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ORIGINAL ARTICLE

Role of Plasma Amino Acid and Urine Organic Acid for the Diagnosis of Inborn Errors of Metabolism in Children with Developmental Impairment

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ABSTRACT

Objective: The inborn errors of metabolism (IEM) can lead to developmental impairment in children. Aim of the study is to find out the role of plasma amino acid and urine organic acid in diagnosing IEM.

Study Design: In this cross sectional prospective study, children from 6 months to 6 years of age either with developmental delay or developmental regression were included. Plasma amino acid and urine organic acid test were advised in all these children.

Place and Duration of Study: Pediatric Neurology OPD of Izzat Ali Shah Hospital in Wah Cantt from January 2019 to September 2020

Material and Methods: Plasma amino acids and urine organic acid analysis was performed at Aga Khan University Hospital Karachi.

Results: A total of 54 children were enrolled who presented with either developmental delay (n=44) or developmental regression (n=10). There were 35 male and 19 female children. Neuro-imaging findings were abnormal in 20 (37%) while EEG was abnormal in 9 (17%) children. Plasma amino acid showed metabolic abnormality in 3 (6%) cases (p value= 0.506) and urine organic acid identified metabolic disorder in 9 (16.6%) children (p value =0.218). The diagnostic yield of combined plasma amino acid and urine organic test was 20%.

Conclusion: Plasma amino acid and urine organic acid are useful tests in diagnosing inborn errors of metabolism responsible for developmental delay or developmental regression. Further studies in large scale are required to look for its usefulness.

Key Words: *Plasma amino acid, Urine organic acid, Developmental impairment, Inborn errors of metabolism*

INTRODUCTION

Developmental delay is one of the major problems in young children and its incidence is around 5-10% in childhood population.[1] Clinicians come across developmental impairment either in the form of delay or regression. There is wide variety of reasons behind the developmental impairment and multi-disciplinary approach is required to diagnose these patients. Developmental delay can be global or specific. Global developmental delay is defined as significant (>2 standard

deviation) delay in 2 or more developmental domains (gross motor, fine motor and vision, hearing and speech and social skills). The incidence of global developmental delay is 1-3% in children aged less than 5 years.[1,2] Developmental regression is loss of developmental mile stones which child had previously achieved.

Inborn error of metabolism (IEM) is rarely a cause of isolated developmental delay.[1] It usually affects other systems of body as well. IEM are single gene disorders resulting from the defects in the biochemical pathway of the body. The specific enzyme defect interfere with the metabolism of protein, carbohydrate or fats. IEM leads to abnormal synthesis and catabolism of metabolites.[3]

The prevalence of IEM in different countries varies between 1:300 to 1:5000 neonates. The overall incidence of recorded IEM was 10 folds greater among Pakistani children as compared to Caucasian population.[4] The main reason behind is the consanguineous marriage. In Pakistan there is no national newborn metabolic screen program available so most of these disorder go undiagnosed until someone specifically investigate for IEM. In developmentally delayed children, the prevalence of IEM is around 1% to 5%. It is important to diagnose IEM in children with developmental impairment as genetic counseling can be offered to the family. Specific therapies may be initiated to the child. The associated risk of acute metabolic compensation can be highlighted. Timely diagnosis of IEM reduces the morbidity and mortality in such children. Neuro-metabolic disorders account for nearly half of all IEM presents in the clinical practice.

There are many types of inborn errors of metabolism but common ones are disorders of carbohydrate metabolism, amino acid metabolism, urea cycle, fatty acid oxidation or porphyrin. There are also disorders of mitochondrial function, peroxisomal function and lysosomal storage which can present with developmental impairment. In this study, we decided to check the role of plasma amino acid (AA) and urine for organic acids (OA) in diagnosing IEM. Plasma AA is performed by ion exchange chromatography method, which is a process in which different chemicals are separated based on their affinity towards ion exchange resin. Urine OA is performed by gas chromatography on mass spectroscopy (GCMS). The diagnosis can be confirmed by enzyme or genetic studies. The current study is designed to look at the frequency of IEM in children with developmental impairment. The primary objective of the study to assess the role of plasma AA and urine OA in diagnosing inborn error of metabolism in children with developmental delay or regression.

MATERIAL AND METHODS

This cross sectional prospective study was carried out in the Pediatric Neurology out-patient department of Izzat Ali Shah Hospital in Wah Cantt from January 2019 to September 2020. The patients were consecutively enrolled from the age of 6 months to 16 years who presented with developmental delay or developmental regression. All the children were evaluated comprehensively and advised plasma amino acid and urine organic acid test where diagnosis was not clearly established by neuro-imaging and other biochemical tests. All the children who had developmental impairment due to hypothyroidism, congenital heart disease, Trisomy 21 and any other non-neurological medical condition were excluded from the study.

All the metabolic results were recorded in designed proforma. In addition, the demographic details, consanguinity, family history of metabolic disorder, history of epilepsy, other associated medical disorders, EEG and Neuro-imaging findings were also recorded.

Plasma amino acids and urine organic acid analysis was performed at the Biochemical Genetic Laboratory (BGL) of the Aga University Hospital Karachi. 3-4 ml of blood samples was collected in lithium heparin tube and random spot urine samples were collected for plasma amino acids and urine organic acids analysis respectively. Samples were transported in dry ice to the BGL from outreach phlebotomy centre in Wah Cantt and Islamabad/Rawalpindi region. All samples were stored at -20°C prior to analysis.

High performance liquid chromatography method was used for plasma amino acid test and Gas chromatography mass spectrometry method was

used for urine organic acid analysis. Internal and external quality control for amino acids and organic acids were performed, according to Institution guidelines.

All data was entered and analyzed using SPSS version 25. For quantitative variables mean and S.D was calculated. Qualitative variables like consanguinity, family history of similar disorder, history of epilepsy, EEG and Neuro-imaging findings were measured as frequency and percentage.

RESULTS

A total of 82 patients were enrolled for the study and given request for the plasma amino acid and urine organic acid but only 54 patients reported with the test results. The cost of the test was the main reason due to which they declined the test. Some patients were residing very far from the lab available for the tests. The children who were enrolled either presented with developmental delay (n=44) or developmental regression (n=10). Table 1 shows the frequency distribution of male children (n=35) as compared to female children (n=19). Family history of metabolic disorder was present in 7 (13%) children only while consanguinity among parents was present in 46 (85%) children. Thirty eight (70%) of children with developmental impairment also had history of epilepsy while 9 (16.6%) children had other associated medical disorders. Neuro-imaging finding either in the form of CT scan or MRI Brain were abnormal in 25 (46%) children while EEG was abnormal in 9 (17%) children. Plasma AA showed metabolic abnormality in 3 (6%) cases and urine OA was able to identify metabolic disorder in 9 (16.6%) children. Overall yield of combined plasma amino acid and urine organic test was 20% in the diagnosis of inborn errors of metabolism.

Table 2 and 3 shows the relation of plasma AA and urine OA with age categories. Most of the enrolled children were under 2 years of age, hence one case of abnormal plasma AA was identified in each of three categories which was not statistically significant. In terms of urine organic acid, it was found abnormal mostly in under 2 years of age.

TABLE 1: Frequency of demographic and clinical factors in children

Demographic and clinical factors		Number of children	Percentage
Developmental Delay		44	81.0
Developmental Regression		10	19.0
Male		35	64.0
Female		19	36.0
Family history of Metabolic disorder	Yes	7	13.0
	No	47	87.0
Consanguinity among parents	Yes	46	85.0
	No	08	15.0
History of Epilepsy	Yes	38	70.0
	No	16	30.0
Other associated medical disorder	Yes	09	17.0
	No	45	83.0
Neuro-Imaging (CT /MRI)	Normal	29	53.0
	Abnormal	25	46.0
EEG Findings	Normal	07	13.0
	Abnormal	09	17.0
	Not done	037	70.0
Plasma amino acid	Normal	51	94.0
	Abnormal	03	06.0
Urine Organic acid	Normal	45	83.0
	Abnormal	09	17.0

TABLE 2: Chi square test on relation of age with plasma amino acid

Age categories	Normal	Abnormal	Total	p value
< 2 years	29	1	30	
2 to 5 years	05	1	06	
>5 years	17	1	18	
Total	**51**	**3**	**54**	**0.529**

TABLE 3: Chi square test on relation of age with urine organic acid

Age categories	Normal	Abnormal	Total	p value
< 2 years	23	7	30	
2 to 5 years	6	0	6	
> 5 years	16	2	18	
Total	**45**	**9**	**54**	**0.173**

Table 4 and 5 shows that in 44 children with developmental delay, plasma AA was abnormal in 2 cases. In 10 children with developmental regression one had abnormal plasma AA (p value =0.446). Regarding urine OA, it was abnormal in 6 cases of developmental delay and 3 cases of developmental regression (p value=0.342).

TABLE 4: Correlation of developmental delay and regression with plasma amino acid

Development	Normal	Abnormal	Total	p value
Delay	42	2	44	
Regression	09	1	10	
Total	**51**	**3**	**54**	**0.446**

TABLE 5: Correlation of developmental delay and regression with urine organic acid

Development	Normal	Abnormal	Total	p value
Delay	38	6	44	
Regression	07	3	10	
Total	**45**	**9**	**54**	**0.342**

Table 6 shows the different diseases diagnosed on plasma AA and urine OA. There were two cases of multiple carboxylase deficiency, methylmelonic acidemia and glutaruic acidemia identified while single case of Phenylketonuria, Fumaric aciduria, Propionic aciduria, Non-ketotic hyperglycenemia and 3-methyl-glutaconic acidemia were diagnosed. Fumaric aciduria was the only condition which was confirmed on both plasma AA and urine OA.

TABLE 6: **Diseases identified on plasma AA and urine OA**

Diseases identified	Developmental delay (DD) or Developmental Regression (DR)	Family history of metabolic disorder	Consan-guinity	History of Epilepsy	MRI findings
Multiple Carboxylase Deficiency	DD	No	No	No	Normal
Methylmelonic acidemia	DD	No	Yes	Yes	Abnormal
Phenylketonuria	DD	No	Yes	Yes	Normal
Multiple Carboxylase Deficiency	DR	No	Yes	Yes	Normal
Fumaric aciduria	DR	No	Yes	Yes	Abnormal
Glutaric aciduria	DD	No	Yes	No	Abnormal
Glutaric aciduria	DD	Yes	Yes	Yes	Abnormal
Methylmelonic acidemia	DD	No	Yes	Yes	Normal
Propionic academia	DD	No	Yes	No	Abnormal
Non ketotic Hyperglycenemia	DD	No	Yes	No	Abnormal
3-methyl glutaconic aciduria	DR	Yes	Yes	No	Normal

DISCUSSION

Developmental impairment in the form of delay or regression is one of the major problems that come across in neurology outpatient department. Inborn errors of metabolism are sometimes responsible for this problem. Plasma AA and urine OA can detect some of the IEMs and sometimes it also needs confirmation by genetic testing.

In our study, the importance of these tests were evaluated. The diagnostic yield of these tests together was found to be nearly 20%. There is no single diagnosis which was more common in our study. In Satwani et al study, organic acidemias were found to be more common though the test was performed in children with all types of possible symptoms leading to IEM. Vomiting and respiratory distress were commonest symptoms.[5] In a quite similar study, Hafeez et al examined 805 samples for metabolic screening. Inherited metabolic diseases were found in 49 (6%) patients. Seizures were found to be the commonest symptom. Aminoacidopathies accounted for 57% and organic acidurias were found in 24.5% of positive cases. Non-ketotic hyperglycinemia was the most common disease found.[6] In our study epilepsy was present in 5 out of 11 diagnosed cases. There are several other Pakistani studies on inherited metabolic disorders with variable results. Cheema et al found 19

cases of IMD out of 239 samples. Disorders of carbohydrate metabolism were found to be the most common ones. Their sample size in not particularly related to neurological problems and all possible symptoms leading to IEM were entertained.[7] In another study, 10 patients were diagnosed with Methylmelonic acidemia being the most common one.[8] Afroz et al found 85 cases of IMD in 426 patients evaluated. Methylmelonic acidemia was the most common diagnosed case in their study as well.[9] In another study at AKU, Organic acidemia was the most common diagnosed case.[10]

Chiong et al looked at the biochemical marker in plasma AA and urine OA and could not find the relation with neuro-developmental outcome in cases of Maple syrup urine disease.[11] No case of MSUD was diagnosed in our study. Kiykim et al performed metabolic screening in 300 Turkish children with autistic spectrum disorder and found different inherited metabolic diseases. Nine children with phenylketonuria (PKU) were diagnosed which was the commonest disorder.[12] Larger sample size is required to get more spectrum of metabolic disorder.

Nizon et al concluded that organic acidurias, especially propionic aciduria leads to severe neurological outcome. Plasma methylmelonic acid (MMA) levels can be checked during follow up of these patients to monitor the progress.[13] In the absence of newborn screening in developing country, plasma AA and urine OA has important role to diagnose IEM. Karam et al found the diagnostic yield of these tests as 10%. Plasma AA and urine OA were diagnostic in 8.8% and 3.9% of analyzed cases, respectively.[14]

In one of the Malaysian study, 25 patients were diagnosed to have MSUD on 12,728 plasma amino acid and urine organic acid samples of suspected IEM.[15] It shows that MSUD is not as easy and common to detect and it was present in 0.2 percent of all the samples sent.

Karimzadeh et al used spectrum tandem mass spectrometry method to diagnose IEM in children with seizures, developmental delay and regression but couldn't find the significant relationship between the positive results and the neurological issues of the children.[16] In our study developmental delay was found in 8 out of 11 diagnosed cases while developmental regression was present in 3 cases which was significant. Tandem mass spectrometry method is not very sensitive to pick up some of the neuro-metabolic disorders.

In cases of mitochondrial respiratory chain disorder, Alban et al tried to see the relationship of metabolites in plasma and urine. Urine organic acid in combination of plasma FGF21 was found useful.[17] This study failed to detect any mitochondrial respiratory chain disorder because it needs other specified tests which are not mostly available in Pakistan. Shatla et al concluded that plasma AA and urine OA is useful test to diagnose mitochondrial disease (MD) specially in resource restricted countries.[18] They also monitor serum and urine lactate in all cases of suspected mitochondrial disorders. MD scoring system was used to diagnose mitochondrial disorders.

CONCLUSION

Plasma amino acid and urine organic acid are useful tests in the diagnosis of inborn errors of metabolism responsible for developmental delay or developmental regression. Awareness among Pediatricians should be raised so that these tests should be carried out in all cases of developmental impairment where diagnosis is not established by routine tests. Further studies in large scale are required to look for its usefulness.

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