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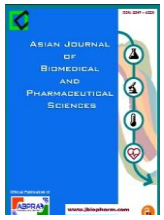

RESEARCH ARTICLE

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Spectrum of Clinical Symptoms in Children with Elevated Lactate: Pyruvate Ratio in a Tertiary Care Setting

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Abstract

Introduction: This study is designed to study clinical symptoms in children with elevated lactate: pyruvate ratio to understand the magnitude of respiratory chain disorder.

Materials and Method: Children attending pediatric outpatient department in our center who showed clinical symptoms suggestive of inherited metabolic disorder were enrolled into the study. Arterial blood gas, lactate and lactate: pyruvate ratio were analysed by collecting arterial blood from selected children. Clinical symptoms of those children with elevated lactate: pyruvate ratio was evaluated.

Result: 304 patients who showed clinical symptoms of either developmental delay without asphyxia, seizures, vomiting, poor suck or weak cry at birth were evaluated. Of them 147 showed one or more of positive biochemical findings such as metabolic acidosis, elevated lactate or lactate: pyruvate ratios which were the basic laboratory data to suspect energy metabolic disorder. Elevated lactate: pyruvate ratio was found in 81 patients, which is one of the biochemical markers to narrow down diagnosis of respiratory chain disorder.

Conclusion: Our study showed that suspected energy metabolic disorders are not uncommon. Clinical suspicion is crucial to start further diagnostic profile. Children with delay in attaining milestones despite being born at term without asphyxia need to be referred for metabolic work up. There is a need to create awareness in primary health care providers about this in order to initiate further diagnosis and intervention. Elevated lactate: pyruvate ratio suggests this and warrants further work up. Serum alanine and beta-hydroxy butyrate: acetoacetate ratio may help us to know more about energy metabolic disorders like respiratory chain disorders.

Keywords: Developmental delay, inborn errors of metabolism, lactate: pyruvate ratio, respiratory chain disorder

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INTRODUCTION

Approximately 111 years ago in 1902 the term Inborn Errors of Metabolism (IEM) was first used by Sir Archibald Garrod [1]. They are typically caused by a deficiency of one or more metabolic enzymes [2]. Based on their pathogenesis they are classified into three groups namely Group 1: Which gives rise to intoxication, Group 2: Disorders involving energy metabolism, Group 3: Disorders involving complex molecules[3].

Within the cell except erythrocytes, mitochondria is the powerhouse where energy related processes take place. These processes occur in the presence of oxygen. The energy metabolic disorders may be due to partial or complete deficiency of enzyme involved in energy production or utilisation within liver, myocardium, muscle or other tissues. Further this group can be divided as mitochondrial respiratory chain and cytoplasmic energy defects. Mitochondrial disorder is one of the energy metabolic disorders which may lead to lack of ATP generation [3].

Disorders of mitochondrial respiratory chain were first introduced in 1962 while describing a case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control by Luft and his coworkers [4]. Since then a number of clinical symptoms were added to this and knowledge of this disorder increased to the molecular level. RC disorder represents most common group of IEM and approximately one third of IEM in children are attributable to this category. Earlier epidemiological studies indicated that respiratory chain disorder is not rare and its estimated birth prevalence around 1 in 5,000- 10,000 children [5] - [10]. Mutations in the genes encoding subunits of respiratory chain enzyme complexes can produce a wide variety of oxidative phosphorylation diseases[11],[12]. Skeletal muscle, heart and brain are the organs which requires more energy and depend on aerobic oxidation. Decrease in energy production due to respiratory chain disorder lead to neuronal depolarisation and cell death[12] - [15]. Respiratory chain disorder may present with non specific symptoms such as respiratory distress, lethargy, limb and truncal hypotonia, poor sucking reflex, psychomotor regression, mental retardation, cardiomyopathy, dehydration, seizures etc [3], [8]. Highly variable phenotypes causes delay in clinical suspicion of these disorders and misdiagnosis. Routine biochemical parameters may warrant but results must be interpreted in conjunction with clinical symptoms. In the initial stages the laboratory techniques employed for IEM were simple. Now due to extensive study and research it relies on sophisticated tests like molecular analysis, enzymatic analysis requiring biopsies or cell culture which are either not available, expensive and unaffordable or unreliable[3],[8],[10],[16].

Immediately after clinical examination biochemical parameters assessed to narrow down the disorder are arterial blood gas analysis, plasma glucose, , ammonia, lactate and lactate: pyruvate ratio. Some other useful serum diagnostic markers include creatinine kinase and alanine. A recent study showed an additional value for creatine compared to alanine and lactate. Study revealed that extracellular creatine increases in response to respiratory chain inhibition in cultured cells and in human plasma of individual with respiratory chain disorder. But they are not specific or sensitive for this disorder [6],[10],[17],[18]. Other biomarkers which are elevated in respiratory chain disorders are amino acids like glycine, tyrosine, proline and sarcosine, organic acids like intermediates of tricarboxylic acid cycle, methylmalonic acid, methylglutaconic acid and dicarboxylic acids in blood, urine, and CSF. Plasma carnitine and acyl carnitine profile help in differentiating these disorders from fatty acid oxidation defects. Finding specific reliable single serum biomarker is challenging and has not been defined, which indicates difficulty in interpreting clinical and biochemical findings[17].

There is paucity of study regarding respiratory chain disorders in our Country. Nonavailability of less expensive and noninvasive biomarkers may be one of the causes for limited study about this. Before selecting profile for further diagnosis it is needed to know the magnitude of respiratory chain disorders. Lactate: pyruvae ratio is correlated with the cytoplasmic NADH:NAD^+ ratio and is used as a surrogate measure of cytosolic oxido-reduction state. Lactate :pyruvate molar ratio >25 is considered increased and suggestive of a primary respiratory chain dysfunction[3],[9],[10]. In the present study we studied lactate: pyruvate ratio in children with clinical symptoms suspicious of energy metabolic disorder and studied clinical symptoms in pediatric patients with elevated lactate: pyruvate ratio.

Objective: To study clinical symptoms in patients with elevated lactate: pyruvate ratio

Subjects: Inclusion criteria: Patients showing any of the following:

Age: Newborns, infants

i. History of sibling deaths with similar symptoms in the absence of significant perinatal insults.

ii. Myoclonic seizures in a child with no significant perinatal insults.

iii. Weak cry or poor suck from birth despite being delivered at term with no asphyxia.

iv. Regression of milestones following a minor intercurrent illness.

vi. Cardiomegaly with no structural malformation on 2DEcho
vii. Hypotonia with difficult to elicit deep tendon reflexes

Older children : Motor-mental retardation with or without seizures in the absence of dysmorphology pointing to a syndromic constellation or significant perinatal insult, microcephaly, cataract especially congenital, subluxation of lenses and epilepsy of unknown etiology attending the outpatient department at Kasturba Hospital Manipal.

Exclusion criteria: Developmental delay due to birth asphyxia or seizures due to CNS involvement or meningitis.

MATERIALS AND METHODS

The study was conducted for a period of 2 years 6 months starting from July 2010 to December 2012 in a tertiary hospital of coastal Karnataka, India. Institutional ethical clearance was obtained for the study. Neonatal, infants, older children who showed one or more of neurodegenerative symptoms were enrolled in our study after obtaining informed consent from their parents. Detailed family histories regarding sibling death, stillbirth or abortion, affected siblings with similar disorder, parental consanguinity were collected from the parents. Clinical symptoms either developmental delay without asphyxia, seizures, regression of milestones, vomiting, hypotonia, truncal weakness, poor suck or weak cry at birth were evaluated. 304 children included in the study, of them 63.8% male and 36.2% were female.

The initial evaluative work up for inborn errors of metabolism consisted of metabolic screening tests which included arterial blood gas analysis, estimation of pyruvate and lactate. Arterial blood was collected from individuals who showed symptoms suggestive of inborn errors of metabolism for screening tests. Vaccutainers with grey top which contained sodium fluoride as antiglycolytic agent and EDTA as anticoagulant was used to collect blood for estimation of lactate and it's levels were measured using in Cobas 6000 autoanalyser using lactate assay kit by Roche Diagnostics. Pyruvate is highly unstable, blood was deproteinised with 1M perchloric acid immediately after sampling. LDH and NADH obtained from Sisco and pyruvate levels were estimated by enzymatic method using standard protocol [19],[20]. Heparin rinsed syringe was used to collect sample for blood gas analysis. Lactate: pyruvate ratio >25, lactate level >22 mg/dl (normal 5-20mg/dl) and ammonia more than 80 µg/dl (normal 20-70 µg/dl) were considered as elevated and within the range as normal by referring normal range for that age group. Significant elevation in lactate: pyruvate ratio > 25 is considered strongly suggestive of RC. Finally all patients with elevated lactate: pyruvate ratios were evaluated for clinical symptoms. For data analysis, score 1 was given for normal biochemical parameter or absence of symptoms and score 2 for elevated biochemical parameters or presence of symptoms. All data gathered were analysed by using Statistical Package for Social Science Version 16. Clinical features and biochemical parameters were reported as frequency with percentage.

RESULTS

During two and half year's period of study we have evaluated 304 samples which consisted of 63.8% male and 36.2% female. About 90 % of the participants were from Karnataka. More than 50 % of study group was of 1 month to 4 yrs age group as given in Table 1. Of total, 147 showed positive biochemical findings either metabolic acidosis, elevated lactate or lactate: pyruvate ratio. 81 participants were with elevated lactate: pyruvate ratio (Table 2).

Characteristics	**n=304 (%)**
Sex	
Male	63.8%
Female	36.2%
Region	
Udupi district	37.8%
Outside Udupi within Karnataka	50%
Outside Karnataka	12.2%
Age	
Less than 1 month	13.5%
1 month to 1 yr	37.2%
1 yr to 4 yrs	24%
4 yrs to 12 yrs	21.7%
Above 12 yrs	3.6%
Family history	
Affected siblings	65 (Alive : 26; succumbed : 39)(21.38%)
Consanguinity	60 (19.73%)
H/O stillbirth/abortion	30 (9.8%)

Table 1: Region, age, sex-wise distribution and family history of total participants

Test	**n= 304**
L:P ratio	Elevated in 81(26.6 %)
Lactate	Elevated in 77 (25.32%)
ABG	Acidosis in 65 (21 %)

Table 2: Results of metabolic screening tests

Symptoms	**n=81**
Developmental delay	50 (61.7%)
Seizures	37 (45.6%)
Hypotonia	21 (26%)
Truncal weakness	18 (22%)
Poor feeding	15 (18.5%)
Vomiting	12 (14.8%)
Weak cry at birth	09 (11.1%)
Development regression	05 (0.05%)

Table 3: Clinical symptoms in patients with elevated lactate: pyruvate ratio

DISCUSSION

We evaluated 304 children with suspected mitochondrial disorder. Among them 81 had elevated lactate: pyruvate ratio and were analysed further. Our study showed suspected mitochondrial disorder in more than 50% of study population based on elevated lactate, lactate: pyruvate ratio and metabolic acidosis. Of total 77 had elevated lactate level. Plasma lactate level reflects equilibrium between its production and consumption by various tissues. Lactate is the end product of anaerobic glycolysis, which serves as the main source of energy for heart, muscles and kidney. Hyperlactatemia can be observed in inherited metabolic disorder [19]. Scaglia et al reported elevated lactate level in about 68% of patients with definite mitochondrial disorder. They also commented that all diagnostic criteria equally contribute and there is no gold standard to confirm presence of mitochondrial disorder [8], [12]. Elevated lactate is a nonspecific marker for mitochondrial disorder and it causes metabolic acidosis, 65 patients showed metabolic acidosis by arterial blood gas analysis.

Of total 81 showed elevated lactate: pyruvate ratio. Lactate: pyruvate molar ratios in plasma are an index of oxidation /reduction status in cytoplasm [3]. A recent study observed a strong positive association between lactate: pyruvate ratio and blood lactate in non-pyruvate dehydrogenase congenital lactic acidosis. Lactate: pyruvate ratio >25 is suggestive of a primary respiratory chain dysfunction. This supports finding of mitochondrial disorder in our center based on biochemical tests along with clinical symptoms. Normal lactate, pyruvate level will not exclude mitochondrial disorder as it rises following metabolic crisis or exercise. Further study about ketone body ratio and blood alanine may help to narrow down the disorder [3],[17],[21]. Final diagnosis of mitochondrial disorder relies on enzyme studies, muscle biopsy and molecular analysis.

Based on underlying pathogenesis of the diseases, mitochondrial disorder may show variety of clinical symptoms. In our study, children with elevated lactate: pyruvate ratio developmental delay was predominant symptom, followed by seizures, hypotonia, muscle weakness, poor feeding, vomiting, weak cry at birth and developmental regression. Neurological and neuromuscular disorders are the most common symptoms of mitochondrial disorder and many study reported high incidence of same. Identifying the clinical symptom is one of the markers to start further work up and needed for initiating early intervention as some disorders cause irreversible damage to brain. Parents would identify weak cry at birth and poor sucking and seek medical advice and primary healthcare provider needs to direct them for further metabolic workup. Creating awareness about basic information of disorders in parents and establishing guidelines for primary care physicians may help in initiating early interventions and reducing sequelae.

CONCLUSION

Inborn Errors of Metabolism are individually rare but collectively common. Diagnosis of mitochondrial disorder a type of IEM is challenging as there is no single sensitive biomarker to exclude or confirm it. In spite of getting molecular knowledge regarding mitochondrial disorder, there is no specific noninvasive less expensive diagnostic tool. Positive symptoms or biochemical parameters may help in diagnosis but negative does not lower the suspicion of metabolic disorder. Each child with suspected clinical symptoms should undergo basic diagnostic panel, which may help in treatment. Children with delay in attaining milestones despite being born at term without asphyxia need to be referred for metabolic work up. Further there is a need to study plasma alanine, lactate: pyruvate ratio and beta-hydroxy butyrate: acetoacetate ratio simultaneously when there is a symptom in patients which may narrow down the diagnosis.

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