metabolic acidosis with increased anion gap Multiple fractures Child mortality in the family from diseases with similar symptoms
Additional criteria (symptoms)	Treatment-resistant seizures Abnormal muscle tone: dystonia, hyperkinesis, hypotension Speech retardation Mental retardation of unknown cause Cardiomyopathy Tachypnea Frequent spitting up (vomiting) Osteoarticular anomalies (joint stiffness, chest deformity, rickets-like changes) Hernias (umbilical, inguinal-scrotal) Persistent or recurrent hypoglycemia Metabolic alkalosis An increase in ketone bodies in the blood and (or) urine Hyperammonemia An increase in the level of liver enzymes (ALAT, AST) more than 1.5 times Increase in the level of creatine phosphokinase (CPK) by more than 2 times Decrease in the level of alkaline phosphatase (AP) below the age norm Imaging or electrophysiological examinations indicating a metabolic disorder Leukopenia Thrombocytopenia Abnormal odor of urine, body, earwax, any unusual odor Hair growth disorders, alopecia Ophthalmic anomalies Unusual appearance, dysmorphic features History of death of a previous sibling of unknown cause Parents' consanguinity Positive family history with metabolic disorders

Exclusion criteria:

Patients having the following conditions will be excluded:

1. perinatal brain injury,
2. brain injury,
3. infections of the central nervous system,
4. toxicological diseases,
5. tumors,
6. chromosomal abnormalities,
7. symptoms specified in the Inclusion criteria, but with a confirmed diagnosis of any disease other than amino acid disorders (AAD), fatty acid oxygenation disorders (FAOD), or organic acidemias (OA).

Mass Spectrometry Analysis

Specimen Collection and Storage

Neonatal whole blood samples were collected from infants no earlier than 3 hours after feeding by heel prick using a heel stick. Five drops of whole blood (each ~75 µl) were applied to Guthrie cards, Ahlstrom 226 filter paper, and PerkinElmer 226 Five-Spot Card (PerkinElmer Health Sciences, Greenville, USA) to form dried blood spots (DBSs) for LC-MS/MS analysis. Samples were dried for 4 hours at room temperature and then stored at 4°C in labeled individual zip-lock plastic envelopes with desiccants until analyzed by LC-MS/MS. Samples were sent to the laboratory within five days. In the case of long-term storage of samples, it was carried out at a temperature of –20°C.

Specimen Preparation and LC-MS/MS Analysis

The Neobase2 TM Non-derivatized MSMS kit (PerkinElmer, Wallac Oy, Turku, Finland) was used to quantify 15 amino acids, free carnitine, 35 acylcarnitines, and succinylacetone in DBS according to the manufacturer's instructions. Metabolites to be measured: Amino acids: Alanine (Ala), Arginine (Arg), Citrulline (Cit), Glutamine (Gln), Glutamic acid (Glu), Glycine (Gly), Leucine (Leu), Isoleucine (Ile), Hydroxyproline (Pro-OH), Methionine (Met), Ornithine (Orn), Phenylalanine (Phe), Proline (Pro), Tyrosine (Tyr), Valine (Val).

Acylcarnitines: Free carnitine (C0), Acetylcarnitine (C2), Propionylcarnitine (C3), Malonylcarnitine+3-Hydroxybutyrylcarnitine (C3DC/C4OH), Butyrylcarnitine (C4), 2H9-C5-Methylmalonylcarnitine+3-Hydroxyisovalerylcarnitine (C4DC/C5OH), Isovalerylcarnitine (C5), Tiglylcarnitine (C5:1), Glutarylcarnitine (C5DC), Hexanoylcarnitine (C6), Octanoylcarnitine (C8), Octenoylcarnitine (C8:1), Decanoylcarnitine (C10), Decenoylcarnitine (C10:1), Decadienoylcarnitine (C10:2), Dodecanoylcarnitine (C12), Hydroxydodecenoylcarnitine (C12:1), Myristoylcarnitine (C14), Tetradecenoylcarnitine (C14:1), Tetradecadienyl-carnitine (C14:2), Hydroxytetradecanoylcarnitine (C14OH), Palmitoylcarnitine (C16), Hexadecenoylcarnitine (C16:1), 2H3-C16-3-Hydroxy-Hexadecanoylcarnitine (C16OH), 2H3-C16-3-Hydroxypalmitoleylcarnitine (C16:1OH), 2H3-Stearoylcarnitin (C18), 2H3-C18-Octadecenoylcarnitine (C18:1), 2H3-C18-Linoleylcarnitine (C18:2), 2H3-C18-3-Hydroxystearoylcarnitine (C18OH), 2H3-C18-3-Hydroxyoleoylcarnitine (C18:1OH), 2H3-C18-3-Hydroxylinoleoylcarnitine (C18:2OH).
Succinylacetone (SUAC) (13C5-MPP IS).

DBS were analyzed using a Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer (Shimadzu Corporation, Kyoto, Japan). Sample preparation was based on extraction followed by derivatization into oil esters. Level I and Level II (low standard and high standard) dried blood drops were included with each assay lot of the Neobase2 TM Non-derivatized MSMS kit to monitor system accuracy and precision. To analyze amino acids and acylcarnitines, stored DBS card samples are brought to room temperature (+18 to +25°C) before extraction. A 3.2 mm disc (equivalent to ~3.1 µl of whole blood) is punched out of one dried blood spot with a diameter of 3.2 mm using a Wallac DBS Puncher (PerkinElmer, Wallac Oy, Mustionkatu 6, FI-20750 Turku, Finland) into the well of the 96-well polystyrene U-bottom microplate supplied with the Neobase2 TM Non-derivatized MSMS kit. After adding 125 µL of working extraction solution to each well of the microplate, the plate is covered with an adhesive aluminum film and incubated for 30 minutes at room temperature on a microplate shaker with a shaking speed of 650 rpm. After incubation, 100 µL of the supernatant is transferred to a new 96-well U-bottom microplate, covered with aluminum foil to reduce evaporation, and incubated for 1 hour. The plate is then placed into the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer autosampler, and 5 µL of supernatant is injected into the LCMS for analysis.

Statistical Analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to check the normality of the distribution. The data obtained in the study demonstrated that the distribution of amino acids and acylcarnitines in DBS differs from normal. Me (median) and quartiles (IQR interquartile range) were used for descriptive statistics of the samples. Nonparametric tests (Mann-Whitney U test, Kruskal-Wallis H test) were used to test differences in amino acids and acylcarnitines concentrations depending on various factors (gender, place of residence). Two-sided levels <0.05 are assumed to be statistically significant. Statistical analysis will be carried out using the statistical packages IBM SPSS v. 23.0 (IBM, Armonk, NY, USA), Statistica (StatSoft, Inc., Tulsa, OK, USA, v. 10), and R 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

The study is currently in the recruitment stage. In total, samples from 130 study participants were collected. Demographic and anthropometric data of newborns with suspected hereditary metabolic diseases are presented in Table 2.

Table 2. Demographic and anthropometric data of newborns with suspected hereditary metabolic diseases

	Newborns with suspected IEM (n=130)
Weight in grams, Median (IQR)	3007 (2522;3489)
Gender	
Male, n, %	66 (50.8 %)
Female, n, %	64 (49.2 %)
Geographic distribution	
Urban population, n, %	89 (68.5 %)
Rural population, n, %	41 (31.5 %)

Table 3 describes the primary clinical manifestations and main diagnostic markers of IEM and their frequency in high-risk newborns. Currently, these clinical manifestations have not been confirmed by LC-MS/MS in the majority of children examined. Only a small proportion of patients (5) showed deviations from the reference values of amino acids and acylcarnitines established earlier in our study.

Table 3. Common features encountered in newborns with suspected IEM (n = 130).

Variables	Number of patients (percent)
Developmental delay	78 (60.0 %)
Neurological abnormalities	82 (63.1 %)
Disturbed consciousness level	8 (6.15 %)
Vomiting/dehydration	13 (10.0 %)
Hyperammonemia	11 (8.46 %)
Metabolic acidosis	24 (18.5 %)
MRI brain abnormalities	7 (5.38 %)
Infections	61 (46.9 %)
Hypoglycemia	15 (11.5 %)
Organomegaly	9 (6.92 %)
Micro/macrocephaly	38 (29.2 %)
Ophthalmic abnormalities	7 (5.38 %)
Cardiopathy	18 (13.8 %)
Seizures	48 (36.9 %)
Tachypnea	24 (18.5 %)
Thrombocytopenia	6 (4.6 %)
Abnormal smell	1 (0.07 %)
Hair growth disorders	6 (4.61 %)
Osteoarticular anomalies	17 (13.08 %)
Abnormal muscle tone	82 (63.1 %)

Conclusion

The data of selective screening conducted among newborns at high risk of IEM, given the inclusion in the study of the largest pediatric hospitals in western Kazakhstan, can serve as a basis for calculating the relative frequencies of various IEMs in the child population of the region. The research will further develop the national as selective as expanded newborn screening programs.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Acknowledgements or Notes

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