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Diagnostic Workup of Inherited Metabolic Disorders using Amino Acid Profiling

Muhammad Usman Munir, Nayab Zehra, Muhammad Qaiser Alam Khan, Nasir Ahmed

Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS), Rawalpindi Pakistan

ABSTRACT

Objective: To assess amino acid profiling-appropriate utility for diagnosing inherited metabolic diseases.
Study Design: Retrospective observational study.
Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jan 2021 to Dec 2022.
Methodology: The study comprised of a total of 1128 cases enrolled over the period of two years. The analysis of plasma amino acids was done on Biochrome 30+ by Ion Exchange Chromatography using a lithium column diameter of 4.6 mm. Our study included infants and children of either gender from 3 days to 18 months of age having symptoms of developmental delay, deranged laboratory investigations and suspicion of inherited metabolic diseases (IMDs). Chi-Square test was applied to check the correlation between age of patients and different diagnosed parameters.
Results: Out of 1128 cases, 46 cases (4.0%) came out to be positive for IMDs over the period of two years with more percentage of females 24(52.2%) than males 22(47.8%). The maximum number of cases 14(30.4%) reported were of Maple Syrup Urine Disorder (MSUD), a branched chain metabolic disorder, followed by Non-Ketotic Hyperglycinemia (NKH) 21.7%. All these cases had deranged biochemical profiles with a history of developmental delay.
Conclusion: Diagnosis of inherited metabolic diseases (IMDs) should involve plasma amino acid analysis keeping in view the biochemical findings and differential diagnosis to avoid unnecessary financial burden on the patient's family and the diagnostic laboratories.

Keywords: High Performance Liquid Chromatography, Inherited Metabolic Diseases, Plasma Amino Acids.

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INTRODUCTION

Plasma amino acids are a group of compounds that serve as the building blocks of proteins in the human body. They play essential roles in various physiological processes like growth, development, production of enzymes, hormones, and various neurotransmitters, and can be influenced by factors such as dietary intake, metabolic processes, and any pathological process in the body. The deficiency or imbalance in specific amino acids can lead to impaired growth, development, and compromised immune function resulting in various metabolic disorders.[1,2] These genetic disorders are mostly inherited in an autosomal recessive pattern and are mainly frequent in countries where consanguineous marriages are prevalent.[3]

The occurrence of inherited metabolic diseases (IMDs) in Pakistan is not exactly known.[4] Due to these constraints, children born with IMDs are usually diagnosed on the basis of symptoms with which they present as hypotonia, developmental delay, lethargy, poor feeding, and seizures.[5] Plasma amino acid analysis is a diagnostic tool used in clinical settings to assess amino acid levels in the blood. This test can help identify amino acid deficiencies, excesses, or abnormalities that may indicate underlying health conditions. However, before proceeding to treatment, an accurate genetic analysis is mandatory to confirm the diagnosis.[6,7] Moreover, the population selected for screening also contributes to determining the frequency of these IMDs.[8]

The treatment of most of the IMDs primarily includes dietary restriction of that specific amino acid and later on some medications have proved to be very effective in treating such patients. The substituted ingredients necessary for growth and development should be included in the diet.[9] The purpose of our study was to assess the best utility of amino acid profiling to diagnose various IMDs on the basis of their clinical and biochemical correlation and also to screen unnecessary testing among our population.

METHODOLOGY

This retrospective observational study was conducted at the Armed Forces Institute of Pathology, Rawalpindi Pakistan, from January 2021 to December

Correspondence: Dr Muhammad Usman Munir, Department of Chemical Pathology and Endocrinology, AFIP, Rawalpindi Pakistan
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2022, after ethical approval from institutional review board (No. Cons-CHP-6/READ-IRB/23/1762).

Inclusion Criteria: Our study included infants and children of either gender from 3 days to 15 months of age, having symptoms of vomiting, metabolic acidosis, hypotonia, persistent hypoglycemia and development delay with seizures were included.

Exclusion Criteria: Patients without neuroimaging reports of computerized tomography (CT) scan and magnetic resonance imaging (MRI brain) to relate any CNS disorder with their clinical symptoms were excluded.

Total number of 1128 cases were enrolled. Sample size of 782 was calculated using World Health Organization sample size calculator taking prevalence of 1 case of IMDs in 5000 live births with margin of error of 0.001.[10] Data was extracted from laboratory information management system and inherited metabolic disease workup sheet.

Investigations conducted at the hospital laboratory included complete blood picture, arterial blood gases, C-reactive protein, liver and kidney function tests, blood glucose random, serum electrolytes, serum ammonia, plasma lactate, urine for reducing substances and ketones. Complete history of any unexplained death of the siblings, feeding, medication or any significant antenatal history was also taken from the parents. The suspected cases of IMDs based on plasma amino acid levels were included in the study.

About 3-4 ml of venous blood in lithium heparin tube was used for analysis. Samples were then centrifuged and sample extraction done according to the standard protocol. Plasma amino acid analysis was done on Biochrome 30+ by Ion Exchange Chromatography using lithium column diameter 4.6 mm. The column elute was mixed with Ninhydrin reagent and the Ninhydrin reacts with amino acids present in the elute to form colored complexes. The amount of colored complex produced was directly proportional to the concentration of that particular amino acid present in the original mixture. The measurement is taken at 2 wave lengths. The photometer forms peaks showing amino acids present in the original mixture in relation with the retention time.

Statistical Package for Social Sciences (SPSS) version 23 was used to analyze the data. Frequencies and percentages were calculated for gender, consanguinity, sibling deaths, biochemical and clinical findings. Different IMDs were identified and frequencies and percentages were analyzed for each specific IMD. Chi-Square test was applied to check the association between age of patients and different diagnosed parameters. A *p*-value of ≤0.05 was considered statistically significant.

RESULTS

Out of 1128 cases, 46 were reported positive for IMDs over a period of two years. From these, females were 24(52.2%) and males were 22(47.8%). The mean age was 10.0±3.4 months and 8.6±6.5 months for males and females respectively. More cases were reported among males and females in age groups ≤3 months (Table-I). The maximum number of cases 14(30.4%) reported were of Maple Syrup Urine Disorder (MSUD), a branched chain metabolic disorder, followed by Non-Ketotic Hyperglycinemia (NKH) 21.7%.

Table-I: Gender Distribution in different Age Groups (n=46)

Age	Female n(%)	Male n(%)	Total n(%)
≤3 months	17(70.8%)	15(68.2%)	32(69.6%)
>3 months	7(29.2%)	7(31.8%)	14(30.4%)

All IMDs were more significant in the age group of ≤3 months except Phenylketonuria and Citrullinemia in which children presented at more than 3 months of age (Table-II).

Table-II: Distribution of Inherited Metabolic Diseases according to Age (n=46)

Types of IMDs	Age		n(%) (Total=46)	*p*-value
	≤3 months n(%)	>3 months n(%)		
Non-ketotic hyperglycinemia	9(1.00%)	1(7.1%)	10(21.7%)	0.019
Urea Cycle Defects	8(88.9%)	1(11.1%)	9(19.6%)	
Maple Syrup Urine Disease	10(71.4%)	4(28.6%)	14(30.4%)	
Phenyl Ketone Urea	0(0.0%)	2(100.0%)	2(4.3%)	
Tyrosinemia	2(100.0%)	0(0.0%)	2(4.3%)	
Citrullinemia	0(0.0%)	1(100.0%)	1(2.2%)	
Others	3(37.5%)	5(62.5%)	8(17.4%)	

There were 4 cases of Methyl Malonic Acid (MMA) and 4 cases of Alkaptonuria placed in this group. Among the clinical symptoms, hypotonia was the most common presenting feature in all cases followed by seizures.

There was a significant increase (>150 mmol/L) of ammonia in 34(80.4%) cases, however in 12(19%) cases the ammonia levels were not markedly raised though raised from normal level (Table-III). Metabolic acidosis was found in 36(78%) cases. Consanguineous

marriages were found in most of the cases 42(91%). However, there was no significant relation found between neuroimaging findings and metabolic disorders. Electrolytes were found deranged in 30(65%) of positive patients. Figure-1 shows the distribution of different types of Inherited Metabolic Diseases.

Table-III: Biochemical and Clinical Presentation of different Inherited Metabolic Diseases IMDs (n=46)

IMDs		Age n(%)		(Total=46) n(%)	*p*-value
		≤3 months	>3 months		
Family History	Positive	17(89.5%)	2(10.5%)	19(41.3%)	0.014
	Not Significant	15(55.6%)	12(44.4%)	27(58.7%)	
Consanguinity	Positive	30(71.4%)	12(28.6%)	42(91.3%)	0.373
	Negative	2(50.0%)	2(50.0%)	4(8.7%)	
Clinical Symptoms	Hypotonic	26(70.3%)	11(29.7%)	37(80.4%)	0.833
	Seizures	6(66.7%)	3(33.3%)	9(19.6%)	
Neuro-imaging	Positive Findings	2(33.3%)	4(66.7%)	6(13.0%)	0.039
	Not Significant	30(75.0%)	10(25.0%)	40(87.0%)	
Ammonia Levels	Markedly Raised	26(76.5%)	8(23.5%)	34(80.4%)	0.087
	Not Markedly Raised	6(50.0%)	6(50.0%)	12(19.6%)	
Metabolic Acidosis	Present	28(77.8%)	8(22.2%)	36(78.3%)	0.022
	Not Significant	4(40.0%)	6(60.0%)	10(21.7%)	
Electrolytes	Deranged	21(70.0%)	9(30.0%)	30(65.2%)	0.930
	Not Significant	11(68.7%)	5(31.3%)	16(34.8%)	

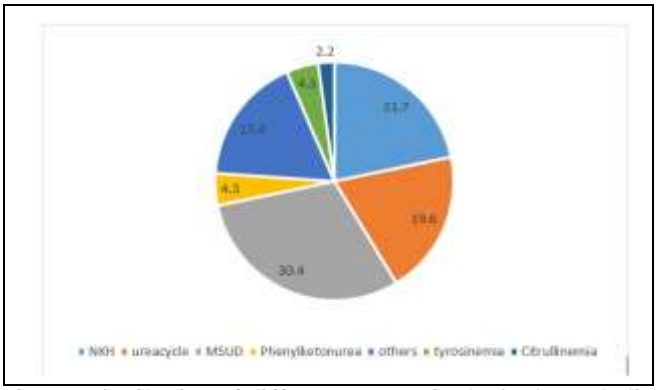

Figure: Distribution of different Types of Inherited Metabolic Diseases (n-46)

DISCUSSION

The prevalence of amino acid disorders is increasing day by day, as is the awareness of testing and its importance is increasing among the population. These metabolic disorders have multiple long-term effects on the family of the patients in terms of social, economic, and psychological background. The IMDs can be classified into two major categories. One has severe metabolic/neurological effects with fatal outcomes and the other has less severe effects with less harmful outcomes. Despite the category of IMD, it is important to diagnose them as early as possible to halt the disease at its initial stage and counsel the parents on the future development of that child as well as the chances of these disorders in further siblings.[11]

Few studies have been conducted in the past among our local population to see the frequency of IMDs. One such study was conducted by Afroze et al., among these patients, 426 patients were diagnosed with IMDs, showing a high percentage of 41%.[12] A similar study was conducted in Islamabad in 2017 by Gul et al. in which 30 positive cases were diagnosed over a period of one year showing G6PD Deficiency being the highest in the list of amino acid metabolic disorders. The commonest clinical presentation was vomiting followed by persistent jaundice.[13]

The purpose of our study was to correlate the clinical findings and biochemical investigations with the diagnosis of inborn metabolic disorder, which would be helpful for mapping the essential diagnostic workup of amino acid analysis. This would help in declining the unnecessary workload avoiding financial burden on the family of the patients, and on the institute as well. It has been observed that amino acid profiling is prescribed without clear indication of IMDs sought from clinical presentation and basic biochemical profile, which results in undue expenditure to patient's family and inappropriate usage of laboratory tests. We identified the patients having markedly raised ammonia, lactate levels, deranged arterial blood gases (ABGs) either having metabolic acidosis or metabolic alkalosis, elevated liver function tests with clinical findings have more probability of deranged amino acids as compared to those with no findings.

Of all the samples we received for amino acid profiling only 46(4.0%) of patients had markedly disturbed biochemical profiles along with deranged amino acid profiles. That shows the importance of initial workup before going to amino acid testing while cases with mildly raised or within the normal range of these parameters did not have a significant increase of any amino acid on ion exchange chromatography. The treatment of such patients is crucial due to dietary restrictions and lifelong medications which could have an impact on the child's health otherwise. So, diagnosing such cases is very important by considering all aspects of the patient.[14] We concluded that amino acid analysis could be reduced by considering the differential diagnosis in light of biochemical findings. This in turn would reduce the financial burden and unnecessary psychological pressure on the patient's family.

Some international studies showing the prevalence and distribution of IMDs in different geographical areas also disclosed that among thousands of cases reported at different timings, only few came out to be positive having significant biochemical findings. A study conducted by Dunne E, from January 2018 to December 2020 showed a prevalence of only 2.4% indicating that thoughts should be given to the usefulness of each targeted metabolic test.[15] One study conducted in China over the period of 2009-2016 in which total of 1,861,262 newborns were screened for amino acid metabolic disorders. Only 164 cases were diagnosed with IMDs, hyperphenylalaninemia (83 cases) being the most common disorder.[16] This is consistent with another study as well.[17]

On the other side, advances in genetic testing and understanding of metabolic pathways have significantly improved the diagnosis and management of IMDs. However, these disorders can still present significant challenges and require lifelong management and support for affected individuals and their families.[18] These disorders require strict dietary restrictions and supportive therapies for management. The parents of the affected child should also be counseled and guided for the child's future developmental challenges. Moreover, ongoing research in this field aims to develop new therapies and interventions to further improve outcomes for individuals with IEMs. However, till now genetic testing and enzyme estimation assays are the confirmatory tests for diagnosing metabolic disorders.[19]

LIMITATION OF STUDY

Being a single-centre study, the generalizability of our findings is limited.

CONCLUSION

Diagnosis of IMDs involves clinical evaluation, biochemical analysis, and genetic testing. Plasma amino acid analysis should be performed, keeping in view the biochemical findings and differential diagnosis to avoid unnecessary (financial) burden on the patient's family and the diagnostic laboratories.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MUM & NZ: Data acquisition, data analysis, critical review, approval of the final version to be published.

MQAK & NA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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