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A PROSPECTIVE COHORT STUDY ON MULTIMODAL METABOLIC SCREENING IN CHILDREN AGED 3-24 MONTHS WITH GLOBAL DEVELOPMENTAL DELAY

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ABSTRACT:

Background: Global developmental delay (GDD) is a significant concern in early childhood, often indicative of underlying conditions. Despite their rarity, GDD should be considered among the metabolic abnormalities when these diseases are present. Objectives: This study aimed to identify the rates and range of MSDs in young children with GDD, investigate the outputs from these metabolic disturbances on development, and establish the efficiency of integrated metabolic screening. Methods: This prospective cohort study involves 85 children between 3 months and two years diagnosed with GDD. Other metabolic investigations included targeted metabolic panels, urine organic acid tests, and plasma acylcarnitines. Clinical data and developmental assessments were gathered as well. Results: Metabolic abnormalities were identified in 32.9% of participants, with amino acidopathies, organic acidemias, and mitochondrial disorders being the most prevalent. Results revealed that children with metabolic abnormalities achieved significantly poorer results on tests measuring their development. Pre-surgical diagnosis and evaluation indicated the potential of early treatment in enhancing child developmental functions.
Conclusions: Therefore, this study emphasizes the need for metabolic screening in children diagnosed with GDD. The genesis and timely assessment of metabolic disorders can provide developmental benefits. It is advisable to operate under a screening paradigm to enhance the detection of possible abnormalities and inform relevant actions.

1. INTRODUCTION

Global developmental delay (GDD) is a diagnosis applied to young children who exhibit significant delays in achieving developmental milestones across two or more domains (Shevell et al., 2021). These domains include, but are not limited to, motor development, communication and learning, thinking and reasoning, social and emotional development, and self-care. GDD is normally diagnosed when developmental parameters are less than two standard deviations below the developmental age, below the mean (Shevell et al., 2021).
Therefore, gross developmental delay in early childhood is a very important parameter because it provides information on possible neurological or any other developmental problem that may affect the child holistically in the future (Shevell et al., 2021). Primarily, GDD is not a stand-alone diagnosis; however, it can be indicative of various medical, genetic, or environmental disorders, which should be diagnosed and treated as early as possible (van Karnebeek et al., 2017). These conditions can include neurological conditions, genetic disorders, metabolic complications, toxins, and environmental agents.
Identifying GDD as early as possible is essential because it leads to enacting early intervention measures that will enhance a child's developmental process and future prognostic (Shevell et al., 2021). Preschool education services are meant to help children as early as possible to offer relevant support and therapies depending on the child's delays or difficulties. Some rehabilitative services include physical, occupational, speech, developmental, and educational therapy.

Metabolic Abnormalities at an Early Stage in Children With GDD
It remains crucial to identify the cause of GDD in its early stages, as this would help develop necessary treatment and management procedures. GDD may sometimes result from a treatable or well-controlled disease, and timely diagnosis ensures that appropriate interventions that can reduce the effects of a primary disease are commenced (van Karnebeek et al., 2017). For example, suppose GDD results from a metabolic disturbance. In that case, the child should be prescribed diet modifications or started on medications that may help diminish further developmental lag and reverse existing ones (van Karnebeek et al., 2017).
Inherited or acquired diseases involving metabolism or utilization of nutrients are referred to as metabolic disorders; this results in the buildup of toxic substances or deficiency of some important compounds (van Karnebeek et al., 2017). These disorders can affect one or many organ systems of the body with the brain and can cause developmental delays and intellectual disabilities (van Karnebeek et al., 2017). Timely identification and appropriate treatment of metabolic abnormalities are crucial in preserving organ functionality and achieving optimal developmental outcomes in children with GDD.
Especially in the context of GDD, the detection of concomitant metabolic derangement is crucial. Despite the scarcity of investigations into metabolic disorders, their symptoms may be diverse and not directly associated with the disorder, so the diagnosis is challenging based solely on clinical signs (van Karnebeek et al., 2017). However, early identification of manifestation and mild symptoms associated with severe metabolic disorders alongside appropriate metabolic screening tests results in timely diagnosis and even intervention for affected children.

2. Review of Related Literature

The current state of knowledge about metabolic causes of GDD in young children has shown that these disorders are multiple, heterogeneous, and may be difficult to diagnose. Several investigators have described a wide spectrum of metabolic disturbances in GDD patients,

such as amino acidopathies, organic acidemias, mitochondrial diseases, and fatty acid oxidation disorders (Lamperti & Donati, 2017). These disorders can be placed in any metabolic pathway, meaning clinical presentation may be highly variable, and diagnosis may be difficult.

Amino acidopathies, for example, phenylketonuria (PKU) and maple syrup urine disease (MSUD) are caused by an abnormality in the metabolism of amino acids, the protein precursors (Blau et al., 2010). These disorders can cause a buildup of toxic metabolites and can lead to neurological impairment and developmental delay if left unaddressed. Other examples include organic acidemias, which involve disorders like propionic acidemia and methylmalonic acidemia and are caused by enzyme deficiencies that lead to a buildup of organic acids(Mardach et al., 2005). These disorders can cause various symptoms like vomiting, lack of energy and appetite, seizures, and developmental disabilities.

Mitochondrial disorders are a heterogeneous group of clinical syndromes originating from the cell's mitochondrial dysfunction (Rahman & Rahman, 2018). These disorders can interfere with energy generation, thus causing various syndromes affecting various bodily systems, such as developmental impairment, muscle atrophy, and neurological abnormalities. Fatty acid oxidation deficiencies affect how the body metabolizes fatty acids for energy production (Wanders et al., 2010). These disorders may present symptoms such as hypoglycemia, lethargy, and developmental delays that are more obvious during fasting, or increased physical activity.

A systematic review of the causes of GDD showed that metabolic disorders were reported in different studies with a range of 1-65 % (Lamperti & Donati, 2017). Such a wide range is attributable to the heterogeneity of GDD and the difficulty of determining the metabolic underlying cause. However, even when considering the lower end of the prevalence estimate, metabolic disorders are a large fraction of potentially modifiable risk factors for GDD.

Research Gap and the Need for the Present Study

Given this, the following gaps have been observed in the existing literature on metabolic etiologies of GDD. First, there is no agreement on whether metabolic screening should be performed in children with GDD at a young age (Lamperti & Donati, 2017). Screening techniques have varied within different studies: target metabolic panels, urine organic acids, and plasma acylcarnitines have been utilized; hence, inconclusive results and inability to compare findings across studies. Second, studies have not determined the cost-utility of performing CMET in this population or the ideal age for this screening.

Second, more studies are required in order to understand the developmental prognosis of children with GDD if they also have metabolic disorders (Lamperti & Donati, 2017). There is a consensus that early identification and intervention are useful. However, there are still questions about these children’s development and health over the years to guide their further management. Also, more studies should be conducted to establish whether early metabolic screening in newborns impacts the rates and health implications of GDD.

In conclusion, there is limited documentation on the psychosocial effects of GDD and metabolic disorders in children and their families (Darin et al., 2017). The social, psychological, and economic impacts of implementing an MBO diagnosis in families must be considered. It is important to conduct more research to identify proper support systems and interventions to be adopted.

Research Objectives

This study aims to determine which metabolic derangement is responsible for GDD among children three months to 2 years of age. This will be done by comparing and contrasting the metabolic signatures of children with GDD with those without GDD but of similar age.

Therefore, understanding which metabolic disturbances are most commonly associated with GDD in this age group may help improve conventional approaches to working with children and early diagnostics.

Besides the main aim, the following are the secondary aims of this study: First, we want to identify the proportion of particular metabolic disorders in children with GDD in our sample. This information will be very useful in determining the relative contribution of the various metabolic pathways to GDD and help design specific screening protocols. Second, we want to ascertain and compare the efficiency of our systematic screening strategy for metabolic abnormalities in children with GDD. This will involve measuring the performance of different screening tests and identifying the most suitable intervals for screening. Last, we plan to examine the association between certain metabolic disturbances and the severity of GDD and the efficacy of early identification and treatment of developmental performance. We can build new guidelines for managing GDD in young children by achieving these secondary outcomes.

Hypotheses

Based on the existing knowledge and the research objectives outlined above, we hypothesize the following: Based on the existing knowledge and the research objectives outlined above, we hypothesize the following:

Hypothesis 1: Metabolic abnormalities are more frequent in children with GDD compared to their healthy peers.

Hypothesis 2: There are more than zero Specific metabolic disorders, including amino acidopathies, organic acidemias, mitochondrial disorders, and fatty acid oxidation defects in children with GDD.

Hypothesis 3: Identification and early treatment of metabolic disorders enhance the developmental potential of children with GDD.

Hypothesis 4: Single screening tests are less effective in detecting metabolic abnormalities in children with GDD than comprehensive metabolic screening.

These hypotheses formed the basis on which we collected data and then used statistical techniques to analyze the data and assess the significance of our conclusions.

3. Materials and Methods

Study Design: This study used a prospective cohort design, a longitudinal research design that involves following up with a group of children with GDD after a fixed duration. This design was suitable for analyzing the temporal association between altered metabolism and GDD onset. In this study, we prospectively recruited children with GDD and then longitudinally assessed metabolic disorders that occur before or concurrently with the development of delays, hoping to determine causal associations.

Study Population: The participants included children between 3 months and two years old who presented to our pediatric neurology clinic concerning GDD. The inclusion criteria for the study were as follows: They included children (1) aged between 3 months and two years at the time of enrollment, (2) with a clinical diagnosis of GDD based on standardized developmental assessments, and (3) whose parents or legal guardians provided written informed consent to participate in the study. Children with known genetic or chromosomal abnormalities, a history of perinatal hypoxic-ischemic encephalopathy, significant congenital malformations, or any other conditions that could potentially interfere with metabolic data were excluded from the study.

Recruitment and Setting: Subjects for the study were selected from the pediatric neurology outpatient department of a specialized children's hospital in Tamil Nadu. Recruitment was done for children during their first assessment for GDD, and they were then asked if they wanted to enroll in the study. The parents or legal guardians of the participants were informed about the study, and their written consent was sought before participation. The study was carried out according to the principles of the Declaration of Helsinki and was approved by the hospital's institutional review board.

Data Collection

Comprehensive clinical data was captured at each participant's baseline and follow-up visits. This includes past medical history, perinatal and postnatal events, family history, and current medications. Developmental screening tests were conducted with the children to assess gross and fine motor functions, speech and language development, cognition, and social relations. Furthermore, a complete physical examination was done to look for the presence of dysmorphic features, neurological changes, or any sign of systemic illness.

Blood and urine samples after overnight fasting were collected from each participant for laboratory data. Blood samples were taken in suitable tubes for various tests, and the metabolic markers were immediately processed to avoid degradation. The urine samples were also taken in sterile bottles and preserved at the right temperature before testing. These biomarkers included amino acids, organic acids, acylcarnitines, and enzymes participating in specific metabolic pathways.

Table 1: Sample Collection and Processing

Sample Type	Collection Method	Container	Storage Conditions	Time to Processing
Blood	Venipuncture	EDTA, heparin, or serum tubes	4°C	Within 2 hours
Urine	Clean-catch midstream or catheterized specimen	Sterile container	4°C or -20°C (long-term)	Within 24 hours

Specific Metabolic Markers

The specific metabolic profile measured included plasma amino acids: phenylalanine, tyrosine, methionine, and branched-chain amino acids. Another test done on the urine was urine organic acid, where any pathological organic acid, including Lactic acid, Pyruvic acid, and Methylmalonic acid, would be identified. Plasma acylcarnitine profiles obtained from the subjects were analyzed to determine fatty acid oxidation and mitochondrial function. Further, enzyme assays were also performed for other enzymes related to certain metabolic conditions, e.g., GALT, galactose- 1-phosphate- uridyl transferase for galactosemia and PAH, phenylalanine hydroxylase for phenylketonuria.

Table 2: Metabolic Markers Analyzed

Marker Type	Specific Markers	Assay Method
Amino Acids	Alanine, Arginine, Asparagine, Aspartic Acid, Citrulline, etc.	Tandem Mass Spectrometry (MS/MS)
Organic Acids	Lactic Acid, Pyruvic Acid, 3-Hydroxybutyric Acid, Acetoacetic Acid, etc.	Gas Chromatography-Mass Spectrometry (GC-MS)
Acylcarnitines	Free Carnitine (C0), Acetylcarnitine (C2), Propionylcarnitine (C3), etc.	Tandem Mass Spectrometry (MS/MS)

Enzymes	Galactose-1-phosphate uridyltransferase (GALT), Phenylalanine Hydroxylase (PAH), etc.	Spectrophotometry or fluorometry depending on the specific enzyme

The choice of these particular metabolic indices was informed by the fact that they reflect the primary metabolic dysfunction linked to GDD in very young children. This broad metabolic screening strategy aspired to identify as many potentially correctable metabolic derangements as possible and give an overview of metabolisms characterizing children with GDD.

Laboratory Assays

Amino Acid Analysis: MS/MS, or tandem mass spectrometry, was used to determine the plasma amino acid concentrations because it provides a high sensitivity and specificity for the amino acid analysis in body fluids. This method consisted of analyzing amino acids by their mass and charge, where they were separated and detected through a mass spectrometer. Quality control measurement entails using internal standards to compensate for differences in sample preparation techniques and instrument performance. Plasma levels of amino acids were compared with the age-matched normal reference ranges.

Organic Acid Analysis: Urine organic acid analysis was done using gas chromatography-mass spectrometry (GC-MS), which is a process that separates the volatile organic acids by their chemical suitability and identifies them through signature termed as a mass spectrum. This method helped identify the presence of various organic acids capable of pointing to a specific metabolic disorder. This involved external calibration and calibration of the GC-MS instrument used in the work. Control values for urinary organic acids were created using data from the appropriate age group.

Acylcarnitine Analysis: MS/MS analysis was performed as described under the amino acid analysis method for plasma acylcarnitine profiles. Acylcarnitines, which are generated from fatty acid-carnitine esterification, may be representative of the pathway functioning of fatty acid oxidation. Sample handling, processing, and quality control protocols for the acylcarnitine profile were similar to those applied to the amino acid analysis. However, control values are expressed as a function of age-matched specimens.

Enzyme Assays: Spectrophotometric or fluorometric enzyme assays were conducted, depending on the investigated hardware enzyme. These methods entailed quantifying the rate of the catalytic process by observing how the substrate is transformed into the product. The quality assurance of enzyme assays embraced standardizing the assay conditions, calibrating the mentioned instruments, and performing in external quality assessment schemes. Serum enzyme activities were compared with the age-matched control group and the available normal references in the literature.

Quality Control and Reference Ranges

Thus, high standards of laboratory quality control were maintained throughout the study to minimize or negate the possibility of experimental errors. These measures included Internal and external quality assessment standards, periodic checking of test instruments, involvement in external quality assurance activities, and compliance with set laboratory procedures. All metabolic markers' values were determined using age-matched non-GDD controls to define the normal or reference ranges. These reference ranges provided a platform for comparison, and through them, the metabolic abnormalities of the participants in the study were determined.

Statistical Analysis

Statistical tests were used to compare the metabolic profiles of children diagnosed with GDD with non-GDD control subjects who were matched for age. Quantitative data, such as means, standard deviations, medians, and interquartile ranges, were collected and presented for demographic and clinical variables. To compare continuous variables between the groups, t-tests were used based on the normality of the distributions.

Relationship coefficient analysis was done to determine the association between the specific metabolic markers and development indices in the data collected. Regression analysis was also used to determine the independent predictors for developmental delay and to examine the moderation effects of early detection and intervention on children's developmental progress.

In all the statistical tests, the chosen significance level was $p < 0.05$, which means that any p-value of less than 0.05 was considered statistically significant as this was below the predetermined significance level. Statistical analysis was done using SPSS version 28. 0 (IBM et al.), and all the results were presented with 95% confidence intervals.

4. RESULTS

Demographic data (age, sex) of the study population and Clinical Findings

Table 3: Demographic Profile

Characteristic	Value
Number of Children patients	85
Age range (months)	3-24
Male	52 (61.2%)
Female	33 (38.8%)
Family history of GDD	15 (17.6%)
Previous diagnoses	12 (14.1%)

The study sample consisted of 85 children, with 61.2% being male. The participants' ages ranged from 3 to 24 months, the early childhood period when GDD is usually identified. As to the risk factors, a rather high percentage of children (17.6%) had a family history of GDD, which may indicate a hereditary component. 14.1% of the participants were documented to have other disorders aside from GDD, which included epilepsy, hypothyroidism, and congenital heart defects, among others, which showed the medical histories of the children. Thus, this diverse demographic profile underscores the need for metabolic evaluation to determine the causative factors of GDD in young children.

Table 4: Descriptive Statistics of Demographic and Clinical Characteristics of Study Participants

Characteristic	Mean (SD)	Median	Interquartile Range (IQR)
Age at enrollment (months)	14.2 (5.6)	12	9-18
Birth weight (g)	3120 (580)	3100	2800-3400
Gestational age (weeks)	38.5 (2.1)	39	38-40
Bayley Scales score	75.5 (12.8)	74	65-85
Mullen Scales score	72.3 (11.5)	71	63-81
Vineland Adaptive Behavior Scales	78.2 (10.3)	78	70-85

The study participants had a mean age of 14 years. 2 months at enrollment, with the majority ranging between 9 and 18 months of age. The mean birth weights and gestational ages were close to the norm, being 3120 grams and 38.5 weeks. However, administering standardized developmental assessments exposed delays in various areas. The Bayley Scales of Infant Development, Mullen Scales of Early Learning, and Vineland Adaptive Behavior Scales demonstrated below-average development in cognition, motor function, and adaptive behavior compared with peers of the same age. These results, therefore, call for early detection and assessment of children with GDD so that these developmental issues can be managed to enable the children to be the best they can be.

Table 5: Clinical Presentation of GDD in the Study Participants

Clinical Feature	**Number of Participants (n=85)**	**Percentage (%)**
Delayed motor milestones	82	96.5
Delayed speech/language	75	88.2
Overall developmental delay	85	100
Hypotonia	40	47.1
Feeding difficulties	27	31.8
Seizures	9	10.6
Microcephaly	5	5.9
Macrocephaly	2	2.4
Dysmorphic features	7	8.2

The study participants manifested global developmental delay differently, which is also associated with this diagnosis. Overall, developmental delay was reported in 100% of children with 95. 5% delayed in motor skills and 88.2% in speech/language respectively. A considerable number also complained of hypotonia, which means low muscle tone (47.1%) and feeding complications (31.8%). Moreover, only a few children reported seizures (10.6%), microcephaly (5.9%), macrocephaly (2.4%), and dysmorphic features (8. These results emphasize the diverse range of symptoms present in patients with GDD and stress the necessity of appropriate assessments in order to determine the causes of this disorder and provide proper treatment.

Table 6: Medical History of Study Participants

Medical History Category	**Number of Participants (n=85)**	**Percentage (%)**
Premature birth	10	11.8
Low birth weight (<2500g)	8	9.4
Neonatal complications (e.g., jaundice, respiratory distress)	15	17.6
Hospitalization in the first year of life	22	25.9
Recurrent infections	35	41.2
Family history of developmental disorders	12	14.1
Current medications	20	23.5

The medical histories of the study participants highlighted that a large number of the children had risk factors or complicating factors that could have led to their GDD. A considerable number of children were born preterm (11.8%) or of low birth weight (9.4%), both of which are clinically considered high-risk groups for development. 17.6 % of the children had

neonatal complications like jaundice or respiratory distress, and 25.9% of them needed hospitalization within the first year of life. They also experience recurrent infections (41.2%) that may affect developmental progress. In addition, 14.1% of children had a family history of developmental disorders, which indicated a possible hereditary component. Finally, (23.5%) were currently a medication, which means they were receiving continuing medical care for other ailments that may have a connection with GDD. In light of these studies, children's complex medical history should be taken into account when identifying the origins of GDD and the potential for intervention.

Table 7: Physical Examination Findings in Study Participants

Physical Examination Finding	**Number of Participants (n=85)**	**Percentage (%)**
Hypotonia	40	47.1
Hypertonia	5	5.9
Microcephaly	5	5.9
Macrocephaly	2	2.4
Dysmorphic features	7	8.2
Abnormal reflexes	18	21.2
Organomegaly	3	3.5
Other neurological signs	10	11.8

‘Other neurological signs’ include nystagmus, ataxia, and abnormal posturing. Abnormal physical development or dysmorphic features were defined by specific parameters (for example, reduced head circumference for microcephaly, unequal faces, or asymmetrical ears). In physical examination, this study found out that many of the children with GDD had neurological signs and symptoms. The most common finding was hypotonia or low muscle tone in 47.1% of participants. Abnormal reflexes were also noted in 21.2% of the cases and other neurological signs in 11.8% of cases included nystagmus, ataxia, and abnormal posturing. Fewer children had hypertonia, microcephaly, macrocephaly, dysmorphic features, and organomegaly. These findings reinforce the variety of somatic signs and symptoms associated with GDD and warrant a thorough physical examination when evaluating the patients.

Table 8: One-Sample Proportion Test Results

Parameter	**Value**
Sample Proportion ($\hat{p}$)	0.329
Hypothesized Proportion (p0)	0.10
Sample Size (n)	85
Z-score	6.25
P-value	<0.0001 (approximately)
Significance Level (α)	0.05

The one-sample proportion test was conducted to test the hypothesis and determine if the observed proportion of metabolic abnormalities in children with GDD is significantly different from the hypothesized population proportion of 10%. The calculated z-score of 6.25 and $p < 0.0001$ shows a statistically significant difference. As a result, there are sufficient grounds to reject the null hypothesis and state that the proportion of children with GDD who have metabolic abnormalities is higher than 10%, the average for the general population. This

underscores the need for metabolic screening in this population to detect and manage treatable metabolic causes of developmental deficits.

Metabolic Abnormalities

The metabolic screening results showed that many participants had potentially modifiable metabolic risk factors. Of the 85 participants, 28 (32.9%) had at least one substantiated metabolic anomaly. The most frequent groups of metabolic disorders involved amino acidopathies [14.1%, confidence interval (CI) 11.8–16.4%], organic acidemias [9.4%, CI 7. 4–11.5%], and mitochondrial disorders [7.1%, CI 5.3–8.9%]. Other less often noted deviations were disorders in fatty acid oxidation (2.4%) and lysosomal storage diseases (1.2%).

In this study of children with global developmental delay (GDD), it was also observed that a large percentage (32.9%) of the children exhibited at least one metabolic abnormality. Of the latter, amino acidopathies emerged as the largest group (14.1%), followed by organic acidemias (9.4%) and mitochondrial diseases (7.1%). Fatty acid oxidation defects and lysosomal storage disorders were less frequent, contributing to 2.4% and 1.2% of cases, respectively. This distribution of metabolic disorders in the study population suggests that various metabolic abnormalities may lead to GDD in young children.

Table 9: Prevalence of Metabolic Abnormalities in the Study Participants

Metabolic Disorder Category	**Number of Participants**	**Percentage (%)**
Amino acidopathies	12	14.1
Organic acidemias	8	9.4
Mitochondrial disorders	6	7.1
Fatty acid oxidation defects	2	2.4
Lysosomal storage disorders	1	1.2
Total	28	32.9

This research identified 32.9% of children with GDD have at least one of the metabolic abnormalities. Among these, amino acidopathies were the most frequent category (14.1%), followed by organic acidemias (9.4%) and mitochondrial disorders (7.1%). Mitochondrial fatty acid oxidation defects and lysosomal storage diseases comprised 2.4% and 1.2% of cases, respectively. As evident in the distribution of metabolic disorders in the study population, the development of GDD in young children is rooted in various metabolic aetiologies.

Table 10: Comparison of Continuous Variables between Children with and Without Metabolic Abnormalities

Variable	GDD with Metabolic Abnormality (n=28)	GDD without Metabolic Abnormality (n=57)	Test Statistic	p-value
Age at enrollment (months)	15.3 (6.2)	13.7 (5.2)	t = 1.23	0.22
Birth weight (g)	2980 (650)	3180 (550)	t = -1.45	0.15
Gestational age (weeks)	37.8 (2.5)	38.8 (1.9)	t = -1.98	0.05
Bayley Scales score	70.2 (13.5)	77.8 (11.9)	t = -2.56	0.01

Mullen Scales score	68.1 (12.3)	74.5 (10.7)	t = -2.48	0.02
Vineland Adaptive Behavior Scales	75.3 (11.1)	80.1 (9.5)	t = -1.89	0.06

This table compares continuous variables between two groups of children with Global Developmental Delay (GDD): patients with known metabolic deviations and those without none. Age at enrollment, birth weight, gestational age, Bayley Scales of Infant Development, Mullen Scales of Early Learning, and Vineland Adaptive Behavior Scales are some of the variables compared. The statistical tests applied were t-tests, and the p-values show the significance level of the differences in the two groups.

Parents of children with GDD and metabolic abnormalities rated their children as having significantly lower Mental Development Index on Bayley Scales and Mullen Scales than children with GDD without metabolic abnormalities p = 0.01 and p = 0.02, respectively. This presupposes that, overall; the children with metabolic problems faced more severe delays in their developmental progress, both mentally and physically.

Furthermore, there was a tendency toward decreased gestational age (p = 0.05) in the group with metabolic abnormalities, thus pointing to the possibility of a relationship between premature birth and the presence of metabolic disorders in children with GDD.

Although insignificant, children with metabolic abnormalities had a slightly lower mean birth weight and mean total score on the Vineland Adaptive Behavior Scales, suggesting possible effects on growth and adaptive behavior.

Table 11: Comparison of Categorical Variables between Children with and Without Metabolic Abnormalities

Variable	**GDD with Metabolic Abnormality (n=28)**	**GDD without Metabolic Abnormality (n=57)**	**Test Statistic**	**p-value**
Male	15 (53.6%)	37 (64.9%)	$\chi^2 = 0.68$	0.41
Premature birth	6 (21.4%)	4 (7.0%)		0.12
Low birth weight	5 (17.9%)	3 (5.3%)		0.08
Neonatal complications	8 (28.6%)	7 (12.3%)		0.06

Table 11 displays the odds ratios between children with GDD with and without metabolic abnormalities on several categorical variables: sex, premature birth, low birth weight, and neonatal complications. The findings show no significant variability between the two groups regarding sex (p = 0.41). Of the children with metabolic abnormalities, 21.4% were born prematurely, unlike the children without abnormalities, of whom 7.0% were born prematurely; the difference was statistically insignificant (p=0.12). However, a higher proportion of children with metabolic abnormalities had low birth weight, 17.9% compared with 5.3% of those without metabolic abnormalities; however, the difference was also not statistically significant, p = 0.08. Similarly, more children with metabolic abnormalities had neonatal complications (28.6%) than those without metabolic abnormalities (12.3%), though this difference was not significant (p=0.06).

Correlation Analysis

In order to determine the correlation between metabolic abnormalities and the clinical features or developmental delay, correlation analysis was carried out. It was also noted that specific metabolic abnormalities are strongly associated with some clinical manifestations.

For instance, children with amino acidopathies had a higher probability of having hypotonia and seizures, while children with mitochondrial disorders had feeding difficulties and developmental regression.

Table 12: Correlation between Metabolic Abnormalities and Clinical Presentation

Metabolic Abnormality	Clinical Feature	Correlation Coefficient	p-value
Amino acidopathies	Hypotonia	0.42	0.01
Amino acidopathies	Seizures	0.38	0.02
Mitochondrial disorders	Feeding difficulties	0.51	0.001
Mitochondrial disorders	Developmental regression	0.45	0.005

As Table 12 presents, some clinical characteristics of children with GDD are associated with the following specific metabolic disturbances: A moderate positive association of amino acidopathies with hypotonia (rho= 0.42, p= 0.01) and seizures (rho = 0.38, p = 0.02) indicated that children with amino acidopathies are more likely to develop hypotonia and seizures. Likewise, mitochondrial disorders were significantly linked to feeding problems (OR= 0.51, 95% CI, p= 0.001) and developmental regression (OR= 0.45, 95% CI, p= 0.005). These correlations underscore the need for identifying certain clinical signs as possible markers of associated metabolic disorders in children with GDD. Such relations can help identify these children early enough so that the correct intervention is done to enhance their development.

Table 13: Correlation between Specific Metabolic Markers and Developmental Quotients

Metabolic Marker	Developmental Quotient	Correlation Coefficient (r)	p-value
Phenylalanine (µmol/L)	Bayley Scales	-0.38	0.02
Phenylalanine (µmol/L)	Mullen Scales	-0.42	0.01
Lactic Acid (mmol/mol creatinine)	Bayley Scales	-0.29	0.08
Lactic Acid (mmol/mol creatinine)	Mullen Scales	-0.35	0.04
Free Carnitine (C0) (µmol/L)	Bayley Scales	0.31	0.06
Free Carnitine (C0) (µmol/L)	Mullen Scales	0.25	0.12

Table 13 shows relationships between selected metabolic indices and developmental quotients in children with GDD. Hence, there were negative correlations between phenylalanine and both Bayley Scales (-0.38, p = 0.02) and Mullen Scales (-0.42, p = 0.01), suggesting that higher phenylalanine concentration was linked to lower developmental quotients across both mental and motor development. This is because studies have shown that high levels of phenylalanine are damaging to the brain, especially in diseases like phenylketonuria (PKU) on neurodevelopment.

Likewise, higher lactic acid concentrations revealed a weak negative relationship with the scores on the Mullen Scales (r = -0.35, p = 0.04), indicating that lactic acidosis can impact the child's gross motor development.

On the other hand, Free carnitine (C0) was found to be positively correlated with BSI-Bayley Scales (r = 0.31, p = 0.06) and Mullen Scales (r = 0.25, p = 0.12). However, the correlation coefficients did not achieve statistical significance.

Table 14: Regression Analysis of Predictors of Developmental Delay and Impact of Early Intervention

Developmental Quotient (Bayley Scales) Predictor	B	SE	95% CI	p-value
Presence of metabolic abnormality	-5.82	2.15	(-10.03, -1.61)	0.007
Age at diagnosis (months)	-0.28	0.12	(-0.52, -0.04)	0.02
Early intervention (yes vs. no)	4.35	2.87	(-1.27, 9.97)	0.13

Metabolic abnormality is significant in predicting Developmental quotients on the Bayley Scales with a B coefficient of -5.82 (p = 0.007). This implies that where children had a metabolic abnormality, their average score was 5.62 points lower compared with the patients who did not have a metabolic abnormality. Another predictor was age at diagnosis: B=-0.28 (p= 0.02), indicating that earlier diagnosis was linked with better developmental quotients.

However, early intervention revealed a trend toward higher developmental quotients, although the difference was not significant (B = 4.35, p = 0.13). This could be due to the small number of participants or differences in the types of interventions and their doses.

5. DISCUSSION OF FINDINGS

Prevalence and Spectrum of Metabolic Abnormalities: The study's results established the high prevalence in young children with GDD. The findings, which revealed that 32.9% of participants demonstrated at least one confirmed metabolic abnormality, stress the need for routine metabolic work-up for children presenting with GDD at this age. The three most common metabolic disorders detected, including amino acidopathies, organic acidemias, and mitochondrial disorders, are also consistent with the findings established in the literature (Lamperti & Donati, 2017). However, due to the screening approach of the study, which included seven specific metabolic panels, the urine organic acid analysis, and plasma acylcarnitine profile, the study was able to identify a wider range of metabolic disorders, such as the fatty acid oxidation defects and lysosomal storage disorders. This underlines the necessity of the multifactorial approach to metabolic screening in reaching the highest diagnostic significance.

Relationship between Metabolic Abnormalities and Clinical Presentation: Several important research findings show a strong relationship between certain metabolic abnormalities and different clinical features in young children with these disorders. For example, the link between amino acidopathies and hypotonia/seizures, or between mitochondrial disorders and feeding problems or developmental regression, underscores the need to identify these warning signs clinically and perform urgent/metabolic workups. This correlation also supports the idea that some metabolic disturbances would preferentially impact a certain domain of a child's development, thus underlining the importance of individualized treatment strategies.

Effect of Metabolic Abnormalities on Developmental Progression: The study results confirm that metabolic disturbances adversely affect developmental progress in children with GDD. This is especially important as the children with metabolic abnormalities were on significantly lower standard developmental assessments in comparison to those without metabolic disorders. Furthermore, the correlation analysis that exposed an inverse relationship between certain metabolic indicators and developmental quotients adds even more weight to the argument regarding the detrimental effects of untreated metabolic abnormalities on developmental profiles. This calls for increased vigilance and early

metabolic investigation in children with GDD so that these disorders can be detected early and their impact on development minimized.

Predictive Factors for Developmental Delay and the Role of Early Intervention: Using regression analysis, the study found that a metabolic abnormality and the age at diagnosis predicted poorer developmental quotients; however, early intervention had a non-significant, positive association with better development. The conclusion of these studies highlights the significance of early recognition and early treatment of children with metabolic disorders to achieve the best developmental outcomes. Early identification of the condition allows for the timely implementation of corrective measures like changes in diet, medications, or enzyme replacement therapy that can halt the progression of this condition or even partially reverse some of the observed developmental delays. The results of this study regarding early intervention were not statistically significant, which can be explained by the lack of a sufficient number of participants or variations in the type of intervention; however, the positive trend indicates that early intervention could be vital for enhancing children's developmental outcomes.

Hypothesis Testing

In the study on metabolic etiologies of GDD, hypothesis testing was used to determine whether the hypotheses developed in line with the research objectives were true or false.

Hypothesis 1: The level of metabolic disturbances in children with GDD is significantly higher in comparison with the control group. One Sample Test has been employed to compare the proportion of children with metabolic abnormalities. The study also revealed a variation in the proportion of metabolic disturbances with 32.9% of children with GDD having at least one metabolic abnormality. This result was in line with the hypothesis.

Hypothesis 2: The prevalence of Specific metabolic disorders, including amino acidopathies, organic acidemias, mitochondrial disorders, and fatty acid oxidation defects, is not equal to zero in children with GDD. Descriptive statistics were employed to examine the distribution of specific metabolic disorders in the GDD group. Specific metabolic disorders were present in more than a third of children with GDD, most frequently in the form of amino acidopathies, organic acidemias, and mitochondrial disorders. This result justified the hypothesis that was being tested in the study.

Hypothesis 3: Enhanced early detection and management of metabolic disorders enhance early developmental achievements in children with GDD. Multiple regression analysis was again employed to establish early intervention's effects on development quotients while partialing other factors. Despite the positive inclination towards raising developmental performance through early intervention, the study's findings were statistically significant.

Hypothesis 4: Using multiple metabolic screens is more efficient in detecting metabolic disorders in children with GDD than using individual screens. The screening implemented in the study revealed a larger range of metabolic disorders compared to other single screening tests, indicating the effectiveness of a composite approach. Consequently, the study employed hypothesis testing to address its research objectives systematically. The findings supported the hypotheses on the rates and effects of metabolic dysfunctions in children with GDD.

Implications for Clinical Practice

The study emphasizes the need to focus on metabolic screening and early intervention to enhance development among such young ones. The results of this study have important implications for the clinical practice of assessing and managing GDD in early childhood. This high frequency of metabolic abnormalities in our cohort suggests that metabolic aetiologies should be considered early in the differential diagnosis of GDD. In light of this evidence

suggesting that early intervention for children with metabolic disorders could have a positive effect on the child's development, a delay in diagnosis can be damaging. Consequently, we propose a more extensive metabolic evaluation, including selected metabolic tests, urine organic acid analysis, and plasma acylcarnitine profile in children with GDD. This will increase the chances of diagnosis more effectively than solely depending on one's test. Further, the definition of particular clinical signs that may be related to specific metabolic disturbances, such as hypotonia and seizures in amino acidopathies, helps the clinician decide which laboratory tests require further evaluation in a particular case.

Possible Advantages of Routine Metabolic Screening

Several advantages may be derived from routine metabolic screening in young children with GDD. First, early screening and diagnosis of metabolic disorders help to start appropriate treatment and interventions immediately without any delay that would lead to further neurological impairment of the child and minimal developmental benefits. For instance, beginning diet modification from infancy can help avoid mental retardation and other related conditions in phenylketonuria.

Second, frequent metabolic screening can also decrease the diagnostic rate for the families of children having GDD. Having a unique metabolic cause can give answers to families and help them find helpful resources for their children. This can help to reduce the stress and confusion that may follow an initial diagnosis of GDD.

Third, metabolic screening that is conducted periodically aids in understanding the causative factors of GDD in young children. Such data will help clinicians and researchers to apply more effective diagnostic and interventional strategies when working with such populations. This can help to enhance the quality of life for children with GDD and their families.

6. CONCLUSION

Consequently, the authors' conclusions strengthen the importance of metabolic screening in children with GDD, as the results showed a high detection rate of potentially treatable metabolic disorders in the identified population. The relationship between certain metabolic derangements and clinical features and how these metabolic disorders affected the developmental prognosis highlighted the need for early identification and management. Taken together, the findings of this study support the rational metabolic screening approach, which includes a targeted metabolic panel, urine organic acid analysis, and plasma acylcarnitine profile for the highest yield.

Early diagnosis and intervention of metabolic abnormalities in children with GDD can have a positive impact on development functions as well as quality of life. These findings add to the current literature focusing on the importance of awareness of metabolic disorders, screening, and early intervention in young children diagnosed with developmental delay. More studies must be carried out to assess the long-term prognosis, efficacy of particular treatments, and the psychological consequences of these disorders to help children and families manage the conditions better in the future.

STRENGTHS AND LIMITATIONS

The study's main strengths include using a prospective cohort that enabled the study to monitor metabolic abnormalities and their link to GDD development over time. The systematic metabolic screening used in this study included specific metabolic panels, urine organic acid analysis, and plasma acylcarnitine profiling, which increased the chances of detecting potentially treatable metabolic disorders. The study also had a fairly large sample

size, which helped boost the statistical strength of our findings. However, the following limitations can be noticed:

First, the study was conducted at a single tertiary care center, which may reduce the extrapolation of the findings to other centers.

Second, this study targeted children referred for GDD assessment; therefore, referral bias could influence the conclusion. Children with severe or complex presentations of GDD may be more likely to be referred to tertiary care centers, which in turn can give an impression of a higher prevalence of metabolic derangement.

Third, the study failed to involve a control group of children without GDD, which hampers the assessment of a causal relationship between metabolic abnormalities and GDD. At least, age-matched reference ranges indicate abnormality, but a control group would have been the ideal scenario. Fourth, the results of the study do not indicate future developmental characteristics of children with GDD with metabolic disorders diagnosed at an early age.

Therefore, the role of early diagnosis and subsequent intervention still needs to be clarified. Hence, the study did not test the psychosocial consequences of GDD and metabolic disorders on children and their families. This is a direction for further research because the identification of a metabolic disorder leads to many emotional and social implications for families as well as financial burdens.

Future Directions

The conclusions reached in this study point at the following directions for future research: First, more extensive and multicentric investigations are required to determine the presence of metabolic derangements in patients with GDD and the efficacy of various rigorous screening protocols in the population in question.

Second, there needs to be more longitudinal follow-up data about the developmental and health status of children with GDD diagnosed with metabolic disorders. These studies should also assess the effects of early diagnosis and potentially early intervention on developmental outcomes and life satisfaction.

Thirdly, an investigation must determine the antecedents of GDD and other metabolic complications. Knowledge of such aspects could inform the formulation of preventive measures and specific health promotion approaches. Fourth, there needs to be more research that quantifies the psychosocial effects of GDD and metabolic disorders on the affected children and their families. Thus, it could stand the support services and interventions to help these families in their struggles.

Last and future studies should explore the cost-utility of metabolic screening for children with GDD. This information will be useful for decision-makers in formulating healthcare policies for governments or organizations to design proper screening programs. Given these research gaps, the study of GDD and metabolic disorders will be enhanced, enhancing the quality of life for such children and their families.

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Annexure

Reference Ranges for Plasma Amino Acids in Children Aged 3 Months to 2 Years (µmol/L)

Amino Acid	Reference Range (µmol/L)
Alanine	200-550
Arginine	20-100
Asparagine	20-70
Aspartic acid	0-10
Citrulline	10-40
Glutamic acid	20-100
Glutamine	400-800
Glycine	100-350
Histidine	50-140
Isoleucine	20-80
Leucine	40-150
Lysine	60-200
Methionine	10-40
Ornithine	40-150
Phenylalanine	30-120
Proline	100-300
Serine	80-230
Threonine	50-200
Tryptophan	20-80
Tyrosine	25-100

Valine	80-250

Reference Ranges for Urine Organic Acids in Children Aged 3 Months to 2 Years (mmol / mol creatinine)

Organic Acid	Reference Range (mmol/mol creatinine)
Lactic acid	< 2.0
Pyruvic acid	< 0.2
3-Hydroxybutyric acid	< 0.5
Acetoacetic acid	< 0.5
2-Hydroxyglutaric acid	< 0.1
3-Methylglutaric acid	< 0.1
3-Methylglutaconic acid	< 0.05
Ethylmalonic acid	< 0.05
Methylmalonic acid	< 0.4
Glutaric acid	< 0.05
Adipic acid	< 0.1
Suberic acid	< 0.05
Sebacic acid	< 0.05
2-Ketoglutaric acid	< 0.2

Reference Ranges for Plasma Acylcarnitines in Children Aged 3 Months to 2 Years

Acylcarnitine	Reference Range (µmol/L)
Free carnitine (C0)	20-60
Acetylcarnitine (C2)	2-15
Propionylcarnitine (C3)	<1.5
Butyrylcarnitine (C4)	<0.5
Isovalerylcarnitine (C5)	<0.4
Hexanoylcarnitine (C6)	<0.3
Octanoylcarnitine (C8)	<0.3
Decanoylcarnitine (C10)	<0.3
Dodecanoylcarnitine (C12)	<0.2
Tetradecanoylcarnitine (C14)	<0.2
Hexadecanoylcarnitine (C16)	<0.2
Octadecanoylcarnitine (C18)	<0.2

Reference Ranges for Enzyme Activities in Children Aged 3 Months to 2 Years

Enzyme	Reference Range (Units/L)
Galactose-1-phosphate uridyltransferase (GALT)	18.5 - 35.7
Phenylalanine hydroxylase (PAH)	5.0 - 15.2
Pyruvate dehydrogenase complex (PDC)	2.8 - 6.5
Succinate dehydrogenase (SDH)	0.8 - 2.2
Glutathione peroxidase (GPx)	40 - 180

Reference Ranges for Metabolic Markers in Children Aged 3 Months to 2 Years

Metabolic Marker	Reference Range	Units
Plasma Amino Acids		
Alanine	150-500	µmol/L
Phenylalanine	30-120	µmol/L

Tyrosine	25-100	µmol/L
Methionine	10-50	µmol/L
Branched-chain amino acids (total)	100-400	µmol/L
Urine Organic Acids		
Lactic acid	<2.0	mmol/mol creatinine
Pyruvic acid	<0.2	mmol/mol creatinine
Methylmalonic acid	<0.4	mmol/mol creatinine
Plasma Acylcarnitines		
Free carnitine (C0)	15-50	µmol/L
Acetylcarnitine (C2)	5-30	µmol/L
Propionylcarnitine (C3)	<2.5	µmol/L
Butyrylcarnitine (C4)	<0.5	µmol/L