Role of baseline biochemical work up in the diagnosis of possible respiratory chain disorder in clinically suspected children

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ABSTRACT

Introduction and Aim: Respiratory chain disorder is a genetic defect in electron transport chain which involves ATP generation. There is no single screening or confirmatory test for its diagnosis, hence combination of clinical symptoms and biomarkers are utilized despite the advancement of techniques. The present study conducted to investigate the usefulness of baseline biochemical metabolic workup in clinically suspected children for diagnosis of possible respiratory chain disorder.

Materials and Methods: Clinical and baseline metabolic workup results of 385 children with clinically suspected inborn errors of metabolism was collected from their medical records. These results were utilized to classify them as possible respiratory chain disorder.

Results: Of the 385 children with clinically suspected to have inborn errors of metabolism, 99 were classified as possible respiratory chain disorder based on the applied criteria. Nearly 50 % of the cases had positive family history however; the majority of them visited the hospital only after the appearance of symptoms such as developmental delay, seizures, hypotonic, etc.

Conclusion: Baseline metabolic work up may be used to identify possible respiratory chain in children with family history and or initial clinical symptoms to initiate further diagnosis and timely intervention. Educating parents and primary care physician in this regard may be useful.

Keywords: Mitochondrial disorder; liquid chromatography; elevated lactate; lactate: pyruvate ratio.

INTRODUCTION

The mitochondrial disorder occurs due to the genetic defects of energy metabolism and includes pyruvate dehydrogenase complex, the Kreb's cycle and the respiratory electron transport chain- involved in the final steps of ATP generation. The occurrence of respiratory chain disorder is 1 in 5000 to 10,000 births (1-3). The first case of respiratory chain disorder was identified about 56 years ago by Luft and later Theoder Leber in the year 1981 described in adult-onset blindness unknowingly. In the subsequent years, a tremendous development has occurred in the field of mitochondrial disorder which has led to the detection of more than 200 mutations of the mitochondrial genome (2, 4, 5). Mitochondrial respiratory chain disorder may be due to acquired or mutations in mitochondrial DNA or nuclear DNA (6).

As the knowledge increased in the area of mitochondrial disorders, the spectrum of clinical symptoms also has expanded dramatically. They usually involve multisystem, progressively disturbing the total functioning of the organism. Being the powerhouse, pathologic changes in the mitochondria deplete the adenosine triphosphate (ATP) and affects the activity of the cell. As metabolic rate is high in skeletal muscle, brain, and heart they are more vulnerable organs for ATP depletion. Depletion in ATP may also accelerate alternative pathways, resulting in the formation of elevated lactate, which may be injurious to the organism.

Respiratory chain disorders (RCD) manifest with signs and symptoms of a highly variable pattern of organ dysfunction. Detection requires a knowledge of their characteristic signs and symptoms as well as mitochondrial biochemistry and genetics. Studies found that serum lactate is elevated in most of the cases with RCD (3, 7-9) and it is also evident that sometime it varies (10-13). Preprandial and postprandial measurement of lactate (14), serum lactate: pyryvate ratio, alanine, carnitine profile, urine and plasma amino

acids and organic acids are the useful biochemical markers for diagnosis and differential diagnosis of mitochondrial disorder (15-17). There is no single diagnostic marker hence work-up may be difficult. Studies have used mitochondrial disorder criteria for diagnosing the definite mitochondrial disorder using scoring system. The scoring system was established and utilized several clinical symptoms, laboratory investigation and MRI findings for this purpose. The Walker criteria modified by Bernier et al considered family history such as stillbirth related with a decreased intrauterine movement, neonatal death, pediatric features such as movement disorder, neonatal hypertonia, neonatal hypotonia, weakness, muscle wasting, exercise intolerance, severe failure to thrive, ptosis and cardiomyopathy as clinical criteria for respiratory chain disorder. One or more metabolic markers of respiratory chain dysfunction are considered the minor laboratory criteria for respiratory chain disorder. G.Oliviera used Bernier's major and minor criteria for the classification of the Mitochondrial disorder. Mitochondrial respiratory chain (MRC) disorder was considered 'definite' if two major or one major and two minor criteria were present. It was considered as 'probable' if one major and one minor or at least three minor criteria were present and as 'possible' if one major or two minor criteria (one of which must be laboratory) were present (7,8,18). In our present study, we utilized these criteria partially and attempted to detect possible respiratory chain disorder based on clinical symptoms and baseline metabolic work-up which includes lactate, lactate:pyruvate ratio, ammonia and arterial blood gas analysis.

MATERIALS AND METHODS

This retrospective study was conducted after obtaining Institutional ethical committee clearance. Children of age group 0 to 14 years with clinical suspicion of IEM who attended Paediatric outpatient department of Kasturba Hospital, Manipal, Manipal Academy of Higher Education were included for this study. Symptoms included weak cry at birth, poor suck at the breast, history of sibling death, early breath holding spells, seizures, regression of milestones, excessive lethargy, hypotonia, developmental delay. Children in the above age group with perinatal brain injury, brain trauma/tumor, infections to central nervous system and chromosomal anomalies were excluded from the study. During the 3 ½ years period from July 2010 to December 2013, we have included data from 385 children as per our criteria, using convenient sampling method. Results of baseline biochemical metabolic workup which included arterial lactate, pyruvate(done on perchlorate sample), ammonia and arterial blood gas analysis were collected from the medical record of selected children and analysed. Results were considered abnormal if lactate: pyruvate ratio was more than 25 (normal <25), arterial lactate level as more than 22 mg/dL (normal 5-20 mg/dL) and ammonia as more than 90 µg/dL (normal 20-70 µg/dL).

We have defined children as possible respiratory chain disorder based on clinical symptoms, history, and baseline metabolic workup result in two categories. a. Clinical symptoms or history suggestive of IEM with elevated lactate, elevated lactate: pyruvate ratio, normal ammonia with or without metabolic acidosis. b. Clinical symptoms or history suggestive of IEM with elevated lactate, elevated lactate: pyruvate ratio, elevated ammonia with or without metabolic acidosis

Statistical analysis

Statistical analysis included descriptive statistics was done using SPSS version 15.

RESULTS

Of the 385 children with clinical symptoms suggestive of inborn errors of metabolism 99 were considered as possible respiratory chain disorder based on our criteria. Among them 74 cases with possible respiratory chain disorder and 15 cases with possible RCD associated with organic aciduria (Table 1).

Table 1: Baseline biochemical metabolic work-up in possible respiratory chain disorder: n= 99

Lactate	L: P ratio	Ammonia	Metabolic acidosis	Frequency (%)
+	+	-	+	73 (73.7%)
+	+	-	-	11 (11%)
+	+	+	+	12 (12%)
+	+	+	-	03 (3%)

Table 2: Demographic characteristics of children with possible RCD: n=99

Age at diagnosis	
0-30 days	23 (23%)
1 month to 6 months	08 (8%)
6 months to 1 yr.	30 (30%)
1 yr. to 4 yrs.	29 (29%)
>4 years	09 (9%)
Gender-wise distribution	
Male	82 (82.8%)
Female	17 (17.2%)
Region-wise distribution	
Udupi District	29 (29.3%)
Outside Udupi within Karnataka	53 (53.5%)
Outside Karnataka	17 (17.2%)

The data presented in the above table 2 shows that the majority of children are males and 61% of children with possible RCD reported before one year of age.

Table 3: Family history, physical signs and symptoms at presentation in possible RCD: n=99

Symptoms at presentation	Birth history	Family history	Physical signs	Frequency
+	+	+	+	3
+	+	-	+	3
+	-	+	+	22
+	-	-	+	15
-	+	-	+	2
-	-	+	+	2
-	-	-	+	1
+	+	+	-	2
+	+	-	-	10
+	-	+	-	11
+	-	-	-	20
-	-	+	-	8

Of the 99 cases, 48 cases of possible RCD had a positive family history. Of them, 8 cases visited the hospital only because of family history suggestive of IEM, the remaining 40 cases who had a positive history, visited the hospital only after the appearance of physical signs and symptoms.

Table 4: Detailed family history in children with possible RCD: n=99

Family history	Frequency (%)
Affected sibling	25 (25.3%)
Consanguinity	22 (22.2%)
Abortion	12 (12.1%)
Other family members affected	06 (6.1%)

The family history of sibling affection was obtained in 25 cases of possible RCD. Of them, 8 cases of possible RCD were asymptomatic with a positive work-up.

Table 5: Symptoms at presentation in children with possible RCD: n=99

Symptoms at presentation	Frequency (%)
Developmental delay	52 (52.5%)
Seizures	50 (50.5%)
Poor feeding at the breast	15 (15.2%)
Weak cry at birth	10 (10.1%)
Respiratory distress	11 (11.1%)
Vomiting	09 (9.1%)
Regression	07 (7.1%)
Failure to thrive	08 (8.1%)
Early breath holding spells	02 (2%)

Table 6: Physical signs in children with possible RCD: n=99

Physical signs	Frequency (%)
Hypotonia	32 (32.3%)
Hypertonia	20 (20.2%)
Absent DTRs	10 (10.1%)
DTR brisk	20 (20.2%)
Muscle weakness	27 (27.2%)
Truncal weakness	09 (09.1%)
Lethargy	05 (05.1%)
Calf muscle Firm	05 (05.1%)
Ptosis	02 (02.0%)

DISCUSSION

The mitochondrial disorder is associated with decreased energy production, which is heterogeneous by genetically, clinically and biochemically; respiratory chain disorder is one among this. By applying partial diagnostic criteria of this disorder in our study, we found that 22% (99 out of 385) of the studied cases belonged to possible respiratory chain disorder. A retrospective study from Australia reported that 20% of the referred cases were definite respiratory chain disorder, according to Bernier criteria (8). We could not further classify our cases as definite respiratory chain disorder for lack of further confirmatory results. In our study of the 99 cases of possible RCD, 15 cases had elevated plasma ammonia level with associated metabolic acidosis in 12, which may be due to the association of organic aciduria along with respiratory chain disorder.

Almost all our cases with possible RCD were born at term. The majority of (90.9%) cases reported below four years of age, which is in accordance with other studies, which found that age at diagnosis for most of the cases ranges from 0 to 4 yrs. A study by Scalgia et al. found the mean age at presentation of the mitochondrial disorder as 3.3 years and by Kim *et al.* as 1.5 yrs. A study by Debray *et al.*, documented the median age at presentation to be seven months (3, 19, 20).

We found male predominance in children diagnosed with possible RCD which t male, which is similar to other studies (9, 20, 21). In our cases with possible RCD, about 50% cases had a family history suggestive of IEM. Among them, an affected sibling was the most common (25) followed by consanguinity, history of abortion and history of other affected family members. Eight of them with positive biochemical workup were

asymptomatic and screened in view of the affected sibling, which suggests the importance of screening children for baseline metabolic workup when there is the presence of strong positive family history and initiation of therapy before the appearance of symptoms. Studies showed that positive family history in children RCD varies from 10% to 47.4 % (3, 9). Our finding suggests that whenever there is family history it warrants the requirement of further metabolic workup.

Skeletal muscle, brain and heart are the major organs- highly energy dependent and susceptible to energy deficiency. Based on the underlying pathogenesis of the diseases, the mitochondrial disorder may show a variety of clinical symptoms. Neurological and neuromuscular symptoms are most common in mitochondrial disorders, and many studies have reported a high incidence of these symptoms (3, 20-27).

In our study, nearly 70% of cases were symptomatic at presentation. Developmental delay was most common followed by seizures, poor feeding, weak cry at birth, respiratory distress, vomiting, neuro-regression and early onset of breath holding spells. 75% of the cases had physical signs such as hypotonia, DTR brisk/ absent DTRs, muscle weakness, truncal weakness, lethargy, calf muscle firmness and ptosis. Identifying the clinical symptom is one of the markers to undertake further biochemical workup and initiate early intervention- as some disorders can cause irreversible damage to the brain.

A significant number of children were diagnosed as possible respiratory chain disorder in our study based on clinical criteria and baseline metabolic workup. Some of them showed signs such as weak cry at birth, poor sucking at the breast early breath holding spell which are easily identified by the parents. Establishing guidelines and creating awareness about basic information of disorders among parents, relatives and health care provider may be useful for timely detection and initiation of therapy if available.

Limitation of the study: We could not get the data about these investigations in healthy children as the collection of arterial blood gas is invasive, and routinely these tests are not done in healthy children. Most of the children in our study never underwent definitive testing hence we could not confirm the diagnosis.

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